Handbook of Experimental Pharmacology 197

Monika Schäfer-Korting
Editor

Drug Delivery

Springer

Handbook of Experimental Pharmacology

Volume 197

For further volumes:
http://www.springer.com/series/164

Monika Schäfer-Korting
Editor

Drug Delivery

Springer

Editor
Prof. Dr. Monika Schäfer-Korting
Freie Universität Berlin
Institut für Pharmazie
Königin-Luise-Str. 2-4
14195 Berlin
Germany
msk@zedat.fu-berlin.de

ISSN 0171-2004 e-ISSN 1865-0325
ISBN 978-3-642-00476-6 e-ISBN 978-3-642-00477-3
DOI 10.1007/978-3-642-00477-3
Springer Heidelberg Dordrecht London New York

Library of Congress Control Number: 2009933605

Cover design: SPi Publisher Services

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

The *Handbook of Experimental Pharmacology* is regarded as "one of the most authoritative and influential book series in pharmacology". It is said to provide "critical and comprehensive discussions of the most significant areas of pharmacological research". Obviously, this mission has already been followed for quite a while, the present volume being number 197. It all started in the first half of the twentieth century as *Handbuch der Experimentellen Pharmakologie*, founded by Dr. A. Heffter who, among other things, acted as the first Chairman of the German Society of Pharmacologists and as Rector of Berlin University in 1922. His pharmacological interests included among other things a crystalline glycoside from *Strophanthus kombe*. Accordingly, most volumes so far have covered various active pharmaceutical ingredients (principles), or rather groups thereof.

Recently, the scope has become broader. This is reflected by the flanking volumes and their subjects, namely Volume 196 which is on "Adverse Drug Reactions" and Volume 198 on "Fertility Control". Nevertheless, "Drug Delivery" is certainly a topic which might not have been included in the series during the first decades of the existence of the handbook. The problem in this context would not be "drug", although this would appear to be too broad a term in the context of a particular volume of a handbook on pharmacology. The point really is "delivery". Certainly, today everybody and every institution has to deliver, the word being used in the meaning of fulfilling expectations or reaching goals. The latter, in fact, comes close to what is meant here. The term "delivery" in general life is used in many contexts. In particular, it is related to commercial activity where goods have to be transported, essentially from the producer to the user. In this context, for centuries a major role has been played by vehicles, which can therefore be dubbed delivery vehicles. While as late as the nineteenth century such vehicles were still carriages moved by horse power, in a stricter sense today we might rather think in this context of fast self-propelled objects such as rather small goods vehicles which virtually dominate today's motorways.

Indeed, in modern drug treatment also, routes (of application) and exact time frames have become critical for success in an era in which surrogate parameters for efficacy are increasingly replaced by true outcome parameters.

The "Fundamentals" section of the table of contents of this present volume introduces three terms which can be called key words of our time. In first place is "targeting". Active principles must definitely get to where they should be. This has been familiar to the military for thousands of years, and more recently been has highlighted in this field, coining the term "surgical warfare". The idea is to increase benefit-to-risk ratios by preventing what soldiers now call "collateral damage".

The next term is "nanomedicine". This word reflects a broader term, which is "nanotechnology". The term and the concept behind it have been greatly influenced by the book *The Coming Era of Nanotechnology*, published in 1986 by Dr. E. Drexler. This work reflected an earlier one by Dr. N. Taniguchi, who had defined nanotechnology as early as 1974. The point in our context is that nanotechnology can, among other things, provide minute particles called nanoparticles as potential carriers for active pharmaceutical ingredients. This approach in a way reflects a natural approach used by the mammalian body, which is protein targeting. Whatever the primary material for production might look like, we are talking about carriers. One of the meanings of a "carrier" is a human being carrying goods for third parties, another term being "porter". Today, the term carrier is used in many different contexts, one being aircraft carrier, essentially a ship from which military fighter aircraft can be launched. In this way targets can be reached which would be beyond the range of the aircraft alone.

The third term is "biosensing". This means the use of biosensors, biological structures capable of identifying particular analytes. Using metaphors from the military field for the final time, we might think of the radar systems which help both the aircraft carrier and the carried aircraft to perform their mission.

By now it has become obvious that very specific devices are essential to perform the task of drug delivery as well as possible – often to save lives and quality of life. Indeed, today there is a wide spectrum to be covered here, ranging from liposomes to drug-delivering medical devices. When it comes to details, it is certainly important whether drug delivery is considered in the context of therapeutics for systemic or topical use. At least with one particular organ, namely the skin, there is something which could be called the dual use option: while carriers can be required simply to optimize topical treatment of skin disease, transdermal delivery is also a relevant option in everyday drug treatment today when aiming for systemic drug effects.

When planning the present volume, I was impressed by the breadth and depth of current knowledge on drug delivery. Shortly before finally handing over the manuscript to the publisher for printing, I have to admit that I am even now most impressed – and I do hope that this enthusiasm will also be felt by the relevant scientific community when looking at this new book. Certainly, the book would not in the least have been possible without the support of 16 pre-eminent contributors (and in some cases their collaborators) from various specialties or sub-specialties of the life sciences. This could only happen because of the invitation and the

continuing support from the series editor-in-chief, my distinguished colleague Dr. Walter Rosenthal from Berlin, to whom I am indeed most grateful. Moreover, thanks are due to Ms. Susanne Dathe from Springer Heidelberg for continuous editorial support. Finally, I would like to thank for technical assistance Ms. Barbara Brüggener, who helped me with the handling of the manuscripts.

Berlin, November 2009 *M. Schäfer-Korting*

Contents

Contributors

Frank Alexis
Laboratory of Nanomedicine and Biomaterials and Department of Anesthesiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA; MIT-Harvard Center for Cancer Nanotechnology Excellence, Cambridge, MA 02139, USA; Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA 02139, USA

Andreas Bernkop-Schnürch
Institute of Pharmacy, Universität Innsbruck, Josef-Moeller-Haus, Innrain 52, 6020 Innsbruck, Austria

Michael Bur
Biopharmaceutics and Pharmaceutical Technology, Saarland University, 66123 Saarbrücken, Germany

Jonathan J. Elsner
Faculty of Engineering, Department of Biomedical Engineering, Tel Aviv University, Tel Aviv 69978, Israel

Omid C. Farokhzad
Laboratory of Nanomedicine and Biomaterials and Department of Anesthesiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA; MIT-Harvard Center for Cancer Nanotechnology Excellence, Cambridge, MA 02139, USA

Christian Fillafer
Department of Pharmaceutical Technology and Biopharmaceutics, Althanstrasse 14, 1090 Vienna, Austria

Franz Gabor
Institut für Pharm. Technologie, Universität Wien, Athanstr. 4, 1090 Wien, Austria

Orly Grinberg
Faculty of Engineering, Department of Biomedical Engineering, Tel Aviv University, Tel Aviv 69978, Israel

Richard H. Guy
Department of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath, BA2 7AY, UK

Rainer Haag
Institut für Chemie und Biochemie – Organische Chemie, Freie Universität Berlin, Takustr. 3, 14195 Berlin, Germany

Regine Heilbronn
Institut für Virologie, Charité – Universitätsmedizin Berlin, Hindenburgdamm 27, (HBD 27), 12203 Berlin, Germany, regine.heilbronn@charite.de

Stephanie Hein
Biopharmaceutics and Pharmaceutical Technology, Saarland University, 66123 Saarbrücken, Germany

Andreas Henning
Biopharmaceutics and Pharmaceutical Technology, Saarland University, 66123 Saarbrücken, Germany

Juliane Hombach
University of Innsbruck, Innrain 52, 6020 Innsbruck, Austria

Mark Kendall
Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, Building 75 – Cnr of College and Cooper Road, Brisbane Qld 4072, Australia

Jayant Khandare
Institut für Chemie und Biochemie, Freie Universität Berlin, Takustrasse 3, 14195 Berlin, Germany

Hans Christian Korting
Klinik und Poliklinik für Dermatologie und Allergologie, Ludwig-Maximilians-Universität München (LMU), Frauenlobstraße 9-11, 80337 München, Germany

Amir Kraitzer
Faculty of Engineering, Department of Biomedical Engineering, Tel Aviv University, Tel Aviv 69978, Israel

Randy Kurz
Center for Biotechnology and Biomedicine, University of Leipzig, Deutscher Platz 5, 04103 Leipzig, Germany

Robert S. Langer
MIT-Harvard Center for Cancer Nanotechnology Excellence, Cambridge, MA 02139, USA; Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA 02139, USA; Department of Chemical Engineering, Massachusetts Institute of Technology MIT, Cambridge, MA 02139, USA

Claus-Michael Lehr
Department of Biopharmaceutics and Pharmaceutical Technology, Im Stadtwald, 66123 Saarbrücken, Germany

Rainer H. Müller
Department of Pharmaceutics and Biopharmaceutics, Free University of Berlin, Kelchstrasse 31, 12169 Berlin, Germany

Lukas Neutsch
Department of Pharmaceutical Technology and Biopharmaceutics, Universität Wien, Althanstrasse 14, 1090 Vienna, Austria

Yuichiro Ogura
Department of Ophthalmology and Visual Science, Nagoya City University, Graduate School of Medical Sciences, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya, Aichi 467-8601, Japan

Heidrun Potschka
Tierärztliche Fakultät, Ludwig-Maximilians-Universität, Veterinärstr. 13, 80539 München, Germany; Institute of Pharmacology, Toxicology, and Pharmacy Ludwig-Maximilians-University Munich, München, Germany

Eric M. Pridgen
MIT-Harvard Center for Cancer Nanotechnology Excellence, Cambridge, MA 02139, USA; Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Gerda Ratzinger
Department of Pharmaceutical Technology and Biopharmaceutics, Universität Wien, Althanstrasse 14, 1090 Vienna, Austria

Andrea Robitzki
Institut für Biochemie, Universität Leipzig, Brüderstraße 34, 04103 Leipzig

Monika Schäfer-Korting
Institut für Pharmazie (Pharmakologie und Toxikologie), Freie Universität Berlin, Königin-Luise-Straße 2-4, 14195, Berlin, Germany

Marc Schneider
Pharmaceutical Nanotechnology, Saarland University, Im Stadtwald, 66123 Saarbrücken, Germany

Eliana B. Souto
Faculty of Health Sciences, Fernando Pessoa University, Rua Carlos da Maia, 296, 4200-150 Porto, Portugal

Vladimir P. Torchilin
Department of Pharmaceutical Sciences and Center for Pharmaceutical Biotechnology and Nanomedicine, Northeastern University, 360 Huntington Avenue, Boston, MA 02115, USA

Stefan Weger
Institute of Virology, Charité – Universitätsmedizin Berlin, Hindenburgdamm 27, 12203 Berlin, Germany

Dirk Wildemeersch
CONTREL RESEARCH, Technology Park, University of Gent, 9052 Gent (Zwijnaarde), Belgium

Michael Wirth
Department of Pharmaceutical Technology and Biopharmaceutics, Universität Wien, Althanstrasse 14, 1090 Vienna, Austria

Tsutomu Yasukawa
Department of Ophthalmology and Visual Science, Nagoya City University, Graduate School of Medical Sciences, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya, Aichi 467-8601, Japan

Meital Zilberman
Faculty of Engineering, Department of Biomedical Engineering, Tel Aviv University, Tel Aviv 69978, Israel

Passive and Active Drug Targeting: Drug Delivery to Tumors as an Example

Vladimir P. Torchilin

Contents

Abstract The paradigm of using nanoparticulate pharmaceutical carriers has been well established over the past decade, both in pharmaceutical research and in the clinical setting. Drug carriers are expected to stay in the blood for long time, accumulate in pathological sites with affected and leaky vasculature (tumors, inflammations, and infarcted areas) via the enhanced permeability and retention (EPR) effect, and facilitate targeted delivery of specific ligand-modified drugs and drug carriers into poorly accessible areas. Among various approaches to specifically target drug-loaded carrier systems to required pathological sites in the body, two seem to be most advanced – passive (EPR effect-mediated) targeting, based on the longevity of the pharmaceutical carrier in the blood and its accumulation in pathological sites with compromised vasculature, and active targeting, based on the attachment of specific ligands to the surface of pharmaceutical carriers to recognize and bind pathological cells. Here, we will consider and discuss these two targeting approaches using tumor targeting as an example.

V.P. Torchilin
Department of Pharmaceutical Sciences and Center for Pharmaceutical Biotechnology and Nanomedicine, Northeastern University, 360 Huntington Avenue, Boston, MA, 02115, USA
e-mail: v.torchilin@neu.edu

M. Schäfer-Korting (ed.), *Drug Delivery*,
Handbook of Experimental Pharmacology 197,
DOI 10.1007/978-3-642-00477-3_1,

Keywords Drug delivery · Drug targeting · Tumors · Liposomes · Polymeric micelles

1 Drug Targeting: General Considerations

By drug targeting, one usually understands an increased accumulation of an active pharmaceutical ingredient (API) in the required area of the body compared to other tissues and organs mediated by a spontaneous or external force or targeting moiety. For the majority of pharmaceuticals currently in use, the specificity and activity of pharmaceuticals towards disease sites or individual diseases is usually based on the API's ability to interfere with local pathological processes or with defective biological pathways, but not on its selective accumulation in the specific intracellular compartment or in the target cell, organ or tissue. Usually, pharmaceutical agents, practically independently on the route of administration, are distributed within the body rather evenly, proportionally to the regional blood flow. Moreover, to reach the site of action, the API has to cross many biological barriers, such as other organs, cells and intracellular compartments, where it can be inactivated or express undesirable effects on organs and tissues that are not involved in the pathological process. As a result, to achieve a required therapeutic concentration of an API in a certain body compartment or certain tissue, one has to administer the drug in large quantities (thus increasing the cost of the therapy), the great part of which, even in the best case scenario, is just wasted in normal tissues, while cytotoxic and/or antigenic/immunogenic agents can become the cause of many negative side-effects.

Drug targeting can bring a solution to all these problems. In a very general sense, one understands drug targeting as the ability of the API to accumulate in the target organ or tissue selectively and quantitatively, independent of the site and method of its administration. Ideally, under such conditions, the local concentration of the agent at the disease site(s) should be high, while its concentration in other nontarget organs and tissues should be below certain minimal levels to prevent any negative side-reactions. The following advantages of drug targeting are evident: drug administration protocols may be simplified; the drug quantity required to achieve a therapeutic effect may be greatly reduced, as well as the cost of therapy; and drug concentration in the required sites can be sharply increased without negative effects on nontarget compartments. The same is true, to a great extent, for the use of many diagnostic agents.

Although the concept of drug targeting, suggested by Paul Ehrlich early in the twentieth century, considered a hypothetical "magic bullet" as an entity consisting of two components – the first one should recognize and bind the target, while the second one should provide a therapeutic action in this target – currently, the whole set of suggested targeting protocols includes many different approaches to targeted drug delivery. These approaches do not necessarily involve the use of specific targeting moieties. In certain cases various physical principles and/or some physiological

features of the target area may be utilized for a successful targeting of pharmaceuticals and pharmaceutical carriers.

The concept of targeted pharmaceuticals includes a coordinated interaction of several components: pharmaceutical agent, targeting moiety, pharmaceutical carrier (soluble polymers, microcapsules, microparticles, cells, cell ghosts, liposomes, and micelles) used to load many drug molecules per single targeting moiety, and a target. The recognition of the target may proceed on various levels: on the level of a whole organ, certain cells specific for this organ, or even individual components of these cells (cell surface antigens). Recognition on the molecular level is certainly the most universal form of target recognition, because in each organ or tissue certain components can be found specific only to that organ or tissue.

Currently, numerous approaches for drug targeting have been described, permitting the specific delivery of therapeutic and diagnostic pharmaceutical agents to a variety of tissues and organs; some of them are discussed in many review-type publications (see for example Francis and Delgado 2000; Gregoriadis 1977; Muzykantov and Torchilin 2003; Torchilin 1995).

Attempts have been made to directly administer drugs into the affected zone, to load APIs into certain carriers sensitive to specific values of pH or temperature in pathological areas, or even use certain external forces, such as magnetic fields or ultrasound, to guide drugs to required targets or to release APIs there. The passive accumulation of many drugs and drug carriers due to their extravasation through leaky vasculature (named the Enhanced Permeability and Retention [EPR] effect) works very well for tumors, infarcts, and inflammation areas. The use of specific delivery vectors (certain moieties possessing high specific affinity towards the target areas) can target drugs and drug carriers almost everywhere.

Direct drug administration into an affected area was used in the intracoronary infusion of thrombolytic enzymes for therapy of coronary thrombosis (Chazov et al. 1976), and in the intra-articular administration of nonhormonal drugs for the treatment of arthritis (Williams et al. 1996). However, the direct administration of a drug into an affected organ or tissue is technically quite difficult in a majority of cases; in addition, many diseases are spread over a variety of cells or tissues. All this limits the applicability of this approach to a very few clinical situations.

Various endogenous and/or exogenous physical factors have been shown to mediate targeted delivery of pharmaceuticals. This approach utilizes, for example, the differences in pH and temperature values between normal tissues and pathological areas (tumors, inflammation, etc.) that are characterized by acidosis (decreased pH) and hyperthermia. With this in mind, it was suggested to load various pharmaceutical agents onto pH- or temperature-responsive drug carriers that can change their properties and release an encapsulated agent when they are brought to the areas with lower (compared to normal) pH or higher temperature. The advantage of this approach is that even though the drug-loaded carrier is evenly distributed within the circulation, it degrades and releases the drug only in the target area. Moreover, the target area can be additionally heated from the outside by applying external heat or ultrasound irradiation. Thus, it was shown that on intravenous administration, the anti-cancer drug methotrexate accumulated in tumors in mice

several times faster when it was incorporated into temperature-sensitive liposomes and external heat was applied locally onto the tumor area (Weinstein et al. 1979). Drug-loaded pH-sensitive liposomes are also frequently used for experimental delivery of API and genetic material into a variety of compromised tissues (see Budker et al. 1996; Torchilin et al. 1993 for just a few of many reviews). In many cases, however, a pathological site does not differ much from normal tissues in terms of temperature or local pH value, which makes the use of targeting based on pH or temperature differences inapplicable.

An intentionally applied external magnetic field can also be used for the targeted delivery of pharmaceuticals. In this case, the API of choice has to be attached to a drug carrier possessing ferromagnetic properties. As a result, one can expect the accumulation of a drug-loaded ferromagnetic carrier in the area to which an external magnetic field is applied. High magnetic field gradient together with high blood flow velocity and accompanying high shear strength in large blood vessels do not allow this approach to work in blood vessels such as the aorta; however, magnetic field-mediated drug accumulation in smaller blood vessels with slower blood flow and located closer to the body surface was clearly demonstrated (Widder et al. 1983). Dextran-coated microparticles of iron oxide have been used to couple the thrombolytic enzyme streptokinase, and the preparation was successfully used for the targeted thrombus lysis of artificially formed thrombi in carotid arteries of experimental dogs when a small strong permanent magnet was implanted into the tissues next to a vessel in the area of thrombus (Torchilin et al. 1988). The local prevention of thrombosis in experimental dogs and rabbits was achieved by the intravenous application of autologous red blood cells loaded with ferromagnetic colloid compound and aspirin, when a strong magnet was positioned externally to the blood vessel where the thrombus was initiated (Orekhova et al. 1990). Quite a few examples exist (McBain et al. 2008; Pauwels and Erba 2007; Sun et al. 2008) of magnetic targeting of various anticancer drugs to tumors, when such drugs have been co-loaded together with magnetically sensitive nanoparticles into various pharmaceutical carriers and concentrated in tumors under the action of an external magnetic field. However, magnetic drug delivery has its limitations connected with the blood flow rate in the target, and is virtually impossible in large vessels or in "deep" tissues.

In this chapter, we will concentrate on drug targeting based on the EPR effect (passive targeting) and on the use of targeting moieties (active targeting) and will use as examples the studies related to drug delivery into tumors, which are the most numerous and advanced.

2 Concepts of Passive and Active Targeting

It is now a well-established phenomenon that under certain circumstances the endothelial lining of the blood vessel wall becomes more permeable than in the normal state. This was clearly demonstrated in many tumors (Hobbs et al. 1998; Jain 1999) and in infarcted areas (Palmer et al. 1984; Torchilin et al. 1992). As a

result, in such areas, large molecules and even relatively large particles, such as micelles and liposomes ranging from 10 to 500 nm in size, can leave the vascular bed and accumulate inside the interstitial space. Assuming these large (polymeric) molecules/particles are loaded with a pharmaceutical agent, they can bring this agent into the area with the increased vascular permeability, where the API can be eventually released from a carrier. Because the cut-off size of the permeabilized vasculature varies from case to case (Hobbs et al. 1998; Yuan et al. 1995), the size of a drug-carrying particle may be used to control the efficacy of such spontaneous "passive" drug delivery or EPR effect (Maeda 2003; Maeda et al. 2000; Fig. 1). This type of targeting requires drug delivery systems to be long-circulating (i.e., to stay in the blood for extended periods of time) in order to provide a sufficient level of accumulation in the target. The most usual way to keep drug carriers in the blood long enough is to "mask" them by modifying (grafting) their surface with certain water-soluble polymers with a well-solvated and flexible main chain, such as polyethylene glycol (PEG) (Klibanov et al. 1990; Torchilin and Trubetskoy 1995). The surface-grafted "protective" polymers effectively prevent (slow down) the opsonization of drug carriers and their clearance by the reticuloendothelial system. The approach is best developed for liposomes (Lasic and Martin 1995; Lasic and Papahadjopoulos 1998), although it has a rather broad applicability (Torchilin 1998). The anticancer drug doxorubicin incorporated into long-circulating PEG-coated liposomes, which is currently used in clinical conditions, demonstrates

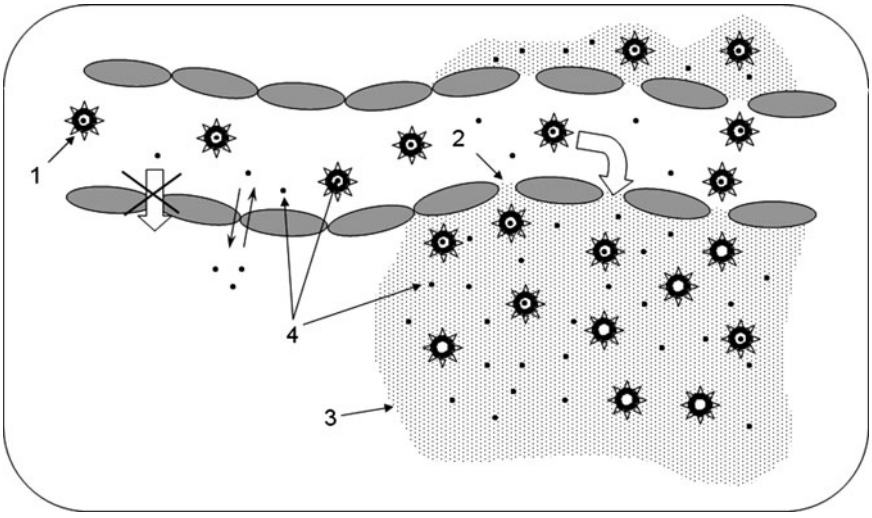


Fig. 1 Schematics of the Enhanced Permeability and Retention (EPR) effect or "passive" targeting. (1) Drug-loaded nanocarrier; it cannot extravasate through normal endothelium and only small molecules of free drug (4) can traverse normal endothelium to a certain extent in both directions; (2) gaps between endothelial cells appear in pathological areas (3) (such as tumors, infarcts, and inflammations), through which nanoparticles can extravasate and accumulate in such areas creating high local drug concentrations

high efficacy in EPR-based tumor therapy and strongly diminishes the side-effects (Gabizon 1995, 2001) characteristic of free doxorubicin. Long-circulating polymeric micelles (Torchilin 2001) may be used as carriers for drug delivery into tumors with a smaller cut-off size (Hobbs et al. 1998; Yuan et al. 1995), as was shown in Lewis lung carcinoma-bearing mice (Weissig et al. 1998).

Important advantages of prolonged circulation of drugs and drug carriers in the blood flow include the possibility of maintaining a required concentration of an API or drug carrier in the blood for a long time after a single administration; the ability to utilize the EPR effect for the accumulation of pharmaceuticals in the areas with leaky vasculature; and the possibility of enhancing targeting of drugs and drug carriers into the areas with a limited blood supply and/or low concentration of a target antigen, where an extended time is required to allow for a sufficient quantity of a drug in the target zone. More information regarding the importance of drug carrier longevity is given below.

It is important to mention, however, that in many pathological situations the integrity of vascular endothelium remains unaffected and there is no opportunity for EPR.

Many of the approaches to drug targeting described so far are not universal. Thus, direct administration of a drug into an affected organ or tissue may be technically difficult, or the disease site may be delocalized. Often, the affected area does not differ much from normal tissues in terms of vascular permeability, temperature or local pH value. Magnetic drug delivery also has limitations connected with the blood flow rate in the target. The most natural and universal way to impart to a nonspecific drug affinity towards its target is the binding of this drug with another molecule (usually referred to as a targeting moiety or vector molecule) capable of specific recognition and binding to a target site (Fig. 2). The following substances can be used as targeting moieties: antibodies and their fragments, lectins, other proteins, lipoproteins, hormones, charged molecules, mono-, oligo- and polysaccharides, and some low-molecular-weight ligands, such as folate. Monoclonal antibodies against characteristic components of target organs or tissues are the most frequently used vector molecules.

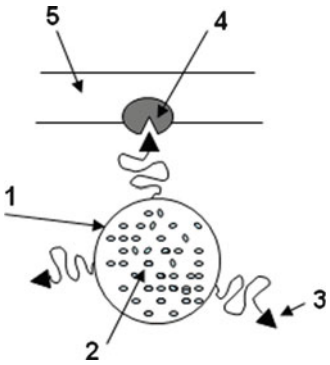


Fig. 2 Schematics of the specific ligand-mediated active targeting. Nanocarrier (1) loaded with the drug (2) is modified with the moieties of a specific ligand (3) capable of recognizing certain binding sites (4) on the cell surface (5). As a result, the carrier remains attached to the cell surface and releases its drug load there or can be internalized bringing the drug inside target cells

Direct coupling of a drug to a targeting moiety seems the simplest way to prepare a targeted drug. Immunotoxins represent the most vivid examples of this approach (Vitetta et al. 1983). A natural toxin can be "cut" into active moiety (the toxic one) and recognizing moiety, and then the latter is separated and the former is conjugated with an antibody. As a result, a toxic unit may be delivered only in those cells that express an appropriate antigen (usually cancer cells), while antigen-free cells will not be recognized by the immunotoxin and damaged. However, in this case every single antibody molecule is able to carry just one active moiety. Since toxic moieties of toxins/immunotoxins are extremely active (just one catalytic moiety of the plant toxin ricin can kill a cell if gets inside and destroys thousands of ribosomes), immunoxins may still find clinical application, primarily for cancer treatment (Goldmacher et al. 2002; Vitetta et al. 1983).

Another example of this kind is the attachment of various thrombolytic enzymes to some antibodies specific towards different components of thrombi. Thus, it was clearly demonstrated in hamsters and baboons that effective thrombolysis may be achieved by using the conjugate between single-chain urokinase-type plasminogen activator and a bispecific monoclonal antibody against this activator and fibrin (Imura et al. 1992). The data on enzyme–antibody conjugates for thrombolysis, as well as on a variety of antibodies used to deliver the thrombolytic therapy directly to the occlusion site, are numerous and well reviewed (Haber 1994; Khaw 2002).

Certain attempts have been also made to use direct drug–antibody conjugates for targeted treatment of malignant diseases, such as human small cell lung cancer (SCLC). Antibody against the proliferative compartment of mammalian squamous carcinomas was conjugated with daunomycin and sharply enhanced drug potency in the murine model (Ding et al. 1990). Murine monoclonal antibody NCC-LU-243 was conjugated with mitomycin C and used for the targeted therapy of nude mice with the transplanted antigen-positive cell line of human SCLC (Kubota et al. 1992).

In general, however, the load of a pharmaceutical agent onto a single targeting moiety should be much higher than a simple 1:1 ratio to make the whole approach beneficial and practically applicable. Alternatively, some soluble or insoluble carrier can be loaded with multiple active moieties and then conjugated additionally with the targeting unit according to the scheme suggested by Ringsdorf in the mid-1970s (Ringsdorf 1975). Different reactive and biocompatible soluble polymers can be used as soluble carriers, whereas the family of insoluble carriers includes microcapsules, nanoparticles, liposomes, micelles and cell ghosts. Various reservoir-type systems, such as liposomes or microcapsules, demonstrate the following important advantages over other drug carriers: (a) maximum volume at a given surface (i.e., maximum load of the drug); (b) few targeting moieties can carry multiple drug moieties loaded into the reservoir; (c) the possibility to control size and permeability.

To date, it has already been shown that body compartments and pathologies that can be successfully targeted via different mechanisms include components of cardiovascular system (blood pool, vascular walls, lungs, heart), reticuloendothelial system (liver and spleen); lymphatic system (lymph nodes and lymphatic vessels), tumors, infarcts, inflammations, infections, and transplants.

The parameters determining the efficacy of drug targeting include: the size of the target, blood flow through the target, number of binding sites for the targeted drug/drug carrier within the target, number and affinity of targeting moieties on an API molecule (drug carrier particle), and multipoint interaction of a drug/drug carrier with the target.

3 Pharmaceutical Carriers: Liposomes and Micelles as Examples

Numerous drug delivery and drug targeting systems, such as synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins, liposomes, and micelles (Cohen and Bernstein 1996; Müller 1991), are currently developed or under development. Their use aims to minimize drug degradation upon administration, prevent undesirable side-effects, and increase drug bioavailability and the fraction of the drug accumulated in the pathological area. To better achieve these goals, all listed drug carriers can be made slowly degradable, stimuli-reactive (for example, pH- or temperature-sensitive), and targeted (for example, by conjugating them with ligands specific towards certain characteristic components/receptors of the area of interest). In addition, drug carriers are expected to stay in the blood for prolonged time intervals (Lasic and Martin 1995; Torchilin and Trubetskoy 1995) in order to maintain the required therapeutic level of pharmaceuticals in the blood over an extended period, to allow for their slow accumulation in pathological sites with affected and leaky vasculature (tumors, inflammations, and infarcted areas) via the enhanced permeability and retention (EPR) effect (Maeda et al. 2000; Palmer et al. 1984), and facilitate targeted delivery of specific ligand-modified drugs and drug carriers into poorly accessible areas (Torchilin 1998).

Pharmaceutical drug carriers, especially those for parenteral administration, are expected to be easy and reasonably cheap to prepare, biodegradable, have small particle size, possess high loading capacity, demonstrate prolonged circulation, and, ideally, accumulate specifically or nonspecifically in required pathological sites in the body (Gref et al. 1994).

The paradigm of using nanoparticulate pharmaceutical carriers to enhance the in vivo efficiency of many drugs, anti-cancer drugs first of all, has been well established over the past decade both in pharmaceutical research and in the clinical setting and does not need any additional proofs. Various pharmaceutical nanocarriers, such as nanospheres, nanocapsules, liposomes, micelles, cell ghosts, lipoproteins and some others are widely used for experimental (and even clinical) delivery of therapeutic and diagnostic agents (Alonso 2004; Gregoriadis 1988; Müller 1991; Rolland 1993). Surface modification of these carriers is often used to control their properties in a desirable fashion and make them simultaneously perform several different functions. The most important results of such modification(s) include increased longevity and stability in the circulation, changed biodistribution, targeting effect, sensitivity to

stimuli (pH or temperature), and contrast properties. Frequent surface modifiers (used separately or simultaneously) include: soluble synthetic polymers (to achieve carrier longevity); specific ligands, such as antibodies, peptides, folate, transferrin, sugar moieties (to achieve targeting effect); pH- or temperature-sensitive copolymers (to impart stimuli sensitivity); and chelating compounds, such as EDTA, DTPA or deferoxamine (to add a diagnostic/contrast moiety onto a drug carrier). Evidently, different modifiers can be combined on the surface of the same nanoparticular drug carrier providing it with a combination of useful properties (for example, longevity and targetability, targetability and stimuli sensitivity, or targetability and contrast properties).

Liposomes, artificial phospholipid vesicles, can be obtained by various methods from lipid dispersions in water. Preparation of liposomes, their physico-chemical properties and possible biomedical application have already been extensively discussed in several monographs (Gregoriadis 2007; Lasic and Martin 1995; Lasic and Papahadjopoulos 1998; Torchilin and Weissig 2003; Woodle and Storm 1998). To date, many different methods have been suggested for preparing liposomes of different sizes, structure and size distribution. To increase liposome stability towards the action of the physiological environment, cholesterol is incorporated into the liposomal membrane (sometimes up to 50% mol). The size of liposomes depends on their composition and preparation method and can vary from around 50 nm to greater than 1 μm in diameter. MLVs (multilamellar vesicles) range from 500 to 5,000 nm and consist of several concentric bilayers. LUVs (large unilamellar vesicles) range from 200 to 800 nm. SUVs (small unilamellar vesicles) are around 100 nm (or even smaller) in size and are formed by a single bilayer (see Fig. 3). The encapsulation efficacy for different substances is also variable depending on the liposome composition, size, charge, and preparation method. The use of the reverse phase evaporation method (Szoka and Papahadjopoulos 1980) permits the inclusion of 50% or more of the substance to be encapsulated from the water phase into the liposomes. Besides, a variety of methods have been developed to obtain lyophilized liposomal preparations possessing good storage stability (Madden et al. 1985). The in vitro release rate of different compounds from liposomes, including proteins of moderate molecular weight, such as lysozyme or insulin, is usually under 1% per hour (assuming that the incubation temperature sufficiently differs from the phase transition temperature of a given phospholipid).

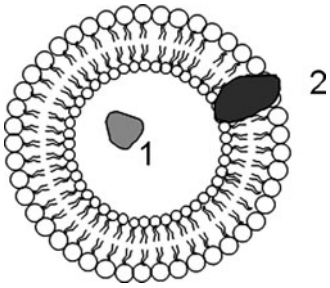


Fig. 3 Liposomes can vary in size between 50 and 1000 nm. Structures and drug loading: soluble hydrophilic drugs are entrapped into the aqueous interior of the liposome (1), while poorly soluble hydrophobic drugs are localized in the liposomal membrane (2)

In vivo, this parameter can vary within wide limits (minutes to hours) and depends on the liposome membrane composition and cholesterol content, and location of the liposome in the body.

Liposomes are biocompatible, cause no or very little antigenic, pyrogenic, allergic and toxic reactions; they easily undergo biodegradation; they protect the host from any undesirable effects of the encapsulated API, at the same time protecting an entrapped API from the inactivating action of the physiological medium; and, last but not least, liposomes are capable of delivering their content inside many cells. Different methods of liposomal content delivery into the cytoplasm have been elaborated (Connor and Huang 1986). According to one of these methods, the liposome is made of pH-sensitive components and, after being endocytosed in the intact form, it fuses with the endovacuolar membrane under the action of lowered pH inside the endosome, releasing its content into the cytoplasm. In addition, liposomes have been shown to fuse with the microscopic pores on the cell surface (which appear, for example, as a result of ischemia) (Khaw et al. 2001, 1995) and deliver their contents including DNA into the cell cytoplasm. Liposomes modified on the surface with TAT-peptide (Torchilin and Levchenko 2003) (or other cell-penetrating peptides, such as Antp, penetratin, or synthetic polyarginines; see the review in Torchilin 2008) are also capable of delivering their cargo inside cells (Torchilin et al. 2003a).

Liposomes have been considered promising drug carriers for over two decades (Ringsdorf 1975). However, upon intravenous administration, plain liposomes are very quickly (usually within 15–30 min) opsonized and sequestered by cells of the reticuloendothelial system (RES), primarily by the liver (Ringsdorf 1975). From this point of view, the use of targeted liposomes, i.e., liposomes with a specific affinity for the affected organ or tissue, may both increase the efficacy of liposomal drug, and decrease the loss of liposomes and their contents in RES.

To obtain targeted liposomes, different methods have been developed to bind corresponding vectors (antibodies) to the liposome surface. These methods are relatively simple and allow binding of sufficient numbers of antibody molecules to a liposome surface without affecting the liposome integrity and antibody affinity and specificity. At present, over 100 antibody molecules can be bound to a single 200 nm liposome, allowing for firm multi-point liposome binding with a target. The routine methods for antibody coupling to liposomes include covalent binding to a reactive group on the liposome membrane, and hydrophobic interaction of proteins specifically modified with hydrophobic residues with the membrane (Francis and Delgado 2000; Ringsdorf 1975).

A potentially important problem with liposomes (or any other microparticulate drug carrier) is their inability to reach extravascular targets.

Despite some promising results with immunoliposomes as pharmaceutical carriers, the whole approach is limited because of the short lifetime of liposomes and immunoliposomes in the circulation. The majority of antibody-modified liposomes still end up in the liver as a consequence of an insufficient time for the interaction between the target and targeted liposome. This is certainly the case when the target has a diminished blood supply (ischemic or necrotic areas). Even high liposome

affinity towards the target cannot provide substantial liposome accumulation because of the small quantity of liposomes passing through the target with the blood during the time period when liposomes are still present in the circulation. The same lack of targeting can happen if the concentration of the target antigen is very low, and even sufficient blood flow (and liposome passage) through the target does not result in good accumulation due to the small number of "productive collisions" between antigens and immunoliposomes. In both cases, better accumulation can be achieved if liposomes can remain in the circulation long enough. This is why long-circulated (usually PEGylated) liposomes have attracted so much attention over the last decade.

Micelles represent colloidal dispersions with particle size from 5 to 50–100 nm. At a certain concentration and temperature, such colloids are spontaneously formed by amphiphilic or surface-active agents (surfactants), molecules of which consist of two clearly distinct regions with opposite affinities towards a given solvent (Mittal and Lindman 1991). At low concentrations, these amphiphilic molecules exist separately as unimers; however, as their concentration is increased, aggregation begins to take place at a certain concentration called the critical micelle concentration (CMC). The aggregates known as micelles include several dozens of amphiphilic molecules and usually have a shape close to spherical. Hydrophobic fragments of amphiphilic molecules form the core of a micelle, which can solubilize poorly soluble pharmaceuticals (Lasic 1992). This solubilization phenomenon was extensively investigated and reviewed in many publications (see, for example, Attwood and Florence 1983). In aqueous systems, nonpolar molecules will be solubilized within the micelle core, polar molecules will be adsorbed on the micelle surface, and substances with intermediate polarity will be distributed along surfactant molecules in certain intermediate positions (Fig. 4).

Polymeric micelles are usually prepared of amphiphilic block-copolymers of hydrophilic PEG and various hydrophobic blocks. Numerous studies (see, for example, Gao and Eisenberg 1993; Hunter 1991; Kabanov et al. 1992) have been published on polymeric micelle formation and properties. Many good recent reviews exist dealing with various aspects of polymeric micelle preparation, physicochemical and biological properties, and possible applications as pharmaceutical carriers (Adams et al. 2003; Jones and Leroux 1999; Kabanov et al. 2002a, b; Kakizawa and Kataoka 2002; Kwon 1998, 2003; Lukyanov and Torchilin 2004; Otsuka et al. 2003; Torchilin 2001).

In the majority of cases, amphiphilic unimers include PEG blocks with a molecular weight from 1 to 15 kDa as corona-forming blocks, and the length of a hydrophobic core-forming block is close to or somewhat lower than that of a hydrophilic block (Cammas et al. 1997). Though some other hydrophilic polymers may be used as hydrophilic blocks (Torchilin et al. 1995), PEG still remains the corona block of choice. At the same time, a variety of polymers may be used to build hydrophobic core-forming blocks: propylene oxide (Miller et al. 1997), L-lysine (Katayose and Kataoka 1998), aspartic acid (Harada and Kataoka 1998), β-benzoyl-L-aspartate (La et al. 1996), γ-benzyl-L-glutamate (Jeong et al. 1998), caprolactone (Allen et al. 1998), and D, L-lactic acid (Hagan et al. 1996). In certain

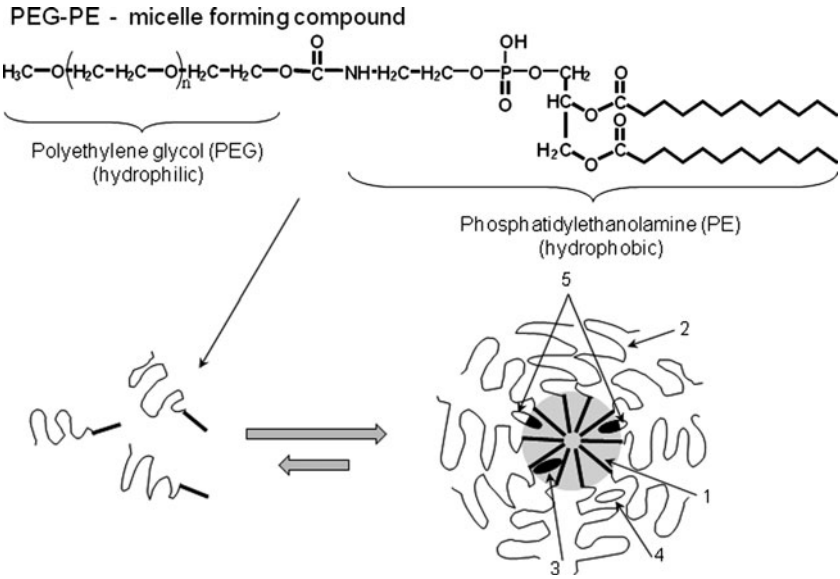


Fig. 4 A micelle as it self-assembles in the aqueous medium from amphiphilic unimers (such as polyethylene glycol–phosphatidylethanolamine conjugate, PEG–PE; see on the top) with the hydrophobic core (1) and hydrophilic corona (2). In water, nonpolar molecules will be solubilized within the micelle core (3), polar molecules will be adsorbed on the micelle surface (4), and substances with intermediate polarity will be distributed along surfactant molecules in certain intermediate positions (5)

cases, the starting copolymers can be prepared from two hydrophilic blocks and then one of those blocks is modified by the attachment of a hydrophobic pharmaceutical agent (such as paclitaxel, cisplatin, antracyclin antibiotics, hydrophobic diagnostic units, etc.) yielding amphiphilic micelle-forming copolymers (Katayose and Kataoka 1998; Kwon and Kataoka 1995; Trubetskoy et al. 1997).

In some cases, phospholipid residues – short but extremely hydrophobic due to the presence of two long-chain fatty acyl groups – can also be successfully used as hydrophobic core-forming groups (Trubetskoy and Torchilin 1995). The use of lipid moieties as hydrophobic blocks capping hydrophilic polymer (such as PEG) chains can provide additional advantages for particle stability when compared with conventional amphiphilic polymer micelles, due to the existence of two fatty acid acyls which might contribute considerably to an increase in the hydrophobic interactions between the polymeric chains in the micelle's core. Similar to other PEG-containing amphiphilic block-copolymers, diacyllipid–PEG conjugates (such as PEG–phosphatidyl ethanolamine, PEG–PE) were found to form very stable micelles in an aqueous environment (Klibanov et al. 1990; Lasic et al. 1991b). Their CMC values can be as low as 10^{-6} M (Kabanov et al. 2002a; Torchilin 2001), which is at least 100-fold lower than those of conventional detergents (Rowe et al. 2003), so that micelles prepared from these polymers will maintain their integrity even upon strong dilution (for example, in the blood during a therapeutic application).

The high stability of polymeric micelles also allows for good retention of encapsulated drugs in the solubilized form upon parenteral administration.

Three targeting mechanisms can be seen for micelles, as for any other pharmaceutical long-circulating drug carrier. The first one is based on spontaneous penetration of micelles into the interstitium through the leaky vasculature (EPR effect) and is considered "passive targeting" (Gabizon 1995; Maeda et al. 2000; Palmer et al. 1984). Thus, it was repeatedly shown that micelle-incorporated anticancer drugs (such as adriamycin, see, for example, Kwon and Kataoka 1999) accumulate much better in tumors than in nontarget tissues (such as the heart muscle), which minimizes undesired drug toxicity. In certain cases, it is the small size of micelles which makes them superior to other nanoparticulates, including liposomes. The transport efficacy and accumulation of microparticulates, such as liposomes and/or micelles, in the tumor interstitium is to a great extent determined by their ability to penetrate the leaky tumor vascular endothelium (Jain 1999; Yuan et al. 1995); see the schematic representation of this phenomenon in Fig. 1. Diffusion and accumulation parameters were recently shown to be strongly dependent on the cut-off size of tumor blood vessel wall, and the cut-off size varies for different tumors. As a result, the use of PEG–PE micelles for the delivery of a model protein drug to a murine solid tumor with a low permeability, Lewis lung carcinoma, provided the best results compared to other particulate carriers (Weissig et al. 1998).

The second targeting mechanism is based on the fact that many pathological processes in various tissues and organs are accompanied by local temperature increase and/or acidosis (Helmlinger et al. 1997). Micelles made of temperature- or pH-sensitive components, such as poly(*N*-isopropylacrylamide) and its copolymers with poly(D,L-lactide) and other blocks, can disintegrate in such areas releasing the micelle-incorporated drug (Jones and Leroux 1999).

Finally, specific ligands can be attached to the water-exposed termini of hydrophilic blocks, such as antibodies and/or certain sugar moieties (Rammohan et al. 2001). In this case, in order to make the micelles targeted without creating any steric hindrances for the antibody, the antibody of choice or its fragment can be chemically attached to an activated water-exposed free terminus of a hydrophilic block of micelle-forming polymer. For this purpose, relatively simple chemistry can be applied similar to that developed earlier for liposomes (Torchilin et al. 2001a) and involving the use of amphiphilic PEG–PE with a protein-reactive *p*-nitrophenylcarbonyl (pNP) group on the distal tip of the hydrophilic PEG block.

4 Chemistry Used to Provide Pharmaceutical Nanocarriers with Various Functions

Preparing various functional nanocarriers with controlled properties requires the conjugation of proteins, peptides, polymers, cell-penetrating moieties, reporter groups and other functional ligands to the carrier surface (although in certain

cases functional components may be loaded inside the nanocarrier or distributed within the nanocarrier structure: thus, for example, fine ferromagnetic particles can be loaded inside liposomes or polymeric nanoparticles to make them magnetic). This attachment can proceed noncovalently, via the hydrophobic adsorption of certain intrinsic or specially inserted hydrophobic groups in the ligands to be attached onto or into the surface of the nanocarrier. Thus, amphiphilic polymers or hydrophobically modified proteins can adsorb on the hydrophobic surface of polystyrene nanoparticles (Yuan et al. 1995) or incorporate into the phospholipid membrane of liposomes (Torchilin 1998) or hydrophobic core of micelles (Torchilin 2001). More frequently, the attachment is performed chemically, via the interaction of reactive groups generated on the carrier surface and certain groups in the molecule to be attached. In the case of liposomes, the most popular drug delivery system and convenient example of the techniques used, the conjugation methodology, is based on three main reactions, which are quite efficient and selective: reaction between activated carboxyl groups and amino groups yielding an amide bond; reaction between pyridyldithiols and thiols yielding disulfide bonds; and reaction between maleimide derivatives and thiols yielding thioether bonds (Torchilin and Klibanov 1993). Some other approaches also exist, for example yielding the carbamate bond via the reaction of the *p*-nitrophenylcarbonyl groups introduced onto the surface of nanocarriers with amino group of various ligands (Torchilin et al. 2001b). The detailed review of numerous coupling procedures and protocols used for attaching a whole variety of surface modifiers to drug carriers can be found in Klibanov et al. (2003) and Torchilin et al. (2003c).

It was shown, for example, that carboxylic groups of immunoglobulins can be activated by water-soluble carbodiimide; activated protein can then be bound to free amino-group-containing surfaces, such as PE-containing liposomes (Dunnick et al. 1975). For further ligand attachment, corresponding reactive groups on the surface of nanocarriers can be premodified with the aid of heterobifunctional cross-linking reagents, such as the popular *N*-succinimidyl-3(2-pyridyldithio)-propionate (SPDP) used to synthesize a PE derivative further used for coupling to SH-containing proteins (Leserman et al. 1980). Another possibility is to rely on the reaction of the thiol groups on a ligand (protein) with the maleimide-carrying surfaces (phospholipid molecules, in the case of liposomes). This approach (Martin and Papahadjopoulos 1982) is now one of the most widely used in research and practical applications. Different commercially available maleimide reagents can be used for the preparation of maleimide-carrying phospholipids in a simple single-step procedure. Various high and low molecular weight compounds have been attached to liposomes by using pyridyldithiopropionyl-PE or maleimide reagents (Klibanov et al. 2003; Torchilin et al. 2003c). The application of free thiol groups located on immunoglobulin Fab fragments is also attractive. It is believed that these SH groups are positioned far from the antigen-binding sites, enabling the nanocarrier-bound antibody fragments to retain their specific interaction with antigens.

Some ligands carry carbohydrate residues, which can be easily oxidized to yield aldehyde groups that can react with surface aminogroups with the formation of Schiff bases (Heath et al. 1980). Nanocarriers (such as liposomes) containing

surface-exposed carboxylic groups were used for the attachment of different ligands (Kung and Redemann 1986). In the case of liposomes, they can be prepared by various techniques and activated with water-soluble carbodiimide directly prior to ligand addition. The same chemical reactions can be used to attach nonmodified proteins and peptides to various nanocarriers, including preformed liposomes, containing membrane-incorporated reactive lipid derivatives, such as *N*-glutaryl-PE or glutaryl-cardiolipin (Bogdanov et al. 1988; Weissig and Gregoriadis 1992; Weissig et al. 1990). The use of a four-tailed hydrophobic cardiolipin derivative instead of a two-tailed PE derivative allows for a decrease in the number of amino groups involved in the conjugation reaction at the same degree of hydrophobicity. This results in better preservation of the activity of the hydrophobized and liposome-attached protein (Niedermann et al. 1991; Weissig et al. 1986). Some current methods for attaching various (mainly, targeting) ligands to nanocarriers are reviewed in Nobs et al. (2004).

Some special methods are designed to attach various sterically protective polymers to the surface of nanocarriers (see below). Thus, for example, to make PEG capable of incorporation into the liposomal membrane, the reactive derivative of hydrophilic PEG is single terminus-modified with a hydrophobic moiety (usually, the residue of PE or long-chain fatty acid is attached to PEG-hydroxysuccinimide ester) (Klibanov et al. 1991, 1990). In the majority of protocols, PEG–PE is used, which must be added to the lipid mixture prior to liposome formation. Alternatively, it was suggested to synthesize single-end-reactive derivatives of PEG able to be coupled with certain reactive groups (such as maleimide) on the surface of already prepared liposomes, referred to as the postcoating method (Maruyama et al. 1995). Currently, numerous studies on the preparation and properties of polymer-modified liposomes are well reviewed in several important books (Gregoriadis 1993; Lasic and Barenholz 1996; Lasic and Martin 1995). Spontaneous incorporation of PEG-lipid conjugates into the liposome membrane from PEG-lipid micelles was also shown to be very effective and did not disturb the vesicles (Sou et al. 2000).

5 Longevity of Nanocarriers in the Blood and its Importance for Drug Delivery

Longevity in the blood is one of the key properties of nanoparticulate drug delivery systems for both passive and active targeting, and long-circulating pharmaceuticals and pharmaceutical carriers currently represent an important and still growing area of biomedical research (see, for example, Cohen and Bernstein 1996; Lasic and Martin 1995; Moghimi and Szebeni 2003; Torchilin 1996b, 1998; Trubetskoy and Torchilin 1995). There are quite a few important reasons for making long-circulating drugs and drug carriers. One of them is to maintain a required level of a pharmaceutical agent (both therapeutic and diagnostic) in the blood for an extended

time interval. Long-circulating diagnostic agents are of primary importance for blood pool imaging, which helps in evaluating the current state of blood flow and discovering its irregularities caused by pathological lesions. Blood substitutes represent another important area for the use of long-circulating pharmaceuticals, when artificial oxygen carriers should be present in the circulation for long enough (Winslow et al. 1996). Then, as was discussed above, long-circulating drug-containing microparticulates or large macromolecular aggregates can slowly accumulate ("passive" targeting: Maeda 2001; Maeda et al. 2000) in pathological sites with affected and leaky vasculature (primarily tumors) and improve or enhance drug delivery in those areas (Gabizon 1995; Maeda 2001; Maeda et al. 2000). In addition, the prolonged circulation can help to achieve a better targeting effect for targeted (specific ligand-modified) drugs and drug carriers, allowing more time for their interaction with the target (Torchilin 1996b) due to larger number of passages of targeted pharmaceuticals through the target.

Chemical modification of drugs and drug carriers with certain synthetic polymers is the most frequent way to add in vivo longevity to other functions of drugs and drug carriers. Hydrophilic polymers have been shown to protect individual molecules and solid particulates from interaction with different solutes. This phenomenon relates to the stability of various aqueous dispersions (Molyneux 1984), and within the pharmaceutical field it helps to protect APIs and drug carriers from undesirable interactions with components of the biological milieu. The term "steric stabilization" has been introduced to describe the phenomenon of polymer-mediated protection (Naper 1983). The most popular and successful method to obtain long-circulating biologically stable nanoparticles is coating with certain hydrophilic and flexible polymers, primarily with poly(ethylene glycol) (PEG), as was first suggested for liposomes (Allen et al. 1991; Klibanov et al. 1990; Maruyama et al. 1991; Senior et al. 1991). On the biological level, coating nanoparticles with PEG sterically hinders interactions of blood components with their surface and reduces the binding of plasma proteins with PEG particles, as was demonstrated for liposomes (Allen 1994; Chonn et al. 1991, 1992; Lasic et al. 1991a; Senior et al. 1991; Woodle 1993). This prevents drug carrier interaction with opsonins and slows down their fast capture by RES (Senior 1987; Fig. 5). The mechanisms of preventing opsonization by PEG include shielding of the surface

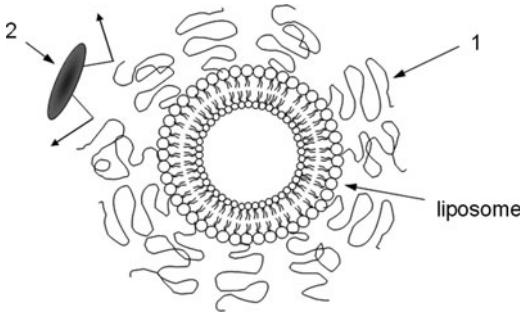


Fig. 5 The mechanism of steric protection of pharmaceutical nanocarriers by surface-grafted polymers. As an example, PEG chains (1) on the liposome surface prevent opsonin (2) from being adsorbed on the liposome and allow for its prolonged circulation

charges, increased surface hydrophilicity (Gabizon and Papahadjopoulos 1992), enhanced repulsive interaction between polymer-coated nanocarriers and blood component (Needham et al. 1992), and formation of a polymeric layer over the particle surface which is impermeable for other solutes even at relatively low polymer concentrations (Gabizon and Papahadjopoulos 1992; Torchilin et al. 1994).

Although quite a few polymers have been tried as steric protectors for nanoparticular drug carriers (Torchilin and Trubetskoy 1995), which will be discussed further, the majority of research on long-circulating drugs and drug carriers was performed with the use of PEG as a sterically protecting polymer because of the very attractive combination of properties of PEG: its excellent solubility in aqueous solutions and its ability to bind many water molecules, high flexibility of its polymer chain, very low toxicity, immunogenicity, and antigenicity, lack of accumulation in the RES cells, and minimum influence on specific biological properties of modified pharmaceuticals (Pang 1993; Powell 1980; Yamaoka et al. 1994; Zalipsky 1995). It is also important that PEG is not biodegradable and subsequently does not form any toxic metabolites. On the other hand, PEG molecules with molecular weight below 40 kDa are readily excreted from the body via the kidneys. From the practical point of view, PEG is easily commercially available in a variety of molecular weights. PEGs which are normally used for the modification of drug and drug carriers have a molecular weight from 1,000 to 20,000 Da. Single-terminus reactive (semitelehelic) PEG derivatives are often used for modification of pharmacologically important substances without the formation of cross-linked aggregates and heterogenic products. Currently, there exist many chemical approaches to synthesize activated derivatives of PEG and to couple these derivatives with a variety of drugs and drug carriers. Extensive reviews of these methods and their applicability for solving various problems in the drug delivery area were done by several authors (Torchilin 2002; Veronese 2001; Zalipsky 1995). Despite well-developed chemistry of PEG coupling, the search for alternative sterically protecting polymers is quite active. These polymers should be biocompatible, soluble, hydrophilic, and have a highly flexible main chain (see some data in Chonn et al. 1992; Lasic et al. 1991a; Maruyama et al. 1994; Takeuchi et al. 1999; Torchilin 1996b; Torchilin et al. 2001b, 1995; Torchilin and Trubetskoy 1995; Trubetskoy and Torchilin 1995; Woodle et al. 1994).

The most important biological consequence of nanocarrier modification with protecting polymers is a sharp increase in their circulation time and decrease in their RES (liver) accumulation (Klibanov et al. 1990; Lasic and Martin 1995; Torchilin et al. 1994). From the clinical point of view, it is extremely important that various long-circulating liposomes of a relatively small size (100–200 nm) were shown to effectively accumulate in many tumors via the "impaired filtration" mechanism (Gabizon and Papahadjopoulos 1988; Gabizon 1995; Maeda 2001; Maeda et al. 2000). As a result, PEG-coated and other long-circulating liposomes were prepared containing a variety of anticancer agents, such as doxorubicin, arabinofuranosylcytosine, adriamycin, and vincristin (Allen et al. 1992; Boman et al. 1994; Gabizon et al. 1994; Huang et al. 1994). The biggest success was achieved with PEG-liposome-incorporated doxorubicin, which has already demonstrated very good

clinical results (Ewer et al. 2004; Gabizon 1995; Rose 2005). An analysis of the pharmacokinetics of long-circulating nanocarriers (using PEG-liposomes) was performed by Allen (Allen et al. 1995b). In general, the association of drugs with nanocarriers has pronounced effects on pharmacokinetics: delayed drug absorption, restricted drug biodistribution, decreased volume of drug biodistribution, delayed drug clearance, and retarded drug metabolism (Hwang 1987). All these effects are determined by hindered interstitial penetration of a drug and lesser drug accessibility for the biological milieu because of entrapment into the drug carrier. The presence of protective polymer on the carrier surface changes all these parameters still further (Klibanov et al. 1990; Senior et al. 1991). Thus, while "plain" liposomes have nonlinear, saturable kinetics, long-circulating liposomes demonstrate dose-independent, nonsaturable, and log-linear kinetics (Allen and Hansen 1991; Huang et al. 1992; Mayhew et al. 1992). All pharmacokinetic effects depend on the route of liposome administration and their size and composition, and are always less expressed for sterically protected PEG-carriers (Allen et al. 1989; Liu et al. 1991, 1992; Maruyama et al. 1992).

An additional function can be added to long-circulating PEGylated pharmaceutical carriers, which allows for the detachment of PEG chains under the action of certain local stimuli characteristic of pathological areas, such as the decreased pH value or increased temperature usually noted in inflamed and neoplastic areas. The problem is that the stability of PEGylated nanocarriers may not always be favorable for drug delivery. In particular, if drug-containing nanocarriers accumulate inside the tumor, they may be unable to easily release the API to kill the tumor cells. Likewise, if the carrier has to be taken up by a cell via an endocytic pathway, the presence of the PEG coat on its surface may preclude the contents from escaping the endosome and being delivered in the cytoplasm. In order to solve these problems, for example, in the case of long-circulating liposomes, the chemistry was developed to detach PEG from the lipid anchor in the desired conditions. Labile linkage that would degrade only in the acidic conditions characteristic of the endocytic vacuole or the acidotic tumor mass are well-known from the area of controlled drug release. Such linkages can be based, e.g., on diortho ester acid-labile chemistry (Guo and Szoka 2001), or vinyl ester chemistry (Boomer and Thompson 1999). The latter reference describes the preparation of an acidic medium-cleavable PEG-lipid. Cysteine-cleavable lipopolymers were also described (Zalipsky et al. 1999). When the PEG brush is cleaved (e.g., from the liposome surface), membrane destabilization should occur, and the liposome contents would be delivered to its target (e.g., by escaping from the primary endosome into the cell cytoplasm). Polymeric components with pH-sensitive (pH-cleavable) bonds are widely used to produce stimuli-responsive drug delivery systems that are stable in the circulation or in normal tissues. However, they acquire the ability to degrade and release the entrapped agents in body areas or cell compartments with lowered pH, such as tumors, infarcts, inflammation zones or cell cytoplasm or endosomes (Roux et al. 2002a, 2004; Simoes et al. 2004). Since in "acidic" sites the pH drops from the normal physiological value of 7.4 to pH 6 and below, chemical bonds used so far to prepare the acidic pH-sensitive carriers have included vinyl esters, double esters,

and hydrazones that are quite stable at pH around 7.5 but hydrolyze relatively fast at pH values of 6 and below (Guo and Szoka 2001; Kratz et al. 1999; Zhang et al. 2004). To date, a variety of liposomes (Leroux et al. 2001; Roux et al. 2002b) and micelles (Lee et al. 2003a, b; Sudimack et al. 2002) have been described that include components with the above-mentioned bonds, as well as a variety of drug conjugates capable of releasing such drugs as adriamycin (Jones et al. 2003), paclitaxel (Suzawa et al. 2002), doxorubicin (Potineni et al. 2003; Yoo et al. 2002), and DNA (Cheung et al. 2001; Venugopalan et al. 2002) in acidic cell compartments (endosomes) and pathological body areas under acidosis. New detachable PEG conjugates are also described in Zalipsky et al. (1999), where the detachment process is based on the mild thiolysis of the dithiobenzylurethane linkage between PEG and the amino-containing substrate (such as phosphatidyl ethanol amine). Serum stable, long-circulating PEGylated pH-sensitive liposomes were also prepared using, on the same liposome, the combination of PEG and pH-sensitive terminally alkylated copolymer of *N*-isopropylacrylamide and methacrylic acid (Roux et al. 2004). The attachment of the pH-sensitive polymer to the surface of liposomes might facilitate liposome destabilization and drug release in compartments with decreased pH values. Numerous in vitro and in vivo experiments have shown great potential for improved efficiency of drug delivery and targeting.

6 Passive Accumulation of Liposomes and Micelles in Tumors

Since it was repeatedly shown that, like macromolecules, long-circulating liposomes are capable of accumulating in various pathological areas with affected vasculature via the EPR effect (Maeda et al. 2001; Yuan et al. 1994), long-circulating polymer (PEG)-coated liposomes have been repeatedly used for drug delivery into tumors via passive accumulation. An important feature of protective polymers is their flexibility, which allows a relatively small number of surface-grafted polymer molecules to create an impermeable layer over the liposome surface (Torchilin et al. 1994; Torchilin and Trubetskoy 1995). Although PEG remains the gold standard in liposome steric protection for passively targeted preparations, attempts continue to identify other polymers that could be used to prepare long-circulating liposomes. Earlier studies with various water-soluble flexible polymers have been summarized in Torchilin and Trubetskoy (1995) and Woodle (1998). More recent papers describe long-circulating liposomes prepared using poly[*N*-(2-hydroxypropyl)methacrylamide)] (Whiteman et al. 2001), poly-*N*-vinylpyrrolidones (Torchilin et al. 2001b), L-amino acid-based biodegradable polymer-lipid conjugates (Metselaar et al. 2003), and polyvinyl alcohol (Takeuchi et al. 2001).

As was already mentioned, long-circulating liposomes demonstrate dose-independent, nonsaturable, log-linear kinetics, and increased bioavailability (Allen and Hansen 1991). The relative role of the liposome charge and protective polymer molecular size was investigated, showing that opsonins with different

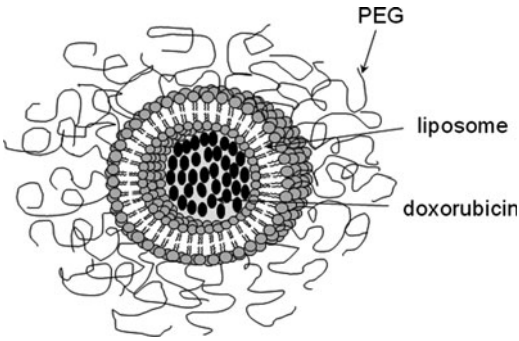


Fig. 6 Schematic structure of Doxil® – doxorubicin in PEG-coated liposomes

molecular sizes may be involved in the clearance of liposomes containing different charged lipids (Levchenko et al. 2002). PEG was also attached to the liposome surface in a removable fashion to facilitate liposome capture by the cell after PEG-liposomes accumulate in target site via the EPR effect (Maeda et al. 2001), and the PEG coating is detached under the action of local pathological conditions (decreased pH in tumors). New detachable PEG conjugates are described in Zalipsky et al. (1999), where the detachment process is based on the mild thiolysis of the dithiobenzylurethane linkage between PEG and an amino-containing substrate (such as PE). Low pH-degradable PEG-lipid conjugates based on the hydrazone linkage between PEG and lipid have also been described (Kale and Torchilin 2007; Sawant et al. 2006).

Doxorubicin in PEG-coated liposomes (Doxil® and Caelyx®; see the schematic structure in Fig. 6) is successfully used for the treatment of solid tumors in patients with breast carcinoma metastases, with subsequent survival improvement (O'Shaughnessy 2003; Perez et al. 2002; Symon et al. 1999). The same set of indications was targeted by the combination therapy involving liposomal doxorubicin and paclitaxel (Schwonzen et al. 2000) or Doxil/Caelyx and carboplatin (Goncalves et al. 2003). Caelyx is currently also in Phase II studies for patients with squamous cell cancer of the head and neck (Harrington et al. 2001) and ovarian cancer (Johnston and Gore 2001). Clinical data showed the impressive effect of doxorubicin in PEG-liposomes against unresectable hepatocellular carcinoma (Schmidinger et al. 2001), cutaneous T-cell lymphoma (Wollina et al. 2003), and sarcoma (Skubitz 2003). The recent review on the successful use of Caelyx in the treatment of ovarian cancer can be found in Perez-Lopez et al. (2007). It should, however, be noted here that recent evidence showed that PEG-liposomes, previously considered biologically inert, could still induce certain side-reactions via activation of the complement system (Moein Moghimi et al. 2006; Moghimi and Szebeni 2003).

Different methods of liposomal content delivery into the cytoplasm have been elaborated by adding the pH-sensitivity function to liposomal preparations (Torchilin 1991). When the liposome is made of pH-sensitive components then, after being endocytosed, it fuses with the endovacuolar membrane under the action of lowered pH inside the endosome and destabilizes it, releasing its content into the cytoplasm (Torchilin et al. 1993). Thus, endosomes become the gates from

the outside into the cell cytoplasm (Sheff 2004). This approach has been reviewed many times in various publications (in 2004, endosomal escape by pH-sensitive drug delivery systems was specifically discussed in a special issue of *Advanced Drug Delivery Reviews* #56, J.C. Leroux, ed.). It is usually assumed that inside the endosome, the low pH and some other factors destabilize the liposomal membrane, which, in turn, interacts with the endosomal membrane provoking its secondary destabilization and drug release into the cytoplasm. The presence of fusogenic lipids in the liposome composition, such as unsaturated DOPE (dioleoyl-*sn*-glycero-3-phosphatidylethanolamine), is usually required to render pH sensitivity to liposomes (Shalaev and Steponkus 1999). Multifunctional long-circulating PEGylated DOPE-containing pH-sensitive liposomes, although having a decreased pH sensitivity, still effectively deliver their contents into cytoplasm after being passively accumulated in the tumor (Varga et al. 2000). Antisense oligonucleotides (ODN) were delivered into cells by anionic pH-sensitive PE-containing liposomes, which are stable in the blood but undergo phase transition at acidic endosomal pH and facilitate oligonucleotide release into cell cytoplasm (Fattal et al. 2004). New pH-sensitive liposomal additives were recently described including oleyl alcohol (Sudimack et al. 2002) and pH-sensitive morpholine lipids (mono-stearoyl derivatives of morpholine) (Asokan and Cho 2003).

In the case of micellar preparations of anticancer drugs, passive micelle targeting to pathological organs or tissues can further increase the pharmaceutical efficiency of a micelle-encapsulated drug. Direct correlations between the longevity of a particulate drug carrier in the circulation and its ability to reach its target site have been observed on multiple occasions (Gabizon 1995; Maeda et al. 2001). The results of the blood clearance study of various PEG–PE micelles clearly demonstrated their longevity: the micelle formulations studied had circulation half-lives in mice, rats, and rabbits from 1.2 to 2.0 h depending on the molecular size of the PEG block (Lukyanov et al. 2002). The increase in the size of a PEG block increases the micelle circulation time in the blood probably by providing a better steric protection against opsonin penetration to the hydrophobic micelle core. However, circulation times for PEG–PE micelles are somewhat shorter than those for PEG-coated long-circulating liposomes (Klibanov et al. 1990), which could be explained in part by the more rapid extravasation of the micelles from the vasculature associated with their considerably smaller size compared to liposomes (Weissig et al. 1998). Slow dissociation of micelles under physiological conditions due to continuous clearance of unimers, with a micelle–unimer equilibrium being shifted towards the unimer formation (Trubetskoy et al. 1997), can also play its role.

As with long-circulating liposomes (Gabizon 1992, 2001; Papahadjopoulos et al. 1991), PEG–PE-based micelles formed by PEG_{750}–PE, PEG_{2000}–PE, and PEG_{5000}–PE accumulate efficiently in tumors via the EPR effect. It is worth mentioning that micelles prepared with several different PEG–PE conjugates demonstrated much higher accumulation in tumors compared to nontarget tissue (muscle), even in the case of an experimental Lewis lung carcinoma (LLC) in mice known to have a relatively small vasculature cut-off size (Hobbs et al. 1998; Weissig et al. 1998).

In other words, because of their smaller size, micelles may have additional advantages as a tumor drug-delivery system which utilizes the EPR effect compared to particulate carriers with larger sizes of individual particles. Thus, the micelle-incorporated model protein (soybean trypsin inhibitor or STI, MW 21.5 kDa) accumulates to a higher extent in subcutaneously established murine Lewis lung carcinoma than the same protein in larger liposomes (Weissig et al. 1998).

The accumulation pattern of PEG–PE micelles prepared from all versions of PEG–PE conjugates is characterized by peak tumor accumulation times of about 3–5 h. The largest total tumor uptake of the injected dose 5 h post-injection (as AUC) was found for micelles formed by the unimers with a relatively large PEG block (PEG_{5000}–PE). This may be explained by the fact that these micelles have the longest circulation time and a lesser extravasation into the normal tissue than micelles prepared from the smaller PEG–PE conjugates. Micelles prepared from PEG–PE conjugates with shorter versions of PEG, however, might be more efficient carriers of poorly soluble drugs because they have a greater hydrophobic-to-hydrophilic phase ratio and can be loaded with drug more efficiently on a weight-to-weight basis. Similar results have been obtained with another murine tumor model, EL4 T-cell lymphoma (Lukyanov et al. 2002). Some other recent data also clearly indicate spontaneous targeting of PEG–PE-based micelles into other experimental tumors (Torchilin et al. 2003b) in mice, as well as into the damaged heart areas in rabbits with experimental myocardial infarction (Lukyanov et al. 2004b).

Among drugs delivered by passively targeted micelles, one can name paclitaxel, which was shown to accumulate in tumors much better than its commercial formulation Taxol®, when loaded into micelles made of PEG-*b*-poly(4-phenyl-1-butanoate)-l-aspartamide conjugates (Hamaguchi et al. 2005). With this preparation, an almost 100-fold increase in the AUC, a 15-fold decrease in the volume of distribution and a significant decrease of drug clearance was achieved, which resulted in 25-fold improved drug accumulation in C-26 tumor in mice and corresponding increase in antitumor activity. Some other micellar preparations for passive targeting of paclitaxel have also been tested with variable degrees of success (Hamaguchi et al. 2005; Kim et al. 2004).

PEG-*b*-poly(amino acid)-based micelles loaded with cisplatin (CDDP) were designed for passive drug targeting into tumors and are undergoing clinical trials (Uchino et al. 2005). Among other micellar preparations for passive drug targeting in clinical trials, one can also mention doxorubicin in micelles made of PEG-*block*-poly(*l*-aspartate)–doxorubicin conjugate (these micelles contain both free and hydrophobic block-conjugated drug) (Matsumura et al. 2004) as well as doxorubicin in micelles made of Pluronic® (Danson et al. 2004).

Another targeting mechanism is based on the fact that many pathological processes in various tissues and organs are accompanied by local temperature increase and/or acidosis (Vutla et al. 1996; Yerushalmi et al. 1994). Hence, the efficiency of the micellar carriers can be further improved by making micelles capable of disintegration under the increased temperature or decreased pH values in pathological sites, i.e., by combining the EPR effect with stimuli responsiveness.

For this purpose, micelles are made of temperature- or pH-sensitive components, such as poly(*N*-isopropylacrylamide) and its copolymers with poly(D,L-lactide) and other blocks, and acquire the ability to disintegrate in target areas, releasing the micelle-incorporated drug (Cammas et al. 1997; Chung et al. 1998; Kohori et al. 1998; Kwon and Okano 1999; Meyer et al. 1998). pH-responsive polymeric micelles loaded with phthalocyanine seem to be promising carriers for photodynamic cancer therapy (Le Garrec et al. 2002), while doxorubicin-loaded polymeric micelles containing acid-cleavable linkages provided an enhanced intracellular drug delivery into tumor cells and thus higher efficiency (Yoo et al. 2002). Thermo-responsive polymeric micelles were shown to demonstrate an increased drug release upon temperature changes (Chung et al. 1999).

Passively targeted micelles (polymeric micelles) can also demonstrate pH sensitivity and ability to escape from endosomes. Thus, micelles prepared from PEG-poly(aspartate hydrazone adriamycin) easily release an active drug at lowered pH values typical for endosomes and facilitate its cytoplasmic delivery and toxicity against cancer cells (Bae et al. 2005). Alternatively, micelles for intracellular delivery of antisense ODN were prepared from ODN–PEG conjugates complexed with a cationic fusogenic peptide, KALA, and provided much higher intracellular delivery of the ODN than could be achieved with free ODN (Jeong et al. 2003). One could also enhance an intracellular delivery of drug-loaded micelles by adding to their composition the lipid components used in membrane-destabilizing Lipofectin®. Thus, PEG–lipid micelles, for example, carry a net negative charge (Lukyanov et al. 2004b), which might hinder their internalization by cells. On the other hand, it is known that the net positive charge usually enhances the uptake of various nanoparticles by cells, and after endocytosis the drug/DNA-loaded particles could escape from the endosomes and enter a cell's cytoplasm through disruptive interaction of the cationic lipid with endosomal membranes (Hafez et al. 2001). The compensation of this negative charge by the addition of positively charged lipids to PEG–PE micelles could improve the uptake by cancer cells of drug-loaded mixed PEG–PE/positively charged lipid micelles. It is also possible that after the enhanced endocytosis, such micelles could escape from the endosomes and enter the cytoplasm of cancer cells. With this in mind, an attempt was made to increase the intracellular delivery and, thus, the anticancer activity of micellar paclitaxel by preparing paclitaxel-containing micelles from a mixture of PEG–PE and Lipofectin® lipids (LL) (Wang et al. 2005). Multifunctional polymeric micelles capable of pH-dependent dissociation and drug release when loaded with doxorubicin and supplemented with biotin as cancer cell-interacting ligand were also described in Lee et al. (2005).

The problems with drug delivery using micelles for passive targeting are usually associated with too fast drug release from the micelles and with the difficulties of intracellular drug delivery (Aliabadi and Lavasanifar 2006). In order to minimize drug release from the micelles, the drug can be chemically conjugated with the hydrophobic blocks of micelle-forming components or drug-loaded micelles can be additionally chemically cross-linked (Kang et al. 2005; Lavasanifar et al. 2002; Shuai et al. 2004; Yuan et al. 2005).

7 Active Tumor Targeting with Drug-Loaded Liposomes

Current development of liposomal carriers often involves the attempt to combine the properties of long-circulating liposomes and targeted liposomes in one preparation (Abra et al. 2002; Blume et al. 1993; Torchilin et al. 1992). To achieve better selectivity of PEG-coated liposomes, it is advantageous to attach the targeting ligand via a PEG spacer arm, so that the ligand is extended outside of the dense PEG brush, excluding steric hindrances for the ligand binding to the target. Various advanced technologies are used for this purpose, and the targeting moiety is usually attached above the protecting polymer layer, by coupling it with the distal water-exposed terminus of an activated liposome-grafted polymer molecule (Blume et al. 1993; Torchilin et al. 2001a), see Fig. 7.

Since PEG–lipid conjugates used for the steric protection of liposomes and other pharmaceutical nanocarriers, and for the preparation of polymeric micelles, are derived from methoxy-PEG (mPEG) and carry nonreactive methoxy terminal groups, several attempts have been made to functionalize PEG tips in PEG–lipid conjugates. For this purpose several types of end-group functionalized lipopolymers were introduced of general formula X-PEG–PE (Zalipsky 1995; Zalipsky et al. 1998), where X represents a reactive functional group-containing moiety, and PEG–PE represents the conjugate of PE and PEG.

An interesting approach to couple various ligands, such as antibodies, to liposomes, including PEGylated liposomes, involves a so-called "postinsertion" technique (Ishida et al. 1999). This technique is based on the preliminary activation of ligands with any reactive PEG–PE derivative and subsequent co-incubation of unstable micelles formed by the modified ligand–PEG–PE conjugates with preformed drug-loaded plain or PEGylated liposomes. Eventually, modified ligands spontaneously incorporate from their micelles into the more thermodynamically favorable surroundings of the liposome membrane. This method was used, in particular, to prepare immuno-Doxil by modifying it with *p*-nitrophenylcarbonyl

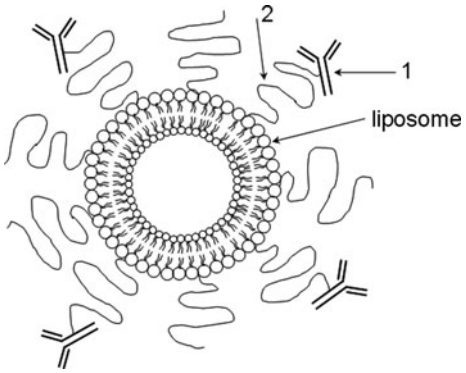


Fig. 7 The attachment of the targeting moiety (mostly monoclonal antibody) to PEGylated pharmaceutical nanocarrier (with liposome as an example). Although the targeting ligand could be co-immobilized on the surface together with PEG (1), the targeting moiety is usually attached above the protecting polymer layer, by coupling it with the distal water-exposed terminus of the activated liposome-grafted polymer molecule (2)

(pNP)–PEG–PE-modified anticancer 2C5 monoclonal antibody (Elbayoumi and Torchilin 2007; Lukyanov et al. 2004a).

Since antibodies are the most diverse and broadly used specific ligands for experimental targeted chemotherapy of various tumors with drug-loaded liposomes, there exist multiple original papers and reviews on antibody-targeted drug-loaded liposomes in cancer (see for reviews Kontermann 2006; Park et al. 2004; Sapra and Allen 2003; Sofou and Sgouros 2008; Torchilin 1996a, 2000; Vingerhoeds et al. 1994).

Antibody-modified liposomes of the "first generation" have been used to estimate certain parameters of their interaction with target cells in vitro (Klibanov et al. 1985) and also perform liposome targeting to certain model and real targets both in vitro and in vivo, such as extracellular matrix antigens or infarcted areas in the myocardium (Chazov et al. 1981; Torchilin et al. 1985). Importantly, it was noted that the modification of antibody-bearing liposomes with PEG (to make them long-circulating) usually results in decreased binding efficacy because of steric shielding of surface-attached antibodies by the liposome-grafted PEG (Klibanov et al. 1991; Torchilin et al. 1992). This eventually led to the development of multiple methods to attach antibodies onto the surface of the PEG layer in PEGylated liposomes.

In general, antibody attachment can decrease the circulating time of liposomes because of increased uptake of the modified liposomes via Fc receptors of circulating or liver macrophages, or opsonization of the liposome-tagged antibody molecules (Allen et al. 1995a; Kamps and Scherphof 1998). Whole antibodies can also trigger complement-mediated cytotoxicity and antibody-dependent cellular cytotoxicity (Sapra and Allen 2003). These effects could be minimized by using antibody Fab fragments instead of whole antibodies (Flavell et al. 1997). Although Fab fragments can also accelerate liposome clearance (Maruyama et al. 1997), in general Fab-liposomes circulate significantly longer than full antibody-modified liposomes (Maruyama et al. 1997). In the case of antibody-modified PEGylated liposomes, even a certain decrease in the circulation time still allows for their sufficiently long circulation, permitting good target accumulation. Clearly, attention should be paid not to over-modify PEG-liposomes with the antibody to the level at which their longevity is seriously compromised.

Interestingly, in some cases tumor accumulation of antibody-modified long-circulating liposomes is comparable with the accumulation of long-circulating liposomes without antibody attached (Moreira et al. 2001; Park et al. 2002, 1997, 2001). However, therapeutic activity is higher for antibody-targeted liposomes. As explained in Kirpotin et al. (2006) using PEGylated liposomes modified or nonmodified with anti-HER2 antibody, although intratumoral accumulation is similar for both preparations, antibody-modified preparations are much better internalized by tumor cells, which allows for higher drug doses delivered inside cancer cells, i.e., for more efficient cancer cell killing.

In some other cases, however, the liposome internalization seems not to be important. Thus, it was shown in Sapra and Allen (2004) that PEGylated liposomes loaded with vincristine or doxorubicin and modified (or nonmodified) with antibodies against internalizing CD19 antigen or noninternalizing CD20 antigen

demonstrate therapeutic effects which depended more on the type of drug used than on its ability to be internalized. As expected, the cytotoxicity of targeted liposomes depended also on the rate of drug release from the liposomes (Allen et al. 2005).

An interesting phenomenon was described in Hosokawa et al. (2003), the authors of which have demonstrated that, while nontargeted doxorubicin-containing liposomes were toxic to various cancer cells to the extent reflecting cell sensitivity to the drug, the cytotoxicity of antibody-targeted liposomes was proportional to the surface density of the surface antigen against which liposomes were targeted. The critical antigen surface concentration was about 4×10^4 sites per single cell, and after this value a further increase in antigen density was not important any more. Similar observations have also been made in Lopes de Menezes et al. (1998) and Park et al. (2002). Since cancer cells are often rather heterogenous in respect to antigens they express, it was suggested in Sapra and Allen (2003) to use a combination of antibodies against different antigens on a single liposome to provide better and more uniform targeting of all cells within the tumor. Alternatively, the "bystander" effect can also be relied upon (Sapra and Allen 2003), i.e., the action of the drug released from the liposomes attached to a certain cancer cell on the neighboring cancer cells devoid of a similar receptor.

An antibody which has gained popularity in cancer targeting is the monoclonal antibody against HER2, the antigen frequently over-expressed on various cancer cells. Monoclonal anti-HER2 antibodies including the humanized ones as well as currently clinically used Herceptin antibodies have been used to render drug-loaded liposomes (long-circulating liposomes) specific for HER2-positive cancer cells (Kirpotin et al. 1997, 2006; Park et al. 1995, 1997, 2001; Yang et al. 2007). This antibody was successfully used to deliver doxorubicin, both in plain and long-circulating liposomes, to breast tumors xenografts in mice, which resulted in significantly enhanced therapeutic activity of the drug. PEGylated liposomes decorated with anti-HER2 antibody were shown to undergo effective endocytosis by HER2-positive cancer cells, allowing for better drug (doxorubicin) accumulation inside tumor cells with better therapeutic outcome. Compared to doxorubicin in plain PEGylated liposomes (Doxil®), which normally accumulates in the tumor interstitial space, in the case of antibody-targeted Doxil, more drug molecules were discovered inside cancer cells, i.e., targeting with the antibody increases drug internalization by target cells.

Another promising antibody to target tumors with drug-loaded liposomes is the monoclonal antibody against CD19 antigen, which is also frequently over-expressed on various cancer cells. Anti-CD19 antibody-modified liposomes loaded with doxorubicin demonstrated clearly enhanced targeting and therapeutic efficacy both in vitro and in vivo in mice with human CD19+ B-lymphoma cells (Lopes de Menezes et al. 1998). Similar results have also been obtained with doxorubicin-loaded liposomes modified with antibodies against internalizable C19 antigen and against noninternalizable CD20 antigen (Sapra and Allen 2004). Anti-CD19 antibodies have also been used to target doxorubicin-loaded liposomes with variable drug release rates to experimental tumors (Allen et al. 2005). Recently, a successful attempt was made to target doxorubicin-loaded long-circulating liposomes to

CD19-expressing cancer cells with single chain Fv fragments of CD19 antibodies (Cheng and Allen 2008; Cheng et al. 2007).

Since neuroblastoma cells usually over-express disialoganglioside GD2, antibodies against GD2 and their Fab′ fragments have been suggested to target drug-loaded liposomes to corresponding tumors (Brignole et al. 2003; Pastorino et al. 2006, 2003). Fab′ fragments of anti-GD2 antibodies covalently coupled to long-circulating liposomes loaded with doxorubicin allowed for increased binding and higher cytotoxicity against target cells both in vitro and in vivo, including in models of human tumors in nude mice and in metastatic models. GD2-targeted immunoliposomes with the novel antitumoral drug, fenretinide, inducing apoptosis in neuroblastoma and melanoma cell lines, have also demonstrated strong anti-neuroblastoma activity both in vitro and in vivo in mice (Raffaghello et al. 2003). The combination of doxorubicin-loaded PEGylated liposomes targeted with anti-GD2 and with NGR-peptides specifically binding with the tumor vasculature, produced an improved therapeutic effect by acting on both tumor cells and tumor blood vessels (Pastorino et al. 2006).

An interesting novel target for anti-tumor drug delivery by means of targeted liposomes is the membrane type-1 matrix metalloproteinase (MT1-MMP), playing an important role in tumor neoangiogenesis and over-expressed both on tumor cells and on neoangiogenic endothelium. The modification of doxorubicin-loaded long-circulating liposomes with anti-MT1-MMP antibody resulted in an increased uptake of the targeted liposomes by MT1-MMP-over-expressing HT1080 fibrosarcoma cells in vitro and in more effective inhibition of tumor growth in vivo compared to antibody-free doxorubicin-loaded PEGylated liposomes (Hatakeyama et al. 2007). It was demonstrated that anti-MM1-MMP antibody enhances the endocytic internalization of drug-loaded liposomes, thus increasing their cytotoxicity (Atobe et al. 2007). Strong action of such preparation on tumor endothelial cells was noted.

Epidermal growth factor receptor (EGFR) and its variant EGFRvIII can serve as valuable targets for intracellular drug delivery into tumor cells over-expressing these receptors. Fab′ fragments of the monoclonal antibody C225, which binds both EGFR and EGFRvIII, and scFv fragment of the monoclonal antibody, which binds only to EGFR, were coupled to drug-loaded liposomes and allowed for substantially enhanced binding of such targeted liposomes with cancer cells over-expressing corresponding receptors, such as glioma cells U87 and carcinoma cells A0431 and MDA-MB-468. The better binding resulted in enhanced internalization and increased cytotoxicity (Mamot et al. 2003). In vivo therapy with such targeted drug-loaded liposomes (doxorubicin, epirubicin and vinorelbine were used as drugs) always resulted in better tumor growth inhibition than therapy with nontargeted liposomal drugs (Mamot et al. 2005). Fab′ fragment derived from the humanized anti-EGFR monoclonal antibody EMD72000 was shown to provide efficient intracellular delivery of the liposomal drugs into colorectal tumor cells (Mamot et al. 2006). The authors of this study have also shown that the attachment of the targeting moiety to PEGylated liposomes requires the length of the spacer arm sufficient to overcome possible steric shielding of antibody fragments by sterically-protecting PEG chains. An interesting method to construct anti-EGFR-targeted

liposomes was suggested in Pan and Lee (2007), where the anti-EFGR antibody (cetuximab or C225) was covalently linked to the folate-binding protein via a thioester bond and then coupled to the preformed folate-containing liposomes. Cetuximab-liposomes loaded with boron derivatives for boron neutron capture therapy were also prepared using the cholesterol-based anchor and micelle transfer technology (Pan et al. 2007).

Various proteins of the extracellular matrix expressed on the surface of cancer cells have also been used as targets for the antibody-mediated delivery of liposomal drugs. Thus, β_1-integrins expressed on the surface of human nonsmall cell lung carcinomas were targeted by doxorubicin-loaded liposomes modified with Fab′ fragments of anti-β_1-integrin monoclonal antibodies (Sugano et al. 2000). Treatment of SCID mice with lung tumor xenografts with such liposomes resulted in significant suppression of tumor growth compared to all controls and also inhibited metastases. The idea of targeting various antigens (preferably, the internalizable ones) on the endothelial cells by antibody–liposome conjugates was tested long ago (Trubetskaya et al. 1988). However, the approach attracted real attention only in the last few years. Thus, liposomes modified with anti-E-selectin antibodies were successfully internalized by activated endothelial cells in vitro through E-selectin-mediated endocytosis (Asgeirsdottir et al. 2008). Another possible target for antibody-mediated cancer therapy with drug-loaded liposomes is the epithelial cell adhesion molecule (EpCAM), which is expressed in many tumors but not in normal cells (Hussain et al. 2007). EpCAM-targeted immunoliposomes were generated by covalent attachment of the humanized scFv fragment of the 4D5MOCB monoclonal antibody to the surface of PEGylated doxorubicin-loaded liposomes and demonstrated significantly improved binding, internalization and cytotoxicity with EpCAM-positive cancer cells. Similarly, liposomes coupled with antibodies against vascular cell adhesion molecule-1 (VCAM-1) can be effectively targeted to activated endothelial cells over-expressing VCAM-1 (Voinea et al. 2005). Liposomes loaded with cytotoxic drugs were also targeted to ED-B fibronectin using scFv fragments of the corresponding antibody (Marty and Schwendener 2005). Proliferating endothelial cells have been targeted with doxorubicin-loaded liposomes modified with scFv fragments of the antibody against endoglin over-expressed on such cells (Volkel et al. 2004).

Lipid-based drug carriers have also been conjugated with antibodies (or their fragments) against transferrin receptor (TfR), frequently over-expressed on the surface of various cancer cells. For example, such carriers were modified with the OX26 monoclonal antibody against TfR via liposome-incorporated maleimide-modified PEG_{2000}–PE molecules and demonstrated strong binding with cells over-expressing TfR (Beduneau et al. 2007). The same antibody was attached to daunomycin-loaded liposomes noncovalently via the avidin–biotin couple, and the modified liposomes demonstrated good accumulation in multidrug-resistant RBE4 brain capillary endothelial cells both in vitro and in vivo (Schnyder et al. 2005).

Liposomes loaded with a lipophilic prodrug 5-fluorodeoxyuridine and modified with the monoclonal antibody CC531 against rat colon carcinoma demonstrated good binding with target cells (Koning et al. 1999) and effective intracellular drug

delivery compared to all controls (Koning et al. 2002). Antibody CC52 against rat colon adenocarcinoma CC531 attached to PEGylated liposomes provided specific accumulation of liposomes in a rat model of metastatic CC531 tumors (Kamps et al. 2000).

Nonpathogenic antinuclear autoantibodies (ANAs), frequently detected in cancer patients and in healthy elderly individuals, represent a subclass of natural anticancer antibodies. Earlier, we have shown that certain monoclonal ANAs (such as mAbs 2C5 and 1G3) recognize the surface of numerous tumor, but not normal, cells (Iakoubov et al. 1995a, b; Iakoubov and Torchilin 1997). Nucleosome-restricted specificity was shown for some of these monoclonal ANAs, and tumor cell surface-bound nucleosomes (NSs) have been shown to be their universal molecular target on the surface of a variety of tumor cells (Iakoubov and Torchilin 1997, 1998). Because these antibodies can effectively recognize a broad variety of tumors, they may serve as specific ligands to deliver other drugs and drug carriers into tumors. These antibodies were used to prepare drug-loaded tumor-targeted long-circulating immunoliposomes (with doxorubicin), which demonstrated highly specific binding with various cancer cells (murine Lewis lung carcinoma, 4T1, C26, and human BT-20, MCF-7, PC3 cells) in vitro (Elbayoumi and Torchilin 2007; Lukyanov et al. 2004a), significantly increased tumor accumulation in model tumors in mice including intracranial human brain U-87 MG tumor xenografts in nude mice, decreased side-effects, and superior antitumor activity in vivo (Elbayoumi and Torchilin 2006, 2008; Gupta and Torchilin 2007).

Doxorubicin-loaded PEGylated liposomes were also modified with Fab′ fragments of an anti-CD74 antibody via a PEG-based heterobifunctional coupling reagent and demonstrated significantly accelerated and enhanced accumulation in Raji human B-lymphoma cells in vitro (Lundberg et al. 2007). Anti-CD166 scFv attached to drug-loaded liposomes facilitated doxorubicin internalization by several prostate cancer cell lines (Du-145, PC3, LNCaP) (Roth et al. 2007). scFv fragments of antibodies against leukemia stem cells and oncogenic molecules participating in acute myeloid leukemia pathogenesis were used to target acute leukemia stem cells (Wang et al. 2008). Doxorubicin-loaded liposomes were successfully targeted to the kidney by using Fab′ fragments of the monoclonal OX7 antibody directed against Thy1.1 antigen in rats (Tuffin et al. 2005). Since fibroblast activation protein (FAP) represents a cell surface antigen expressed by the tumor stromal fibroblasts in different cancers, scFv from the antibody cross-reacting with human and mouse FAP was used to target PEGylated liposomes to tumor stromal cells (Baum et al. 2007). Tumor necrotic zones were effectively targeted by doxorubicin-loaded liposomes modified with chimeric TNT-3 monoclonal antibody specific towards degenerating cells located in necrotic regions of tumors and demonstrated enhanced therapeutic efficacy in nude mice bearing H460 tumors (Pan et al. 2008). The combination of immunoliposome and endosome-disruptive peptide improves cytosolic delivery of liposomal drugs, increases cytotoxicity, and opens new approaches to constructing targeted liposomal systems, as shown with diphtheria toxin A chain incorporated together with pH-dependent fusogenic peptide diINF-7 into liposomes specific towards ovarian carcinoma (Mastrobattista et al. 2002).

Early clinical trials of antibody-targeted drug-loaded liposomes have already demonstrated some promising results. Thus, doxorubicin-loaded PEGylated liposomes (with a size of approx. 140 nm) modified with $F(ab')_2$ fragments of the GAH monoclonal antibody specific for stomach cancer were tested in a Phase I clinical study and demonstrated pharmacokinetics similar to that of Doxil® (Matsumura et al. 2004).

Since transferrin (Tf) receptors (TfR) are over-expressed on the surface of certain tumor cells, antibodies against TfR as well as Tf itself are among popular ligands for liposome targeting to tumors and inside tumor cells (Hatakeyama et al. 2004) (although TfR expression in normal cells, particularly in the liver, can compete with tumor targeting of Tf-liposomes). Recent studies involve the coupling of Tf to PEG on PEGylated liposomes in order to combine longevity and targetability for drug delivery into solid tumors (Ishida et al. 2001). A similar approach was applied to deliver into tumors agents for photodynamic therapy, including hypericin (Derycke and De Witte 2002; Gijsens et al. 2002), and for intracellular delivery of cisplatin into gastric tumors (Iinuma et al. 2002). Tf-coupled doxorubicin-loaded liposomes demonstrate increased binding and toxicity against C6 glioma (Eavarone et al. 2000). Interestingly, the increase in the expression of the TfR was also discovered in post-ischemic cerebral endothelium, and was used to deliver Tf-modified PEG-liposomes to post-ischemic brain in rats (Omori et al. 2003). Tf (Joshee et al. 2002) as well as anti-TfR antibodies (Tan et al. 2003; Xu et al. 2002) were also used to facilitate gene delivery into cells by cationic liposomes. Tf-mediated liposome delivery was also successfully used for brain targeting. Immunoliposomes with OX26 monoclonal antibody to rat TfR were found to concentrate on brain microvascular endothelium (Huwyler et al. 1996).

Targeting tumors with folate-modified liposomes represents a very popular approach, since folate receptor (FR) is frequently over-expressed in many tumor cells. After early studies demonstrated the possibility of delivery of macromolecules (Leamon and Low 1991) and then liposomes (Lee and Low 1994) into living cells utilizing FR endocytosis, which could bypass multidrug resistance, the interest in folate-targeted drug delivery by liposomes grew quickly (see important reviews in Gabizon et al. 2004; Lu and Low 2002a). Liposomal daunorubicin (Ni et al. 2002) as well as doxorubicin (Pan et al. 2003) and 5-fluorouracil (Gupta et al. 2007) were delivered into various tumor cells both in vitro and in vivo via FR and demonstrated increased cytotoxicity. Recently, the application of folate-modified doxorubicin-loaded liposomes for the treatment of acute myelogenous leukemia was combined with the induction of FR using all-*trans*-retinoic acid (Pan et al. 2002). Folate-targeted liposomes have been suggested as delivery vehicles for boron neutron capture therapy (Stephenson et al. 2003) and used also for targeting tumors with haptens for tumor immunotherapy (Lu and Low 2002b). Within the frame of gene therapy, folate-targeted liposomes were utilized for both gene targeting to tumor cells (Reddy et al. 2002) as well as for targeting tumors with antisense ODN (Leamon et al. 2003).

The search for new ligands for liposome targeting concentrates on specific receptors over-expressed on target cells (particularly cancer cells) and certain

specific components of pathological cells. Thus, liposome targeting to tumors has been achieved by using vitamin and growth factor receptors (Drummond et al. 2000). Vasoactive intestinal peptide (VIP) was used to target PEG-liposomes with radionuclides to VIP-receptors of the tumor, which resulted in an enhanced breast cancer inhibition in rats (Dagar et al. 2003). PEG-liposomes were also targeted by RGD-peptides to integrins of the tumor vasculature and, being loaded with doxorubicin, demonstrated increased efficiency against C26 colon carcinoma in a murine model (Schiffelers et al. 2003). RGD-peptide was also used for targeting liposomes to integrins on activated platelets and, thus, could be used for specific cardiovascular targeting (Lestini et al. 2002) as well as for selective drug delivery to monocytes/neutrophils in the brain (Qin et al. 2007). A similar angiogenic homing peptide was used for targeted delivery to vascular endothelium of drug-loaded liposomes in experimental treatment of tumors in mice (Asai et al. 2002). Epidermal growth factor receptor (EGFR)-targeted immunoliposomes were specifically delivered to a variety of tumor cells over-expressing EGFR (Mamot et al. 2003). Mitomycin C in long-circulating hyaluronan-targeted liposomes increases its activity against tumors over-expressing hyaluronan receptors (Peer and Margalit 2004). The ability of galactosylated liposomes to concentrate in parenchymal cells was applied for gene delivery to these cells; see Hashida et al. (2001) for review. Cisplatin-loaded liposomes specifically binding chondroitin sulfate, over-expressed in many tumor cells, were used for successful suppression of tumor growth and metastases in vivo (Lee et al. 2002). Tumor-selective targeting of PEGylated liposomes was also achieved by grafting these liposomes with basic fibroblast growth factor-binding peptide (Terada et al. 2007).

8 Active Tumor Targeting with Drug-Loaded Micelles

As with other delivery systems, the drug delivery potential of polymeric micelles may also be still further enhanced by attaching targeting ligands to the micelle surface (Fig. 8). Among those ligands one can name various sugar moieties

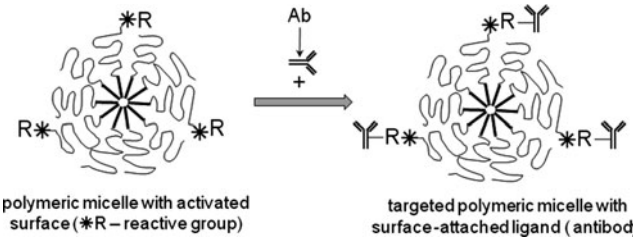


Fig. 8 As with other delivery systems, the drug delivery potential of polymeric micelles may be still further enhanced by attaching targeting ligands to the micelle surface

(Nagasaki et al. 2001), transferrin (Vinogradov et al. 1999), and folate residues (Ota et al. 2002), since many target cells, especially cancer cells, over-express appropriate receptors (such as transferrin and folate receptors) on their surface. Thus, it was shown that galactose- and lactose-modified micelles made of PEG–polylactide copolymer specifically interact with lectins, thus modeling targeting delivery of the micelles to hepatic sites (Jule et al. 2003; Nagasaki et al. 2001). Transferrin-modified micelles based on PEG and polyethyleneimine with a size between 70 and 100 nm are expected to target tumors with over-expressed transferrin receptors (Vinogradov et al. 1999). Mixed micelle-like complexes of PEGylated DNA and polyethyleneimine modified with transferrin (Dash et al. 2000; Ogris et al. 1999) were designed for enhanced DNA delivery into cells over-expressing the same transferrin receptors. A similar targeting approach was successfully tested with folate-modified micelles (Leamon and Low 2001). Poly(L-histidine)/PEG and poly (L-lactic acid)/PEG block copolymer micelles carrying folate residue on their surface were shown to be efficient for the delivery of adriamycin to tumor cells in vitro demonstrating potential for solid tumor treatment and combined targetability and pH sensitivity (Lee et al. 2003a).

Among all specific ligands, antibodies provide the broadest opportunities in terms of diversity of targets and specificity of interaction. Several attempts to covalently attach an antibody to surfactant or polymeric micelles (i.e., to prepare immunomicelles) have been described (Kabanov et al. 1989; Torchilin 2001; Torchilin et al. 2003b; Vinogradov et al. 1999). Thus, micelles modified with fatty acid-conjugated Fab fragments of antibodies to antigens of brain glia cells (acid gliofibrillar antigen and α_2-glycoprotein) loaded with neuroleptic trifluoperazine increasingly accumulated in the rat brain upon intracarotide administration (Chekhonin et al. 1991; Kabanov et al. 1989).

By adapting the coupling technique developed for attaching specific ligands to liposomes (Leamon and Low 2001), PEG–PE-based immunomicelles modified with monoclonal antibodies have been prepared. The approach uses PEG–PE with the free PEG terminus activated with a *p*-nitrophenylcarbonyl (pNP) group. Diacyllipid fragments of such a bifunctional PEG derivative firmly incorporate into the micelle core, while the water-exposed pNP group, stable at pH values below 6, efficiently interacts with amino-groups of various ligands (such as antibodies and their fragments) at pH values above 7.5 yielding a stable urethane (carbamate) bond. All nonreacted pNP groups spontaneously hydrolyze at the same pH values. To prepare immunotargeted micelles, the antibody to be attached was simply incubated with drug-loaded micelles at pH around 8.0 (Lee et al. 2003a; Torchilin et al. 2003b). Both the original and antibody-modified micelles have a spherical shape, and a uniform size of about 20 nm. The micelle-attached protein was quantified using fluorescent labels or by SDS-PAGE (Gao et al. 2003; Torchilin et al. 2003b). It was calculated that 10 to 20 antibody molecules could be attached to a single micelle. Antibodies attached to the micelle corona preserve their specific binding ability. Blood clearance data in mice showed similar pharmacokinetic profiles for 2C5-modified and plain PEG–PE micelles, confirming the long circulation of prepared immunomicelles.

To specifically enhance the tumor accumulation of PEG–PE-based micelles, the latter have been modified with tumor-specific anti-nucleosome monoclonal antibodies, such as mAb 2C5 (Lee et al. 2003a; Torchilin et al. 2003b). Rhodamine-labeled 2C5-immunomicelles effectively bind to the surface of several unrelated tumor cell lines: human BT20 (breast adenocarcinoma) and murine LLC (Lewis lung carcinoma) and EL4 (T-lymphoma) cells. Paclitaxel-loaded 2C5-immunomicelles also demonstrated the same specific properties as "empty" immunomicelles and effectively bound various tumor cells. In studies in vivo, ^{111}In-labeled 2C5-immunomicelles demonstrated significantly higher accumulation in LLC-tumor-bearing female C57BL/6J mice than plain micelles, were able to bring more micelle-incorporated drug into the tumor, and demonstrated significantly higher ability to inhibit tumor growth (Lee et al. 2003a; Torchilin et al. 2003b).

A few other specific ligands (glycoproteins, lipoproteins, carbohydrates, peptides) have also been used to achieve active targeting by polymeric micelles (Jule et al. 2003; Vinogradov et al. 1999; Wakebayashi et al. 2004). Polymeric micelles modified with sugar moieties (glucose, galactose, mannose, lactose) have been particularly successful (Jule et al. 2003; Nagasaki et al. 2001). Folate-targeted mixed block-copolymer micelles have been prepared consisting of folate-PEG-poly (DL-lactic-glycolic acid) and folate-free copolymers bearing a single doxorubicin moiety per polymeric chain (Yoo and Park 2004). Such folate-targeted doxorubicin-loaded micelles demonstrated better uptake by folate receptor over-expressing human squamous carcinoma cells of oral cavity and higher cytotoxicity against these cells both in vitro and in vivo compared to folate-free micelles. There also exist quite a few other examples of drug-loaded targeted polymeric micelles for cancer therapy (see, for example, Park et al. 2005; Vinogradov et al. 1998, 1999; Xiong et al. 2007).

In case of targeted micelles, a local release of a free drug from micelles in the target organ should lead to the increased efficacy of the drug, while the stability of the micelles en route to the target organ or tissue should contribute drug solubility and toxicity reduction due to less interaction with nontarget organs.

9 Conclusion

Summing up this section, one must note that there are several clear aims when using antibody-mediated tumor targeting of drug-loaded nanocarriers compared to more traditional dosage forms: (1) such delivery systems should accumulate in target tumors fast and effectively; (2) the quantity of the drug delivered into the tumor by such systems should be higher than in the case of other delivery systems; (3) ideally, drugs in nanocarriers should not only accumulate in the interstitial space inside tumors but also be internalized by the target cells creating high intracellular drug concentration and allowing multidrug resistance to be bypassed.

To achieve these goals, certain considerations should be taken into account when developing targeted preparations for chemotherapy. First, a target should be

identified which is present (over-expressed) on the surface of tumor cells in sufficient quantity providing good opportunity for the targeted liposomes to firmly bind with cancer cells (Hosokawa et al. 2003). Second, the specific ligand (antibody or its fragment) should be attached to the surface of the drug-loaded nanocarrier in a way which does not affect its specific binding properties (optimal choice should be made from the variety of coupling methods available, keeping in mind that the method suitable for one antibody will not necessarily be suitable for another one), and in sufficient quantity to provide multipoint binding with the target; and in the case of PEGylated long-circulating carriers the quantity of the attached antibodies should not be excessive so as not to compromise the longevity too much (Lukyanov et al. 2004a; Moreira et al. 2002). Third, it is highly desirable that the targeting antibody is internalizable and facilitates the internalization of the carrier and carrier-incorporated anti-cancer drug (Kirpotin et al. 2006; Mamot et al. 2005). Fourth, drug release from the carrier inside the tumor or inside the tumor cell should deliver the therapeutic concentration of the drug in the target and maintain it for a reasonable period of time (a few hours) (Allen et al. 2005; Sapra and Allen 2004).

References

Abra RM, Bankert RB, Chen F, Egilmez NK, Huang K, Saville R, Slater JL, Sugano M, Yokota SJ (2002) The next generation of liposome delivery systems: recent experience with tumor-targeted, sterically-stabilized immunoliposomes and active-loading gradients. J Liposome Res 12:1–3

Adams ML, Lavasanifar A, Kwon GS (2003) Amphiphilic block copolymers for drug delivery. J Pharm Sci 92:1343–1355

Aliabadi HM, Lavasanifar A (2006) Polymeric micelles for drug delivery. Expert Opin Drug Deliv 3:139–162

Allen C, Yu Y, Maysinger D, Eisenberg A (1998) Polycaprolactone-*b*-poly(ethylene oxide) block copolymer micelles as a novel drug delivery vehicle for neurotrophic agents FK506 and L-685,818. Bioconjug Chem 9:564–572

Allen TM (1994) The use of glycolipids and hydrophilic polymers in avoiding rapid uptake of liposomes by the mononuclear phagocyte system. Adv Drug Deliv Rev 13:285–309

Allen TM, Brandeis E, Hansen CB, Kao GY, Zalipsky S (1995a) A new strategy for attachment of antibodies to sterically stabilized liposomes resulting in efficient targeting to cancer cells. Biochim Biophys Acta 1237:99–108

Allen TM, Hansen C (1991) Pharmacokinetics of stealth versus conventional liposomes: effect of dose. Biochim Biophys Acta 1068:133–141

Allen TM, Hansen C, Martin F, Redemann C, Yau-Young A (1991) Liposomes containing synthetic lipid derivatives of poly(ethylene glycol) show prolonged circulation half-lives in vivo. Biochim Biophys Acta 1066:29–36

Allen TM, Hansen C, Rutledge J (1989) Liposomes with prolonged circulation times: factors affecting uptake by reticuloendothelial and other tissues. Biochim Biophys Acta 981:27–35

Allen TM, Hansen CB, de Menezes DEL (1995b) Pharmacokinetics of long-circulating liposomes. Adv Drug Deliv Rev 16:267–284

Allen TM, Mehra T, Hansen C, Chin YC (1992) Stealth liposomes: an improved sustained release system for 1-beta-D-arabinofuranosylcytosine. Cancer Res 52:2431–2439

Allen TM, Mumbengegwi DR, Charrois GJ (2005) Anti-CD19-targeted liposomal doxorubicin improves the therapeutic efficacy in murine B-cell lymphoma and ameliorates the toxicity of liposomes with varying drug release rates. Clin Cancer Res 11:3567–3573

Alonso MJ (2004) Nanomedicines for overcoming biological barriers. Biomed Pharmacother 58:168–172

Asai T, Shimizu K, Kondo M, Kuromi K, Watanabe K, Ogino K, Taki T, Shuto S, Matsuda A, Oku N (2002) Anti-neovascular therapy by liposomal DPP-CNDAC targeted to angiogenic vessels. FEBS Lett 520:167–170

Asgeirsdottir SA, Zwiers PJ, Morselt HW, Moorlag HE, Bakker HI, Heeringa P, Kok JW, Kallenberg CG, Molema G, Kamps JA (2008) Inhibition of proinflammatory genes in anti-GBM glomerulonephritis by targeted dexamethasone-loaded AbEsel liposomes. Am J Physiol Renal Physiol 294:F554–F561

Asokan A, Cho MJ (2003) Cytosolic delivery of macromolecules II. Mechanistic studies with pH-sensitive morpholine lipids. Biochim Biophys Acta 1611:151–160

Atobe K, Ishida T, Ishida E, Hashimoto K, Kobayashi H, Yasuda J, Aoki T, Obata K, Kikuchi H, Akita H, Asai T, Harashima H, Oku N, Kiwada H (2007) In vitro efficacy of a sterically stabilized immunoliposomes targeted to membrane type 1 matrix metalloproteinase (MT1-MMP). Biol Pharm Bull 30:972–978

Attwood D, Florence AT (1983) Surfactant systems: their chemistry, pharmacy, and biology. Chapman and Hall, London, p 794

Bae Y, Nishiyama N, Fukushima S, Koyama H, Yasuhiro M, Kataoka K (2005) Preparation and biological characterization of polymeric micelle drug carriers with intracellular pH-triggered drug release property: tumor permeability, controlled subcellular drug distribution, and enhanced in vivo antitumor efficacy. Bioconjug Chem 16:122–130

Baum P, Muller D, Ruger R, Kontermann RE (2007) Single-chain Fv immunoliposomes for the targeting of fibroblast activation protein-expressing tumor stromal cells. J Drug Target 15:399–406

Beduneau A, Saulnier P, Hindre F, Clavreul A, Leroux JC, Benoit JP (2007) Design of targeted lipid nanocapsules by conjugation of whole antibodies and antibody Fab' fragments. Biomaterials 28:4978–4990

Blume G, Cevc G, Crommelin MD, Bakker-Woudenberg IA, Kluft C, Storm G (1993) Specific targeting with poly(ethylene glycol)-modified liposomes: coupling of homing devices to the ends of the polymeric chains combines effective target binding with long circulation times. Biochim Biophys Acta 1149:180–184

Bogdanov AA Jr, Klibanov AL, Torchilin VP (1988) Protein immobilization on the surface of liposomes via carbodiimide activation in the presence of *N*-hydroxysulfosuccinimide. FEBS Lett 231:381–384

Boman NL, Masin D, Mayer LD, Cullis PR, Bally MB (1994) Liposomal vincristine which exhibits increased drug retention and increased circulation longevity cures mice bearing P388 tumors. Cancer Res 54:2830–2833

Boomer JA, Thompson DH (1999) Synthesis of acid-labile diplasmenyl lipids for drug and gene delivery applications. Chem Phys Lipids 99:145–153

Brignole C, Marimpietri D, Gambini C, Allen TM, Ponzoni M, Pastorino F (2003) Development of Fab' fragments of anti-GD(2) immunoliposomes entrapping doxorubicin for experimental therapy of human neuroblastoma. Cancer Lett 197:199–204

Budker V, Gurevich V, Hagstrom JE, Bortzov F, Wolff JA (1996) pH-sensitive, cationic liposomes: a new synthetic virus-like vector. Nat Biotechnol 14:760–764

Cammas S, Suzuki K, Sone C, Sakurai Y, Kataoka K, Okano T (1997) Thermo-responsive polymer nanoparticles with a core-shell micelle structure as site-specific drug carriers. J Control Release 48:157–164

Chazov EI, Alexeev AV, Antonov AS, Koteliansky VE, Leytin VL, Lyubimova EV, Repin VS, Sviridov DD, Torchilin VP, Smirnov VN (1981) Endothelial cell culture on fibrillar collagen: model to study platelet adhesion and liposome targeting to intercellular collagen matrix. Proc Natl Acad Sci USA 78:5603–5607

Chazov EI, Matveeva LS, Mazaev AV, Sargin KE, Sadovskaia GV, Ruda MI (1976) Intracoronary administration of fibrinolysin in acute myocardial infarct. Ter Arkh 48:8–19

Chekhonin VP, Kabanov AV, Zhirkov YA, Morozov GV (1991) Fatty acid acylated Fab-fragments of antibodies to neurospecific proteins as carriers for neuroleptic targeted delivery in brain. FEBS Lett 287:149–152

Cheng WW, Allen TM (2008) Targeted delivery of anti-CD19 liposomal doxorubicin in B-cell lymphoma: a comparison of whole monoclonal antibody, Fab' fragments and single chain Fv. J Control Release 126:50–58

Cheng WW, Das D, Suresh M, Allen TM (2007) Expression and purification of two anti-CD19 single chain Fv fragments for targeting of liposomes to CD19-expressing cells. Biochim Biophys Acta 1768:21–29

Cheung CY, Murthy N, Stayton PS, Hoffman AS (2001) A pH-sensitive polymer that enhances cationic lipid-mediated gene transfer. Bioconjug Chem 12:906–910

Chonn A, Semple SC, Cullis PR (1991) Separation of large unilamellar liposomes from blood components by a spin column procedure: towards identifying plasma proteins which mediate liposome clearance in vivo. Biochim Biophys Acta 1070:215–222

Chonn A, Semple SC, Cullis PR (1992) Association of blood proteins with large unilamellar liposomes in vivo. Relation to circulation lifetimes. J Biol Chem 267:18759–18765

Chung JE, Yokoyama M, Aoyagi T, Sakurai Y, Okano T (1998) Effect of molecular architecture of hydrophobically modified poly(*N*-isopropylacrylamide) on the formation of thermoresponsive core-shell micellar drug carriers. J Control Release 53:119–130

Chung JE, Yokoyama M, Yamato M, Aoyagi T, Sakurai Y, Okano T (1999) Thermo-responsive drug delivery from polymeric micelles constructed using block copolymers of poly(*N*-isopropylacrylamide) and poly(butylmethacrylate). J Control Release 62:115–127

Cohen S, Bernstein H (1996) Microparticulate systems for the delivery of proteins and vaccines. Drugs and the pharmaceutical sciences, Vol 77. Marcel Dekker, New York, p 525

Connor J, Huang L (1986) pH-sensitive immunoliposomes as an efficient and target-specific carrier for antitumor drugs. Cancer Res 46:3431–3435

Dagar S, Krishnadas A, Rubinstein I, Blend MJ, Onyuksel H (2003) VIP grafted sterically stabilized liposomes for targeted imaging of breast cancer: in vivo studies. J Control Release 91:123–133

Danson S, Ferry D, Alakhov V, Margison J, Kerr D, Jowle D, Brampton M, Halbert G, Ranson M (2004) Phase I dose escalation and pharmacokinetic study of pluronic polymer-bound doxorubicin (SP1049C) in patients with advanced cancer. Br J Cancer 90:2085–2091

Dash PR, Read ML, Fisher KD, Howard KA, Wolfert M, Oupicky D, Subr V, Strohalm J, Ulbrich K, Seymour LW (2000) Decreased binding to proteins and cells of polymeric gene delivery vectors surface modified with a multivalent hydrophilic polymer and retargeting through attachment of transferrin. J Biol Chem 275:3793–3802

Derycke AS, De Witte PA (2002) Transferrin-mediated targeting of hypericin embedded in sterically stabilized PEG-liposomes. Int J Oncol 20:181–187

Ding L, Samuel J, MacLean GD, Noujaim AA, Diener E, Longenecker BM (1990) Effective drug-antibody targeting using a novel monoclonal antibody against the proliferative compartment of mammalian squamous carcinomas. Cancer Immunol Immunother 32:105–109

Drummond DC, Hong K, Park JW, Benz CC, Kirpotin DB (2000) Liposome targeting to tumors using vitamin and growth factor receptors. Vitam Horm 60:285–332

Dunnick JK, McDougall IR, Aragon S, Goris ML, Kriss JP (1975) Vesicle interactions with polyamino acids and antibody: in vitro and in vivo studies. J Nucl Med 16:483–487

Eavarone DA, Yu X, Bellamkonda RV (2000) Targeted drug delivery to C6 glioma by transferrin-coupled liposomes. J Biomed Mater Res 51:10–14

Elbayoumi TA, Torchilin VP (2006) Enhanced accumulation of long-circulating liposomes modified with the nucleosome-specific monoclonal antibody 2C5 in various tumours in mice: gamma-imaging studies. Eur J Nucl Med Mol Imaging 33:1196–1205

Elbayoumi TA, Torchilin VP (2007) Enhanced cytotoxicity of monoclonal anticancer antibody 2C5-modified doxorubicin-loaded PEGylated liposomes against various tumor cell lines. Eur J Pharm Sci 32:159–168

Elbayoumi TA, Torchilin VP (2008) Tumor-specific antibody-mediated targeted delivery of Doxil® reduces the manifestation of auricular erythema side effect in mice. Int J Pharm 357:272–279

Ewer MS, Martin FJ, Henderson C, Shapiro CL, Benjamin RS, Gabizon AA (2004) Cardiac safety of liposomal anthracyclines. Semin Oncol 31:161–181

Fattal E, Couvreur P, Dubernet C (2004) "Smart" delivery of antisense oligonucleotides by anionic pH-sensitive liposomes. Adv Drug Deliv Rev 56:931–946

Flavell DJ, Noss A, Pulford KA, Ling N, Flavell SU (1997) Systemic therapy with 3BIT, a triple combination cocktail of anti-CD19, -CD22, and -CD38-saporin immunotoxins, is curative of human B-cell lymphoma in severe combined immunodeficient mice. Cancer Res 57: 4824–4829

Francis GE, Delgado C (2000) Drug targeting: strategies, principles, and applications. Humana Press, Totowa, N.J

Gabizon A, Catane R, Uziely B, Kaufman B, Safra T, Cohen R, Martin F, Huang A, Barenholz Y (1994) Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. Cancer Res 54:987–992

Gabizon A, Papahadjopoulos D (1988) Liposome formulations with prolonged circulation time in blood and enhanced uptake by tumors. Proc Natl Acad Sci USA 85:6949–6953

Gabizon A, Papahadjopoulos D (1992) The role of surface charge and hydrophilic groups on liposome clearance in vivo. Biochim Biophys Acta 1103:94–100

Gabizon A, Shmeeda H, Horowitz AT, Zalipsky S (2004) Tumor cell targeting of liposome-entrapped drugs with phospholipid-anchored folic acid-PEG conjugates. Adv Drug Deliv Rev 56:1177–1192

Gabizon AA (1992) Selective tumor localization and improved therapeutic index of anthracyclines encapsulated in long-circulating liposomes. Cancer Res 52:891–896

Gabizon AA (1995) Liposome circulation time and tumor targeting: implications for cancer chemotherapy. Adv Drug Deliv Rev 16:285–294

Gabizon AA (2001) Pegylated liposomal doxorubicin: metamorphosis of an old drug into a new form of chemotherapy. Cancer Invest 19:424–436

Gao Z, Eisenberg AA (1993) A model of micellization for block copolymers in solution. Macromolecules 26:7353–7360

Gao Z, Lukyanov AN, Chakilam AR, Torchilin VP (2003) PEG-PE/phosphatidylcholine mixed immunomicelles specifically deliver encapsulated taxol to tumor cells of different origin and promote their efficient killing. J Drug Target 11:87–92

Gijsens A, Derycke A, Missiaen L, De Vos D, Huwyler J, Eberle A, de Witte P (2002) Targeting of the photocytotoxic compound AlPcS4 to Hela cells by transferrin conjugated PEG-liposomes. Int J Cancer 101:78–85

Goldmacher VS, Blatter WA, Lambert JM, Chari RVJ (2002) Immunotoxins and antibody-drug conjugates for cancer treatment. In: Muzykantov V, Torchilin VP (eds) Biomedical aspects of drug targeting. Kluwer, Dordrecht, pp 291–309

Goncalves A, Braud AC, Viret F, Genre D, Gravis G, Tarpin C, Giovannini M, Maraninchi D, Viens P (2003) Phase I study of pegylated liposomal doxorubicin (Caelyx) in combination with carboplatin in patients with advanced solid tumors. Anticancer Res 23:3543–3548

Gref R, Minamitake Y, Peracchia MT, Trubetskoy V, Torchilin V, Langer R (1994) Biodegradable long-circulating polymeric nanospheres. Science 263:1600–1603

Gregoriadis G (1977) Targeting of drugs. Nature 265:407–411

Gregoriadis G (1988) Liposomes as drug carriers: recent trends and progress. Wiley, New York, p 910

Gregoriadis G (1993) Liposome technology, 2nd edn. CRC Press, Boca Raton, FL

Gregoriadis G (2007) Liposome technology: liposome preparation and related techniques, 3rd edn, Vol 1. Taylor & Francis, London, UK, p 352
Guo X, Szoka FC Jr (2001) Steric stabilization of fusogenic liposomes by a low-pH sensitive PEG-diortho ester-lipid conjugate. Bioconjugate Chem 12:291–300
Gupta B, Torchilin VP (2007) Monoclonal antibody 2C5-modified doxorubicin-loaded liposomes with significantly enhanced therapeutic activity against intracranial human brain U-87 MG tumor xenografts in nude mice. Cancer Immunol Immunother 56:1215–1223
Gupta Y, Jain A, Jain P, Jain SK (2007) Design and development of folate appended liposomes for enhanced delivery of 5-FU to tumor cells. J Drug Target 15:231–240
Haber E (1994) Antibody targeting as a strategy in thrombolysis. In: Khaw BA, Narula J, Strauss HW (eds) Monoclonal antibodies in cardiovascular diseases. Lea & Febiger, Malvern, pp 187–197
Hafez IM, Maurer N, Cullis PR (2001) On the mechanism whereby cationic lipids promote intracellular delivery of polynucleic acids. Gene Ther 8:1188–1196
Hagan SA, Coombes AGA, Garnett MC, Dunn SE, Davies MC, Illum L, Davis SS (1996) Polylactide-poly(ethylene glycol) copolymers as drug delivery systems 1. Characterization of water dispersible micelle-forming systems. Langmuir 12:2153–2161
Hamaguchi T, Matsumura Y, Suzuki M, Shimizu K, Goda R, Nakamura I, Nakatomi I, Yokoyama M, Kataoka K, Kakizoe T (2005) NK105, a paclitaxel-incorporating micellar nanoparticle formulation, can extend in vivo antitumour activity and reduce the neurotoxicity of paclitaxel. Br J Cancer 92:1240–1246
Harada A, Kataoka K (1998) Novel polyion complex micelles entrapping enzyme molecules in the core: preparation of narrowly-distributed micelles from lysozyme and poly(ethylene glycol)-poly(aspartic acid) block copolymer in aqueous medium. Macromolecules 31:288–294
Harrington KJ, Lewanski C, Northcote AD, Whittaker J, Peters AM, Vile RG, Stewart JS (2001) Phase II study of pegylated liposomal doxorubicin (Caelyx) as induction chemotherapy for patients with squamous cell cancer of the head and neck. Eur J Cancer 37:2015–2022
Hashida M, Nishikawa M, Yamashita F, Takakura Y (2001) Cell-specific delivery of genes with glycosylated carriers. Adv Drug Deliv Rev 52:187–196
Hatakeyama H, Akita H, Ishida E, Hashimoto K, Kobayashi H, Aoki T, Yasuda J, Obata K, Kikuchi H, Ishida T, Kiwada H, Harashima H (2007) Tumor targeting of doxorubicin by anti-MT1-MMP antibody-modified PEG liposomes. Int J Pharm 342:194–200
Hatakeyama H, Akita H, Maruyama K, Suhara T, Harashima H (2004) Factors governing the in vivo tissue uptake of transferrin-coupled polyethylene glycol liposomes in vivo. Int J Pharm 281:25–33
Heath TD, Robertson D, Birbeck MS, Davies AJ (1980) Covalent attachment of horseradish peroxidase to the outer surface of liposomes. Biochim Biophys Acta 599:42–62
Helmlinger G, Yuan F, Dellian M, Jain RK (1997) Interstitial pH and pO_2 gradients in solid tumors in vivo: high-resolution measurements reveal a lack of correlation. Nat Med 3:177–182
Hobbs SK, Monsky WL, Yuan F, Roberts WG, Griffith L, Torchilin VP, Jain RK (1998) Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. Proc Natl Acad Sci USA 95:4607–4612
Hosokawa S, Tagawa T, Niki H, Hirakawa Y, Nohga K, Nagaike K (2003) Efficacy of immunoliposomes on cancer models in a cell-surface-antigen-density-dependent manner. Br J Cancer 89:1545–1551
Huang SK, Mayhew E, Gilani S, Lasic DD, Martin FJ, Papahadjopoulos D (1992) Pharmacokinetics and therapeutics of sterically stabilized liposomes in mice bearing C-26 colon carcinoma. Cancer Res 52:6774–6781
Huang SK, Stauffer PR, Hong K, Guo JW, Phillips TL, Huang A, Papahadjopoulos D (1994) Liposomes and hyperthermia in mice: increased tumor uptake and therapeutic efficacy of doxorubicin in sterically stabilized liposomes. Cancer Res 54:2186–2191
Hunter RJ (1991) Foundations of colloid science. Oxford University Press, New York

Hussain S, Pluckthun A, Allen TM, Zangemeister-Wittke U (2007) Antitumor activity of an epithelial cell adhesion molecule targeted nanovesicular drug delivery system. Mol Cancer Ther 6:3019–3027

Huwyler J, Wu D, Pardridge WM (1996) Brain drug delivery of small molecules using immunoliposomes. Proc Natl Acad Sci USA 93:14164–14169

Hwang KJ (1987) Liposome pharmacokinetics. In: Ostro MJ (ed) Liposomes: from biophysics to therapeutics. Dekker, New York, pp 109–156

Iakoubov L, Mongayt D, Torchilin VP (1995a) Monoclonal anti-nuclear autoantibody from the aged effectively suppresses tumor development in vivo. Cancer Biother Radiopharm 8:299–310

Iakoubov L, Rokhlin O, Torchilin V (1995b) Anti-nuclear autoantibodies of the aged reactive against the surface of tumor but not normal cells. Immunol Lett 47:147–149

Iakoubov LZ, Torchilin VP (1997) A novel class of antitumor antibodies: nucleosome-restricted antinuclear autoantibodies (ANA) from healthy aged nonautoimmune mice. Oncol Res 9: 439–446

Iakoubov LZ, Torchilin VP (1998) Nucleosome-releasing treatment makes surviving tumor cells better targets for nucleosome-specific anticancer antibodies. Cancer Detect Prev 22:470–475

Iinuma H, Maruyama K, Okinaga K, Sasaki K, Sekine T, Ishida O, Ogiwara N, Johkura K, Yonemura Y (2002) Intracellular targeting therapy of cisplatin-encapsulated transferrin-polyethylene glycol liposome on peritoneal dissemination of gastric cancer. Int J Cancer 99:130–137

Imura Y, Stassen JM, Kurokawa T, Iwasa S, Lijnen HR, Collen D (1992) Thrombolytic and pharmacokinetic properties of an immunoconjugate of single-chain urokinase-type plasminogen activator (u-PA) and a bispecific monoclonal antibody against fibrin and against u-PA in baboons. Blood 79:2322–2329

Ishida O, Maruyama K, Tanahashi H, Iwatsuru M, Sasaki K, Eriguchi M, Yanagie H (2001) Liposomes bearing polyethyleneglycol-coupled transferrin with intracellular targeting property to the solid tumors in vivo. Pharm Res 18:1042–1048

Ishida T, Iden DL, Allen TM (1999) A combinatorial approach to producing sterically stabilized (Stealth) immunoliposomal drugs. FEBS Lett 460:129–133

Jain RK (1999) Transport of molecules, particles, and cells in solid tumors. Annu Rev Biomed Eng 1:241–263

Jeong JH, Kim SW, Park TG (2003) Novel intracellular delivery system of antisense oligonucleotide by self-assembled hybrid micelles composed of DNA/PEG conjugate and cationic fusogenic peptide. Bioconjug Chem 14:473–479

Jeong YI, Cheon JB, Kim SH, Nah JW, Lee YM, Sung YK, Akaike T, Cho CS (1998) Clonazepam release from core-shell type nanoparticles in vitro. J Control Release 51:169–178

Johnston SR, Gore ME (2001) Caelyx: phase II studies in ovarian cancer. Eur J Cancer 37(Suppl 9): S8–S14

Jones M, Leroux J (1999) Polymeric micelles – a new generation of colloidal drug carriers. Eur J Pharm Biopharm 48:101–111

Jones MC, Ranger M, Leroux JC (2003) pH-sensitive unimolecular polymeric micelles: synthesis of a novel drug carrier. Bioconjug Chem 14:774–781

Joshee N, Bastola DR, Cheng PW (2002) Transferrin-facilitated lipofection gene delivery strategy: characterization of the transfection complexes and intracellular trafficking. Hum Gene Ther 13:1991–2004

Jule E, Nagasaki Y, Kataoka K (2003) Lactose-installed poly(ethylene glycol)-poly(d,l-lactide) block copolymer micelles exhibit fast-rate binding and high affinity toward a protein bed simulating a cell surface. A surface plasmon resonance study. Bioconjug Chem 14:177–186

Kabanov AV, Batrakova EV, Alakhov VY (2002a) Pluronic block copolymers as novel polymer therapeutics for drug and gene delivery. J Control Release 82:189–212

Kabanov AV, Batrakova EV, Melik-Nubarov NS, Fedoseev NA, Dorodnich TY, Alakhov VY, Chekhonin VP, Nazarova IR, Kabanov VA (1992) A new class of drug carriers: micelles of

poly(oxyethylene)-poly(oxypropylene) block copolymer as microcontainers for drug targeting from blood in brain. J Control Release 22:141–157

Kabanov AV, Chekhonin VP, Alakhov V, Batrakova EV, Lebedev AS, Melik-Nubarov NS, Arzhakov SA, Levashov AV, Morozov GV, Severin ES et al (1989) The neuroleptic activity of haloperidol increases after its solubilization in surfactant micelles. Micelles as microcontainers for drug targeting. FEBS Lett 258:343–345

Kabanov AV, Lemieux P, Vinogradov S, Alakhov V (2002b) Pluronic block copolymers: novel functional molecules for gene therapy. Adv Drug Deliv Rev 54:223–233

Kakizawa Y, Kataoka K (2002) Block copolymer micelles for delivery of gene and related compounds. Adv Drug Deliv Rev 54:203–222

Kale AA, Torchilin VP (2007) Design, synthesis, and characterization of pH-sensitive PEG-PE conjugates for stimuli-sensitive pharmaceutical nanocarriers: the effect of substitutes at the hydrazone linkage on the pH stability of PEG-PE conjugates. Bioconjug Chem 18:363–370

Kamps JA, Koning GA, Velinova MJ, Morselt HW, Wilkens M, Gorter A, Donga J, Scherphof GL (2000) Uptake of long-circulating immunoliposomes, directed against colon adenocarcinoma cells, by liver metastases of colon cancer. J Drug Target 8:235–245

Kamps JA, Scherphof GL (1998) Receptor versus non-receptor mediated clearance of liposomes. Adv Drug Deliv Rev 32:81–97

Kang N, Perron ME, Prud'homme RE, Zhang Y, Gaucher G, Leroux JC (2005) Stereocomplex block copolymer micelles: core-shell nanostructures with enhanced stability. Nano Lett 5:315–319

Katayose S, Kataoka K (1998) Remarkable increase in nuclease resistance of plasmid DNA through supramolecular assembly with poly(ethylene glycol)-poly(L-lysine) block copolymer. J Pharm Sci 87:160–163

Khaw BA (2002) Targeting the pathological myocardium. In: Muzykantov V, Torchilin VP (eds) Biomedical aspects of drug targeting. Kluwer, Dordrecht, pp 47–67

Khaw BA, daSilva J, Vural I, Narula J, Torchilin VP (2001) Intracytoplasmic gene delivery for in vitro transfection with cytoskeleton-specific immunoliposomes. J Control Release 75:199–210

Khaw BA, Torchilin VP, Vural I, Narula J (1995) Plug and seal: prevention of hypoxic cardiocyte death by sealing membrane lesions with antimyosin-liposomes. Nat Med 1:1195–1198

Kim TY, Kim DW, Chung JY, Shin SG, Kim SC, Heo DS, Kim NK, Bang YJ (2004) Phase I and pharmacokinetic study of Genexol-PM, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies. Clin Cancer Res 10:3708–3716

Kirpotin D, Park JW, Hong K, Zalipsky S, Li WL, Carter P, Benz CC, Papahadjopoulos D (1997) Sterically stabilized anti-HER2 immunoliposomes: design and targeting to human breast cancer cells in vitro. Biochemistry 36:66–75

Kirpotin DB, Drummond DC, Shao Y, Shalaby MR, Hong K, Nielsen UB, Marks JD, Benz CC, Park JW (2006) Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models. Cancer Res 66:6732–6740

Klibanov AL, Maruyama K, Beckerleg AM, Torchilin VP, Huang L (1991) Activity of amphipathic poly(ethylene glycol) 5000 to prolong the circulation time of liposomes depends on the liposome size and is unfavorable for immunoliposome binding to target. Biochim Biophys Acta 1062:142–148

Klibanov AL, Maruyama K, Torchilin VP, Huang L (1990) Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes. FEBS Lett 268:235–237

Klibanov AL, Muzykantov VR, Ivanov NN, Torchilin VP (1985) Evaluation of quantitative parameters of the interaction of antibody-bearing liposomes with target antigens. Anal Biochem 150:251–257

Klibanov AL, Torchilin VP, Zalipsky S (2003) Long-circulating sterically protected liposomes. In: Torchilin VP, Weissig V (eds) Liposomes: a practical approach, 2nd edn. Oxford; New York, Oxford University Press, pp 231–265

Kohori F, Sakai K, Aoyagi T, Yokoyama M, Sakurai Y, Okano T (1998) Preparation and characterization of thermally responsive block copolymer micelles comprising poly(*N*-isopropylacrylamide-*b*-DL-lactide). J Control Release 55:87–98

Koning GA, Kamps JA, Scherphof GL (2002) Efficient intracellular delivery of 5-fluorodeoxyuridine into colon cancer cells by targeted immunoliposomes. Cancer Detect Prev 26:299–307

Koning GA, Morselt HW, Velinova MJ, Donga J, Gorter A, Allen TM, Zalipsky S, Kamps JA, Scherphof GL (1999) Selective transfer of a lipophilic prodrug of 5-fluorodeoxyuridine from immunoliposomes to colon cancer cells. Biochim Biophys Acta 1420:153–167

Kontermann RE (2006) Immunoliposomes for cancer therapy. Curr Opin Mol Ther 8:39–45

Kratz F, Beyer U, Schutte MT (1999) Drug-polymer conjugates containing acid-cleavable bonds. Crit Rev Ther Drug Carrier Syst 16:245–288

Kubota T, Yamamoto T, Takahara T, Furukawa T, Ishibiki K, Kitajima M, Shida Y, Nakatsubo H (1992) Targeting cancer chemotherapy using a monoclonal antibody (NCC-LU-243) conjugated with mitomycin C. J Surg Oncol 51:75–80

Kung VT, Redemann CT (1986) Synthesis of carboxyacyl derivatives of phosphatidylethanolamine and use as an efficient method for conjugation of protein to liposomes. Biochim Biophys Acta 862:435–439

Kwon GS (1998) Diblock copolymer nanoparticles for drug delivery. Crit Rev Ther Drug Carrier Syst 15:481–512

Kwon GS (2003) Polymeric micelles for delivery of poorly water-soluble compounds. Crit Rev Ther Drug Carrier Syst 20:357–403

Kwon GS, Kataoka K (1995) Block copolymer micelles as long-circulating drug vehicles. Adv Drug Deliv Rev 16:295–309

Kwon GS, Kataoka K (1999) Block copolymer micelles as long-circulating drug vehicles. Adv Drug Delivery Rev 16:295–309

Kwon GS, Okano T (1999) Soluble self-assembled block copolymers for drug delivery. Pharm Res 16:597–600

La SB, Okano T, Kataoka K (1996) Preparation and characterization of the micelle-forming polymeric drug indomethacin-incorporated poly(ethylene oxide)-poly(beta-benzyl L-aspartate) block copolymer micelles. J Pharm Sci 85:85–90

Lasic DD (1992) Mixed micelles in drug delivery. Nature 355:279–280

Lasic DD, Barenholz Y (1996) Handbook of nonmedical applications of liposomes. CRC Press, Boca Raton

Lasic DD, Martin FJ (1995) Stealth liposomes. CRC Press, Boca Raton, p 320

Lasic DD, Martin FJ, Gabizon A, Huang SK, Papahadjopoulos D (1991a) Sterically stabilized liposomes: a hypothesis on the molecular origin of the extended circulation times. Biochim Biophys Acta 1070:187–192

Lasic DD, Papahadjopoulos D (1998) Medical applications of liposomes. Elsevier, Amsterdam, New York, p 779

Lasic DD, Woodle MC, Martin FJ, Valentincic T (1991b) Phase behavior of "stealth-lipid" decithin mixtures. Period Biol 93:287–290

Lavasanifar A, Samuel J, Kwon GS (2002) The effect of fatty acid substitution on the in vitro release of amphotericin B from micelles composed of poly(ethylene oxide)-block-poly (*N*-hexyl stearate-L-aspartamide). J Control Release 79:165–172

Le Garrec D, Taillefer J, Van Lier JE, Lenaerts V, Leroux JC (2002) Optimizing pH-responsive polymeric micelles for drug delivery in a cancer photodynamic therapy model. J Drug Target 10:429–437

Leamon CP, Cooper SR, Hardee GE (2003) Folate-liposome-mediated antisense oligodeoxynucleotide targeting to cancer cells: evaluation in vitro and in vivo. Bioconjug Chem 14:738–747

Leamon CP, Low PS (1991) Delivery of macromolecules into living cells: a method that exploits folate receptor endocytosis. Proc Natl Acad Sci USA 88:5572–5576

Leamon CP, Low PS (2001) Folate-mediated targeting: from diagnostics to drug and gene delivery. Drug Discov Today 6:44–51

Lee CM, Tanaka T, Murai T, Kondo M, Kimura J, Su W, Kitagawa T, Ito T, Matsuda H, Miyasaka M (2002) Novel chondroitin sulfate-binding cationic liposomes loaded with cisplatin efficiently suppress the local growth and liver metastasis of tumor cells in vivo. Cancer Res 62:4282–4288

Lee ES, Na K, Bae YH (2003a) Polymeric micelle for tumor pH and folate-mediated targeting. J Control Release 91:103–113

Lee ES, Na K, Bae YH (2005) Super pH-sensitive multifunctional polymeric micelle. Nano Lett 5:325–329

Lee ES, Shin HJ, Na K, Bae YH (2003b) Poly(L-histidine)-PEG block copolymer micelles and pH-induced destabilization. J Control Release 90:363–374

Lee RJ, Low PS (1994) Delivery of liposomes into cultured KB cells via folate receptor-mediated endocytosis. J Biol Chem 269:3198–3204

Leroux J, Roux E, Le Garrec D, Hong K, Drummond DC (2001) *N*-isopropylacrylamide copolymers for the preparation of pH-sensitive liposomes and polymeric micelles. J Control Release 72:71–84

Leserman LD, Barbet J, Kourilsky F, Weinstein JN (1980) Targeting to cells of fluorescent liposomes covalently coupled with monoclonal antibody or protein A. Nature 288:602–604

Lestini BJ, Sagnella SM, Xu Z, Shive MS, Richter NJ, Jayaseharan J, Case AJ, Kottke-Marchant K, Anderson JM, Marchant RE (2002) Surface modification of liposomes for selective cell targeting in cardiovascular drug delivery. J Control Release 78:235–247

Levchenko TS, Rammohan R, Lukyanov AN, Whiteman KR, Torchilin VP (2002) Liposome clearance in mice: the effect of a separate and combined presence of surface charge and polymer coating. Int J Pharm 240:95–102

Liu D, Mori A, Huang L (1991) Large liposomes containing ganglioside GM1 accumulate effectively in spleen. Biochim Biophys Acta 1066:159–165

Liu D, Mori A, Huang L (1992) Role of liposome size and RES blockade in controlling biodistribution and tumor uptake of GM1-containing liposomes. Biochim Biophys Acta 1104:95–101

Lopes de Menezes DE, Pilarski LM, Allen TM (1998) In vitro and in vivo targeting of immunoliposomal doxorubicin to human B-cell lymphoma. Cancer Res 58:3320–3330

Lu Y, Low PS (2002a) Folate-mediated delivery of macromolecular anticancer therapeutic agents. Adv Drug Deliv Rev 54:675–693

Lu Y, Low PS (2002b) Folate targeting of haptens to cancer cell surfaces mediates immunotherapy of syngeneic murine tumors. Cancer Immunol Immunother 51:153–162

Lukyanov AN, Elbayoumi TA, Chakilam AR, Torchilin VP (2004a) Tumor-targeted liposomes: doxorubicin-loaded long-circulating liposomes modified with anti-cancer antibody. J Control Release 100:135–144

Lukyanov AN, Gao Z, Mazzola L, Torchilin VP (2002) Polyethylene glycol-diacyllipid micelles demonstrate increased acculumation in subcutaneous tumors in mice. Pharm Res 19:1424–1429

Lukyanov AN, Hartner WC, Torchilin VP (2004b) Increased accumulation of PEG-PE micelles in the area of experimental myocardial infarction in rabbits. J Control Release 94:187–193

Lukyanov AN, Torchilin VP (2004) Micelles from lipid derivatives of water-soluble polymers as delivery systems for poorly soluble drugs. Adv Drug Deliv Rev 56:1273–1289

Lundberg BB, Griffiths G, Hansen HJ (2007) Cellular association and cytotoxicity of doxorubicin-loaded immunoliposomes targeted via Fab' fragments of an anti-CD74 antibody. Drug Deliv 14:171–175

Madden TD, Bally MB, Hope MJ, Cullis PR, Schieren HP, Janoff AS (1985) Protection of large unilamellar vesicles by trehalose during dehydration: retention of vesicle contents. Biochim Biophys Acta 817:67–74

Maeda H (2001) The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. Adv Enzyme Regul 41:189–207

Maeda H (2003) Enhanced permeability and retention (EPR) effect: basis for drug targeting to tumors. In: Muzykantov V, Torchilin VP (eds) Biomedical aspects of drug targeting. Kluwer, Dordrecht, pp 211–228

Maeda H, Sawa T, Konno T (2001) Mechanism of tumor-targeted delivery of macromolecular drugs, including the EPR effect in solid tumor and clinical overview of the prototype polymeric drug SMANCS. J Control Release 74:47–61

Maeda H, Wu J, Sawa T, Matsumura Y, Hori K (2000) Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J Control Release 65:271–284

Mamot C, Drummond DC, Greiser U, Hong K, Kirpotin DB, Marks JD, Park JW (2003) Epidermal growth factor receptor (EGFR)-targeted immunoliposomes mediate specific and efficient drug delivery to EGFR- and EGFRvIII-overexpressing tumor cells. Cancer Res 63:3154–3161

Mamot C, Drummond DC, Noble CO, Kallab V, Guo Z, Hong K, Kirpotin DB, Park JW (2005) Epidermal growth factor receptor-targeted immunoliposomes significantly enhance the efficacy of multiple anticancer drugs in vivo. Cancer Res 65:11631–11638

Mamot C, Ritschard R, Kung W, Park JW, Herrmann R, Rochlitz CF (2006) EGFR-targeted immunoliposomes derived from the monoclonal antibody EMD72000 mediate specific and efficient drug delivery to a variety of colorectal cancer cells. J Drug Target 14:215–223

Martin FJ, Papahadjopoulos D (1982) Irreversible coupling of immunoglobulin fragments to preformed vesicles. An improved method for liposome targeting. J Biol Chem 257:286–288

Marty C, Schwendener RA (2005) Cytotoxic tumor targeting with scFv antibody-modified liposomes. Methods Mol Med 109:389–402

Maruyama K, Okuizumi S, Ishida O, Yamauchi H, Kikuchi H, Iwatsuru M (1994) Phosphatidyl polyglycerols prolong liposome circulation in vivo. Int J Pharm 111:103–107

Maruyama K, Takahashi N, Tagawa T, Nagaike K, Iwatsuru M (1997) Immunoliposomes bearing polyethyleneglycol-coupled Fab' fragment show prolonged circulation time and high extravasation into targeted solid tumors in vivo. FEBS Lett 413:177–180

Maruyama K, Takizawa T, Yuda T, Kennel SJ, Huang L, Iwatsuru M (1995) Targetability of novel immunoliposomes modified with amphipathic poly(ethylene glycol)s conjugated at their distal terminals to monoclonal antibodies. Biochim Biophys Acta 1234:74–80

Maruyama K, Yuda T, Okamoto A, Ishikura C, Kojima S, Iwatsuru M (1991) Effect of molecular weight in amphipathic polyethyleneglycol on prolonging the circulation time of large unilamellar liposomes. Chem Pharm Bull (Tokyo) 39:1620–1622

Maruyama K, Yuda T, Okamoto A, Kojima S, Suginaka A, Iwatsuru M (1992) Prolonged circulation time in vivo of large unilamellar liposomes composed of distearoyl phosphatidylcholine and cholesterol containing amphipathic poly(ethylene glycol). Biochim Biophys Acta 1128:44–49

Mastrobattista E, Koning GA, van Bloois L, Filipe AC, Jiskoot W, Storm G (2002) Functional characterization of an endosome-disruptive peptide and its application in cytosolic delivery of immunoliposome-entrapped proteins. J Biol Chem 277:27135–27143

Matsumura Y, Hamaguchi T, Ura T, Muro K, Yamada Y, Shimada Y, Shirao K, Okusaka T, Ueno H, Ikeda M, Watanabe N (2004) Phase I clinical trial and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin. Br J Cancer 91:1775–1781

Mayhew EG, Lasic D, Babbar S, Martin FJ (1992) Pharmacokinetics and antitumor activity of epirubicin encapsulated in long-circulating liposomes incorporating a polyethylene glycol-derivatized phospholipid. Int J Cancer 51:302–309

McBain SC, Yiu HH, Dobson J (2008) Magnetic nanoparticles for gene and drug delivery. Int J Nanomedicine 3:169–180

Metselaar JM, Bruin P, de Boer LW, de Vringer T, Snel C, Oussoren C, Wauben MH, Crommelin DJ, Storm G, Hennink WE (2003) A novel family of L-amino acid-based biodegradable polymer-lipid conjugates for the development of long-circulating liposomes with effective drug-targeting capacity. Bioconjug Chem 14:1156–1164

Meyer O, Papahadjopoulos D, Leroux JC (1998) Copolymers of *N*-isopropylacrylamide can trigger pH sensitivity to stable liposomes. FEBS Lett 421:61–64

Miller DW, Batrakova EV, Waltner TO, Alakhov V, Kabanov AV (1997) Interactions of pluronic block copolymers with brain microvessel endothelial cells: evidence of two potential pathways for drug absorption. Bioconjug Chem 8:649–657

Mittal KL, Lindman B (1991) Surfactants in solution, vol 1–3. Plenum Press, New York

Moein Moghimi S, Hamad I, Bunger R, Andresen TL, Jorgensen K, Hunter AC, Baranji L, Rosivall L, Szebeni J (2006) Activation of the human complement system by cholesterol-rich and PEGylated liposomes-modulation of cholesterol-rich liposome-mediated complement activation by elevated serum LDL and HDL levels. J Liposome Res 16:167–174

Moghimi SM, Szebeni J (2003) Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonization and protein-binding properties. Prog Lipid Res 42:463–478

Molyneux P (1984) Water-soluble synthetic polymers: properties and behavior. CRC Press, Boca Raton

Moreira JN, Gaspar R, Allen TM (2001) Targeting Stealth liposomes in a murine model of human small cell lung cancer. Biochim Biophys Acta 1515:167–176

Moreira JN, Ishida T, Gaspar R, Allen TM (2002) Use of the post-insertion technique to insert peptide ligands into pre-formed stealth liposomes with retention of binding activity and cytotoxicity. Pharm Res 19:265–269

Müller RH (1991) Colloidal carriers for controlled drug delivery and targeting: modification, characterization, and in vivo distribution. CRC Press, Stuttgart, Boca Raton, Wissenschaftliche Verlagsgesellschaft

Muzykantov VR, Torchilin V (2003) Biomedical aspects of drug targeting. Kluwer, Dordrecht

Nagasaki Y, Yasugi K, Yamamoto Y, Harada A, Kataoka K (2001) Sugar-installed block copolymer micelles: their preparation and specific interaction with lectin molecules. Biomacromolecules 2:1067–1070

Naper DH (1983) Polymeric stabilization of colloidal dispersions. Academic Press, New York

Needham D, McIntosh TJ, Lasic DD (1992) Repulsive interactions and mechanical stability of polymer-grafted lipid membranes. Biochim Biophys Acta 1108:40–48

Ni S, Stephenson SM, Lee RJ (2002) Folate receptor targeted delivery of liposomal daunorubicin into tumor cells. Anticancer Res 22:2131–2135

Niedermann G, Weissig V, Sternberg B, Lasch J (1991) Carboxyacyl derivatives of cardiolipin as four-tailed hydrophobic anchors for the covalent coupling of hydrophilic proteins to liposomes. Biochim Biophys Acta 1070:401–408

Nobs L, Buchegger F, Gurny R, Allemann E (2004) Current methods for attaching targeting ligands to liposomes and nanoparticles. J Pharm Sci 93:1980–1992

O'Shaughnessy JA (2003) Pegylated liposomal doxorubicin in the treatment of breast cancer. Clin Breast Cancer 4:318–328

Ogris M, Brunner S, Schuller S, Kircheis R, Wagner E (1999) PEGylated DNA/transferrin-PEI complexes: reduced interaction with blood components, extended circulation in blood and potential for systemic gene delivery. Gene Ther 6:595–605

Omori N, Maruyama K, Jin G, Li F, Wang SJ, Hamakawa Y, Sato K, Nagano I, Shoji M, Abe K (2003) Targeting of post-ischemic cerebral endothelium in rat by liposomes bearing polyethylene glycol-coupled transferrin. Neurol Res 25:275–279

Orekhova NM, Akchurin RS, Belyaev AA, Smirnov MD, Ragimov SE, Orekhov AN (1990) Local prevention of thrombosis in animal arteries by means of magnetic targeting of aspirin-loaded red cells. Thromb Res 57:611–616

Ota T, Maeda M, Tatsuka M (2002) Cationic liposomes with plasmid DNA influence cancer metastatic capability. Anticancer Res 22:4049–4052

Otsuka H, Nagasaki Y, Kataoka K (2003) PEGylated nanoparticles for biological and pharmaceutical applications. Adv Drug Deliv Rev 55:403–419

Palmer TN, Caride VJ, Caldecourt MA, Twickler J, Abdullah V (1984) The mechanism of liposome accumulation in infarction. Biochim Biophys Acta 797:363–368

Pan H, Han L, Chen W, Yao M, Lu W (2008) Targeting to tumor necrotic regions with biotinylated antibody and streptavidin modified liposomes. J Control Release 125:228–235

Pan X, Lee RJ (2007) Construction of anti-EGFR immunoliposomes via folate-folate binding protein affinity. Int J Pharm 336:276–283

Pan X, Wu G, Yang W, Barth RF, Tjarks W, Lee RJ (2007) Synthesis of cetuximab-immunoliposomes via a cholesterol-based membrane anchor for targeting of EGFR. Bioconjug Chem 18:101–108

Pan XQ, Wang H, Lee RJ (2003) Antitumor activity of folate receptor-targeted liposomal doxorubicin in a KB oral carcinoma murine xenograft model. Pharm Res 20:417–422

Pan XQ, Zheng X, Shi G, Wang H, Ratnam M, Lee RJ (2002) Strategy for the treatment of acute myelogenous leukemia based on folate receptor beta-targeted liposomal doxorubicin combined with receptor induction using all-trans retinoic acid. Blood 100:594–602

Pang SNJ (1993) Final report on the safety assessment of polyethylene glycols (PEGs) -6, -8, -32, -75, -150, -14M, -20M. J Am Coll Toxicol 12:429–457

Papahadjopoulos D, Allen TM, Gabizon A, Mayhew E, Matthay K, Huang SK, Lee KD, Woodle MC, Lasic DD, Redemann C et al (1991) Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. Proc Natl Acad Sci USA 88:11460–11464

Park EK, Kim SY, Lee SB, Lee YM (2005) Folate-conjugated methoxy poly(ethylene glycol)/poly(epsilon-caprolactone) amphiphilic block copolymeric micelles for tumor-targeted drug delivery. J Control Release 109:158–168

Park JW, Benz CC, Martin FJ (2004) Future directions of liposome- and immunoliposome-based cancer therapeutics. Semin Oncol 31:196–205

Park JW, Hong K, Carter P, Asgari H, Guo LY, Keller GA, Wirth C, Shalaby R, Kotts C, Wood WI et al (1995) Development of anti-p185HER2 immunoliposomes for cancer therapy. Proc Natl Acad Sci USA 92:1327–1331

Park JW, Hong K, Kirpotin DB, Colbern G, Shalaby R, Baselga J, Shao Y, Nielsen UB, Marks JD, Moore D, Papahadjopoulos D, Benz CC (2002) Anti-HER2 immunoliposomes: enhanced efficacy attributable to targeted delivery. Clin Cancer Res 8:1172–1181

Park JW, Hong K, Kirpotin DB, Meyer O, Papahadjopoulos D, Benz CC (1997) Anti-HER2 immunoliposomes for targeted therapy of human tumors. Cancer Lett 118:153–160

Park JW, Kirpotin DB, Hong K, Shalaby R, Shao Y, Nielsen UB, Marks JD, Papahadjopoulos D, Benz CC (2001) Tumor targeting using anti-her2 immunoliposomes. J Control Release 74:95–113

Pastorino F, Brignole C, Di Paolo D, Nico B, Pezzolo A, Marimpietri D, Pagnan G, Piccardi F, Cilli M, Longhi R, Ribatti D, Corti A, Allen TM, Ponzoni M (2006) Targeting liposomal chemotherapy via both tumor cell-specific and tumor vasculature-specific ligands potentiates therapeutic efficacy. Cancer Res 66:10073–10082

Pastorino F, Brignole C, Marimpietri D, Sapra P, Moase EH, Allen TM, Ponzoni M (2003) Doxorubicin-loaded Fab' fragments of anti-disialoganglioside immunoliposomes selectively inhibit the growth and dissemination of human neuroblastoma in nude mice. Cancer Res 63:86–92

Pauwels EK, Erba P (2007) Towards the use of nanoparticles in cancer therapy and imaging. Drug News Perspect 20:213–220

Peer D, Margalit R (2004) Loading mitomycin C inside long circulating hyaluronan targeted nano-liposomes increases its antitumor activity in three mice tumor models. Int J Cancer 108:780–789

Perez-Lopez ME, Curiel T, Gomez JG, Jorge M (2007) Role of pegylated liposomal doxorubicin (Caelyx) in the treatment of relapsing ovarian cancer. Anticancer Drugs 18:611–617

Perez AT, Domenech GH, Frankel C, Vogel CL (2002) Pegylated liposomal doxorubicin (Doxil) for metastatic breast cancer: the Cancer Research Network, Inc. experience. Cancer Invest (Suppl 2) 20:22–29

Potineni A, Lynn DM, Langer R, Amiji MM (2003) Poly(ethylene oxide)-modified poly(beta-amino ester) nanoparticles as a pH-sensitive biodegradable system for paclitaxel delivery. J Control Release 86:223–234

Powell GM (1980) Polyethylene glycol. In: Davidson RL (ed) Handbook of water-soluble gums and resins. McGraw-Hill, New York, pp 1–31

Qin J, Chen D, Hu H, Cui Q, Qiao M, Chen B (2007) Surface modification of RGD-liposomes for selective drug delivery to monocytes/neutrophils in brain. Chem Pharm Bull (Tokyo) 55:1192–1197

Raffaghello L, Pagnan G, Pastorino F, Cosimo E, Brignole C, Marimpietri D, Bogenmann E, Ponzoni M, Montaldo PG (2003) Immunoliposomal fenretinide: a novel antitumoral drug for human neuroblastoma. Cancer Lett 197:151–155

Rammohan R, Levchenko T, Weissig V, Chakilam A, Torchilin V (2001) Immunomicelles: attachment of specific ligands including monoclonal antibodies to polymeric micelles. 28th International Symposium on Controlled Release of Bioactive Materials, San Diego 2001. Control Release Society, Inc., pp 484–485

Reddy JA, Abburi C, Hofland H, Howard SJ, Vlahov I, Wils P, Leamon CP (2002) Folate-targeted, cationic liposome-mediated gene transfer into disseminated peritoneal tumors. Gene Ther 9:1542–1550

Ringsdorf H (1975) Structure and properties of pharmacologically active polymers. J Polym Sci 51:135–153

Rolland A (1993) Pharmaceutical particulate carriers: therapeutic applications carriers. Marcel Dekker, New York

Rose PG (2005) Pegylated liposomal doxorubicin: optimizing the dosing schedule in ovarian cancer. Oncologist 10:205–214

Roth A, Drummond DC, Conrad F, Hayes ME, Kirpotin DB, Benz CC, Marks JD, Liu B (2007) Anti-CD166 single chain antibody-mediated intracellular delivery of liposomal drugs to prostate cancer cells. Mol Cancer Ther 6:2737–2746

Roux E, Francis M, Winnik FM, Leroux JC (2002a) Polymer based pH-sensitive carriers as a means to improve the cytoplasmic delivery of drugs. Int J Pharm 242:25–36

Roux E, Passirani C, Scheffold S, Benoit JP, Leroux JC (2004) Serum-stable and long-circulating, PEGylated, pH-sensitive liposomes. J Control Release 94:447–451

Roux E, Stomp R, Giasson S, Pezolet M, Moreau P, Leroux JC (2002b) Steric stabilization of liposomes by pH-responsive *N*-isopropylacrylamide copolymer. J Pharm Sci 91:1795–1802

Rowe RC, Sheskey PJ, Weller PJ (2003) Handbook of pharmaceutical excipients, 4th edn. Pharmaceutical Press, American Pharmaceutical Association, London, Chicago Washington, DC

Sapra P, Allen TM (2003) Ligand-targeted liposomal anticancer drugs. Prog Lipid Res 42: 439–462

Sapra P, Allen TM (2004) Improved outcome when B-cell lymphoma is treated with combinations of immunoliposomal anticancer drugs targeted to both the CD19 and CD20 epitopes. Clin Cancer Res 10:2530–2537

Sawant RM, Hurley JP, Salmaso S, Kale A, Tolcheva E, Levchenko TS, Torchilin VP (2006) "SMART" drug delivery systems: double-targeted pH-responsive pharmaceutical nanocarriers. Bioconjug Chem 17:943–949

Schiffelers RM, Koning GA, ten Hagen TL, Fens MH, Schraa AJ, Janssen AP, Kok RJ, Molema G, Storm G (2003) Anti-tumor efficacy of tumor vasculature-targeted liposomal doxorubicin. J Control Release 91:115–122

Schmidinger M, Wenzel C, Locker GJ, Muehlbacher F, Steininger R, Gnant M, Crevenna R, Budinsky AC (2001) Pilot study with pegylated liposomal doxorubicin for advanced or unresectable hepatocellular carcinoma. Br J Cancer 85:1850–1852

Schnyder A, Krahenbuhl S, Drewe J, Huwyler J (2005) Targeting of daunomycin using biotinylated immunoliposomes: pharmacokinetics, tissue distribution and in vitro pharmacological effects. J Drug Target 13:325–335

Schwonzen M, Kurbacher CM, Mallmann P (2000) Liposomal doxorubicin and weekly paclitaxel in the treatment of metastatic breast cancer. Anticancer Drugs 11:681–685

Senior J, Delgado C, Fisher D, Tilcock C, Gregoriadis G (1991) Influence of surface hydrophilicity of liposomes on their interaction with plasma protein and clearance from the circulation: studies with poly(ethylene glycol)-coated vesicles. Biochim Biophys Acta 1062:77–82

Senior JH (1987) Fate and behavior of liposomes in vivo: a review of controlling factors. Crit Rev Ther Drug Carrier Syst 3:123–193

Shalaev EY, Steponkus PL (1999) Phase diagram of 1, 2-dioleoylphosphatidylethanolamine (DOPE): water system at subzero temperatures and at low water contents. Biochim Biophys Acta 1419:229–247

Sheff D (2004) Endosomes as a route for drug delivery in the real world. Adv Drug Deliv Rev 56:927–930

Shuai X, Merdan T, Schaper AK, Xi F, Kissel T (2004) Core-cross-linked polymeric micelles as paclitaxel carriers. Bioconjug Chem 15:441–448

Simoes S, Moreira JN, Fonseca C, Duzgunes N, de Lima MC (2004) On the formulation of pH-sensitive liposomes with long circulation times. Adv Drug Deliv Rev 56:947–965

Skubitz KM (2003) Phase II trial of pegylated-liposomal doxorubicin (Doxil) in sarcoma. Cancer Invest 21:167–176

Sofou S, Sgouros G (2008) Antibody-targeted liposomes in cancer therapy and imaging. Expert Opin Drug Deliv 5:189–204

Sou K, Endo T, Takeoka S, Tsuchida E (2000) Poly(ethylene glycol)-modification of the phospholipid vesicles by using the spontaneous incorporation of poly(ethylene glycol)-lipid into the vesicles. Bioconjug Chem 11:372–379

Stephenson SM, Yang W, Stevens PJ, Tjarks W, Barth RF, Lee RJ (2003) Folate receptor-targeted liposomes as possible delivery vehicles for boron neutron capture therapy. Anticancer Res 23:3341–3345

Sudimack JJ, Guo W, Tjarks W, Lee RJ (2002) A novel pH-sensitive liposome formulation containing oleyl alcohol. Biochim Biophys Acta 1564:31–37

Sugano M, Egilmez NK, Yokota SJ, Chen FA, Harding J, Huang SK, Bankert RB (2000) Antibody targeting of doxorubicin-loaded liposomes suppresses the growth and metastatic spread of established human lung tumor xenografts in severe combined immunodeficient mice. Cancer Res 60:6942–6949

Sun C, Lee JS, Zhang M (2008) Magnetic nanoparticles in MR imaging and drug delivery. Adv Drug Deliv Rev 60:1252–1265

Suzawa T, Nagamura S, Saito H, Ohta S, Hanai N, Kanazawa J, Okabe M, Yamasaki M (2002) Enhanced tumor cell selectivity of adriamycin-monoclonal antibody conjugate via a poly (ethylene glycol)-based cleavable linker. J Control Release 79:229–242

Symon Z, Peyser A, Tzemach D, Lyass O, Sucher E, Shezen E, Gabizon A (1999) Selective delivery of doxorubicin to patients with breast carcinoma metastases by stealth liposomes. Cancer 86:72–78

Szoka F Jr, Papahadjopoulos D (1980) Comparative properties and methods of preparation of lipid vesicles (liposomes). Annu Rev Biophys Bioeng 9:467–508

Takeuchi H, Kojima H, Toyoda T, Yamamoto H, Hino T, Kawashima Y (1999) Prolonged circulation time of doxorubicin-loaded liposomes coated with a modified polyvinyl alcohol after intravenous injection in rats. Eur J Pharm Biopharm 48:123–129

Takeuchi H, Kojima H, Yamamoto H, Kawashima Y (2001) Evaluation of circulation profiles of liposomes coated with hydrophilic polymers having different molecular weights in rats. J Control Release 75:83–91

Tan PH, Manunta M, Ardjomand N, Xue SA, Larkin DF, Haskard DO, Taylor KM, George AJ (2003) Antibody targeted gene transfer to endothelium. J Gene Med 5:311–323

Terada T, Mizobata M, Kawakami S, Yamashita F, Hashida M (2007) Optimization of tumor-selective targeting by basic fibroblast growth factor-binding peptide grafted PEGylated liposomes. J Control Release 119:262–270

Torchilin V, Klibanov A (1993) Coupling and labeling of phospholipids. In: Cevc G (ed) Phospholipid Handbook. Marcel Dekker, New York, pp 293–322
Torchilin VP (1991) Immobilized enzymes in medicine. Progress in clinical biochemistry and medicine Vol 11. Springer, Berlin, p 206
Torchilin VP (1995) Handbook of targeted delivery of imaging agents. CRC Press, Boca Raton, FL
Torchilin VP (1996a) Affinity liposomes in vivo: factors influencing target accumulation. J Mol Recognit 9:335–346
Torchilin VP (1996b) How do polymers prolong circulation times of liposomes. J Liposome Res 9:99–116
Torchilin VP (1998) Polymer-coated long-circulating microparticulate pharmaceuticals. J Microencapsul 15:1–19
Torchilin VP (2000) Drug targeting. Eur J Pharm Sci 11(Suppl 2):S81–S91
Torchilin VP (2001) Structure and design of polymeric surfactant-based drug delivery systems. J Control Release 73:137–172
Torchilin VP (2002) Strategies and means for drug targeting: an overview. In: Muzykantov V, Torchilin VP (eds) Biomedical aspects of drug targeting. Kluwer, Boston, pp 3–26
Torchilin VP (2008) Tat peptide-mediated intracellular delivery of pharmaceutical nanocarriers. Adv Drug Deliv Rev 60:548–558
Torchilin VP, Klibanov AL, Huang L, O'Donnell S, Nossiff ND, Khaw BA (1992) Targeted accumulation of polyethylene glycol-coated immunoliposomes in infarcted rabbit myocardium. FASEB J 6:2716–2719
Torchilin VP, Klibanov AL, Ivanov NN, Gluckhova MA, Koteliansky VE, Kleinman HK, Martin GR (1985) Binding of antibodies in liposomes to extracellular matrix antigens. J Cell Biochem 28:23–29
Torchilin VP, Levchenko TS (2003) TAT-liposomes: a novel intracellular drug carrier. Curr Protein Pept Sci 4:133–140
Torchilin VP, Levchenko TS, Lukyanov AN, Khaw BA, Klibanov AL, Rammohan R, Samokhin GP, Whiteman KR (2001a) p-Nitrophenylcarbonyl-PEG-PE-liposomes: fast and simple attachment of specific ligands, including monoclonal antibodies, to distal ends of PEG chains via p-nitrophenylcarbonyl groups. Biochim Biophys Acta 1511:397–411
Torchilin VP, Levchenko TS, Rammohan R, Volodina N, Papahadjopoulos-Sternberg B, D'Souza GG (2003a) Cell transfection in vitro and in vivo with nontoxic TAT peptide-liposome-DNA complexes. Proc Natl Acad Sci USA 100:1972–1977
Torchilin VP, Levchenko TS, Whiteman KR, Yaroslavov AA, Tsatsakis AM, Rizos AK, Michailova EV, Shtilman MI (2001b) Amphiphilic poly-*N*-vinylpyrrolidones: synthesis, properties and liposome surface modification. Biomaterials 22:3035–3044
Torchilin VP, Lukyanov AN, Gao Z, Papahadjopoulos-Sternberg B (2003b) Immunomicelles: targeted pharmaceutical carriers for poorly soluble drugs. Proc Natl Acad Sci USA 100:6039–6044
Torchilin VP, Omelyanenko VG, Papisov MI, Bogdanov AA Jr, Trubetskoy VS, Herron JN, Gentry CA (1994) Poly(ethylene glycol) on the liposome surface: on the mechanism of polymer-coated liposome longevity. Biochim Biophys Acta 1195:11–20
Torchilin VP, Papisov MI, Orekhova NM, Belyaev AA, Petrov AD, Ragimov SE (1988) Magnetically driven thrombolytic preparation containing immobilized streptokinase-targeted transport and action. Haemostasis 18:113–116
Torchilin VP, Trubetskoy VS (1995) Which polymers can make nanoparticulate drug carriers long-circulating? Adv Drug Deliv Rev 16:141–155
Torchilin VP, Trubetskoy VS, Whiteman KR, Caliceti P, Ferruti P, Veronese FM (1995) New synthetic amphiphilic polymers for steric protection of liposomes in vivo. J Pharm Sci 84:1049–1053
Torchilin VP, Weissig V (2003) Liposomes: a practical approach. Practical approach series, Vol 264, 2nd edn. Oxford University Press, Oxford, p 396

Torchilin VP, Weissig V, Martin FJ, Heath TD, New RRC (2003c) Surface modifications of liposomes. In: Torchilin VP, Weissig V (eds) Liposomes: a practical approach, 2nd edn. Oxford University Press, Oxford, pp 193–229

Torchilin VP, Zhou F, Huang L (1993) pH-sensitive liposomes. J Liposome Res 3:201–255

Trubetskaya OV, Trubetskoy VS, Domogatsky SP, Rudin AV, Popov NV, Danilov SM, Nikolayeva MN, Klibanov AL, Torchilin VP (1988) Monoclonal antibody to human endothelial cell surface internalization and liposome delivery in cell culture. FEBS Lett 228:131–134

Trubetskoy VS, Gazelle GS, Wolf GL, Torchilin VP (1997) Block-copolymer of polyethylene glycol and polylysine as a carrier of organic iodine: design of long-circulating particulate contrast medium for X-ray computed tomography. J Drug Target 4:381–388

Trubetskoy VS, Torchilin VP (1995) Use of polyoxyethylene-lipid conjugates as long-circulating carriers for delivery of therapeutic and diagnostic agents. Adv Drug Deliv Rev 16:311–320

Tuffin G, Waelti E, Huwyler J, Hammer C, Marti HP (2005) Immunoliposome targeting to mesangial cells: a promising strategy for specific drug delivery to the kidney. J Am Soc Nephrol 16:3295–3305

Uchino H, Matsumura Y, Negishi T, Koizumi F, Hayashi T, Honda T, Nishiyama N, Kataoka K, Naito S, Kakizoe T (2005) Cisplatin-incorporating polymeric micelles (NC-6004) can reduce nephrotoxicity and neurotoxicity of cisplatin in rats. Br J Cancer 93:678–687

Varga CM, Wickham TJ, Lauffenburger DA (2000) Receptor-mediated targeting of gene delivery vectors: insights from molecular mechanisms for improved vehicle design. Biotechnol Bioeng 70:593–605

Venugopalan P, Jain S, Sankar S, Singh P, Rawat A, Vyas SP (2002) pH-sensitive liposomes: mechanism of triggered release to drug and gene delivery prospects. Pharmazie 57:659–671

Veronese FM (2001) Peptide and protein PEGylation: a review of problems and solutions. Biomaterials 22:405–417

Vingerhoeds MH, Storm G, Crommelin DJ (1994) Immunoliposomes in vivo. Immunomethods 4:259–272

Vinogradov S, Batrakova E, Li S, Kabanov A (1999) Polyion complex micelles with protein-modified corona for receptor-mediated delivery of oligonucleotides into cells. Bioconjug Chem 10:851–860

Vinogradov SV, Bronich TK, Kabanov AV (1998) Self-assembly of polyamine-poly(ethylene glycol) copolymers with phosphorothioate oligonucleotides. Bioconjug Chem 9:805–812

Vitetta ES, Krolick KA, Miyama-Inaba M, Cushley W, Uhr JW (1983) Immunotoxins: a new approach to cancer therapy. Science 219:644–650

Voinea M, Manduteanu I, Dragomir E, Capraru M, Simionescu M (2005) Immunoliposomes directed toward VCAM-1 interact specifically with activated endothelial cells–a potential tool for specific drug delivery. Pharm Res 22:1906–1917

Volkel T, Holig P, Merdan T, Muller R, Kontermann RE (2004) Targeting of immunoliposomes to endothelial cells using a single-chain Fv fragment directed against human endoglin (CD105). Biochim Biophys Acta 1663:158–166

Vutla NB, Betageri GV, Banga AK (1996) Transdermal iontophoretic delivery of enkephalin formulated in liposomes. J Pharm Sci 85:5–8

Wakebayashi D, Nishiyama N, Yamasaki Y, Itaka K, Kanayama N, Harada A, Nagasaki Y, Kataoka K (2004) Lactose-conjugated polyion complex micelles incorporating plasmid DNA as a targetable gene vector system: their preparation and gene transfecting efficiency against cultured HepG2 cells. J Control Release 95:653–664

Wang GP, Qi ZH, Chen FP (2008) Treatment of acute myeloid leukemia by directly targeting both leukemia stem cells and oncogenic molecule with specific scFv-immunolipoplexes as a deliverer. Med Hypotheses 70:122–127

Wang J, Mongayt D, Torchilin VP (2005) Polymeric micelles for delivery of poorly soluble drugs: preparation and anticancer activity in vitro of paclitaxel incorporated into mixed micelles based on poly(ethylene glycol)-lipid conjugate and positively charged lipids. J Drug Target 13:73–80

Weinstein JN, Magin RL, Yatvin MB, Zaharko DS (1979) Liposomes and local hyperthermia: selective delivery of methotrexate to heated tumors. Science 204:188–191
Weissig V, Gregoriadis G (1992) Coupling of aminogroup bearing ligands to liposomes. In: Gregoriadis G (ed) Liposome technology, vol 3. CRC Press, Boca Raton, pp 231–248
Weissig V, Lasch J, Gregoriadis G (1990) Covalent binding of peptides at liposome surfaces. Pharmazie 45:849–850
Weissig V, Lasch J, Klibanov AL, Torchilin VP (1986) A new hydrophobic anchor for the attachment of proteins to liposomal membranes. FEBS Lett 202:86–90
Weissig V, Whiteman KR, Torchilin VP (1998) Accumulation of protein-loaded long-circulating micelles and liposomes in subcutaneous Lewis lung carcinoma in mice. Pharm Res 15:1552–1556
Whiteman KR, Subr V, Ulbrich K, Torchilin VP (2001) Poly(HPMA)-coated liposomes demonstrate prolonged circulation in mice. J Liposome Res 11:153–164
Widder KJ, Marino PA, Morris RM, Senyei AE (1983) Targeting antineoplastic agents using magnetic albumin microspheres. In: Goldberg EP (ed) Targeted Drugs. Wiley, New York, pp 201–230
Williams AS, Camilleri JP, Goodfellow RM, Williams BD (1996) A single intra-articular injection of liposomally conjugated methotrexate suppresses joint inflammation in rat antigen-induced arthritis. Br J Rheumatol 35:719–724
Winslow RM, Vandegriff KD, Intaglietta M (1996) Blood substitutes: new challenges. Birkhäuser, Boston
Wollina U, Dummer R, Brockmeyer NH, Konrad H, Busch JO, Kaatz M, Knopf B, Koch HJ, Hauschild A (2003) Multicenter study of pegylated liposomal doxorubicin in patients with cutaneous T-cell lymphoma. Cancer 98:993–1001
Woodle MC (1993) Surface-modified liposomes: assessment and characterization for increased stability and prolonged blood circulation. Chem Phys Lipids 64:249–262
Woodle MC (1998) Controlling liposome blood clearance by surface-grafted polymers. Adv Drug Deliv Rev 32:139–152
Woodle MC, Engbers CM, Zalipsky S (1994) New amphipatic polymer-lipid conjugates forming long-circulating reticuloendothelial system-evading liposomes. Bioconjug Chem 5:493–496
Woodle MC, Storm G (1998) Long circulating liposomes: old drugs, new therapeutics. Biotechnology intelligence unit. Springer, Berlin, p 301
Xiong XB, Mahmud A, Uludag H, Lavasanifar A (2007) Conjugation of arginine-glycine-aspartic acid peptides to poly(ethylene oxide)-*b*-poly(epsilon-caprolactone) micelles for enhanced intracellular drug delivery to metastatic tumor cells. Biomacromolecules 8:874–884
Xu L, Huang CC, Huang W, Tang WH, Rait A, Yin YZ, Cruz I, Xiang LM, Pirollo KF, Chang EH (2002) Systemic tumor-targeted gene delivery by anti-transferrin receptor scFv-immunoliposomes. Mol Cancer Ther 1:337–346
Yamaoka T, Tabata Y, Ikada Y (1994) Distribution and tissue uptake of poly(ethylene glycol) with different molecular weights after intravenous administration to mice. J Pharm Sci 83:601–606
Yang T, Choi MK, Cui FD, Kim JS, Chung SJ, Shim CK, Kim DD (2007) Preparation and evaluation of paclitaxel-loaded PEGylated immunoliposome. J Control Release 120:169–177
Yerushalmi N, Arad A, Margalit R (1994) Molecular and cellular studies of hyaluronic acid-modified liposomes as bioadhesive carriers for topical drug delivery in wound healing. Arch Biochem Biophys 313:267–273
Yoo HS, Lee EA, Park TG (2002) Doxorubicin-conjugated biodegradable polymeric micelles having acid-cleavable linkages. J Control Release 82:17–27
Yoo HS, Park TG (2004) Folate-receptor-targeted delivery of doxorubicin nano-aggregates stabilized by doxorubicin-PEG-folate conjugate. J Control Release 100:247–256
Yuan F, Dellian M, Fukumura D, Leunig M, Berk DA, Torchilin VP, Jain RK (1995) Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. Cancer Res 55:3752–3756

Yuan F, Leunig M, Huang SK, Berk DA, Papahadjopoulos D, Jain RK (1994) Microvascular permeability and interstitial penetration of sterically stabilized (stealth) liposomes in a human tumor xenograft. Cancer Res 54:3352–3356

Yuan X, Harada A, Yamasaki Y, Kataoka K (2005) Stabilization of lysozyme-incorporated polyion complex micelles by the omega-end derivatization of poly(ethylene glycol)-poly(alpha, beta-aspartic acid) block copolymers with hydrophobic groups. Langmuir 21:2668–2674

Zalipsky S (1995) Chemistry of polyethylene glycol conjugates with biologically active molecules. Adv Drug Deliv Rev 16:157–182

Zalipsky S, Gittelman J, Mullah N, Qazen MM, Harding JA (1998) Biologically active ligand-bearing polymer-grafted liposomes. In: Gregoriadis G (ed) Targeting of drugs 6: strategies for stealth therapeutic systems. Plenum Press, New York, pp 131–139

Zalipsky S, Qazen M, Walker JA 2nd, Mullah N, Quinn YP, Huang SK (1999) New detachable poly(ethylene glycol) conjugates: cysteine-cleavable lipopolymers regenerating natural phospholipid, diacyl phosphatidylethanolamine. Bioconjug Chem 10:703–707

Zhang JX, Zalipsky S, Mullah N, Pechar M, Allen TM (2004) Pharmaco attributes of dioleoylphosphatidylethanolamine/cholesterylhemisuccinate liposomes containing different types of cleavable lipopolymers. Pharmacol Res 49:185–198

Nanoparticle Technologies for Cancer Therapy

Frank Alexis, Eric M. Pridgen, Robert Langer, and Omid C. Farokhzad

Contents

Abstract Nanoparticles as drug delivery systems enable unique approaches for cancer treatment. Over the last two decades, a large number of nanoparticle delivery systems have been developed for cancer therapy, including organic and inorganic materials. Many liposomal, polymer–drug conjugates, and micellar formulations are part of the state of the art in the clinics, and an even greater number of nanoparticle platforms are currently in the preclinical stages of development. More recently developed nanoparticles are demonstrating the potential sophistication of

O.C. Farokhzad (✉)
Harvard Medical School, Laboratory of Nanomedicine and Biomaterials, Brigham and Women's Hospital, 75 Francis St, MRB-5th Floor, Boston, MA, 02115, USA
e-mail: ofarokhzad@zeus.bwh.harvard.edu

M. Schäfer-Korting (ed.), *Drug Delivery*,
Handbook of Experimental Pharmacology 197,
DOI 10.1007/978-3-642-00477-3_2,

these delivery systems by incorporating multifunctional capabilities and targeting strategies in an effort to increase the efficacy of these systems against the most difficult cancer challenges, including drug resistance and metastatic disease. In this chapter, we will review the available preclinical and clinical nanoparticle technology platforms and their impact for cancer therapy.

Keywords Nanoparticle · Drug delivery · Targeted · Metastatic cancer · Cancer therapy

Abbreviations

BBB	Blood–brain barrier
DSPC	1,2-Distearoyl-glycero-3-phosphocholine
DSPE	1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine
EggPG	Egg yolk phosphatidylglycerol
EPR	Enhanced Permeability and Retention effect
FDA	Food and Drug Administration
HPMA	*N*-(2-Hydroxypropyl)methacrylamide
HSPC	Hydrogenated phosphatidylcholine from soybean lecithin
LPS	Lipopolysaccharide
MTD	Maximum tolerated dose
NCI	National Cancer Institute
NIR	Near infrared
NSCL cancer	Non-small-cell lung cancer
PAMAM	Polyamidoamine
PDLLA	Poly-DL-lactic acid
PEG	Polyethylenglycol
PLA	Polylactic acid
PLA2	Phospholipase A2
PLGA	Poly(lactic-*co*-glycolic acid)
SEM	Scanning electron microscope

1 Introduction

Nanotechnology is a multidisciplinary field that uses principles from chemistry, biology, physics, and engineering to design and fabricate nanoscale devices (Farokhzad and Langer 2009; Ferrari 2005; Fox 2000; Jiang et al. 2007; Peppas 2004; Sinha et al. 2006; Uchegbu 2006). In its strictest definition, nanotechnology refers to structures with a size range of 1–100 nm in at least one dimension.

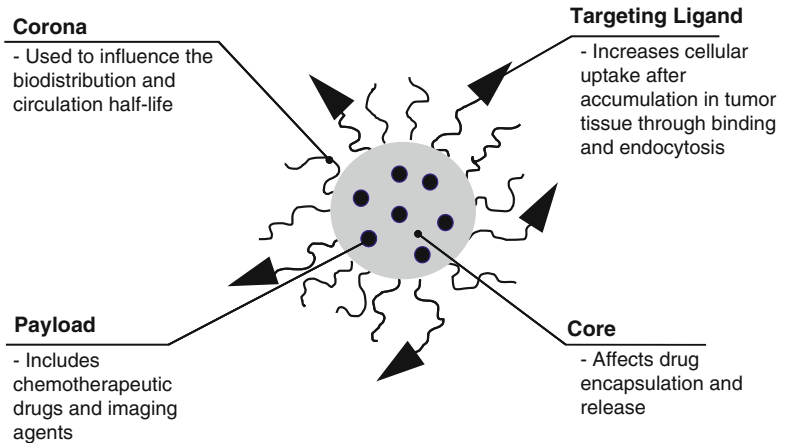


Fig. 1 Schematic of physicochemical structure of nanoparticle platforms for drug delivery, including core, corona, payload, and targeting ligand

However, it more commonly refers to materials up to several hundred nanometers that are developed using top-down or bottom-up engineering. The resulting nanomaterials demonstrate unique capabilities based on intrinsic properties such as shape and size as well as functional properties conferred through surface modifications (Fig. 1).

The field of medicine stands to be a significant benefactor of advances in nanotechnology, with oncology already starting to reap the benefits of novel nanoscale technologies (Alexis et al. 2008b; Alexis et al. 2008c; Davis et al. 2008; Euliss et al. 2006; Farokhzad 2008; Farokhzad et al. 2006b; Farokhzad and Langer 2006; Freitas 2005; Jain 2008; Kawasaki and Player 2005; Lanza et al. 2006; Levy-Nissenbaum et al. 2008; Moghimi et al. 2005; Peer et al. 2007; Pridgen et al. 2007; Riehemann et al. 2009; Rosen and Abribat 2005; Salvador-Morales et al. 2009a; Venugopal et al. 2008; Zhang et al. 2008b). These benefits have included advances in detection, imaging, and therapy of disease. The National Cancer Institute (NCI) has identified nanotechnology as having the potential to make paradigm-changing impacts on the detection, treatment, and prevention of cancer. The level of interest in nanotechnology by both academic and industrial investigators has led to increased development of novel nanotechnology platforms for medical applications, sharp increases in government funding, and venture capital investment. The combination of funding and early clinical success has provided the resources and opportunities for nanotechnology to solve important medical challenges. The early success in oncology has already been a catalyst for the application of nanotechnology to other medical problems, such as cardiovascular disease and vaccines.

One area where nanotechnology has the potential to make a significant impact is drug delivery (Farokhzad and Langer 2009; Pridgen et al. 2007). This impact has

already been felt with the translation of several nanoscale drug delivery systems into the clinic, although the full potential of these systems is only starting to be explored. Nanoscale drug delivery vehicles have shown the ability to encapsulate a variety of therapeutic agents such as small molecules (hydrophilic and/or hydrophobic), peptides, protein-based drugs, and nucleic acids. By encapsulating these molecules inside a nanocarrier, the solubility and stability of the drugs can be improved, providing an opportunity to reevaluate potential drugs previously ignored because of poor pharmacokinetics (Langer 1998). Encapsulated molecules can be released from nanocarriers in a controlled manner over time to maintain a drug concentration within a therapeutic window or the release can be triggered by some stimulus unique to the delivery site (Moghimi 2006). The surface of the nanocarrier can be engineered to increase the blood circulation half-life and influence the biodistribution, while attachment of targeting ligands to the surface can result in enhanced uptake by target tissues (Gref et al. 1994; Moghimi et al. 2001). The small size allows nanocarriers to overcome biological barriers and achieve cellular uptake (Brigger et al. 2002). The net result of these properties is to lower the systemic toxicity of the therapeutic agent while increasing the concentration of the agent in the area of interest, resulting in a higher therapeutic index for the therapeutic agent. In addition to therapeutic drugs, imaging agents can also incorporated into nanocarriers to improve tumor detection and imaging (Kim et al. 2006; Montet et al. 2006). Finally, nanoparticles can be engineered to be multifunctional with the ability to target diseased tissue, carry imaging agents for detection, and deliver multiple therapeutic agents for combination therapy (Nasongkla et al. 2006). The multimodal capabilities of nanoparticle delivery systems offer the opportunity to develop novel approaches to deliver drugs that may result in alternative or complementary therapeutic options for the treatment of disease.

In this chapter, we will focus on nanoparticle technologies (Fig. 2), with a particular emphasis on the development of nanocarrier drug delivery systems for cancer therapy applications. These technologies include polymeric nanoparticles, dendrimers, nanoshells, liposomes, inorganic/metallic nanoparticles, hybrid nanoparticles, micelles, and magnetic and bacterial nanoparticles. Nucleic acid delivery technologies will not be included, but are extensively reported elsewhere (Chen and Huang 2008; Gao and Huang 2008; Gary et al. 2007; Juliano et al. 2008; Li and Huang 2008b; Luten et al. 2008; Tseng et al. 2009; Whitehead et al. 2009). A discussion of how improvements in the understanding of the tumor microenvironment have guided the design of both non-targeted and targeted nanocarriers as therapeutic vehicles for cancer will follow (Bierie and Moses 2006; Bissell and Labarge 2005; Cairns et al. 2006; Fesik 2005; Fidler 1995; Galon et al. 2007; Overall and Kleifeld 2006; Siclari et al. 2006; Zetter 2008). The breakthrough potential of nanoparticle delivery systems is becoming increasingly recognized, with several examples of first generation nanocarriers approved by the FDA for therapy, and targeted nanocarriers in clinical phase development. Many of the nanocarrier systems in clinical phase development will be highlighted in this

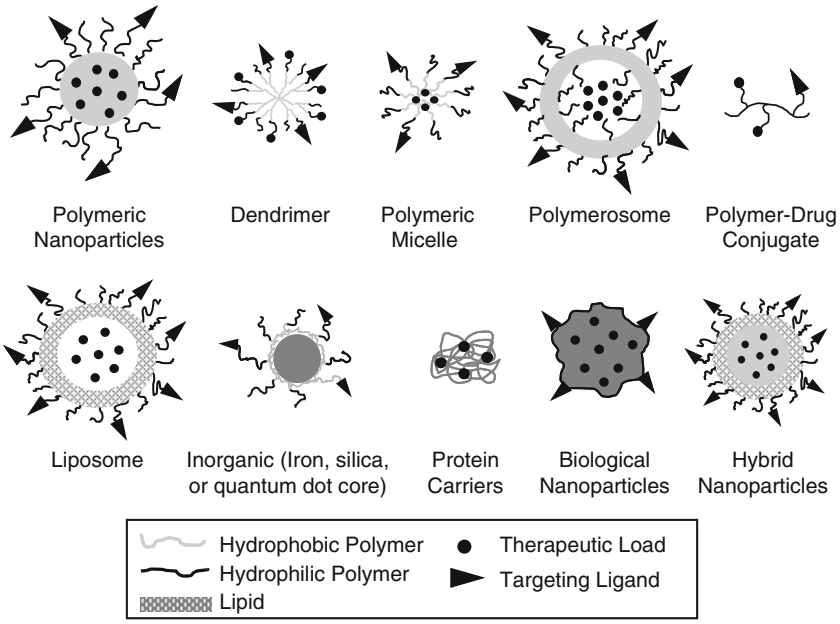


Fig. 2 Nanoparticle platforms for drug delivery. Nanoparticle platforms are characterized by their physicochemical structures, including polymer–drug conjugates, lipid-based nanoparticles, polymeric nanoparticles, protein-based nanoparticles, biological nanoparticles, and hybrid nanoparticles

chapter to demonstrate how these systems are being translated to the clinic and the advantages they provide for cancer therapy.

2 Nanoparticle Technologies

The first nanoscale drug delivery systems were lipid vesicles, which were first described in the 1960s and later became known as liposomes (Bangham et al. 1965). Since then, there have been several key developments that have paved the way for current nanoparticle technologies. In 1976, the first controlled-release polymer systems for the delivery of macromolecules were demonstrated (Langer and Folkman 1976). This was followed in 1980 with the first application of targeted liposomes (Heath et al. 1980; Leserman et al. 1980). The surface modification of liposomes and polymeric nanoparticles with polyethylene glycol (PEG) in 1990 and 1994, respectively, led to increases in circulation time, or "stealth" properties (Gref et al. 1994; Klibanov et al. 1990). These developments culminated in the approval of Doxil (James 1995a, b), a vesicle delivery system encapsulating doxorubicin that has proven to be a potent treatment for multiple types of cancer (Porche 1996;

Tejada-Berges et al. 2002). Since then, research has led to tremendous progress in the development of nanoparticles engineered to have multifunctional capabilities as well as "smart" properties such as the ability to respond to the environment to facilitate more effective drug delivery strategies. Currently, there are 70 reported clinical trials evaluating nanoparticle carriers, 208 evaluating drug conjugates, and 361 evaluating vesicle-based carriers (http://www.clinicaltrials.gov). The clinical trials include combination therapies and treatments through various administration routes, such as pulmonary and oral.

Nanoparticle technologies for cancer therapy include polymeric nanoparticles (Moghimi 2006; Pridgen et al. 2007), vesicle-based carriers such as liposomes (Kaneda 2000; Torchilin 2005), micelles (Fan 2008; Liggins and Burt 2002; Matsumura 2008), dendrimers (Florence and Hussain 2001; Lee et al. 2005; McCarthy et al. 2005; Najlah and D'Emanuele 2007), polymer conjugates (Greco and Vicent 2008; Li and Wallace 2008; Thanou and Duncan 2003), protein carriers (Hawkins et al. 2008; Wang and Uludag 2008), inorganic nanoparticles (Murakami and Tsuchida 2008), and bacterial nanocarriers. The diversity of delivery systems, each of which is discussed below, allows nanoparticles to be developed with a diverse array of shapes, sizes, and components that enables them to be tailored for specific applications. However, the primary consideration when designing any drug delivery system is to achieve more effective therapies by controlling the drug

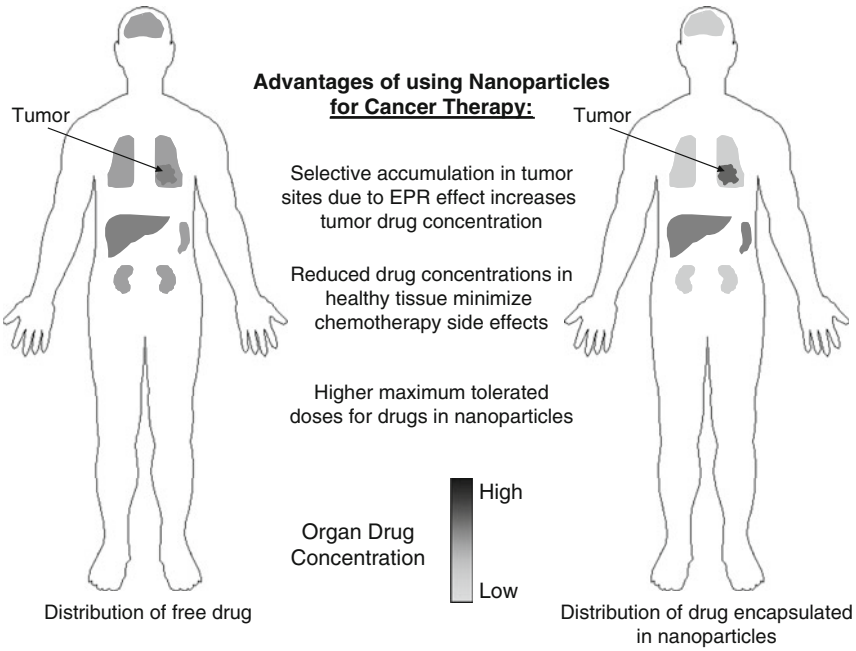


Fig. 3 Advantages of using nanoparticles as drug delivery system for cancer therapy compared to free drug

concentration in the therapeutic window, reducing cytotoxic effects, and improving patient compliance (Fig. 3). This allows effective treatment cycles to be maintained while reducing damage to healthy cells and minimizing the recovery period.

2.1 Liposome Nanoparticles

Lipids form nanoparticle vesicles through the self-assembly of amphiphilic lipids and excipients. The lipids form a bilayer based on hydrophobic interactions in continuous parallel packing, with the hydrophilic head groups positioned towards the aqueous environment. Hydrophilic molecules can be encapsulated in the inner aqueous phase while hydrophobic molecules can be carried in the hydrophobic domains of the lipid bilayer. Physicochemical properties of liposomes can be precisely changed to control surface charge, functionality, and size by simply mixing commercially available lipid molecules. This offers a significant advantage over other carriers that require much more controlled synthesis steps and additional chemical modifications. Generally, lipids used to prepare vesicular formulations are found in the human body and approved by the FDA, such as DSPE (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine), HSPC (hydrogenated phosphatidylcholine from soybean lecithin), EggPG (egg yolk phosphatidylglycerol) and DSPC (1,2-distearoyl-glycero-3-phosphocholine). Each of these lipids can be obtained with or without PEG, which can be used to modify the surface of the resulting liposome.

Doxil, a pegylated liposome clinically used to treat multiple types of cancer, is a landmark for liposomal drug delivery systems. Doxil consists of a packed pegylated surface (2 kDa PEG chains) and is loaded with doxorubicin through drug diffusion based on an ammonium salt gradient. This method achieves a stable drug entrapment in a crystal form with reduced leakage over a long period of time. Doxil liposomes have a size of ~100 nm, surface charge of ~−10 mV, and a long-term shelf stability of ~2 years at ~4°C. Recently, Aphios Corp. developed nanosomes (small liposomes, <100 nm) carrying multiple drugs such as docetaxel, camptothecin, bryostatin-1 and vitamin D analog for treatment of multiple cancer types (Castor 2005) using a manufacturing technology based on a super-critical fluid process. In addition, Novosom AG uses amphoteric liposomes to deliver nucleic acids. The liposomal formulation is able to change surface charge properties (zeta potential) with changes in solution pH. The charge switch at acidic pH results in fusion with the cell membrane during endocytosis uptake, allowing escape of the nanocarriers into the cytoplasm to deliver the therapeutic load.

Liposomal formulations have demonstrated multiple benefits as drug delivery vehicles. However, they must be used to carry very potent drugs due to their low encapsulated load. Lipid-based vesicles pose several other challenges such as instability in the bloodstream, poor solubility of many drugs in the lipid/surfactant solution, and a rapid, burst release of drug. Liposomal formulations are also associated with severe side effects due to their accumulation in skin tissue. While

prolonged drug release kinetics are difficult to control using liposomal systems, alternatives such as environmentally triggered release can be easily engineered by inserting destabilizing lipids with amine head groups into the vesicle membrane or including additives such as morpholine in the lipid formulation (Cullis and Chonn 1998; Guo et al. 2003; Kocer 2007; Sudimack et al. 2002; Vial et al. 2005). There are currently no liposomal formulations with triggered drug release approved for clinical use or in early phases of clinical trials. However, LiPlasome Pharma developed non-targeted liposomes consisting of lipids designed to be degraded by phospholipase A2 (PLA2), which is up-regulated in the tumor microenvironment (Andresen et al. 2004; Andresen et al. 2005; Jensen et al. 2004; Jorgensen et al. 2002). The lipid degradation products are converted into anticancer drugs, resulting in local delivery of cytotoxic drugs in the tumor. In-vivo results showed a delay in colon cancer progression using a human tumor xenograft mice model (Tribler et al. 2007). This approach also provides the possibility of multi-drug delivery. Protein stabilization of liposomes is being investigated by Azaya Therapeutics to deliver hydrophobic drugs such as docetaxel for cancer therapy. Docetaxel is encapsulated into the liposome bi-layer and stabilized by albumin to prevent rapid drug leakage (ATI-1123). The results of ATI-1123 efficacy studies in human xenograft mice models for prostate, pancreatic, and non-small-cell lung cancer (NSCL cancer) showed partial tumor regression in 90% of the PC3 tumor xenograft model and improved efficacy in the pancreas model when compared to groups treated with docetaxel at equal doses (25 mg kg^{-1}). This may be explained by the slower plasma elimination and higher bioavailability of ATI-1123 relative to free docetaxel (Zamboni 2008).

2.2 *Polymer–Drug Conjugates Nanoparticles*

Polymer–drug conjugates are one of the most investigated types of nanocarriers and are currently in clinical trials as advanced as phase III. Polymer–drug conjugates are formed through side-chain grafting of drugs to polymer chains, allowing them to deliver high doses of chemotherapeutic drugs. Although the physicochemical properties of a number of formulations are not disclosed, the size of polymer–drug conjugates is generally below 20 nm. HPMA-doxorubicin (*N*-(2-hydroxypropyl) methacrylamide) copolymer (PK1) was the first synthetic polymer–anticancer drug conjugate to enter clinical trials more than a decade ago and the clinical phase II trial for women with advanced breast cancer is still ongoing (Vasey et al. 1999). Similarly, Prolindac (AP5346) is composed of a HPMA backbone copolymer with platinum grafted to the side chains through a pH-sensitive chelator designed for drug release in the tumor environment (Sood et al. 2006). Preclinical data shows superior efficacy of the polymer–drug conjugates using multiple cancer models including a M5076 sarcoma platinum-resistant tumor xenograft mice model, multiple colon xenograft models, L1210 leukemia, and 0157 hybridoma models (Rice et al. 2006). Oxaliplatin drug loading was ~10% (w/w) using a polymer chain of 25 kDa and the drug release was slow. Formulations were injected once a week for

three weeks and the polymer–drug conjugates significantly retarded tumor growth over one month due to higher intracellular concentration of Pt. In the clinical phase I trial conducted in Europe (Campone et al. 2007), systemic injection of 640 mg Pt m^{-2} weekly for 3 weeks resulted in a response by platinum-resistant ovarian cancer. Recently, Access Pharmaceuticals Inc. reported the results of the clinical phase II trial showing that 66% of the patients with ovarian cancer experienced meaningful disease stabilization and limited side effects.

Polyamino acids grafted with drugs on the side chains are another class of polymer–drug conjugates that have demonstrated high drug loading and efficacy (Li 2002; Matsumura 2008). In the case of polyglutamate-glycine-campthotecin (CT-2106), degradable linkers have allowed drug loadings ranging from 5% to 50%. Using a glycine linker, drug loadings were increased threefold over polyglutamate-campthotecin alone due to reduced steric hindrance. However, a formulation with a drug load of ~30% was selected for clinical trials due to superior stability and efficacy in human tumor xenograft mice models (Homsi et al. 2007). Meanwhile, Xyotax, a similar polymer–drug conjugate (polyglutamate-paclitaxel), is in 22 clinical trials at the moment for multiple cancer therapies including prostate cancer, metastatic breast cancer, neck cancer, metastatic colorectal cancer, and recurrent NSCL (Phase III). Paclitaxel is grafted to polyglutamic acid (30–40 kDa) to reach a drug load of 20–40% by weight (Singer 2005; Singer et al. 2003). The clinical data shows an improvement in median survival in Xyotax patients compared with the control group, although there were no differences in the overall survival. One benefit of the treatment was the reduction of multiple side effects including neurotoxicity (Boddy et al. 2005). Overall, polymer–drug conjugates are considered simple nanocarrier systems, but tuning the optimal formulation might require extensive development. For example, small changes in the polymer–drug conjugation efficiency may significantly modify the pharmacokinetic parameters and tissue biodistribution. The resulting formulation could also be considered a new chemical entity, complicating regulatory approval.

2.3 Polymeric Nanoparticles

Polymeric nanoparticles may represent the most effective nanocarriers for prolonged drug delivery. The early in vitro and in vivo development of polymeric nanoparticles loaded with drugs in the 1980s using polyalkylcyanoacrylate-based nanoparticles releasing doxorubicin (Couvreur et al. 1979) led to multiple reports using polymer-based materials for drug delivery. Langer and Folkman (Langer and Folkman 1976) demonstrated the first controlled release of macromolecules using polymers, which allowed the development of anti-angiogenic drug delivery systems for cancer therapy and opened new areas for the delivery of macromolecules. In 1994, Langer et al. described nanoparticles composed of poly(lactic acid)/poly (lactic-*co*-glycolic acid) (PLA/PLGA) and PEG block copolymer as "long-circulating nanoparticles" due to their stealth properties (Gref et al. 1994), leading to an increased

interest in polymeric nanoparticles and their therapeutic applications. Only a few papers per year were published using polymeric nanoparticles as a drug delivery system in the 1990s in contrast to ~200 papers in 2008.

Polymeric nanoparticles provide significant flexibility in design because polymers can be biodegradable or nonbiodegradable, and can be made synthetically or derived from natural sources. Some common polymers used for nanoparticle formation include poly(lactic acid) (PLA), dextran, and chitosan. Biodegradable polymers are typically degraded into individual monomers, which are metabolized and removed from the body via normal metabolic pathways. Degradation and drug release kinetics can be precisely controlled by the physicochemical properties of the polymer, such as molecular weight, dispersity index, hydrophobicity, and crystallinity. In general, drugs can be released in a controlled manner with first-order kinetics due to drug diffusion through the polymeric matrix or triggered in response to the local environment. The nanoparticle surface is usually sterically stabilized by grafting, conjugating, or adsorbing hydrophilic polymers such as PEG to its surface, which can also reduce hepatic uptake and improve circulation half-life (Gref et al. 2000; Peracchia et al. 1999).

Several polymeric nanoparticles are now in various stages of preclinical and clinical development. For example, Nanolymf Ltd. developed microparticles carrying encapsulated nanocapsules loaded with drugs. Drug-loaded polymethacrylate nanocapsules (~400 nm) are encapsulated in 2–10 μm cellulose-based microspheres and given orally, resulting in uptake by M-cells and a drug blood bioavailability of ~5%. DeSimone et al. (Euliss et al. 2006; Gratton et al. 2008a; Gratton et al. 2008b; Kelly and DeSimone 2008; Rolland et al. 2005) have shown that physicochemical properties of particles such as shape, size and mechanical flexibility contribute to their interactions with cell membranes and control their internalization pathways. This has led to the preclinical development of polymeric nanoparticles using a "PRINT" technology (Particle Replication In Non-wetting Templates) for cancer therapy and other diseases.

2.4 Micelle Nanoparticles

Micelles are composed of lipids or other amphiphilic molecules, such as polymers or polyamino acids, and self-assemble into small nanoparticles composed of a hydrophobic core. Micelles have been developed as drug delivery carriers for hydrophobic drugs (Aliabadi et al. 2008; Liggins and Burt 2002; Matsumura 2008). There are multiple examples of micellar formulations under investigation or in clinical trials, such as Genexol-PM (Kim et al. 2007a; Kim et al. 2004; Lee et al. 2008), NC-6004 (Uchino et al. 2005), NK105 (Hamaguchi et al. 2007), and NK911 (Matsumura et al. 2004; Tsukioka et al. 2002). Genexol-PM is the first non-targeted polymeric micellar formulation approved for cancer therapy. It was approved in Korea in 2006 as a first-line therapy for metastatic breast and NSCL cancer (currently in Phase III). It is currently being evaluated in a clinical phase II

trial in the USA for metastatic pancreatic cancer therapy. Genexol-PM is composed of a block copolymer PDLLA (1.75 kDa)–mPEG (2 kDa) forming micelles with a size of ~60 nm and paclitaxel loading of ~15% (w/w). The maximum tolerated dose (MTD) of Genexol-PM is threefold higher than Taxol (60 mg kg^{-1} vs. 20 mg kg^{-1}, respectively) and the median lethal tolerated dose (LD_{50}) using Sprague–Dawley rats was reported to be ~20 times higher than Taxol. Interestingly, the area under the plasma concentration (AUC) was similar for both formulations. However, paclitaxel had more significant accumulation in tissues such as the liver and tumor with the Genexol-PM formulation, leading to differential tumor cytotoxicity and reduction of tumor volume (Kim et al. 2001). Results of a clinical phase I trial showed that while the MTD was almost double (390 mg m^{-2}) for Genexol-PM compared to Taxol with similar toxicological profiles, the recommended dose was determined to be 300 mg m^{-2} (Kim et al. 2004). The clinical phase II trial in Korea evaluated Genexol-PM as a co-therapy with cisplatin for advanced NSCL in contrast to a single agent therapy (Kim et al. 2007a). The clinical phase II results showed ~30% of the patients had stable disease status and 60% of the patients had an increased survival of one year using slightly lower doses of cisplatin than with the combined treatment of Taxol with cisplatin (60 mg m^{-2} versus 75 mg m^{-2}, respectively) (Kim et al. 2007a). Other companies such as Labopharm and Intezym are also developing micelle systems for the delivery of a myriad of anticancer agents using formulations with sizes ranging from 10 to 200 nm using polyamino acids and synthetic polymers.

2.5 Dendrimer Nanoparticles

Dendrimers are globular macromolecules (5–10 nm) with well-defined branching architectures and surface functional groups available for further modification. The multifunctional capabilities possible through controlled synthesis methods are leading to new classes of dendrimers that can carry drug molecules, diagnostic agents, and targeting molecules. Dendrimers have remarkable molecular monodispersity and suitable pharmacokinetic properties for systemic drug delivery with cleavable chemistry for drug dissociation (Lee et al. 2005). Amphiphilic dendrimers are able to form micelles by self-assembly with hydrophilic groups on the surface for functionalization. Drug release kinetics are controlled through the properties of the polymer chains, which can be designed to be degraded for release of a payload.

Baker et al. have developed “avidimers” (Majoros et al. 2005, 2006; Myc et al. 2008), which are dendrimers targeted to tumor vasculature using a methotrexate-polyamidoamine (PAMAM) bioconjugate platform functionalized with small targeting ligands (Quintana et al. 2002). Non-targeted and folate-targeted G5-PAMAM dendrimers differentially accumulated into a human KB cell line xenograft tumor model within a day (8%–10% targeted versus 2% non-targeted I.D./g of tissues) (Kukowska-Latallo et al. 2005). Higher accumulation in the tumor resulted

in the inhibition of tumor growth, lower toxicity, and longer survival time compared to free drug at equal dosage. More importantly, recent efficacy studies using targeted transferrin-cyclodextrin-siRNA nanoparticles (CALAA-01, ~70 nm) in animal models of human epithelial cancer showed tumor size reduction and differential distribution in tumors (Bartlett et al. 2007; Davis 2009; Davis and Brewster 2004). The preclinical data motivated further development of CALAA-01. The toxicological results reported in April 2007 for CALAA-01, which was the first targeted, polymeric nanoparticle platform in non-human primates (Heidel et al. 2007), led to the submission of an investigational new drug application and human clinical trials for solid tumor therapy in May 2008.

2.6 Polymersome Nanoparticles

Polymersomes have a structure similar to liposomes, but are composed of synthetic polymer/polypeptide amphiphiles and self-assemble to form polymer shell vesicles (~100 nm) when hydrated and extruded. Discher et al (Discher et al. 1999) described vesicles made of amphiphilic diblock copolymers with low water permeability. The hydrophilicity/hydrophobicity ratio is used to control the morphology of the nanoparticle, which can range from spherical to cylindrical. The membrane core thickness can be controlled by the molecular weight of the diblock copolymer. Polymersomes show higher stability and lateral fluidity than liposomes and the release is triggered by the degradation of the polymer chain and destabilization of the shell layer. Incubation of polymersomes in the blood showed adherence and uptake by white blood cells within 10 h. In vivo results using a breast cancer tumor xenograft model showed therapeutic efficacy after a single i.v. injection using polymersomes loaded with paclitaxel and doxorubicin at the maximum tolerated dose (2.5 mg kg^{-1} for each drug). The tumor size was reduced within five days postinjection in contrast to the free drug formulations (Ahmed et al. 2006).

2.7 Protein Nanoparticles

Protein-based drug delivery systems have recently made a big impact with albumin-bound drug nanoparticles (~130 nm). The recent approval of albumin-bound paclitaxel (Abraxane, ABI-008, January 2005) by the Food and Drug Administration (FDA) for metastatic breast cancer therapy, as well as multiple clinical trials currently in progress for other types of cancer, has now opened the possibility of using protein-based nanoparticles for delivery of therapeutic agents (Gradishar 2006). Given the limiting pharmacokinetic properties and numerous side effects of Taxol (hypersensitivity), the albumin-bound paclitaxel allows the formulation of the hydrophobic drug in a solvent-free solution. Albumin is a natural noncovalent physiological transporter of molecules across endothelial barriers through a

transcytosis-mediated mechanism (caveolae vesicle). Preclinical studies have shown that the concentration of paclitaxel bound to albumin in endothelial cells and in the extravascular space was significantly increased (3–10 fold) (Desai et al. 2006; Nyman et al. 2005). Data suggests that albumin may have intrinsic targeting abilities to tumors, although the enhanced permeability and retention (EPR) effect may play an additional role in tumor accumulation. Overall, the albumin-bound paclitaxel formulation allowed higher dosages than the Taxol formulation (260 mg m^{-2} vs. 175 mg m^{-2}, respectively) and demonstrated improved efficacy and safety (Nyman et al. 2005). Abraxane is currently being tested as a first-line therapy or in combination with other drugs (rapamycin, verinostat, etc.) for metastatic breast cancer and other cancers that have been shown to be sensitive to taxane drugs, such as ovarian and prostate. In addition, albumin is now being tested as a platform for delivery of other molecules that have reduced water solubility, such as rapamycin (~2.5 μg ml^{-1}). Albumin-bound rapamycin (ABI-009) has been in a clinical phase trial for the treatment of non-hematologic malignancies since January 2008.

2.8 *Biological Nanoparticles*

Biological nanoparticles such as bacteria are unicellular microorganisms with different shapes and sizes that encapsulate essential components of the cytoplasm as well as hydrophobic and hydrophilic molecules. One example of biological nanoparticles being evaluated for cancer therapy is a drug delivery system developed by EnGeneIC Pty Ltd called a "nanocell", which consists of anucleate globular bacteria (~400 nm). The absence of DNA prevents endogenous mutations and replication originally reported in 1967 (Adler et al. 1967). It has been demonstrated that a nanocell can be efficiently loaded with molecules of different solubility and charge, such as doxorubicin, paclitaxel, and siRNA, through drug diffusion into the bacteria within a few hours (MacDiarmid et al. 2007). No signs of toxicity have been reported in large animals such as pigs and monkeys with repeated dosages at high titers, although there is the potential for an immunological response to the carrier due to the presence of lipopolysaccharide (LPS).

2.9 *Inorganic Nanoparticles*

Inorganic nanoparticles are primarily metal-based and have the potential to be synthesized with near monodispersity. Inorganic materials have been extensively studied for imaging using magnetic resonance and high-resolution superconducting quantum interference devices while their intrinsic properties have been explored for therapy. Several types of metal nanoparticles (Cheng et al. 2008; Paciotti et al. 2004; Visaria et al. 2007) are able to convert energy into heat at levels up to 70°C

through near-infrared light excitation or oscillating magnetic field stimulation (Johannsen et al. 2005). Iron oxide nanoparticles coated with aminosilane (Nanotherm M01) are in clinical phase II trials in Germany for brain cancer therapy and recurrent prostate cancer therapy using hyperthermia as well as thermoablation methods. The phase I results showed that prostate tumor cells can be locally killed by magnetic iron oxide nanoparticles (Johannsen et al. 2007). Nanoparticles were injected locally using ultrasound to guide tumor injections and patients were treated once a week for 1 h over two months. The small nanoparticles (~20 nm) are able to penetrate tumors, enter cancer cells, and generate heat under magnetic fields (50 and 100 kHz), allowing treatment width between 20 and 30 cm and within a circular area of 20 cm of diameter. The authors report no dose-limiting toxicities and mild discomfort from internal heating. Similarly, silica nanoparticles coated with gold that absorb near-infrared laser energy and covert it into heat to kill solid tumors are currently under investigation in a pilot study for head and neck cancer therapy. In vivo results (Hirsch et al. 2003) of nanoshell-mediated NIR (near infrared) thermal therapy using human breast cancer xenograft models showed that the nanoparticles induced irreversible cancer tissue damage at a temperature ~40°C. However, the temperature variance between different mice treated was quite significant (28–60°C) and was suggested to be due to differential distribution of nanoshells in the treated volume of the tumor. In addition, the maximum recorded temperature was only ~1 mm under the skin. Recently, the same nanoparticles (150 nm) were used for brain cancer treatment in an orthotopic canine model (Schwartz et al. 2009). Tumors were killed using percutaneous infiltrated NIR fibers reaching a temperature of ~70°C in tumor tissues and ~50°C in normal white and grey matter, which is expected to significantly damage non-diseased areas of the brain.

Surface properties and functionalities of gold nanoparticles have also been used for the delivery of surface-bound therapeutics. Aurimune (CYT-6091) is an example of tumor necrosis factor (TNF)-alpha bound to PEG-coated gold nanoparticles (~27 nm) developed by CytImmune Sciences, Inc. for solid tumor therapy (Paciotti et al. 2004). TNF-alpha is a potent cytokine with antitumor cytotoxicity which requires incorporation into a nanocarrier formulation to reduce systemic toxicity. The results show that nanoparticle formulations delayed the tumor growth with local heating (42°C for 1 h) using a SCK mammary tumor xenograft mouse model. However, the combined treatment showed a higher efficacy and suppression of intratumor blood flow (Visaria et al. 2006). Preliminary SEM micrographs of nanoparticles accumulated in breast tumor tissue sections in contrast to healthy tissues showed possible targeting of the nanoparticles by the EPR effect. Many other formulations are still in the discovery stage using combinations of drugs such as TNF with paclitaxel, doxorubicin or interleukin-12. However, the load of therapeutic agent is reported to be several hundreds of molecules due to the surface adsorption density, which may limit the effect of the therapeutic agent. Recently, Adair's group (Kester et al. 2008; Morgan et al. 2008) has reported the encapsulation of organic molecules in calcium phosphate nanocomposite particles (~27 nm) for intracellular imaging and delivery. Calcium

phosphate-based nanoparticles are biocompatible and their pH dissolution properties can be used for controlled release of molecules in the acidic tumor environment. In vitro studies show high uptake of the nanoparticles in bovine aortic endothelial cells and the delivery of hexanoyl-ceramide (Cer-6) to human vascular smooth muscle cells showed 100% inhibition of cell growth at 200 nM of drug (Kester et al. 2008). This technology is now being developed by Keystone Nano for imaging and delivery of therapeutic agents.

Non-specific accumulation into healthy tissues is always a concern for nanoparticle drug delivery systems. Using local sensitization through light or temperature may reduce overall toxicity, but it is expected to damage adjacent healthy tissues as well. Ultimately, inorganic particles may not provide advantages over other types of nanoparticles for systemic targeting of cancer cells because they are not biodegradable, have low payloads, and have no controlled release properties.

2.10 Hybrid Nanoparticles

Hybrid nanoparticles are recently developed nanocarriers that combine advantages from existing systems with well-characterized properties to form lipid–polymer nanoparticles and solid liposomal nanoparticles. Hybrid nanoparticles are composed of at least two different materials to form the core and the corona structure. In general, metallic and polymeric materials form the core and are coated with a single or multiple lipid layers to form a protecting membrane (corona) similar to a liposome or micelle. We (Chan et al. 2009; Zhang et al. 2008a) and others (Al-Jamal et al. 2008; Kim et al. 2007b; Sengupta et al. 2005; Thevenot et al. 2007; Wong et al. 2007; Wong et al. 2006a; Wong et al. 2006b) have developed hybrid nanoparticles for cancer therapy. Sasisekharan and co-workers (Sengupta et al. 2005) have reported PLGA-core nanoparticles coated with a bi-phospholipid layer to carry multiple drugs for cancer therapy using melanoma and Lewis lung carcinoma models. In their system, doxorubicin is conjugated to PLGA to form the core of the nanoparticle (~1% load by weight of doxorubicin, 70% encapsulation efficiency) while an anti-angiogenesis drug, combrestatin, is mixed with phospholipids and encapsulated in the lipid bi-layer during the self-assembly process to form nanoparticles (~200 nm) described as "nanocells". The drugs were release at different rates over a period of ~3 days, with combrestatin released first to reduce vascular density in the tumor followed by the release of doxorubicin to kill the cancer cells. The results showed a significant delay in tumor growth and increased survival time in both cancer models, suggesting accumulation of the nanocell by the EPR effect and added therapeutic value by delivering multiple drugs. The nanocell technology is now in preclinical development by Cerulean Pharma.

Others have reported solid-lipid nanoparticles using different polymers and formulations in vitro and in vivo for combination therapy. Recently, Thevenot et al. (2007) described a mechanism for the encapsulation of a hydrophobic

polymer core (PLA) in PEG-liposomes. As part of the work, the importance of PEG chain length to sterically stabilize lipoparticles with optimal colloidal stability was demonstrated (PEG (5 kDa) at 10% of lipid content). Our group has reported (Chan et al. 2009; Zhang et al. 2008a) a one-step formulation for self-assembly of a single layer of lipid on the hydrophobic surface of PLA nanoparticles (size < 100 nm). Surface functionalization using different lipid constituents allows the precise control of the charge and targeting ligand density, leading to stable hybrid nanoparticle formulations (Chan et al. 2009). In addition, drug loading was significantly increased up to ~8% by weight and the release kinetics of docetaxel was shown to be controlled by the lipid layer on the surface of the nanoparticles. Multifunctional nanoparticle technologies (Bertin et al. 2006; Schneider et al. 2009; Wang et al. 2008b) are now able to combine multiple therapeutic approaches that are the state of the art for cancer therapy, including the delivery of multiple drugs (Ahmed et al. 2006; Sengupta et al. 2005) or radiation sensitizers (van Vlerken et al. 2008), combined therapeutic approaches such as photothermal and drug delivery (Park et al. 2008; Rapoport et al. 2007), and simultaneous delivery of therapeutic drugs and imaging agents (Gao et al. 2008; McCarthy and Weissleder 2008; Shin et al. 2009).

3 Strategies for Cancer Therapy Using Nanoparticles

3.1 Metastatic Cancer

Metastatic cancer is a clinical description for the spread of cancer cells from the primary tumor site to distant organs, establishing secondary tumor sites. Detachment of cancer cells from the primary tumor site and circulation in the blood allows the cells to arrest in organs such as the lungs, liver, lymph nodes, skin, kidneys, brain, colon, and bones, where they can extravasate and proliferate (Chambers et al. 2002; Fidler 2003). Despite significant increases in the understanding of metastatic cancer pathogenesis, early diagnosis, surgical methods, and irradiation treatment, most cancer deaths are due to metastases that are not curable. Reasons for this include resistance to treatments, difficulty accessing the tumor sites and removing all cancer cells during surgery, or physiological barriers for drug access such as the blood–brain barrier (BBB). Therefore, improving therapy of metastatic cancer is still a challenge even though multiple therapeutic approaches are approved or in clinical development.

An improved understanding of cancer biology, including microenvironment functions, signaling pathways, and metastasis evolution, has resulted in clear advances in cancer therapy. Drugs have now been developed against a range of targets including matrix metalloproteinase inhibitors, epidermal growth-factor receptor inhibitors, transferase inhibitors, migration inhibitors, and angiogenesis inhibitors. However, due the complexity of tumor progression, tumor composition,

blood vessel structures, and drug resistance mechanisms, most of the current therapies have provided limited extension of survival time across multiple cancer types with the exception of imatinib (tyrosine kinase inhibitor) for gastrointestinal stromal tumor (Sawaki and Yamao 2004). Knowledge of drug action pathways and cellular drug resistance mechanisms to specific drugs has allowed the development and evaluation of promising drug combinations (Kim et al. 2008; Szakacs et al. 2006). Trials of combinations of agents are usually designed to enhance the activity of the primary agent or to inhibit different pathways to circumvent drug resistance to the primary agent. The critical advantage of using drug combinations is to prevent drug resistance development during cancer therapy without increasing the known side effects of each drug. Although it is believed that tumor growth and metastases are adaptable mechanisms, higher doses of single drugs are able to prevent resistance mechanisms in vitro in some cases (Kim et al. 2008). However, multi-drug regimens with synergistic combinations have been shown to be more successful in patients, probably due to cell heterogeneity in tumors and between patients. Unfortunately, multi-drug treatment requires complicated dosing regimens. Nanoparticle delivery systems offer solutions to both of these approaches. Delivery of single drugs in nanoparticles results in increased drug concentrations in the tumor, allowing higher doses compared with free drug using both non-targeted and targeted delivery. Nanoparticles can also be engineered to carry multiple drugs that are delivered together in one particle with control over the release rate of each drug, preventing the need for complicated multi-drug dosing regimens and improving patient compliance.

3.2 Non-Targeted Nanoparticles

Non-targeted nanoparticles circulating in the blood have been shown to significantly improve drug bioavailability and accumulation in tumors through the enhanced permeability and retention effect (EPR) (Fig. 4). The EPR effect allows the passive targeting of nanoparticles to tumors due to pathological abnormalities in the tumor vasculature (Maeda 2001; Minko et al. 2000). Interendothelial gap defects increase vascular permeability in tumors, allowing extravasation of nanoparticles up to 400 nm (Hobbs et al. 1998). Accumulation of nanoparticles is further enhanced due to poor lymphatic drainage in tumors. The local release of anti-cancer drugs from nanocarriers in the extravascular space results in an increased intra-tumoral drug concentration. In general, hydrophobic drugs released extracellularly will diffuse and be taken up by cancer cells, leading to enhanced tumor cytotoxicity. Since cancer cell populations, cell density, antigen expression, microenvironment, and vasculature density are significantly different across different cancers and even within primary and secondary metastatic sites, nanoparticle biodistribution and circulation time represent critical parameters for cancer therapy.

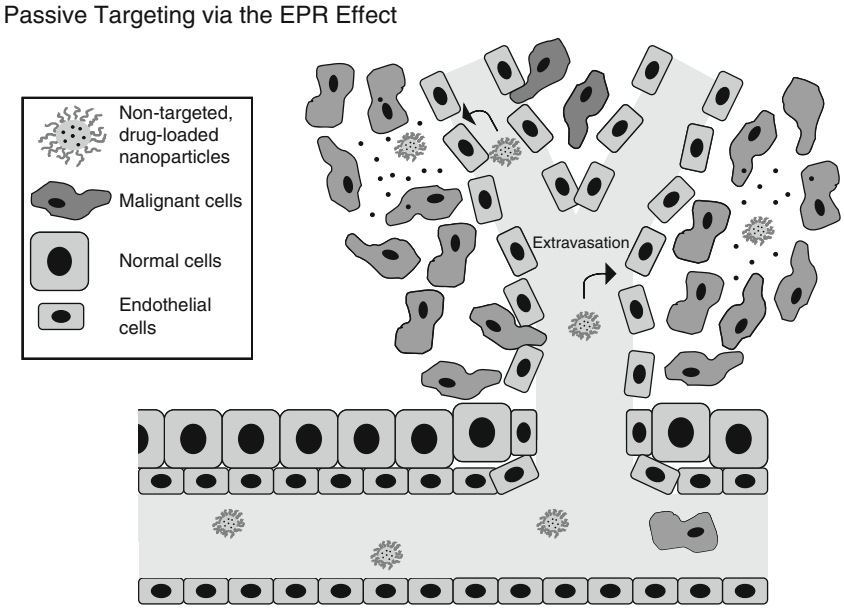


Fig. 4 Schematic of "passive targeting" via enhanced permeability and retention effect (EPR). The small size of nanoparticles allows them to circulate for a long period of time, extravasate, and accumulate into tumor tissues through leaky tumor vasculature

Multiple factors affect the pharmacokinetic behavior of nanoparticles, but the surface charge, size, nanoparticle shape and stealth properties are among the most critical (Alexis et al. 2008b; Li and Huang 2008a). As described in the nanoparticle technologies section above, five common types of nanoparticles are approved or in late stage of clinical trials, including polymer–drug conjugates, micelles, protein-based carrier, liposomes, and polymeric nanoparticles. Overall, non-targeted nanoparticles accumulate in tumor xenograft mice models in the range of 1–4% of I.D./g of tissue, although these numbers are difficult to compare due to different post-injection time assessments (Alexis et al. 2008b; Soepenberg et al. 2005). Polymer–drug conjugates are the smallest (1–20 nm) and have a circulation half-life in human ranging from hours to days depending on the system. To our knowledge, dextran–camptothecin (DE-310) has the longest circulation half-life (~300 h) in humans and has been shown to have no major toxicity compared to the free drug formulation in clinical phase II trials (Soepenberg et al. 2005). However, its therapeutic efficacy might be limited by its dosage regimen compared to PEG–camptothecin and polyglutamate–camptothecin conjugates (7,000 and 25 mg m^{-2}, respectively) (Homsi et al. 2007). These results underline the significant differences of pharmacokinetic parameters using different polymer–drug conjugates due to different loading, release profiles, and molecular weights of the carrier. This is also true for the circulation half-life of other

polymer–drug conjugates such as HPMA–drug conjugates, polyglutamate–drug conjugates, dextran–drug conjugates and pegylated drugs such as PEG–arginine deaminase (Hepacid, 7 days) and PEG–camptothecin (Prothecan, 40 h) (Ascierto et al. 2005; Posey et al. 2005). In general, larger nanoparticles such as micelles and liposomes seem to have a shorter circulation half-life in the blood (2–50 h) but higher maximum tolerated doses. The Genexol-PM formulation of paclitaxel is given at a twofold higher dosage than HPMA–paclitaxel (PNU166945) and polyglutamate–paclitaxel (Xyotax). However, it is not clear whether circulation half-life or maximum tolerated dose is the most critical for optimum accumulation in tumor tissues. For example, polycyclodextrin–camptothecin micelles (IT-101) and PEG–camptothecin conjugates show similar circulation half-life but significantly different accumulation of drug in tumor xenograft models. However, this may be due to the different xenograft models used. Unfortunately, it is difficult to compare the therapeutic efficacy of different systems in humans due to different patient populations and disease stages. Clinical data suggests that the circulation half-life and biodistribution of nanoparticles are related to the physicochemical properties of the vehicle. This is consistent with the in vivo biodistribution and circulation half-life results using animal models (Alexis et al. 2008b). In addition, it is well established that hydrophilic polymers such as PEG can be grafted, conjugated, or absorbed onto the surface of nanoparticles to form a corona, which provides steric stabilization and confers "stealth" properties by reducing protein absorption and rapid clearance.

Recently, we (Salvador-Morales et al. 2009b) and others (Cedervall et al. 2007a; Cedervall et al. 2007b; Lindman et al. 2007) investigated nanoparticle surface properties and adsorption of proteins present in the blood. Lindman et al. (Cedervall et al. 2007b) found that protein adsorption kinetics and composition depends on particle size and surface hydrophobicity. The results show that albumin adsorbed more on the surface of 200 nm nanoparticles than on smaller nanoparticles (70 nm). Nanoparticles with hydrophilic surfaces significantly prevented protein adsorption. It was suggested that smaller nanoparticles (70 nm) have higher curvature which reduce protein adsorption of larger proteins. Interestingly, the results show a binding competition leading to adsorption exchanges between proteins despite different concentrations and affinities. Lundqvist et al. have shown that protein adsorption (Lundqvist et al. 2008) depends significantly on the size and charge of the nanoparticles. Identification of protein compositions bound to the nanoparticles showed a mixture of proteins with different functions such as immunoglobulin, lipoproteins, complement pathways proteins, and coagulation factor proteins. Similarly, our group investigated complement activation, blood clotting, and protein adsorption properties of hybrid nanoparticles with precise control of the charge (Salvador-Morales et al. 2009b).

DeSimone's group has investigated internalization pathways (Gratton et al. 2008b) and in-vivo biodistribution of polymeric nanoparticles with different size and shapes (Gratton et al. 2007). Nanoparticles were more efficiently taken up by Hela cells than microparticles. Rod-like nanoparticles were internalized much more

efficiently than their spherical counterpart in vitro but there was no clear evidence of the effect of shape affecting the biodistribution and circulation half-life of the nanoparticles in vivo. Other groups have also shown differential uptake of nanoparticles with different shapes (Chithrani and Chan 2007; Chithrani et al. 2006; Ferrari 2008). These findings are highlighted by the mechanical modeling reported by Decuzzi (Decuzzi and Ferrari 2006; Decuzzi et al. 2007; Decuzzi et al. 2005; Decuzzi et al. 2009; Gentile et al. 2008a; Gentile et al. 2008b) showing that nanoparticle geometry and physicochemical properties contribute to the cellular internalization rate and adhesion forces on the surface of the cells. Mathematical models suggest that nanoparticle size will control its interaction with cells, especially the endothelial wall of vasculatures through a margination dynamic mechanism (Decuzzi and Ferrari 2008). Finally, the surface structure of the nanoparticle can affect its cellular uptake. Recent studies have shown that nanoparticles coated with sub-nanometer striations demonstrate enhanced uptake compared with random surface structures (Verma et al. 2008).

3.3 Targeted Nanoparticles

The concept of targeted therapy appeared in the late 1970s with the development of antibodies (Schrama et al. 2006), whereas the application of targeted nanoparticles appeared later using immunoliposomes (Heath et al. 1980; Leserman et al. 1980). Advances in cancer proteomics and bioinformatics have allowed the development of targeted therapies, which were referred to as a "magic bullet" by the visionary Paul Ehrlich (Strebhardt and Ullrich 2008). Nanocarriers may be surface functionalized with biomolecules for "active" tumor targeting. Surface ligands include antibodies, aptamers, peptides, or small molecules which recognize tumor-specific or tumor-associated antigens in the tumor microenvironment (Alexis et al. 2008b,c; Bareford and Swaan 2007; Farokhzad et al. 2006a,c; Sudimack and Lee 2000; van Vlerken and Amiji 2006). The active targeting mechanism takes advantage of highly specific interactions between the targeting ligand and certain tissues or cell surface antigens to increase cellular uptake and increase tumor retention. Conjugation approaches have been developed to control the amount of targeting ligands on the surface of the nanoparticles. In the case of weak binding ligands, multivalent functionalization on the surface of the nanoparticles provides sufficient avidity. In general, small molecule ligands such as peptides, sugars, and small molecules are more attractive than antibodies due to higher stability, higher purity, ease of production through synthetic routes, and non-immunogenicity.

There are two common approaches for receptor-mediated targeting. This first approach is to target the tumor microenvironment, including the extracellular matrix or surface receptors on tumor blood vessel endothelial cells (Fig. 5), which is usually most efficient for the delivery of immune induction or anti-angiogenesis molecules. The second approach is to target tumor cell surface

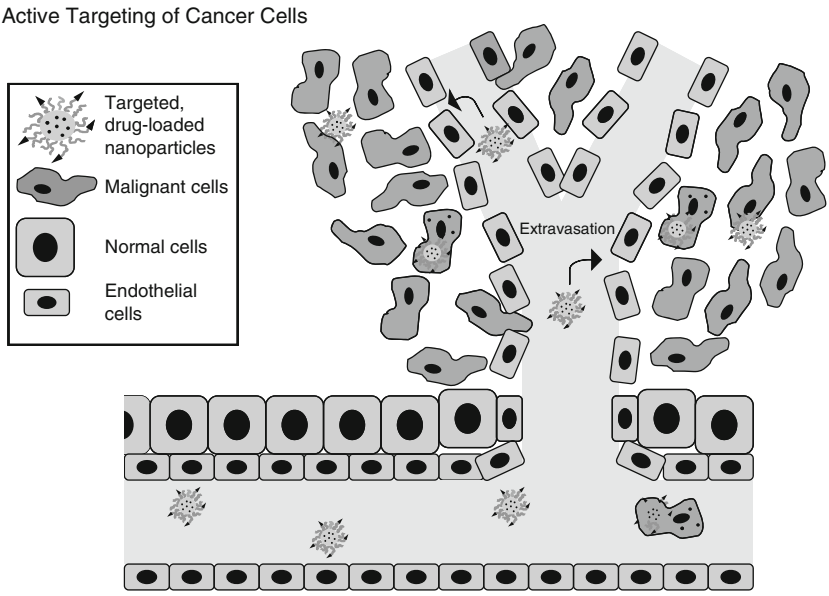


Fig. 5 Schematic of "active targeting" of functionalized nanoparticles to cancer cells. Targeting ligands on the surface of nanoparticles are able to bind to receptors on malignant cells, causing local drug delivery or uptake through receptor-mediated endocytosis

receptors for intracellular delivery (Fig. 6) of cytotoxic agents or signal-pathway inhibitors. Nanocarriers targeted to the extracellular portion of transmembrane tumor antigens are generally specifically taken up by cancer cells through receptor-mediated endocytosis for efficient delivery of therapeutic loads intracellularly. Although it is not clear which approach will provide the highest therapeutic efficacy for treatment of cancer metastases, a recent report using integrin receptor targeted nanoparticles delivering a cytotoxic drug (doxorubicin) showed promising data in primary and metastatic sites of human renal and pancreatic carcinoma mouse xenograft models (Murphy et al. 2008). Targeted nanoparticles showed tumor accumulation and decreased the tumor weight in the primary tumor and hepatic lymph node metastasis. We (Alexis et al. 2008a; Bagalkot et al. 2006, 2007; Dhar et al. 2008; Farokhzad et al. 2004; Gu et al. 2008; Wang et al. 2008a; Zhang et al. 2007) and others (Brannon-Peppas and Blanchette 2004; Peppas 2004) have developed targeted nanoparticles for multiple cancer types. Our group has developed nucleic acid aptamer functionalized nanoparticles for controlled drug delivery. Aptamers are able to bind to specific targets with high affinity and specificity, resulting in clinical development for multiple applications. We are developing multiple technologies using targeted nanoparticle–aptamer bioconjugates for drug delivery to prostate cancer. In a proof-of-concept study, polymeric nanoparticles utilizing aptamers as the targeting ligand showed

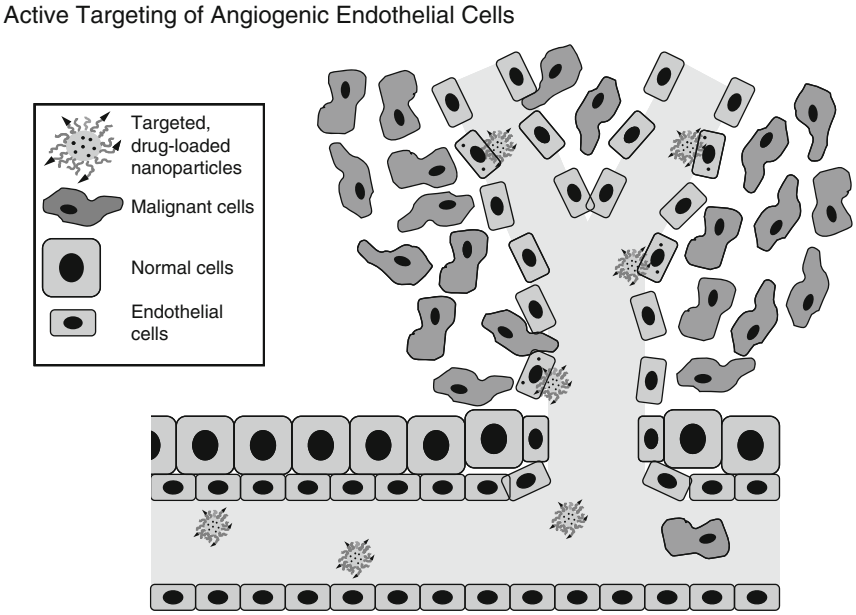


Fig. 6 Schematic of "active targeting" of functionalized nanoparticles to endothelial wall. Targeting ligands on the surface of nanoparticles are able to bind to receptors on endothelial cells or basement membrane matrix, causing local drug delivery on the endothelial wall for anti-angiogenesis therapy

almost complete reduction in tumor growth in a human prostate cancer tumor xenograft mice model (Farokhzad et al. 2004, 2006a). All the treated mice survived more than three months in contrast to other controls. Subsequently, we reported a novel strategy for formulating targeted nanoparticles that was tested in vivo (Gu et al. 2008). We also engineered hydrophilic cisplatin drugs for efficient encapsulation into PLGA–PEG nanoparticles (Dhar et al. 2008).

4 Summary

Metastasis is still an extremely complex disease with multiple questions still remaining. While 90% of human cancer deaths are due to cancer metastases, the hope for fighting cancer is sustained by the fact that there were more than 50 new agents approved in the past 10 years for cancer treatment and hundreds of new agents in clinical development. The development of nanoparticle drug delivery systems is expected to have a big impact on the clinical approaches for cancer therapy. The ability to specifically target nanoparticles along with the controlled delivery of a therapeutic payload provides powerful new ways to treat cancer which are only starting to be realized. By rationally designing nanoparticles based on

improved knowledge of cancer biology and the tumor microenvironment, improved efficacy can be achieved. In addition, multifunctional nanoparticles able to carry imaging agents and deliver multiple drugs are now being developed for enhanced detection and treatment of cancer. The application of nanotechnology to cancer has already produced some exciting results and holds even greater promise for cancer patients in the future.

Acknowledgements This work was supported by National Institute of Health Grants CA119349 and EB003647 and a Koch-Prostate Cancer Foundation Award in Nanotherapeutics. EMP is supported by a National Defense Science and Engineering Graduate Fellowship (NDSEG).

References

Adler HI, Fisher WD, Cohen A, Hardigree AA (1967) Miniature Escherichia coli cells deficient in DNA. Proc Natl Acad Sci USA 57:321–326

Ahmed F, Pakunlu RI, Srinivas G, Brannan A, Bates F, Klein ML, Minko T, Discher DE (2006) Shrinkage of a rapidly growing tumor by drug-loaded polymersomes: pH-triggered release through copolymer degradation. Mol Pharm 3:340–350

Al-Jamal WT, Al-Jamal KT, Bomans PH, Frederik PM, Kostarelos K (2008) Functionalized-quantum-dot-liposome hybrids as multimodal nanoparticles for cancer. Small 4:1406–1415

Alexis F, Basto P, Levy-Nissenbaum E, Radovic-Moreno AF, Zhang L, Pridgen E, Wang AZ, Marein SL, Westerhof K, Molnar LK, Farokhzad OC (2008a) HER-2-targeted nanoparticle-affibody bioconjugates for cancer therapy. ChemMedChem 3:1839–1843

Alexis F, Pridgen E, Molnar LK, Farokhzad OC (2008b) Factors affecting the clearance and biodistribution of polymeric nanoparticles. Mol Pharm 5:505–515

Alexis F, Rhee JW, Richie JP, Radovic-Moreno AF, Langer R, Farokhzad OC (2008c) New frontiers in nanotechnology for cancer treatment. Urol Oncol 26:74–85

Aliabadi HM, Shahin M, Brocks DR, Lavasanifar A (2008) Disposition of drugs in block copolymer micelle delivery systems: from discovery to recovery. Clin Pharmacokinet 47:619–634

Andresen TL, Davidsen J, Begtrup M, Mouritsen OG, Jorgensen K (2004) Enzymatic release of antitumor ether lipids by specific phospholipase A2 activation of liposome-forming prodrugs. J Med Chem 47:1694–1703

Andresen TL, Jensen SS, Kaasgaard T, Jorgensen K (2005) Triggered activation and release of liposomal prodrugs and drugs in cancer tissue by secretory phospholipase A2. Curr Drug Deliv 2:353–362

Ascierto PA, Scala S, Castello G, Daponte A, Simeone E, Ottaiano A, Beneduce G, De Rosa V, Izzo F, Melucci MT, Ensor CM, Prestayko AW, Holtsberg FW, Bomalaski JS, Clark MA, Savaraj N, Feun LG, Logan TF (2005) Pegylated arginine deiminase treatment of patients with metastatic melanoma: results from phase I and II studies. J Clin Oncol 23:7660–7668

Bagalkot V, Farokhzad OC, Langer R, Jon S (2006) An aptamer-doxorubicin physical conjugate as a novel targeted drug-delivery platform. Angew Chem Int Ed Engl 45:8149–8152

Bagalkot V, Zhang L, Levy-Nissenbaum E, Jon S, Kantoff PW, Langer R, Farokhzad OC (2007) Quantum dot-aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on bi-fluorescence resonance energy transfer. Nano Lett 7:3065–3070

Bangham AD, Standish MM, Watkins JC (1965) Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol 13:238–252

Bareford LM, Swaan PW (2007) Endocytic mechanisms for targeted drug delivery. Adv Drug Deliv Rev 59:748–758

Bartlett DW, Su H, Hildebrandt IJ, Weber WA, Davis ME (2007) Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles measured by multimodality in vivo imaging. Proc Natl Acad Sci USA 104:15549–15554

Bertin PA, Gibbs JM, Shen CK, Thaxton CS, Russin WA, Mirkin CA, Nguyen ST (2006) Multifunctional polymeric nanoparticles from diverse bioactive agents. J Am Chem Soc 128:4168–4169

Bierie B, Moses HL (2006) Tumour microenvironment: TGFβ: the molecular Jekyll and Hyde of cancer. Nat Rev Cancer 6:506–520

Bissell MJ, Labarge MA (2005) Context, tissue plasticity, and cancer: are tumor stem cells also regulated by the microenvironment? Cancer Cell 7:17–23

Boddy AV, Plummer ER, Todd R, Sludden J, Griffin M, Robson L, Cassidy J, Bissett D, Bernareggi A, Verrill MW, Calvert AH (2005) A phase I and pharmacokinetic study of paclitaxel poliglumex (XYOTAX), investigating both 3-weekly and 2-weekly schedules. Clin Cancer Res 11:7834–7840

Brannon-Peppas L, Blanchette JO (2004) Nanoparticle and targeted systems for cancer therapy. Adv Drug Deliv Rev 56:1649–1659

Brigger I, Dubernet C, Couvreur P (2002) Nanoparticles in cancer therapy and diagnosis. Adv Drug Deliv Rev 54:631–651

Cairns R, Papandreou I, Denko N (2006) Overcoming physiologic barriers to cancer treatment by molecularly targeting the tumor microenvironment. Mol Cancer Res 4:61–70

Campone M, Rademaker-Lakhai JM, Bennouna J, Howell SB, Nowotnik DP, Beijnen JH, Schellens JH (2007) Phase I and pharmacokinetic trial of AP5346, a DACH-platinum-polymer conjugate, administered weekly for three out of every 4 weeks to advanced solid tumor patients. Cancer Chemother Pharmacol 60:523–533

Castor TP (2005) Phospholipid nanosomes. Curr Drug Deliv 2:329–340

Cedervall T, Lynch I, Foy M, Berggard T, Donnelly SC, Cagney G, Linse S, Dawson KA (2007a) Detailed identification of plasma proteins adsorbed on copolymer nanoparticles. Angew Chem Int Ed Engl 46:5754–5756

Cedervall T, Lynch I, Lindman S, Berggard T, Thulin E, Nilsson H, Dawson KA, Linse S (2007b) Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. Proc Natl Acad Sci USA 104:2050–2055

Chambers AF, Groom AC, MacDonald IC (2002) Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer 2:563–572

Chan JM, Zhang L, Yuet KP, Liao G, Rhee JW, Langer R, Farokhzad OC (2009) PLGA-lecithin-PEG core-shell nanoparticles for controlled drug delivery. Biomaterials 30:1627–1634

Chen Y, Huang L (2008) Tumor-targeted delivery of siRNA by non-viral vector: safe and effective cancer therapy. Expert Opin Drug Deliv 5:1301–1311

Cheng Y, CS A, Meyers JD, Panagopoulos I, Fei B, Burda C (2008) Highly efficient drug delivery with gold nanoparticle vectors for in vivo photodynamic therapy of cancer. J Am Chem Soc 130:10643–10647

Chithrani BD, Chan WC (2007) Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. Nano Lett 7:1542–1550

Chithrani BD, Ghazani AA, Chan WC (2006) Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. Nano Lett 6:662–668

Couvreur P, Kante B, Roland M, Speiser P (1979) Adsorption of antineoplastic drugs to polyalkylcyanoacrylate nanoparticles and their release in calf serum. J Pharm Sci 68:1521–1524

Cullis PR, Chonn A (1998) Recent advances in liposome technologies and their applications for systemic gene delivery. Adv Drug Deliv Rev 30:73–83

Davis M (2009) The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: from concept to clinic. Mol Pharm 6:659–668

Davis ME, Brewster ME (2004) Cyclodextrin-based pharmaceutics: past, present and future. Nat Rev Drug Discov 3:1023–1035
Davis ME, Chen ZG, Shin DM (2008) Nanoparticle therapeutics: an emerging treatment modality for cancer. Nat Rev Drug Discov 7:771–782
Decuzzi P, Ferrari M (2006) The adhesive strength of non-spherical particles mediated by specific interactions. Biomaterials 27:5307–5314
Decuzzi P, Ferrari M (2008) Design maps for nanoparticles targeting the diseased microvasculature. Biomaterials 29:377–384
Decuzzi P, Gentile F, Granaldi A, Curcio A, Causa F, Indolfi C, Netti P, Ferrari M (2007) Flow chamber analysis of size effects in the adhesion of spherical particles. Int J Nanomedicine 2:689–696
Decuzzi P, Lee S, Bhushan B, Ferrari M (2005) A theoretical model for the margination of particles within blood vessels. Ann Biomed Eng 33:179–190
Decuzzi P, Pasqualini R, Arap W, Ferrari M (2009) Intravascular delivery of particulate systems: does geometry really matter? Pharm Res 26:235–243
Desai N, Trieu V, Yao Z, Louie L, Ci S, Yang A, Tao C, De T, Beals B, Dykes D, Noker P, Yao R, Labao E, Hawkins M, Soon-Shiong P (2006) Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of cremophor-free, albumin-bound paclitaxel, ABI-007, compared with cremophor-based paclitaxel. Clin Cancer Res 12:1317–1324
Dhar S, Gu FX, Langer R, Farokhzad OC, Lippard SJ (2008) Targeted delivery of cisplatin to prostate cancer cells by aptamer functionalized Pt(IV) prodrug-PLGA-PEG nanoparticles. Proc Natl Acad Sci USA 105:17356–17361
Discher BM, Won YY, Ege DS, Lee JC, Bates FS, Discher DE, Hammer DA (1999) Polymersomes: tough vesicles made from diblock copolymers. Science 284:1143–1146
Euliss LE, DuPont JA, Gratton S, DeSimone J (2006) Imparting size, shape, and composition control of materials for nanomedicine. Chem Soc Rev 35:1095–1104
Fan H (2008) Nanocrystal-micelle: synthesis, self-assembly and application. Chem Commun (Camb) 28:1383–1394
Farokhzad OC (2008) Nanotechnology for drug delivery: the perfect partnership. Expert Opin Drug Deliv 5:927–929
Farokhzad OC, Cheng J, Teply BA, Sherifi I, Jon S, Kantoff PW, Richie JP, Langer R (2006a) Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. Proc Natl Acad Sci USA 103:6315–6320
Farokhzad OC, Dimitrakov JD, Karp JM, Khademhosseini A, Freeman MR, Langer R (2006b) Drug delivery systems in urology–getting "smarter". Urology 68:463–469
Farokhzad OC, Jon S, Khademhosseini A, Tran TN, Lavan DA, Langer R (2004) Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. Cancer Res 64:7668–7672
Farokhzad OC, Karp JM, Langer R (2006c) Nanoparticle-aptamer bioconjugates for cancer targeting. Expert Opin Drug Deliv 3:311–324
Farokhzad OC, Langer R (2006) Nanomedicine: developing smarter therapeutic and diagnostic modalities. Adv Drug Deliv Rev 58:1456–1459
Farokhzad OC, Langer R (2009) Impact of nanotechnology on drug delivery. ACS Nano 3:16–20
Ferrari M (2005) Cancer nanotechnology: opportunities and challenges. Nat Rev Cancer 5: 161–171
Ferrari M (2008) Nanogeometry: beyond drug delivery. Nat Nanotechnol 3:131–132
Fesik SW (2005) Promoting apoptosis as a strategy for cancer drug discovery. Nat Rev Cancer 5:876–885
Fidler IJ (1995) Modulation of the organ microenvironment for treatment of cancer metastasis. J Natl Cancer Inst 87:1588–1592
Fidler IJ (2003) The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer 3:453–458

Florence AT, Hussain N (2001) Transcytosis of nanoparticle and dendrimer delivery systems: evolving vistas. Adv Drug Deliv Rev 50(Suppl 1):S69–S89

Fox JL (2000) Researchers discuss NIH's nanotechnology initiative. Nat Biotechnol 18:821

Freitas RA Jr (2005) What is nanomedicine? Dis Mon 51:325–341

Galon J, Fridman WH, Pages F (2007) The adaptive immunologic microenvironment in colorectal cancer: a novel perspective. Cancer Res 67:1883–1886

Gao J, Liang G, Cheung JS, Pan Y, Kuang Y, Zhao F, Zhang B, Zhang X, Wu EX, Xu B (2008) Multifunctional yolk-shell nanoparticles: a potential MRI contrast and anticancer agent. J Am Chem Soc 130:11828–11833

Gao K, Huang L (2008) Nonviral methods for siRNA delivery. Mol Pharm 6:651–658

Gary DJ, Puri N, Won YY (2007) Polymer-based siRNA delivery: perspectives on the fundamental and phenomenological distinctions from polymer-based DNA delivery. J Control Release 121:64–73

Gentile F, Chiappini C, Fine D, Bhavane RC, Peluccio MS, Cheng MM, Liu X, Ferrari M, Decuzzi P (2008a) The effect of shape on the margination dynamics of non-neutrally buoyant particles in two-dimensional shear flows. J Biomech 41:2312–2318

Gentile F, Curcio A, Indolfi C, Ferrari M, Decuzzi P (2008b) The margination propensity of spherical particles for vascular targeting in the microcirculation. J Nanobiotechnology 6:9

Gradishar WJ (2006) Albumin-bound paclitaxel: a next-generation taxane. Expert Opin Pharmacother 7:1041–1053

Gratton SE, Napier ME, Ropp PA, Tian S, DeSimone JM (2008a) Microfabricated particles for engineered drug therapies: elucidation into the mechanisms of cellular internalization of PRINT particles. Pharm Res 25:2845–2852

Gratton SE, Pohlhaus PD, Lee J, Guo J, Cho MJ, Desimone JM (2007) Nanofabricated particles for engineered drug therapies: a preliminary biodistribution study of PRINT nanoparticles. J Control Release 121:10–18

Gratton SE, Ropp PA, Pohlhaus PD, Luft JC, Madden VJ, Napier ME, DeSimone JM (2008b) The effect of particle design on cellular internalization pathways. Proc Natl Acad Sci USA 105:11613–11618

Greco F, Vicent MJ (2008) Polymer–drug conjugates: current status and future trends. Front Biosci 13:2744–2756

Gref R, Luck M, Quellec P, Marchand M, Dellacherie E, Harnisch S, Blunk T, Muller RH (2000) 'Stealth' corona-core nanoparticles surface modified by polyethylene glycol (PEG): influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. Colloids Surf B Biointerfaces 18:301–313

Gref R, Minamitake Y, Peracchia MT, Trubetskoy V, Torchilin V, Langer R (1994) Biodegradable long-circulating polymeric nanospheres. Science 263:1600–1603

Gu F, Zhang L, Teply BA, Mann N, Wang A, Radovic-Moreno AF, Langer R, Farokhzad OC (2008) Precise engineering of targeted nanoparticles by using self-assembled biointegrated block copolymers. Proc Natl Acad Sci USA 105:2586–2591

Guo X, MacKay JA, Szoka FC Jr (2003) Mechanism of pH-triggered collapse of phosphatidylethanolamine liposomes stabilized by an ortho ester polyethyleneglycol lipid. Biophys J 84:1784–1795

Hamaguchi T, Kato K, Yasui H, Morizane C, Ikeda M, Ueno H, Muro K, Yamada Y, Okusaka T, Shirao K, Shimada Y, Nakahama H, Matsumura Y (2007) A phase I and pharmacokinetic study of NK105, a paclitaxel-incorporating micellar nanoparticle formulation. Br J Cancer 97:170–176

Hawkins MJ, Soon-Shiong P, Desai N (2008) Protein nanoparticles as drug carriers in clinical medicine. Adv Drug Deliv Rev 60:876–885

Heath TD, Fraley RT, Papahdjopoulos D (1980) Antibody targeting of liposomes: cell specificity obtained by conjugation of F(ab')2 to vesicle surface. Science 210:539–541

Heidel JD, Yu Z, Liu JY, Rele SM, Liang Y, Zeidan RK, Kornbrust DJ, Davis ME (2007) Administration in non-human primates of escalating intravenous doses of targeted

nanoparticles containing ribonucleotide reductase subunit M2 siRNA. Proc Natl Acad Sci USA 104:5715–5721
Hirsch LR, Stafford RJ, Bankson JA, Sershen SR, Rivera B, Price RE, Hazle JD, Halas NJ, West JL (2003) Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance. Proc Natl Acad Sci USA 100:13549–13554
Hobbs SK, Monsky WL, Yuan F, Roberts WG, Griffith L, Torchilin VP, Jain RK (1998) Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. Proc Natl Acad Sci USA 95:4607–4612
Homsi J, Simon GR, Garrett CR, Springett G, De Conti R, Chiappori AA, Munster PN, Burton MK, Stromatt S, Allievi C, Angiuli P, Eisenfeld A, Sullivan DM, Daud AI (2007) Phase I trial of poly-L-glutamate camptothecin (CT-2106) administered weekly in patients with advanced solid malignancies. Clin Cancer Res 13:5855–5861
Jain KK (2008) Nanomedicine: application of nanobiotechnology in medical practice. Med Princ Pract 17:89–101
James JS (1995a) DOXIL approved by FDA. AIDS Patient Care 9:306
James JS (1995b) DOXIL approved for KS. AIDS Treat News 236:6
Jensen SS, Andresen TL, Davidsen J, Hoyrup P, Shnyder SD, Bibby MC, Gill JH, Jorgensen K (2004) Secretory phospholipase A2 as a tumor-specific trigger for targeted delivery of a novel class of liposomal prodrug anticancer etherlipids. Mol Cancer Ther 3:1451–1458
Jiang W, Kim BY, Rutka JT, Chan WC (2007) Advances and challenges of nanotechnology-based drug delivery systems. Expert Opin Drug Deliv 4:621–633
Johannsen M, Gneveckow U, Eckelt L, Feussner A, Waldofner N, Scholz R, Deger S, Wust P, Loening SA, Jordan A (2005) Clinical hyperthermia of prostate cancer using magnetic nanoparticles: presentation of a new interstitial technique. Int J Hyperthermia 21:637–647
Johannsen M, Gneveckow U, Thiesen B, Taymoorian K, Cho CH, Waldofner N, Scholz R, Jordan A, Loening SA, Wust P (2007) Thermotherapy of prostate cancer using magnetic nanoparticles: feasibility, imaging, and three-dimensional temperature distribution. Eur Urol 52:1653–1661
Jorgensen K, Davidsen J, Mouritsen OG (2002) Biophysical mechanisms of phospholipase A2 activation and their use in liposome-based drug delivery. FEBS Lett 531:23–27
Juliano R, Alam MR, Dixit V, Kang H (2008) Mechanisms and strategies for effective delivery of antisense and siRNA oligonucleotides. Nucleic Acids Res 36:4158–4171
Kaneda Y (2000) Virosomes: evolution of the liposome as a targeted drug delivery system. Adv Drug Deliv Rev 43:197–205
Kawasaki ES, Player A (2005) Nanotechnology, nanomedicine, and the development of new, effective therapies for cancer. Nanomedicine 1:101–109
Kelly JY, DeSimone JM (2008) Shape-specific, monodisperse nano-molding of protein particles. J Am Chem Soc 130:5438–5439
Kester M, Heakal Y, Fox T, Sharma A, Robertson GP, Morgan TT, Altinoglu EI, Tabakovic A, Parette MR, Rouse S, Ruiz-Velasco V, Adair JH (2008) Calcium phosphate nanocomposite particles for in vitro imaging and encapsulated chemotherapeutic drug delivery to cancer cells. Nano Lett 8:4116–4121
Kim D, Lee ES, Oh KT, Gao ZG, Bae YH (2008) Doxorubicin-loaded polymeric micelle overcomes multidrug resistance of cancer by double-targeting folate receptor and early endosomal pH. Small 4:2043–2050
Kim DW, Kim SY, Kim HK, Kim SW, Shin SW, Kim JS, Park K, Lee MY, Heo DS (2007a) Multicenter phase II trial of Genexol-PM, a novel Cremophor-free, polymeric micelle formulation of paclitaxel, with cisplatin in patients with advanced non-small-cell lung cancer. Ann Oncol 18:2009–2014
Kim JS, Rieter WJ, Taylor KM, An H, Lin W, Lin W (2007b) Self-assembled hybrid nanoparticles for cancer-specific multimodal imaging. J Am Chem Soc 129:8962–8963

Kim K, Lee M, Park H, Kim JH, Kim S, Chung H, Choi K, Kim IS, Seong BL, Kwon IC (2006) Cell-permeable and biocompatible polymeric nanoparticles for apoptosis imaging. J Am Chem Soc 128:3490–3491

Kim SC, Kim DW, Shim YH, Bang JS, Oh HS, Wan Kim S, Seo MH (2001) In vivo evaluation of polymeric micellar paclitaxel formulation: toxicity and efficacy. J Control Release 72: 191–202

Kim TY, Kim DW, Chung JY, Shin SG, Kim SC, Heo DS, Kim NK, Bang YJ (2004) Phase I and pharmacokinetic study of Genexol-PM, a Cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies. Clin Cancer Res 10:3708–3716

Klibanov AL, Maruyama K, Torchilin VP, Huang L (1990) Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes. FEBS Lett 268:235–237

Kocer A (2007) A remote controlled valve in liposomes for triggered liposomal release. J Liposome Res 17:219–225

Kukowska-Latallo JF, Candido KA, Cao Z, Nigavekar SS, Majoros IJ, Thomas TP, Balogh LP, Khan MK, Baker JR Jr (2005) Nanoparticle targeting of anticancer drug improves therapeutic response in animal model of human epithelial cancer. Cancer Res 65:5317–5324

Langer R (1998) Drug delivery and targeting. Nature 392:5–10

Langer R, Folkman J (1976) Polymers for the sustained release of proteins and other macromolecules. Nature 263:797–800

Lanza G, Winter P, Cyrus T, Caruthers S, Marsh J, Hughes M, Wickline S (2006) Nanomedicine opportunities in cardiology. Ann N Y Acad Sci 1080:451–465

Lee CC, MacKay JA, Frechet JM, Szoka FC (2005) Designing dendrimers for biological applications. Nat Biotechnol 23:1517–1526

Lee KS, Chung HC, Im SA, Park YH, Kim CS, Kim SB, Rha SY, Lee MY, Ro J (2008) Multicenter phase II trial of Genexol-PM, a Cremophor-free, polymeric micelle formulation of paclitaxel, in patients with metastatic breast cancer. Breast Cancer Res Treat 108:241–250

Leserman LD, Barbet J, Kourilsky F, Weinstein JN (1980) Targeting to cells of fluorescent liposomes covalently coupled with monoclonal antibody or protein A. Nature 288:602–604

Levy-Nissenbaum E, Radovic-Moreno AF, Wang AZ, Langer R, Farokhzad OC (2008) Nanotechnology and aptamers: applications in drug delivery. Trends Biotechnol 26:442–449

Li C (2002) Poly(L-glutamic acid)–anticancer drug conjugates. Adv Drug Deliv Rev 54:695–713

Li C, Wallace S (2008) Polymer–drug conjugates: recent development in clinical oncology. Adv Drug Deliv Rev 60:886–898

Li SD, Huang L (2008a) Pharmacokinetics and biodistribution of nanoparticles. Mol Pharm 5:496–504

Li SD, Huang L (2008b) Targeted delivery of siRNA by nonviral vectors: lessons learned from recent advances. Curr Opin Investig Drugs 9:1317–1323

Liggins RT, Burt HM (2002) Polyether-polyester diblock copolymers for the preparation of paclitaxel loaded polymeric micelle formulations. Adv Drug Deliv Rev 54:191–202

Lindman S, Lynch I, Thulin E, Nilsson H, Dawson KA, Linse S (2007) Systematic investigation of the thermodynamics of HSA adsorption to *N*-iso-propylacrylamide/*N*-tert-butylacrylamide copolymer nanoparticles. Effects of particle size and hydrophobicity. Nano Lett 7:914–920

Lundqvist M, Stigler J, Elia G, Lynch I, Cedervall T, Dawson KA (2008) Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. Proc Natl Acad Sci USA 105:14265–14270

Luten J, van Nostrum CF, De Smedt SC, Hennink WE (2008) Biodegradable polymers as non-viral carriers for plasmid DNA delivery. J Control Release 126:97–110

MacDiarmid JA, Mugridge NB, Weiss JC, Phillips L, Burn AL, Paulin RP, Haasdyk JE, Dickson KA, Brahmbhatt VN, Pattison ST, James AC, Al Bakri G, Straw RC, Stillman B, Graham RM, Brahmbhatt H (2007) Bacterially derived 400 nm particles for encapsulation and cancer cell targeting of chemotherapeutics. Cancer Cell 11:431–445

Maeda H (2001) The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. Adv Enzyme Regul 41:189–207

Majoros IJ, Myc A, Thomas T, Mehta CB, Baker JR Jr (2006) PAMAM dendrimer-based multifunctional conjugate for cancer therapy: synthesis, characterization, and functionality. Biomacromolecules 7:572–579

Majoros IJ, Thomas TP, Mehta CB, Baker JR Jr (2005) Poly(amidoamine) dendrimer-based multifunctional engineered nanodevice for cancer therapy. J Med Chem 48:5892–5899

Matsumura Y (2008) Poly (amino acid) micelle nanocarriers in preclinical and clinical studies. Adv Drug Deliv Rev 60:899–914

Matsumura Y, Hamaguchi T, Ura T, Muro K, Yamada Y, Shimada Y, Shirao K, Okusaka T, Ueno H, Ikeda M, Watanabe N (2004) Phase I clinical trial and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin. Br J Cancer 91:1775–1781

McCarthy JR, Weissleder R (2008) Multifunctional magnetic nanoparticles for targeted imaging and therapy. Adv Drug Deliv Rev 60:1241–1251

McCarthy TD, Karellas P, Henderson SA, Giannis M, O'Keefe DF, Heery G, Paull JR, Matthews BR, Holan G (2005) Dendrimers as drugs: discovery and preclinical and clinical development of dendrimer-based microbicides for HIV and STI prevention. Mol Pharm 2:312–318

Minko T, Kopeckova P, Pozharov V, Jensen KD, Kopecek J (2000) The influence of cytotoxicity of macromolecules and of VEGF gene modulated vascular permeability on the enhanced permeability and retention effect in resistant solid tumors. Pharm Res 17:505–514

Moghimi SM (2006) Recent developments in polymeric nanoparticle engineering and their applications in experimental and clinical oncology. Anticancer Agents Med Chem 6:553–561

Moghimi SM, Hunter AC, Murray JC (2001) Long-circulating and target-specific nanoparticles: theory to practice. Pharmacol Rev 53:283–318

Moghimi SM, Hunter AC, Murray JC (2005) Nanomedicine: current status and future prospects. Faseb J 19:311–330

Montet X, Weissleder R, Josephson L (2006) Imaging pancreatic cancer with a peptide-nanoparticle conjugate targeted to normal pancreas. Bioconjug Chem 17:905–911

Morgan TT, Muddana HS, Altinoglu EI, Rouse SM, Tabakovic A, Tabouillot T, Russin TJ, Shanmugavelandy SS, Butler PJ, Eklund PC, Yun JK, Kester M, Adair JH (2008) Encapsulation of organic molecules in calcium phosphate nanocomposite particles for intracellular imaging and drug delivery. Nano Lett 8(12):4108–4115

Murakami T, Tsuchida K (2008) Recent advances in inorganic nanoparticle-based drug delivery systems. Mini Rev Med Chem 8:175–183

Murphy EA, Majeti BK, Barnes LA, Makale M, Weis SM, Lutu-Fuga K, Wrasidlo W, Cheresh DA (2008) Nanoparticle-mediated drug delivery to tumor vasculature suppresses metastasis. Proc Natl Acad Sci USA 105:9343–9348

Myc A, Douce TB, Ahuja N, Kotlyar A, Kukowska-Latallo J, Thomas TP, Baker JR Jr (2008) Preclinical antitumor efficacy evaluation of dendrimer-based methotrexate conjugates. Anticancer Drugs 19:143–149

Najlah M, D'Emanuele A (2007) Synthesis of dendrimers and drug-dendrimer conjugates for drug delivery. Curr Opin Drug Discov Devel 10:756–767

Nasongkla N, Bey E, Ren J, Ai H, Khemtong C, Guthi JS, Chin SF, Sherry AD, Boothman DA, Gao J (2006) Multifunctional polymeric micelles as cancer-targeted, MRI-ultrasensitive drug delivery systems. Nano Lett 6:2427–2430

Nyman DW, Campbell KJ, Hersh E, Long K, Richardson K, Trieu V, Desai N, Hawkins MJ, Von Hoff DD (2005) Phase I and pharmacokinetics trial of ABI-007, a novel nanoparticle formulation of paclitaxel in patients with advanced nonhematologic malignancies. J Clin Oncol 23:7785–7793

Overall CM, Kleifeld O (2006) Tumour microenvironment - opinion: validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. Nat Rev Cancer 6:227–239

Paciotti GF, Myer L, Weinreich D, Goia D, Pavel N, McLaughlin RE, Tamarkin L (2004) Colloidal gold: a novel nanoparticle vector for tumor directed drug delivery. Drug Deliv 11:169–183

Park H, Yang J, Seo S, Kim K, Suh J, Kim D, Haam S, Yoo KH (2008) Multifunctional nanoparticles for photothermally controlled drug delivery and magnetic resonance imaging enhancement. Small 4:192–196

Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R (2007) Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol 2:751–760

Peppas NA (2004) Intelligent therapeutics: biomimetic systems and nanotechnology in drug delivery. Adv Drug Deliv Rev 56:1529–1531

Peracchia MT, Harnisch S, Pinto-Alphandary H, Gulik A, Dedieu JC, Desmaele D, d'Angelo J, Muller RH, Couvreur P (1999) Visualization of in vitro protein-rejecting properties of PEGylated stealth polycyanoacrylate nanoparticles. Biomaterials 20:1269–1275

Porche DJ (1996) Liposomal doxorubicin (Doxil). J Assoc Nurses AIDS Care 7:55–59

Posey JA 3rd, Saif MW, Carlisle R, Goetz A, Rizzo J, Stevenson S, Rudoltz MS, Kwiatek J, Simmons P, Rowinsky EK, Takimoto CH, Tolcher AW (2005) Phase 1 study of weekly polyethylene glycol-camptothecin in patients with advanced solid tumors and lymphomas. Clin Cancer Res 11:7866–7871

Pridgen EM, Langer R, Farokhzad OC (2007) Biodegradable, polymeric nanoparticle delivery systems for cancer therapy. Nanomed 2:669–680

Quintana A, Raczka E, Piehler L, Lee I, Myc A, Majoros I, Patri AK, Thomas T, Mule J, Baker JR Jr (2002) Design and function of a dendrimer-based therapeutic nanodevice targeted to tumor cells through the folate receptor. Pharm Res 19:1310–1316

Rapoport N, Gao Z, Kennedy A (2007) Multifunctional nanoparticles for combining ultrasonic tumor imaging and targeted chemotherapy. J Natl Cancer Inst 99:1095–1106

Rice JR, Gerberich JL, Nowotnik DP, Howell SB (2006) Preclinical efficacy and pharmacokinetics of AP5346, a novel diaminocyclohexane-platinum tumor-targeting drug delivery system. Clin Cancer Res 12:2248–2254

Riehemann K, Schneider SW, Luger TA, Godin B, Ferrari M, Fuchs H (2009) Nanomedicine–challenge and perspectives. Angew Chem Int Ed Engl 48:872–897

Rolland JP, Maynor BW, Euliss LE, Exner AE, Denison GM, DeSimone JM (2005) Direct fabrication and harvesting of monodisperse, shape-specific nanobiomaterials. J Am Chem Soc 127:10096–10100

Rosen H, Abribat T (2005) The rise and rise of drug delivery. Nat Rev Drug Discov 4:381–385

Salvador-Morales C, Gao W, Ghatalia P, Murshed F, Aizu W, Langer R, Farokhzad OC (2009a) Multifunctional nanoparticles for prostate cancer therapy. Expert Rev Anticancer Ther 9:211–221

Salvador-Morales C, Zhang L, Langer R, Farokhzad OC (2009b) Immunocompatibility properties of lipid–polymer hybrid nanoparticles with heterogeneous surface functional groups. Biomaterials 30:2231–2240

Sawaki A, Yamao K (2004) Imatinib mesylate acts in metastatic or unresectable gastrointestinal stromal tumor by targeting KIT receptors–a review. Cancer Chemother Pharmacol 54(Suppl 1): S44–S49

Schneider GF, Subr V, Ulbrich K, Decher G (2009) Multifunctional cytotoxic stealth nanoparticles. A model approach with potential for cancer therapy. Nano Lett 9:636–642

Schrama D, Reisfeld RA, Becker JC (2006) Antibody targeted drugs as cancer therapeutics. Nat Rev Drug Discov 5:147–159

Schwartz JA, Shetty AM, Price RE, Stafford RJ, Wang JC, Uthamanthil RK, Pham K, McNichols RJ, Coleman CL, Payne JD (2009) Feasibility study of particle-assisted laser ablation of brain tumors in orthotopic canine model. Cancer Res 69:1659–1667

Sengupta S, Eavarone D, Capila I, Zhao G, Watson N, Kiziltepe T, Sasisekharan R (2005) Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system. Nature 436:568–572

Shin J, Anisur RM, Ko MK, Im GH, Lee JH, Lee IS (2009) Hollow manganese oxide nanoparticles as multifunctional agents for magnetic resonance imaging and drug delivery. Angew Chem Int Ed Engl 48:321–324

Siclari VA, Guise TA, Chirgwin JM (2006) Molecular interactions between breast cancer cells and the bone microenvironment drive skeletal metastases. Cancer Metastasis Rev 25:621–633
Singer JW (2005) Paclitaxel poliglumex (XYOTAX, CT-2103): a macromolecular taxane. J Control Release 109:120–126
Singer JW, Baker B, De Vries P, Kumar A, Shaffer S, Vawter E, Bolton M, Garzone P (2003) Poly-(L)-glutamic acid-paclitaxel (CT-2103) [XYOTAX], a biodegradable polymeric drug conjugate: characterization, preclinical pharmacology, and preliminary clinical data. Adv Exp Med Biol 519:81–99
Sinha R, Kim GJ, Nie S, Shin DM (2006) Nanotechnology in cancer therapeutics: bioconjugated nanoparticles for drug delivery. Mol Cancer Ther 5:1909–1917
Soepenberg O, de Jonge MJ, Sparreboom A, de Bruin P, Eskens FA, de Heus G, Wanders J, Cheverton P, Ducharme MP, Verweij J (2005) Phase I and pharmacokinetic study of DE-310 in patients with advanced solid tumors. Clin Cancer Res 11:703–711
Sood P, Thurmond KB 2nd, Jacob JE, Waller LK, Silva GO, Stewart DR, Nowotnik DP (2006) Synthesis and characterization of AP5346, a novel polymer-linked diaminocyclohexyl platinum chemotherapeutic agent. Bioconjug Chem 17:1270–1279
Strebhardt K, Ullrich A (2008) Paul Ehrlich's magic bullet concept: 100 years of progress. Nat Rev Cancer 8:473–480
Sudimack J, Lee RJ (2000) Targeted drug delivery via the folate receptor. Adv Drug Deliv Rev 41:147–162
Sudimack JJ, Guo W, Tjarks W, Lee RJ (2002) A novel pH-sensitive liposome formulation containing oleyl alcohol. Biochim Biophys Acta 1564:31–37
Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM (2006) Targeting multidrug resistance in cancer. Nat Rev Drug Discov 5:219–234
Tejada-Berges T, Granai CO, Gordinier M, Gajewski W (2002) Caelyx/Doxil for the treatment of metastatic ovarian and breast cancer. Expert Rev Anticancer Ther 2:143–150
Thanou M, Duncan R (2003) Polymer–protein and polymer–drug conjugates in cancer therapy. Curr Opin Investig Drugs 4:701–709
Thevenot J, Troutier AL, David L, Delair T, Ladaviere C (2007) Steric stabilization of lipid/polymer particle assemblies by poly(ethylene glycol)-lipids. Biomacromolecules 8:3651–3660
Torchilin VP (2005) Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov 4:145–160
Tribler L, Jensen LT, Jorgensen K, Brunner N, Gelb MH, Nielsen HJ, Jensen SS (2007) Increased expression and activity of group IIA and X secretory phospholipase A_2 in peritumoral versus central colon carcinoma tissue. Anticancer Res 27:3179–3185
Tseng YC, Mozumdar S, Huang L (2009) Lipid-based systemic delivery of siRNA. Adv Drug Deliv Rev 61:721–731
Tsukioka Y, Matsumura Y, Hamaguchi T, Koike H, Moriyasu F, Kakizoe T (2002) Pharmaceutical and biomedical differences between micellar doxorubicin (NK911) and liposomal doxorubicin (Doxil). Jpn J Cancer Res 93:1145–1153
Uchegbu IF (2006) Pharmaceutical nanotechnology: polymeric vesicles for drug and gene delivery. Expert Opin Drug Deliv 3:629–640
Uchino H, Matsumura Y, Negishi T, Koizumi F, Hayashi T, Honda T, Nishiyama N, Kataoka K, Naito S, Kakizoe T (2005) Cisplatin-incorporating polymeric micelles (NC-6004) can reduce nephrotoxicity and neurotoxicity of cisplatin in rats. Br J Cancer 93:678–687
van Vlerken LE, Amiji MM (2006) Multi-functional polymeric nanoparticles for tumour-targeted drug delivery. Expert Opin Drug Deliv 3:205–216
van Vlerken LE, Duan Z, Little SR, Seiden MV, Amiji MM (2008) Biodistribution and pharmacokinetic analysis of Paclitaxel and ceramide administered in multifunctional polymer-blend nanoparticles in drug resistant breast cancer model. Mol Pharm 5:516–526
Vasey PA, Kaye SB, Morrison R, Twelves C, Wilson P, Duncan R, Thomson AH, Murray LS, Hilditch TE, Murray T, Burtles S, Fraier D, Frigerio E, Cassidy J (1999) Phase I clinical and

pharmacokinetic study of PK1 [*N*-(2-hydroxypropyl)methacrylamide copolymer doxorubicin]: first member of a new class of chemotherapeutic agents-drug-polymer conjugates. Cancer Research Campaign Phase I/II Committee. Clin Cancer Res 5:83–94

Venugopal J, Prabhakaran MP, Low S, Choon AT, Zhang YZ, Deepika G, Ramakrishna S (2008) Nanotechnology for nanomedicine and delivery of drugs. Curr Pharm Des 14:2184–2200

Verma A, Uzun O, Hu Y, Hu Y, Han HS, Watson N, Chen S, Irvine DJ, Stellacci F (2008) Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles. Nat Mater 7:588–595

Vial F, Rabhi S, Tribet C (2005) Association of octyl-modified poly(acrylic acid) onto unilamellar vesicles of lipids and kinetics of vesicle disruption. Langmuir 21:853–862

Visaria R, Bischof JC, Loren M, Williams B, Ebbini E, Paciotti G, Griffin R (2007) Nanotherapeutics for enhancing thermal therapy of cancer. Int J Hyperthermia 23:501–511

Visaria RK, Griffin RJ, Williams BW, Ebbini ES, Paciotti GF, Song CW, Bischof JC (2006) Enhancement of tumor thermal therapy using gold nanoparticle-assisted tumor necrosis factor-alpha delivery. Mol Cancer Ther 5:1014–1020

Wang AZ, Gu F, Zhang L, Chan JM, Radovic-Moreno A, Shaikh MR, Farokhzad OC (2008a) Biofunctionalized targeted nanoparticles for therapeutic applications. Expert Opin Biol Ther 8:1063–1070

Wang G, Uludag H (2008) Recent developments in nanoparticle-based drug delivery and targeting systems with emphasis on protein-based nanoparticles. Expert Opin Drug Deliv 5:499–515

Wang L, Bai J, Li Y, Huang Y (2008b) Multifunctional nanoparticles displaying magnetization and near-IR absorption. Angew Chem Int Ed Engl 47:2439–2442

Whitehead KA, Langer R, Anderson DG (2009) Knocking down barriers: advances in siRNA delivery. Nat Rev Drug Discov 8:129–138

Wong HL, Bendayan R, Rauth AM, Li Y, Wu XY (2007) Chemotherapy with anticancer drugs encapsulated in solid lipid nanoparticles. Adv Drug Deliv Rev 59:491–504

Wong HL, Bendayan R, Rauth AM, Xue HY, Babakhanian K, Wu XY (2006a) A mechanistic study of enhanced doxorubicin uptake and retention in multidrug resistant breast cancer cells using a polymer–lipid hybrid nanoparticle system. J Pharmacol Exp Ther 317:1372–1381

Wong HL, Rauth AM, Bendayan R, Manias JL, Ramaswamy M, Liu Z, Erhan SZ, Wu XY (2006b) A new polymer–lipid hybrid nanoparticle system increases cytotoxicity of doxorubicin against multidrug-resistant human breast cancer cells. Pharm Res 23:1574–1585

Zamboni WC (2008) Concept and clinical evaluation of carrier-mediated anticancer agents. Oncologist 13:248–260

Zetter BR (2008) The scientific contributions of M. Judah Folkman to cancer research. Nat Rev Cancer 8:647–654

Zhang L, Chan JM, Gu FX, Rhee JW, Wang AZ, Radovic-Moreno AF, Alexis F, Langer R, Farokhzad OC (2008a) Self-assembled lipid–polymer hybrid nanoparticles: a robust drug delivery platform. ACS Nano 2:1696–1702

Zhang L, Gu FX, Chan JM, Wang AZ, Langer RS, Farokhzad OC (2008b) Nanoparticles in medicine: therapeutic applications and developments. Clin Pharmacol Ther 83:761–769

Zhang L, Radovic-Moreno AF, Alexis F, Gu FX, Basto PA, Bagalkot V, Jon S, Langer RS, Farokhzad OC (2007) Co-delivery of hydrophobic and hydrophilic drugs from nanoparticle-aptamer bioconjugates. ChemMedChem 2:1268–1271

Biosensing and Drug Delivery at the Microscale

Novel Devices for Controlled and Responsive Drug Delivery

Andrea A. Robitzki and Randy Kurz

Contents

Abstract An overall objective of pharmaceutical research is the controlled release or delivery of drugs at the biological target site in a therapeutically and pharmacodynamically optimal amount. In relation to "intelligent" drug delivery, several basic aspects are important, i.e., release of active pharmaceutical ingredients from the formulation, transport to and penetration across biological barriers, and subsequent biotransformation depending on a controlled release process. Future development of advanced and/or controlled drug releasing systems, e.g. polymeric or particulate drug targeting systems, nano-carbon tube related and/or nano-pillar based drug release, or electronically mediated molecule delivery, is expected to take advantage of progress in molecular cell biology, cell and tissue engineering,

A.A. Robitzki (✉)
University of Leipzig, Center for Biotechnology and Biomedicine, Deutscher Platz 5, 04103 Leipzig, Germany
e-mail: andrea.robitzki@bbz.uni-leipzig.de

M. Schäfer-Korting (ed.), *Drug Delivery*,
Handbook of Experimental Pharmacology 197,
DOI 10.1007/978-3-642-00477-3_3, © Springer-Verlag Berlin Heidelberg 2010

membrane nano-biophysics, and bioelectronic properties (Bramstedt et al. 2005; Gardner et al. 2006). In this chapter novel aspects of the development of innovative drug delivery systems described and are categorized into polymeric, lipid-based or electronically mediated delivery systems (De la Heras et al. 2004).

Keywords Responsive drug delivery · Micro-implants · Polymeric release systems · Electronic delivery device

1 Progress and Challenges in Controlled Drug Delivery

The treatment of acute disease or chronic illness has been achieved by – among other means – delivery of drugs to the patient. For many years conventional drug delivery systems included tablets, injections, suspensions, creams, ointments, liquids and aerosols, still widely used for therapeutic approaches. The ultimate aim of pharmacy and medicine is the delivery of any drug at the right time, within the ideal therapeutic window and outside the systemic toxic range, in a safe and reproducible manner, to a specific target, at the required level. Conventional types of dosage such as oral delivery and/or injection are the predominant routes of administration. However, this approach is only rarely useful to control the rate of drug delivery, is often associated with an immediate, rapid drug release, and does not regulate the target area of the applied drugs. Consequently the initial concentration of the delivered drug in the organism rises and may reach the level of toxicity, decreasing over time to a sub-therapeutic level. The duration of therapeutic efficiency is then dependent on the frequency of administration and the half-life of the drug. High dosages of non-targeted drugs are often administered in order to achieve an effective concentration. At the site of disease, the plasma concentration range of many novel peptide, recombinant protein or nucleic acid based therapeutic compounds is rather small, and varies with time. Toxicity is observed for peaks of drug concentrations, rendering traditional methods of drug delivery ineffective. With a controlled drug release system, the rate of compound delivery should match the rate of drug elimination and, therefore, the drug concentration remains in the therapeutic window for the vast majority of the 24 h period. Clinically, temporal control can initiate a significant improvement in drug-mediated therapy, avoiding most common side effects and guaranteeing efficacy, safety and bioavailability (Santini et al. 2000).

Controlled drug release should be a delivery at a rate and/or at a location determined by needs over a specified period of time. Therefore, two main objectives exist for these release systems: (1) temporally acting drug delivery over an extended time period during treatment and (2) spatial or local delivery. The efficiency relates to a high concentration of the drug at the therapeutic site combined with a low systemic drug concentration and, therefore, a lower occurence of side effects.

Nowadays a number of mechanisms that can provide such a controlled drug release processes include transdermal patches, chemotherapeutic wafers, osmotic micro-pumps, bio-adhesive systems, micro-encapsulations, nano-particles and micro-implants (De la Heras Alarcón et al. 2004; Gardner 2006).

The main foci described here will be polymeric systems, followed by micro-chips. Most of the drug molecules need to be dissolved in the aqueous environment of the patient and freely diffuse within that medium before they can act on their target receptors. Polymeric devices that achieve a temporally controlled release employ protection of the molecules in aqueous solutions or cellular cytoplasmic areas according to pre-programmed periods of time. This protective effect can affect or inhibit the diffusion of molecules and the discharge of the drug compartment by controlling the flow of the drug solution or suspension. Polymers employed to delay drug dissolution aim to decelerate the flux rate of the drug delivery system in relation to the aqueous environment. This might be achieved by a polymer coating or matrix that dissolves or degrades at a slower rate than the drug diffuses and releases. However diffusion-controlled drug release can also be blocked by using insoluble polymer matrices with resuspended drug molecules which must migrate and actively exit the device. Other releasing devices contain

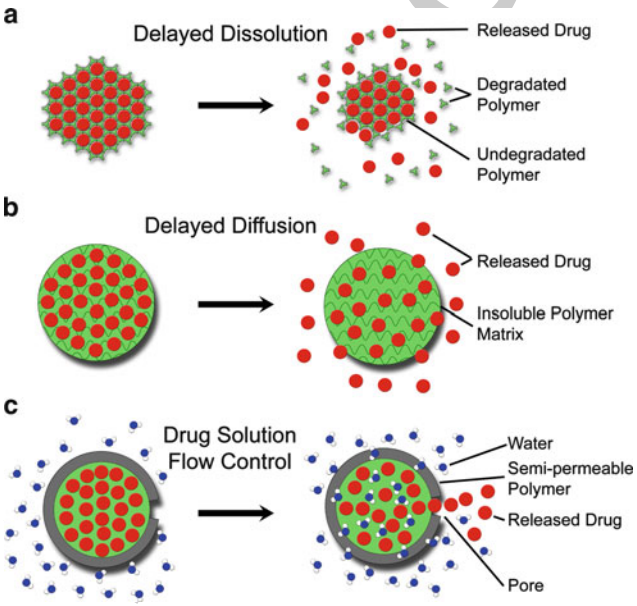


Fig. 1 Polymer-based drug release mechanisms. Scheme showing several mechanisms for temporally controlled polymer-based drug release systems. (**a**) Delayed dissolution mediated by a polymer which dissolves or degrades slowly, (**b**) Diffusion-controlled release through voids in polymeric devices, and (**c**) Controlled flow of the drug solution utilising an osmotic potential gradient across a semi-permeable membrane

cross-linked polymer chains in hydrogels and form so-called diffusion barriers. The diffusion barrier can be decreased by swelling of the hydrogel, resulting in generated voids in the gel structure. Such hydrogels also possess bio-adhesive characteristics which allow them to reside within the gastrointestinal tract for extended time periods. Polymers used for diffusion-controlled release can be fabricated either as matrices in which the active agent is distributed or as a rate-limiting membrane that protects the drug reservoir from the environment (Santini et al. 2000; Hilt and Peppas 2005; Al Malyan et al. 2007; Saylor et al. 2007; Angelos et al. 2008; Fig. 1).

Delivery systems that control the flow of drug solutions utilize osmotic potential gradients across semi-permeable polymer barriers to generate osmotic chambers containing aqueous solutions of the drug. In these containers, pressure is relieved by the flow of the solution out of the compartment. The rate of flow is controlled by restricted fluid transport through pores of micrometer-scale or larger diameter (Bry et al. 2001).

2 Polymer Actuator for Controlled Drug Delivery

Innovative biodegradable Micro-Electro-Mechanical Systems (MEMS) for drug delivery have been developed to prolong the time period of release. These responsive polymeric devices consist of a drug reservoir and micro-channels positioned between the reservoir and the open end of the micro-channels (Staples et al. 2006). They operate as osmotic or diffusion pumps depending on the choice of geometric parameters and water permeability of polymers. Therefore, stronger osmosis is induced by enlarging the area of the water-permeable membrane and incorporating highly potent osmotic agents (e.g., polyethylene glycol). Conversely, the diffusion process of the agent's delivery is suppressed by elongating the micro-channels (Ryu et al. 2007).

Such a micro-device comprises a micro-fabricated reservoir and micro-channels micro-molded in a biodegradable polymer layer sealed by a semi-permeable biodegradable polymer layer. Active pharmaceutical ingredients and osmotic agents are stored in the reservoir. For the molecule release, water must flow into the reservoir mediating an osmotic effect, finally causing a hydrostatic pressure which leads to inflation. The internal pressure now begins to propel the drug molecules out through the micro-channels (Ryu et al. 2007; Fig. 2).

Micro-molds of the structures were fabricated by a combination of wet and reactive ion etching processes of the bottom polymer layer. A 85/15 poly(L-lactid-co-glycolid) acts as semi-permeable top layer. The micro-structured bottom layer and the semi-permeable membrane were thermally bonded. A temporary inserted teflon sheet leaves the reservoir area open for drug loading. For example, a mixture of a PEG-coated active pharmaceutical ingredient was loaded into the reservoir using a syringe (volume = 20 µl per device). Afterwards the open loading edge of

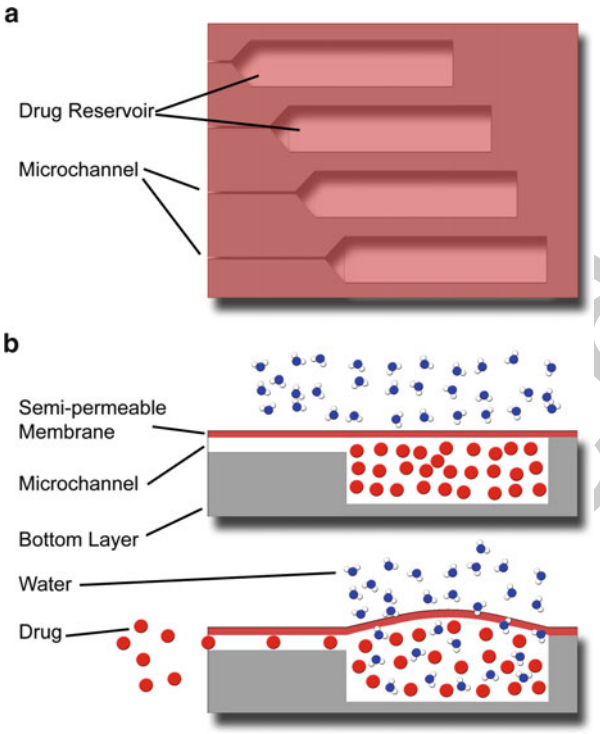


Fig. 2 Drug delivery via micro-channels. (**a**) A schematic drawing and design of a micro-device consisting of micro-channels with a width of 50 μm and various geometries in length. (**b**) Scheme showing the release mechanism before (*top*) and after inflation (*bottom*) of water (modified according to Ryu et al. 2007)

micro-device was sealed by a heat sealer. Osmotic drug release is regulated mainly by the parameters of the semi-permeable membran like the water permeability, the size, and the thickness of the membrane (Ryu et al. 2007). A further modulation of the release rate is possible by modification of the exit orifice if the osmotic influx of water is decreased by either a relatively large hydrostatic pressure or a pressure leak through the membrane resulting in a constant pressure within the reservoir. In order to understand the effect of the state of the exit orifice, the lengths of micro-channels were made 2, 4 and 8 mm. The profiles of the releasing process for micro-channels with three different lengths show a strong correlation to the lengths of the channels for a time period of two weeks. The device with the shortest channel released, e.g., a dye at a rate almost comparable to the estimated value from the osmotic release equation. The release rate decreased when the channels became longer concerning of the increased hydrostatic resistance that slows down the transport of active pharmaceutical ingredients. Devices with micro-channels of different lengths also show three different plateaus of the release rate after one week, indicating that the change in length affects the steady state condition of the

device (Fig. 2). One possible interpretation is that the presence of air trapped in the reservoir attenuates a further creation of pressure in the device by permeating through the membrane of the reservoir. This allows a constant pressure difference between inside and outside of the device; longer channels could induce higher flow resistance and decrease the overall release rate in the steady state (Juengst and Siegel 1988; Dorski et al. 1997; Ryu et al. 2007).

3 Complex Drug Releasing Systems for Synchronous Drug Delivery

More complex drug releasing systems for synchronous or serial delivery of multiple therapeutic compounds demand comprehensive control or regulation. The release profile can be pre-programmed to the device by designing a proper micro-geometry for one type of dose schedule without a need to develop a new specific formulation. When a larger dose rate is needed, the increased rate can be achieved either by enlarging the reservoir membrane of an osmotic-driven device or by increasing the cross-sectional area of the channels of a diffusion-driven device. Unlike conventional delivery systems made from biodegradable polymers, the release mechanism of biodegradable osmotic pumps is not directly coupled to the degradation of the polymer. The release is directly controlled either by the geometry of the micro-shape of the device or by the permeability of the polymer. Such a device can be used for one month before the degradation process of the device begins. This decoupling of the releasing and the degradation step offers a great advantage and benefit, e.g. for realizing (1) a more specified and accurate modulation of release, (2) an avoidance of drug exposure to an acidic environment that might occur during a degradation-mediated release, (3) a reduction of an adverse inflammatory response during the release, and (4) a minimization of any possible reaction between drugs and the degraded polymers. A biodegradable polymer-based microchip of an implantable multi-reservoir drug delivery device incorporates an array of reservoirs capped with resorbable membranes that may differ from other membranes in the array by thickness or chemical composition. The main component of the device consists of a reservoir-containing substrate that is fabricated from a degradable polymer. Truncated conical reservoirs in the substrate are loaded with the chemical to be released and sealed with polymeric degradable reservoir membranes at one end and a sealant layer (polyester tape) at the opposite end (Santini et al. 1999; Jager et al. 2000; Low et al. 2000; Wang et al. 2006; Randall et al. 2007).

These polymeric devices and their reservoirs are formed by compression molding of poly-lactic acid (PLA). The degradable polymer membranes are prepared using various ratios of lactic acid to glycolic acid and different molecular weight polymers to control release. First prototypes show geometries of approximately 11.9 mm in diameter, 480–560 mm thickness, and contain 36 single 120-nl to 130-nl reservoirs. A PLGA (poly(lactide-*co*-glycolide)) polymer-based membrane

with a molecular weight range of 4.4–64.0 kDa can be used to control the releasing rate of, e.g., heparin immobilized in the polymer matrix (Grayson et al. 2003; De la Heras Alarcón et al. 2004; Staples et al. 2006; Fig. 3).

4 A Novel Nanoscale Valve Responding to pH Changes May Allow a Targeted Drug Release

The main focus of the actual development of "intelligent" polymer-based releasing systems is based on the process of a pulsatile release for delivery of, e.g., human insulin directed to diabetes. The requirement for insulin fluctuates during the day according to the metabolism and blood glucose level. Current insulin formulations require repeated injections daily and careful control of glucose intake. Novel drug delivery devices consist of feedback control loops and might revolutionize insulin therapy via a controlled and regulated release of insulin in response to an increased blood glucose level. These micro-devices in general comprise two components: (1) a sensor that detects the environmental physiological parameters and stimulates the drug release, and (2) a delivery device or drug container (Dorski et al. 1997; De la Heras Alarcón et al. 2004). For diabetes treatment, responsive drug delivery systems have been proposed using the enzyme glucose oxidase as a biochemical sensor. When blood glucose level rises, glucose oxidase converts glucose to glucuronic acid resulting in a lower pH. This pH decrease acts as a signal for the required insulin release. The delivery process is achieved by a pH-sensitive polymer as a "molecular gate" that either swells or degrades in an acidic environment,

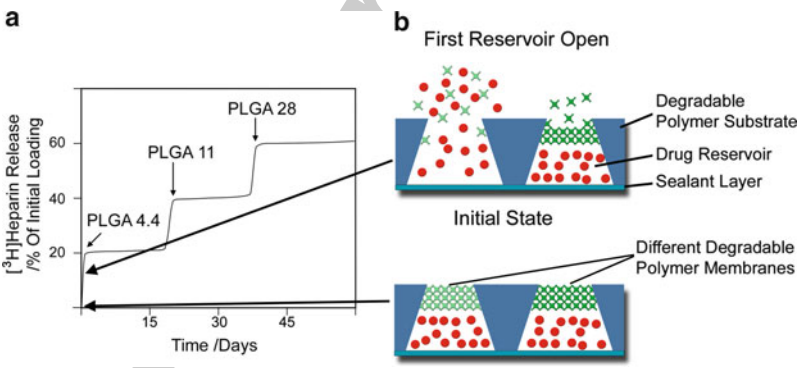


Fig. 3 A polymeric controlled drug release without electronics. (**a**) Schematic view of cumulative release results for devices loaded with [^{3}H]heparin and consisting of different PLGA membrane types (modified according to Grayson et al. 2003). (**b**) A design of a polymeric micro-chip device consisting of reservoirs containing a substrate fabricated using a degradable polymer. The truncated conical reservoirs are loaded with chemical compounds to be released via degradable reservoir membranes. The device is shown in its initial state (*bottom*) and with the degradation of the PLGA4.4 membrane type (*top*)

e.g. poly(methacrylic acid)-g-poly(ethylene glycol), P(MAA-g-EG) copolymer with immobilized and distributed glucose oxidase. This gel expands at higher pH values (physiological pH = 7.4) by closing the gates, and shrinks at low pH values (pH = 4.0, due to the interaction of glucose with immobilized glucose oxidase) by opening the gates. The real control of the released insulin concentration depends on the size and the response rate of the gates. The ability of a polymer to swell is not only coupled with environmental pH change but also depends on temperature, ionic interactions, magnetic, thermal and electrical forces. For most of these polymers, the structural changes are reversible and show a memory effect according to additional changes in the external environment (Fig. 4).

5 pH-Responsive Supramolecular Nano-valves

A nano-valve-based system was developed for drug delivery mediated by an opening process in response to pH changes. The principle of that release system is based on a procedure realized by filling a tiny, porous silica sphere with a

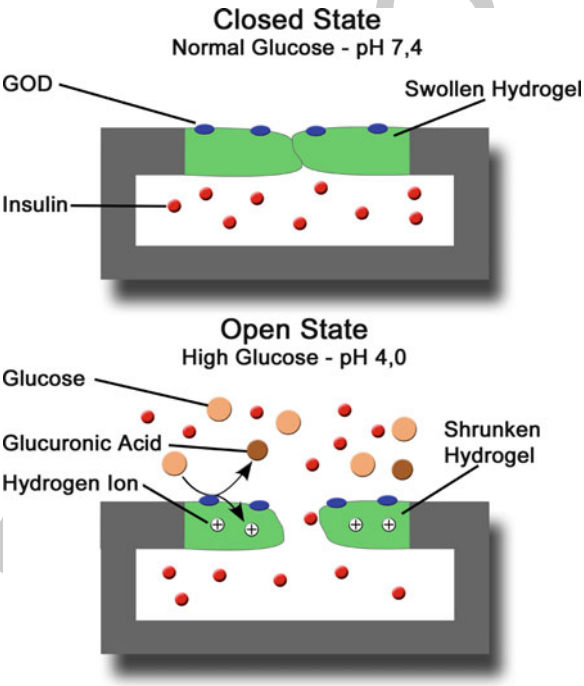


Fig. 4 Responsive polymer-based hormone release. Release mechanism of P(MAA-g-EG) pH-responsive hydrogel system coupled with glucose oxidase (GOD) for controlled insulin release depending on glucose concentration (modified according to De la Heras Alarcón et al. 2004)

compound, followed by plugging the pores with the valves. The delivery process can be determined or controlled by changing the pH (Angelos et al. 2008). The appeal of this technique is that the pH of healthy and diseased tissue often differs, and from this it follows that spheres could be designed to release the drugs specifically in diseased tissue only. Previous types of these valves functioned only in organic solvents and were finally activated by elaborate oxidation reactions. Now, by switching to the described pH-activated mechanism, the valves were made functional in water. Of course this seems to be a critical feature for any drug delivery system and a lot of emphasis was put on systems that are biocompatible. Thus the new valve has already been prototyped and tested for use in water. The core of the drug delivery system is a rigid 400 nm diameter silica nano-sphere structured like a honeycomb network of pores, which were filled with "reference molecules" that can be selectively released. A stalk is inserted into each pore and protrudes from the surface of the sphere, impaling a molecule of so-called cucurbit[6]uril. This donut-shaped molecule, which is effectively plugging the nano-pores, indirectly prevents the leaking out of the molecules or drugs. A further advantage is that these tiny spheres just 400 nm in diameter could easily be taken up by biological cells. After endocytosis they could respond to the internal cytoplasmic pH of the cell and either retain or release their contents, giving a controlled drug release. Angelos et al. observed that at neutral to acidic pH, cucurbit[6]uril is bound to the stalk by electrostatic forces, and the plug remains in place. When the pH turns basic, these electrostatic forces are disrupted, the cucurbit[6]uril plug is removed and the drug is free to leak out of the pores of these silica spheres. Current research in this field is focusing on more specific mechanisms for triggering the release of the compounds. If diseased cells express a particular enzyme not present in their healthy counterparts, thus breaking a particular chemical bond, this aspect can be introduced into the machinery. Applications might be in cancer therapy for releasing chemotherapy drugs directly to tumour cells and finally avoiding side effects. The nano-valve system could also be optimized and adapted for release of compounds and/or small peptides directed to targets correlated to degenerative diseases in cases where a particular cell type is affected. Although the valves were tested according to their proof of delivery of a dye in a test tube, their safety and efficacy in living biological cells, tissues, and animals have yet to be demonstrated (Fig. 5).

6 Electronically Controlled "Smart" Pill

The pill includes a housing and a drug reservoir for storage, with an electronically controlled release valve or hatch for dispensing one or more different compounds stored in the reservoir while transiting the gastrointestinal area. The device comprises autonomous control and timing circuitry responsible for opening and closing the valve. The advantage is that the control and timing circuitry is able to open and close the valve throughout a dispensing time period in correlation with a preset

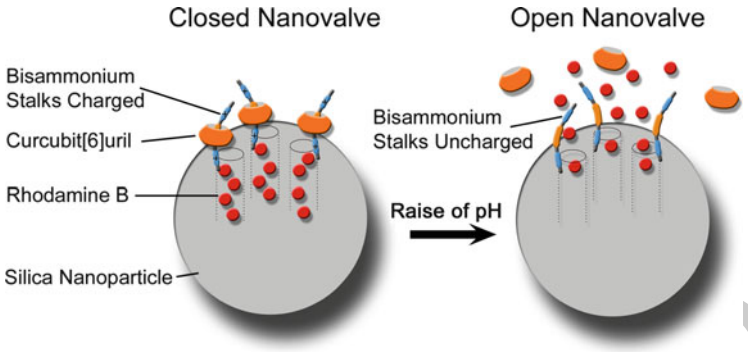


Fig. 5 Graphical scheme of operating supramolecular nano-valves. The alkyne-functionalized mesoporous silica nanoparticles MCM-41 are loaded with rhodamine B molecules, and capped with curcubit[6]uril on a positively charged bisammonium stalk during the CB[6]-catalyzed alkyne-azide 1,3 dipolar cycloaddition (*left*). After rise in pH, the bisammonium stalks become uncharged and the curcubit[6]uril caps detaches (*right*) (modified according to Angelos et al. 2008)

dispensing timing pattern programmed within the circuitry (PCT/IB05/52771, 2007 Philips Patent). A radio-frequency communication circuit receives control signals for setting the dispensing timing pattern remotely, reprogramming the control and timing circuitry, or terminating the dispensing of the drug and/or compounds within the organism. Thus this medication delivery system relates to an electronically controlled pill for releasing one active pharmaceutical ingredient. Demonstrating the advantage of this novel delivery system, one might reflect that objects typically pass through the gastrointestinal tract in 20–40 h. Several medicaments are still available as time-release capsules for delivering portions of the drug at different times. Time-release capsules require chemical reactions mediated by chemical compounds in the gastrointestinal area, and the coating of the drug release capsule is influenced and disturbed by proteins and fats. Therefore, an exact and precise dispensing or dissolving pattern is not possible. A patient may have more than a "normal" amount of chemical compounds in the gastrointestinal tract due to a condition or a previously-administered drug, and, therefore, cause the coating of the time-release capsule to react faster than normal. Accordingly, the active pharmaceutical ingredient is released by the time-release capsule at a faster rate than intended. However, another patient may have less than the "normal" amount of chemical agents in the gastrointestinal tract and cause the coating of the time-release capsule to react slower than normal, thereby releasing the active agent at a slower rate than intended. Thus for a controlled drug release some novel inventions provide an electronically controlled pill or drug delivery system for releasing or dispensing a compound according to a preset dispensing timing pattern while transiting, e.g., the gastrointestinal tract. The preset dispensing timing pattern is fixed and is not susceptible to a patient's physiological processes and conditions, mood, previously-administered drugs, etc. The electronically controlled pill

includes control and timing circuitry for controlling the opening and closing of a valve or hatch according to the preset dispensing timing pattern for dispensing a compound stored within a reservoir of the "smart" pill. The electronically controlled pill allows a patient to take all pills more or less simultaneously. Medication that does not fit into one electronically controlled pill can be coordinated with other electronically controlled pills for the full day's payload regimen.

7 Novel Micro- and Nano-Mechanical Drug Delivery Implants

Micro- and Nano-Electromechanical Systems (MEMS or NEMS)-based drug delivery devices offer opportunities to address unmet medical needs related to dosing. Such devices should be considered when conventional dosing methods perform suboptimally in terms of safety, efficacy, pain, or convenience (Staples et al. 2006). In addition, applications of these technologies may create totally new drug delivery paradigms. MEMS technologies may create new therapies with existing molecular entities. Such a MEMS consists of a micro-battery, micro-controller with programs that control the drug release in response to a sensor signal, using an actuator (Banghmann 1996, Santini et al. 1999; Bay et al. 2001; Grayson et al. 2003; Teymoori and Abbaspour-Sani 2005; Valsesia et al. 2006; Xu et al. 2006; Tsai and Madou 2007). Innovative delivery devices have the capability for complete control of drug release: doses may be administered in pulses or continuously for periods of months to years, or can be stored in a device pending an immediate need for emergency administration. Today novel technologies contributing to advanced drug delivery designs including MEMS or NEMS need new aspects of material science, data management (recruiting, housing, and communicating data), and biological science (Shimoda and Smela 1998; Pernant and Reynolds 2000; Smela 2003; Finkenstadt 2005; Tabard-Cossa et al. 2005; Tsai et al. 2007). New technologies might be useful only if they can be commercialized, and if drug delivery devices follow the regulatory systems. In 2002, the Food and Drug Administration (FDA) in the USA established the Office of Combination Products (OCP) to provide an appropriate regulatory framework for products that do not fit the established categories of drugs, devices, and biologicals. In Germany in 2002 the law on medical products was updated to take into account this new class of implants. Therefore, already available drug releasing systems are combined with novel innovative aspects to improve, e.g., such releasing systems like pulsatile release from polymeric matrices, already described before. An alternative method for achieving an improved and controlled pulsatile release involves micro-fabrication technology to develop active devices that incorporate micrometer-scale pumps, valves and flow channels. The release mechanism of this solid-state silicon microchip is based on the electrochemical dissolution of thin anode membranes covering micro-reservoirs filled with chemical compounds in solid, liquid or gel form. Such concepts are microchips with gold and saline solutions as electrode materials.

Release from a particular reservoir is initiated by applying an electric potential between the anode, a membrane covering the reservoir, and a cathode. The devices used in an previous study show a geometry and configuration of 17 mm × 17 mm × 310 µm and contain 34 reservoirs. A practical and optimal size of the medical release device should and could be reduced to less than 2 mm side length, depending on the particular application and implantation. As a point of reference, a device of the size used in these studies (17 mm) has enough surface area to accommodate over 1000 reservoirs. Devices were fabricated by a sequential process using silicon wafers and microelectronic processing techniques including ultraviolet photolithography, chemical vapour deposition, electron beam evaporation and reactive ion etching (Santini et al. 1999; Low et al. 2000; De la Heras Alarcón et al. 2004). Each device contains reservoirs that extend completely through the wafer. Each reservoir has a square pyramidal shape and contains one large and one small exit; it represents a volume of 25 nl, and is sealed on the small square end by a 0.3-µm-thick, gold membrane anode. Here gold (Au) is chosen as a conducting but biocompatible membrane material because it can be easily deposited and patterned, and has a low reactivity with other substances and resists spontaneous corrosion in many solutions over the entire pH range. However, the presence of a small amount of chloride ion creates an electric potential region which favours the formation of soluble gold chloride complexes. Holding the anode potential in this corrosion region enables reproducible gold dissolution (Santini et al. 1999; Fig. 6). Other metals such as copper or titanium tend to dissolve spontaneously under these conditions or do not form soluble materials on application of an electric potential.

Inkjet printing in combination with a computer-controlled alignment apparatus is capable of depositing less than 0.2 nl of a liquid or gel solution of a known concentration in each reservoir. So-called micro-syringe pumps also have the ability to deposit nanolitre quantities of solutions into device reservoirs (Low et al. 2000). The reservoirs are then covered with squares of a thin adhesive plastic and sealed with a waterproof epoxy. The objective of initial release experiments with this chip was to determine if pulsatile release of a single compound could be obtained from a microchip device. Release of a compound was achieved from a reservoir of a prototype immersed in phosphate-buffered saline (PBS) by applying a potential of +1.04 V. Samples were taken every few minutes from the release medium, analyzed for dye content in a fluorimeter.

These studies demonstrate that the activation of each reservoir can be controlled individually, creating a possibility for achieving many complex release patterns (Teymoori and Abbaspour-Sani 2005; Tsai and Madou 2007). Varying amounts of chemical compounds in solid, liquid or gel form can be released in either a pulsatile manner, a continuous manner, or a combination of both, sequentially or simultaneously from a single device. Such a device has additional potential advantages including small size, fast response and low power consumption. In addition, all chemical agents to be released are stored in the reservoirs of the microchip itself, creating a possibility for the future development of autonomous devices. “MicroCHIPS” has been developed as a device similar in size and

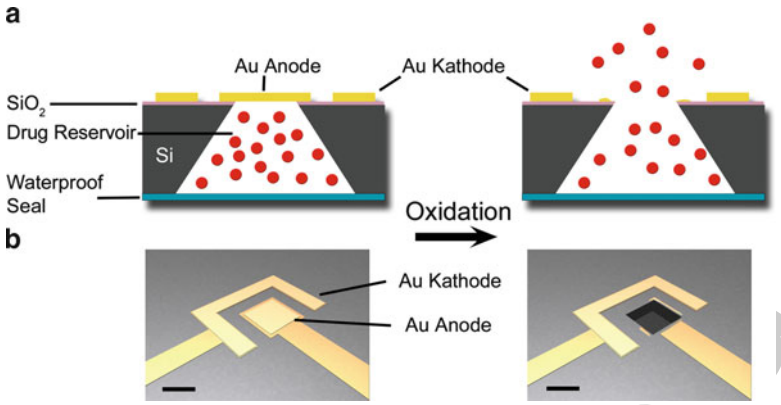


Fig. 6 Drug delivery microchip. (**a**) Design of a microchip based on electrochemical dissolution. (**b**) Illustration of the removal of a gold anode membrane to initiate drug release from a reservoir in a microchip device (according to Santini et al. 2000); before (*left*) and after (*right*) the application of +1.04 V versus SCE for several seconds in phosphate-buffered saline (scale bar 50 μm)

appearance to an implantable cardiac defibrillator, although the volume of future devices can be significantly reduced with the incorporation of custom electronic components. The microchip, wireless communication hardware, power supply, and electrical components are embedded and hermetically sealed inside the device. Each 15 mm × 15 mm × 1.0 mm microchip consists of a silicon/glass-bonded substrate containing 100 individually addressable, 300 nl capacity reservoirs. The membranes over each reservoir, composed of platinum and titanium layers, are removed by local resistive heating from an applied current. This electro-thermal method is independent of the chemistry of the surrounding medium and is many times faster than an electro-chemical method. The implantable "MicroCHIPS" device has been shown to realize a controlled, pulsatile release of the polypeptide leuprolide from discrete, individually addressable reservoirs in vivo for nearly six months. The devices were implanted into the subcutaneous tissue of male beagle dogs and each reservoir contained 25 μg lyophilized leuprolide in a matrix of solid polyethylene glycol. The mean pharmacokinetic profile of the release of leuprolide from the device in vivo generated throughout the study period corresponded to in vitro release kinetics. The maximum serum leuprolide concentration (C_{max}), the area under the pharmacokinetic curve (AUC) and the time to reach the maximum serum leuprolide concentration (T_{max}) after a release event were constant for six months within experimental variability. The averaged AUC values ranged from 37 to 50 ng × h × ml^{-1}, the averaged C_{max} values ranged from 5 to 11 ng × ml^{-1} and the average T_{max} values ranged from 2.0 to 3.2 h. The bioavailability of leuprolide was estimated at 60% compared to subcutaneous injections of solution-phase leuprolide. This device was the first demonstration of a fully self-contained microchip implant that provides chronic programmed delivery of a therapeutic active agent and also shows that

such a system can be used to release stability-optimized solid-phase drug formulations (Prescott et al. 2006; Staples et al. 2006).

The membrane activation process and, therefore, the release are similar to the operation of an electrical fuse. Current is passed through the connections and membrane to rapidly heat the membrane to the point of failure in order to expose the contents of a particular reservoir. Heating occurs preferentially in the membrane for several reasons. First, the membrane is suspended in an environment with a lower thermal conductivity than the silicon substrate. Second, the membrane has a smaller cross-sectional area than the traces, resulting in increased current density and resistive heating. Third, the membrane may be made from a material that is more resistive than the trace material, increasing heat generation. Membrane failure occurs as fast as 5 μs after the application of the activation current, briefly exposing tissue, the active agent, or biosensor components to elevated temperatures. The magnitude of the instantaneous current (range 0.3–2 A) depends on the applied voltage, the membrane material and dimensions of the devices used (Prescott et al. 2006). There is no longer a barrier between the contents of the reservoir and the external environment after the membrane is removed. Stored APIs are released by dissolution and diffusion through the recently created exit or, alternatively, molecules in physiological fluid can diffuse into the reservoir to contact a "stored biosensor". Successful reservoir activation is confirmed by observing an increase in total circuit resistance caused by the partial or complete removal of the membrane current path. A disadvantage of using the same material and cross-sectional area for both the membranes and the traces is a relative low power efficiency, since the traces are much longer than the membrane. In this hypothetical case, most of the input power during activation dissipates in the traces and does not contribute to the electro-thermal membrane removal process. Decoupling the membrane and fabrication processes of the trace provides the opportunity to incorporate different materials and vary cross-sectional areas to improve power efficiency. This is especially important for implantable electronic devices, where the size of the device might be a critical parameter. High power efficiency can reduce the size of a micro-implant allowing the use of a smaller battery and electronic components. Such a micro-fabricated device can be the core component of a fully implantable, intelligent system for controlled and completely monitored release of potent therapeutic compounds.

8 Highlights in Micro-Machined Biosensing Drug Delivery Devices

Another example for a controlled release system with actuating miniature valves in a drug reservoir has been demonstrated. The prototype represents a micro-machined drug delivery system with a battery, control circuitry, a biosensor, and a drug reservoir equipped with drug release actuator holes. ChipRx Inc. (Lexington, KY,

USA) has proposed this implantable, single-reservoir device that, in theory, could be adjusted to deliver drugs according to targeted pharmacokinetics and bioavailability. The release mechanism employs polymeric artificial muscles that ring micrometer-sized diameter holes which open for drug release. The polymer ring expands or contracts in response to an electrical signal transmitted through a conducting polymer that contacts a swelling hydrogel (Shimoda and Smela 1998; Pernant and Reynolds 2000; Smela 2003; Tabard-Cossa et al. 2005; Staples et al. 2006).

So how do such artificial muscles of this smart pill work? The basic materials for these artificial muscles are redox polymers and hydrogels. Redox polymers and/or conducting polymers such as polyaniline, polypyrrole, polythiophene and their derivatives have been extensively studied in the last decade. These polymers can be oxidized and reduced at moderate potentials causing changes in their physical properties such as conductivity or chemical properties such as hydrophobicity. The movement of counter ions and water in and out of the conducting polymer leads to simultaneous variations in physical and electrochemical properties such as the oxidation depth, and changes in conductivity, 30% of their volume, colour, etc. These polymers are organic semiconductors characterized by alternating single and double bonds along the polymer backbone, a structure that results in a delocalized positive charge if an electron is removed from the polymer. The removal of the electrons can be achieved electrochemically by the application of a sufficient positive potential (Xu et al. 2006; Tsai et al. 2007). Therefore, the introduction of positive charge transfers the material to an electrically conducting substrate. To maintain the charge neutrality, negative charged anions are incorporated into the polymer backbone. The electrons can be reversibly returned by applying more negative potential to reduce the polymer. The primary mechanism for volume change is mass transport. If ions or solvents enter the polymer, it expands, and if these agents exit again, the polymer contracts. In polymers prepared with small mobile anions, the process on, e.g., the left side dominates and the polymer expands in the oxidized state. In polymers with large immobile anions that cannot be expelled upon reduction, charge compensation occurs primarily mediated by the incorporation of cations and the polymer expands in the reduced state. Hydrogels are considered to be the most efficient polymers in transforming molecular energy into mechanical energy. A hydrogel is a water-swollen three-dimensional network of hydrophilic homopolymers or copolymers with cross-links formed by covalent or ionic bonds. Hydrogel-based actuator systems constitute an active research area in the field of robotics for building robots with noiseless and life-like movements.

Significant aspects of recent research have focused on redox polymers and hydrogels. However, the practical applications of these polymers have been limited due to some inherent difficulties. The low mechanical strength of redox polymers limits the shape and size of products that can be created. Other problems with the fabrication processes and stability in ambient conditions have also restricted their use. Hydrogels are not electrical conductors; therefore, electro-osmotic and diffusion processes act as intermediate steps for converting electrical energy to mechanical energy in gel-based actuators. These facts result in low actuation

rates and undesirably high working potentials. The combination of hydrogels and conducting polymers can circumvent some of the problems mentioned above and create a new class of fast and efficient actuator materials. These actuators can be used in reversible polymeric valves in responsive drug delivery systems. A design for opening and closing holes or pores in a drug reservoir using the artificial muscle concept is illustrated here in Fig. 7 (Low et al. 2000).

A transmission electron microscopy gold grid with each hole 38.5 μm × 38.5 μm was used as a pattern and a working electrode for the artificial muscle material that acts in a sphincter-like mode. The conducting redox polymer in the blend is the "electronic backbone" of the artificial muscle and is sensitive to pH, applied electrical potential, and chemical potential in its micro-environment. Redox polymers have been shown to attain length variations of about 30% with high reversibility in time ranges between 3 s and 50 s. The "ionic body" of the muscle is a hydrogel that exhibits dramatic effects of swelling and shrinking upon changes in pH, solvent, temperature or electric field. On the other hand, hydrogels are capable of large swelling up to 250%; however it takes as long as 10–100 s to complete the overall volume changes. Unlike the redox polymers, hydrogels are not redox active. Hence, a combination of both the hydrogel and the redox polymer could result in a voltage controlled material with a more impressive degree of swelling procedures compared to a redox polymer alone. Furthermore, an enhancement in the rate of swelling and shrinking in this combination could be expected because of the distribution of protons from the electronic backbone of the redox polymer throughout the hydrogel (Low et al. 2000). The in-situ monitoring of the artificial muscle blend of polyaniline as hydrogel and poly(2-hydroxyethyl methacrylate) synthesized by a parallel coating showed a significant change in the size of the opening when the blend was subjected to a cyclic potential scan. The change in the maximum cord length between the largest opening state and the smallest swollen state was observed to be approximately 150%. The novel material obtained from

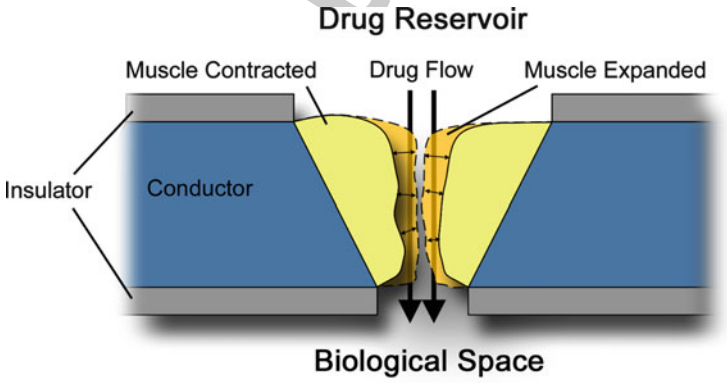


Fig. 7 Design of an artifical muscle valve as a part of a drug release device, with a open state (*muscle contracted*) and a closed state (*muscle expanded*)

blending a redox polymer and a hydrogel possesses qualities of a high swelling hydrogel and a voltage controlled redox polymer. Due to these conditions, this material demonstrates a more intensive swelling and shrinking process than the redox polymer itself, and thus a faster responsive time than the hydrogel. It was found that to open a same-size reservoir, 75% less energy was consumed in this approach compared with an earlier approach involving the corrosion of gold membrane. In addition, multiple drug pulsatile release and continuous linear release patterns were successfully implemented by controlling the activations of an array of valves.

In conclusion, drug delivery micro-chip technology shows a potential to benefit patients by delivering accurate, appropriate time-dependent dosing, as well as site-targeted dosing with reduced side effects. However, microchip implantation can be viewed as "bodily invasion". The additional possible patient monitoring and tracking functions of such devices can be useful in the monitoring of patients who wander or escape due to cognitive dysfunction but further surveillance functions of drug delivery devices can impede the acceptance of this implantable microchips. Also the forced drug treatment could be useful in some situations (e.g. patients who are noncompliant or mentally ill), but anyway ethical and legal reflection is essential. These legal and ethical issues should be considered when evaluating bundled drug chips for marketing approval. Any new regulations that result should reflect guiding principles of ethics, and the benefits and burdens of the technology (Juengst and Siegel 1988; Fig. 8).

9 Novel Technological Challenges in Drug Delivery – Nano-Micro-Implants

Controlled drug release in the human body within a defined time range can be realized using various systems. It is known that drugs can be embedded in polymers

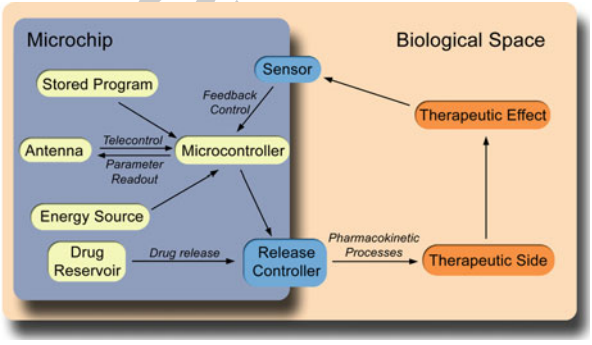


Fig. 8 Basic composition of a drug delivery microchip with implemented biosensing modules and feedback control units

and released within a specific time frame. Furthermore, polymers which are biodegradable within a predicted time can be used for controlled drug release. This delivery system is only able to release one dose into a therapeutically interesting area. These implantable devices enable the restriction of the drug release to specific parts of the organism, so that the necessary amount of systemic drug load as well as the degradation of large amounts of xenobiotics by the body can be reduced (Schierholz and Beuth 2001). Many physiological and clinical situations do not allow the release of a temporarily constant rate of drugs, e.g. the delivery of insulin, hormone release, cancer therapies, etc., and therapeutic needs for a given drug can vary considerably with the severity of symptoms over time, e.g. anti-arrhythmic agents.

For optimized controlled drug release, polymers have been developed which can be directed by external environmental situations, e.g. ultrasound, electric and magnetic fields, light, enzymes, changed pH or temperature (Kost and Langer 1992). Unfortunately, a significant disadvantage of these polymer-based systems is their low biocompatibility and their toxicity, their slow release after stimuli, their short activity time, and any representative reproducibility of drug delivery. The amount of containers or the surface for immobilization of compounds is too small. Several patents (e.g. US 4/003/779, US 4/146/029, US 3/692/027, and US 4/360/019) for micro-systems describe controlled drug delivery by modules with mechanical mechanisms by specially miniaturized electrically controlled components for drug release from a reservoir. However these mechanical systems show many problems in the release of small amounts of highly active agents. A further mechanism for controlled release of drugs from a reservoir or container is described in the US patent 5/556/528. This micro-device consists of a nano-porous membrane (e.g. P2VB) which is combined on its surface with a thin layer of molecules showing a dipole moment higher than 5 Debye. This thin control layer changes its permeability for active substances depending on the strength of the electric field used.

This process of electro poration controls the permeability and impermeability of this delivery system (Lurquin 1997). Nevertheless, an implantable smart module could not be described. A further micro-system based on a planar microchip (US 5/797/898) comprises cavities used as drug containments. These micro-containers are closed and protected by conductive slashes which can be electrochemically degraded after setting an electric potential. The micro-system design or the chip has a thickness of 300 μm and consists of several cavities arranged in an array (opening area 30 × 30 μm; electronic elements are implemented for wiring the electrodes). The disadvantage of this micro-device is limited or lack of repeat drug delivery from these containers. Thus a rather large number of reservoirs would be necessary in a smart device for release of a representative amount of drug, so the translation to a micro-implant might not be possible. Another problem is the release of a large amount of active substance at one specific time point, resulting in undesired side effects e.g. irritation, physiological toxicity etc.

10 Novel Aspects of Electronically Controlled Drug Delivery Systems

Progress in designing and developing drug delivery systems with a high degree of novelty, efficiency and release control relates to micro-system concepts for controlling the release of a bioactive substance from a reservoir comprising a thin carrier substrate which is made of a material which is impermeable with respect to the active compound and has more pores for drug penetration. Electrodes can be arranged and altered in the region of these pores and can be controlled by means of an electronic system which is integrated into the carrier substrate. The pores are preformed in the shape of micro-gaps or micro-channels comprising electrodes on both sides and covered by a layer of an electro-porous material on one side of the carrier substrate. Finally the micro-system can be used for a controlled release of an active substance in applications where space restrictions in vivo are critical (e.g. spinal cord, CNS etc.). The application could be a stand-alone system for blood vessels or a compartment of a prosthesis. In the case of a flexible design, the micro-system concept described can be adapted to the form of prostheses to match the space in the organism (minimal space requirement). The micro-system is also useful for implementation in smart prostheses, i.e. stents. The electrodes for a controlled release of substance can be directed by an electronic unit which can be triggered by physiological events. In this case a drug can be released depending on physiological demands. Repeated delivery of bioactive substances from one reservoir might be possible if relaxation and remodelling of the electro-porous substrate is possible (DE 01/04874; WO 02/05 6862A2) in a micro-system for controlled dispensation of an active substance from a reservoir (Fig. 9).

Another research aspect focuses on a novel drug delivery device based on a Au/PPy(DBS) (gold/polypyrrole (dodecylbenzoatsulfonate)) bilayer. PPy is a conducting polymer and acts as an electro-active actuator. Opening of a single valve was externally actuated by the application of a small electrical potential. Different release patterns, such as multiple drug pulsatile release and continuous linear release, were constructed by opening several valves in preprogrammed sequences. A single chip consisted of a high number of these drug reservoirs and valves would feature flexibility for achieving more complicated drug release patterns to meet different clinical demands. A conceptual model of the drug delivery device and a schematic of its cross-section are shown in (Fig. 10). The drug reservoir in the silicon substrate is covered by a polypyrrole (DBS)/Au bilayer flap on the top and by waterproof sealing plastic from the bottom. It is designed to release drugs by bending of the redox polymer/Au away from the substrate when activated by the application of a small voltage. With standard micro-fabrication processes a silicon wafer was coverd with a silicon dioxide layer as an insulating film for both the polymerization and drug release steps. Then, a chromium adhesion layer is deposited on top of the wafer and patterned by UV photolithography. After that, a diluted polyimide precursor is spin-coated onto the patterned Cr to form a polyimide layer 150 nm in thickness. The Cr layer is used as an adhesion promoting layer, which is

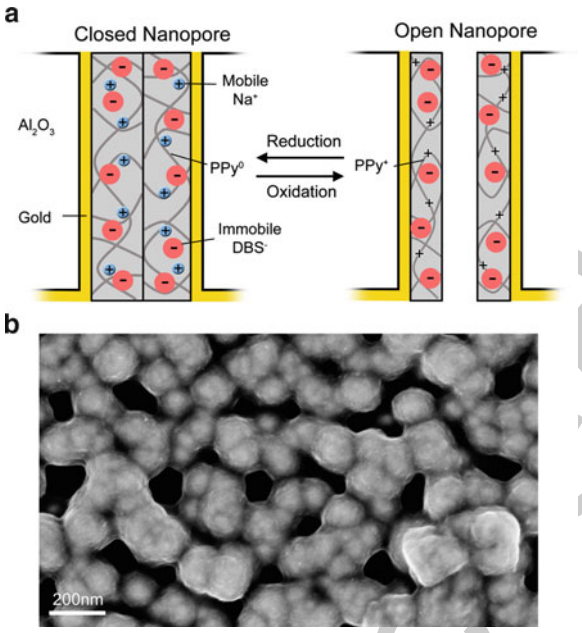


Fig. 9 (**a**) Schematic drawing of an electronic polymer-based drug delivery micro-implant for active pharmaceutical ingredient release via controlled opening and closing nano-pores. (**b**) PPy(DBS)-polymer layer conducted by a gold layer for electrically induced generation of nano-pores (EM)

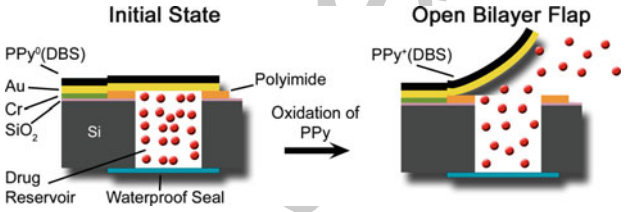


Fig. 10 Illustration of the cross-section of a single reservoir of a Au/PPy(DBS) bilayer drug release system. In its initial state, the PPy(DBS) is relaxed (electrochemically neutral) and the bilayer flap is closed (*left*). After oxidation, the PPy(DBS) shortens and therefore the bilayer bends outwards and opens the drug reservoir (*right*)

used to "glue" the gold layer down, since the gold adheres weakly to polyimide. The bending generated by the voltage-actuated PPy(DBS) film is sufficient to cause the release of the bilayer from the surface without the use of any other chemical or mechanical means. After the polyimide process, a structural gold layer (250 nm) is deposited and patterned by UV photolithography. The gold film in the bilayer functions as both a working electrode for the electrochemical deposition of PPy (DBS) and a structural layer/conducting layer during the actuation process (Fig. 10).

Afterwards the drug reservoir is created from the back side of the silicon wafer using deep reactive ion etching, until the gold layer on the other side of the wafer is

reached. A PPy(DBS) film with a thickness of around 5 μm is electropolymerized from monomer pyrrole on top of the patterned gold layer. Finally, the active agents to be released are loaded and the reservoir is sealed with a thin adhesive plastic tape reinforced by waterproof epoxy glue from the back side. During the preliminary drug release test, it was observed that the valve was successfully actuated to open after one triangular voltage cycle was applied and the specimen a colored dye was released from the reservoir.

11 Biosensing of Drug Delivery in In Vitro Tissue Models

The focus of this aspect of biosensing in real time for verifying drug release and drug efficacy and/or mechanisms is based on a recent development of a novel 3D microcavity array for the ultrafast positioning and bioelectronical characterization of biopsy material and/or 3D tissue samples (Kloß et al. 2008a, b). The advantage of this novel tissue-based chip is a microcavity structure for a positioning of viable tissue samples in vitro for real time measurements of physiological and morphological changes. Furthermore, this microarray can be used for high content and high throughput screening of applied and released drugs simulating the situation in the organism and minimizing animal testing. The measurement method used is impedance spectroscopy – cellular dielectric spectroscopy (CDS) or electric impedance spectroscopy (EIS). Impedance spectroscopy can be used to measure frequency-dependent changes of passive electrical properties of single cells and/or complex tissues by applying a defined alternating current. For this purpose, an alternating voltage current can be applied to a biological sample whereby the current flows from an active working electrode through and beneath the cell or tissue/extracellular space to a counter electrode. For these non-invasive conditions, the cell or tissue sample or model itself acts as a resistor and capacitor affecting the recorded impedance. Depending on the frequency and the dielectric properties of sub-cellular structures, it might be possible to analyze different cellular processes occurring according to native conditions or after drug release. Thus, drugs and their mechanisms, toxic effects etc. can be detected in real time and online by such biosensing drug delivery systems depending on the therapeutic and systemic window according to efficacy and safety. Up to now, a comparable screening system of spherical in vivo equivalents is not known. It was the aim to provide a fast, reliable, highly sensitive, and cost-effective chip-based screening technology for three-dimensional organotypic tissue equivalents to be used in basic and applied science as well as pharmaceutical industry. This approach is a novel innovative sensing system which can be integrated in principle into drug release systems. The novel micro-cavity-multi-electrode array can be miniaturized and translated as well as transferred into a concept for multi-well-microimplant systems for controlled drug release. Up to now, the microarray can be coupled with various 3D tissues or biopsy materials for a non-invasive, labeling-free and continuous screening and/or diagnosis of cellular alterations under real-time conditions, and works as

a lab-on-a-chip model. In this way, an automated high-content and/or high throughput screening of biologically active compounds on 3D tissues derived from biopsies of tumours, ischemic cardiomyocytes etc. can be achieved. Thus, the technique presented can be of great benefit for controlled implantable drug delivery systems in respect to individualized therapies and safe drug delivery processes. Future challenges are the miniaturization and implementation of the cavity-based chip system into an automated release system to provide an efficient biosensing and release mode.

Fast, labeling-free, and non-destructive processes are important requirements for improved screening and monitoring systems in drug release in representative tissue models. For real-time monitoring of inter- and intracellular parameters of cell cultures under in vivo-like conditions, a 3D multifunctional electrode-based Micro-Cavity Array (MCA) was designed, fabricated and validated. Such a sensory unit consists of 15 individual square micro-cavities with implemented gold microelectrodes. Planar multi-electrode biosensors are widely used for the characterization of monolayer cell cultures. Their electrical properties, monitored by impedance spectroscopy, also give feedback about cell motility, proliferation, and adherence. For in vitro testing of physiological parameters, multicellular spheroids are used. A scaffold-free, rotation-mediated generation of sphere-like histotypic in vitro tissues enables the proof of cellular changes under 3D in vivo-like conditions since adequate cell–cell and cell–extracellular matrix contacts are created. These contacts, which are essential for cellular communication, play an important role in fundamental cellular processes such as growth, differentiation, migration and programmed cell death (apoptosis). Spheroids are formed via a self-assembly process and mimic avascular tissue models. Therefore, spheres represent excellent in vivo organotypic equivalents. Their well-defined reproducible size and structure enables the construction of optimally adapted arrays for the coupling of spheroids to a sensor. Alterations in morphology, penetration of drugs and the effects of cytotoxic agents can then be monitored easily. Testing those properties is a main aspect of any drug screening assay. When comparing different cell types from three different species and tissues, we found similar amplitudes ($|\Delta Z|$) of 23–44%. All biological samples varied in peak position (characteristic frequency). Spheres from, e.g., human melanoma (Bro) have the most compact and spheric shape, but a rather rough surface morphology. CHO and COS-7 spheroids stand out due to their rough surface. Zeolite beads with a perfectly smooth surface but high porosity showed a lower impedance value, below the range of the biological tissues, whereas recording with glass beads resulted in significantly higher magnitudes with more than 80% of normalized impedance.

12 Biosensing of Drug Delivery In Vivo – Microelectrodes in Endoluminal Sensors

Interventional techniques for monitoring and drug delivery are necessary and allow the characterization as well as the treatment of intravascular pathological processes. Thus, electric impedance spectroscopy (EIS) can provide cellular information on

biological tissue. Acute coronary syndromes, for example, are frequently triggered by rupture of atherosclerotic plaques presenting according to an angiographically determined diameter stenosis of less than 50% (Fuster et al. 1992; Falk et al. 1995; Rössig et al. 2001). Coronary atherosclerosis not resulting in symptomatic coronary disease may still constitute a source of vulnerable lesions, which cannot be monitored by X-ray angiography (Little et al. 1988). Recent studies reported that progress in intravascular ultrasound enabled the characterisation of different plaque types by mechano-elastography and radiofrequency in vitro and in vivo (De Korte et al. 2000, 2002; Nair et al. 2002; Stahr et al. 2002). Furthermore, thermometry could demonstrate local temperature heterogeneity in animal experiments and patients with stable angina pectoris (Stefanadis 1999, 2001; Verheye et al. 2002). For more detailed or specific atherosclerotic plaque detection and characterization, however, alternative or adjunct techniques are deemed to be necessary, which might allow determination of plaque growth, tissue differentiation and cellular characterization. This early monitoring might be coupled and/or integrated with endoluminal delivery systems, e.g. drug-coated stents or micro-implants for drug release including biosensing systems.

Electric impedance is a complex quantity combining resistance and reactance, depending on the frequency of the alternating current employed. Biological tissues represent a complex electrical impedance, because they consist of components that have both resistive and charge storage properties. By recording the electric impedance of a tissue over a frequency range – electric impedance spectroscopy (EIS) – its frequency-dependent electric and dielectric behavior can be detected (Foster and Schwan 1989). The electrical properties of biological tissues are related to their physiological and morphological properties, and therefore impedance spectroscopy is suitable for detection of tissue composition. Indeed, invasive EIS could successfully detect carcinoma cells for the diagnosis of cervical neoplasia (Brown et al. 2000; Wilkinson et al. 2002). Progress in nano-bioengineering provides the option to design and fabricate micro-electrodes which allow monitoring impedance spectroscopy inside coronary arteries. In a recent study, the feasibility of intravascular EIS was tested using a new impedance catheter system with integrated microelectrodes applied and/or transplanted in an experimental animal model. Eighteen stents were implanted into the iliac arteries of female New Zealand White rabbits to induce intimal proliferation. After 14, 28 and 56 days post-transplantation, the electric impedance was measured inside and outside the stented arterial segments using a balloon catheter with four integrated micro-electrodes oriented axially. The impedance was recorded at a frequency range from 10 Hz to 1 MHz. The impedance inside and outside the stent was analyzed and correlated with histomorphometric data of explanted stented arterial segments. Fourteen 28 and 56 days post-stent implantation, the difference in electrical impedance between native and stented iliac artery segments increased significantly, reflecting an potential increase of proliferation of endothelial cells. Obviously the increase in the electrical impedance corresponded to an increased neo-intimal proliferation in the stented arterial segment. Intravascular EIS can be performed by a balloon catheter with integrated microelectrodes and allows the detection of neo-intimal proliferation after stent implantation (Süselbeck et al. 2005).

References

Al Malyan M, Becchi C, Boncinelli S, Ashammakhi N (2007) Novel drug delivery systems in pain therapy. Minerva Anestesiol 73:173–179

Angelos S, Yang Y-W, Patel K, Stoddart JF, Zink JI (2008) pH-Responsive supramolecular nanovalves based on cucurbit[6]uril pseudorotaxanes. Angew Chem Int Ed 47:2222–2226

Banghmann RH (1996) Conducting polymer artificial muscles. Synthetic Metals 78:339–353

Bay L, Jacobsen T, Shaarup S (2001) Mechanism of actuation in conducting polymers: osmotic expansion. J Phys Chem B105:8492–8497

Bramstedt KA (2005) When microchip implants do more than drug delivery: blending, blurring, and bundling of protected health information and patient monitoring. Technology Health Care 13:193–198

Brown BH, Tidy JA, Boston K, Blackett AD, Smallwood RH, Sharp F (2000) Relation between tissue structure and imposed electrical current flow in cervical neoplasia. Lancet 355:892–895

Bry L, Jacobsen T, Shaarup S, West K (2001) Mechanism of actuation in conducting polymers: osmotic expansion. J Phys Chem B105:8492–8497

De Korte CL, Pasterkamp G, van der Steen AF, Woutman HA, Bom N (2000) Characterization of plaque components with intravascular ultrasound elastography in human femoral and coronary arteries. Circulation 102:617–623

De Korte CL, Sierevogel MJ, Mastik F, Strijder C, Schaar JA, Velema E, Pasterkamp G, Serruys PW, van der Steen AF (2002) Identification of atherosclerotic plaque components with intravascular ultrasound elastography in vivo: a Yucatan pig study. Circulation 105:1627–1630

De la Heras Alarcón C, Pennadam S, Alexander C (2004) Stimuli responsive polymers for biomedical applications. Chem Soc Rev 34:276–285

Dorski CM, Doyle FJ, Peppas NA (1997) Preparation and characterization of glucose-sensitive P (MAA-g-EG) hydrogels. Polym Mater Sci Eng Proceed 76:281–282

Falk E, Shah PK, Fuster V (1995) Coronary plaque disruption. Circulation 92:657–671

Finkenstadt VL (2005) Natural polysaccharides as electroactive polymers. Appl Microbiol Biotechnol 67:735–745

Foster KR, Schwan HP (1989) Dielectric properties of tissue and biological material: a critical review. Crit Rev Biomed Eng 17:25–104

Fuster V, Badimon L, Badiman JJ, Chesobro JH (1992) The pathogenesis of coronary artery disease and the acute coronary syndromes. N Engl J Med 326:242–250

Gardner P (2006) Microfabricated nanochannel implantable drug delivery devices: trends, limitations and possibilities. Exp Opin Drug Deliv 3:479–487

Grayson ACR, Choi LS, Tyler BM, Wang PP, Brem H, Cima MJ, Langer R (2003) Multi-pulse drug delivery from a resorbable polymeric microchip device. Nature Mater 2:767–772

Hilt JZ, Peppas NA (2005) Microfabricated drug delivery devices. Int J Pharm 306:15–23

Jager EWH, Smela E, Inganäs O (2000) Microfabricating conjugated polymer actuators. Science 290:1540–1545

Juengst E, Siegel R (1988) Substrating injury from insult: ethical issues in the use of pharmaceutical implants. Hastings Center Report 18:41–46

Kloß D, Kurz R, Jahnke H-G, Fischer M, Rothermel A, Anderegg U, Simon JC, Robitzki AA (2008a) Microcavity array (MCA)-based biosensor chip for functional drug screening of 3D tissue models. Biosens Bioelectron 23:1473–1480

Kloß D, Fischer M, Rothermel A, Simon JC, Robitzki AA (2008b) Drug testing on 3D in vitro tissues trapped on a microcavity chip. Lab Chip 8:879–884

Kost J, Langer R (1992) Responsive polymer systems for controlled delivery of therapeutics. Trends Biotech 10:127–131

Little WC, Constantinescu M, Applegate RJ et al (1988) Can coronary angiography predict the site of subsequent myocardial infarction in patients with mild-to-moderate coronary artery disease? Circulation 78:1157–1166

Low L-M, Seetharaman S, He K-Q, Madou MJ (2000) Microactuators towards microvalves for responsive controlled drug delivery. Sensors Actuators B67:149–160

Lurquin PF (1997) Gene transfer by electroporation. Mol Biotechnol 7:5–35

Nair A, Kuban BD, Tuzcu EM Schoenhagen P, Nissen SE, Vince DG (2002) Coronary plaque classification with intravascular ultrasound radiofrequency data analysis. Circulation 106: 2200–2206

Pernant J-M, Reynolds JR (2000) Use of conducting electroactive polymers for drug delivery and sensing of bioactive molecules: A redox chemistry approach. J Phys Chem B104:4080–4090

Prescott JH, Lipha S, Baldwin S, Sheppard NF Jr, Maloney JM, Coppeta J, Yomotov B, Staples MA, Santini JT Jr (2006) Chronic programmed polypeptide delivery from an implanted multireservoir microchip device. Nature Biotech 24:437–438

Randall CL, Leong TG, Bassik N, Gracias DH (2007) 3D lithographically fabricated nanoliter containers for drug delivery. Adv Drug Deliv Rev 59:1547–1561

Rössig l, Dimmeler S, Zeiher AM (2001) Apoptosis in the vascular wall and atherosclerosis. Basic Res Cardiol 96:11–22

Ryu W, Huang Z, Prinz FB, Goodman SB, Fasching R (2007) Biodegradable micro-osmotic pump for long-term and controlled release of basic fibroblast growth factor. J Control Release 124:98–105

Santini JT Jr, Cima MJ, Langer R (1999) A controlled-release microchip. Nature 397:335–337

Santini JT Jr, Richards AC, Scheidt R, Cima MJ, Langer R (2000) Microchip as controlled drug-delivery devices. Angew Chem Int Ed 39:2396–2407

Saylor DM, Kim C-S, Patwardhan DV, Warren JA (2007) Diffuse-interface theory for structure formation and release behavior in controlled drug release systems. Acta Biomat 3: 851–864

Schierholz JM, Beuth J (2001) Sophisticated medical devices as local drug-delivery systems. Med Device Technol 11:12–17

Schwan HP (1993) Mechanisms responsible for electric properties of tissues and cell suspensions. Med Prog Technol 19:163–165

Shimoda S, Smela E (1998) The effect of pH on polymerization and volume change in PPy(DBS). Electrochim Acta 44:219–238

Smela E (2003) Conjugated polymer actuators for biomedical applications. Adv Mater 15: 481–494

Stahr PM, Hofflinghaus T, Voigtlander T et al (2002) Discrimination of early/intermediate and advanced/complicated coronary plaque types by radiofrequency intravascular ultrasound analysis. Am J Cardiol 90:19–23

Staples M, Daniel K, Cima MJ, Langer R (2006) Application of micro- and nano-electromechanical devices to drug delivery. Pharm Res 23:847–863

Stefanadis C, Diamantopoulos L, Vlachopoulos C et al (1999) Thermal heterogeneity within atherosclerotic coronary arteries detected in vivo: A new method of detection by application of a special thermography catheter. Circulation 99:1965–1971

Stefanadis C, Toutouzas K, Tsiamis E et al (2001) Increased local temperature in human coronary atherosclerotic plaques: an independent predictor of clinical outcome in patients undergoing a percutaneous coronary intervention. J Am Coll Cardiol 37:1277–1283

Süselbeck T, Thielecke H, Weinschenk I, Reininger-Mack A, Stieglitz T, Metz J, Borggrefe M, Robitzki A, Haase KK (2005) In vivo intravascular electric impedance spectroscopy using a new catheter with integrated microelectrodes. Basic Res Cardiol 10:28–34

Tabard-Cossa V, Godin M, Grütter P (2005) Redox-induced surface stress of poly-pyrrole-based actuators. J Phys Chem B109:17531–17537

Teymoori MM, Abbaspour-Sani E (2005) Design and simulation of a novel electrostatic peristaltic micromachined pump for drug delivery applications. Sensors Actuators A117:222–229

Tsai H-KA, Madou M (2007) Microfabrication of bilayer polymer actuator valves for controlled drug delivery. J Assoc Lab Automat 12:291–295

Tsai H-KA, Ma K-S, Wang C, Xu H, Wang C, Zoval J, Kulinsky L, Madou M (2007) Development of integrated protection for a miniaturized drug delivery system. Smart Mater Struct 16:5295–5299

Valsesia A, Lisboa P, Colpo P, Rossi F (2006) Polypyrrole nanopillars – a new generation for biosensor development. Anal Chem 78:75887591

Verheye S, De Meyer GR, Van Langenhove G, Knaapen MW, Kockx MM (2002) In vivo temperature heterogeneity of atherosclerotic plaques is determined by plaque composition. Circulation 105:1596–1601

Wang HC, Zoval J, Madou MJ (2006) Polymer actuator valves towards controlled drug delivery application. Biosens Bioelec 21:2094

Wilkinson BA, Smallwood RH, Keshtar A, Lee JA, Hamdy FC (2002) Electrical impedance spectroscopy and the diagnosis of bladder pathology: a pilot study. J Urol 168:1563–1567

Xu H, Wang C, Wang C, Zoval J, Madou M (2006) Polymer actuator valves toward controlled drug delivery application. Biosens Bioelec 21:2094–2099

Lipid Nanoparticles: Effect on Bioavailability and Pharmacokinetic Changes

Eliana B. Souto and Rainer H. Müller

Contents

Abstract The main aim of pharmaceutical technology research is the design of successful formulations for effective therapy, taking into account several issues including therapeutic requirements and patient compliance. In this regard, several achievements have been reported with colloidal carriers, in particular with lipid nanoparticles, due to their unique physicochemical properties. For several years these carriers have been showing potential success for several administration routes, namely oral, dermal, parenteral, and, more recently, for pulmonary and brain targeting. The present chapter provides a review of the use of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) to modify the release profile and the pharmacokinetic parameters of active pharmaceutical ingredients (APIs) incorporated in these lipid matrices, aiming to modify the API

E.B. Souto (✉)
Faculty of Health Sciences, Fernando Pessoa University, Rua Carlos da Maia, 296, P-4200-150, Porto, Portugal
e-mail: eliana@ufp.edu.pt

M. Schäfer-Korting (ed.), *Drug Delivery*,
Handbook of Experimental Pharmacology 197,
DOI 10.1007/978-3-642-00477-3_4,

bioavailability, either upwards or downwards depending on the therapeutic requirement. Definitions of the morphological characteristics, surface properties, and polymorphic structures will also be given, emphasizing their influence on the incorporation parameters of the API, such as yield of production, loading capacity, and encapsulation efficiency.

Keywords Lipid nanoparticles · Lipid polymorphism · Pharmacokinetics · Bioavailability · API release · Orals · Dermalics · Parenterals · Pulmonary delivery · Brain delivery

Abbreviations

AFM	Atomic force microscopy
API	Active pharmaceutical ingredient
AUC	Area under the curve
BBB	Blood–brain barrier
BSC	Biopharmaceutical classification system
CNS	Central nervous system
DSC	Differential scanning calorimetry
EE	Encapsulation efficiency
ESR	Electron spin resonance
FFF	Field flow fractionation
GIT	Gastrointestinal tract
HLB	Hydrophilic–lipophilic balance
HPH	High pressure homogenization
IES	Inter-endothelial cell slits
LC	Loading capacity
LD	Laser diffractometry
LDL	Low density lipoproteins
LHRH	Luteinizing hormone releasing hormone
MPS	Mononuclear phagocytic system
NLC	Nanostructured lipid carriers
NMR	Nuclear magnetic resonance
PCS	Photon correlation spectroscopy
PEG	Polyethylene glycol
RES	Reticulo-endothelial system
SAXS	Small angle X-ray scattering
SEM	Scanning electron microscopy
SFEE	Supercritical fluid extraction of emulsion
SLN	Solid lipid nanoparticles
TEM	Transmission electron microscopy

TPGS D-α-Tocopheryl polyethylene glycol 1,000 succinate
WAXS Wide angle X-ray scattering
YP Yield of production

1 Introduction

The success of drug therapy with is highly dependent on the design of active pharmaceutical ingredients (APIs) delivery. A properly designed delivery system aims to achieve an optimized concentration of the API at the site of action in order to produce a therapeutic response with minimum adverse effects. Nevertheless, individual variations in the pharmacokinetic and pharmacodynamic parameters makes the dosage regimens somewhat difficult to establish. Therefore, novel approaches are being developed e.g. within the field of lipid-based colloidal carriers in order to achieve proper clinical response.

Most conventional formulations are designed to release the API immediately to obtain its rapid and complete systemic absorption. Recently, however, various modified API delivery systems have been developed to release the API at a controlled/well-defined rate. Within those novel delivery systems, the lipid-based colloidal carriers, such as solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC), receive particular attention. A variety of modified-release SLN and NLC designed for different administration routes have been formulated for several APIs, based on their physicochemical and pharmacokinetic properties as well as the effect induced.

Lipid nanoparticles (SLN and NLC) combine advantages of other colloidal carriers, e.g., polymeric nanoparticles, liposomes, and conventional oil-in-water (o/w) emulsions. It has been reported (Kaur et al. 2008; Müller et al. 2000) that: (1) small particles ranging between 120 and 200 nm only rarely undergo blood clearance by the cells of the reticulo-endothelial system (RES), therefore liver and spleen filtration is avoided (Chen et al. 2004); (2) modified release profiles can be obtained when the API is incorporated within the lipid matrix (Hu et al. 2006; Manjunath et al. 2005; Pople and Singh 2006; Saupe et al. 2006; Schwarz and Mehnert 1999; Schwarz et al. 1994); and (3) API targeting can be achieved by means of ligands placed onto the surface of lipid nanoparticles (Lockman et al. 2003). Furthermore, high loadings (for both hydrophilic and lipophilic APIs) (Chen et al. 2001; Fundaro et al. 2000; Reddy and Venkateswarlu 2004), long-term shelf stability (Freitas and Müller 1998, 1999a, b), and the possibility of sterilization and large-scale production (in particular avoiding organic solvents) (Gohla and Dingler 2001; Kuntsche and Bunjes 2007; Manjunath et al. 2005), have also been pointed out. To improve handling and stability, lipid nanoparticle dispersions can be spray-dried, maintaining their colloidal size after reconstitution, and exhibiting good redispersibility (Varia et al. 2008). Other advantages include the lipid composition of SLN and NLC, making them biocompatible, biodegradable, and safe.

2 Definition of Lipid Nanoparticles (SLN vs. NLC)

SLN and NLC are composed of pure lipids or a mixture of lipid compounds (triacylglycerols, fatty acids, steroids, waxes, and oils), and a single surfactant (or in association with a co-surfactant) surrounding the particles. Lipid composition, as well as the production method, will define several nanoparticle characteristics, including the type of surfactant to be selected for SLN/NLC stabilization (anionic, cationic, or non-ionic), the particle size and size distribution, the yield of production (YP), the loading capacity (LC), and the encapsulation efficiency (EE). Obviously, the amount of API that lipid nanoparticles can carry and deliver will also be dependent on its lipophilicity, i.e., the ability of the API to be dissolved in the lipid matrix.

The YP can be measured in terms of nanoparticles produced per dispersion, or as a function of the EE and LC, which are determined as follows:

$$\mathrm{YP} = \frac{W_\mathrm{L}}{V_\mathrm{D}} \times 100 \tag{1}$$

$$\mathrm{EE} = \frac{W_\mathrm{a} - W_\mathrm{s}}{W_\mathrm{a}} \times 100 \tag{2}$$

$$\mathrm{LC} = \frac{W_\mathrm{a} - W_\mathrm{s}}{W_\mathrm{a} - W_\mathrm{s} + W_\mathrm{L}} \times 100 \tag{3}$$

where W_L is the weight of lipid added in the formulation, V_D is the volume of the aqueous phase, W_a is the weight of API added in the formulation, and W_s is the weight of API analyzed in the supernatant (after separation of lipid and aqueous phases by centrifugation). EE is thus defined as the ratio between the mass of entrapped API and the total mass of API, whereas LC is the ratio between the mass of entrapped API and the total mass of lipid. Factors determining LC and EE are: (1) the solubility and miscibility of the API in the melted lipid phase, (2) the physico-chemical structure of the solid lipid matrix, and (3) the polymorphic state of the lipid material (Kaur et al. 2008).

High encapsulation parameters are obviously desirable, since they can reduce the number of particles required to achieve therapeutic levels. Depending on their lipophilicity and hydrophilicity, APIs will be located in the lipid nanoparticles in a particular way. To achieve a high EE and LC for a particular API, its sufficiently high solubility in the melted lipid is the main requisite (Wissing et al. 2004). Therefore, hydrophilic molecules are hardly incorporated due to their low affinity with the lipid matrix. Moreover, API solubility should in general be higher in the melted lipid state that in the solid state, since the solubility usually decreases when the melt cools down, and it might even be lower in the solid lipid. However, biotechnological APIs have successfully been loaded into SLN (Almeida et al. 1997; Müller and Keck 2004a). To enhance solubility in the melted lipid,

solubilizers can be added. Examples of these are non-ionic surfactants such as polysorbates and polyoxyls, covering a hydrophilic–lipophilic balance (HLB) range between 2 and 18, which can be used in combination with lipids to promote self-emulsification (Gibson 2007). Furthermore, when using mono- and di-acylglycerols as lipid matrix composition, API solubility might increase in comparison to very pure lipids, such as monoacid triacylglycerols. Naturally occurring oils and fats comprise mixtures of mono-, di- and tri-acylglycerols, containing fatty acids of varying chain length and degree of unsaturation (Hauss 2007). The melting point of these lipids increases with the length of the fatty acid chain, and decreases with the degree of unsaturation. The chemical nature of the lipid is also important because lipids which form highly crystalline particles with a perfect lattice (e.g., monoacid triacylglycerols) lead to API expulsion during storage time. Mixtures of lipids containing fatty acids of different chain length form less perfect crystals with many imperfections offering space to accommodate guest molecules. Therefore, an important issue to be addressed in the lipid nanoparticle formulation is the selection of the lipid excipients. Although a systematic procedure to select an appropriate lipid composition has not been published yet, there are a number of criteria to be kept in mind. These are the API lipophilicity (Log *P*), in particular solubility in pharmaceutically acceptable lipids, which should be sufficient to allow the required therapeutic dose of API to be administered.

Physicochemically stable lipid nanoparticles will be obtained only when the right surfactant and adjusted concentration have been employed. For a particular lipid matrix, the surfactant composition is usually chosen according to its HLB, which is based on packing parameter theory (P) (Israelachvili et al. 1980).

SLN/NLC dispersions have been stabilized with surfactants having HLB values below 12. Nevertheless, one needs to keep in mind that lipid molecular characteristics, bulk, and surface properties strongly affect physicochemical stability and suitability of SLN/NLC as nanoscaled API delivery systems (Bummer 2004; Wissing et al. 2004).

Another critical situation is the risk of peroxidation of the materials used to produce SLN/NLC. It is well known that a number of lipids and surfactants are susceptible to oxidation, and may create highly-reactive peroxide species (Mead et al. 1986). Lipid peroxidation can be deleterious to the physicochemical stability of both the API and the SLN/NLC dispersion. Nevertheless, such phenomena can be limited and rationally controlled using anti-oxidants.

Polymorphism is also an important issue determining both EE and LC (2 and 3). To create a solid matrix, crystallization of the lipid occurs differently in SLN/NLC than in bulk material, i.e., the lipid matrix recrystallizes at least partially in the α-form (unstable polymorphic form) or in the β′-form (metastable polymorphic form), while the lipid as a bulk tends to recrystallize preferentially in the β′-form, which transforms quickly into the β-form (Westesen et al. 1993). During organization into more stable polymorphic forms, the number of imperfections in the lipid lattice decreases, i.e., formation of β′/β-modification promoting API leakage. Generally, the transformation is slower for long-chain than for short-chain triacylglycerols. An optimized SLN/NLC formulation can be generated in a controlled

way when a certain fraction of the β′-form is created and preserved during the storage time. Within this concept, SLN/NLC can be considered intelligent API delivery systems achieving a built-in triggering mechanism to initiate transformation from β′- to β-forms and consequently controlled API release (Jenning and Gohla 2001). The connection between the physical properties of SLN/NLC and their in vitro and in vivo performance should always be addressed (Kristl et al. 2003; Westesen et al. 1997), and therefore studies on the inner structure should always be carried out, since their lack can cause misinterpretation of the in vivo results (Westesen and Bunjes 1995).

Finally, the production procedures critically influence the bioavailability of loaded APIs since they affect the design and the structure of the system itself.

To produce SLN and NLC, the high pressure homogenization (HPH) procedure is typically applied, either the hot or the cold technique (Souto et al. 2007). For the hot HPH the lipid phase is previously heated 5–10°C above its melting point, followed by API dissolution or fine dispersion in the melted phase. Stirring this melted phase in a hot surfactant solution, a pre-emulsion will be produced. The pre-emulsion is homogenized under high pressure producing a hot nanoemulsion, which is further cooled, recrystallizing the lipid and forming SLN or NLC. The cold HPH technique requires a previous step of melting the solid lipid so that the API can be dissolved and/or admixed in this phase. By applying liquid nitrogen or dry ice, the lipid phase cools down rapidly, solidifying, and then by means of mortar milling it is ground to obtain microparticles. These microparticles are further dispersed in a cold aqueous surfactant solution producing a pre-suspension that is homogenized at or below room temperature using the HPH.

Other methods reported in the literature include those that require also the melting of lipid phase: i.e., the microemulsion (Bondi et al. 2007, 2003; Brioschi et al. 2008; Cavalli et al. 1997, 1998, 2001; Fontana et al. 2005; Mandawgade and Patravale 2008; Miglietta et al. 2000; Ugazio et al. 2002), the phase-inversion (Anton et al. 2008, 2007; Jayagopal et al. 2008; Malzert-Freon et al. 2006), and the extrusion techniques (El-Harati et al. 2006; Joshi and Patravale 2008), and those based on the previous dissolution of the lipid in an organic solvent (non-polar, semi-polar, or polar) (Cortesi et al. 2002; Hu et al. 2002, 2005, 2006, 2008; Trickler et al. 2008). In comparison to the HPH technique, these methods are reported to achieve low lipid nanoparticle YP (1) (Mehnert and Mäder 2001; Müller et al. 2000).

More recently, supercritical fluid technology has also been adapted to produce lipid nanoparticles (Chattopadhyay et al. 2007; de Sousa et al. 2006, 2007; Young et al. 2004). In particular, supercritical fluid extraction of emulsions (SFEE) has been reported to show high YP (Chattopadhyay et al. 2007). The method allowed the production of stable SLN of a narrow size distribution, with a mean diameter below 30 nm. Thus, the particle size obtained was significantly smaller than that reported by other techniques. The residual solvent content in the final suspension was shown to be below 20 ppm. When the o/w emulsion containing the lipid and the API is introduced into the supercritical CO_2 phase, parallel processes of solvent extraction into the supercritical CO_2 phase and inverse flux of CO_2 into the emulsion droplets occur, leading to expansion of the organic phase of the emulsion.

This leads to precipitation of lipid-API material dissolved in the organic phase producing the solid matrix. The solvent extraction efficiency using supercritical CO_2 is much higher than for the conventional methods such as evaporation, liquid extraction, and dilution, providing a more uniform particle size distribution, because of the fast removal of the organic solvent. Supercritical CO_2 also tends to extract other low-molecular weight impurities, purifying the lipids. In addition, supercritical CO_2 typically results in a depression of the lipid melting point and plasticization of the amorphous lipid structures. This plasticization can be beneficial in establishing a thermodynamically stable lipid form, such as β-polymorph of the triacylglycerol, facilitating as well a more uniform distribution of the API within the lipid phase. The size of SLN obtained in the SFEE process is directly related to the emulsion droplet size and is therefore dependent upon the method of formulation and the stability of the emulsions employed for precipitation.

With regard to the design and structure of the systems, basically the structure of both SLN and NLC is composed of a solid core covered by a layer of surfactant molecules. In the following sections the different types of each will be described.

2.1 Solid Lipid Nanoparticles (SLN)

The SLN Type I is defined as the homogeneous matrix model, because the API is molecularly dispersed in the lipid core or is present in form of amorphous clusters (Mehnert and Mäder 2001; Müller et al. 2000; Souto et al. 2007; Souto and Müller 2007). This model is obtained when using optimized ratios of API and lipid passing through the HPH at above the melting point of the lipid, or when using the cold HPH technique. As consequence of their structure, SLN Type I can show controlled release properties. The SLN Type II, or API-enriched shell model (Lukowski and Werner 1998), is obtained when the API concentration in the melted lipid is low. After applying the hot HPH technique, during the cooling of the homogenized nanoemulsion, the lipid phase precipitates first, leading to a steadily increasing concentration of API in the remaining lipid melt with increased fraction of solidified lipid. An API-free (or API-reduced) lipid core is formed; when the API reaches its saturation solubility in the remaining melt, an outer shell containing both API and lipid will solidify around this core which contains low amount of API. This model is not suitable for prolonged API release; nevertheless, it may be used to obtain a burst release of API, in addition to the occlusive properties of the lipid core. The SLN Type III, or API-enriched core model (Souto et al. 2004b; Westesen et al. 1997), is formed when the API concentration is relatively close to or at its saturation solubility in the lipid melt. On cooling the nanoemulsion, the solubility of the API will decrease; when the saturation solubility is exceeded the API precipitates, and is covered by a shell of lipid almost free of API. This SLN type is useful for achieving a prolonged release of API since it is immobilized within the lipid core.

2.2 Nanostructured Lipid Carriers (NLC)

NLC are also composed of a solid core covered by the surfactant used during the production procedure. For these carriers also, three incorporation models have been proposed, mainly differing in the type of lipid compounds used for their production.

The NLC Type I is termed the imperfect crystal model, and consists of a matrix with many voids and vacancies that are able to accommodate the API. These particles are obtained when mixing solid lipids with a sufficient amount of liquid lipids (oils). Due to the different chain length of the fatty acids and the mixture of mono-, di- and triacylglycerols, the matrix of NLC is not able to form a highly ordered structure (Müller et al. 2002), thus creating available spaces (structural imperfections). The NLC Type II, or the amorphous model, is obtained when mixing special lipids (e.g., hydroxyoctacosanylhydroxystearate, isopropylmyristate, dibutyl adipate) that do not recrystallize after homogenization and cooling of the nanoemulsion. These lipids create amorphous matrices, which avoid/delay the recrystallization phenomenon of lipids on cooling and during shelf life, thus minimizing API expulsion during storage time. The NLC Type III is defined as the multiple model because it is composed of very small oily nanocompartments created inside the solid lipid matrix of the nanoparticles by a phase separation process (Müller et al. 2002). It results when mixing solid lipids with oils (e.g., medium (Hu et al. 2006) and long-chain triacylglycerols (Souto et al. 2004a), oleic acid (Hu et al. 2005) in such a ratio that the solubility of the oil molecules in the solid lipid is exceeded. During the cooling of the nanoemulsion the lipid droplets reach the miscibility gap (40°C), and the oil precipitates forming tiny oil droplets. Subsequent solidification of the solid lipid surrounding these droplets leads to fixation of the oily nanocompartments. The advantage of this model is the increase of LC for APIs of higher solubility in liquid lipids than in solid lipids (Jenning et al. 2000). The structure of NLC Type III defined by the presence of nanocompartments or nanostructures within the matrix is still a controversial subject (Castelli et al. 2005; Jores et al. 2003, 2004, 2005; Müller et al. 2002). The precise structure may be intrinsically dependent on the composition of the formulation (i.e., lipid, surfactant, and API), as well as on the production procedure (Schäfer-Korting et al. 2007). These theoretical NLC models have been established based on analytical data, which can be used to physicochemically characterize NLC matrices.

Several techniques have been applied to outline the physical and chemical inner organization of SLN/NLC, such as differential scanning calorimetry (DSC), nuclear magnetic resonance (NMR), electron spin resonance (ESR), and small angle and wide angle X-ray scattering techniques (SAXS, WAXS) (Castelli et al. 2005; Jores et al. 2003; Mayer and Lukowski 2000; Zimmermann et al. 2005). DSC, WAXS and SAXS are useful for characterizing the polymorphic forms of lipid molecules of the nanoparticle matrix, which are dependent on the lipid and surfactant composition. NMR and ESR are useful for evaluating the dynamic phenomena and the presence of oily nanocompartments, which are characteristic of NLC Type III (Müller et al. 2000). Other analytical procedures for assessing morphology, surface

characteristics, and particle size include microscopic analysis, e.g., scanning (SEM) and transmission (TEM) electron microscopy, and atomic force microscopy (AFM) (Mehnert and Mäder 2001; zur Mühlen et al. 1996), as well as photon correlation spectroscopy (PCS), laser diffractometry (LD), and field flow fractionation (FFF).

3 Effects of Lipid Polymorphism on API Bioavailability

When the lipid bulk material is formulated as nanoparticles (solid lipid core surrounded by surfactant molecules) the formulation will show altered properties (Bummer 2004). These properties are due to (1) the changes involved in the physical state of lipid molecules, (2) the level of molecular interaction within the lipid core and with the aqueous surfactant environment, and (3) the energies involved. When decreasing the particle size below a submicrometer range, a relative increase of the surface area will occur, with a high curvature radius followed by higher energy of interaction between the lipid/surfactant/API molecules. This will clearly influence the bioavailability of API-loaded SLN/NLC, since the nanoparticle dose administered is proportional to the loading capacity (2) as well as to the number of particles per volume.

The inner structure is another important parameter that dramatically changes when decreasing the particle size (Bunjes et al. 2000; Lippacher et al. 2002). Since SLN/NLC are composed of pure lipids or mixtures of short, medium or long mono-, di- and triacylglycerols, their inner structure will be very different in comparison to the bulk material.

As mentioned previously, to transform the bulk lipid into nanoparticles, the lipid has to be either melted or solubilized in an organic solvent, followed by cooling down or solvent removal, respectively, so that the lipid recrystallizes, becoming solid again. Generally, recrystallization of melted lipid molecules creates an unstable hexagonal α-form which is converted, via a metastable orthorhombic β′-form, into a more stable triclinic β-form upon reheating and storage (Bunjes et al. 1996; Freitas and Müller 1999a; Westesen and Siekmann 1997). The particle size is the main factor affecting the transition rate from α to β′ to β, which is much faster in colloidal lipid particles that in the bulk lipid. Furthermore, the occurrence of such transitions is higher when using lipids of lower melting points. The LC and EE (2 and 3) are intrinsically dependent on these transition rates. The changes in the physical structure of the lipid matrix also influence both the particle shape and morphology. In general, a platelet-like shape is observed when the content of the β-form is higher. Depending on the particle size, different shapes will be observed with the increase of the α-form. Larger nanoparticles (>200 nm) are usually more spherical, while smaller nanoparticles (<100 nm) are characterized by a blocky isometric layered shape (Bunjes et al. 2003). Polymorphic transitions followed by changes in the particle surface area will obviously influence the physical stability of the lipid nanoparticle dispersions (Westesen and Siekmann 1997; Lukowski et al. 2000).

According to the lipid chain length, the melting and crystallization temperatures of the SLN/NLC dispersions are very different from the bulk materials (Bunjes et al. 1996). Depending on the lipid structure, crystallization does not always occur, creating so-called supercooled melts (Westesen et al. 1997). Since it is difficult to predict and to characterize the actual physical state of the lipid matrix, in vitro or in vivo performance of the systems might be easily misunderstood. In fact, supercooled melts behave mainly as emulsions. A comparison study has been run between SLN composed of different triacylglycerols varying in their chain length. Due to their higher melting point, tristearin and tripalmitin SLN were crystalline at room temperature, whereas trimyristin and trilaurin nanoparticles maintained their liquid status, behaving as emulsions for several months upon storage under the same conditions. By DSC analysis it was observed that trimyristin SLN started recrystallizing at 10°C while trilaurin SLN were still liquid at 4°C (Bunjes et al. 1996). Such a phenomenon was attributed to the small size of the particles, which can reduce their melting point by several degrees in comparison to the bulk material. The same effect may also happen for the crystallization temperature. Thus supercooled melts may often occur, especially for lipid mixtures, short-chain lipids or less pure ones. The possibility of polymorph coexistence strongly influences lipid nanoparticle stability. Trilaurin exhibits four different polymorphs, i.e., α, β', β_1, and β_2 (Lippacher et al. 2000). Upon fast cooling, trilaurin SLN recrystallized directly into the metastable α-form. Other than with NLC, this factor strongly affects API loading in SLN. Although high EE (3) have been reported, especially for lipophilic APIs, the LC (2) in SLN is limited by their small size. Lipid polymorphic structures often undergo modification upon API loading as a result of the intercalation of the API between lipid layers (Westesen et al. 1997). An acylglycerol behenate SLN formulation showed small amounts of the unstable α-form that disappeared upon heating or when loading the system with the API (Hou et al. 2003).

Generally, the presence of guest molecules in the lipid matrix also influences its crystallization degree, decreasing the lipid layer organization. In fact, depending on the lipid chain length, a depression of melting and crystallization temperatures is usually reported, indicating a strong tendency towards supercooling (Westesen et al. 1997; Bunjes et al. 1996). Moreover, the LC and EE (2 and 3) are generally higher in the case of mixtures of acylglycerols as a result of their lower crystallinity in comparison to pure lipids (Westesen et al. 1997). Such a characteristic influences API distribution and motility and also the pharmacokinetics and biodistribution. An increase of LC from 1% up to 50% caused dramatic changes in the lipid structure; API leakage from the lipid matrix occurred upon storage. The rate of API expulsion was dependent on the lipid matrices' composition and this feature was correlated to the rate of polymorphic transformation (Westesen et al. 1997). Nevertheless, stable mifepristone-loaded SLN formulations could be produced with less ordered crystalline organizations (Siekmann and Westesen 1994). This has been attributed to the less rigid and unordered structures which can provide vacancies to guest molecules, and their expulsion is less likely to occur upon storage. Furthermore, lipid nanoparticles of spherical shape are usually of lower crystalline status. If the formulation

is not intended for controlled/prolonged API release, supercooled melts may be a suitable alternative, since in some cases they can enhance the solubility of poorly soluble APIs and increase both LC and EE. Nevertheless, these melts are not thermodynamically stable, having the risk of long-term recrystallization.

4 Lipid Nanoparticles Applications

SLN and NLC have been proposed as alternative carriers to well-known liposomes and polymeric nanoparticles in order to overcome some of their common problems, achieving API bioavailability enhancement, controlled release, and API targeting. Due to the high lipid biocompatibility, virtually all the existing administration routes are possible and many of them have been investigated, namely the oral, ocular, topical, dermal and transdermal, pulmonary, and parenteral delivery. Several examples will be given in the following sections.

4.1 Oral Delivery

Oral delivery of poorly soluble APIs remains a significant challenge in pharmaceutical technology. Nevertheless, the ability of lipid-based formulations to facilitate absorption from the gastrointestinal tract (GIT) is well documented, and the pharmacological activity of API is not impaired.

Lipids are considered to be safe materials in the development of API delivery systems (Müller et al. 1997a; Schwarz et al. 1994; Wissing et al. 2004). This is easily exemplified by emulsions and microemulsions, which have widely been used to enhance the absorption and bioavailability of APIs belonging, respectively, to class III and class II of the Biopharmaceutical Classification System (BCS, Table 1) (Bummer 2004). The stability of such systems is strictly related to particle size distribution, lipid content, and presence of a surfactant able to stabilize the dispersion. The molecular properties of the phases involved deeply influence the lipid organization and its assembly.

Clinical applications of very potent agents are in general difficult to assess because of the high risk of API toxicity, poor oral bioavailability, insolubility,

Table 1 Biopharmaceutical classification system (BCS)

Class	Solubility	Permeability	In vitro/In vivo correlations
I	High	High	Easy to establish bioequivalence
II	Low	High	In vitro dissolution is similar to in vivo dissolution
III	High	Low	Absorption is the limiting factor
IV	Low	Low	Difficult to establish bioequivalence

and poor physicochemical stability. One possibility to overcome such limitations is the incorporation of those APIs in lipid nanoparticles. Micro- and nanoencapsulation in lipid-based colloidal delivery systems is usually applied to enhance API stability, increase oral bioavailability, reduce adverse side effects and/or API toxicity, and also has the possibility to modify the API release profile.

Cyclosporine A is an example of a hydrophobic cyclic peptide that shows low oral bioavailability, about 30% (Fahr 1993; Noble and Markham 1995). In addition, the absorption rate and extent is limited by several factors, such as food intake, bile production, and GIT motility. Many attempts have been made to enhance cyclosporine bioavailability using different dosage forms. The commercial microemulsion Sandimmun Neoral/Optoral®, commonly administered in many therapies, consists of oil, propylene glycol and, as surfactant, polyoxyl-40 hydrogenated castor oil; the amount of cyclosporine in this microemulsion is about 10%. With the purpose of the development of an improved oral cyclosporine delivery system to treat autoimmune diseases and to prevent transplant rejection, this immunosuppressive API has been formulated into SLN using several production procedures, e.g., HPH (Müller et al. 2006, 2008; Varia et al. 2008), via the microemulsion method (Ugazio et al. 2002), or by means of organic solvent diffusion (Hu et al. 2004b). The effect of lipid composition and particle size on the oral cyclosporine bioavailability has been assessed. The formulations composed of API, acylglycerol monostearate as solid lipid and a combination of surfactant/cosurfactant (Tagat/sodium cholate), resulted in physicochemically stable SLN of approx. 160 nm (PCS mean diameter) (Müller et al. 2006). The oral bioavailability of the peptide was determined in pigs following the cyclosporine blood levels after oral administration of the SLN formulation, in comparison to the commercial Sandimmun Neoral/Optoral®. Administration of cyclosporine-loaded SLN led to a mean plasma profile with almost similarly low variations in comparison to the commercial formulation, however, no initial blood peak was observed with the Sandimmun Neoral/Optoral®.

SLN composed of stearylamine as solid matrix and produced by a solvent diffusion method showed a burst release of 18% cyclosporine over the first 12 h, followed by a sustained release over 16 days when about 4% of the peptide was released per day (Hu et al. 2004b). The release kinetics were dependent on the composition of the lipid matrix (Varia et al. 2008).

Despite of the high EE (3) achieved for cyclosporine in SLN, e.g., 100% with the optimized lipid and surfactant composition (Varia et al. 2008), the bioavailability ranged from 20 to 60%. Concerning colloidal carriers, a correlation between the particle size and the oral bioavailability of cyclosporine formulations has been reported. Nanoparticles composed mainly of solid triacylglycerols (e.g., tricaprin, trilaurin, tristearin) and a certain amount of hydrogenated vegetable oil, stabilized by egg or soybean phosphatidylcholine, revealed higher cyclosporine bioavailability when the particle size was below 60 nm (Bekerman et al. 2004). In fact, several examples emphasize that the GIT uptake of APIs loaded on nanoparticles is greater when compared to microparticles (Bekerman et al. 2004; Desai et al. 1996, 1997; Pescovitz et al. 1992).

Attempts have also been made to incorporate hydrophilic peptides/proteins within lipid matrices. Successful examples in SLN are gonadorelin (Hu et al. 2004a), insulin (Battaglia et al. 2007; Gallarate et al. 2008; Sarmento et al. 2007; Zhang et al. 2006a, b), and salmon calcitonin (Garcia-Fuentes et al. 2003; Martins et al. 2009).

An EE of 70% was achieved for gonadorelin in SLN, and the peptide-loaded SLN revealed a PCS diameter of about 420 nm with a zeta potential of −22 mV (dispersed in distilled water) (Hu et al. 2004a). The in vitro release assay was performed in simulated GIT conditions revealing a biphasic profile, i.e., after a burst release of 24.4% of loaded gonadorelin within the first 6 h, a distinctly prolonged release over a monitored period of 12 days was observed and nearly 3.81% gonadorelin was released per day. Insulin was incorporated in SLN by a modified double-emulsion procedure, achieving an EE of approx. 40% (Gallarate et al. 2008; Sarmento et al. 2007). Cetylpalmitate-based SLN were orally administered to diabetic rats and a considerable hypoglycemic effect over 24 h was observed (Sarmento et al. 2007). Trimyristin-based SLN showed a mean diameter of 200 nm with a calcitonin EE of approx. 86% (Martins et al. 2009). This protein was released at a rate up to 8 h, under both gastric and intestinal simulated pH conditions. Being hydrophilic in nature, salmon calcitonin is not soluble in SLN matrix, therefore a novel production procedure based on a double w/o/w emulsion technique has been developed (Martins et al. 2009).

The pharmacological activity of calcitonin was evaluated following oral dosage of protein-loaded SLN in rats. When loaded into SLN, calcitonin decreased the basal blood calcium levels by up to 20% with 500 IU/kg for at least 8 h (Martins et al. 2009). The minimum calcium serum level was obtained 1 h after administration. In contrast, the serum calcium levels increased due to the stress induced in the rats during administration following calcitonin solution testing for reference (Martins et al. 2009). The efficacy of calcitonin-loaded SLN was attributed to SLN uptake through Peyer's patches. In fact, the ileum is an ideal site for nanoparticle uptake, where abundant Peyer's patches exist with proteolytic enzyme activity (des Rieux et al. 2006). The paracellular pathway has also been shown to contribute to protein absorption; most protein and polypeptide APIs diffuse through the aqueous-filled tight junctional pathway due to their hydrophilic nature (Salamat-Miller and Johnston 2005). Thus salmon calcitonin released from SLN within GIT might be immediately absorbed. However, due to the tightness of the junctions of the intercellular spaces, the calcitonin absorption rate might be somewhat reduced (Salamat-Miller and Johnston 2005).

Another example of enhanced API uptake from GIT is tobramycin, which is not absorbed following oral administration. Loaded into SLN and administered duodenally, tobramicin was targeted to the lymph, showing a high availability and a sustained release profile (Bargoni et al. 1998, 2001; Cavalli et al. 2000b).

The poorly soluble fenofibrate formulated in SLN and as API nanocrystals (so-called DissoCubes®) was investigated in rats following oral administration; two nanosuspensions of micronized fenofibrate were used as reference (Hanafy et al. 2007). Both colloidal delivery systems showed approximately two-fold bioavailability enhancement in terms of rate and extent compared to the reference

formulations. Between SLN and nanocrystals no significant differences were found in AUC, C_{max} and t_{max}.

Factors increasing solubility of APIs in GIT are solubilising agents, bile salts, and lecithin from intestinal fluid making contact with the lipid nanoparticles (Dressman and Reppas 2000). Moreover, the surfactant vitamin E TPGS figuring in the SLN composition can enhance the solubility as reported for spironolactone (Langguth et al. 2005). A 5.7-fold bioavailability enhancement was observed for the spironolactone-loaded SLN composed of of 9.5% vitamin E TPGS and 10% vitamin E6-100. The small particle size was not the major factor for bioavailability improvement, but the type of surfactant used in the formulation. The greater improvement in bioavailability for spironolactone formulated with vitamin E TPGS could be explained by an additional P-glycoprotein inhibition (Dintaman and Silverman 1999). Since spironolactone has affinity to the P-glycoprotein efflux pump (Wu and Benet 2005), combining the P-glycoprotein substrate with an inhibitor may improve and enhance absorption and API bioavailability. Developing SLN/NLC with vitamin E TPGS may be a very interesting approach to increase oral uptake for other poorly soluble drugs and also those which are P-glycoprotein substrates.

Liquid dosage forms are extremely important, in particular for elderly people and children, due to their difficulties in swallowing solid dosage forms. API-loaded SLN/NLC dispersions show multiple advantages to overcome such limitations, since they can be added to fruit juices or yogurts, to syrups simplex, and can even be loaded into soft gelatine capsules which are easy to swallow. Furthermore, the latter approach can also take advantage of using phospholipids as surfactants surrounding the particles. After oral administration of soft capsules, their content is released to gastric juices and the phospholipid molecules may adhere onto the GIT membrane enhancing oral API absorption. Although the small particle size seems to significantly improve bioavailability of APIs, the composition, and particularly the surface properties of the nanoparticles, may also affect the oral bioavailability (Andrysek 2003, 2006).

4.2 Pulmonary Delivery

Increasing attention has also been given to the potential of the pulmonary route as an alternative for non-invasive systemic delivery of therapeutic agents for both local and systemic API delivery (Scheuch et al. 2006). Advantages of pulmonary delivery using lipid nanoparticles rely on the possibility of site-specific application and controlled release to the lung. Since several advantages can be pointed out for this route (Hussain et al. 2004; Patton et al. 2004), e.g., large absorption area, extensive vasculature, easily permeable membrane, low extracellular and intracellular enzyme activity, pulmonary delivery of APIs becomes an opened and relatively unexplored field, in particular for peptides and proteins (Hussain et al. 2004; Malik et al. 2007). Nevertheless, for successful development of pulmonary delivery systems several challenges still remain, a major issue being the formulation of APIs into inhalable forms with sufficient stability and appropriate size (Abu-Dahab

et al. 2001; Dailey et al. 2003). Inhalation devices as well as the physicochemical characteristics of the formulation may influence aerodynamic particle size and thereafter affect the localization of aerosolized nanoparticles.

The pharmaceutical industry provides several inhalation devices, including metered-dose inhalers and API powder inhalers. Aqueous dispersions of lipid nanoparticles can be lyophilized to obtain powders, which may then be administered by means of these inhalers. Nevertheless, the particle size obtained after passing the sample through these devices is usually very large and thus might not be suitable for efficient deposition due to inertial impaction in the upper respiratory tract. More appropriate inhalers would be those generating a mist of small particles, which could penetrate the lung regions readily, and are better fitted for pulmonary delivery of APIs (Roche and Huchon 2000).

Colloidal carriers have also been pointed out as a suitable alternative for effectiveness of pulmonary API delivery. Examples include liposomes (Huang and Wang 2006; Karathanasis et al. 2005) and nanoparticles (Kawashima et al. 1999; Zhang et al. 2001), which exhibit some well-defined characteristics, especially for proteins. Higher bioavailability, controlled release properties, and enzymatic tolerance may be obtained (Chattopadhyay et al. 2007). SLN have also been recently proposed as a non-toxic API delivery system for pulmonary administration due to their unique physicochemical characteristics (e.g., small size, long-term physicochemical stability, biocompatibility and biotolerability, deep-lung deposition). By controlling the aerosolized particle size populations (mist of small particles versus larger particles) a dual effect of prolonged API release and rapid API transport could be achieved by means of SLN (Pandey and Khuller 2005; Videira et al. 2002). However lung targeting using nanoparticles has not been fully accepted yet. Most published data are limited to in vitro characterization of the nanoparticles for pulmonary delivery, and most of the reports address the treatment of local diseases, instead of systemic treatment by means of proteins or gene delivery (Almeida and Souto 2007; Rudolph et al. 2004).

To develop SLN-based formulations for such purposes, one needs to make sure the physicochemical stability of the aerosolized nanoparticles can be guaranteed. Chattopadhyay et al. have loaded triacylglycerols-based SLN with ketoprofen and indomethacin using the SFEE technique (Chattopadhyay et al. 2007). They successfully aerosolized the API-loaded SLN formulations using micron-sized nozzle devices. The particle size of aerosolized SLN dispersion was assessed by cascade impactor and by laser diffractometry, and it was shown to be similar to the size of aerosolized droplets usually obtained when administering API solution formulations using these devices. When using the micron-sized nozzles, the emitted dose was shown to be relatively higher and superior to those using the larger size API suspensions (Yim et al. 2005). Such results were easily attributed to the fact that smaller particles are less likely to clog the nozzle holes, and therefore aerosolization was close to the typical emitted dose of 65–70% observed with solution formulations (Boyd et al. 2004). The authors reported that aerosolized indomethacin-loaded SLN revealed more narrow size distribution and smaller mean particle size in comparison to ketoprofen-loaded SLN (Chattopadhyay et al. 2007). SLN

formulations were very stable during the SFEE with small emulsion droplet size leading to very uniform particles.

In another report, insulin-loaded SLN for pulmonary delivery were developed by a reverse micelle-double emulsion method, using a mixture of stearic and palmitic acids as solid lipid matrix, stabilized by sodium cholate and soybean phosphatidylcholine in aqueous dispersion (Liu et al. 2007, 2008). SLN remained stable under aerosolization achieving approx. 97% of EE, with the respirable fraction and nebulization efficiency of 82% and 63%, respectively. Pulmonary administration of 20 IU/kg SLN formulation reduced fasting plasma glucose within the first 4 h by about 40%, with an increased insulin level of approx. 170 μIU/ml. Pharmacological bioavailability was 24% and relative bioavailability 22% relative to subcutaneous injection as a reference. Aerosolized SLN were effectively and homogeneously distributed in the lung alveoli, with improved in vitro and in vivo stability, and prolonged hypoglycemic effect.

4.3 Parenteral Delivery and Drug Distribution

The major limiting factor for the parenteral delivery of lipid nanoparticles is their rapid clearance from the systemic circulation by the RES, which is dependent on the particle size, surface charge, and hydrophilic/lipophilic surface characteristics (Borm et al. 2006; Hoet et al. 2004). Colloidal API carriers usually depict a lipophilic surface, being therefore recognized as foreign elements by specific plasma components (opsonins), such as immunoglobulins (IgG), albumin, the elements of the complement system, fibronectin, and others, and then cleared from the blood stream by the phagocytic cells within minutes (Furumoto et al. 2004; Moghimi et al. 2001, 2005). Following intravenous (i.v.) injection, approx. 60–90% of the particles are distributed to the liver, and the remaining ones into spleen (2–10%), lungs (3–20% and more), and bone marrow (> 1%) (Kreuter 1994). The distribution in the body is also affected by the extravasation of nanoparticles from the peripheral capillary walls of these organs due to their large interendothelial gaps of about 150 nm. Thus, the passive targeting strongly limits the use of nanoparticles in API delivery to sites other than those belonging to the RES (Wolburg and Lippoldt 2002). To overcome such limitations, nanoparticles are usually surface-modified by hydrophilic molecules (e.g., surfactants and hydrophilic polymers or proteins) to avoid recognition by the mononuclear phagocytic system (MPS). Furthermore, it is also generally accepted that negative surfaces activate the complement system and coagulation factors (Moghimi et al. 2001). In addition to particle size reduction, changes in API biodistribution will occur, enhancing the systemic time circulation of the carriers and their deposition in non-RES organs (Kreuter 2001). In fact, one of the current approaches to achieve site-specific delivery is to bypass the normal physiological defense processes by reducing the particle size, thereby remaining for a prolonged period of time in the systemic circulation.

It is also known that the size and deformability of nanoparticles are of major importance in their clearance by the sinusoidal spleens of humans and rats, i.e., to avoid the splenic filtration at the inter-endothelial cell slits (IES) in the walls of venous sinuses, nanoparticles must be sufficiently small or deformable (Moghimi et al. 1993, 1991). It has been reported that ideally the size of an engineered long-circulatory particle should not exceed 200 nm (Groom 1987). Otherwise, the nanoparticle must be deformable enough to bypass IES filtration. Alternatively, long-circulating rigid particles of greater than 200 nm may act as splenotropic agents and be removed later on, if they are not rigid (Moghimi et al. 1991). If SLN are below 200 nm they will show an increased systemic circulation and thus an increase in the time for which the API remains in contact with the target site.

SLN have been proposed as a suitable system for parenteral delivery of hydrophilic APIs, such as diminazine, as well as of other BCS class IV APIs, e.g., paclitaxel, vinblastine, camptothecin, etoposide, and cyclosporine (Cavalli et al. 2000a; Chen et al. 2001; Yang et al. 1999a, b). Due to their lipophilic nature, SLN can be rapidly taken up by the RES, which may result in therapeutic failure due to insufficient API concentration in the plasma.

Steric stabilization is also an option because it creates a dense conformational cloud surrounding the particles, reducing opsonization and phagocytosis as well as the uptake by neutrophilic granulocytes. The result will be an increase in the systemic half-life of the API. An example of steric stabilization is the lipid nanoparticle stability provided by polyethylene glycol (PEG) molecules. PEG is a hydrophilic and electrically neutral polymer with a high chain flexibility. Its lack of functional groups prevents it from physicochemical interaction with the biological surroundings. PEG molecules with a molecular weight between 2,000 and 5,000 kDa are usually required to suppress plasma protein adsorption, and those creating thicker hydrophilic layers surrounding the particles will also contribute to the reduction of liver clearance (Chen et al. 2001).

To increase selectivity of SLN to a particular target, ligands or homing devices (which specifically bind to surface epitopes or receptors on the target sites) could be coupled onto their surface. It is known that cancer cells over-express specific receptors, such as folic acid receptors (over-expressed in cells of cancers with epithelial origin), low density lipoproteins (LDL) receptors (i.e., B16 melanoma cell line shows higher expression of LDL receptors), and peptide receptors (e.g., for somatostatin, vasoactive intestinal peptide, gastrin related peptides, cholecystokinin, gonadotropin releasing hormone). Therefore, attaching suitable ligands for these particular receptors onto the SLN surface may increase selectivity (Pardridge 2007b).

4.4 Brain Targeting

In the last decade, there has been emerging interest in API targeting to the brain (Blasi et al. 2007; Göppert and Müller 2005; Kreuter 2001; Pardridge 2005, 2007b, c, d, e). The lack of knowledge regarding the physiology of the central

nervous system (CNS) is one of the limiting factors in the development of effective APIs and appropriate API delivery systems for brain targeting and delivery (Pardridge 2003, 2007a,c,d). The specific blood–brain barrier (BBB) tightly regulates the exchange between the peripheral blood circulation and the cerebrospinal fluid circulatory system. Thus, these physiological features of the brain microvasculature restrict enormously the number of APIs that can enter the brain upon systemic administration. In fact, more than 98% of the new potential CNS active drugs are unable to cross the BBB (Pardridge 2007a). A drug molecule with a high lipophilicity and a molecular weight below 500 Da can pass through the BBB. Several strategies have been tried to effectively achieve API delivery and deposition to the CNS (Badruddoja and Black 2006; Johanson et al. 2005; Vyas et al. 2005), in particular the use of API carrier systems (Tiwari and Amiji 2006).

One possibility for access to the brain is receptor-mediated transport, because the BBB at the luminal side expresses receptors for endogenous large molecules (e.g., insulin, transferrin, leptin, ApoE, thiamin). The receptor-mediated transport of these molecules can be used for specific delivery into the brain (Cornford and Hyman 1999). The binding of the drug or the carrier (e.g., liposomes and nanoparticles) to specific ligands (peptides) or peptidomimetic monoclonal antibodies will shuttle the API directly into the brain (Pardridge 2003). These monoclonal antibodies act as Trojan horses for delivery of nanoparticles to the brain. The use of peptidomimetic antibodies which can bind to BBB transcytosis receptor, brain-targeted pegylated immuno-nanoparticles, has also been proposed. The delivery of entrapped APIs into the brain parenchyma can be achieved without inducing alteration in BBB permeability (Harris and Chess 2003). Yet some transporters such as P-glycoprotein existing in the BBB may also limit brain API delivery and can prevent the accumulation of various agents including APIs in the brain (Stouch and Gudmundsson 2002). To overcome this limitation, P-glycoprotein inhibition has been proposed using the generally accepted pharmaceutical surfactants (Batrakova et al. 1999; Miller et al. 1999).

Polymeric nanoparticles have been considered particularly useful to overcome the BBB (Garcia-Garcia et al. 2005; Müller and Keck 2004b), which seems to be high if nanoparticles are coated with polysorbate 80 (Tween 80) (Göppert and Müller 2005; Koziara et al. 2003). SLN have also been tested for brain targeting (Garcia-Garcia et al. 2005; Göppert and Müller 2005; Müller and Keck 2004a, b). The potential advantages of SLN over polymeric nanoparticles for brain targeting are based on their lower cytotoxicity, higher API loading capacity, and better production scalability. The surfactant-coated technology designed for brain targeting has been transferred to SLN and related carriers with relatively high success.

Göppert and Müller developed polysorbate-surfaced SLN to deliver several APIs to the brain. These studies demonstrated in addition that ApoC and ApoCII adsorbed onto SLN surface inhibit the receptor-mediated binding of β-VLDl expressing ApoE at the particle surface to the LDL receptor (Goppert and Müller 2005). The authors have emphasized the advantage of having a high ApoE/ApoCII ratio absorbed on the particles to achieve brain targeting. Furthermore, they found

that stealth SLN with polysorbate 80 adsorbed the lowest amount of ApoCII onto the particle surface. The pathfinder technology, i.e., differential protein adsorption, exploits plasma proteins which adsorb onto the surface of intravenously injected SLN for targeting. ApoE is such a moiety for SLN targeting to the endothelial cells of the BBB (Müller and Keck 2004a).

Zara and colleagues developed SLN and PEG-coated SLN containing increasing amounts of this stealth agent, for brain delivery of doxorubicin following i.v. administration (Kaur et al. 2008). The brain concentration of doxorubicin increased when increasing the stealth agent. The amount of doxorubicin in the rabbit brain ranged from 27.5 ng g^{-1} for non-stealth SLN to 242.0 ng g^{-1} for stealth SLN (surfaced with PEG molecules).

Thole et al. reported improved interaction with brain endothelial cells and higher intracellular accumulation of sterically stabilized liposomes coupled to cationized albumin in comparison to bovine serum albumin nanoparticles (Thole et al. 2002). Positively charged albumin nanoparticles were taken up into the brain endothelia via a caveolae-mediated endocytic pathway.

The effect of the surface charge of SLN on brain delivery was also assessed following administration of etoposide-loaded tripalmitin SLN. Brain levels were compared to the etoposide solution. Positively charged etoposide-loaded SLN achieved the highest brain concentration (0.07% of injected dose/g) clearly exceeding the uptake compared to negatively charged etoposide-loaded SLN (0.02%) and etoposide solution (0.01%) (Reddy and Venkateswarlu 2004).

Moreover, nitrendipine-loaded SLN composed of different acylglycerols (tripalmitin, trimyristin, tristearin), surfactants (soy lecithin, poloxamer 188), and charge modifiers (dicetyl phosphate, stearylamine) were produced aiming to compare the systemic half-life of API upon i.v. administration, in comparison to a conventional nitrendipine suspension (Manjunath and Venkateswarlu 2006). SLN formulation was found to be taken up to a greater extent by the brain and maintained high API levels for 6 h, whereas nitrendipine suspension achieved such levels only for 3 h. A 3.2-, 7.3- and 9.1-fold enhancement in C_{max} was shown when using SLN composed of tripalmitin, tripalmitin dicetyl phosphate, or tripalmitin stearylamine, respectively, in comparison to the API suspension. Similar findings were reported with 3′,5′-dioctanoyl-5-fluoro-2′-deoxyuridine-loaded SLN (Wang et al. 2002).

Stearic acid-based SLN loaded with camptothecin were administered i.v. to mice (1.3 mg kg^{-1}) resulting in a significantly prolonged drug residence time in the body in comparison to the camptothecin solution (Yang et al. 1999a). A fivefold increase in plasma AUC and a tenfold increase in brain AUC was observed on increasing the dose of camptothecin from 1.3 to 3.3 mg kg^{-1}.

In addition to the advantages of SLN for enhancing drug uptake by the brain, the very low brain cytotoxicity of SLN makes these carriers very attractive candidates for brain delivery (Müller et al. 1997b). It is important to underline that the toxicity of SLN is not only related to the lipid type, but also to the surfactant employed to stabilize the particle in aqueous dispersion. The most common surfactant exploited for nanoparticle brain targeting is polysorbate 80. Interestingly, free polysorbate 80 was more toxic than when bound (Koziara et al. 2006), which has been attributed to

the fact that this surfactant is more likely to be incorporated into SLN matrix rather than adsorbed, and thus its minimal release will also decrease toxicity.

5 Conclusions and Perspectives

The present chapter reviews current achievements in modifying the API pharmacokinetic parameters and bioavailability by means of lipid nanoparticles (SLN and NLC). These carriers are composed of materials compatible with the biological environment. SLN and NLC have been exploited for oral, dermal, pulmonary, and parenteral administration. Obviously, the in vivo behavior and consequently therapeutic potential of these nanoparticles are defined by their physicochemical properties as well as by the administration route. The type of lipid nanoparticle system (SLN versus NLC) should be critically selected according to the administration route, e.g., NLC are less likely to be used for brain delivery. Nevertheless, both systems can be used to decrease API toxicity.

The pharmaceutical industry is interested in the development of a delivery system that could be sufficiently versatile to be exploited for several administration routes. Changes in the carrier surface properties (electric charge, hydrophilicity) and matrix composition may be required to minimize or overcome limitations associated with more conventional colloidal carriers (e.g., liposomes, polymeric nanoparticles, nanoemulsions). SLN and NLC can be designed according to the physicochemical properties of API molecules, as well as to the administration route and target/delivery purposes.

References

Abu-Dahab R, Schäfer UF, Lehr CM (2001) Lectin-functionalized liposomes for pulmonary drug delivery: effect of nebulization on stability and bioadhesion. Eur J Pharm Sci 14:37–46

Almeida AJ, Runge S, Müller RH (1997) Peptide-loaded solid lipid nanoparticles (SLN): influence of production parameters. Int J Pharm 149:255–265

Almeida AJ, Souto E (2007) Solid lipid nanoparticles as a drug delivery system for peptides and proteins. Adv Drug Deliv Rev 59:478–490

Andrysek T (2003) Impact of physical properties of formulations on bioavailability of active substance: current and novel drugs with cyclosporine. Mol Immunol 39:1061–1065

Andrysek T (2006) Excipients and their role in absorption: Influencing bioavailability of cyclosporine by triglycerides and polyglycerol esters. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 150:227–233

Anton N, Benoit JP, Saulnier P (2008) Design and production of nanoparticles formulated from nano-emulsion templates-a review. J Control Release 128:185–199

Anton N, Gayet P, Benoit JP, Saulnier P (2007) Nano-emulsions and nanocapsules by the PIT method: an investigation on the role of the temperature cycling on the emulsion phase inversion. Int J Pharm 344:44–52

Badruddoja MA, Black KL (2006) Improving the delivery of therapeutic agents to CNS neoplasms: a clinical review. Front Biosci 11:1466–1478

Bargoni A, Cavalli R, Caputo O, Fundaro A, Gasco MR, Zara GP (1998) Solid lipid nanoparticles in lymph and plasma after duodenal administration to rats. Pharm Res 15:745–750

Bargoni A, Cavalli R, Zara GP, Fundaro A, Caputo O, Gasco MR (2001) Transmucosal transport of tobramycin incorporated in solid lipid nanoparticles (SLN) after duodenal administration to rats. Part II–tissue distribution. Pharmacol Res 43:497–502

Batrakova EV, Li S, Miller DW, Kabanov AV (1999) Pluronic P85 increases permeability of a broad spectrum of drugs in polarized BBMEC and Caco-2 cell monolayers. Pharm Res 16:1366–1372

Battaglia L, Trotta M, Gallarate M, Carlotti ME, Zara GP, Bargoni A (2007) Solid lipid nanoparticles formed by solvent-in-water emulsion-diffusion technique: development and influence on insulin stability. J Microencapsul 24:660–672

Bekerman T, Golenser J, Domb A (2004) Cyclosporin nanoparticulate lipospheres for oral administration. J Pharm Sci 93:1264–1270

Blasi P, Giovagnoli S, Schoubben A, Ricci M, Rossi C (2007) Solid lipid nanoparticles for targeted brain drug delivery. Adv Drug Deliv Rev 59:454–477

Bondi ML, Azzolina A, Craparo EF, Lampiasi N, Capuano G, Giammona G, Cervello M (2007) Novel cationic solid-lipid nanoparticles as non-viral vectors for gene delivery. J Drug Target 15:295–301

Bondi ML, Fontana G, Carlisi B, Giammona G (2003) Preparation and characterization of solid lipid nanoparticles containing cloricromene. Drug Deliv 10:245–250

Borm PJ, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, Schins R, Stone V, Kreyling W, Lademann J, Krutmann J, Warheit D, Oberdörster E (2006) The potential risks of nanomaterials: a review carried out for ECETOC. Part Fibre Toxicol 3:11

Boyd B, Noymer P, Liu K, Okikawa J, Hasegawa D, Warren S, Taylor G, Ferguson E, Schuster J, Farr S, Gonda I (2004) Effect of gender and device mouthpiece shape on bolus insulin aerosol delivery using the AERx pulmonary delivery system. Pharm Res 21:1776–1782

Brioschi A, Zara GP, Calderoni S, Gasco MR, Mauro A (2008) Cholesterylbutyrate solid lipid nanoparticles as a butyric acid prodrug. Molecules 13:230–254

Bummer PM (2004) Physical chemical considerations of lipid-based oral drug delivery–solid lipid nanoparticles. Crit Rev Ther Drug Carrier Syst 21:1–20

Bunjes H, Koch MH, Westesen K (2003) Influence of emulsifiers on the crystallization of solid lipid nanoparticles. J Pharm Sci 92:1509–1520

Bunjes H, Koch MHJ, Westesen K (2000) Effect of particle size on colloidal solid triglycerides. Langmuir 16:5234–5241

Bunjes H, Westesen K, Koch MHJ (1996) Crystallization tendency and polymorphic transitions in triglyceride nanoparticles. Int J Pharm 129:159–173

Castelli F, Puglia C, Sarpietro MG, Rizza L, Bonina F (2005) Characterization of indomethacin-loaded lipid nanoparticles by differential scanning calorimetry. Int J Pharm 304:231–238

Cavalli R, Caputo O, Carlotti ME, Trotta M, Scarnecchia C, Gasco MR (1997) Sterilization and freeze-drying of drug-free and drug-loaded solid lipid nanoparticles. Int J Pharm 148:47–54

Cavalli R, Caputo O, Gasco MR (2000a) Preparation and characterization of solid lipid nanospheres containing paclitaxel. Eur J Pharm Sci 10:305–309

Cavalli R, Caputo O, Marengo E, Pattarino F, Gasco MR (1998) The effect of the components of microemulsions on both size and crystalline structure of solid lipid nanoparticles (SLN) containing a series of model molecules. Pharmazie 53:392–396

Cavalli R, Gasco MR, Barresi AA, Rovero G (2001) Evaporative drying of aqueous dispersions of solid lipid nanoparticles. Drug Dev Ind Pharm 27:919–924

Cavalli R, Zara GP, Caputo O, Bargoni A, Fundaro A, Gasco MR (2000b) Transmucosal transport of tobramycin incorporated in SLN after duodenal administration to rats. Part I – A pharmacokinetic study. Pharmacological Research 42:541–545

Chattopadhyay P, Shekunov BY, Yim D, Cipolla D, Boyd B, Farr S (2007) Production of solid lipid nanoparticle suspensions using supercritical fluid extraction of emulsions (SFEE) for pulmonary delivery using the AERx system. Adv Drug Deliv Rev 59:444–453

Chen DB, Yang TZ, Lu WL, Zhang Q (2001) In vitro and in vivo study of two types of long-circulating solid lipid nanoparticles containing paclitaxel. Chem Pharm Bull 49:1444–1447

Chen Y, Dalwadi G, Benson HA (2004) Drug delivery across the blood-brain barrier. Curr Drug Deliv 1:361–376

Cornford EM, Hyman S (1999) Blood-brain barrier permeability to small and large molecules. Adv Drug Deliv Rev 36:145–163

Cortesi R, Esposito E, Luca G, Nastruzzi C (2002) Production of lipospheres as carriers for bioactive compounds. Biomaterials 23:2283–2294

Dailey LA, Schmehl T, Gessler T, Wittmar M, Grimminger F, Seeger W, Kissel T (2003) Nebulization of biodegradable nanoparticles: impact of nebulizer technology and nanoparticle characteristics on aerosol features. J Control Release 86:131–144

de Sousa ARS, Calderone M, Rodier E, Fages J, Duarte CMM (2006) Solubility of carbon dioxide in three lipid-based biocarriers. J Supercrit Fluids 39:13–19

de Sousa ARS, Simplicio AL, de Sousa HC, Duarte CMM (2007) Preparation of glyceryl mono stearate-based particles by PGSS® – application to caffeine. J Supercrit Fluids 43:120–125

des Rieux A, Fievez V, Garinot M, Schneider Y-J, Preat V (2006) Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach. J Control Release 116:1–27

Desai MP, Labhasetwar V, Amidon GL, Levy RJ (1996) Gastrointestinal uptake of biodegradable microparticles: effect of particle size. Pharm Res 13:1838–1845

Desai MP, Labhasetwar V, Walter E, Levy RJ, Amidon GL (1997) The mechanism of uptake of biodegradable microparticles in Caco-2 cells is size dependent. Pharm Res 14:1568–1573

Dintaman JM, Silverman JA (1999) Inhibition of P-glycoprotein by D-alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS). Pharm Res 16:1550–1556

Dressman JB, Reppas C (2000) In vitro-in vivo correlations for lipophilic, poorly water-soluble drugs. Eur J Pharm Sci 11(Suppl 2):S73–S80

El-Harati AA, Charcosset C, Fessi H (2006) Influence of the formulation for solid lipid nanoparticles prepared with a membrane contactor. Pharm Dev Technol 11:153–157

Fahr A (1993) Cyclosporin clinical pharmacokinetics. Clin Pharmacokinet 24:472–495

Fontana G, Maniscalco L, Schillaci D, Cavallaro G, Giammona G (2005) Solid lipid nanoparticles containing tamoxifen characterization and in vitro antitumoral activity. Drug Deliv 12:385–392

Freitas C, Müller RH (1998) Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLN™) dispersions. Int J Pharm 168:221–229

Freitas C, Müller RH (1999a) Correlation between long-term stability of solid lipid nanoparticles (SLN™) and crystallinity of the lipid phase. Eur J Pharm Biopharm 47:125–132

Freitas C, Müller RH (1999b) Stability determination of solid lipid nanoparticles (SLN™) in aqueous dispersion after addition of electrolyte. J Microencapsul 16:59–71

Fundaro A, Cavalli R, Bargoni A, Vighetto D, Zara GP, Gasco MR (2000) Non-stealth and stealth solid lipid nanoparticles (SLN) carrying doxorubicin: pharmacokinetics and tissue distribution after i.v. administration to rats. Pharmacol Res 42:337–343

Furumoto K, Nagayama S, Ogawara K, Takakura Y, Hashida M, Higaki K, Kimura T (2004) Hepatic uptake of negatively charged particles in rats: possible involvement of serum proteins in recognition by scavenger receptor. J Control Release 97:133–141

Gallarate M, Trotta M, Battaglia L, Chirio D (2008) Preparation of solid lipid nanoparticles from W/O/W emulsions: Preliminary studies on insulin encapsulation. J Microencapsul 3:1–9

Garcia-Fuentes M, Torres D, Alonso MJ (2003) Design of lipid nanoparticles for the oral delivery of hydrophilic macromolecules. Colloids Surf B-Biointerfaces 27:159–168

Garcia-Garcia E, Andrieux K, Gil S, Couvreur P (2005) Colloidal carriers and blood-brain barrier (BBB) translocation: a way to deliver drugs to the brain? Int J Pharm 298:274–292

Gibson L (2007) Lipid-based excipients for oral drug delivery. In: Hauss DJ (ed) Oral lipid-based formulations: enhancing the bioavailability of poorly water-soluble drugs. Informa Healthcare, Inc., New York, pp 43–51

Gohla SH, Dingler A (2001) Scaling up feasibility of the production of solid lipid nanoparticles (SLN^{TM}). Pharmazie 56:61–63

Göppert TM, Müller RH (2005) Polysorbate-stabilized solid lipid nanoparticles as colloidal carriers for intravenous targeting of drugs to the brain: comparison of plasma protein adsorption patterns. J Drug Target 13:179–187

Groom AC (1987) The Microcirculatory Society Eugene M. Landis award lecture. Microcirculation of the spleen: new concepts, new challenges. Microvasc Res 34:269–289

Hanafy A, Spahn-Langguth H, Vergnault G, Grenier P, Tubic Grozdanis M, Lenhardt T, Langguth P (2007) Pharmacokinetic evaluation of oral fenofibrate nanosuspensions and SLN in comparison to conventional suspensions of micronized drug. Adv Drug Deliv Rev 59:419–426

Harris JM, Chess RB (2003) Effect of pegylation on pharmaceuticals. Nat Rev Drug Discov 2:214–221

Hauss DJ (2007) Oral lipid-based formulations. Adv Drug Deliv Rev 59:667–676

Hoet PH, Bruske-Hohlfeld I, Salata OV (2004) Nanoparticles – known and unknown health risks. J Nanobiotechnol 2:12

Hou D, Xie C, Huang K, Zhu C (2003) The production and characteristics of solid lipid nanoparticles (SLNs). Biomaterials 24:1781–1785

Hu FQ, Hong Y, Yuan H (2004a) Preparation and characterization of solid lipid nanoparticles containing peptide. Int J Pharm 273:29–35

Hu FQ, Jiang SP, Du YZ, Yuan H, Ye YQ, Zeng S (2005) Preparation and characterization of stearic acid nanostructured lipid carriers by solvent diffusion method in an aqueous system. Colloids Surf B Biointerfaces 45:167–173

Hu FQ, Jiang SP, Du YZ, Yuan H, Ye YQ, Zeng S (2006) Preparation and characteristics of monostearin nanostructured lipid carriers. Int J Pharm 314:83–89

Hu FQ, Wu M, Yuan H, Zhang HH (2004b) A novel preparation of solid lipid nanoparticles with cyclosporin A for prolonged drug release. Pharmazie 59:683–685

Hu FQ, Yuan H, Zhang HH, Fang M (2002) Preparation of solid lipid nanoparticles with clobetasol propionate by a novel solvent diffusion method in aqueous system and physicochemical characterization. Int J Pharm 239:121–128

Hu FQ, Zhang Y, Du YZ, Yuan H (2008) Nimodipine loaded lipid nanospheres prepared by solvent diffusion method in a drug saturated aqueous system. Int J Pharm 348:146–152

Huang YY, Wang CH (2006) Pulmonary delivery of insulin by liposomal carriers. J Control Release 113:9–14

Hussain A, Arnold JJ, Khan MA, Ahsan F (2004) Absorption enhancers in pulmonary protein delivery. J Control Release 94:15–24

Israelachvili JN, Marcelja S, Horn RG (1980) Physical principles of membrane organization. Q Rev Biophys 13:121–200

Jayagopal A, Sussman EM, Shastri VP (2008) Functionalized solid lipid nanoparticles for transendothelial delivery. IEEE Trans Nanobioscience 7:28–34

Jenning V, Gohla SH (2001) Encapsulation of retinoids in solid lipid nanoparticles (SLN). J Microencapsul 18:149–158

Jenning V, Schäfer-Korting M, Gohla S (2000) Vitamin A-loaded solid lipid nanoparticles for topical use: drug release properties. J Control Release 66:115–126

Johanson CE, Duncan JA, Stopa EG, Baird A (2005) Enhanced prospects for drug delivery and brain targeting by the choroid plexus-CSF route. Pharm Res 22:1011–1037

Jores K, Haberland A, Wartewig S, Mäder K, Mehnert W (2005) Solid lipid nanoparticles (SLN) and oil-loaded SLN studied by spectrofluorometry and Raman spectroscopy. Pharm Res 22:1887–1897

Jores K, Mehnert W, Drechsler M, Bunjes H, Johann C, Mäder K (2004) Investigations on the structure of solid lipid nanoparticles (SLN) and oil-loaded solid lipid nanoparticles by photon correlation spectroscopy, field-flow fractionation and transmission electron microscopy. J Control Release 95:217–227

Jores K, Mehnert W, Mäder K (2003) Physicochemical investigations on solid lipid nanoparticles and on oil-loaded solid lipid nanoparticles: a nuclear magnetic resonance and electron spin resonance study. Pharm Res 20:1274–1283

Joshi M, Patravale V (2008) Nanostructured lipid carrier (NLC) based gel of celecoxib. Int J Pharm 346:124–132

Karathanasis E, Ayyagari AL, Bhavane R, Bellamkonda RV, Annapragada AV (2005) Preparation of in vivo cleavable agglomerated liposomes suitable for modulated pulmonary drug delivery. J Control Release 103:159–175

Kaur IP, Bhandari R, Bhandari S, Kakkar V (2008) Potential of solid lipid nanoparticles in brain targeting. J Control Release 127:97–109

Kawashima Y, Yamamoto H, Takeuchi H, Fujioka S, Hino T (1999) Pulmonary delivery of insulin with nebulized DL-lactide/glycolide copolymer (PLGA) nanospheres to prolong hypoglycemic effect. J Control Release 62:279–287

Koziara JM, Lockman PR, Allen DD, Mumper RJ (2003) In situ blood-brain barrier transport of nanoparticles. Pharm Res 20:1772–1778

Koziara JM, Lockman PR, Allen DD, Mumper RJ (2006) The blood-brain barrier and brain drug delivery. J Nanosci Nanotechnol 6:2712–2735

Kreuter J (1994) Nanoparticles. Marcel Dekker, New York, pp 219–342

Kreuter J (2001) Nanoparticulate systems for brain delivery of drugs. Adv Drug Deliv Rev 47:65–81

Kristl J, Volk B, Ahlin P, Gombac K, Sentjurc M (2003) Interactions of solid lipid nanoparticles with model membranes and leukocytes studied by EPR. Int J Pharm 256:133–140

Kuntsche J, Bunjes H (2007) Influence of preparation conditions and heat treatment on the properties of supercooled smectic cholesteryl myristate nanoparticles. Eur J Pharm Biopharm 67:612–620

Langguth P, Hanafy A, Frenzel D, Grenier P, Nhamias A, Ohlig T, Vergnault G, Spahn-Langguth H (2005) Nanosuspension formulations for low-soluble drugs: pharmacokinetic evaluation using spironolactone as model compound. Drug Dev Ind Pharm 31:319–329

Lippacher A, Müller RH, Mäder K (2000) Investigation on the viscoelastic properties of lipid based colloidal drug carriers. Int J Pharm 196:227–230

Lippacher A, Müller RH, Mäder K (2002) Semisolid SLN^{TM} dispersions for topical application: influence of formulation and production parameters on viscoelastic properties. Eur J Pharm Biopharm 53:155–160

Liu J, Gong T, Fu H, Wang C, Wang X, Chen Q, Zhang Q, He Q, Zhang Z (2008) Solid lipid nanoparticles for pulmonary delivery of insulin. Int J Pharm 356:333–344

Liu J, Gong T, Wang C, Zhong Z, Zhang Z (2007) Solid lipid nanoparticles loaded with insulin by sodium cholate-phosphatidylcholine-based mixed micelles: preparation and characterization. Int J Pharm 340:153–162

Lockman PR, Oyewumi MO, Koziara JM, Roder KE, Mumper RJ, Allen DD (2003) Brain uptake of thiamine-coated nanoparticles. J Control Release 93:271–282

Lukowski G, Kasbohm J, Pflegel P, Illing A, Wulff H (2000) Crystallographic investigation of cetylpalmitate solid lipid nanoparticles. Int J Pharm 196:201–205

Lukowski G, Werner U (1998) Investigation of surface and drug release of solid lipid nanoparticles loaded with acyclovir. Intern Symp Control Rel Bioact Mater 25:425–428

Malik DK, Baboota S, Ahuja A, Hasan S, Ali J (2007) Recent advances in protein and peptide drug delivery systems. Curr Drug Deliv 4:141–151

Malzert-Freon A, Vrignaud S, Saulnier P, Lisowski V, Benoit JP, Rault S (2006) Formulation of sustained release nanoparticles loaded with a tripentone, a new anticancer agent. Int J Pharm 320:157–164

Mandawgade SD, Patravale VB (2008) Development of SLNs from natural lipids: application to topical delivery of tretinoin. Int J Pharm 363:132–138

Manjunath K, Reddy JS, Venkateswarlu V (2005) Solid lipid nanoparticles as drug delivery systems. Methods Find Exp Clin Pharmacol 27:127–144

Manjunath K, Venkateswarlu V (2006) Pharmacokinetics, tissue distribution and bioavailability of nitrendipine solid lipid nanoparticles after intravenous and intraduodenal administration. J Drug Target 14:632–645

Martins S, Silva AC, Ferreira DC, Souto EB (2009) Improving oral absorption of Salmon calcitonin by mucoadhesive solid lipid nanoparticles (SLN). J Biomed Nanotech 5:76–83

Mayer C, Lukowski G (2000) Solid state NMR investigations on nanosized carrier systems. Pharm Res 17:486–489

Mead J, Alfin-Slater R, Howton D, Popjak G (1986) Peroxidation of fatty acids lipids: chemistry, biochemistry and nutrition. Plenum Press, New York, pp 83–99

Mehnert W, Mäder K (2001) Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev 47:165–196

Miglietta A, Cavalli R, Bocca C, Gabriel L, Gasco MR (2000) Cellular uptake and cytotoxicity of solid lipid nanospheres (SLN) incorporating doxorubicin or paclitaxel. Int J Pharm 210:61–67

Miller DW, Batrakova EV, Kabanov AV (1999) Inhibition of multidrug resistance-associated protein (MRP) functional activity with pluronic block copolymers. Pharm Res 16:396–401

Moghimi SM, Hedeman H, Muir IS, Illum L, Davis SS (1993) An investigation of the filtration capacity and the fate of large filtered sterically-stabilized microspheres in rat spleen. Biochim Biophys Acta 1157:233–240

Moghimi SM, Hunter AC, Murray JC (2001) Long-circulating and target-specific nanoparticles: theory to practice. Pharmacol Rev 53:283–318

Moghimi SM, Hunter AC, Murray JC (2005) Nanomedicine: current status and future prospects. Faseb J 19:311–330

Moghimi SM, Porter CJ, Muir IS, Illum L, Davis SS (1991) Non-phagocytic uptake of intravenously injected microspheres in rat spleen: influence of particle size and hydrophilic coating. Biochem Biophys Res Commun 177:861–866

Müller RH, Keck CM (2004a) Challenges and solutions for the delivery of biotech drugs–a review of drug nanocrystal technology and lipid nanoparticles. J Biotechnol 113:151–170

Müller RH, Keck CM (2004b) Drug delivery to the brain–realization by novel drug carriers. J Nanosci Nanotechnol 4:471–483

Müller RH, Maassen S, Schwarz C, Mehnert W (1997a) Solid lipid nanoparticles (SLN) as potential carrier for human use: interaction with human granulocytes. J Control Release 47:261–269

Müller RH, Mäder K, Gohla S (2000) Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art. Eur J Pharm Biopharm 50:161–177

Müller RH, Radtke M, Wissing SA (2002) Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev 54 (Suppl 1):S131–S155

Müller RH, Rühl D, Runge S, SchulzeForster K, Mehnert W (1997b) Cytotoxicity of solid lipid nanoparticles as a function of the lipid matrix and the surfactant. Pharm Res 14:458–462

Müller RH, Runge S, Ravelli V, Mehnert W, Thünemann AF, Souto EB (2006) Oral bioavailability of cyclosporine: solid lipid nanoparticles (SLN) versus drug nanocrystals. Int J Pharm 317:82–89

Müller RH, Runge SA, Ravelli V, Thünemann AF, Mehnert W, Souto EB (2008) Cyclosporine-loaded solid lipid nanoparticles (SLN): drug-lipid physicochemical interactions and characterization of drug incorporation. Eur J Pharm Biopharm 68:535–544

Noble S, Markham A (1995) Cyclosporin. A review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation (Neoral). Drugs 50:924–941

Pandey R, Khuller GK (2005) Solid lipid particle-based inhalable sustained drug delivery system against experimental tuberculosis. Tuberculosis 85:227–234

Pardridge WM (2003) Blood-brain barrier drug targeting: the future of brain drug development. Mol Interv 3(90–105):51

Pardridge WM (2005) The blood-brain barrier: bottleneck in brain drug development. NeuroRx 2:3–14

Pardridge WM (2007a) Blood-brain barrier delivery. Drug Discov Today 12:54–61
Pardridge WM (2007b) Blood-brain barrier delivery of protein and non-viral gene therapeutics with molecular Trojan horses. J Control Release 122:345–348
Pardridge WM (2007c) Brain drug development and brain drug targeting. Pharm Res 24: 1729–1732
Pardridge WM (2007d) Drug targeting to the brain. Pharm Res 24:1733–1744
Pardridge WM (2007e) shRNA and siRNA delivery to the brain. Adv Drug Deliv Rev 59:141–152
Patton JS, Fishburn CS, Weers JG (2004) The lungs as a portal of entry for systemic drug delivery. Proc Am Thorac Soc 1:338–344
Pescovitz MD, Book BK, Pollard SG, Milgrom ML, Leapman SB, Filo RS (1992) An evaluation of the cyclosporine parent-compound-specific whole blood TDx assay. Clin Transplant 6: 43–47
Pople PV, Singh KK (2006) Development and evaluation of topical formulation containing solid lipid nanoparticles of vitamin A. AAPS PharmSciTech 7(4):91
Reddy JS, Venkateswarlu V (2004) Novel delivery systems for drug targeting to the brain. Drugs Future 29:63–83
Roche N, Huchon GJ (2000) Rationale for the choice of an aerosol delivery system. J Aerosol Med 13:393–404
Rudolph C, Schillinger U, Ortiz A, Tabatt K, Plank C, Müller RH, Rosenecker J (2004) Application of novel solid lipid nanoparticle (SLN)-gene vector formulations based on a dimeric HIV-1 TAT-peptide in vitro and in vivo. Pharm Res 21:1662–1669
Salamat-Miller N, Johnston TP (2005) Current strategies used to enhance the paracellular transport of therapeutic polypeptides across the intestinal epithelium. Int J Pharm 294:201–216
Sarmento B, Martins S, Ferreira D, Souto EB (2007) Oral insulin delivery by means of solid lipid nanoparticles. Int J Nanomedicine 2:743–749
Saupe A, Gordon KC, Rades T (2006) Structural investigations on nanoemulsions, solid lipid nanoparticles and nanostructured lipid carriers by cryo-field emission scanning electron microscopy and Raman spectroscopy. Int J Pharm 314:56–62
Schäfer-Korting M, Mehnert W, Korting HC (2007) Lipid nanoparticles for improved topical application of drugs for skin diseases. Adv Drug Deliv Rev 59:427–443
Scheuch G, Kohlhaeufl MJ, Brand P, Siekmeier R (2006) Clinical perspectives on pulmonary systemic and macromolecular delivery. Adv Drug Deliv Rev 58:996–1008
Schwarz C, Mehnert W (1999) Solid lipid nanoparticles (SLN) for controlled drug delivery II. Drug incorporation and physicochemical characterization. J Microencapsul 16:205–213
Schwarz C, Mehnert W, Lucks JS, Müller RH (1994) Solid lipid nanoparticles (SLN) for controlled drug-delivery.1. Production, characterization and sterilization. J Control Release 30:83–96
Siekmann B, Westesen K (1994) Thermoanalysis of the recrystallization process of melt-homogenized glyceride nanoparticles. Colloids Surf B Biointerfaces 3:159–175
Souto EB, Almeida AJ, Müller RH (2007) Lipid nanoparticles (SLN (R), NLC (R)) for cutaneous drug delivery: structure, protection and skin effects. J Biomed Nanotechnol 3:317–331
Souto EB, Müller RH (2007) Lipid nanoparticles (SLN and NLC) for drug delivery. In: Domb AJ, Tabata Y, Ravi Kumar MNV, Farber S (eds) Nanoparticles for pharmaceutical applications, Chap. 5. American Scientific Publishers, Stevenson Ranch, CA, pp 103–122
Souto EB, Wissing SA, Barbosa CM, Müller RH (2004a) Comparative study between the viscoelastic behaviors of different lipid nanoparticle formulations. J Cosmet Sci 55:463–471
Souto EB, Wissing SA, Barbosa CM, Müller RH (2004b) Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. Int J Pharm 278:71–77
Stouch TR, Gudmundsson O (2002) Progress in understanding the structure-activity relationships of P-glycoprotein. Adv Drug Deliv Rev 54:315–328
Thole M, Nobmanna S, Huwyler J, Bartmann A, Fricker G (2002) Uptake of cationzied albumin coupled liposomes by cultured porcine brain microvessel endothelial cells and intact brain capillaries. J Drug Target 10:337–344

Tiwari SB, Amiji MM (2006) A review of nanocarrier-based CNS delivery systems. Curr Drug Deliv 3:219–232
Trickler WJ, Nagvekar AA, Dash AK (2008) A novel nanoparticle formulation for sustained paclitaxel delivery. AAPS PharmSciTech 9:486–493
Ugazio E, Cavalli R, Gasco MR (2002) Incorporation of cyclosporin A in solid lipid nanoparticles (SLN). Int J Pharm 241:341–344
Varia JK, Dodiya SS, Sawant KK (2008) Cyclosporine a loaded solid lipid nanoparticles: optimization of formulation, process variable and characterization. Curr Drug Deliv 5:64–69
Videira MA, Botelho MF, Santos AC, Gouveia LF, de Lima JJ, Almeida AJ (2002) Lymphatic uptake of pulmonary delivered radiolabelled solid lipid nanoparticles. J Drug Target 10:607–613
Vyas TK, Shahiwala A, Marathe S, Misra A (2005) Intranasal drug delivery for brain targeting. Curr Drug Deliv 2:165–175
Wang JX, Sun X, Zhang ZR (2002) Enhanced brain targeting by synthesis of 3′, 5′-dioctanoyl-5-fluoro-2′-deoxyuridine and incorporation into solid lipid nanoparticles. Eur J Pharm Biopharm 54:285–290
Westesen K, Bunjes H (1995) Do nanoparticles prepared from lipids solid at room-temperature always possess a solid lipid matrix. Int J Pharm 115:129–131
Westesen K, Bunjes H, Koch MHJ (1997) Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. J Control Release 48:223–236
Westesen K, Siekmann B (1997) Investigation of the gel formation of phospholipid-stabilized solid lipid nanoparticles. Int J Pharm 151:35–45
Westesen K, Siekmann B, Koch MHJ (1993) Investigations on the physical state of lipid nanoparticles by synchrotron-radiation X-ray-diffraction. Int J Pharm 93:189–199
Wissing SA, Kayser O, Müller RH (2004) Solid lipid nanoparticles for parenteral drug delivery. Adv Drug Deliv Rev 56:1257–1272
Wolburg H, Lippoldt A (2002) Tight junctions of the blood-brain barrier: development, composition and regulation. Vascul Pharmacol 38:323–337
Wu CY, Benet LZ (2005) Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. Pharm Res 22:11–23
Yang S, Zhu J, Lu Y, Liang B, Yang C (1999a) Body distribution of camptothecin solid lipid nanoparticles after oral administration. Pharm Res 16:751–757
Yang SC, Lu LF, Cai Y, Zhu JB, Liang BW, Yang CZ (1999b) Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. J Control Release 59:299–307
Yim D, Cipolla D, Shekunov BY, Chattopadhyay P, Boyd B (2005) Feasibility of pulmonary delivery of nano-suspension formulations using the AERx System. J Aerosol Med 18:101–102
Young TJ, Johnson KP, Pace GW, Mishra AK (2004) Phospholipid-stabilized nanoparticles of cyclosporine A by rapid expansion from supercritical to aqueous solution. AAPS PharmSciTech 5:E11
Zhang N, Ping Q, Huang G, Han X, Cheng Y, Xu W (2006a) Transport characteristics of wheat germ agglutinin-modified insulin-liposomes and solid lipid nanoparticles in a perfused rat intestinal model. J Nanosci Nanotechnol 6:2959–2966
Zhang N, Ping Q, Huang G, Xu W, Cheng Y, Han X (2006b) Lectin-modified solid lipid nanoparticles as carriers for oral administration of insulin. Int J Pharm 327:153–159
Zhang Q, Shen Z, Nagai T (2001) Prolonged hypoglycemic effect of insulin-loaded polybutylcyanoacrylate nanoparticles after pulmonary administration to normal rats. Int J Pharm 218:75–80
Zimmermann E, Souto EB, Müller RH (2005) Physicochemical investigations on the structure of drug-free and drug-loaded solid lipid nanoparticles (SLN) by means of DSC and 1H NMR. Pharmazie 60:508–513
zur Mühlen A, zur Mühlen E, Niehus H, Mehnert W (1996) Atomic force microscopy studies of solid lipid nanoparticles. Pharm Res 13:1411–1416

Viral Vectors for Gene Transfer: Current Status of Gene Therapeutics

Regine Heilbronn and Stefan Weger

Contents

R. Heilbronn (✉)
Institute of Virology, Charité – Universitätsmedizin Berlin, Hindenburgdamm 27 12203 Berlin, Germany
e-mail: regine.heilbronn@charite.de

M. Schäfer-Korting (ed.), *Drug Delivery*,
Handbook of Experimental Pharmacology 197,
DOI 10.1007/978-3-642-00477-3_5, © Springer-Verlag Berlin Heidelberg 2010

Abstract Gene therapy for the correction of inherited or acquired disease has gained increasing importance in recent years. Successful treatment of children suffering from severe combined immunodeficiency (SCID) was achieved using retrovirus vectors for gene transfer. Encouraging improvements of vision were reported in a genetic eye disorder (LCA) leading to early childhood blindness. Adeno-associated virus (AAV) vectors were used for gene transfer in these trials.

This chapter gives an overview of the design and delivery of viral vectors for the transport of a therapeutic gene into a target cell or tissue. The construction and production of retrovirus, lentivirus, and AAV vectors are covered. The focus is on production methods suitable for biopharmaceutical upscaling and for downstream processing. Quality control measures and biological safety considerations for the use of vectors in clinical trials are discussed.

Keywords Viral vectors · Gene therapy · Adeno-associated virus · Retrovirus · Lentivirus · Production methods · Safety considerations

Abbreviations

AAV	Adeno-associated virus
AAVS1	Adeno-associated virus integration site 1
Ad	Adenovirus
ADA	Adenosine deaminase
cDNA	Complementary DNA
CMV	Cytomegalovirus
CNS	Central nervous system
cPPT	Central polypurine tract
CsCl	Caesium chloride
DOC	Deoxycholate
EIAV	Equine infectious anemia virus
ELISA	Enzyme-linked immunosorbent assay
FGFR	Fibroblast growth factor receptor
GFP	Green fluorescent protein
GMP	Good manufacturing practice
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
ITR	Inverted terminal repeat
lacZ	β-Galactosidase
LCA	Leber congenital amaurosis
LMO2	LIM domain only 2 (rhombotin-like 1)
MoMLV	Moloney murine leukemia virus
PCR	Polymerase chain reaction
PIC	Pre-integration complex
RCV	Replication-competent virus

RRE	*Rev* responsive element
scAAV	Self-complementary AAV
SCID	Severe combined immunodeficiency
SDS-PAGE	SDS polyacrylamide gel electrophoresis
SF9	*Spodoptera frugiperda* cell line
SIN	Self-inactivating
U3 region	Unique 3′ region
VA-RNA	Viral associated RNA
VP	Viral protein
VSV	Vesicular stomatitis virus

1 Gene Therapy: Definition and State of the Art

The original concept of gene therapy was to complement or replace the function of a defective gene by introduction of a functional gene copy into a target cell for protein expression. Variations of this original concept have led to the development of gene therapy approaches to induce, harness, or modulate gene expression by various DNA or RNA molecules. Full-length genes leading to expression of a particular protein, but also truncated versions thereof, are being expressed that either complement or interfere with gene expression. In addition, various modulatory and interfering RNA molecules, like antisense RNA, shRNA, etc. are being expressed that lead to more sophisticated regulation at the transcriptional or post-transcriptional level of gene expression. In view of this extended repertoire of potential applications, gene therapy should be defined today as a means of drug delivery, where the drug is a nucleic acid, either DNA or RNA, delivered to a target cell by a specific pharmaceutical formulation that has to fulfill two goals. The active principle, DNA or RNA, has to be protected in a way that allows safe travel to the site of action. Unprotected, naked DNA or RNA is prone to degradation. In addition, the protective shield must be efficient in attaching to the cell membrane and must enable cell entry, followed by safe travel and efficient delivery to the intracellular site of action, mostly the cell nucleus.

To solve the problems associated with protection of the DNA or RNA molecules and ensure efficient and safe delivery across cell membranes, strategies are being exploited that viruses have evolved over millions of years. Viral nucleic acids are protected by a protein core, which is encoded by the virus itself. A number of viruses carry an additional envelope, a coat formed of a cell-derived lipid bilayer with embedded viral proteins. The three-dimensional structure and the binding characteristics of exposed viral proteins of the outer capsid or envelope ensure attachment to cell receptors. This initiates entry of the virus into the cell. Due to the evolved efficiency of viral entry, the majority of gene therapy approaches rely on engineered human or animal viruses that transport a foreign gene of interest.

In gene therapy the drug DNA or RNA is used to treat a wide spectrum of diseases covering all major disease groups. This is reflected by the numbers of current clinical trials of gene therapy in the year 2008: 65% aim at treating cancer, 9% cardiovascular diseases, 8% monogenic disorders, 7% infectious diseases, 2% neurological and eye diseases and the remaining few percent cover a variety of additional applications. Gene therapy is generally viewed as an option to treat otherwise untreatable and life-threatening diseases. High hopes and bitter failures have accompanied gene therapy ever since the first trials were initiated more than 15 years ago. In spite of many pitfalls, impressive and lasting successes have been documented, as in the case of children suffering from X-linked SCID disease, a genetic form of severe combined immunodeficiency (SCID). These children can only survive in an absolutely sterile environment. Retrovirus vector-mediated gene replacement of the deficient gene in transduced T-cells led to complete restoration of immune function in the majority of patients (Cavazzana-Calvo et al. 2000; Gaspar et al. 2004). Unfortunately, three to five years after successful therapy, four out of 20 initially treated children developed leukemia. In all cases retrovirus LTR-mediated activation of a nearby cellular oncogene was found. At present 18 of the initial 20 children are alive and well with restored immunity (Kohn and Candotti 2009). Recently, the successful long-term follow-up of retrovirus-mediated gene therapy of the ADA form of SCID was reported. All treated patients are alive and eight out of ten have maintained excellent and persistent immune reconstitution without signs of adverse effects up to 8 years after their initial treatment (Aiuti et al. 2009).

The year 2008 witnessed another impressive success of gene therapy with a different disease and target tissue and achieved by a different kind of vector, derived from adeno-associated virus (AAV). Gene therapy for Leber congential amaurosis, a monogenetic eye disease that leads to early childhood blindness, led to impressive improvement of vision in the treated eyes of juveniles in an advanced stage of the disease. The lasting improvements give hope that treatment in early childhood before the onset of disease may eventually prevent disease progression and maintenance of sight. This was shown previously in a dog model of the disease, where cure of the treated eye has been persistent for over eight years without signs of adverse effects (Bainbridge et al. 2008; Hauswirth et al. 2008; Maguire et al. 2008). AAV vectors are especially efficient in neuronal and retinal gene transfer where they lead to long-term gene expression without apparent decline after months and years of available follow-up, which raises hopes for other central nervous system and eye diseases (Wilson et al. 2008).

A variety of gene therapy vectors are currently used in gene therapy trials, based on a variety of human or animal viruses suitable for the incorporation and transport of a gene of interest into a specific target cell. Although shielding methods have improved over the years to efficiently protect naked or plasmid DNA from premature degradation, viruses are in general superior and the majority of gene therapy protocols make use of recombinant viral vectors. The aforementioned clinical success stories in gene therapy relied on retroviral vectors for the hematopoietic system, and on adeno-associated virus (AAV)-based vectors, respectively.

Additional viral vectors in current use are mostly based on adenovirus, but also on others like vaccinia/poxvirus or herpesvirus-based vectors. All of these vectors are highly immunogenic. Adenovirus, due to its high transgene capacity and ease of vector production, has been widely applied in clinical trials until the disturbing death of a patient following the application of a high dose of adenovirus, which apparently led to overwhelming or inadequate immune stimulation. As long as the inherent problem of high immunogenicity of these vectors remains unsolved, their production and application will remain strictly experimental and academic. This chapter therefore covers drug design and drug delivery for adeno-associated virus vectors and for retrovirus/lentivirus vectors, whose safety profiles are more defined, where bioreactor upscaling methods are already advanced, and, most importantly, where the results from clinical studies appear eventually to be able to fulfill the early promises of gene therapy.

2 AAV Vectors for Gene Therapy

AAV vectors are derived from adeno-associated virus, a small single-stranded DNA virus originally described as a contaminant of adenovirus preparations that gave the virus its name (Muzyczka and Berns 2001).

2.1 Overview of Properties

AAV vectors combine a spectrum of favorite properties that accelerated their widespread use despite the fact that AAV only offers limited transgene capacity with a maximum of 4.5 kb for a conventional AAV vector containing single-stranded DNA. The well-documented advantages of AAV vectors include: AAV wildtype strains infect man and nonhuman primates without evidence of disease or adverse effects. The AAV capsid displays very low immunogenicity combined with a high degree of chemical and physical stability even at temperatures up to 60°C for extended time periods. This allows rigorous methods for virus purification and concentration, a major advantage for bioreactor scale production, as is required for application in the clinic. AAV vector transduction leads to sustained transgene expression in postmitotic, nondividing cells, as shown in brain, retina, muscle, and liver. As a result, long-term gain of function is achieved in mice, dogs, and primates, leading to cure of the underlying disease. Last but not least, an increasing number of AAV subtypes and variants offer the unique possibility to specifically target selected tissues and cell types.

Unfortunately, AAV vector production has long been very laborious. Meanwhile suitable upscaling protocols for clinical-scale production of AAV vectors have been developed. Bioreactor manufacturing will eventually be needed to meet the requirements of gene therapy with AAV vectors in the clinic.

2.2 AAV Structure

AAV represents a nonenveloped, small, single-stranded DNA-containing virus encapsidated by an icosahedral, 20 nm diameter capsid composed of the AAV structural proteins VP1, VP2, and VP3 in a 1:1:10 ratio. To date, at least 12 distinct serotypes have been described, all derived from man and nonhuman primates. The human serotype AAV-2 is considered as the prototype strain and has been used in the majority of early studies that unraveled the molecular properties of AAVs. AAV-2 is also the strain used in the initial development of AAVs as a vector system for mammalian gene transfer. AAV-2 contains a 4.7 kb linear, single-stranded DNA genome with two open reading frames *rep* and *cap*, which are flanked by 145 bp inverted terminal repeats (ITRs). These repeats contain the origins of DNA replication, the packaging signals and also mediate chromosomal integration. The ITRs are the only AAV elements maintained in AAV vector construction. The reading frame in the left-hand side of the genome, called *rep*, codes for four overlapping nonstructural proteins. Two of these, Rep78 and a C-terminal splice variant called Rep68, are expressed from the AAV p5 promotor. In addition, N-terminally truncated versions of either protein, called Rep52 and Rep40, respectively, are expressed from the internal p19 promoter. Rep78/68 are required for most steps of the AAV life cycle, including the regulation of gene expression, chromosomal integration, and AAV DNA replication (Muzyczka and Berns 2001). Rep78/68 initiate AAV DNA replication at the hairpin-structured ITRs, an essential step also for AAV vector production. The *cap* gene is expressed from the p40 promoter and codes for the three capsid proteins, VP1, VP2, and VP3. These are generated by a combination of differential splicing and translation initiation.

2.3 AAV Life Cycle

The characteristic bipartite AAV life cycle has been well characterized in cell culture (Muzyczka and Berns 2001). Productive AAV replication requires co-infection with an unrelated helpervirus such as adenovirus or herpes simplex virus. In the absence of a helpervirus, AAV integrates into the host cell genome with a high preference for a specific region on human chromosome 19, called AAVS1. The AAV Rep78/68 protein appears to mediate the site-specificity of chromosomal integration by the formation of a ternary complex with the Rep binding site on the AAV ITRs and a homologous DNA element in the human genome that facilitates nearby integration. Intact integrated AAV genome copies can be rescued and amplified by superinfection with a helpervirus leading to productive infection with release of progeny AAV. Until recently the in vivo pattern of AAV infection remained largely unknown. Recently, AAV-2 genomes were detected by PCR analysis in human tissue samples and evidence was provided that the AAV genomes persisted as circular nuclear episomes.

2.4 Cell Receptors Used by AAV

AAV infects a wide range of cell types, which is reflected by its interaction with ubiquitous cell surface receptors. Various glycan residues on the cell surface serve as primary receptors. For AAV-2 and AAV-3 these are heparin sulfate proteoglycans. AAV-4 uses O-linked and AAV-5 N-linked sialic acids as primary receptors. The latter also bind to AAV-6 and AAV-1. A variety of coreceptors, which facilitate cell entry by receptor-mediated endocytosis such as fibroblast growth factor receptor 1 (FGFR-1), hepatocyte growth factor receptor, laminin receptor, and various subforms of integrins have also been described. For AAV-5, platelet-derived growth factor receptor appears to serve as co-receptor. For other AAV serotypes, specific cell receptors have not yet been assigned. At present it appears that serotype-specific variations in cell tropism reflect their interaction with distinctive receptor combinations.

2.5 AAV Vector Production

In principle, AAV vectors are designed in such a way that only the inverted terminal repeats (ITR) are retained as *cis*-active DNA elements required for AAV vector replication and packaging. The AAV coding regions with the respective promoters can be replaced by a foreign gene of interest. Up to 4.5 kb of transgene DNA can be incorporated. This includes the promoter and additional regulatory elements. For a more comprehensive overview of the principles of AAV vector design, the reader is referred to a number of excellent reviews (Snyder and Flotte 2002; Grimm and Kay 2003; Choi et al. 2007). To use AAV vectors as drugs in clinical application, a variety of validated AAV vector production protocols have emerged and been adapted to meet the standards of good manufacturing practice (GMP). The suitability of a particular protocol for an intended preclinical or clinical application depends on the scale of production as outlined below.

2.5.1 AAV Vector Production by Cotransfection of Packaging Plasmids

Production of AAV vectors is complicated by the fact that not only the deleted AAV genes *rep* and *cap*, but also the required helpervirus functions, have to be supplied by the cell line. At present, these requirements are met by transfection of plasmids for the well-characterized adenovirus (*Ad*) helper genes, VA-RNA, E2A and E4 together with the AAV *rep* and *cap* genes, either on two separate plasmids or combined on a single helper construct. AAV vector plasmids containing the transgene between the AAV ITRs are cotransfected into 293 cells, a human cell line that constitutively expresses the remaining *Ad* helper genes required, E1A and E1B (Fig. 1). This leads to amplification and packaging of the AAV vector carrying the foreign DNA (Grimm et al. 1998; Xiao et al. 1998). The plasmid transfection

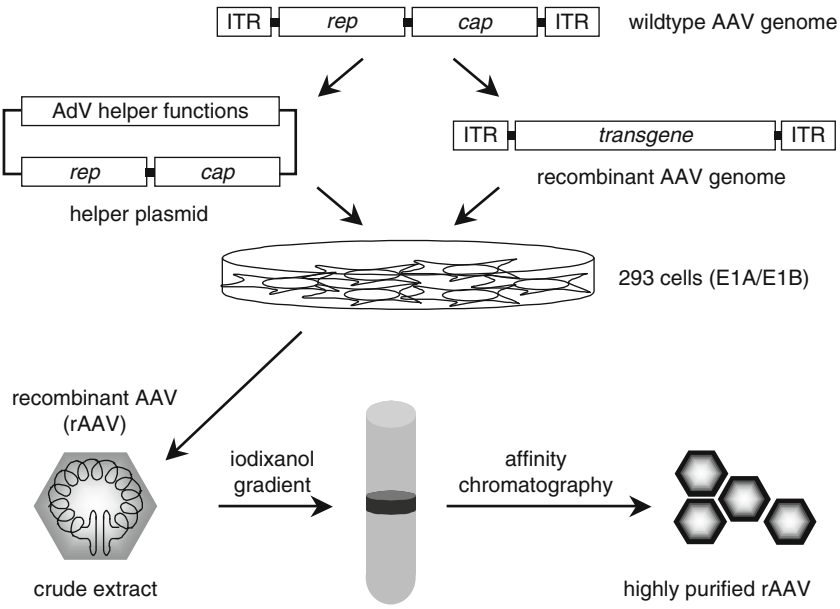


Fig. 1 Generation and purification of AAV-vectors. For the generation of recombinant AAV vectors (rAAV), the AAV genes *rep* and *cap* are replaced by the transgene of interest, including a heterologous promoter and additional regulatory sequences of choice. This only leaves the 145-bp inverted terminal repeats (ITR) at either end of the vector as sole AAV-derived DNA sequences. The Rep and Cap proteins needed for amplification and packaging of the AAV vector genome are provided *in trans* from a transfected helper plasmid, which also contains the required adenovirus type 5-derived helper functions, E2A, E4ORF6 and VA-RNA. Adenovirus E1A and E1B are provided by 293 cells that are used for rAAV packaging. Crude cell lysates are prepared and supernatants containing rAAV vectors are purified by iodixanol density centrifugation and subsequent affinity or ion exchange chromatography

technique eliminates the problem of contamination of the AAV vector preparations by infectious helper adenovirus that had been previously encountered after use of wildtype adenovirus to provide the helper functions. In addition, when compared to the traditional adenovirus infection protocol, AAV vector titres were significantly increased. Sustained cell viability and prolonged viral gene expression add up to this effect. Thus, either one of the two or three plasmid cotransfection protocols constitute the method of choice for laboratory-scale production of AAV vectors. For clinical applications, a considerable scale-up is required, which is difficult to accomplish with protocols that depend on a DNA transfection step.

2.5.2 Upscaling of AAV Vector Production

The need for bioreactor-scale manufacturing processes of AAV vectors for clinical use led to a series of small but cumulative advancements and conceptual changes in the methodology, as will be described in more detail below.

AAV packaging cell lines. An ideal large-scale production method in terms of reproducibility and ease of handling would rely on stable cell lines that continuously express all required components for the packaging process in sufficient quantities. Stable HeLa-, 293- or A549-derived cell lines expressing the AAV *rep*/*cap* genes were extensively tested for bioreactor-scale AAV vector production. Unfortunately most of these cell lines turned out to be unstable. This appeared to be due to the inherent cytotoxicity of the Rep component, which is further enhanced by adenovirus E1A. Elaborate systems were designed to control cytotoxicity by regulating E1A and Rep gene expression. Neveretheless, AAV vector yields remained low and difficult to control with increasing cell passage numbers. It is generally believed that the yields of AAV vectors eventually decline after passage 40–50, which further limits the use of producer cell lines for bioreactor-based AAV vector production.

Recombinant helper virus strains for AAV production. The inherent instability of AAV *rep*/*cap* containing cell lines stimulated the search for alternative large-scale AAV packaging concepts. An obvious and elegant concept appeared to be the incorporation of the required AAV components *rep* and *cap* into a single recombinant "superhelpervirus" based either on adenovirus or herpes simplex virus (HSV). Cell infection with a *rep*/*cap*-expressing helpervirus was thought to provide adequately regulated production of all the required components for AAV vector production. The major advance of the method is the possibility to introduce all components for AAV vector production by an infection step instead of DNA transfection. Infection protocols are superior for bioreactor scale-up with many of the basic production principles already established for vaccine generation. Initially, many labs concentrated on the construction of recombinant adenovirus-based vectors expressing the AAV *rep*/*cap* genes under the control of their cognate promoters. Unfortunately, most of the generated adenovirus recombinants proved to be unstable with regard the AAV *rep* component, which was quickly deleted or otherwise inactivated. This was not entirely unexpected, since Rep had been shown before to considerably interfere with adenovirus replication. HSV serves as an alternative helpervirus, which promotes productive AAV infection with comparably high efficiency as adenovirus. In contrast to recombinant adenoviruses, HSV-based recombinants easily tolerated the incorporation of an AAV *rep*/*cap* gene cassette (Heilbronn et al. 2003). HSV DNA replication is not significantly affected by simultaneous Rep expression and AAV DNA replication, which appears to be an explanation for the observed stability of AAV *rep*/*cap*-expressing HSV recombinants. The first generation of recombinant *rep*/*cap*-expressing wildtype HSVs was further refined by the introduction of mutations in essential HSV genes not required for AAV vector production (Conway et al. 1999). This extended the time frame of the AAV production process and ensured that progeny HSV would not be produced as a contaminant of the AAV vector stocks. Recombinant *rep*/*cap*-expressing HSVs can be used to infect cell lines carrying an integrated AAV vector genome that is rescued upon infection with the recombinant helpervirus (Toublanc et al. 2004). Alternatively, coinfection with a second recombinant herpesvirus carrying the AAV vector backbone with the transgene can be employed to initiate AAV vector

production. The latter design has proven to be more versatile, since only the HSV component and not the cell line has to be redesigned for a new AAV vector to be produced. In addition, variations of the infective doses and the mode of infection allows fine-tuning of the production process. Reported yields of AAV vectors using the recombinant HSV-infection method have risen up to 10,000 viral units per cell (Kang et al. 2009).

An alternative infection-based method for large-scale production of AAV vectors uses infection of insect cells with a combination of recombinant baculoviruses (Urabe et al. 2002). SF9 suspension cells are co-infected with three baculovirus vectors expressing AAV *rep*, AAV *cap* and the recombinant AAV vector backbone. This system was shown to yield high amounts of AAV-2-based vectors in bioreactors. However, the design of the individual components has to be newly and carefully fine-tuned for alternative AAV serotypes and for new vector backbones. It remains to be shown whether the adaptation of the baculovirus-based production system will remain competitive with the HSV-based production scheme.

2.5.3 AAV Vector Purification

A fortunate circumstance for AAV vector production and scale-up is the high stability of AAV virions, which allows rigorous purification schemes. Over the years enormous improvements in AAV vector purification have raised the per cell burst sizes from under 1 up to 100 vector-transducing units per cell with the transfection-based systems. Introduction of the infection-based production methods described above led to improved outputs of 5,000–10,000 transducing units per cell. Purification of AAV vectors not only is required to remove residual cell and viral contaminants, but also enhances the potency of the vector and increases the ratio of AAV infectious units to AAV particles. Traditionally, AAV vectors were purified by caesium chloride gradient centrifugation. Unfortunately, AAV infectivity is lost upon continuous exposure to CsCl and cannot be restored by removal of the CsCl by dialysis or alternative purification steps. Therefore, alternative procedures were explored as an initial purification step. The method of choice today is to band AAV on iso-osmotic iodixonal density gradients (Zolotukhin et al. 1999). This protocol was shown to efficiently eliminate cell-derived contaminations and empty AAV particles leading to more favorable ratios of infectious to non-infectious vector particles. Initially crude cell extracts are generated by deoxycholate (DOC)-mediated cell lysis, freeze–thaw, a combination of both, or an alternative method for mechanical cell lysis. Then, crude cell extracts are treated with benzonase to digest cell-derived and non-encapsidated viral nucleic acids. The extract is loaded on the iodixonal step gradient for subsequent ultracentrifugation (Fig. 2). The banded AAV virions are collected at the interface of the 54 and 40% iodixanol fractions and are sufficiently pure for the subsequent step of heparin sulfate or ion exchange chromatography. The choice of the chromatography matrix is determined by the AAV serotype to be produced. AAV vectors are subsequently dialyzed against the buffer of choice. Alternatively, ultrafiltration can be applied for further

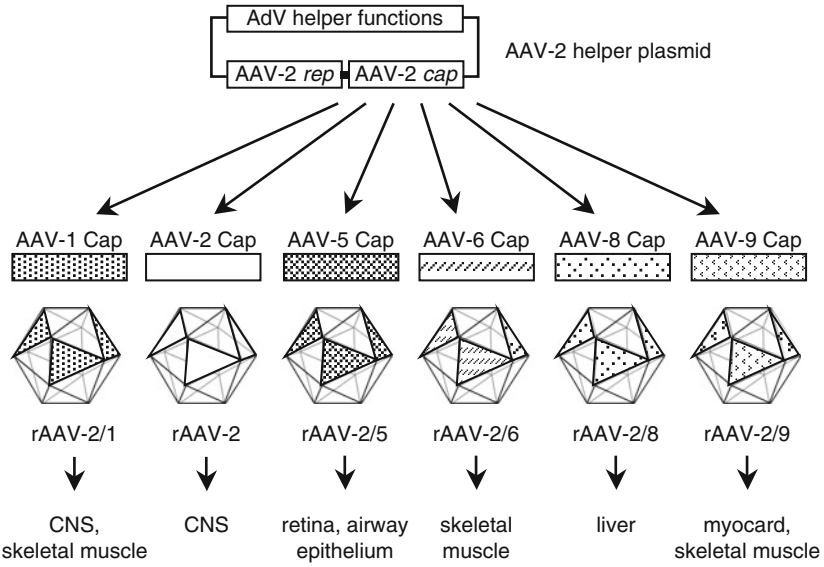


Fig. 2 AAV sero(pseudo)-type vectors. To produce AAV pseudotype vectors harboring the viral capsid derived from a different AAV serotype, the AAV-2 derived vector backbone and the *rep* gene of AAV-2 are maintained. Thus, amplification of rAAV still relies on the AAV-2 *rep* gene and the AAV-2 ITRs. Only the AAV-2 derived *cap* gene is replaced by the *cap* gene of a different AAV serotype, so that the vector genomes are cross-packaged into capsids of the serotype of choice. AAV serotype vectors display improved transduction efficiency for certain tissues or target cells, or variant distribution patterns in target tissues with mixed cell phenotype as compared to first-generation AAV-2 vectors

concentration accompanied by a simultaneous exchange against the buffer of choice. Detailed protocols for the described purification schemes can be found in a recent review (Zolotukhin 2005). Vectors produced according to the latter protocol are highly concentrated and extremely pure, as is required for non-toxic, long-term gene expression in the brain, eye, and other tissues.

2.5.4 Quantification of AAV Vector Yields

To quantify and compare AAV vector yields, quantitative PCR determination of AAV genome copy numbers is the method of choice. Although the amount of infectious virus is often the preferred information, the infectivity of a particular AAV vector preparation may be difficult to evaluate. It is very dependent on the readout of the transgene product. Furthermore, even with a sensitive assay for a particular transgene, the viral titers determined critically depend on the exact conditions used for the transduction assay. These include the seeding density of the cells, the growth rate, and the availability of appropriate receptors on the test

cell line. The increasing use of tissue-specific promoters and of AAV pseudotype capsids further limits the comparability of infectious AAV titers. Therefore to compare AAV vector titers not only from batch to batch but also for divergent transgenes and AAV serotype capsids, quantitative PCR determination of the DNA copy number serves as the gold standard. Reliable and comparable results require AAV vector preparations that are sufficiently purified and free of contaminating DNA that originates from initially transferred packaging plasmids or helper viruses. Therefore a DNAse or benzonase step in the purification scheme is mandatory. In any case, the so-called "DNA titers" of purified AAV vector stocks do not reflect the amount of infectious virus present on a 1:1 scale, due to an excess of defective, non-infectious particles. Extensive purification of prototype AAV-2 vectors using iodixonal gradient centrifugation followed by chromatography generally leads to ratios of infectious to DNA-containing viral particles in the range between 1:50 and 1:100. In crude cell extracts or in the case of partially purified vectors this ratio may be even lower. The detectability of infectious particles is limited by the availability of the required cellular receptors and by the efficiency of intracellular transduction including nuclear entry, uncoating and second-strand DNA synthesis. To evaluate the efficiency of the AAV vector production process, the determination of the burst size, i.e., the amount of infectious, or cell-transducing units per initially infected cell, is a good measure. In summary, biotechnical optimization of AAV production schemes over recent years has led to an enhancement of AAV vector production efficiency from 1–10 transducing units/cell initially to more than 5,000–10,000 transducing units/cell with the more advanced HSV-based or baculovirus-based production schemes followed by sophisticated scale-up purification protocols (Urabe et al. 2002; Zolotukhin 2005; Kang et al. 2009).

2.6 AAV Vector Persistence and Safety

As outlined initially, AAV vectors are characterized by stable long-term gene expression in quiescent or postmitotic cells without discernible decline in transgene expression. For the complementation of a defective gene, this is the ideal gene expression profile. However, questions arise as to the safety of AAV persistence. Numerous studies have shown that wild-type AAV-2 integrates into the host cell genome with a certain preference for a specific region on human chromosome 19q13.42. The specificity of AAV wildtype integration is mediated by AAV Rep, which is absent in AAV vectors. AAV vectors were shown to persist as nuclear episomes, often in the form of large concatameric DNA molecules that rarely integrate into the host cell genome. In the occasional integration events described after cell selection in culture, no site preferences were observed. The predominant episomal state of AAV vectors in quiescent cells ensures an extraordinarily high safety profile for clinical application (Miller et al. 2005).

2.7 AAV Split Vectors

Compared to adenovirus or retro/lentivirus-based vectors, AAV vectors are more limited in packaging capacity. Gene cassettes of up to 4.5 kb including the promoter and other regulatory elements represent the maximum transgene capacity. This is sufficient for many genes, especially if expressed as cDNAs. However, occasionally the expression of larger gene cassettes is inevitable. To meet these needs, so-called AAV split vectors have been developed that divide the transgene of interest into two parts. The first AAV vector carries the promoter and the coding region for the N-terminus of the transgene product, followed by a splice donor site. The second AAV vector carries the coding region for the C-terminal part of the transgene, preceded by a splice acceptor site. Both vectors are packaged separately. Upon co-infection of target cells with the two vectors, recombination events lead to vector multimerization involving head-to-tail concatamerization of vector genomes. In these vectors, the two exons of the split gene are only separated by the splice sites with one AAV ITR in between. Appropriate splicing of the mRNA precursor leads to translation of the precisely fused transgene. In an animal model that exploited this technology, sufficient transgene levels for the correction of a muscular gene defect were demonstrated (Lai et al. 2005). Due to the need for simultaneous infection with two vectors, the split vector technology is not yet as efficient as the traditional method with a single AAV vector, but certainly opens some prospect for the future.

2.8 Dimeric, Self-Complementary (sc) AAV Vectors

A limitation of AAV vector application is the relatively slow onset of transgene expression. This is due to the fact that AAV vectors harbor a single-stranded DNA genome that cannot be transcribed immediately after nuclear entry. The host cell DNA replication machinery is needed to generate the double-stranded DNA template required for mRNA transcription. In the preferred targets for AAV transduction, quiescent or postmitotic cells, the host cell DNA replication machinery is however largely inactive. As a consequence, the onset of gene expression is delayed from days up to weeks until a plateau of transgene expression is reached. To circumvent these problems, so-called “dimeric” or self-complementary (sc) AAV vectors have been developed that are packaged as pseudo-double-stranded genomes. This is attained by a small deletion in the so-called terminal resolution site (trs) in one of the two ITRs so that only one ITR can be properly resolved in the course of DNA replication and packaging of the AAV vector. As a consequence, dimeric genomes are generated in a head-to-head conformation linked by the mutated ITR. These can fold back to a double-stranded form and thus provide a suitable template for immediate transcription after AAV vector transduction. scAAV vectors show an onset of gene expression within hours and reach their plateau level within days after AAV vector transduction of quiescent cells. The increased efficiency comes at the price of a reduced packaging capacity:

the packaging limit of scAAV vectors is 2.15 kb. This is enough for very small proteins and peptides and ideal for the expression of small regulatory RNAs such as those used in RNA interference. To meet the challenges of the reduced transgene capacity, small and optimized promoter and other regulatory elements have to be employed. Despite these limitations, the favorable expression kinetics of scAAV vectors has speeded up AAV vector use and stimulated novel developments for a wide range of even more tailored gene therapy applications (Choi et al. 2007).

2.9 Cell Targeting Strategies for AAV

As outlined initially, a variety of naturally occurring AAV serotypes are available and AAV vectors derived from them have been constructed. The production of AAV variant serotype vectors has gained momentum with the emergence of technologies to package AAV-2-based vector genomes into variant AAV capsids, the so-called AAV pseudotype vectors. Technically this is accomplished by co-transfection of helper plasmids containing the AAV-2 *rep* gene in combination with the *cap* gene from a variant AAV serotype (Fig. 2). AAV-2 Rep is required for DNA replication of the AAV-2-based ITR of the AAV vector backbone. AAV-2 Rep will package the AAV-2 based single-stranded vector DNA into the pre-formed capsids formed by capsid proteins of the variant AAV serotype. Packaging plasmids for variant serotype capsids have been developed for virtually every naturally occurring serotype known today and also for many derivatives thereof (Grimm et al. 2003). This speeded research to find the optimal AAV serotype to target a particular tissue or cell type in vivo. The nomenclature of the pseudotype vectors reflects the origins of the AAV-ITRs in the vector construct and the respective *rep* gene on the one side and the serotype of the capsid proteins on the other. AAV-2/1 vectors for example contain the ITRs from serotype AAV-2 and the capsid from serotype AAV-1. AAV-2/1 vectors show an excellent targeted gene expression profile in the central nervous system and generally lead to higher gene expression levels compared to the traditional AAV-2/2 vectors. Other pseudotype vectors like AAV-2/8 or AAV-2/9 were shown to lead to higher and more widespread gene expression in the CNS, but also in the liver, in skeletal muscle, and in heart muscle, respectively (Fig. 2). Depending on the therapeutic goal, either focussed or widespread and uniform gene expression is desirable. Parkinson disease is an example of the first, the monogenetic disorder α_1-antitrypsin deficiency leading to a general loss of function is an example of the latter. AAV-5 serotype vectors proved to be highly efficient in transducing the retina and the respiratory epithelium. AAV-8 is excellent for liver gene transduction, AAV-9 transduces the myocardium and skeletal muscle. Studies describing the distribution of the various serotype vectors in dependence of the site and mode of application are currently appearing at high speed. For a recent review refer to Li et al. (2008).

The repertoire of AAV capsids has been further refined and extended by technologies leading to the construction of chimeric vectors selected by directed

evolution approaches (Muller et al. 2003; Perabo et al. 2003). This technique uses vector backbones containing capsid protein libraries generated by fragmentation and shuffling of the known spectrum of capsid proteins. These were used for the selection of chimeric AAV pseudotypes optimized for binding to a target cell of choice. The techniques led to the isolation of AAV *cap* gene variants, which enabled the derived vectors to target cardiac endothelium and a variety of other cells and tissues that had formerly been refractory to AAV transduction. Analysis of the chimeric *cap* genes showed that in most cases the preferred cell surface binding of the parent virus, e.g., heparan sulfate proteoglycan for AAV-2, was shifted to alternative receptors.

2.10 Future Directions

Currently, the development of AAV vector technology is proceeding in two main directions. On the one hand, upscaling protocols for GMP-conforming bioreactor production are being increasingly refined to ensure efficient and cost-effective AAV vector production for clinical trials and for the manufacture of AAV vectors as drugs. On the other hand, AAV vectors for improved cell targeting and cell entry are designed based on a rational approach. The gain of specificity often goes along with a loss of infection efficiency, a pressing problem to be solved for future clinical application. A major step in this direction has been reported recently: the detailed study of the vector's fate after AAV entry revealed that AAV vectors bound to the cell surface are an inadequate measure of the amount of virus reaching the cell nucleus for gene expression. Studies with proteasome inhibitors showed that the majority of AAV capsids entering the cell are subject to ubiquitination and degradation and do not reach the nucleus. Based on the crystal structure of the AAV-2 capsid, candidate tyrosines within exposed capsids domains were identified as preferred target sites for ubiquitination. These single amino acids were exchanged by mutations which then led to a significant enhancement of intracellular AAV vector stability with an up to 100-fold enhancement of transgene expression (Zhong et al. 2008). Comparable amino acid exchanges in other serotypes showed the same effect (Petrs-Silva et al. 2009). These and other ongoing developments in AAV vector technology will enhance the ease of manufacturing for production of AAV vectors as drugs. A series of recent clinical trials showed remarkable successes and will further accelerate the development of the field.

3 Retrovirus Vectors for Gene Therapy

The retrovirus family of enveloped RNA viruses comprises six different genera found in birds and mammals (Goff 2001). These are grouped into simple retroviruses, also known as oncoretroviruses, and the so-called complex retroviruses, which

include lentiviruses and spumaviruses. Whereas the oncoretroviruses only harbor the minimal essential genes called *gag*, *pol*, and *env*, complex retroviruses code for additional regulatory and accessory proteins required for the fine-tuning of virus replication and persistence. Retrovirus-derived vectors used in clinical gene therapy are either derived from the oncoretrovirus or from the lentivirus family.

3.1 Retrovirus Structure and Life Cycle

All members of the retrovirus family harbor the so-called *gag*, *pol* and *env* genes. The *gag* gene codes for the capsid, matrix, link, and nucleocapsid proteins. These are components of the retroviral core, which is closely associated with two copies of the viral RNA genome. The *gag*-derived proteins are generated by proteolytic cleavage of the *gag* precursor protein. The *pol* gene encodes the reverse transcriptase, the integrase, and the retroviral protease. Usually a *gag-pol* precursor protein serves as template for further processing. The *env* gene codes for the envelope glycoproteins required for virus attachment and entry. The retrovirus life cycle involves cell entry, reverse transcription of the RNA genome into a complementary (c)DNA, and integration of the cDNA into the host cell genome. Transcription of full-length progeny RNA and of the mRNAs encoding regulatory and structural proteins is initiated from the integrated, proviral DNA copy. Assembly of new viral particles takes place at the plasma membrane. The *cis*-acting sequences required as regulatory signals are all contained within or adjacent to the long terminal repeat sequences (LTR) that flank both sides of the retroviral genome.

3.2 Design and Development of Oncoretroviral Vectors

All viral regulatory proteins engaged in cell entry, reverse transcription and integration are incorporated in the virus particle. Therefore the entire coding regions for viral proteins can be removed in the development of oncoretroviral vectors to accommodate a transgene of interest of up to 8kb. Only the packaging signal, the viral LTRs, and adjacent elements essential for reverse transcription and integration are maintained as viral sequences in the resulting recombinant vector construct. The first generation of oncoretroviral vectors had retained the entire LTRs including the retroviral enhancer and promoter sequences. These can also be partially replaced by various heterologous enhancer/promoter combinations such as inducible or tissue-specific elements.

For the production of oncoretroviral vectors, so-called packaging or producer cell lines are used that express the required retroviral proteins derived from *gag, pol,* and *env*. To prevent unwanted homologous recombination between the transgene-containing vector and the packaging constructs, packaging cell lines were

generated that expressed *gag*/*pol* and *env* from separate chromosomal loci. This reduces the risk of homologous recombination that leads to the generation of replication-competent retroviruses (Fig. 3A).

3.3 Self-Inactivating (SIN) Oncoretroviral Vectors

The application of first-generation oncoretroviral vectors was accompanied by a series of problems. Although the packaging genes were placed on two separate plasmids and cell clones were selected that harbor the genes on different genomic loci (see Section 3.2), the transgene expressing vector backbone was not entirely free of redundant DNA sequences. Occasionally but inevitably this led to the generation of replication-competent virus by homologous recombination. An additional disturbance was the inherent propensity of retrovirus to integrate into active chromosomal sites. Repeatedly this led to unwanted and uncontrollable LTR-mediated host cell gene activation. Both in animal models and in clinical trials, a series of side effects and adverse events due to uncontrolled oncogene activation were encountered. This cumulated in the development of several cases of leukemia in children that had years before been successfully treated for SCID. In all reported cases, retroviral vector-mediated activation of a nearby oncogene was demonstrated. Therefore, measures to control the risk of insertional mutagenesis were mandatory for a safer move forward with retrovirus vector-mediated gene therapy. Self-inactivating (SIN) oncoretroviral vectors were eventually developed, whose concepts and design are described below.

The first step in the development of SIN oncoretroviral vectors was to minimize sequence homologies between vector and helper constructs and simultaneously boost full-length RNA transcription for more efficient vector packaging. Non-retroviral promoters were chosen to replace the U3 promoter region within the 5′LTR. The U3 region typically drives transcription of a full-length RNA for viral encapsidation. Upon replacement of the U3 region within the 5′LTR by a heterologous promoter such as the strong cytomegalovirus (CMV) promoter, the inherent low LTR transcription rate is strongly enhanced. The hybrid CMV/LTR promoter combination leads to a boost in vector titers. When retroviral vectors infect target cells, reverse transcription and second-strand DNA synthesis involves the copying of the U3 region from the 3′LTR to the 5′LTR. This normally leads to reconstitution and repair of the 5′LTR (Fig. 3B). To avoid correction of the LTRs, SIN retroviral vectors were generated that have a deletion in the U3 region of the 3′LTR, comparable to that in the 5′LTR. When the deleted U3 region of the 3′LTR is copied to the 5′LTR it replaces and deletes the CMV promoter. Due to this deletion, the 5′LTR remains transcriptionally inactive after integration and cannot be rescued again. The transgene is expressed from an internal heterologous promoter of choice without further involvement of the LTRs. The SIN concept minimizes two risks of oncoretrovirus vectors: the risk of insertional mutagenesis caused by a functional retroviral LTR, and the risk of vector mobilization.

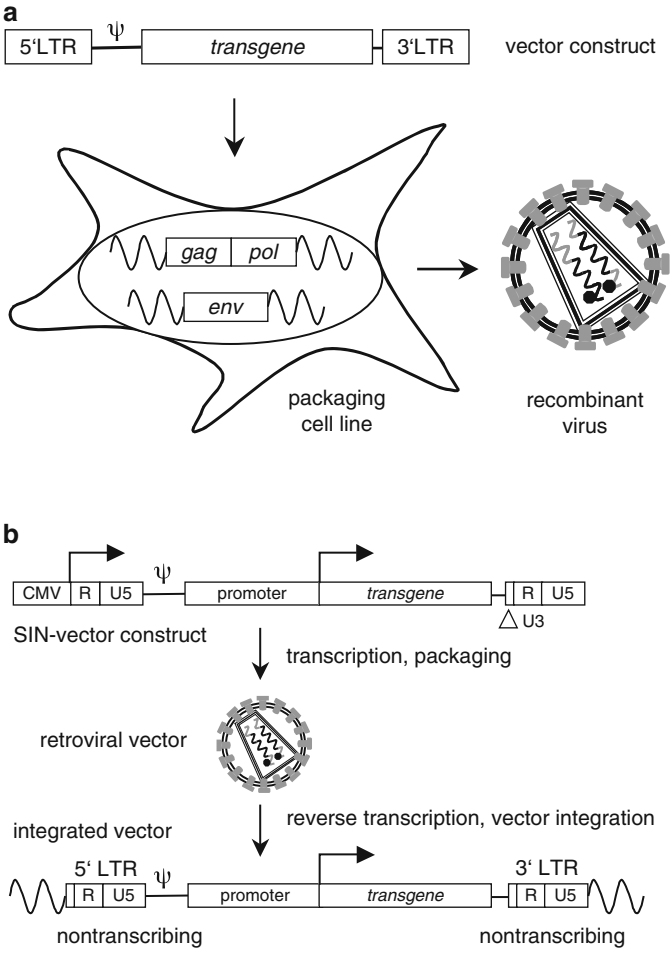


Fig. 3 Retroviral vectors. (**a**) In oncoretroviral vectors, the viral genes *gag*, *pol* and *env* are replaced by the transgene of interest. The retroviral LTRs and the adjacent packaging signal ψ are maintained. The Gag/Pol and Env protein are provided *in trans* from constructs integrated at separate loci in the host cell genome of the packaging cell line. (**b**) In self-inactivating (SIN) vectors, the U3 region of the 5′LTR is replaced by a heterologous enhancer such as that from the human cytomegalovirus (CMV), and the U3 region of the 3′LTR is partially deleted. The CMV/LTR hybrid promoter drives transcription of the vector genome for packaging of the vector. Upon vector entry into the target cell and reverse transcription the partially deleted 3′LTR devoid of promoter and enhancer sequences is copied to the 5′LTR. This leads to deletion of the CMV promoter element and renders the 5′LTR transcriptionally inactive. An internal heterologous promoter mediates transgene expression from the vector integrated into the host cell genome. The defective LTRs will no longer be able to activate cellular genes adjacent to the integration site

3.4 Design and Development of Lentiviral Vectors

Initially, retroviral vectors were derived from simple oncoretroviruses such as Moloney murine leukemia virus (MoMLV), a representative of the gammaretrovirus family. Unfortunately these viruses fail to infect nondividing, differentiated cells. This property is attributed to the inability of the retroviral preintegration complex to enter the nucleus unless the nuclear membrane is disrupted during mitosis (Miller et al. 1990). The preference for dividing cells may be an advantage for cancer therapy or for the treatment of rapidly proliferating cells, e.g., those of the hematopoietic lineage. Since the majority of target cells in the human body are quiescent or slowly dividing, the application spectrum of conventional (onco) retrovirus vectors remains limited.

This shortcoming stimulated interest in the development of integrating retrovirus vectors based on either lentiviruses such as equine infectious anemia virus (EIAV) or primate lentiviruses such as the human immunodeficiency virus (HIV). Research quickly focussed on the HIV-based lentiviral vectors, due to the availability of a comprehensive set of data on their molecular biology. HIV is able to infect nondividing cells that are in either the G0 or the G1 phase of the cell cycle. The HIV preintegration complex (PIC) has the ability to cross an intact nuclear membrane. In addition to the typical retroviral genes *gag*, *pol,* and *env*, HIV-1 synthesizes six accessory proteins Tat, Rev, Nef, Vif, Vpr, and Vpu, which modulate viral transcription, replication, and persistence. Of these, Rev is the only accessory protein that is maintained in lentiviral vector design. Rev is needed for nuclear export of unspliced and single-spliced HIV RNAs.

Despite their high complexity, the development of lentiviral vectors closely mimics the principles established for vectors derived from conventional (onco) retroviruses. On the vector backbone all viral sequences are removed except for the essential *cis*-acting sequences of the LTRs and for the packaging signal residing in the adjacent 5′ untranslated region. Also, the *rev* responsive element (RRE) is maintained to ensure Rev-binding (Fig. 4). The Rev protein is provided on a separate packaging construct. Two separate packaging constructs are used for the *gag*/*pol* and for *env* genes. To overcome the restricted host range of the glycoproteins encoded by HIV-1 *env*, lentiviral vectors can be pseudotyped with the vesicular stomatitis virus glycoprotein (VSV-G) that displays a broad host range. VSV-G-encoding *env* is expressed by replacement of the HIV *env* gene in the corresponding packaging construct (Fig. 4). Similar to the concepts established for oncoretroviral vectors, expression of the viral proteins is driven by a heterologous promoter such as the CMV promoter to ensure high-level protein expression and to minimize sequence homologies between the lentiviral vector genome and the packaging constructs.

A series of improvements to the initial lentiviral vector design have meanwhile led to increased robustness of the vectors. The U3 portion of the 5′LTR could be replaced by the CMV promoter, which resulted in a CMV/LTR hybrid promoter. This increased the efficiency of lentiviral vector production and, more importantly,

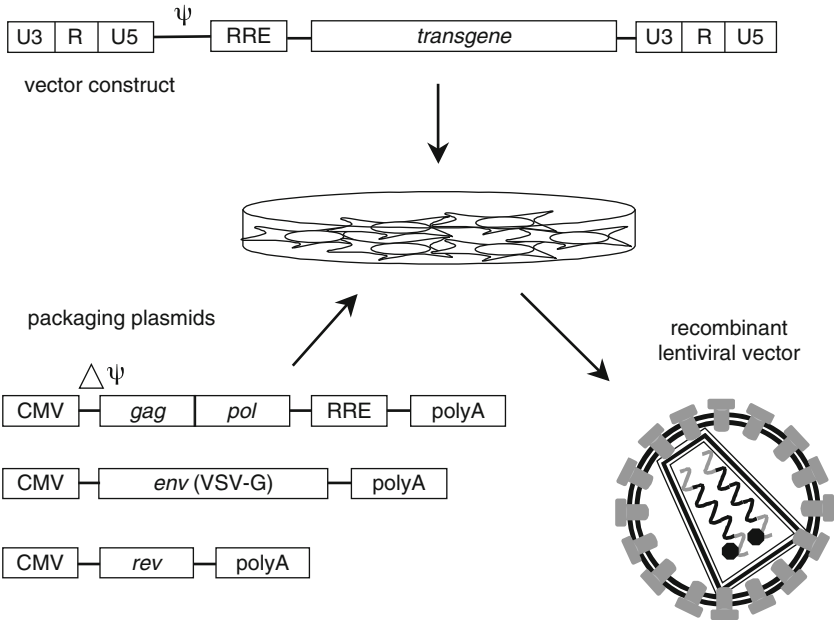


Fig. 4 Lentiviral vectors. As in oncoretroviral vectors, the viral genes *gag*, *pol* and *env* are replaced by the transgene of interest. The LTR and the packaging signal ψ are maintained for vector amplification and packaging. Of the lentiviral accessory proteins, only the *rev* responsive element (RRE) is maintained as an additional *cis*-acting sequence required for the nuclear export of unspliced and single-spliced viral RNAs in the presence of the Rev protein. Rev is expressed by one of the three packaging constructs. The two others encode the Gag/Pol or the Env proteins, respectively. For pseudotyping, the lentiviral Env proteins are often replaced by the vesicular stomatitis virus glycoprotein (VSV-G), which targets a wide variety of cell types

renders the LTR independent of transactivation by the viral Tat protein. To improve the nuclear import of the proviral DNA, the central polypurine tract (cPPT) from HIV was added to the vector as a *cis*-acting sequence. The improved biosafety concept of SIN vectors described above for oncoretroviral vectors was adapted to the lentiviral vector design. Large deletions in the transcriptional activation sequences of the U3 portion of the 3′LTR resulted in inactivation of the 5′LTR after reverse transcription in target cells.

3.5 Safety of Retrovirus Integration

The first successful clinical trial in gene therapy led to a cure of the X1 form of SCID. Unfortunately, years later cases of obviously retrovirus-induced leukemia led to a major setback in the field. Four out of 10 children in the initial Paris study developed a T-cell leukemia around three years after the initial treatment and cure of the disease. In these, chromosomal integration of the retroviral vector was found

to be close to the promoter region of the *LMO2* proto-oncogene. This lead to an upregulation of the LMO2 gene product resulting in uncontrolled stimulation of cell division (Hacein-Bey-Abina et al. 2003). These disturbing findings intensified the development of SIN retrovirus vectors (see 3.3) and prompted intensive investigations of the genome-wide distribution pattern of oncoretroviral integration sites. These screens revealed that transcription start regions are favored target sites for the integration of oncoretroviral vectors based on MoMLV. Integration sites of lentiviral vectors based on HIV also favor genomic transcription units but are more uniformly distributed over the entire gene (Mitchell et al. 2004; Fischer and Cavazzana-Calvo 2005).

The vectors used in the initial clinical trials were first-generation oncoretroviral vectors. Extensive animal studies proved that self-inactivating (SIN) oncoretroviral vectors display a largely improved safety profile. Meanwhile, high-throughput cell culture-based assays were developed to assess the safety profile of retroviral vector integration (Modlich et al. 2006). In addition, lentiviral vectors are being explored for the transduction of hematopoetic stem cells although caution regarding the risk of insertional mutagenesis has to be kept in mind. A recent study documented high frequencies of liver cancers in mice infected as neonates or in utero with EIAV-based lentiviral vectors (Themis et al. 2005).

The other major concern for the safety of retroviral vector preparations refers to vector mobilization and emergence of replication-competent viruses (RCV). These typically arise upon use of first-generation retroviral vectors. The SIN design of oncoretrovirus as well as lentivirus vectors is assumed to eliminate the risk of vector mobilization and also of homologous recombination that leads to the generation of RCV. It will be seen whether SIN vectors maintain their promise of an improved safety profile in ongoing clinical trials.

3.6 Production and Stability of Retroviral Vectors

The major obstacles associated with the application of oncoretroviral and lentiviral vectors are the low titers of the virus stocks produced by the currently available production systems (Rodrigues et al. 2007). This is further aggravated by the inherent instability of the retroviral vector particles released into the cell supernants. By employing either packaging cell lines or transient transfection of plasmid helper constructs, cell supernatants with maximal vector titers in the range of 10^5–10^7 infectious particles per ml can be attained. Whereas these titers may be sufficient for a variety of ex vivo applications, most gene therapy applications require further concentration of the supernatants. In addition, and regardless of their degree of concentration, cell supernatants are inherently impure and require further purification. Undesired side effects such as cell toxicity, inflammation, or immune response, as well as loss of the transduction efficiency of vectors due to the presence of transduction inhibitors, have been reported for unpurified retrovirus containing cell supernatants.

3.7 Purification and Upscaling of Retroviral Vectors

A critical prerequisite for the purification of retroviral vectors is knowledge of the optimal conditions for the maintenance of their stability (Segura et al. 2006). After budding from the producer cell line, retroviral particles quickly lose infectivity at 37°C. Their half-life in culture medium is around eight hours. Thus only a subfraction of the retroviral particles initially produced is still infectious at the time of harvest at 24–48 h after the start of production. Together with the production of defective viral particles, this translates into typical ratios of total to infectious retroviral particles of over 100:1. Consequently, purification schemes have to be short and are usually performed at 4°C. In addition, the inherent instability of retroviral particles outside a pH range of 5.5–8.0 and the sensitivity to high salt concentrations have to be observed.

On the basis of these restrictions, purification schemes for retroviral vectors have been developed that rely on centrifugation-based methods, membrane separation steps and chromatography or a combination thereof. Since retroviral particles are released into the production medium, the initial titers are rather low. On the other hand, disruption of producer cells is not required, which decreases potential contamination with cellular debris. For the separation of the viral particles from detached producer cells and cellular debris, low-speed centrifugation or microfiltration through membranes with a pore width of 0.45 μm may be used. For vector concentration, viral particles can then be pelleted by ultracentrifugation. In contrast to the nonenveloped AAV vectors, the usefulness of ultracentrifugation for retrovirus purification is limited for several reasons. Most importantly, both caesium chloride and sucrose, which are commonly used for density equilibrium centrifugation, lead to significant losses of infectivity (Powell et al. 2000). This especially holds true for nonpseudotyped retroviral vectors with wildtype *env*. These have maintained the authentic surface domains of the wildtype glycoproteins that are easily lost during ultracentrifugation. In addition, ultracentrifugation-based methods are time-consuming and difficult to scale up. Ultrafiltration procedures appear to be attractive alternatives. These are fast, robust, and can be used for the concentration of vector preparations and simultaneous dialysis to adapt buffer conditions required for downstream purification by chromatography. On a laboratory scale, centrifugation-dependent ultrafiltration devices with a molecular weight cut-off in the range of 20–500 kDa or stirred vessels have been used with high recovery rates. The membrane geometries commonly applied for scale-up are tangential flow devices such as flat sheet cassettes or hollow fibers (Rodrigues et al. 2007).

Conditions for further purification of vector preparations by chromatographic methods are quite stringent, since retroviral vectors are sensitive to the agents used for desorption from various chromatography matrices. For example, affinity adsorbents using coupled antibodies directed against the viral Env proteins have not been described for retroviral vectors, obviously due to the harsh conditions required for breaking of the antigen–antibody complex. However, purification of biotinylated

retroviral particles on streptavidin–biotin affinity columns and of particles harboring histidine-tagged Env proteins on metal affinity columns have been described. The standard agents used in elution of proteins from the respective affinity matrices (guanidine-HCl/urea or imidazole/EDTA, respectively) readily inactivate retroviral vectors. Therefore, either alternative desorbents, or immediate removal of the desorbent by dialysis, are employed. Anion-exchange chromatography with desorption at high salt concentrations (1 M NaCl) was successfully applied to the purification of both oncoretroviral and lentiviral vectors. Size exclusion chromatography as an alternative strategy suffers from a low throughput, but is often used as a final polishing step in retroviral vector purification (Rodrigues et al. 2007).

3.8 Vector Quantitation and Quality Assessment

The quality of a purified retroviral vector preparation can be described in terms of dose, potency, purity, and safety standards (Rodrigues et al. 2007). Potency represents the capacity of the target cell to express the transgene after retroviral transduction and can only be measured by assays specifically designed for the transgene and target cell(s) of interest.

Parameters used to describe the vector dose are the number of total particles, their biological activity as measured by the content of infectious particles, and their protein content. For the quantitation of total particles, several methods with different diagnostic values can be employed. A sensitive and fast assay is the measurement of vector RNA molecules by quantitative real-time PCR after reverse transcription. This method usually underestimates the amount of total virus particles, since it does not account for empty particles. Quantitative determination of viral antigens like the p24-Gag protein for HIV and the p30-Gag protein for oncoretroviral vectors by standard ELISA assays or the measurement of reverse transcriptase activity can be used to calculate the number of total particles. In contrast, electron microscopy of negatively-staining virus preparation is a tedious and impractical method for routine quantitation, but can be an important tool to evaluate the integrity of the purified vector preparations (Segura et al. 2005).

A commonly used method to measure infectious, transduction-competent viral particles relies on the determination of the expression of a marker gene such as *GFP* or *lacZ* or an antibiotic resistance gene in transduced cell lines. Most retroviral vectors in developmental phases carry one of these marker genes. However, these determinations often undervalue the actual titers. Due to variable diffusion in the infection media and to decay of viral particles, only a minor proportion of the initially active virus particles in the vector stock eventually transduce target cells (Andreadis et al. 1997). Furthermore, viral titers critically depend on the parameters used for the transduction assay such as the seeding density, growth rate, and receptor availability on the target cell line. In addition, variations in incubation time, incubation volume, and polybrene concentrations (Andreadis and Palsson 1997) influence transgene-expression-based titer measurements. Alternatively,

quantitation of the proviral genomes inserted into the genome of the target cell (Sastry et al. 2002) or the levels of transgene mRNA by real-time PCR (Lizee et al. 2003) can be used as measurement of the biological activity. Generally, a combination of at least two of the above methods should be used, both for the determination of the amount of active, transcription-competent virus and for the measurement of total viral particles in a vector preparation.

Major contaminants of retroviral vector preparations include proteins, DNA, and transduction inhibitors of various chemical compositions. Protein contaminants mainly arise from the serum used to complement growth media or are secreted into the medium by the producer cells. One difficulty in the determination of protein contaminants is the inherent property of retroviruses to incorporate host cell proteins between the lipid bilayer of the viral coat and the inner core structures during the budding process. Determination of protein contaminants is mostly performed by SDS-PAGE followed by silver staining (Segura et al. 2005). Contaminating DNA derived from the producer cells or from transfected packaging plasmids can be assayed by standard DNA hybridization techniques using plasmid or host cell DNA probes. The tolerable limits for DNA contaminations vary depending on the particular vector produced and on the intended application. As a guiding principle for clinical trials, the FDA has recommended an upper limit of 10 ng of foreign DNA per vector dose. To minimize DNA contamination, a DNase digestion step should be included early in the purification process.

A major concern for the safety of retroviral vector preparations is contamination with replication-competent viruses (RCV). Methods to detect RCV in retroviral preparations are technically demanding and time-consuming. They are based on the amplification of the RCV in a permissive cell line over several passages in combination with detection by either cell-based assays or more sensitive PCR-based techniques. For more detailed information the reader is referred to FDA (2001).

3.9 Future Directions of Retroviral Vector Development

Vectors based on classical oncoretroviruses as well as those based on lentiviruses have become potent tools for gene therapy of a variety of disease entities. The successful treatment of the X-linked and the ADA forms of SCID in clinical trials were achieved with classical oncoretrovirus vectors. The reported drawbacks due to leukemia development were shown to be due to oncogene activation by LTR-mediated activation, a well-documented problem of the early vector design. The increased safety and efficiency of the SIN vector concept has been demonstrated in extensive animal studies, such that it is considered safe to proceed to clinical trials. With SIN vectors the safety concerns regarding the formation of replication-competent viruses will hopefully be solved as well. The SIN vector concept can be extended by addition of chromatin insulators or matrix-attachment regions to prevent vectors from transactivation of adjacent cellular genes. To further reduce the risk of insertional mutagenesis, vectors based on lentiviruses,

foamyviruses, and nonmurine retroviruses are being explored, since these were shown to integrate over broader regions of the genome (Kohn and Candotti 2009). Last not least, episomal integrase-deficient vector variants are being explored to circumvent the problem of insertional mutagenesis. Since highly proliferating cells and tissue would quickly lose these vectors, the aim of episomal vector persistence focuses on long-lasting gene expression in postmitotic cells (Philpott and Thrasher 2007), a goal already achieved by AAV vectors with excellent long-term results.

As retroviral vectors are repeatedly moving on to phase III clinical trials, the need to resolve production-associated safety problems has driven retroviral vector research to higher technological levels. The larger vector doses required call for improved scale-up production methods and downstream processing. Two major directions are being explored: vector production in serum-free media to reduce the amount of serum proteins shown previously to evoke unacceptable immunological responses, and the replacement of adherent cells by suspension culture-based production methods. Suspension cultures combine ease of upscaling with reduced contamination by proteoglycans derived from secreted extracellular matrix proteins (Merten 2004). The combination of ongoing retrovirus vector developments will significantly advance both the genetic safety of retrovirus vector application and the ease and safety of bioreactor-scale production for clinical application.

4 Outlook

Gene therapy relies on safe and persistent gene transfer and expression. Since chromosomal integration of a gene therapy vector harbors the inherent risk of insertional mutagenesis, intensive work has been put into methods for in situ gene repair. Two directions are being pursued: one is site-specific targeting of vectors to assumedly safe sites in the human genome; the other is to directly target the defective gene sequence and repair the specific gene defect. DNA sequence-specific designer zinc-finger nucleases are being developed for this purpose. These are linked to suitable endonucleases that induce a double-strand break near the intended target site for gene repair. Simultaneously, a repair matrix with homology sequences surrounding an intact copy of the target sequence is transferred to the cell, which is used as a repair template for homologous recombination at the site of the induced DNA strand break. Gene transfer of the repair matrix can be achieved by a nonintegrating, viral vector, as for example AAV, or by a nonintegrating retroviral or lentiviral vector. Although the efficiency of the described DNA repair process is still relatively low, the technique has improved rapidly over recent years. Animal models for stem cell gene therapy in the hematopoietic system and other rapidly dividing cell types are under intense investigation (Bohne and Cathomen 2008). In situ gene repair exploits the cellular gene repair machinery for template-guided gene repair. Therefore actively proliferating target cells are a prerequisite. However, the majority of cell types in the human body are slowly cycling or do not divide at all and therefore are not accessible to the gene repair approach described

above. To provide expression of an intact copy of a mutated gene in these differentiated cells, episomally persisting vectors like AAV are being employed that lead to a long-lasting and safe gene expression profile.

References

Andreadis S, Palsson BO (1997) Coupled effects of polybrene and calf serum on the efficiency of retroviral transduction and the stability of retroviral vectors. Hum Gene Ther 8:285–291

Andreadis ST, Brott D, Fuller AO, Palsson BO (1997) Moloney murine leukemia virus-derived retroviral vectors decay intracellularly with a half-life in the range of 5.5 to 7.5 h. J Virol 71:7541–7548

Aiuti A, Cattaneo F, Galimberti S, Benninghoff U, Cassani B, Callegaro L, Scaramuzza S, Andolfi G, Mirolo M, Brigida I, Tabucchi A, Carlucci F, Eibl M, Aker M, Slavin S, Al-Mousa H, Al Ghonaium A, Ferster A, Duppenthaler A, Notarangelo L, Wintergerst U, Buckley RH, Bregni M, Marktel S, Valsecchi MG, Rossi P, Ciceri F, Miniero R, Bordignon C, and Roncarolo MG (2009) Gene therapy for immunodeficiency due to adenosine deaminase deficiency. N Engl J Med 360:447–458

Bainbridge JW, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, Viswanathan A, Holder GE, Stockman A, Tyler N, Petersen-Jones S, Bhattacharya SS, Thrasher AJ, Fitzke FW, Carter BJ, Rubin GS, Moore AT, Ali RR (2008) Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med 358:2231–2239

Bohne J, Cathomen T (2008) Genotoxicity in gene therapy: an account of vector integration and designer nucleases. Curr Opin Mol Ther 10:214–223

Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, Gross F, Yvon E, Nusbaum P, Selz F, Hue C, Certain S, Casanova JL, Bousso P, Deist FL, Fischer A (2000) Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science 288:669–672

Choi VW, Asokan A, Haberman RA, Samulski RJ (2007) Production of recombinant adeno-associated viral vectors for in vitro and in vivo use. Curr Protoc Mol Biol Chapter 16: Unit 16 25

Conway J, Rhys C, Zolotukhin I, Zolotukhin S, Muzyczka N, Hayward G, Byrne B (1999) High-titer recombinant adeno-associated virus production utilizing a recombinant herpes simplex virus type I vector expressing AAV-2 rep and cap. Gene Ther 6:986–993

FDA (2001) Supplemental guidance on testing for replication-competent retrovirus in retroviral vector-based gene therapy products and during follow-up of patients in clinical trials using retroviral vectors. Hum Gene Ther 12:315–320

Fischer A, Cavazzana-Calvo M (2005) Integration of retroviruses: a fine balance between efficiency and danger. PLoS Med 2:e10

Gaspar HB, Parsley KL, Howe S, King D, Gilmour KC, Sinclair J, Brouns G, Schmidt M, Von Kalle C, Barington T, Jakobsen MA, Christensen HO, Al Ghonaium A, White HN, Smith JL, Levinsky RJ, Ali RR, Kinnon C, Thrasher AJ (2004) Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector. Lancet 364:2181–2187

Goff S (2001) Retroviridae: the retroviruses and theit replication. In: Knipe DM, Howley PM (eds) Fields Virology, vol 2. Lippincott, Philadelphia, pp 1871–1940

Grimm D, Kay MA (2003) From virus evolution to vector revolution: use of naturally occurring serotypes of adeno-associated virus (AAV) as novel vectors for human gene therapy. Curr Gene Ther 3:281–304

Grimm D, Kay MA, Kleinschmidt JA (2003) Helper virus-free, optically controllable, and two-plasmid-based production of adeno-associated virus vectors of serotypes 1 to 6. Mol Ther 7:839–850

Grimm D, Kern A, Rittner K, Kleinschmidt JA (1998) Novel tools for production and purification of recombinant adenoassociated virus vectors. Hum Gene Ther 9:2745–2760

Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P, Lim A, Osborne CS, Pawliuk R, Morillon E, Sorensen R, Forster A, Fraser P, Cohen JI, de Saint Basile G, Alexander I, Wintergerst U, Frebourg T, Aurias A, Stoppa-Lyonnet D, Romana S, Radford-Weiss I, Gross F, Valensi F, Delabesse E, Macintyre E, Sigaux F, Soulier J, Leiva LE, Wissler M, Prinz C, Rabbitts TH, Le Deist F, Fischer A, Cavazzana-Calvo M (2003) LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science 302:415–419

Hauswirth W, Aleman TS, Kaushal S, Cideciyan AV, Schwartz SB, Wang L, Conlon T, Boye SL, Flotte TR, Byrne B, Jacobson SG (2008) Phase I trial of leber congenital amaurosis due to RPE65 mutations by ocular subretinal injection of adeno-associated virus gene vector: short-term results. Hum Gene Ther 19:979–90

Heilbronn R, Engstler M, Weger S, Krahn A, Schetter C, Boshart M (2003) ssDNA-dependent colocalization of adeno-associated virus Rep and herpes simplex virus ICP8 in nuclear replication domains. Nucleic Acids Res 31:6206–6213

Kang W, Wang L, Harrell H, Liu J, Thomas DL, Mayfield TL, Scotti MM, Ye GJ, Veres G, Knop DR (2009) An efficient rHSV-based complementation system for the production of multiple rAAV vector serotypes. Gene Ther 16:229–39

Kohn DB, Candotti F (2009) Gene therapy fulfilling its promise. N Engl J Med 360:518–521

Lai Y, Yue Y, Liu M, Ghosh A, Engelhardt JF, Chamberlain JS, Duan D (2005) Efficient in vivo gene expression by trans-splicing adeno-associated viral vectors. Nat Biotechnol 23:1435–1439

Li W, Asokan A, Wu Z, Van Dyke T, DiPrimio N, Johnson JS, Govindaswamy L, Agbandje-McKenna M, Leichtle S, Redmond DE Jr, McCown TJ, Petermann KB, Sharpless NE, Samulski RJ (2008) Engineering and selection of shuffled AAV genomes: a new strategy for producing targeted biological nanoparticles. Mol Ther 16:1252–1260

Lizee G, Aerts JL, Gonzales MI, Chinnasamy N, Morgan RA, Topalian SL (2003) Real-time quantitative reverse transcriptase-polymerase chain reaction as a method for determining lentiviral vector titers and measuring transgene expression. Hum Gene Ther 14:497–507

Maguire AM, Simonelli F, Pierce EA, Pugh EN Jr, Mingozzi F, Bennicelli J, Banfi S, Marshall KA, Testa F, Surace EM, Rossi S, Lyubarsky A, Arruda VR, Konkle B, Stone E, Sun J, Jacobs J, Dell'Osso L, Hertle R, Ma JX, Redmond TM, Zhu X, Hauck B, Zelenaia O, Shindler KS, Maguire MG, Wright JF, Volpe NJ, McDonnell JW, Auricchio A, High KA, Bennett J (2008) Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med 358:2240–2248

Merten OW (2004) State-of-the-art of the production of retroviral vectors. J Gene Med 6(Suppl 1): S105–124

Miller DG, Adam MA, Miller AD (1990) Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection. Mol Cell Biol 10:4239–4242

Miller DG, Trobridge GD, Petek LM, Jacobs MA, Kaul R, Russell DW (2005) Large-scale analysis of adeno-associated virus vector integration sites in normal human cells. J Virol 79:11434–11442

Mitchell RS, Beitzel BF, Schroder AR, Shinn P, Chen H, Berry CC, Ecker JR, Bushman FD (2004) Retroviral DNA integration: ASLV, HIV, and MLV show distinct target site preferences. PLoS Biol 2:E234

Modlich U, Bohne J, Schmidt M, von Kalle C, Knoss S, Schambach A, Baum C (2006) Cell-culture assays reveal the importance of retroviral vector design for insertional genotoxicity. Blood 108:2545–2553

Muller OJ, Kaul F, Weitzman MD, Pasqualini R, Arap W, Kleinschmidt JA, Trepel M (2003) Random peptide libraries displayed on adeno-associated virus to select for targeted gene therapy vectors. Nat Biotechnol 21:1040–1046

Muzyczka N, Berns KI (2001) Parvoviridae: the viruses and their replication. In: Knipe DM, Howley PM (eds) Fields virology, vol 2. Lippincott, Philadelphia, pp 2327–2359

Perabo L, Buning H, Kofler DM, Ried MU, Girod A, Wendtner CM, Enssle J, Hallek M (2003) In vitro selection of viral vectors with modified tropism: the adeno-associated virus display. Mol Ther 8:151–157

Petrs-Silva H, Dinculescu A, Li Q, Min SH, Chiodo V, Pang JJ, Zhong L, Zolotukhin S, Srivastava A, Lewin AS, Hauswirth WW (2009) High-efficiency transduction of the mouse retina by tyrosine-mutant AAV serotype vectors. Mol Ther 17:463–471

Philpott NJ, Thrasher AJ (2007) Use of nonintegrating lentiviral vectors for gene therapy. Hum Gene Ther 18:483–489

Powell SK, Kaloss MA, Pinkstaff A, McKee R, Burimski I, Pensiero M, Otto E, Stemmer WP, Soong NW (2000) Breeding of retroviruses by DNA shuffling for improved stability and processing yields. Nat Biotechnol 18:1279–1282

Rodrigues T, Carrondo MJ, Alves PM, Cruz PE (2007) Purification of retroviral vectors for clinical application: biological implications and technological challenges. J Biotechnol 127:520–541

Sastry L, Johnson T, Hobson MJ, Smucker B, Cornetta K (2002) Titering lentiviral vectors: comparison of DNA, RNA and marker expression methods. Gene Ther 9:1155–1162

Segura MM, Kamen A, Trudel P, Garnier A (2005) A novel purification strategy for retrovirus gene therapy vectors using heparin affinity chromatography. Biotechnol Bioeng 90:391–404

Segura MM, Kamen A, Garnier A (2006) Downstream processing of oncoretroviral and lentiviral gene therapy vectors. Biotechnol Adv 24:321–337

Snyder RO, Flotte TR (2002) Production of clinical-grade recombinant adeno-associated virus vectors. Curr Opin Biotechnol 13:418–423

Themis M, Waddington SN, Schmidt M, von Kalle C, Wang Y, Al-Allaf F, Gregory LG, Nivsarkar M, Themis M, Holder MV, Buckley SM, Dighe N, Ruthe AT, Mistry A, Bigger B, Rahim A, Nguyen TH, Trono D, Thrasher AJ, Coutelle C (2005) Oncogenesis following delivery of a nonprimate lentiviral gene therapy vector to fetal and neonatal mice. Mol Ther 12:763–771

Toublanc E, Benraiss A, Bonnin D, Blouin V, Brument N, Cartier N, Epstein AL, Moullier P, Salvetti A (2004) Identification of a replication-defective herpes simplex virus for recombinant adeno-associated virus type 2 (rAAV2) particle assembly using stable producer cell lines. J Gene Med 6:555–564

Urabe M, Ding C, Kotin RM (2002) Insect cells as a factory to produce adeno-associated virus type 2 vectors. Hum Gene Ther 13:1935–1943

Wilson JM, Gansbacher B, Berns KI, Bosch F, Kay MA, Naldini L, Wei YQ (2008) Good news on the clinical gene transfer front. Hum Gene Ther 19:429–430

Xiao X, Li J, Samulski RJ (1998) Production of high-titer recombinant adeno-associated virus vectors in the absence of helper adenovirus. J Virol 72:2224–2232

Zhong L, Li B, Mah CS, Govindasamy L, Agbandje-McKenna M, Cooper M, Herzog RW, Zolotukhin I, Warrington KH Jr, Weigel-Van Aken KA, Hobbs JA, Zolotukhin S, Muzyczka N, Srivastava A (2008) Next generation of adeno-associated virus 2 vectors: point mutations in tyrosines lead to high-efficiency transduction at lower doses. Proc Natl Acad Sci USA 105:7827–7832

Zolotukhin S (2005) Production of recombinant adeno-associated virus vectors. Hum Gene Ther 16:551–557

Zolotukhin S, Byrne BJ, Mason E, Zolotukhin I, Potter M, Chesnut K, Summerford C, Samulski RJ, Muzyczka N (1999) Recombinant adeno-associated virus purification using novel methods improves infectious titer and yield. Gene Ther 6:973–985

Pulmonary Drug Delivery: Medicines for Inhalation

Andreas Henning, Stephanie Hein, Marc Schneider, Michael Bur, and Claus-Michael Lehr

Contents

Abstract Mankind has inhaled substances for medical and other reasons for thousands of years, notably resulting in the cultural manifestations of tobacco and opium smoking. Over the course of time concepts of pulmonary application, including inhalation devices and drug formulations, have been and still are being continuously developed. State of the art instruments even allow for individualized drug application by adaption of the inhalation procedure to the breathing pattern of the patient.

Pulmonary drug delivery offers promising advantages in comparison to "classical" drug administration via the oral or transcutaneous routes, which is also

C.-M. Lehr (✉)
Biopharmaceutics and Pharmaceutical Technology, Saarland University, P.O. Box 15 11 50, 66041 Saarbrücken, Germany
e-mail: lehr@mx.uni-saarland.de

M. Schäfer-Korting (ed.), *Drug Delivery*,
Handbook of Experimental Pharmacology 197,
DOI 10.1007/978-3-642-00477-3_6,

reflected by an increasing interest and number of marketed products for inhalation therapy. However, the lungs' efficient clearance mechanisms still limit the benefit of many therapeutic concepts. In consequence the objective of current research and development in pulmonary drug delivery is to overcome and to control drug clearance from the intended target site. Here, several of the most auspicious future drug delivery concepts are presented and discussed in order to give the reader an insight into this emerging field of medicine.

Keywords Aerosol deposition · Mucociliary clearance · Macrophage clearance · Telomerase inhibition · Inhalation vaccination

Abbreviations

2-OMR	Antisense oligonucleotide 2′-*O*-methyl-RNA
A549	Lung cancer cell line (CCL-185; ATCC)
APCs	Antigen-presenting cells
ATD	Anti-tubercular drugs
AUC	Area under the curve
BALT	Broncho-alveolar lymphoid tissue
Calu-3	Human epithelial-like lung cancer cell line (HTB-55; ATCC)
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CLIJ	Confined liquid impinging jet
COPD	Chronic obstructive pulmonary disease
DOTAP	*N*-[1-(2,3-Dioleoyloxy)]-*N*, *N*, *N*-trimethylammonium propane methylsulphate
DPI	Dry powder inhaler
DPLC	Dipropionate-dilauroylphosphatidylcholine
DPPC	Dipalmitoyl-phosphatidylcholine
DSPC	Distearoyl-phosphatidylcholine
DSPE	Distearoyl-phosphatidylethanolamine
FDA	U.S. Food and Drug Administration
ICRP	International Commission on Radiological Protection
MC	Mucociliary clearance
MDI	Metered-dose inhaler
PEG	Polyethylenglycol
PEI	Polyethylenimine
PLGA	Poly-lactide-*co*-glycolide
PTEN	Phosphatase and tensin homolog: tumor suppressor gene
siRNA	Small interfering RNA
SLIT	Sustained release lipid inhalation targeting
SLM	Solid lipid microparticle
TAT	Human immunodeficiency virus-1 transactivator protein
WHO	World Health Organization

1 Introduction

Several thousand years ago mankind was already employing the respiratory route for drug delivery purposes. Ancient Egyptian physicians used the energy of hot stones to evaporate alkaloids from plants in order to make their patients inhale the active substances (Sanders 2007). Indian and native American shamans knew about the anti-asthmatic effects of *Datura stramonium* when leaves were smoked in a pipe or simply burned within a small room (Dessanges 2001). Moreover, the smoking of opium, containing highly analgesic alkaloids from *Papaver somniferum*, has a long tradition in Chinese culture, although the medical aspect was not of primary relevance in this case.

Hence it is not surprising that over the course of time inhalation technology as well as the general knowledge of therapeutic inhalation has been and today is still being continuously developed. In order to make inhalation more effective, simple smoking pipes from antiquity were soon replaced by more ingenious inhalation instruments. It was in 1654 when the first illustration of an inhaler was depicted in Christopher Bennet's *Theatri Tabidorum* (Bennet 1654). However, the term "inhaler" was introduced in 1778 by John Mudge, an English physician giving advice to treat cough via inhalation of opium vapor (Mudge 1778). Accompanied by continuous technical optimization, the improved understanding of pulmonary drug delivery principles also mediated a more defined and accurate vocabulary in this field. According to Aiache, it was R. Whitlaw and E. Gray Patterson who defined the word "aerosol" in 1932, based on "aer" (air) and "sol" (solution) (Aiache 1990). Hitherto, the terms "mist," "micromist," "fog," and "fume" were used in an imprecise and often even confusing manner. This is astonishing from a current viewpoint, since a defined vocabulary is one of the fundamental requirements in every field of modern science.

The driving force to improve medical inhalation, especially in the nineteenth century, was the treatment of consumption or "pulmonary phthisis", whereas today the focus is more on the treatment of asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis (CF). Over recent decades the number of approved inhalation products has continuously increased and several new therapeutic approaches, such as vaccination via the pulmonary route (Bivas-Benita et al. 2005; Lu and Hickey 2007) and highly specific lung lobe targeting (Selting et al. 2008), already show promising results.

While there are already several excellent reviews and textbooks on pulmonary drug delivery in general (Bechtold-Peters and Luessen 2007; Patton and Byron 2007), this chapter will focus on latest technologies that are beyond the well-known devices and formulations for metered-dose inhalers (MDI), dry powder inhalers (DPI) or nebulizers. The principles of pulmonary drug delivery as well as current developments and concepts in this emerging field will be elucidated and discussed by means of practical examples. Additional references enable the well-disposed reader to find detailed information in comprehensive publications, reviews and

books. Finally, a prospective outlook will reflect the rising demands and future developments in inhalation therapy.

2 Principles of Aerosol Delivery

2.1 Inhalation Therapy

Pulmonary drug delivery is attracting more and more interest, due to the unique barrier properties (Fig. 1) and the huge surface area (~140 m^2) of the lung. Today, several respiratory diseases can be adequately treated by inhalation therapy (Groneberg et al. 2003). For many asthma and COPD patients, drug inhalation is even suitable to serve as the only form of therapeutic intervention. This in fact offers an important advantage regarding the patient's compliance and overall benefit of the therapy. Depending on the actual status and general severity of the disease, drug inhalation is needed once or several times per day. It is of particular importance to note that the correct drug application, in this case the inhalation maneuver, results in a superior therapeutic effect and consequently a lower application rate (Serra-Batlles et al. 2002; Welch et al. 2004; Booker 2005). Because of this fact, every patient receiving inhalative medication should be thoroughly trained in order to optimize drug deposition within the respiratory tract. The crucial

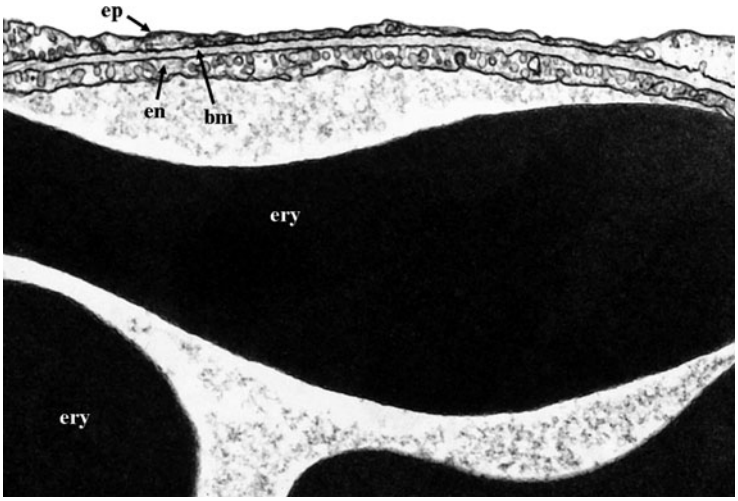


Fig. 1 The alveolar air–blood barrier (TEM image). The lungs' unique barrier properties are correlated with the extremely thin air–blood barrier within the alveolar region. The TEM image (cross-section) shows the epithelium (ep), the endothelium (en), the basal membrane (bm), and erythrocytes (ery) within the alveolar capillary. Courtesy of Prof. Dr. Peter Gehr, Institute of Anatomy, University of Bern, Bern, Switzerland

relevance of a controlled inhalation maneuver is easy to understand by taking the morphological structure into account.

2.2 Lung Structure

The lung consists of at least two dissimilar zones: the centrally located *conducting airways*, and the peripherally located *respiratory zone*. More precisely, the trachea, the main bronchi, and the conducting bronchioles account for the conducting airways, whereas the respiratory bronchioles, alveolar ducts, and, of course, the alveoli account for the lung's respiratory zone (Fig. 2). Based on this tree-like branching pattern, the lung's structure is commonly subdivided into particular airway generations, starting with the trachea as generation 0, and ending with the alveoli as generation 23 (Albertine et al. 2000). As can be assumed from the terminology, the function of the conducting airways is mainly limited to bulk air flow during the active inhaling and exhaling process. The essential and dedicated gas-exchange function of the lungs is realized exclusively within the respiratory

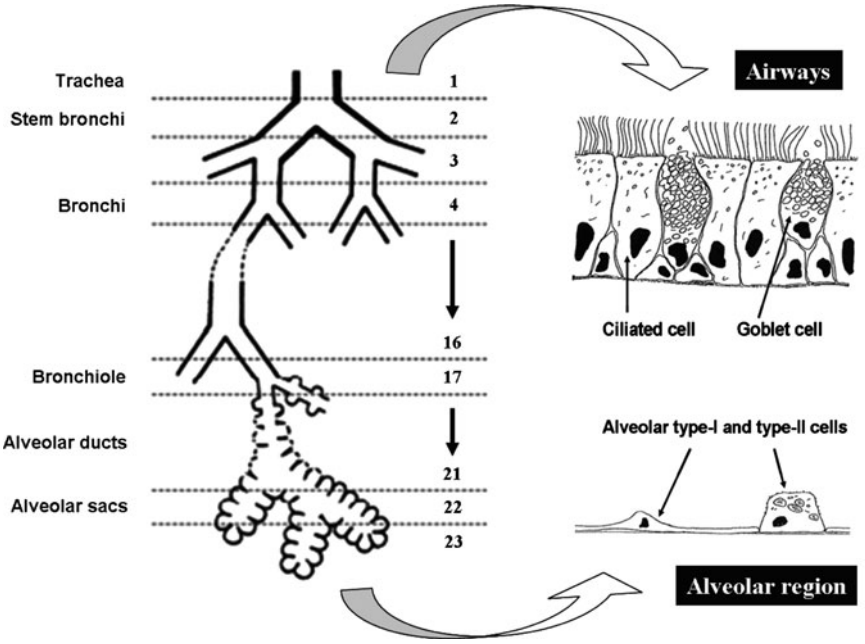


Fig. 2 Schematic overview of human airway structure. Human airway structure exhibits symmetric branching from trachea to the alveolar region. In the upper airways a columnar epithelium mainly consisting of ciliated cells and goblet cells is found, whereas in the alveolar region a flat and monolayer epithelium is formed by alveolar type I and type II cells. Schemes modified after Weibel (1963) and Forbes and Ehrhardt (2005)

zone. Whenever pulmonary drug delivery is the issue to be addressed, these dimensional facts must be seriously taken into account for controlled and efficient drug deposition within the lung.

2.3 Aerosol Deposition

Inside the respiratory system, inhaled material deposits in different lung regions mainly depending on the aerodynamic particle diameter and the overall inhalation maneuver (Fig. 3). Discrimination between the deposition sites is realized due to three different principal mechanisms: *Brownian motion*, *sedimentation* and *impaction* (Heyder 1981). For particles smaller than 1 μm, Brownian motion will be the decisive mechanism for deposition in mainly alveolar regions of the lung. Particles 1–5 μm in size are suitable to enter and sediment within the tracheo-bronchial region, whereas particles larger than 5 μm will mainly be deposited in the oro-pharyngal airways due to impaction (Heyder et al. 1986; Oberdörster et al. 2005). Interestingly, ultrafine particles of 0.005–0.2 μm are efficiently deposited in the deep lungs, whereas most of the particles 0.2–1.0 μm in size are exhaled again (Dolovich et al. 2000; Heyder and Svartengren 2002). However, due to a lack of appropriate formulation technologies that can generate ultra-fine drug particles, and along with intrinsic limitations on the dose of drug that ultra-fine particles can deliver within a reasonable aerosol volume or time of inhalation, this size range is not used for aerosol medicines at the moment.

As mentioned before, incorrect pulmonary application inevitably results in a significantly reduced therapeutic drug effect. However, most inhaled drugs are

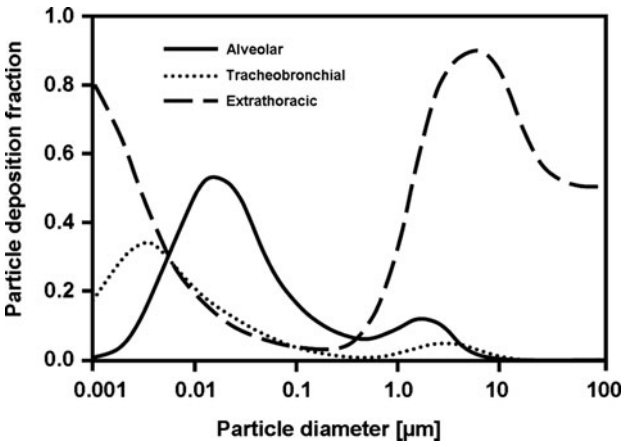


Fig. 3 Regional deposition of particles within the lung. Depending on the particle size and the overall inhalation maneuver, particles deposit within different regions of the lung (Danish EPA, Report No. 12352008)

intended to reach the alveolar region, since the very thin epithelium (0.1–0.5 μm) and the huge alveolar surface (~140 m^2) offer superior conditions for drug absorption. Overall the alveolar surface accounts for more than 95% percent of the lungs' total surface (Weibel 1979). In this context, a defined and controlled inhalation maneuver is of utmost importance for regional targeting and normally intended alveolar deposition of the drug. The inspiratory flow rate as well as total volume act as decisive parameters for regional particle deposition (Bennett et al. 1999; Brand et al. 2000; Brown and Bennett 2004). High flow rates (1,000 mL s^{-1}) are correlated with an increased fraction of particles deposited within the central airways, whereas at slow flow rates (200 mL s^{-1}) the chance for alveolar deposition is increased (Scheuch et al. 2007). Hence, a major paradigm in aerosol drug delivery may be defined as "slow and steady wins the race" (Dhand 2005). So far the efficacy of many commercially available inhalation products still suffers from a large fraction of drug being deposited in the airways (Hochrainer et al. 2005; Pitcairn et al. 2005). It is important to note that several more efficient technologies are available and hopefully will improve future inhalation therapy (Scheuch and Siekmeier 2007; Scheuch and Fischer 2008). However, the growing use of such rising technologies is currently limited by the economic hurdles faced by most new and cost-intensive concepts.

In summary and with regard to pulmonary drug delivery devices, the work from the engineering side is mainly done, the ball is now passed to the "scientific team" to clarify what happens after the controlled deposition of the drug.

2.4 Lung Clearance

In parallel to the dissimilar morphological structure, there are also distinctive differences between the clearance mechanism in the conducting airways and the alveolar region. Deposition in the conducting airways normally occurs with particles adhering to the sticky mucus layer lining the airway surface. Generally, mucosal barriers can be found at various sites of the human body, lining the gastrointestinal tract, intranasal epithelium, airways and more. In all cases the mucosal barrier serves as a shelter intended to protect the human body from exogenous influences such as bacteria, viruses, or fungi spores. These pathogens normally interact with the viscous mucus layer, and hence are immobilized and will be eliminated by clearance mechanisms present at the specific body site.

Airway mucus is a viscous gel composed of highly glycosylated mucus proteins called *mucins* (Desseyn et al. 2000; Thornton et al. 2008). After entrapment by the airway mucus, the particles are subsequently cleared from the deposition site as the mucus continuously moves proximally towards the upper end of the tracheal tube (Fig. 4). Finally, entrapped material and mucus are swallowed and undergo further biochemical processing in the gastrointestinal tract. This clearance mechanism, also termed mucociliary clearance (MC), essentially limits the benefit of many inhalation therapies: after initial inhalation and deposition, drug particles are rapidly

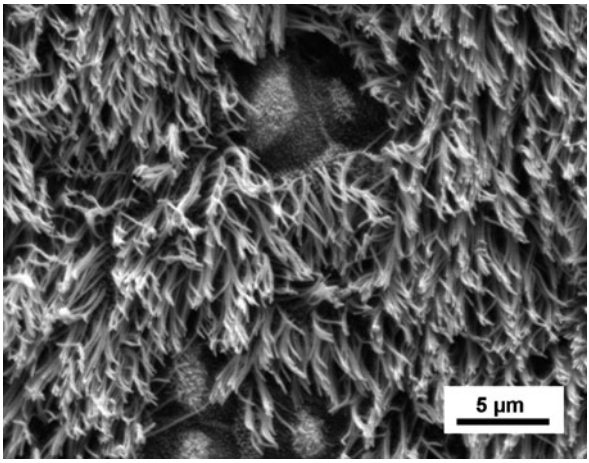


Fig. 4 Top view of airway surface epithelium (SEM image). The airway epithelium is characterized by a densely ciliated surface and mucus-producing goblet cells, bearing no cilia

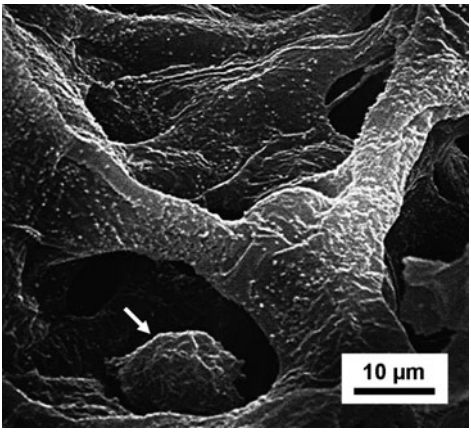


Fig. 5 Top view of alveolar epithelium (SEM image). In the alveolar region, pathogens and inorganic particles are normally eliminated by alveolar macrophages, patrolling on the alveolar surface (*bottom left*). Courtesy of the Lawrence Berkeley National Laboratory

cleared (4–6 mm/min!) and thus removed from the site of therapeutic action (ICRP 1994; Bailey et al. 2007).

Drug particles that have been successfully deposited on the alveolar surface cannot be cleared by MC since no ciliated cells are present in this lung region. Here, alveolar macrophages (Fig. 5) are the decisive mechanism to defeat inhaled pathogens, particles and more (Sibille and Reynolds 1990; Moeller et al. 2005). Adult macrophages, derived from monocytes within the bone marrow, migrate to the alveoli and patrol on the alveolar surface. Geiser et al. were able to show that ultrafine TiO_2 particles deposited in the lung of rats are phagocytosed by the alveolar macrophages within hours (Geiser et al. 2005). Subsequently, alveolar macrophages either undergo MC upon ascending to the airways or actively migrate

into the alveolar lymphatics for further involvement in immunomodulatory processes.

3 Pulmonary Drug Delivery Approaches

3.1 Asthma/COPD

There is a wide range of commercially available inhalation products for the treatment of asthma and COPD. Most of these formulations contain drug particles mixed with lactose as a carrier material and are administered by a dry powder inhaler (DPI) or by nebulizing the drug dissolved/dispersed in a propellant with a pressurized metered-dose inhaler (MDI). Although these formulations achieve efficient pulmonary deposition, they were not designed to provide sustained- or controlled-release characteristics. In the case where such a system shows prolonged drug action, as is the case for salmeterol and formoterol, this effect is based on the drug's pharmacological half-life and not due the delivery system or the formulation concept.

Indeed, particle technology of the early conventional formulations aimed to improve the aerodynamic properties and thus the deposition rate of aerosol particles. However, soon it came clear that a prolonged drug residence time results in a significant improved therapeutic effect and consequently new particulate delivery systems were developed in order to treat asthma, COPD, and other loco-regional lung diseases.

Some of these new approaches try to find formulations exhibiting sustained release properties so as to reduce the dose frequency for patients and improve bioavailability in the lung. Arya et al. (2006) coated budesonide particles with a very thin film of polylactic acid using pulse laser ablation methodology. After intratracheal administration of coated and uncoated budesonide to neonatal rats, higher AUC levels in the lung were observed for the coated budesonide. Moreover the systemic exposure of budesonide was reduced compared to uncoated budesonide. Gaber et al. successfully used PEG-DSPE, a block-co-polymer from polyethylene oxide and distearoyl-phosphatidylethanolamine, to prepare beclomethasone-loaded micelles (Gaber et al. 2006). The lyophilized beclomethasone-loaded polymeric micelles exhibited a high entrapment efficiency (>96%) and showed a sustained release over six days in drug release studies in vitro. Another interesting approach is to incorporate salbutamol acetonide into solid lipid microparticles (SLMs) (Jaspart et al. 2007). It was previously demonstrated that SLMs feature physicochemical stability and no acute in vivo toxicity in rats (Sanna et al. 2004). Subsequent in vitro release studies demonstrated that salbutamol acetonide SLMs had a slower drug release than pure salbutamol acetonide. Thus, SLMs promise to provide sustained pulmonary drug delivery, which in consequence will reduce the number of doses required for an efficient therapy.

Furthermore, liposomes have also been considered as a carrier vehicle for lung delivery of anti-inflammatory drugs. Saari et al. investigated the distribution of ^{99m}Tc-labeled beclomethasone dipropionate-dilauroylphosphatidylcholine (DPLC) and dipalmitoyl-phosphatidylcholine (DPPC) liposomes in healthy volunteers (Saari et al. 1999). They found that the clearance of DPPC liposomes was slower than DPLC liposomes, most likely due to different phase transition temperatures. Interestingly, for both formulations about 80% of the deposited radioactivity was retained in the lungs 24 h after inhalation.

Learoyd et al. produced chitosan-based terbutaline sulfate particles where chitosan acted to modify drug release (Learoyd et al. 2008). Use of low (<190 kDa), middle (190–310 kDa) and high (>310 kDa) molecular weight chitosan, as well as mixtures thereof, resulted in a prolonged duration of terbutaline release for the high molecular weight formulations.

3.2 Immunosuppressives

Pulmonary drug delivery is being evaluated for loco-regional application of immunosuppressive drugs to lung transplant patients. As yet, intravenous and oral tacrolimus formulations are available but suffer from poor tolerance by many patients. Sinswat et al. created nanostructured aggregates containing amorphous (with lactose) or crystalline tacrolimus nanoparticles by an ultra-rapid freezing technique (Sinswat et al. 2008). These aggregates could be successfully delivered by nebulization and showed high drug absorption in the lungs of mice.

Cyclosporin A, another commonly used immunosuppressive drug, exhibits hydrophobic and thus problematic physico-chemical properties. Hitherto aerosol formulations were based on ethanol and propylene glycol dissolutions, inevitably comprising a high irritation potential in animal as well as human lungs (O'Riordan et al. 1995; Iacono et al. 1997). In parallel to beclomethasone, the liposomal encapsulation of cyclosporin A resulted in an optimized drug formulation exhibiting efficient absorption rates into lung tissue and reduced side-effects of the drug (Gilbert et al. 1997; Letsou et al. 1999). In a recent approach, Chiou et al. produced cyclosporin A powders via a confined liquid impinging jet (CLIJ) technique and subsequent spray-drying (Chiou et al. 2008). They optimized this technique to obtain suitable particles for pulmonary delivery of proteins. In summary, all of these advanced formulation technologies for cyclosporin A promise to reduce systemic plasma levels and thus unwanted toxicity to other organs like kidneys, liver, etc.

From the discussion above it is clear that the majority of approaches are intended (1) to improve bioavailability, (2) to control the release properties, or (3) to reduce the dosing frequency for the drug. All of these attempts aim for an improved patient compliance and overall benefit of the therapy. However, a major problem limiting any therapeutic inhalation strategy remains unsolved: to date there is no existing technology available to inhibit or circumvent the efficient clearance mechanisms

within the respiratory tract. Naturally, the potential of such a new drug and/or carrier technology for pulmonary drug delivery would be tremendous. Here it is emphasized that the need for new carrier systems that avoid lung clearance and create a powerful drug depot is urgent and must be considered in future pulmonary drug delivery.

3.3 Vaccines

Delivering vaccines by the pulmonary route is easy, fast and non-invasive, and therefore a powerful strategy in the fight against infectious diseases, particularly in the developing world. Furthermore, this immunization route allows mass vaccination campaigns to be carried out without the need for medical personnel. Many pulmonary vaccines are in development for several infectious diseases such as influenza (Amorij et al. 2007; Garmise et al. 2007), measles (LiCalsi et al. 2001; de Swart et al. 2007; Burger et al. 2008), diphtheria (Amidi et al. 2007), and hepatitis (Lu and Hickey 2007).

Pulmonary vaccination is capable of inducing a locally limited as well as a systemic immune response (Hobson et al. 2003). As mentioned before, the pulmonary mucosa and the underlying epithelial layer act as the primary physical barrier in the lung. Below the epithelium, there is an array of immune cells, such as antigen-presenting cells (APCs), and the broncho-alveolar lymphoid tissue (BALT), which is normally only present in children and elderly people but can be induced by local infection (Tschernig and Pabst 2000). Local activation of the pulmonary immune response has the advantage of targeting pathogens directly at the point of entry. To date, several formulations for intranasal administration of influenza vaccine have been tested and shown to elicit a modest systemic immune response (Read et al. 2005; Garmise et al. 2007). Smith et al. encapsulated inactivated or subunit split influenza virus vaccines in spray-dried microparticles containing dipalmitoyl-phosphatidyl-choline (DPPC) and distearoyl-phosphatidylcholine (DSPC) and administered them intratracheally to mice and rats (Smith et al. 2003). This formulation showed improved local bioavailability to BALT, and increased antigen-loading of APCs, IgG antibodies, and T-cell responses locally as well as systemically. In another approach, an inulin-stabilized influenza vaccine powder was prepared by spray freeze-drying and delivered to the lungs of mice (Amorij et al. 2007). The vaccine powder formulation produced enhanced IgG and IgA levels compared to the conventional intramuscular administered influenza vaccine, proving that the modified vaccine can enhance local as well as systemic antibody production.

Besides influenza, measles is another infectious disease that is transmitted by the airborne route. Several research groups reported a significantly higher immune response in humans after wet mist aerosol administration of live attenuated measles vaccine compared to the injected vaccine formulation (Bennett et al. 2002; Dilraj et al. 2007). However, the vaccine is very sensitive to temperature and maintenance

of the cold-chain from industry to the "patient" or ultimate consumer greatly increasing the price of these products. As yet, different research groups have tried to develop dry powder vaccines of increased stability. De Swart et al. administered two different powder measles vaccines to macaques, though the vaccination was less efficient than intramuscular vaccination or nebulized vaccination (de Swart et al. 2007). Hence, more work is required to develop measles vaccine formulations with acceptable properties for administration by DPI that can boost serum antibody levels.

3.4 Anti-Infectives

One third of the world population is infected by *Mycobacterium tuberculosis*, the causal pathogen of tuberculosis (WHO 2008). Treatment of tuberculosis is a great challenge since *M. tuberculosis* invades and replicates within macrophages. Hitherto drugs against tuberculosis were given orally and over a long period of time. Consequently, side effects and a high dosing frequency result in problems with patient compliance and interruptions of drug therapy. In this context, specific macrophage targeting could improve the overall therapeutic regimen by decreased systemic exposure, reduced total drug doses, and decreased therapeutic side effects. Moreover, a special targeting strategy is needed in this case to both channel the drugs into infected macrophages and provide prolonged drug release once it is delivered.

Pandey et al. produced biodegradable poly-lactide-*co*-glycolide (PLGA) nanoparticles containing three anti-tubercular drugs (ATD), namely rifampicin, isoniazid, and pyrazinamide, and administered the aerosolized nanoparticles to infected guinea pigs (Pandey et al. 2003). The experimental outcome showed that bioavailability of all three drugs was increased compared to intravenous administration. Moreover, the inhaled and ATD-loaded nanoparticles provided lung drug concentrations above the therapeutic concentration for 11 days. Sharma et al. tried to improve the bioavailability of ATDs by producing bioadhesive wheat germ agglutinin-coated PLGA nanoparticles with ATDs (Sharma et al. 2004). Wheat germ agglutinin was used since it is known to bind to the alveolar epithelium (Brueck et al. 2001). Here, in parallel to Pandey's data, the results showed that the plasma concentrations of nebulized ATDs were in a therapeutic range for about 15 days.

As already discussed, liposomes are well suited for administration to the respiratory tract. The similarity of liposome compounds to natural surfactants prevents them from acting as an irritant once deposited in the lungs. Zaru et al. designed different rifampicin-loaded liposomes and reported reduced toxicity for alveolar epithelial cells (A549) as compared to the free drug (Zaru et al. 2007). Today "stealth liposomes," i.e., sterically stabilized liposomes that avoid rapid elimination through the reticuloendothelial system (Allen and Hansen 1991), are already used for intravenous cancer therapy (e.g., Caelyx®/Doxil®). Taking advantage of the "stealth" concept, Deol et al. developed stealth liposomes for pulmonary delivery

by modifying the surface with *O*-stearylamylopectin, to increase the affinity for the lung tissue of mice. The encapsulated drugs, namely isoniazid and rifampicin, showed significantly reduced toxicity for peritoneal macrophages in infected mice compared to the free drugs (Deol and Khuller 1997; Deol et al. 1997). Another targeting strategy exploits the mannose receptors that are expressed on alveolar macrophages through mannosylation of liposomes. Wijagkanalan et al. reported efficient targeting of mannosylated liposomes to alveolar macrophages after intratracheal instillation to rats (Wijagkanalan et al. 2008), as did Chono et al. when they administered ciprofloxacin-loaded mannosylated liposomes for pulmonary intracellular parasitic infections (Chono et al. 2008).

Other groups have developed microspheres to act as carrier systems for anti-infective drugs. Takenaga et al. demonstrated that lipid microspheres loaded with rifampicin could be delivered to alveolar macrophages in vitro as well as in vivo with reduced side effects in the liver (Takenaga et al. 2008). Hirota et al. examined the phagocytic activities of alveolar macrophages to rifampicin-containing PLGA microspheres of different sizes (Hirota et al. 2007). Interestingly, they found 3 μm particles to be most efficient for drug delivery to alveolar macrophages. Capreomycin, which is used for the treatment of multidrug-resistant tuberculosis, shows severe side effects after intravenous administration. Garcia-Contreras et al. developed large porous capreomycin sulfate particles and administered them to the respiratory tract of guinea pigs, reporting a decrease in both inflammation and bacterial burden in the lung tissue (Garcia-Contreras et al. 2007).

However, new approaches for delivering anti-infectives to the respiratory tract are not limited to tuberculosis therapy. Tobramycin is an anti-infective that is used to defeat *Pseudomonas aeruginosa*, a pathogen which is often found in the lungs of CF patients. Pilcer et al. formulated respirable lipid-coated tobramycin particles and reported improved drug deposition due to reduced microparticle agglomeration (Pilcer et al. 2006). Furthermore, moxifloxacin was successfully loaded into chitosan microspheres which were subsequently cross-linked with glutaraldehyde. In vitro testing of the microspheres in Calu-3 cell culture models showed promising and retarded absorption of moxifloxacin compared to the free drug (Ventura et al. 2008).

3.5 Pulmonary Gene Therapy

Cystic fibrosis (CF) is caused by various mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel significantly regulating water and ion flux across epithelial cells. In the pathophysiological state, CF is characterized by abnormal mucus production, inflammation and chronic bacterial infection within the respiratory tract (Sueblinvong et al. 2007). Treatment of CF by gene therapy is an absorbing field since replacing the defective CFTR gene by a functional gene transfer vector results in normalized mucus production and reduced inflammation. Although CFTR gene transfer was one of

the first targets of gene therapy, there are other genetic disorders like α-1 antitrypsin deficiency (Cruz et al. 2007) or hemophilia (Murphy and High 2008), where gene therapy could beneficially affect the etiopathology. Moreover, latest developments hold the promise that treatment of different types of cancer will be one of the biggest gene therapy fields in the near future (Eager et al. 2007).

Nebulization of naked plasmid DNA leads to low transfection rates and poor stability of the DNA (Lentz et al. 2006). Therefore DNA must be delivered to the mucosal surface of the lung by carrier systems that protect the DNA from enzymatic degradation, improve long-term expression of the protein, and enhance transfection efficiency. There are two different types of DNA carriers, viral and non-viral vectors, and both options have clear advantages and disadvantages.

The viral vectors that are used for DNA delivery have a high efficiency in gene transfer even though they have been modified to eliminate their inherent pathogenicity. However, one of the major disadvantages for DNA delivery by viral vectors, compared to non-viral vectors, is their immunogenic potential, strongly limiting the option for multiple dosing therapies (El-Aneed 2004). Thus, the focus in the following section will be on the non-viral vectors. Non-viral vectors allow multiple dose administration but suffer from less efficient gene transfer compared to the viral vectors. Most of the currently used carriers are positively charged to enable complexation and adsorption with the negatively charged DNA via electrostatic interaction. Non-viral vectors need to be biocompatible, non-toxic, and able to carry DNA across various cellular barriers to the nucleus. For these reasons, liposomes and polymeric particles are perfectly suited carriers for DNA delivery that can be produced easily and relatively inexpensively.

Chitosan is a very popular polymer for gene delivery that has been utilized by many research groups (Bivas-Benita et al. 2004; Köping-Höggård et al. 2004; Howard et al. 2006; Issa et al. 2006; Li and Birchall 2006) because of its mucoadhesive properties (Lehr et al. 1992). Li et al. developed lipid/polycation condensed plasmid DNA chitosan particles and could show that in vitro deposition of chitosan-modified powders was higher than for the unmodified powders, and also that the level of reporter gene expression was enhanced (Li and Birchall 2006). Similarly, Tahara et al. demonstrated improved cellular uptake of PLGA nanospheres with a chitosan-modified surface compared to conventional vectors (Tahara et al. 2007). Another polymer that is used for pulmonary gene delivery is polyethylenimine (PEI) (Kleemann et al. 2004; Chen et al. 2007; Tagalakis et al. 2008). Kleemann et al. developed TAT-PEG-PEI conjugates to deliver plasmid DNA and reported enhanced DNA protection and higher transfection efficiencies in vivo compared to unmodified PEI (Kleemann et al. 2005).

Cationic lipids, such as lipofectin, have also been used as carriers for gene delivery. Bhattarai et al. administered lipofectin polymer (poly(*p*-dioxanone-*co*-L-lactide)-block-poly(ethylene glycol) micelles with the tumor suppressor gene *PTEN* to C57BL/6 melanoma-bearing mice. From the experimental results they observed significantly improved gene expression of PTEN in the lungs with no evidence of cell toxicity or acute inflammation and significantly longer survival times (Bhattarai et al. 2007).

In the context of gene therapy it must be noted that many of these particle-based targeting concepts have also been employed to deliver antisense DNA/RNA or siRNA to the lungs. Similar to plasmid DNA, these smaller nucleotide sequences must be formulated with a carrier system that is able to protect antisense nucleotides and enhance overall transfection rates.

3.6 Lung Cancer Therapy

Anti-cancer agents are normally administered to the systemic circulation when high plasma levels may be required in order to provide a suitable drug concentration at the site of action. Hitherto, drugs against lung cancer are administered systemically and, due to the drug's inherent cytotoxicity serving at the same time as the mechanism of drug action, cause serious side effects in healthy organs such as the liver, heart, and kidneys. Considering lung cancer, pulmonary administration offers the opportunity to achieve higher local effects and even sustained release in the lung while reducing the unintended systemic exposure to cancer drugs. Several approaches have been adopted to target different cancer drugs to the lungs and will be discussed below.

Hitzman et al. administered aerosolized lipid-coated nanoparticles loaded with 5-fluorouracil to hamsters with squamous lung cell carcinoma of the lung (Hitzman et al. 2006c). Previous studies in vitro assured sustained release properties for the lipid-coated nanoparticles used (Hitzman et al. 2006a, b). In the animal experiments, 5-fluorouracil levels were much lower in the serum compared to the lungs, indicating effective local exposure and sustained release properties.

The FDA approved injection of paclitaxel-loaded albumin nanoparticles to treat breast cancer in 2005 (Gradishar et al. 2005), and while there are no published studies on their effectiveness in the lungs, the potential of this technology for inhalation therapy deserves to be investigated. In another approach, the toxic effects of cisplatin were reduced by sustained release lipid inhalation targeting (SLIT). SLIT-cisplatin is a dispersion of cisplatin encapsulated in lipid vesicles that releases 50% of the dose immediately while the other 50% remains in liposomes for sustained release (Perkins et al. 2005; Wittgen et al. 2007). Although this phase I study showed that the administration of SLIT-cisplatin is feasible and safe the deposition efficiency (10–15%) still has to be optimized.

With respect to the lungs' unique architecture, cell-specific targeting systems have the potential to further improve cancer therapy in the respiratory tract. For example, lectin-functionalized liposomes specifically bind to the tumor-derived cell line A549 (Abu-Dahab et al. 2001; Brueck et al. 2001) and thus may serve as an effective targeting system. Abu-Dahab et al. successfully investigated the effect of nebulization on the stability of lectin-functionalized liposomes and their binding to A549 cells. A more specific target also may be the transferrin receptor, which is over-expressed in many human tumor cells. Anabousi et al. examined uptake levels and cytotoxicity of transferrin-conjugated liposomes and showed enhanced uptake

as well as increased cytotoxicity for this specific targeting approach (Anabousi et al. 2006a). PEGylation of these liposomes increased stability and will permit promising aerosolization experiments to prove this concept in vivo (Anabousi et al. 2006b).

Last but not least, telomerase is an interesting and emerging target for cancer therapy as this enzyme is present in most human cancers (Hiyama et al. 1995; Shay and Wright 2006). Inhibition of this enzyme may represent a novel therapy for lung cancer, except that specific telomerase inhibitors like the antisense oligonucleotide 2′-*O*-methyl-RNA (2-OMR) need a special carrier system to exert a biological effect in targeted cells. Beisner et al. administered this telomerase inhibitor in different liposomal formulations containing DOTAP (*N*-[1-(2,3-dioleoyloxy)]-*N*, *N*, *N*-trimethylammonium propane methyl sulfate), which is a cationic lipid, or a mixture of DOTAP and cholesterol to A549 cells (Beisner et al. 2008). In the outcome, these formulations enhanced the transfection of A549 cells and efficiently inhibited the telomerase. Nafee et al. recently developed chitosan-coated PLGA nanoparticles, originally developed for plasmid DNA delivery (Ravi Kumar et al. 2004), as a carrier for the 2-OMR antisense oligonucleotide (Nafee et al. 2007). Here, the cationic surface modification by chitosan enables the PLGA particles to form nanoplexes with nucleotide-based drugs. In consequence, the nanoplex structure can protect these molecules from premature degradation and facilitate their cellular uptake. According to this concept, Taetz et al. used cationic chitosan/PLGA nanoparticles to deliver 2-OMR to A549 cells, and observed enhanced uptake of 2-OMR nanoplexes into A549 cells, efficient telomerase inhibition, and significant shortening of telomeres compared to 2-OMR alone (Taetz et al. 2009). Obviously, this kind of nanotechnology-based carrier system represents an interesting new platform for safe and efficient delivery of telomerase inhibitors in the context of lung and possibly other cancers.

4 Future Prospects

Although there is a huge variety of application devices and drugs available, pneumology specialists agree on insufficient therapeutic standards regarding duration of drug effects and regional targeting within the respiratory system. In this chapter we aimed to outline several upcoming and promising concepts in modern inhalation therapy. The technology base exists to produce various advanced drug carrier systems, such as nanoparticles, liposomes, and large porous particles, and efficient lung deposition – even under pathophysiological conditions – seldom limits the therapeutic benefit. Nonetheless, inhalation therapy is still problematic and contains several open questions on drug/particle clearance from the lung.

More work is needed though to clarify and control what happens after the drug is deposited in the respiratory tract. Creating a local depot for prolonged drug action still appears to be rather difficult since the drug/particles must be able to escape from the clearance mechanisms of the lungs, namely macrophage and MC.

Moreover, the effect of any therapeutic intervention must be evaluated with regard to possible effects on these clearance mechanisms, originally developed by evolution to protect the human body from invading pathogens. Lung diseases such as CF and cancer can influence tissue properties in many ways, for example by altering airway mucus characteristics, generating chronic inflammation processes, or producing unventilated lung areas. In consequence, the changed morphological and physiological situation once more underlines the demand for multifunctional concepts which enable treatment of several diseases. Some promising progress is being made in at least one of these areas, namely the penetration of mucus by particulate drug carriers (Lai et al. 2007).

Due to the (understandably) strict emphasis on safety, the complex regulatory hurdles of the drug registration process, and other peculiarities of the pharmaceutical market, the conversion of innovative delivery technologies into marketed drug products is a rather slow process. Setbacks may occur sometimes for economic rather than scientific reasons, such as that seen recently with inhaled insulin. However the progress that has been made in pulmonary drug delivery over the past few years is nevertheless impressive and we are sure new therapies will continue to be developed, even if the road ahead is long and winding.

References

Abu-Dahab R, Schafer UF, Lehr C-M (2001) Lectin-functionalized liposomes for pulmonary drug delivery: effect of nebulization on stability and bioadhesion. Eur J Pharm Sci 14:37–46

Aiache JM (1990) Aerosol therapy in France. J Aerosol Med 3:85–120

Albertine KH, Williams MC, Hyde DM (2000) Anatomy and development of the respiratory tract. In: Murray JF, Nadel JA (eds) Textbook of respiratory medicine. W.B. Saunders CBS Educ. and Professional Publ, New York, NY, pp 3–33

Allen TM, Hansen C (1991) Pharmacokinetics of stealth versus conventional liposomes: effect of dose. BBA Biomembranes 1068:133–141

Amidi M, Pellikaan HC, Hirschberg H, de Boer AH, Crommelin DJA, Hennink WE, Kersten G, Jiskoot W (2007) Diphtheria toxoid-containing microparticulate powder formulations for pulmonary vaccination: preparation, characterization and evaluation in guinea pigs. Vaccine 25:6818–6829

Amorij JP, Saluja V, Petersen AH, Hinrichs WLJ, Huckriede A, Frijlink HW (2007) Pulmonary delivery of an inulin-stabilized influenza subunit vaccine prepared by spray-freeze drying induces systemic, mucosal humoral as well as cell-mediated immune responses in BALB/c mice. Vaccine 25:8707–8717

Anabousi S, Bakowsky U, Schneider M, Huwer H, Lehr CM, Ehrhardt C (2006a) In vitro assessment of transferrin-conjugated liposomes as drug delivery systems for inhalation therapy of lung cancer. Eur J Pharm Sci 29:367–374

Anabousi S, Kleemann E, Bakowsky U, Kissel T, Schmehl T, Gessler T, Seeger W, Lehr CM, Ehrhardt G (2006b) Effect of PEGylation on the stability of liposomes during nebulisation and in lung surfactant. J Nanosci Nanotechnol 6:3010–3016

Arya V, Coowanitwong I, Brugos B, Kim WS, Singh R, Hochhaus G (2006) Pulmonary targeting of sustained release formulation of budesonide in neonatal rats. J Drug Target 14:680–686

Bailey MR, Ansoborlo E, Guilmette RA, Paquet F (2007) Updating the ICRP human respiratory tract model. Radiat Prot Dosim 127:31–34

Bechtold-Peters K, Luessen H (eds) (2007) Pulmonary drug delivery – basics, applications and opportunities for small molecules and biopharmaceutics. Editio Cantor Verlag, Aulendorf, Germany

Beisner J, Dong M, Taetz S, Piotrowska K, Kleideiter E, Friedel G, Schaefer U, Lehr CM, Klotz U, Mürdter TE (2008) Efficient telomerase inhibition in human non-small cell lung cancer cells by liposomal delivery of 2'-O-methyl-RNA. J Pharm Sci 98:1765–1774

Bennet C (1654) Theatri tabidorum vestibulum: seu exercitationes dianoeticae cum historiis et experimentis demonstratives. Newcomb, London, pp 1–126

Bennett JV, De Castro JF, Valdespino-Gomez JL, De Lourdes Garcia-Garcia M, Islas-Romero R, Echaniz-Aviles G, Jimenez-Corona A, Sepulveda-Amor J (2002) Aerosolized measles and measles-rubella vaccines induce better measles antibody booster responses than injected vaccines: randomized trials in Mexican school children. Bull World Health Organ 80:806–812

Bennett WD, Scheuch G, Zeman KL, Brown JS, Kim C, Heyder J, Stahlhofen W (1999) Regional deposition and retention of particles in shallow, inhaled boluses: effect of lung volume. J Appl Physiol 86:168–173

Bhattarai SR, Kim SY, Jang KY, Yi HK, Lee YH, Bhattarai N, Nam SY, Lee DY, Kim HY, Hwang PH (2007) Amphiphilic triblock copolymer poly(p-dioxanone-co-L-lactide)-block-poly(ethylene glycol), enhancement of gene expression and inhibition of lung metastasis by aerosol delivery. Gene Ther 14:476–483

Bivas-Benita M, Ottenhoff THM, Junginger HE, Borchard G (2005) Pulmonary DNA vaccination: concepts, possibilities and perspectives. J Control Release 107:1–29

Bivas-Benita M, Van Meijgaarden KE, Franken KLMC, Junginger HE, Borchard G, Ottenhoff THM, Geluk A (2004) Pulmonary delivery of chitosan-DNA nanoparticles enhances the immunogenicity of a DNA vaccine encoding HLA-A*0201-restricted T-cell epitopes of *Mycobacterium tuberculosis*. Vaccine 22:1609–1615

Booker R (2005) Do patients think that dry powder inhalers can be used interchangeably? Int J Clin Prac 59:30–32

Brand P, Friemel I, Meyer T, Schulz H, Heyder J, Huinger K (2000) Total deposition of therapeutic particles during spontaneous and controlled inhalations. J Pharm Sci 89:724–731

Brown JS, Bennett WD (2004) Deposition of coarse particles in cystic fibrosis: model predictions versus experimental results. J Aerosol Med 17:239–248

Brueck A, Abu-Dahab R, Borchard G, Schaefer UF, Lehr CM (2001) Lectin-functionalized liposomes for pulmonary drug delivery: interaction with human alveolar epithelial cells. J Drug Target 9:241–251

Burger JL, Cape SP, Braun CS, McAdams DH, Best JA, Bhagwat P, Pathak P, Rebits LG, Sievers RE (2008) Stabilizing formulations for inhalable powders of live-attenuated measles virus vaccine. J Aerosol Med 21:1–10

Chen T, Wang Z, Wang R, Lu T, Wang W (2007) Polyethylenimine – DNA solid particles for gene delivery. J Drug Target 15:714–720

Chiou H, Chan HK, Heng D, Prud'homme RK, Raper JA (2008) A novel production method for inhalable cyclosporine A powders by confined liquid impinging jet precipitation. J Aerosol Sci 39:500–509

Chono S, Tanino T, Seki T, Morimoto K (2008) Efficient drug targeting to rat alveolar macrophages by pulmonary administration of ciprofloxacin incorporated into mannosylated liposomes for treatment of respiratory intracellular parasitic infections. J Control Release 127: 50–58

Cruz PE, Mueller C, Flotte TR (2007) The promise of gene therapy for the treatment of α-1 antitrypsin deficiency. Pharmacogenomics 8:1191–1198

de Swart RL, Li Calsi C, Quirk AV, van Amerongen G, Nodelman V, Alcock R, Yuksel S, Ward GH, Hardy JG, Vos H, Witham CL, Grainger CI, Kuiken T, Greenspan BJ, Gard TG, Osterhaus ADME (2007) Measles vaccination of macaques by dry powder inhalation. Vaccine 25:1183–1190

Deol P, Khuller GK (1997) Lung specific stealth liposomes: stability, biodistribution and toxicity of liposomal antitubercular drugs in mice. BBA-Gen Subjects 1334:161–172
Deol P, Khuller GK, Joshi K (1997) Therapeutic efficacies of isoniazid and rifampin encapsulated in lung-specific stealth liposomes against *Mycobacterium tuberculosis* infection induced in mice. Antimicrob Agents Chemother 41:1211–1214
Dessanges JF (2001) A history of nebulization. J Aerosol Med 14:65–71
Desseyn JL, Aubert JP, Porchet N, Laine A (2000) Evolution of the large secreted gel-forming mucins. Mol Biol Evol 17:1175–1184
Dhand R (2005) Aerosol plumes: slow and steady wins the race. J Aerosol Med 18:261–263
Dilraj A, Sukhoo R, Cutts FT, Bennett JV (2007) Aerosol and subcutaneous measles vaccine: measles antibody responses 6 years after re-vaccination. Vaccine 25:4170–4174
Dolovich MA, MacIntyre NR, Anderson PJ, Camargo CA Jr, Chew N, Cole CH, Dhand R, Fink JB, Gross NJ, Hess DR, Hickey AJ, Kim CS, Martonen TB, Pierson DJ, Rubin BK, Smaldone GC (2000) Consensus statement: aerosols and delivery devices. Respir Care 45:589–596
Eager R, Harle L, Nemunaitis JJ (2007) Lung cancer vaccines. Curr Gene Ther 7:469–484
El-Aneed A (2004) An overview of current delivery systems in cancer gene therapy. J Control Release 94:1–14
Forbes B, Ehrhardt C (2005) Human respiratory epithelial cell culture for drug delivery applications. Eur J Pharma Biopharm 60:193–205
Gaber NN, Darwis Y, Peh KK, Tan YTF (2006) Characterization of polymeric micelles for pulmonary delivery of beclomethasone dipropionate. J Nanosci Nanotechnol 6:3095–3101
Garcia-Contreras L, Fiegel J, Telko MJ, Elbert K, Hawi A, Thomas M, VerBerkmoes J, Germishuizen WA, Fourie PB, Hickey AJ, Edwards DA (2007) Inhaled large porous particles of capreomycin for treatment of tuberculosis in a guinea pig model. Antimicrob Agents Chemother 51:2830–2836
Garmise RJ, Staats HF, Hickey AJ (2007) Novel dry powder preparations of whole inactivated influenza virus for nasal vaccination. AAPS PharmSciTech 8:81
Geiser M, Rothen-Rutishauser B, Kapp N, Schurch S, Kreyling W, Schulz H, Semmler M, Im Hof V, Heyder J, Gehr P (2005) Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. Environ Health Perspect 113:1555–1560
Gilbert BE, Knight C, Alvarez FG, Waldrep JC, Rodarte JR, Knight V, Eschenbacher WL (1997) Tolerance of volunteers to cyclosporine A-dilauroylphosphatidylcholine liposome aerosol. Am J Respir Crit Care Med 156:1789–1793
Gradishar WJ, Tjulandin S, Davidson N, Shaw H, Desai N, Bhar P, Hawkins M, O'Shaughnessy J (2005) Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. J Clin Oncol 23:7794–7803
Groneberg DA, Witt C, Wagner U, Chung KF, Fischer A (2003) Fundamentals of pulmonary drug delivery. Respir Med 97:382–387
Heyder J (1981) Mechanisms of aerosol particle deposition. Chest 80:820–823
Heyder J, Gebhart J, Rudolf G, Schiller CF, Stahlhofen W (1986) Deposition of particles in the human respiratory tract in the size range 0.005–15 μm. J Aerosol Sci 17:811–825
Heyder J, Svartengren MU (2002) Basic principles of particle behavior in the human respiratory tract. In: Bisgaard H, O'Callaghan C, Smaldone GC (eds) Drug delivery to the lung. New York, Marcel Dekker, pp 21–45
Hirota K, Hasegawa T, Hinata H, Ito F, Inagawa H, Kochi C, Soma GI, Makino K, Terada H (2007) Optimum conditions for efficient phagocytosis of rifampicin-loaded PLGA microspheres by alveolar macrophages. J Control Release 119:69–76
Hitzman CJ, Elmquist WF, Wattenberg LW, Wiedmann TS (2006a) Development of a respirable, sustained release microcarrier for 5-fluorouracil I: In vitro assessment of liposomes, microspheres, and lipid coated nanoparticles. J Pharm Sci 95:1114–1126
Hitzman CJ, Elmquist WF, Wiedmann TS (2006b) Development of a respirable, sustained release microcarrier for 5-fluorouracil II: In vitro and in vivo optimization of lipid coated nanoparticles. J Pharm Sci 95:1127–1143

Hitzman CJ, Wattenberg LW, Wiedmann TS (2006c) Pharmacokinetics of 5-fluorouracil in the hamster following inhalation delivery of lipid-coated nanoparticles. J Pharm Sci 95:1196–1211
Hiyama K, Hiyama E, Ishioka S, Yamakido M, Inai K, Gazdar AF, Piatyszek MA, Shay JW (1995) Telomerase activity in small-cell and non-small-cell lung cancers. J Natl Cancer Inst 87:895–902
Hobson P, Barnfield C, Barnes A, Klavinskis LS (2003) Mucosal immunization with DNA vaccines. Methods 31:217–224
Hochrainer D, Holz H, Kreher C, Scaffidi L, Spallek M, Wachtel H (2005) Comparison of the aerosol velocity and spray duration of Respimat® Soft Mist™ inhaler and pressurized metered dose inhalers. J Aerosol Med 18:273–282
Howard KA, Rahbek UL, Liu X, Damgaard CK, Glud SZ, Andersen MØ, Hovgaard MB, Schmitz A, Nyengaard JR, Besenbacher F, Kjems J (2006) RNA Interference in vitro and in vivo using a novel chitosan/siRNA nanoparticle system. Mol Ther 14:476–484
Iacono AT, Smaldone GC, Keenan RJ, Diot P, Dauber JH, Zeevi A, Burckart GJ, Griffith BP (1997) Dose-related reversal of acute lung rejection by aerosolized cyclosporine. Am J Respir Crit Care Med 155:1690–1698
ICRP (1994) Human respiratory tract model for radiological protection. Ann ICRP 24:1–120
Issa MM, Köping-Höggård M, Tømmeraas K, Vårum KM, Christensen BE, Strand SP, Artursson P (2006) Targeted gene delivery with trisaccharide-substituted chitosan oligomers in vitro and after lung administration in vivo. J Control Release 115:103–112
Jaspart S, Bertholet P, Piel G, Dogne JM, Delattre L, Evrard B (2007) Solid lipid microparticles as a sustained release system for pulmonary drug delivery. Eur J Pharm Biopharm 65:47–56
Kleemann E, Dailey LA, Abdelhady HG, Gessler T, Schmehl T, Roberts CJ, Davies MC, Seeger W, Kissel T (2004) Modified polyethylenimines as non-viral gene delivery systems for aerosol gene therapy: Investigations of the complex structure and stability during air-jet and ultrasonic nebulization. J Control Release 100:437–450
Kleemann E, Neu M, Jekel N, Fink L, Schmehl T, Gessler T, Seeger W, Kissel T (2005) Nano-carriers for DNA delivery to the lung based upon a TAT-derived peptide covalently coupled to PEG-PEI. J Control Release 109:299–316
Köping-Höggård M, Varum KM, Issa M, Danielsen S, Christensen BE, Stokke BT, Artursson P (2004) Improved chitosan-mediated gene delivery based on easily dissociated chitosan polyplexes of highly defined chitosan oligomers. Gene Ther 11:1441–1452
Lai SK, O'Hanlon DE, Harrold S, Man ST, Wang YY, Cone R, Hanes J (2007) Rapid transport of large polymeric nanoparticles in fresh undiluted human mucus. Proc Natl Acad Sci USA 104:1482–1487
Learoyd TP, Burrows JL, French E, Seville PC (2008) Chitosan-based spray-dried respirable powders for sustained delivery of terbutaline sulfate. Eur J Pharm Biopharm 68:224–234
Lehr C-M, Bouwstra JA, Schacht EH, Junginger HE (1992) In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. Int J Pharm 78:43–48
Lentz YK, Anchordoquy TJ, Lengsfeld CS (2006) Rationale for the selection of an aerosol delivery system for gene delivery. J Aerosol Med 19:372–384
Letsou GV, Safi HJ, Reardon MJ, Ergenoglu M, Li Z, Klonaris CN, Baldwin JC, Gilbert BE, Waldrep JC (1999) Pharmacokinetics of liposomal aerosolized cyclosporine A for pulmonary immunosuppression. Ann Thorac Surg 68:2044–2048
Li HY, Birchall J (2006) Chitosan-modified dry powder formulations for pulmonary gene delivery. Pharm Res 23:941–950
LiCalsi C, Maniaci MJ, Christensen T, Phillips E, Ward GH, Witham C (2001) A powder formulation of measles vaccine for aerosol delivery. Vaccine 19:2629–2636
Lu D, Hickey AJ (2007) Pulmonary vaccine delivery. Expert Rev Vaccines 6:213–226
Möller W, Häußinger K, Kreyling WG, Scheuch G (2005) Particle clearance from the human respiratory tract. Atemw-Lungenkrkh 31:342–351
Mudge J (1778) A radical and expeditious cure for a recent catarrhous cough. Allen, London, pp 1–252

Murphy SL, High KA (2008) Gene therapy for haemophilia. Br J Haematol 140:479–487
Nafee N, Taetz S, Schneider M, Schaefer UF, Lehr CM (2007) Chitosan-coated PLGA nanoparticles for DNA/RNA delivery: effect of the formulation parameters on complexation and transfection of antisense oligonucleotides. Nanomedicine 3:173–183
O'Riordan TG, Iacono A, Keenan RJ, Duncan SR, Burckart GJ, Griffith BP, Smaldone GC (1995) Delivery and distribution of aerosolized cyclosporine in lung allograft recipients. Am J Respir Crit Care Med 151:516–521
Oberdörster G, Oberdörster E, Oberdörster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113:823–839
Pandey R, Sharma A, Zahoor A, Sharma S, Khuller GK, Prasad B (2003) Poly (DL-lactide-co-glycolide) nanoparticle-based inhalable sustained drug delivery system for experimental tuberculosis. J Antimicrob Chemother 52:981–986
Patton JS, Byron PR (2007) Inhaling medicines: delivering drugs to the body through the lungs. Nat Rev Drug Discov 6:67–74
Perkins W, Weers J, Meers P (2005) An inhalation formulation of liposomal cisplatin (SLIT™ Cisplatin) for treatment of lung cancer [abstract]. In: Conference Vancouver: Lipids, Liposomes & Biomembranes
Pilcer G, Sebti T, Amighi K (2006) Formulation and characterization of lipid-coated tobramycin particles for dry powder inhalation. Pharm Res 23:931–940
Pitcairn G, Reader S, Pavia D, Newman S (2005) Deposition of corticosteroid aerosol in the human lung by Respimat® Soft Mist™ inhaler compared to deposition by metered dose inhaler or by Turbuhaler® dry powder inhaler. J Aerosol Med 18:264–272
Ravi Kumar MNV, Bakowsky U, Lehr CM (2004) Preparation and characterization of cationic PLGA nanospheres as DNA carriers. Biomaterials 25:1771–1777
Read RC, Naylor SC, Potter CW, Bond J, Jabbal-Gill I, Fisher A, Illum L, Jennings R (2005) Effective nasal influenza vaccine delivery using chitosan. Vaccine 23:4367–4374
Saari M, Vidgren MT, Koskinen MO, Turjanmaa VMH, Nieminen MM (1999) Pulmonary distribution and clearance of two beclomethasone liposome formulations in healthy volunteers. Int J Pharm 181:1–9
Sanders M (2007) Inhalation therapy: an historical review. Prim Care Respir J 16:71–81
Sanna V, Kirschvink N, Gustin P, Gavini E, Roland I, Delattre L, Evrard B (2004) Preparation and in vivo toxicity study of solid lipid microparticles as carrier for pulmonary administration. AAPS PharmSciTech 5(2):27
Scheuch G, Fischer A (2008) Improved entry into the airways. Manuf Chem Aerosol News 79:39–40
Scheuch G, Siekmeier R (2007) Novel approaches to enhance pulmonary delivery of proteins and peptides. J Physiol Pharmacol 58:615–625
Scheuch G, Zimlich WC, Siekmeier R (2007) Biophysical parameters determining pulmonary drug delivery. In: Bechthold-Peters K, Luessen H (eds) Pulmonary drug delivery: basiscs, applications, and opportunities for small molecules and biopharmaceutics. Editio Cantor Verlag, Aulendorf, pp 46–54
Selting K, Waldrep JC, Reinero C, Branson K, Gustafson D, Kim DY, Henry C, Owen N, Madsen R, Dhand R (2008) Feasibility and safety of targeted cisplatin delivery to a select lung lobe in dogs via the AeroProbe®. J Aerosol Med Pulm Drug Deliv 21:255–268
Serra-Batlles J, Plaza V, Badiola C, Morejon E, Bardagi S, Brotons B, Cabello F, Castillo JA, Hermida JA, Lopez-Vinas A, Marin JM, Tabar A (2002) Patient perception and acceptability of multidose dry powder inhalers: a randomized crossover comparison of Diskus/Accuhaler with Turbuhaler. J Aerosol Med 15:59–64
Sharma A, Sharma S, Khuller GK (2004) Lectin-functionalized poly (lactide-co-glycolide) nanoparticles as oral/aerosolized antitubercular drug carriers for treatment of tuberculosis. J Antimicrob Chemother 54:761–766
Shay JW, Wright WE (2006) Telomerase therapeutics for cancer: challenges and new directions. Nat Rev Drug Discov 5:577–584

Sibille Y, Reynolds HY (1990) Macrophages and polymorphonuclear neutrophils in lung defense and injury. Am Rev Respir Dis 141:471–501

Sinswat P, Overhoff KA, McConville JT, Johnston KP, Williams Iii RO (2008) Nebulization of nanoparticulate amorphous or crystalline tacrolimus – single-dose pharmacokinetics study in mice. Eur J Pharm Biopharm 69:1057–1066

Smith DJ, Bot S, Dellamary L, Bot A (2003) Evaluation of novel aerosol formulations designed for mucosal vaccination against influenza virus. Vaccine 21:2805–2812

Sueblinvong V, Suratt BT, Weiss DJ (2007) Novel therapies for the treatment of cystic fibrosis: new developments in gene and stem cell therapy. Clin Chest Med 28:361–379

Taetz S, Nafee N, Beisner J, Piotrowska K, Baldes C, Mürdter TE, Huwer H, Schneider M, Schaefer UF, Klotz U, Lehr CM (2009) The influence of chitosan content in cationic chitosan/PLGA nanoparticles on the delivery efficiency of antisense 2′-O-methyl-RNA directed against telomerase in lung cancer cells. Eur J Pharm Biopharm 72:358–369

Tagalakis AD, McAnulty RJ, Devaney J, Bottoms SE, Wong JB, Elbs M, Writer MJ, Hailes HC, Tabor AB, O'Callaghan C, Jaffe A, Hart SL (2008) A receptor-targeted nanocomplex vector system optimized for respiratory gene transfer. Mol Ther 16:907–915

Tahara K, Yamamoto H, Takeuchi H, Kawashima Y (2007) Development of gene delivery system using PLGA nanospheres. Yakugaku Zasshi 127:1541–1548

Takenaga M, Ohta Y, Tokura Y, Hamaguchi A, Igarashi R, Disratthakit A, Doi N (2008) Lipid microsphere formulation containing rifampicin targets alveolar macrophages. Drug Deliv 15:169–175

Thornton DJ, Rousseau K, McGuckin MA (2008) Structure and function of the polymeric mucins in airways mucus. Annu Rev Physiol 70:459–486

Tschernig T, Pabst R (2000) Bronchus-associated lymphoid tissue (BALT) is not present in the normal adult lung but in different diseases. Pathobiology 68:1–8

Ventura CA, Tommasini S, Crupi E, Giannone I, Cardile V, Musumeci T, Puglisi G (2008) Chitosan microspheres for intrapulmonary administration of moxifloxacin: interaction with biomembrane models and in vitro permeation studies. Eur J Pharm Biopharm 68:235–244

Weibel ER (ed) (1963) Morphometry of the human lung. Academic Press, New York

Weibel ER (1979) Morphometry of the human lung: the state of the art after two decades. Clin Respir Physiol 15:999–1013

Welch MJ, Nelson HS, Shapiro G, Bensch GW, Sokol WN, Smith JA, Parasuraman BM (2004) Comparison of patient preference and ease of teaching inhaler technique for Pulmicort Turbuhaler® versus pressurized metered-dose inhalers. J Aerosol Med 17:129–139

WHO (2008) http://www.who.int/tb/publications/global_report/2008/summary/en/index.html.

Wijagkanalan W, Kawakami S, Takenaga M, Igarashi R, Yamashita F, Hashida M (2008) Efficient targeting to alveolar macrophages by intratracheal administration of mannosylated liposomes in rats. J Control Release 125:121–130

Wittgen BPH, Kunst PWA, Van Der Born K, Van Wijk AW, Perkins W, Pilkiewicz FG, Perez-Soler R, Nicholson S, Peters GJ, Postmus PE (2007) Phase I study of aerosolized SLIT cisplatin in the treatment of patients with carcinoma of the lung. Clin Cancer Res 13:2414–2421

Zaru M, Mourtas S, Klepetsanis P, Fadda AM, Antimisiaris SG (2007) Liposomes for drug delivery to the lungs by nebulization. Eur J Pharm Biopharm 67:655–666

Needle-Free Vaccine Injection

Mark A.F. Kendall

Contents

Abstract Millions of people die each year from infectious disease, with a main stumbling block being our limited ability to deliver vaccines to optimal sites in the body. Specifically, effective methods to deliver vaccines into outer skin and mucosal layers – sites with immunological, physical and practical advantages that cannot be targeted via traditional delivery methods – are lacking. This chapter investigates the challenge for physical delivery approaches that are primarily needle-free. We examine the skin's structural and immunogenic properties in the context of the physical cell targeting requirements of the viable epidermis, and we review selected current physical cell targeting technologies engineered to meet these needs: needle and syringe, diffusion patches, liquid jet injectors, and microneedle arrays/patches.

M.A.F. Kendall
Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, Building 75 – Cnr of College and Cooper Road The University of Queensland Brisbane, Brisbane, QLD4072, Australia
e-mail: m.kendall@uq.edu.au

M. Schäfer-Korting (ed.), *Drug Delivery*,
Handbook of Experimental Pharmacology 197,
DOI 10.1007/978-3-642-00477-3_7,

We then focus on biolistic particle delivery: we first analyze engineering these systems to meet demanding clinical needs, we then examine the interaction of biolistic devices with the skin, focusing on the mechanical interactions of ballistic impact and cell death, and finally we discuss the current clinical outcomes of one key application of engineered delivery devices – DNA vaccines.

Keywords Biolistics · DNA vaccines · Drug delivery · Gene guns · Immunotherapeutics · Langerhans cells · Skin · Skin mechanical properties · Microneedles · Vaccines

Abbreviations

A	Particle cross-sectional area
APC (APCs)	Antigen-presenting cell(s)
CST	Contoured shock tube
CHMP	Committee for Medicinal Products for Human Use
D	Particle resistive force
dDCs	Dermal dendritic cells
DEM	Discrete element model
DGV	Doppler global velocimetry
GMT	Geometric mean titer
HIV	Human immunodeficiency virus
PIV	Particle image velocimetry
RH	Relative humidity
SC	Stratum corneum
VE	Viable epidermis
V	Particle velocity
$v_{\mathrm{i,ve}}$	Viable epidermis boundary
ρ_{t}	Density of target
σ	Yield stress of target

1 Introduction

Vaccines are most commonly administered using a needle and syringe, a method first invented in 1853. The needle and syringe is effective, but unpopular, and creates a risk of iatrogenic disease from needle-stick injury or needle reuse as a consequence of the billions of administrations each year. Further, the needle and syringe does not deliver the vaccine ingredients optimally to the antigen-presenting cells (APCs), which alone can respond to the combination of antigen and adjuvant (innate immune stimulus) that makes a successful vaccine.

The provision of safe and efficient routes of delivery of vaccines to the immunologically sensitive dendritic cells in the skin (and mucosa) has the potential to

enhance strategies in the treatment of major diseases. The application of physical methods to achieving this goal presents unique engineering challenges in the physical transport of vaccines to these cells.

In this chapter, the physiology, immunology and material properties of the skin are examined in the context of the physical cell targeting requirements of the viable epidermis (as one example). Selected cell targeting technologies engineered to meet these needs are briefly presented. The operating principles of these approaches are described, together with a discussion of their effectiveness for the non-invasive targeting of viable epidermis cells and DNA vaccination against major diseases.

We then focus on one of these needle-free methods, called biolistics, that ballistically delivers millions of microparticles coated with biomolecules to outer skin layers. The engineering of these devices is presented, beginning with earlier prototypes before examining a more advanced system configured for clinical use. Then follows a theoretical and experimental analysis of the ballistic microparticle impact process, including the examination of induced cell death. Finally, the results of applying this technology to key human clinical trials are presented.

2 Targeting Skin and Mucosal Cells: The Immunological Rationale

Why are outer skin cells important targets in the treatment of disease? The answer is found from a consideration of skin structure, shown schematically in Figs. 1 and 2. Human skin can be subdivided into a number of layers: the outer stratum corneum (SC, 10–20 μm in depth), the viable epidermis (VE, 50–100 μm) and the dermis (1–2 mm) (Givens et al. 1993; Fuchs and Raghavan 2002). The SC is the effective physical barrier of dead cells in a "bricks and mortar" structure (Menton and Eisen 1971; Nemes and Steinert 1999). The underlying VE is composed of cells, such as immunologically sensitive Langerhans cells, keratinocytes, stem cells and melanocytes (Fuchs and Raghavan 2002). Unlike the dermis below, the VE lacks blood vessels and sensory nerve endings – important characteristics of a site for pain-free delivery with minimal damage.

In the VE, the skin has evolved a highly competent immunological function, with an abundance of Langerhans cells (500–1,000 cells mm^{-2}) (Berman et al. 1983; Chen et al. 1985; Stenn et al. 1992), often serving as the first line of defense against many pathogens (Babiuk et al. 2000). In particular, Langerhans cells (illustrated in Fig. 2) are extremely effective APCs, responsible for the uptake and processing of foreign materials in order to generate an effective immune response. Such cells are reported to be up to 1,000-fold more effective than keratinocytes, fibroblasts and myoblasts at eliciting a variety of immune responses (McKinney and Streilein 1989; Banchereau and Steinman 1998; Timares et al. 1998; Chen et al. 2002).

Less information is available on underlying, dermal APCs. New populations of dermal dendritic cells (dDCs) that express langerin (originally believed to be an

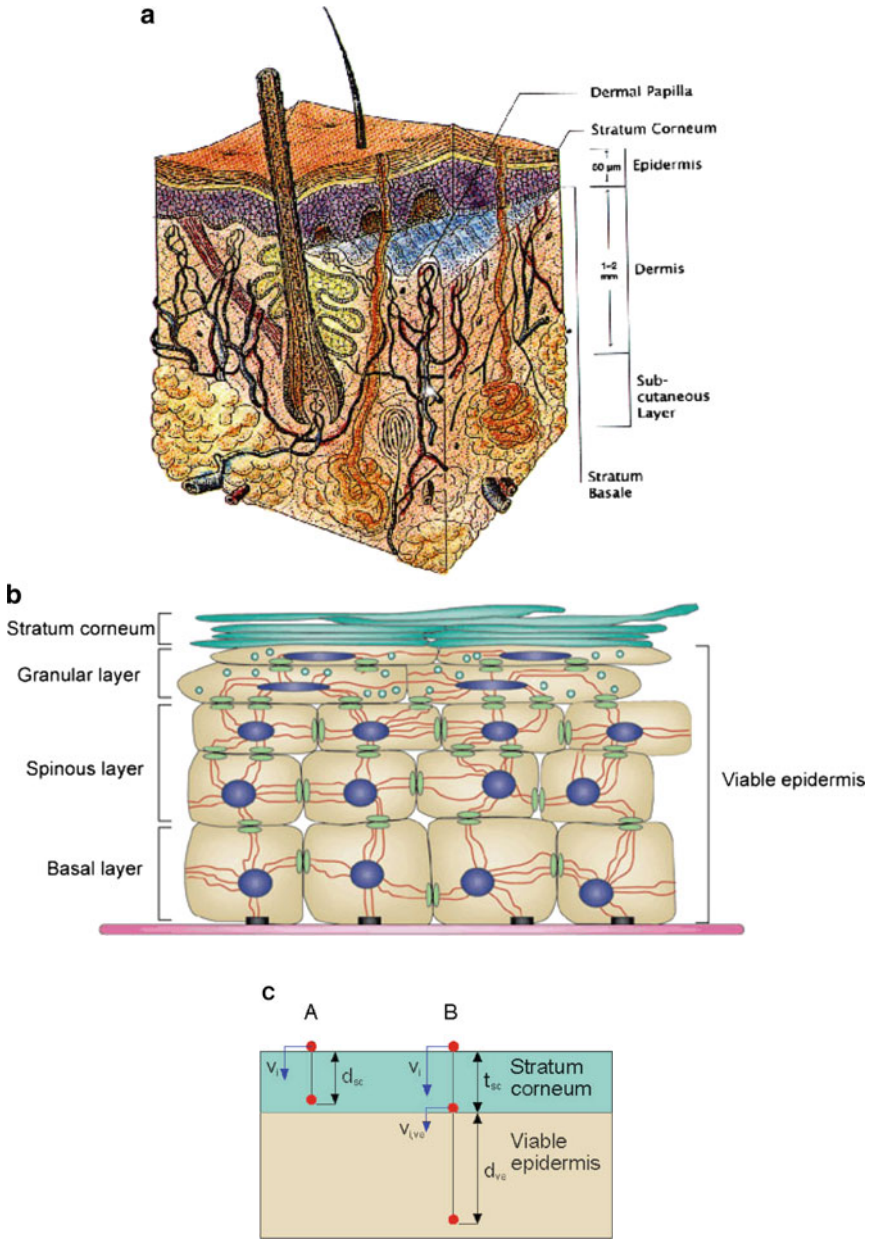


Fig. 1 A schematic diagram of the structure of mammalian skin (**a**), the epidermis of mammalian skin (**b**), and the corresponding bilayer approximation of the epidermis used for the theoretical penetration model (**c**). Penetration case A denotes particle delivery into the stratum corneum (d_{sc}), whereas, in case B, the stratum corneum is fully breached (t_{sc}) and the final particle location is within the viable epidermis (d_{ve}). The impact velocity is v_i, whilst the input velocity for the viable epidermis is $v_{i,ve}$. Adapted from Kendall et al. (2004b)

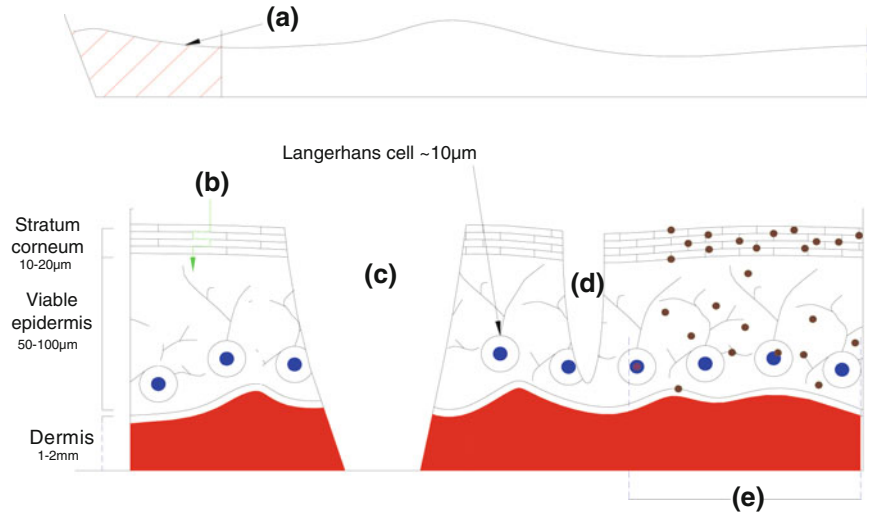


Fig. 2 A schematic cross-section of the skin showing Langerhans cells. Five physical cell targeting approaches are also shown. (**a**) A half-section of a small gauge needle and syringe; (**b**) route of diffusion from patches; (**c**) penetration from a liquid jet injector; (**d**) a hole from a microinjector; and (**e**) distribution of microparticles following biolistic injection. From Kendall (2006)

exclusive marker for Langerhans cells) reportedly exist (Poulin et al. 2007) and have unique immunological functions within the skin (Nagao et al. 2009). Thus, directly targeting specific Langerhans cells or dermal APC populations will allow immune responses to be modulated following vaccination.

For simplicity, this chapter focuses on delivery methods targeting vaccine to the most defined skin APC population, the Langerhans cells, residing in the epidermis. Effective in situ (in vivo) targeting of Langerhans cells and other epidermal cells with polynucleotides or antigens will open up novel applications in disease control (Chen et al. 2002), including vaccination against major viruses/diseases, such as human immunodeficiency virus (HIV) and cancer.

3 Engineering of Physical Approaches for the Targeting of Skin and Mucosal Cells

Within the VE, the location of Langerhans cells – as a delivery target for immunotherapeutics – is tightly defined by:

- A vertical position at a consistent suprabasal location (Hoath and Leahy 2002);
- A spatial distribution in the horizontal plane evenly distributed throughout the skin (Numahara et al. 2001);

- A constitution of 2% of the total epidermal cell population (in human skin) (Bauer et al. 2001).

Despite its recognized potential, the VE has only recently been viewed as a feasible cellular targeting site with the emergence of new biological and physical technologies. The challenge is the effective penetration of the SC and precise targeting of the cells of interest.

3.1 Mechanical Properties of the SC Barrier

The SC is a semi-permeable barrier that, owing to its variable mechanical properties, is challenging to breach in a minimally invasive manner, to target the viable epidermal cells below. Mechanically, the SC is classified as a bio-viscoelastic solid and shows highly variable properties. Obvious differences include the huge variation in thickness and composition with the skin site and the age of an individual (Hopewell 1990). However, there are more subtle and equally important variations in SC properties to consider when configuring targeting methods.

For example, the SC mechanical breaking stress is strongly influenced by the ambient humidity/moisture content (Wildnauer et al. 1971; Christensen et al. 1977; Rawlings et al. 1995; Dobrev 1996; Nicolopoulos et al. 1998) – the relative humidity range from 0% to 100% results in a decrease in excised human SC breaking stress from 22.5 to 3.2 MPa (Kendall et al. 2004b). Similarly, an increase in ambient temperature also results in an SC breaking stress decrease by an order of magnitude (Papir et al. 1975).

More recently, with indentation studies using small probes (diameters of 2 and 5 μm) fitted to a NANO-Indenter (Kendall et al. 2007), we have found more complexity and variation in key SC, and underlying VE, mechanical properties. Specifically:

- The storage modulus and mechanical breaking stress both dramatically decrease through the SC (Fig. 3a, 3b);
- At a given depth within the SC and VE, decreasing the probe size significantly increases the storage modulus (Fig. 3a).

These and other sources of variability in the SC mechanical properties present challenges in configuring approaches to breach the SC in a minimally invasive manner, and effectively deliver vaccines to the underlying cells.

3.2 Biological Approaches

Although the focus of this chapter is on physical approaches to target epidermal cells, it is also important to highlight biological approaches. A powerful biological approach to the transport of biomolecules to epidermal (and other) cells, in vivo, exploits the evolved function of viruses in the transport to cells. In gene delivery,

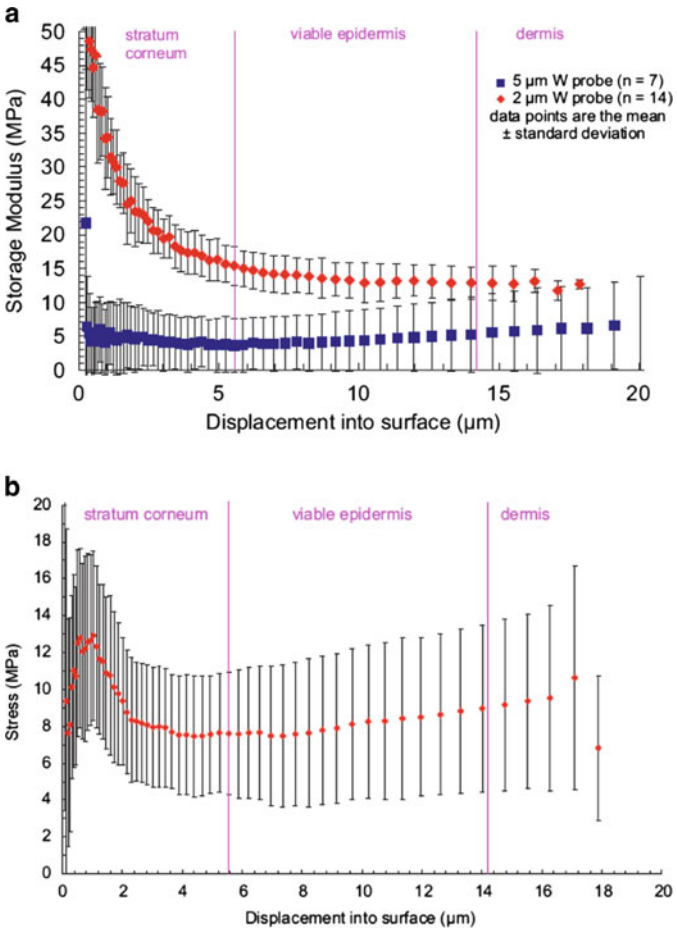


Fig. 3 Mechanical properties (mean ± standard deviation) as a function of displacement obtained with microprobes indented into murine ears. (**a**) Storage modulus with 5 and 2 μm microprobes. (**b**) Stress with a 2 μm microprobe. Adapted from Kendall et al. (2007)

researchers have made use of genetically engineered viruses in DNA vaccination and gene therapy of major diseases with encouraging results; however, viral gene delivery is hindered by safety concerns, a limited DNA-carrying capacity, production and packaging problems and a high cost (Lu et al. 1997; Tang et al. 1997).

3.3 Physical Cell Targeting Approaches

Alternatively, many physical technologies are being developed. Potentially, they can overcome some limitations of biological approaches using needle-free

mechanisms to breach the SC barrier to facilitate drug and vaccine administration directly to epidermal cells. Figure 2 illustrates schematically key physical targeting approaches relative to the scale of typical skin and the Langerhans cell layer of interest.

Needle and syringe. The most common physical delivery method, a small gauge needle and syringe, is shown in half-section in Fig. 2a. Although this approach easily breaches the SC, precise targeting of the Langerhans cell-rich VE cannot be practically achieved. Hence, the needle and syringe is used for intradermal or intramuscular injection. This inefficient, indirect targeting of dendritic cells with DNA has resulted in modest immune responses (Mumper and Ledebur 2001). Other disadvantages of the needle and syringe include risks due to needle-stick injuries (WHO 1999) and needle phobia (Givens et al. 1993).

Diffusion/permeation delivery. The least invasive method of breaching the SC is probably by permeation through it, driven by diffusion from patches applied to the skin (Fig. 2b) (Glenn et al. 2003). However, the current general view is that this mode of delivery is best suited to smaller biomolecules (<500 Da) (Glenn et al. 2003) – considerably smaller than vaccines. This view is being challenged, with a recent study showing that very large recombinant antigens of ~1 MDa can be delivered to elicit systemic responses by diffusion from patches (Guerena-Burgueno et al. 2002). The transport of larger biomolecules through the SC can be further enhanced by simple approaches, including tape stripping with an adhesive tape, brushing with sandpaper (Liu et al. 2001; Watabe et al. 2001) or the application of depilatory agents (Tang et al. 1997; Shi et al. 1999, 2001). Amongst the more advanced technologies are electroporation (Widera et al. 2000; Zucchelli et al. 2000), ablation by laser or heat, radiofrequency high-voltage currents (Sintov et al. 2003), iontophoresis (Alexander and Akhurst 1995; Li and Hoffman 1995; Domashenko et al. 2000), sonophoresis and microporation (Babiuk et al. 2000). Many of these approaches remain untested for complex entities such as vaccines and immunotherapies. Permeation through the SC can also be enhanced by the coating of plasmid DNA on nanoparticles (~100 nm) for DNA vaccination (Cui and Mumper 2001).

Liquid jet injectors. Interest in using high-speed liquid jet injectors arose in the mid-twentieth century because of its needle-free approach (Furth et al. 1995). This technique has seen a recent resurgence, with liquid delivered around the Langerhans cells in gene transfer and DNA vaccination experiments (Furth et al. 1995), and the delivery of drugs (Bremseth and Pass 2001). As shown in Fig. 2c, current liquid jet injectors typically disrupt the skin in the epidermal *and* dermal layer. To target exclusively the viable epidermal cells, such as Langerhans cells, the challenge of more controlled delivery needs to be addressed. With the dermal disruption induced by administration, liquid jet injectors are also reported to cause pain to patients.

Microneedle arrays/patches. Researchers have overcome some of the disadvantages described by fabricating arrays of micrometer-scale projections to breach the SC and to deliver naked DNA to several cells in live animals (Mikszta et al. 2002). Similar microprojection devices are used to increase the permeability of drugs

(Matriano et al. 2002) and "conventional" protein antigen vaccines (Matriano et al. 2002; McAllister et al. 2003). Figure 2d shows that, unlike current liquid jet injectors, these microneedles can accurately target the VE. Furthermore, they are as simple to use as patches, whilst overcoming the SC diffusion barrier to many molecules. Moreover, compared with both the needle and syringe and liquid jet injectors, these microneedle methods are pain-free because of epidermal targeting. By drawing upon a range of manufacturing techniques, McAllister et al. (2003) have shown that these microneedle arrays can be made from a range of materials, including silicon, metal and biodegradable polymers.

Newer devices are now being tailored to the immunological problem of *directly* targeting vaccines to the skin APC. One example is micro-nanoprojection array (otherwise called "nanopatches"). Nanopatches (Chen et al. 2008, 2009; Prow et al. 2008) are very small and densely packed on patches (less than 100 μm in length and over 20,000 MPs cm^{-2}), clearly distinct from the large and sparsely packed ones reported in literature (up to 700 μm in length and less than 321 projections per cm^2) (Matriano et al. 2002; Gill and Prausnitz 2007). These configurations produce highly-targeted and unique immune responses in mice. This includes, in the animal model:

- Orders of magnitude in vaccine dose reduction, compared to the needle and syringe while achieving an equivalent immune response (data submitted for publication) following the delivery of existing conventional vaccines;
- Greatly enhanced immune responses and vaccination protection against disease, obtained by nanopatch delivery of candidate DNA-based vaccines (compared to needle and syringe intradermal delivery).

Importantly, nanopatches are also practical devices – cheap to manufacture, with dry-coated vaccines stable at ambient temperature (i.e., not requiring refrigeration) and easy to use.

4 Biolistic Microparticle Delivery

Currently, the most established physical method of DNA vaccination is biolistic microparticle delivery, otherwise known as gene guns (Fig. 2e); this is the focus of the remainder of the chapter.

4.1 Biolistics Operating Principle

In this needle-free technique, pharmaceutical or immunomodulatory agents, formulated as particles, are accelerated in a supersonic gas jet to sufficient momentum to penetrate the skin (or mucosal) layer and to achieve a pharmacological effect.

Sanford et al. (1987) pioneered this innovation with systems designed to deliver DNA-coated metal particles (of diameter of the order of 1 μm) into plant cells for genetic modification, using pistons accelerated along the barrels of adapted guns (Sanford and Klein 1987). The concept was extended to the treatment of humans with particles accelerated by entrainment in a supersonic gas flow (Bellhouse et al. 1994). Prototype devices embodying this concept have been shown to be effective, painless, and applicable to pharmaceutical therapies ranging from protein delivery (Burkoth et al. 1999), to conventional vaccines (Chen et al. 2000) and DNA vaccines (Roy et al. 2000; Lesinski et al. 2001).

Different embodiments of the concept (e.g., in Figs. 4 and 5) all have a similar operating procedure. Consider the prototype shown schematically in Fig. 4a as one example. Prior to operation, the gas canister is filled with helium or nitrogen to 2–6 MPa and the vaccine cassette, comprising two 20 μm diaphragms, is loaded with a powdered pharmaceutical payload of 0.5–2 mg. The pharmaceutical material is placed on the lower diaphragm surface. Operation commences when the valve in the gas canister is opened to release gas into the rupture chamber, where the pressure builds up until the two diaphragms retaining the vaccine particles sequentially burst. The rupture of the downstream diaphragm initiates a shock which propagates down the converging−diverging nozzle. The ensuing expansion of stored gas results in a short-duration flow in which the drug particles are entrained and accelerated through the device. After leaving the device, particles impact on the skin and penetrate to the epidermis to induce a pharmacological effect.

4.2 Engineering of Hand-Held Biolistic Devices for Clinical Use

Biolistic delivery of immunotherapeutics is an application of transonic flow technology that is otherwise applied to aerospace applications. In this section we introduce prototype devices and discuss the key engineering challenges in applying this aerospace technology to clinical applications. Key parameters used to guide the engineering of biolistic devices are:

- A nominally uniform, controlled and quantified microparticle velocity and spatial distribution impacting the tissue target. Further, the impact momentum is to be within the range needed for delivery to particular locations (e.g., the Langerhans cells for DNA vaccines).
- A sufficient “footprint” on the tissue to deliver sufficient payload and target the appropriate number of cells.
- Noise levels within the user guidelines, for both the operator and patient.
- The device is to be hand-held.
- For long-term stability, the pharmaceutical is to be stored within a sealed environment.
- The device is to be produced from biocompatible materials.

- The devices, manufactured in large numbers, are to be cost-competitive with other relevant technologies.

Earlier generation systems attempting to address these parameters used a prototype device family generated from empirical studies. A schematic of one of these devices, using a convergent−divergent nozzle design is shown in Fig. 4a (Kendall et al. 2004c). Working with these devices, the challenge was to establish the gas−particle dynamics behavior of the systems. A significant research program was directed at this goal.

An array of methods were used to characterize the gas and particle dynamics of these systems. Quinlan et al. (2001) performed static pressure measurements to interrogate the gas flow, together with time-integrated Doppler Global Velocimetry (DGV) measurements of drug particle velocity (Quinlan et al. 2001). These measurements were very useful, but gave an incomplete description of the predominantly unsteady flow in the device.

In subsequent broader studies, the transient gas and particle flow within the device were interrogated with Pitot-static pressure measurement (as instrumented in Fig. 4a), together with Schlieren imaging, and time resolved DGV (Quinlan et al. 2001) and computational fluid dynamics (CFD) modeling (Liu and Kendall 2004b). The findings of this study are summarized with measured axial Mach number profiles through the nozzle (Fig. 4b) and a single Schlieren image (Fig. 4c).

The axial profiles of Mach number at various times after termination of the starting process (based on total-static and Pitot-static pressure measurements) are compared with the theoretical Mach number profile for steady isentropic quasi-one-dimensional supersonic flow (with the assumption of a choked throat) in Fig. 4b. Pitot and static pressure measurements (p_2 and p_3 respectively in Fig. 4a) suggest that 500 μs after diaphragm rupture, the flow 38.5 mm upstream of the nozzle exit is supersonic and close to the isentropic ideal. Further downstream, however, the overexpanded nozzle flow is processed through an oblique shock system which induces flow separation. Consequently, the experimentally determined Mach number (determined from Pitot and static pressure) gradually falls from between 2 and 2.5 (23.5 mm upstream of the exit plane) to 1.5 at the exit plane. The Mach number in the downstream region of the nozzle decays with time as the shock system moves upstream.

Sequences of Schlieren images such as the sample shown in Fig. 4c (t = 132 μs) reveal the structure of the evolving flowfield with greater detail and clarity (Kendall et al. 2004c). The oblique shocks visible have evolved to form at least three oblique shock cells that have interacted with the boundary layer and separated the nozzle flow. DGV images show particles were entrained in the nozzle starting process and the separated nozzle flow – regimes with large variations in gas density and velocity – giving rise to large variations in particle velocity (200–800 m s^{-1}) and spatial distributions (Kendall et al. 2004c). Clearly, the first criterion from above is not satisfied with this geometry.

Furthermore, the gas flow throughout much of the nozzle (Fig. 4c) is highly sensitive to variations in the nozzle boundary condition imposed by inserting a

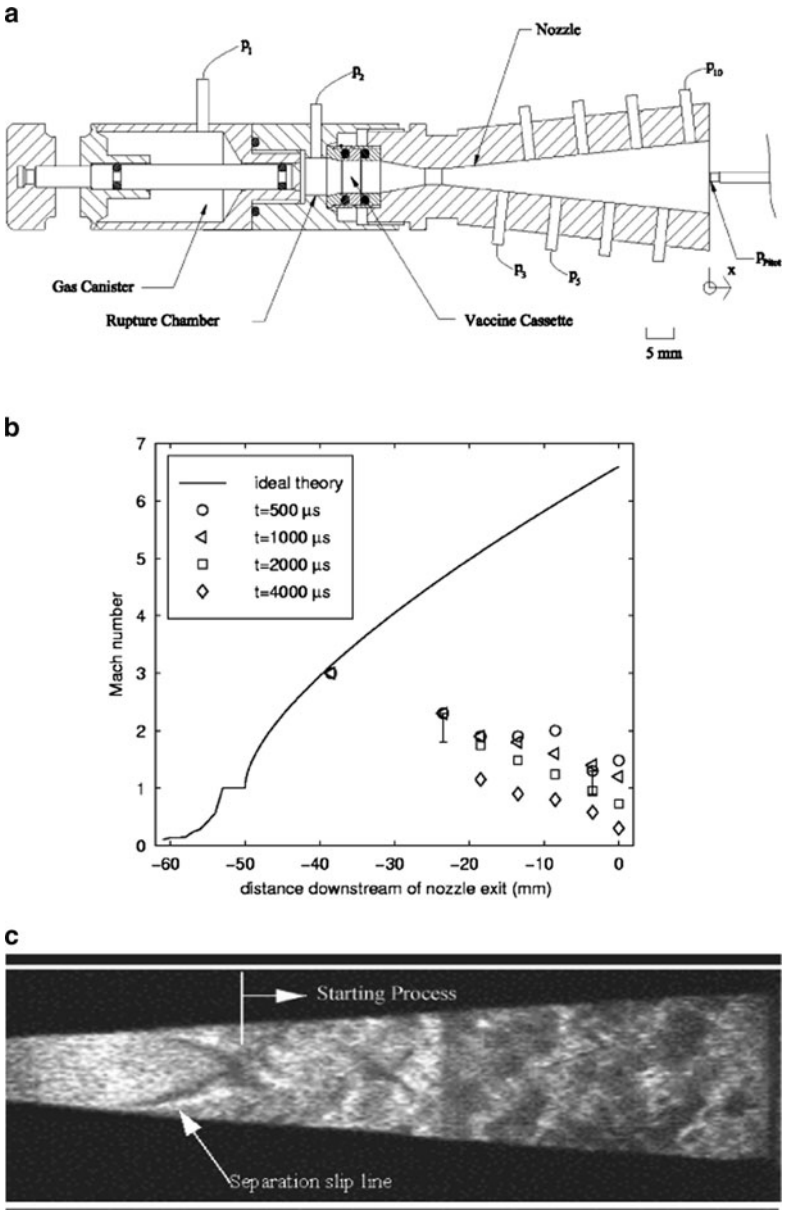


Fig. 4 (**a**) Schematic of a simplified prototype vaccine device instrumented for Pitot and static pressure measurements. The static pressure transducers are labeled p_1–p_{10}. (**b**) Experimental and ideal axial Mach number within the conical nozzle of investigation. The profiles are provided after the starting process. (**c**) A sample Schlieren image within the nozzle. From Kendall et al. (2004c)

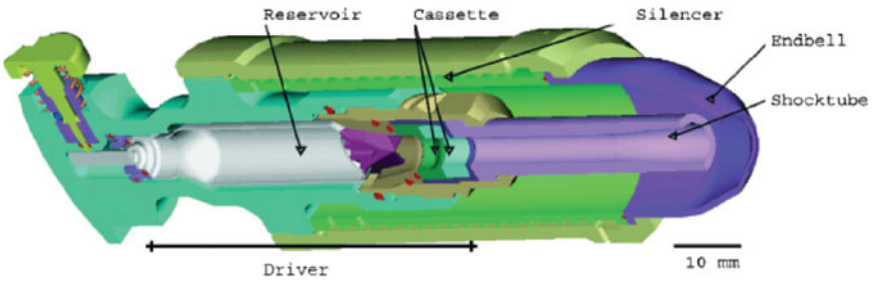


Fig. 5 A contoured shock tube (CST) prototype configured for clinical biolistic delivery. From Kendall (2002)

tissue target and/or a silencer – because the boundary condition information can be communicated upstream. This means that this silenced device applied to the tissue target would have considerably lower and more variable impact velocities. In some cases, it is questionable whether these subsonic nozzle flow silenced devices would deliver particles with a sufficient momentum to reach the target tissue layer.

Improved devices for clinical use to overcome the large variations in particle impact conditions in described earlier devices, and meet the other important criteria of a practical clinical system (outlined above), a next generation biolistic device, called the Contoured Shock Tube (CST) (Fig. 5), was conceived and developed (Kendall 2002; Liu and Kendall 2004a; Hardy and Kendall 2005; Marrion et al. 2005; Truong et al. 2006; Liu et al. 2007). The devices operate with the principle of delivering a payload of microparticles to the skin with a narrow range of velocities, by entraining the drug payload in a quasi-one-dimensional, steady supersonic flowfield.

In experiments with simple prototype CST devices, it was shown that the desired gas flow was achieved repeatedly (Kendall 2002). Importantly, further work with particle payloads measured a variation in free-jet particle velocity of $\pm$ 4% (Kendall et al. 2006). In this research, measurements were made with Particle Image Velocimetry (PIV). A sample PIV result is shown in Fig. 6. Similar PIV images at a range of times after diaphragm rupture were processed to extract the mean centerline axial particle velocity profiles. Importantly, these PIV measurements show particle payloads do achieve near uniform exit-plane velocities at the device exit over the time interval studied. This CST device prototype was a benchtop prototype, not addressing the key criteria for a practical, hand-held clinical immunotherapeutic system.

An embodiment of the CST configured to meet these clinical needs is shown in Fig. 5, with the key components labeled. The device was fabricated from biocompatible materials and the device wall thickness was kept relatively constant to meet autoclave sterilization requirements. To reduce the overall system length, the bottle reservoir (which operates by an actuation pin) is located within the driver annulus. A challenge of this co-axial arrangement was to maintain integrity of transonic gas flow within the driver initiated after diaphragm rupture. This challenge was met by

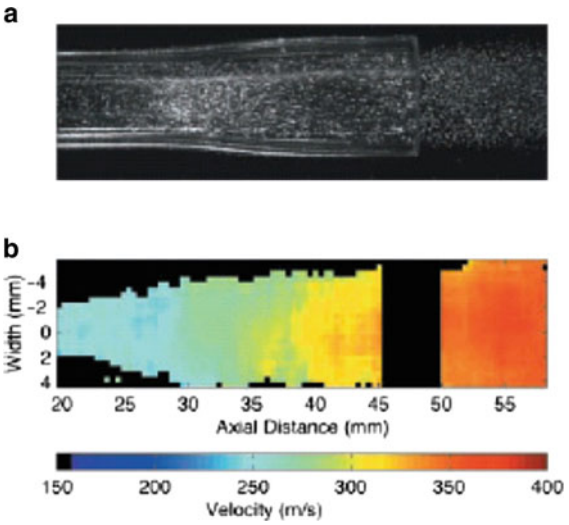


Fig. 6 A raw image (**a**) and derived Particle Image Velocimetry velocity map (**b**) of the instantaneous particle flowfield of a CST prototype, taken 225 μs after diaphragm rupture. The payload was 2.2 mg of 39 μm diameter polystyrene spheres. From Kendall (2002)

carefully contouring the driver and obstacle of the mounting arrangement (Marrion et al. 2005). Possible fragments from opening of the aluminium gas bottle are contained by a sealed filter at the bottle head.

The powdered pharmaceutical is enclosed and sealed by a cassette created by the inclusion of additional diaphragms upstream of the particle payload. In this case, the cassette houses two jets designed to mix the particles into a cloud, hence reducing the dependence on the initial particle location (Kendall 2002; Truong et al. 2006). Therefore, a nominally uniform spatial distribution of particles is released within the quasi-steady flow through the shock tube and nozzle. Repeated in vitro and in vivo experiments show that polycarbonate diaphragm fragments do not damage the target.

Elements of the silencing system are also shown in Fig. 5. The primary shock initiated by diaphragm rupture, reflected from the target, is identified as the main source of sound to be attenuated. This shock is collapsed into compression waves by a series of compressions–expansions induced by an array of orifices and saw-tooth baffles, resulting in appropriate sound levels for the operator and patient.

The device lift-off force is also to be well within user constraints. A peak lift-off force of 13 N is achieved by the careful selection of endbell contact diameter, silencer volume, flow rates through the reservoir and silencer geometry. This peak was for only a very short time within a gas flow lasting only ~200 μs (with a helium driver gas). The point of contact between the device and skin target was selected to maintain a target seal and to minimize the lift-off force, whilst not adversely affecting the impact velocities of the particles. The effect of silencing was also minimized by maintaining a supersonic gas flow transporting particles through the nozzle – so changes in the nozzle boundary condition were not fed upstream. The range of impact conditions for the CST platform was achieved by the selection

of appropriate helium/nitrogen mixtures within the gas bottle driver/driven area ratios.

4.3 Ballistics Microparticle Delivery to Skin

We now examine delivery of microparticles from these quantified and highly-controlled biolistics devices, impacting the skin. Figure 1 shows that skin is a highly variable, bio-viscoelastic material.

The described biolistic devices have been applied to a range of tissue targets for immunotherapeutic applications, including the skin of rodents (Kendall et al. 2006), pigs (Kendall et al. 2004b), dogs (Mitchell 2003), and humans (Kendall et al. 2004a). Typically, two classes of particles are delivered to the tissue. In the powder delivery of conventional vaccines and allergens for allergy immunotherapy, particles of 10–20 μm in radius are delivered to the epidermis of the skin to achieve a therapeutic effect (Kendall 2006). DNA vaccination, however, is an application in which smaller (radius 0.5–2 μm) gold particles coated with a DNA construct are targeted at the nuclei of key immunologically sensitive cells within the epidermis (Lesinski et al. 2001).

4.3.1 Theoretical Model for Ballistic Impact into Skin

In these particle impact studies, the mechanisms of particle impact were explored with a theoretical model, based on a representation first proposed by Dehn (Dehn 1976). The model attributes the particle resistive force (D) to plastic deformation and target inertia

$$D = \frac{1}{2}\rho_t A v^2 + 3A\sigma, \tag{1}$$

where ρ_t and σ are the density and yield stress of the target, A is the particle cross-sectional area and v is the particle velocity. The yield stress (sometimes known as the breaking stress) is the stress at which the tissue begins to exhibit plastic behavior. Equation (1) may be integrated to obtain the penetration depth as a function of particle impact and target parameters. The key parameters of the skin used in the model are summarized in Table 1. Note that these parameters have all been obtained at low, quasi-static strain rates and not the high ballistic strain rates.

The theoretical model of particle penetration into the epidermis using Eq. (1) in a two-layer model is shown in Fig. 1c. Expression (1) shows that the yield stress and density of the SC and VE are important in the ballistic delivery of particles to the epidermis.

In the case of particle delivery only to the SC (labeled "A" in Fig. 1c), the particle depth into the SC (d_{sc}) is obtained by the integration of Eq. (1)

Table 1 Parameters and assigned values used in the theoretical calculations of the particle penetration depth as a function of the relative humidity. From Kendall et al. (2004b)

Skin region	Parameter	Value	Source
Stratum corneum	σ_{sc} (MPa)	22.5–3.2 (0%–100% RH)	Wildnauer et al. (1971)
	ρ_{sc} (kg m^{-3})	1,500	Duck (1990)
	t_{sc} (μm)	10–15.6 (0%–93% RH)	Blank et al. (1984) and measurement
Viable epidermis	σ_{ve} (MPa)	2.2	actin tensile, Kishino and Yanagida (1988)
		10	Epithelium, Mitchell et al. (2003)
	ρ_{ve} (kg m^{-3})	1,150	Duck (1990)

$$d_{sc} = \frac{4\rho r}{3\rho_{sc}}\left\{\ln\left(\frac{1}{2}\rho_{sc}{v_i}^2 + 3\sigma_{sc}\right) - \ln(3\sigma_{sc})\right\}, \tag{2}$$

where the subscript sc denote the SC. Also, v_i and σ_{sc} are, respectively, the particle impact velocity and SC yield stress.

If the particle impact momentum is sufficient to breach the SC (labeled "B" in Fig. 1c), Eq. (2) is rearranged to obtain the velocity of the particle at the SC−VE boundary ($v_{i,ve}$), i.e.,

$$v_{i,ve} = \left\{\left(v_i^2 + \frac{6\sigma_{sc}}{\rho_{sc}}\right)\exp\left(-\frac{3\rho_{sc}t_{sc}}{4\rho r}\right) - \frac{6\sigma_{sc}}{\rho_{sc}}\right\}^{1/2}, \tag{3}$$

where t_{sc} is the thickness of the SC.

The subsequent particle penetration in the VE (d_{ve}) is then calculated using Eq. (2), using instead the material properties of the VE and $v_{i,ve}$. The total particle penetration depth (d_t) is thus

$$d_t = t_{sc} + d_{ve}. \tag{4}$$

An alternative fully numerical Discrete Element Model (DEM) approach has also been applied (Mitchell et al. 2003), but will not be discussed here.

4.3.2 Locations of Microparticles into Skin

As an example, particle delivery to excised human skin is shown for both classes of particles in Fig. 7 (Kendall et al. 2004a). In Fig. 7a, a glass particle of 20 μm radius delivered to the skin at a nominal entry velocity of 260 m s^{-1} is shown. Note the variation in both the SC and epidermal thicknesses. Histological sampling of the three skin sites is from the backs of cadavers. Measured SC and epidermal thickness compared very well with previous reports from the literature. Over 1,800 readings of the deepest particle edge and size of the particles were made on similar

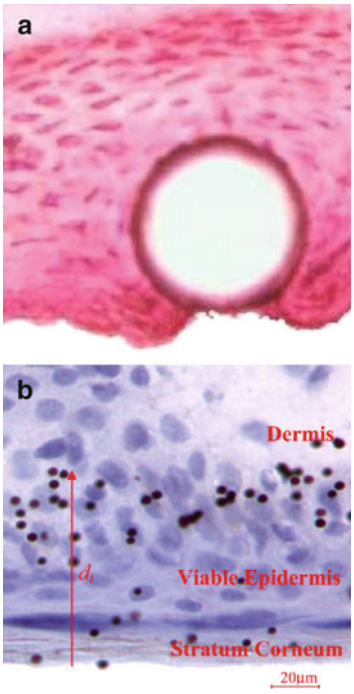


Fig. 7 Photomicrographs of particles delivered to human skin. A 20 μm radius glass sphere delivered at 260 m s^{-1} (**a**) and gold particles (1.0 ± 0.2 μm radius) delivered at 580 ± 50 m s^{-1} (**b**) are shown. From Kendall et al. (2004a)

histological sections with polystyrene, stainless steel and glass particles, selected for different density and size ranges.

In Fig. 7b, a histological section is shown after the impact of gold particles with a measured mean radius of 1 ± 0.2 μm on the skin with a mean calculated impact velocity of 580 ± 50 m s^{-1}. A sample particle depth measurement is labeled as d_i. Over 1,200 readings of the deepest edge and size of the gold particles were made on similar histological sections. All the raw data collected from the histology sections (such as in Fig. 7) are plotted as a function of the particle impact parameter, ρvr, where ρ is the density, v is the velocity and r is the radius, in Fig. 8. The variability of penetration as shown in Fig. 8 is typical of results obtained with other tissues.

Some insights into the sources of scatter in the penetration data of Fig. 8 can be gained when the data are grouped and processed. Consider, for instance, the gold data shown in Fig. 7 grouped by particle radius as shown in Fig. 9. The error bars correspond to one standard deviation in collapsed particle penetration depth and ρvr. Note the trend indicating that for a given value of ρvr, an increase in radius (and hence a decrease in impact velocity) corresponds to a decrease in penetration depth. These data, together with other (unpublished) work show the different particle sizes and the cell matrix results in different penetration depths. For instance, the gold particles are smaller than the average cell size, and during deceleration through the skin tissue, are more likely to penetrate through individual cell membranes. For the larger particles, however, the tissue would primarily fail

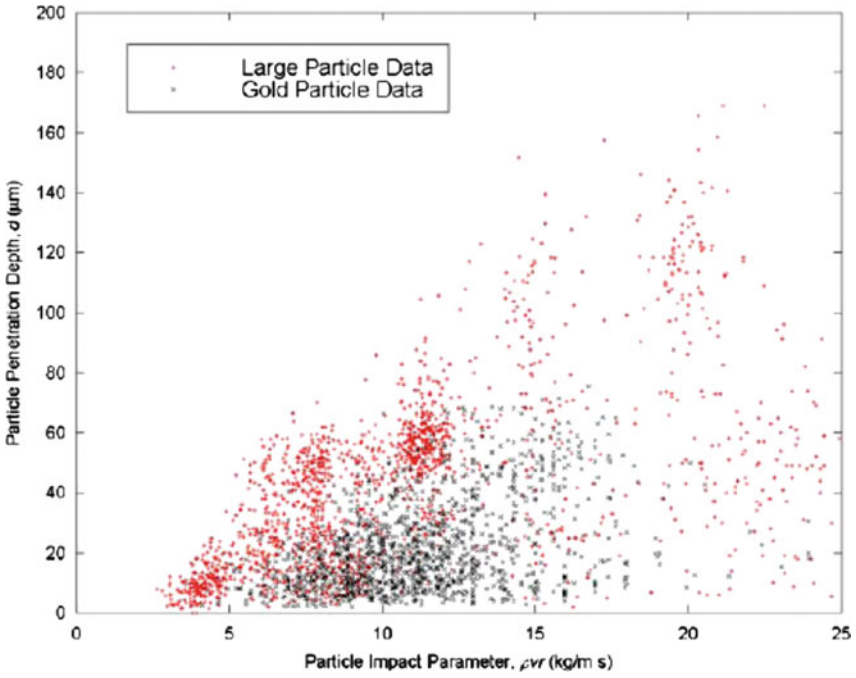


Fig. 8 Raw gold and larger particle penetration into excised human skin as a function of the particle radius, density and impact velocity. From Kendall et al. (2004a)

between the cell boundaries. Indeed, these ballistic penetration data are qualitatively consistent with findings from microprobe indentation studies (Kendall et al. 2007), albeit at considerably higher strain rates.

Corresponding calculated penetration profiles using the theoretical model are also shown in Fig. 9, and illustrate a similar trend with good agreement. Importantly, in this case the yield stress was held constant at 40 MPa, to achieve the closest fit with the data. This is considerably higher than the quasi-static yield stresses reported in the literature (summarized in Table 1 and Fig. 2). This discrepancy is attributed to a huge strain rate effect: the ballistic impact of the microparticle has a peak strain rate of ~10^6 s^{-1}. In a subsequent, more refined study (Kendall et al. 2004b), these strain rate effects are further elucidated.

In addition to the described scale and strain rate effects, another source of variability stems from the high sensitivity in SC mechanical properties to hydration and temperature, deriving from variation in ambient conditions (detailed in Kendall et al. 2004b). Increasing the relative humidity (RH) from 15% to 95% (temperature at 25°C) led to a particle penetration increase by a factor of 1.8. Temperature increases from 20 to 40°C (RH at 15%) enhanced particle penetration two-fold. In both cases, these increases were sufficient to move the target layer from the SC to the VE. In immunotherapeutic applications, this is the difference between the

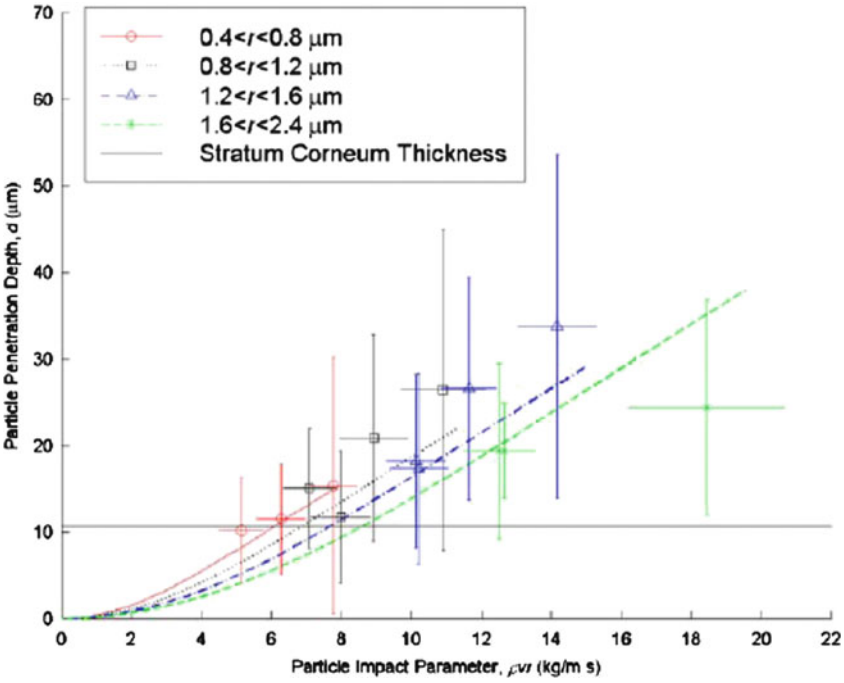


Fig. 9 Impact parameters and penetration depth of gold particles within excised human skin. From Kendall et al. (2004a)

ineffectual delivery of particles to the SC and the targeted delivery of specific cells in the VE.

These collective data show the momentum range obtained from the described biolistic devices primarily translate into delivery within targeted VE and SC. With the precise delivery conditions achieved from these devices, we have obtained new insights into the important biological variability in microparticle impact. This variability, together with more obvious differences in tissue thicknesses (with the tissue site of target, age and gender) must be considered when selecting device conditions for clinical biolistic immunotherapeutic delivery.

4.3.3 Skin Cell Death from Ballistic Impact

The biological responses induced by biolistic impact are of great importance in biolistic applications. When delivered to the tissue surface, the microparticles undergo a tremendous deceleration – peaking at ~10^{10} g – and coming to rest within ~100–200 ns. Such deceleration induces shock and stress waves within the tissue, and it is important to determine under which conditions skin cells are killed. This was investigated in mice, where following the delivery of gold microparticles, the cell death was assayed with mixtures of ethidium bromide and acridine orange

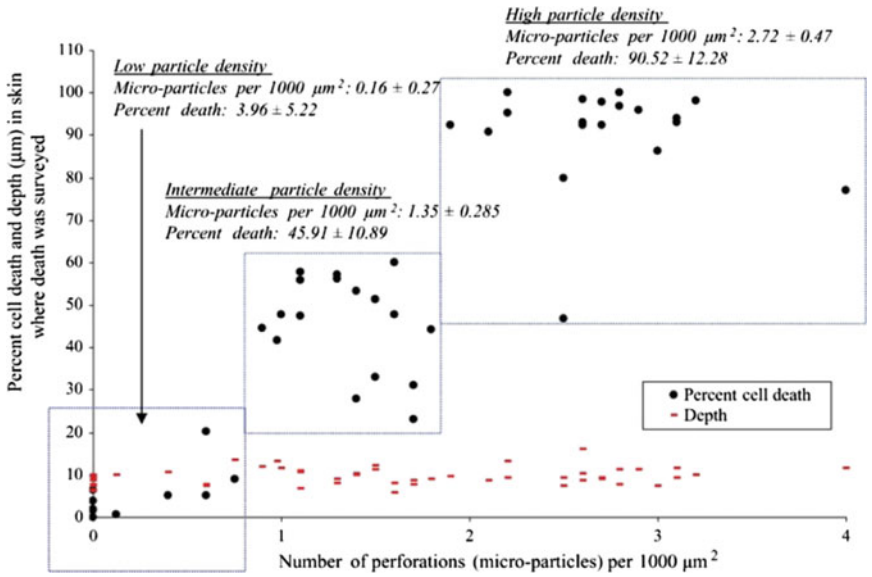


Fig. 10 The viability of epidermal cells targeted by biolistic microparticle delivery (from Raju et al. 2006). Data for this plot was generated by first enumerating the number of perforations per 1,000 μm^2 in the SC caused by microparticle penetration (Fig. 11a). The perforations were equated to microparticles. Second, percent cell death was calculated in the viable epidermis below the stratum corneum using the acridine orange/ethidium bromide assay for discriminating live and dead cells (see Fig. 11b–d)

and imaged non-invasively with Multi-Photon Microscopy (Raju et al. 2006). The data is summarized in Figs. 10 and 11. Each direct impact of a gold microparticle resulted in cell death. Furthermore, even in cases where microparticles passed within ~10 μm of the cell surface – but not touching the cell – cell death resulted. A sufficiently high number density in the tissue can result in complete cell death within the VE. Clearly, this is important when considering the biological responses induced by microparticle impact.

4.4 Clinical Results and Commercial Application

Commercial application. Biolistics is a platform technology for delivering a broad range of drugs and immunotherapeutics. Currently, the technology is progressing commercially in two streams:

- Delivery of lidocaine local anesthetic to the skin (the larger class of particles shown in Fig. 8), approved by the FDA for market application (Zingo™, Anesiva);

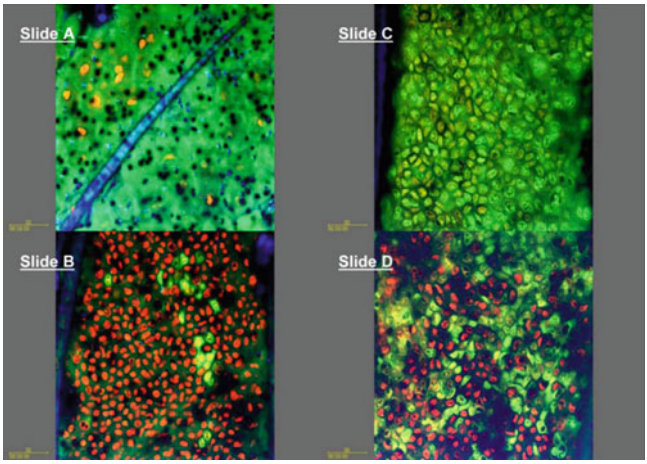


Fig. 11 Murine tissue stained for live–dead cell discrimination with a cocktail of acridine orange/ethidium bromide and imaged with near-infrared two-photon excitation (taken from Raju et al. 2006). Images were collected with LaserSharp software (Carl Zeiss, Hertforshire, UK). Cells emitting red fluorescence are dead whereas cells emitting green fluorescence are alive. (**a**) The dark spots are perforations in the SC caused by microparticle bombardment. (**b**) The corresponding viable epidermis 11.3 μm below the SC. (**a**) and (**b**) represent an image set of "high particle density" shown in Fig. 10. (**c**) The viable epidermis of the control (9.7 μm below the SC) where particle delivery to the tissue was not made. (**d**) Representative of the viable epidermis of tissue with intermediate particle densities (10.5 μm below the SC)

- Delivery of DNA vaccines on gold microparticles (PowderMed^{TM}, Pfizer), undergoing Phase III clinical trials).

Clinical results. Although strong results are achieved in other immunotherapeutics such as allergy immunotherapy of the animal model (Kendall et al. 2006) and lidocaine for anasthesia, the key clinical progress with DNA vaccines is discussed below.

The DNA plasmid that forms the active component of DNA vaccines is precipitated onto microscopic gold particles (typically 2 μg DNA on 1 mg gold). Microscopic elemental gold particles (mean particle diameter ~2 μm) are used as the plasmid DNA carrier, because they are inert and have the appropriate density needed to deliver the vaccine directly into the target epidermal immunologically sensitive cells, including Langerhans cells. Following delivery into the APC, the DNA elutes off the gold particle and is transcribed into RNA. The RNA in turn is translated into the relevant antigen, which is then processed and presented on the cell surface as if it were an intracellular viral protein. An efficient cellular and humoral immune response is thus induced.

A series of clinical trials have been conducted to assess the immunogenicity and safety of a prophylactic hepatitis B virus DNA vaccine (Roy et al. 2000; Rottinghaus et al. 2003; Roberts et al. 2005). These studies have demonstrated that biolistic DNA vaccination can elicit antigen-specific humoral and T cell

responses. In the study by Roy et al. (2000), DNA vaccination with 1–4 μg of hepatitis B surface antigen elicited measurable cytotoxic T cell responses and Th cell responses in 12 healthy adults who had not previously been immunized with a hepatitis B vaccine (Roy et al. 2000). Furthermore, the 12 previously non-vaccinated subjects also seroconverted with levels of hepatitis B-specific antibody ranging from 10 mIU/ml to over 5,000 mIU/ml. This is of particular significance as intramuscular delivery of DNA, using the needle and syringe, with up to 1,000-fold more DNA has generated only low or no antibody responses (MacGregor et al. 1998; MacGregor et al. 2002). The same biolistic hepatitis B DNA vaccine was also shown to increase serum antibody titres in seven of 11 subjects who had previously failed to seroconvert after three or more doses of conventional vaccination with licensed recombinant protein vaccine (Rottinghaus et al. 2003). Finally this plasmid DNA construct has been used to successfully bridge between the earlier bulky experimental device and the simple, hand-held disposable device that will be used for product commercialization (Roberts et al. 2005).

A Phase I study (Drape et al. 2006) has been carried out to investigate the safety and immunogenicity of biolistic administration of an influenza prophylactic plasmid, which encodes a single HA antigen of influenza A/Panama/2007/99 (H3N2). A total of 36 healthy subjects with low pre-existing serological responses to this strain received a vaccination of either 1, 2 or 4 μg DNA at a single administration session. The antibody response was then assessed according to the Committee for Medicinal Products for Human Use (CHMP) criteria for the approval of annual flu vaccines in the European Union. Table 2 summarizes these humoral responses, determined as a haemagglutination inhibition titre elicited on days 0 (predose), 14,

Table 2 Serum antibody responses, seroconversion and seroprotection rate. From Drape et al. (2006)

Group	Day	GMT (range)	Seroconversion[a] (%)	Seroprotection[b] (%)	Mean GMT increase (fold)
1	0	16 (5–40)	–	17 (2/12)	–
	14	23 (5–160)	8 (1/12)	42 (5/12)	1.4
	21	28 (10–240)	17 (2/12)	33 (4/12)	1.7
	56	44 (10–320)	33 (4/12)	58 (7/12)	**2.8**
2	0	17 (5–40)	–	33 (4/12)	–
	14	29 (10–60)	17 (2/12)	50 (6/12)	1.7
	21	36 (20–80)	8 (1/12)	58 (7/12)	2.1
	56	65 (20–320)	**67 (8/12)**	**92 (11/12)**	**3.9**
3	0	12 (5–40)	–	8 (1/12)	–
	14	21 (5–80)	17 (2/12)	25 (3/12)	1.8
	21	40 (10–160)	33 (4/12)	67 (8–12)	3.4
	56	97 (40–640)	**64 (7/11)**	**100 (11/11)**	**8.1**

Values meeting CHMP criteria are bold; geometric mean titer (GMT)

[a]Seroconversion is defined as either a negative pre-vaccination titer ($\leq$10) to a post-vaccination titer $\geq$40, or a significant increase in antibody titer, i.e., at least a four-fold increase between pre- and post-vaccination titers where the pre-vaccination titer is $\geq$10

[b]Seroprotection rate is defined as the proportion of subjects achieving a titer $\geq$40

21 and 56. Time points, where responses met the levels required by the CHMP guidelines for licensing of annual influenza vaccine, are shown in bold.

The 4 μg dose group met the CHMP criteria at day 21, demonstrating the ability of biolistic DNA vaccination to stimulate serological responses equivalent to those seen in protein-based approaches. Furthermore, the responses in all groups continued to increase up to day 56 (the last day monitored) indicating that responses to biolistic vaccination may show a more sustained increase than is typically seen with protein vaccines. By day 56, 100% of those subjects vaccinated with the 4 μg dose were seroprotected.

Overall vaccination was well tolerated and local reactogenicity results were typical of those seen in other biolistic studies.

5 Conclusion

Many vaccines can be radically improved by targeted delivery to particular immunologically-sensitive cells within the outer skin layers. The push is on to develop a range of technologies to meet this need, either using physical or biological targeting approaches. One of these needle-free physical methods, biolistics, delivers biomolecule-coated gold microparticles ballistically to the outer layers of the skin. The method of particle acceleration relies heavily on approaches usually applied to the aerospace industry. Consequently, many unique challenges had to be overcome in engineering biolistic devices for clinical use. Research with the resultant devices has yielded unique insights into the skin at micro-scale dynamic loading – both from mechanical and biological perspectives. Important progress is also being made in clinical trials using biolistic devices to deliver DNA vaccines in the following fields: hepatitis B, influenza, genital herpes, human papilloma virus, HIV/AIDS, Hantaan virus, melanoma and a variety of other cancers.

A newer technology, the Nanopatch (working by a very different principle), is now being developed to address many challenges inherent in the coupling of a ballistic device to skin.

References

Alexander MY, Akhurst RJ (1995) Liposome-medicated gene transfer and expression via the skin. Hum Mol Genet 4:2279–2285

Babiuk S, Baca-Estrada M, Babiuk LA, Ewen C, Foldvari M (2000) Cutaneous vaccination: the skin as an immunologically active tissue and the challenge of antigen delivery. J Control Release 66:199–214

Banchereau J, Steinman RM (1998) Dendritic cells and the control of immunity. Nature 392:245–252

Bauer J, Bahmer FA, Worl J, Neuhuber W, Schuler G, Fartasch M (2001) A strikingly constant ratio exists between Langerhans cells and other epidermal cells in human skin. A stereologic

study using the optical disector method and the confocal laser scanning microscope. J Invest Dermatol 116:313–318
Bellhouse BJ, Sarphie DF, Greenford JC (1994) Needle-less syringe using supersonic gas flow for particle delivery. International patent WO94/24263
Blank IH, Moloney J, 3rd, Emslie AG, Simon I, Apt C (1984) The diffusion of water across the stratum corneum as a function of its water content. J Invest Dermatol 82:188–94
Berman B, Chen VL, France DS, Dotz WI, Petroni G (1983) Anatomical mapping of epidermal Langerhans cell densities in adults. Br J Dermatol 109:553–558
Bremseth DL, Pass F (2001) Delivery of insulin by jet injection: recent observations. Diabetes Technol Ther 3:225–232
Burkoth TL, Bellhouse BJ, Hewson G, Longridge DJ, Muddle AG, Sarphie DF (1999) Transdermal and transmucosal powdered drug delivery. Crit Rev Ther Drug Carrier Syst 16:331–384
Chen D, Endres RL, Erickson CA, Weis KF, McGregor MW, Kawaoka Y, Payne LG (2000) Epidermal immunization by a needle-free powder delivery technology: immunogenicity of influenza vaccine and protection in mice. Nat Med 6:1187–1190
Chen D, Maa YF, Haynes JR (2002) Needle-free epidermal powder immunization. Expert Rev Vaccines 1:265–276
Chen H, Yuan J, Wang Y, Silvers WK (1985) Distribution of ATPase-positive Langerhans cells in normal adult human skin. Br J Dermatol 113:707–711
Chen X, Prow TW, Crichton ML, Jenkins DWK, Roberts MS, Frazer IH, Fernando GJP, Kendall MAF (2009) Dry-coated microprojection array patches for targeted delivery of immunotherapeutics to the skin. J Control Release 3:212–220
Chen XF, Prow TW, Crichton M, Fernando G, Kendall MAF (2008) Novel coating of Micronanoprojections patches for targeted vaccine delivery to skin. In: International Conference on Nanoscience and Nanotechnology, Melbourne Convention Centre, Melbourne, Australia. IEEE Press, pp 105–108
Christensen MS, Hargens CW 3rd, Nacht S, Gans EH (1977) Viscoelastic properties of intact human skin: instrumentation, hydration effects, and the contribution of the stratum corneum. J Invest Dermatol 69:282–286
Cui Z, Mumper RJ (2001) Dendritic cell-targeted genetic vaccines engineered from novel microemulsion precursors. Mol Ther 3:S352
Dehn J (1976) A unified theory of penetration. Int J Impact Eng 5:239–248
Dobrev H (1996) In vivo noninvasive study of the mechanical properties of the human skin after single application of topical corticosteroids. Folia Med (Plovdiv) 38:11–17
Domashenko A, Gupta S, Cotsarelis G (2000) Efficient delivery of transgenes to human hair follicle progenitor cells using topical lipoplex. Nat Biotechnol 18:420–423
Drape RJ, Macklin MD, Barr LJ, Jones S, Haynes JR, Dean HJ (2006) Epidermal DNA vaccine for influenza is immunogenic in humans. Vaccine 24:4475–4481
Duck FA (1990) Physical properties of tissue. Academic Press, London
Fuchs E, Raghavan S (2002) Getting under the skin of epidermal morphogenesis. Nat Rev Genet 3:199–209
Furth PA, Shamay A, Hennighausen L (1995) Gene transfer into mammalian cells by jet injection. Hybridoma 14:149–152
Gill HS, Prausnitz MR (2007) Coating formulations for microneedles. Pharm Res 24:1369–1380
Givens B, Oberle S, Lander J (1993) Taking the jab out of needles. Can Nurse 89:37–40
Glenn GM, Kenney RT, Ellingsworth LR, Frech SA, Hammond SA, Zoeteweij JP (2003) Transcutaneous immunization and immunostimulant strategies: capitalizing on the immunocompetence of the skin. Expert Rev Vaccines 2:253–267
Guerena-Burgueno F, Hall ER, Taylor DN, Cassels FJ, Scott DA, Wolf MK, Roberts ZJ, Nesterova GV, Alving CR, Glenn GM (2002) Safety and immunogenicity of a prototype enterotoxigenic *Escherichia coli* vaccine administered transcutaneously. Infect Immun 70:1874–1880

Hardy MP, Kendall MAF (2005) Mucosal deformation from an impinging transonic gas jet and the ballistic impact of microparticles. Phys Med Biol 50:4567–4580

Hoath SB, Leahy DG (2002) Formation and function of the stratum corneum. In: Marks R, Lévêque J-L, Voegeli R (eds) The essential stratum corneum. Taylor & Francis Group, London, p 31

Hopewell JW (1990) The skin: its structure and response to ionizing radiation. Int J Radiat Biol 57:751–773

Kendall M (2006) Engineering of needle-free physical methods to target epidermal cells for DNA vaccination. Vaccine 24:4651–4656

Kendall M, Mitchell T, Wrighton-Smith P (2004a) Intradermal ballistic delivery of micro-particles into excised human skin for pharmaceutical applications. J Biomech 37: 1733–1741

Kendall M, Mitchell TJ, Costigan G, Armitage M, Lenzo JC, Thomas JA, von Garnier C, Zosky GR, Turner DJ, Stumbles PA, Sly PD, Holt PG, Thomas WR (2006) Downregulation of IgE antibody and allergic responses in the lung by epidermal biolistic microparticle delivery. J Allergy Clin Immunol 117:275–282

Kendall M, Rishworth S, Carter F, Mitchell T (2004b) Effects of relative humidity and ambient temperature on the ballistic delivery of micro-particles to excised porcine skin. J Invest Dermatol 122:739–746

Kendall MA, Chong YF, Cock A (2007) The mechanical properties of the skin epidermis in relation to targeted gene and drug delivery. Biomaterials 28:4968–4977

Kendall MAF (2002) The delivery of particulate vaccines and drugs to human skin with a practical, hand-held shock tube-based system. Shock Waves 12:22–30

Kendall MAF, Quinlan NJ, Thorpe SJ, Ainsworth RW, Bellhouse BJ (2004c) Measurements of the gas and particle flow within a converging–diverging nozzle for high speed powdered vaccine and drug delivery. Exp Fluids 37:128–136

Lesinski GB, Smithson SL, Srivastava N, Chen D, Widera G, Westerink MA (2001) A DNA vaccine encoding a peptide mimic of *Streptococcus pneumoniae* serotype 4 capsular polysaccharide induces specific anti-carbohydrate antibodies in Balb/c mice. Vaccine 19: 1717–1726

Li L, Hoffman RM (1995) The feasibility of targeted selective gene therapy of the hair follicle. Nat Med 1:705–706

Liu LJ, Watabe S, Yang J, Hamajima K, Ishii N, Hagiwara E, Onari K, Xin KQ, Okuda K (2001) Topical application of HIV DNA vaccine with cytokine-expression plasmids induces strong antigen-specific immune responses. Vaccine 20:42–48

Liu Y, Kendall MAF (2004a) Numerical simulation of heat transfer from a transonic jet impinging on skin for needle-free powdered drug and vaccine delivery. Proc Inst Mech Eng – Part C – J Mech Eng Sci 218:1373–1383

Liu Y, Kendall MAF (2004b) Numerical study of a transient gas and particle flow in a high-speed needle-free ballistic particulate vaccine delivery system. J Mech Med Biol 4:559–578

Liu Y, Truong NK, Kendall MA, Bellhouse BJ (2007) Characteristics of a micro-biolistic system for murine immunological studies. Biomed Microdevices 9:465–474

Lu B, Federoff HJ, Wang Y, Goldsmith LA, Scott G (1997) Topical application of viral vectors for epidermal gene transfer. J Invest Dermatol 108:803–808

MacGregor RR, Boyer JD, Ugen KE, Lacy KE, Gluckman SJ, Bagarazzi ML, Chattergoon MA, Baine Y, Higgins TJ, Ciccarelli RB, Coney LR, Ginsberg RS, Weiner DB (1998) First human trial of a DNA-based vaccine for treatment of human immunodeficiency virus type 1 infection: safety and host response. J Infect Dis 178:92–100

MacGregor RR, Ginsberg R, Ugen KE, Baine Y, Kang CU, Tu XM, Higgins T, Weiner DB, Boyer JD (2002) T-cell responses induced in normal volunteers immunized with a DNA-based vaccine containing HIV-1 env and rev. AIDS 16:2137–2143

Marrion M, Kendall MAF, Liu Y (2005) The gas-dynamic effects of a hemisphere-cylinder obstacle in a shock-tube driver. Exp Fluids 38:319–327

Matriano JA, Cormier M, Johnson J, Young WA, Buttery M, Nyam K, Daddona PE (2002) Macroflux microprojection array patch technology: a new and efficient approach for intracutaneous immunization. Pharm Res 19:63–70

McAllister DV, Wang PM, Davis SP, Park JH, Canatella PJ, Allen MG, Prausnitz MR (2003) Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: fabrication methods and transport studies. Proc Natl Acad Sci USA 100:13755–13760

McKinney EC, Streilein JW (1989) On the extraordinary capacity of allogeneic epidermal Langerhans cells to prime cytotoxic T cells in vivo. J Immunol 143:1560–1564

Menton DN, Eisen AZ (1971) Structure and organization of mammalian stratum corneum. J Ultrastruct Res 35:247–264

Mikszta JA, Alarcon JB, Brittingham JM, Sutter DE, Pettis RJ, Harvey NG (2002) Improved genetic immunization via micromechanical disruption of skin-barrier function and targeted epidermal delivery. Nat Med 8:415–419

Mitchell T (2003) The ballistics of micro-particles into the mucosa and skin. DPhill Thesis Engineering science, University of Oxford, Oxford

Mitchell TJ, Kendall MAF, Bellhouse BJ (2003) A ballistic study of micro-particle penetration to the oral mucosa. Int J Impact Eng 28:581–599

Mumper RJ, Ledebur HC Jr (2001) Dendritic cell delivery of plasmid DNA. Applications for controlled genetic immunization. Mol Biotechnol 19:79–95

Nagao K, Ginhoux F, Leitner WW, Motegi S, Bennett CL, Clausen BE, Merad M, Udey MC (2009) Murine epidermal Langerhans cells and langerin-expressing dermal dendritic cells are unrelated and exhibit distinct functions. Proc Natl Acad Sci USA 106:3312–3317

Nemes Z, Steinert PM (1999) Bricks and mortar of the epidermal barrier. Exp Mol Med 31:5–19

Nicolopoulos CS, Giannoudis PV, Glaros KD, Barbenel JC (1998) In vitro study of the failure of skin surface after influence of hydration and preconditioning. Arch Dermatol Res 290:638–640

Numahara T, Tanemura M, Nakagawa T, Takaiwa T (2001) Spatial data analysis by epidermal Langerhans cells reveals an elegant system. J Dermatol Sci 25:219–228

Papir YS, Hsu KH, Wildnauer RH (1975) The mechanical properties of stratum corneum I. The effect of water and ambient temperature on the tensile properties of newborn rat stratum corneum. Biochim Biophys Acta 399:170–180

Poulin LF, Henri S, de Bovis B, Devilard E, Kissenpfennig A, Malissen B (2007) The dermis contains langerin + dendritic cells that develop and function independently of epidermal Langerhans cells. J Exp Med 204:3119–3131

Prow TW, Chen XF, Crichton M, Tiwari Y, Gradissi F, Raphaelli K, Mahony D, Fernando G, Roberts MS, Kendall MAF (2008) Targeted epidermal delivery of vaccines from coated micro-nanoprojection patches. In: International Conference on Nanoscience and Nanotechnology. IEEE, Melbourne Convention Centre, Melbourne, Australia, pp 125–128

Quinlan NJ, Kendall MAF, Bellhouse BJ, Ainsworth RW (2001) Investigations of gas and particle dynamics in first generation needle-free drug delivery devices. Int J Shock Waves 10:395–404

Raju PA, McSloy N, Truong NK, Kendall MA (2006) Assessment of epidermal cell viability by near infrared multi-photon microscopy following ballistic delivery of gold micro-particles. Vaccine 24:4644–4647

Rawlings A, Harding C, Watkinson A, Banks J, Ackerman C, Sabin R (1995) The effect of glycerol and humidity on desmosome degradation in stratum corneum. Arch Dermatol Res 287:457–464

Roberts LK, Barr LJ, Fuller DH, McMahon CW, Leese PT, Jones S (2005) Clinical safety and efficacy of a powdered hepatitis B nucleic acid vaccine delivered to the epidermis by a commercial prototype device. Vaccine 23:4867–4878

Rottinghaus ST, Poland GA, Jacobson RM, Barr LJ, Roy MJ (2003) Hepatitis B DNA vaccine induces protective antibody responses in human non-responders to conventional vaccination. Vaccine 21:4604–4608

Roy MJ, Wu MS, Barr LJ, Fuller JT, Tussey LG, Speller S, Culp J, Burkholder JK, Swain WF, Dixon RM, Widera G, Vessey R, King A, Ogg G, Gallimore A, Haynes JR, Heydenburg Fuller

D (2000) Induction of antigen-specific $CD8^+$ T cells, T helper cells, and protective levels of antibody in humans by particle-mediated administration of a hepatitis B virus DNA vaccine. Vaccine 19:764–778

Sanford JC, Klein MC (1987) Delivery of substances into cells and tissues using a particle bombardment process. Particulate Sci Tech 5:27–37

Shi Z, Curiel DT, Tang DC (1999) DNA-based non-invasive vaccination onto the skin. Vaccine 17:2136–2141

Shi Z, Zeng M, Yang G, Siegel F, Cain LJ, van Kampen KR, Elmets CA, Tang DC (2001) Protection against tetanus by needle-free inoculation of adenovirus-vectored nasal and epicutaneous vaccines. J Virol 75:11474–11482

Sintov AC, Krymberk I, Daniel D, Hannan T, Sohn Z, Levin G (2003) Radiofrequency-driven skin microchanneling as a new way for electrically assisted transdermal delivery of hydrophilic drugs. J Control Release 89:311–320

Stenn KS, Goldenhersh MA, Trepeta RW (1992) Structure and functions of the skin. In: Symmers WSC (ed) The skin, Vol 9. New York, Churchill Livingstone, pp 1–14

Tang DC, Shi Z, Curiel DT (1997) Vaccination onto bare skin. Nature 388:729–730

Timares L, Takashima A, Johnston SA (1998) Quantitative analysis of the immunopotency of genetically transfected dendritic cells. Proc Natl Acad Sci USA 95:13147–13152

Truong NK, Liu Y, Kendall MAF (2006) Gas-particle dynamics characterisation of a preclinical contoured shock tube for vaccine and drug delivery. Shock Waves 15:149–164

Watabe S, Xin KQ, Ihata A, Liu LJ, Honsho A, Aoki I, Hamajima K, Wahren B, Okuda K (2001) Protection against influenza virus challenge by topical application of influenza DNA vaccine. Vaccine 19:4434–4444

WHO (1999) Safety of injections: facts and figures. http://www.who.int/inf-fs/en/fact232.html World Health Organization. Office of Press and Public Relations

Widera G, Austin M, Rabussay D, Goldbeck C, Barnett SW, Chen M, Leung L, Otten GR, Thudium K, Selby MJ, Ulmer JB (2000) Increased DNA vaccine delivery and immunogenicity by electroporation in vivo. J Immunol 164:4635–4640

Wildnauer RH, Bothwell JW, Douglass AB (1971) Stratum corneum biomechanical properties I. Influence of relative humidity on normal and extracted human stratum corneum. J Invest Dermatol 56:72–78

Zucchelli S, Capone S, Fattori E, Folgori A, Di Marco A, Casimiro D, Simon AJ, Laufer R, La Monica N, Cortese R, Nicosia A (2000) Enhancing B- and T-cell immune response to a hepatitis C virus E2 DNA vaccine by intramuscular electrical gene transfer. J Virol 74:11598–11607

Pharmaceutically Used Polymers: Principles, Structures, and Applications of Pharmaceutical Delivery Systems

Jayant Khandare and Rainer Haag

Contents

Abstract This chapter presents a general overview of pharmaceutically used polymers with respect to their physicochemical characteristics and factors affecting drug delivery abilities. Pharmaceutical polymers, chemical structure, and properties are discussed for their applications in controlled drug release systems. An additional focus is on new polymers (dendrimers, hyperbranched polymers), considering their chemical versatility, uniqueness, and future implications. Problems associated with controlled drug release systems are also highlighted. Finally,

R. Haag (✉)
Institut für Chemie und Biochemie, Freie Universität Berlin, Takustrasse 3, 14195 Berlin, Germany
e-mail: haag@chemie.fu-berlin.de

M. Schäfer-Korting (ed.), *Drug Delivery*,
Handbook of Experimental Pharmacology 197,
DOI 10.1007/978-3-642-00477-3_8,

applications of FDA-approved polymers used for oral drug delivery systems are outlined.

Keywords Polymers · Polymer therapeutics · Controlled release system · Novel drug delivery systems · Biomaterials

Abbreviations

Ab	Antibody
API	Active pharmaceutical ingredient
C_{max}	Concentration maximum
CRS	Controlled released system
DDS	Drug delivery system
EPR	Enhanced permeability and retention
FDA	Food and Drug Administration (USA)
G2 (PAMAM)	Generation 2 polyamidoamine dendrimers
GR	Gastro-retentive delivery systems
HPMA	*N*-(2-Hydroxypropyl)methacrylamide
HPMC	Hydroxypropylmethylcellulose
IgG	Human immunoglobulinG
M_w	Molecular weight
mPEG-OH	Polyethylene glycol monomethyl ether
PAA	Poly(acrylic acid)
PAMAM	Polyamidoamine
PDDS	Pulsatile drug delivery system
PDI	Polydispersity index
PEG	Poly(ethylene glycol)
PEO	Poly(ethylene oxide)
PGA	Poly(glutamic acid)
pk_a	Acid dissociation constant
PLA	Polylactic acid
PLGA	poly(lactic-*co*-glycolic acid) copolymer
PMMA	Polymethylmethacrylate
PVA	Poly vinyl alcohol
RES	Reticular endothelium system

1 Introduction

Conventional drug delivery systems often only reach concentrations within the therapeutically effective range (therapeutic window) when taken several times a day (Fig. 1). In contrast, controlled release systems (CRS) are tailored to sustain the

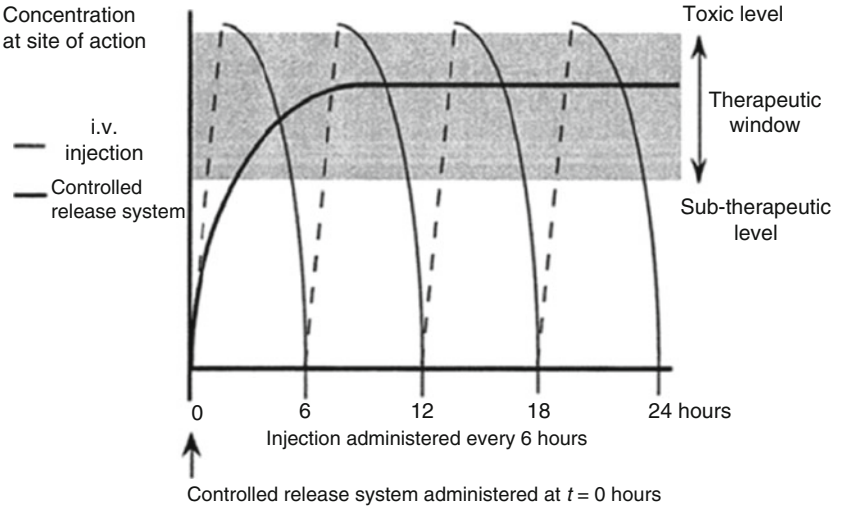


Fig. 1 Therapeutic window of an API: application by a controlled drug delivery system compared with injection (Uhrich et al. 1999)

active pharmaceutical ingredient (API) release at a specific rate over a defined period of time to maintain plasma concentration within the therapeutic range. Thus the release profile of a drug delivery system can allow for a reduction in dosing frequency and thus improve compliance and overall treatment effectiveness. Furthermore, toxic peak plasma levels above the therapeutic window can be avoided and undesired side effects minimized. In particular, implants have the potential for drug delivery for several years which makes them valuable tools in long-term medication.

Constant plasma levels realized by zero-order API release, however, are not always the best profile. In fact, circadian variations in the severity of symptoms may require adaptive API plasma levels. For example, exacerbations of bronchial asthma predominantly occur in the early morning. Therefore efficient bronchodilation can be initiated later in the night. Moreover, bactericidal antibiotics are most efficient when exposing the microorganism to fluctuating drug levels by consumption of several doses a day. However, compliance is best when drug intake is limited to a single daily dose at a convenient time. Therefore, pulsatile release systems are currently being introduced into therapy based on polymer blends.

Moreover, polymers can be used to design delivery systems for parenteral use which allow Ehrlich's principle of the "Magic Bullet" to come into reality. Passive targeting to solid tumors via the Enhanced Permeability and Retention (EPR) effect as well as active targeting by antibodies attached to the particle surface (for details see chapter of Torchilin this volume) can improve clinical efficacy in, for example, cancer therapy. Therefore, the use of CRS and pharmaceutical polymers should increase in the near future.

Although many promising CRS have evolved, challenging physicochemical problems need to be addressed particularly in the areas of polymeric bulk diffusion, membrane permeability, osmotic effect, colloidal aggregation, and polymer dynamics. Although many polymers have found their way into the clinics, there is still a great demand for new polymers. Since the existing polymers do not have sufficient versatility, the physicochemical properties do not fully cover the required spectrum. Moreover, as most of the polymer candidates are under intellectual property rights, it is polymer scientists who introduce and advance new biomaterials. The high standards in safety assessment in drug research, however, limit or at least retard the successful translation of new polymers from laboratory to clinic.

Due to the different chemical functionalities of the polymers it is possible to achieve defined release profiles. This is particularly applicable when polymers are "blended" (used in combined form). The characteristics of the copolymers and their pH-dependent solubility impart new and modified patterns (Ford 1994). Importantly, such systems should be inert, biocompatible, mechanically stable, comfortable for the patient, capable of achieving high drug loading, safe from accidental drug release, simple to administer and – when needed (e.g., implants) – to remove, and easy to fabricate and – when aiming for parenteral use or implantation – to sterilize. Therefore, controlled delivery systems are often more expensive compared to traditional pharmaceutical formulations.

The main disadvantage of CRS, however, is a possible toxicity or non-biocompatibility arising from the polymeric materials used. There could also be undesirable by-products from the polymers due to degradation. Therefore, the production process of the polymer has to be strictly controlled; by-products have to be limited, analytically quantified and subjected to toxicological testing if present in relevant amounts. Moreover, in some instances, surgery may be required to implant or remove the system, which may cause discomfort to the patient.

2 Pharmaceutically Used Polymers and Biomaterials

To adjust the release profile of the API from the formulation, polymer and other excipients are either used in a single or in blended form. In general, the selection of the polymer is based on the release-defining characteristics

- Chemical nature and charge on polymer: anionic, cationic, and neutral
- Hydrophilicity/hydrophobicity of the polymer
- Cross-linking ratios and swelling/de-swelling capacity
- pH-dependency/independency
- Route of administration
- Release pattern and erosion mechanism
- Targeted site for absorption (non-parental application).

For economic reasons, drug delivery research often makes use of a platform technology. As shown in Figs. 2 and 3 the total time line from project concept to

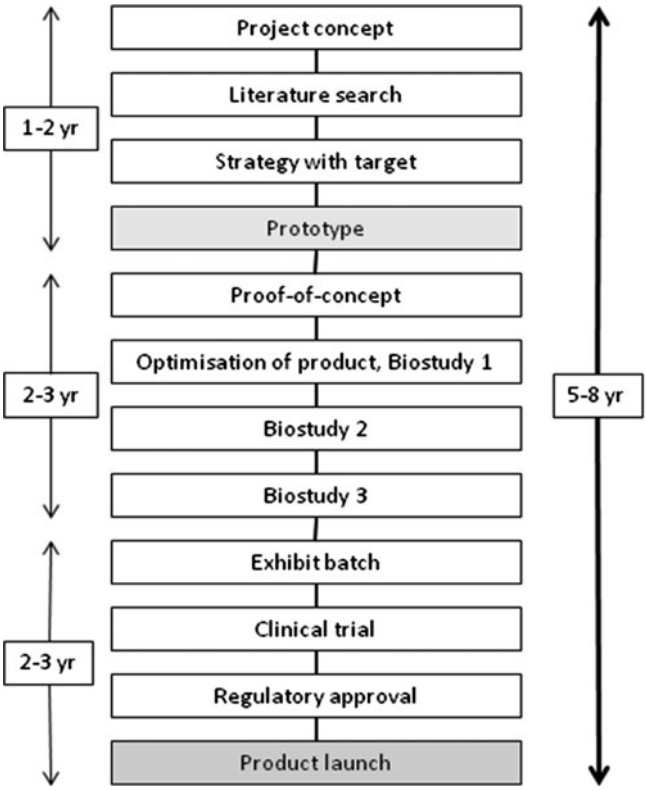


Fig. 2 Drug delivery systems manufacturing pathway leading to estimation of drug bioavailability and product launch

regulatory approval which is mandatory to launch a delivery product requires an average of 5–8 years.

2.1 Classification of Pharmaceutically Used Polymers and Biomaterials – An Overview

A range of pharmaceutical polymers and biomaterials is used to control the release of APIs and other active agents (Brazel and Peppas 2000; Boldhane 2008) and several polymers (Table 1) are currently extensively used for drug delivery (Smolensky and Peppas 2007; Boldhane 2008). A classification of pharmaceutical polymers based on their chemical nature, dissolving capabilities, release mechanism, and applications is given in Table 1. Acrylic acid-based polymers, siloxanes and *N*-vinyl pyrrolidone-based polymers are also used for contact lenses, which in

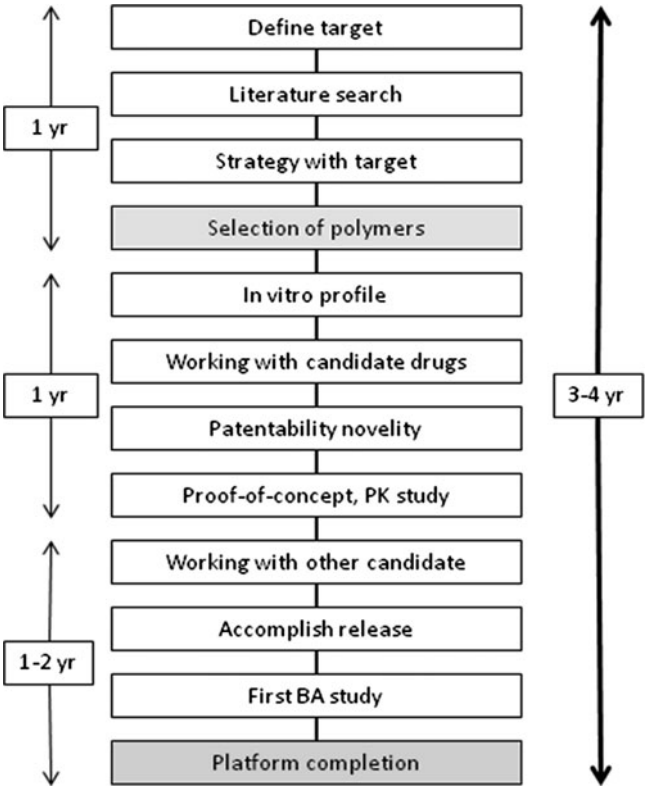


Fig. 3 Major steps in development of platform drug delivery system (BA= Bioavailability)

particular ask for very good local tolerability, wettability, gas transmissibility, physical stability, and clarity.

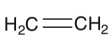
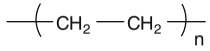
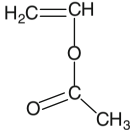
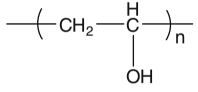
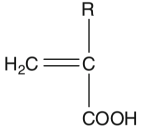
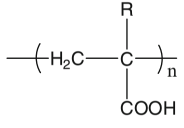
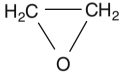
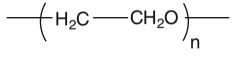
Table 2 gives an overview of the various polymers in pharmaceutical use.

2.2 Orally Applied Polymers: Properties and Applications

2.2.1 Starch

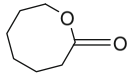
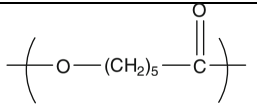
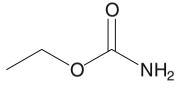
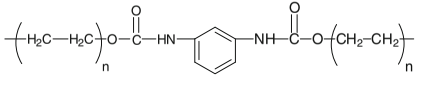
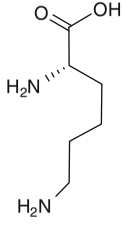
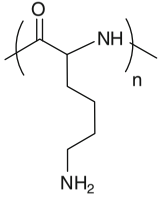
Starch is a complex carbohydrate abundantly found in nature: for example, corn, sorghum, wheat, potato, and rice. Starch comprises a mixture of two polysaccharides, namely, amylose (a linear polysaccharide), and amylopectin (a branched polysaccharide with a molecular mass of up to 200×10^6). Amylose is widely used as a binding agent in tablet manufacturing since it exhibits strong binding and swelling properties. The natural polymer also forms a stable viscous dispersion.

Table 1 Monomers and their polymeric forms and pharmaceutical applications. Polymers 7, 8, and 9 degrade within the physiological environment and are being used in drug delivery (systemic route of application)

	Monomer	Polymer form	Drug delivery applications
1.			Controlled delivery systems
	Ethylene	Poly(ethylene)	
2.			Copolymerization with vinyl acetate is used in microspheres and bio-adhesive hydrogels
	Vinyl acetate	Polyvinyl alcohol (PVA)	
3.			Stimuli-sensitive polymer – pH-sensitive. Reversible swelling hydrogels
	R=H (acrylic acid), R=CH_3 (methacrylic acid)	Poly(acrylic acid) PAA	
4.			Polymer prodrugs to enhance aqueous solubility
	Ethylene oxide	Poly(ethylene glycol)	

(*continued*)

Table 1 (continued)

	Monomer	Polymer form	Drug delivery applications
5.			Used in controlled drug delivery, local delivery, micelles, etc.
	Caprolactone	Polycaprolactone	
6.			Used for temporal controlled release
	Urethane	Polyurethane	
7.			Used in drug delivery systems in various forms including copolymers with polylactic acid (PLA)
	Amino acid e.g., lysine	Poly(lysine)	

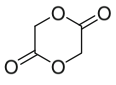
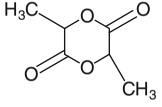
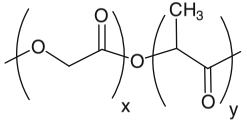
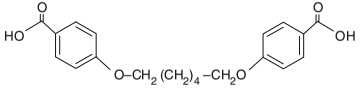
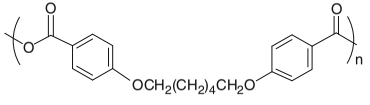
8.	 Glycolide  Lactide	 Poly(lactic-*co*-glycolic acid) (PLGA) copolymer	Nanoparticles and microparticles drug, protein, and peptide delivery
9.	 *p*-(carboxyphenoxy)hexane	 Poly anhydrides *p*-(Carboxyphenoxy)hexane	Used in oral controlled drug delivery systems

Table 2 Polymers in pharmaceutical use

Polymers		Use
Hydrophilic polymers		
Cellulosic	Methyl cellulose	Coating agent, emulsifying agent, suspending agent, tablet and capsule disintegrant, tablet binder, viscosity-increasing agent
	Hypromellose (hydroxypropylmethyl cellulose	Coating agent, film forming agent, rate-controlling polymer for sustained release, stabilizing agent, suspending agent, tablet binder, viscosity-increasing agent
	Hydroxypropyl cellulose	Coating agent, emulsifying agent, suspending agent, stabilizing agent, thickening agent, tablet binder, viscosity-increasing agent
	Hydroxyethyl cellulose	Coating agent, suspending agent, thickening agent, tablet binder, viscosity-increasing agent
	Sodium carboxymethyl cellulose	Coating agent, suspending agent, tablet and capsule disintegrant, tablet binder, viscosity-increasing agent, stabilizing agent, water absorbing agent
Noncellulosic: gums/ polysaccharides	Sodium alginate	Stabilizing agent, suspending agent, tablet and capsule disintegrant, tablet binder, viscosity-increasing agent
	Xanthan gum	Stabilizing agent, suspending agent, viscosity-increasing agent
	Carrageenan	Gel base, suspending agent, sustained-release tablet matrix
	Ceratonia (locust bean gum)	Matrix, binder
	Chitosan	Coating agent, disintegrant, film-forming agent, mucoadhesive, tablet binder, viscosity increasing agent
	Guar gum	Suspending agent, tablet binder, tablet disintegrant, viscosity-increasing agent
	Cross-linked high amylose starch	Gelling agent, thickening agent
Noncellulosic: others	Poly(ethylene oxide)	Mucoadhesive, tablet binder, thickening agent
	Poly(ethylene glycol)	Ointment base, plasticizer, solvent, suppository base, tablet and capsule lubricant
	Poly(vinyl pyrrolidone)	Disintegrant, dissolution aid, suspending agent, tablet binder

(*continued*)

Table 2 (continued)

Polymers		Use
	Homopolymers and copolymers of acrylic acid (Carbomer)	Bioadhesive, emulsifying agent, release modifying agent, suspending agent, tablet binder, viscosity-increasing agent
	Gantrez® maleic acid copolymers	Bioadhesive
Hydrophobic polymers		
Water-insoluble	Ethylcellulose	Coating agent, flavoring fixative, tablet binder, tablet filler, viscosity-increasing agent
	Cellulose acetate	Coating agent, extended release agent, tablet and capsule diluents
	Cellulose acetate propionate	Membrane
	Cellulose acetate phthalate	Enteric coating agent
	Hydroxypropylmethylcellulose phthalate	Enteric coating polymer
	Poly(vinyl acetate) phthalate	Viscosity modifying agent with enteric coating
	Gantrez® AN (copolymers of methyl vinyl ether and maleic anhydride)	Mucosal adhesive resins
	Methacrylic acid copolymers	Film forming agent, tablet binder, tablet diluents
	Eudragit® acrylate copolymers	The wide range of immediate, enteric and sustained release polymers allow the design of any number of combinations to match targeted release profile

2.2.2 Hydroxylpropyl Methylcellulose

The 2-hydroxylpropyl ether of methyl cellulose (HPMC) (Fig. 4) is derived from alkali-treated cellulose which is reacted with methyl chloride and propylene oxide. HPMC swells and dissolves slowly in cold water to produce a viscous colloidal solution. It is also soluble in most polar solvents. Aqueous solutions are surface active, form films upon drying, and undergo reversible transformation from sol to gel upon heating and cooling.

Besides the use as a tabletting agent and as an emulsifier in ointments HPMC is extensively used in oral controlled drug delivery systems. Moreover, the polymer has been widely explored as a matrix-forming agent in the design of patches. Propranolol hydrochloride release from HPMC matrices was fast yet slowed when coated with a protective layer (Guyot and Fawaz 2000). A swellable, floating triple-layer tablet was designed to prolong the gastric residence time of anti-infectives in *Helicobacter pylori* eradication therapy. HPMC and poly(ethylene oxide) (PEO) were used as the rate-controlling polymeric membrane excipients. Tetracycline and metronidazole were incorporated into the core layer of the triple-layer matrix for controlled delivery, while bismuth salt was included in an outer layer for instant

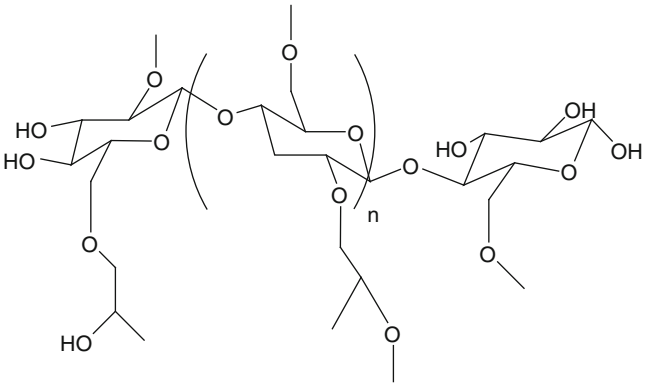


Fig. 4 Chemical structure of hydroxylpropyl methylcellulose (HPMC) polymer

release. Floatation was accomplished by incorporating a gas-generating layer consisting of sodium bicarbonate:calcium carbonate (1:2 ratio) along with the polymers. In vitro testing revealed sustained release of tetracycline and metronidazole over 6–8 h while the tablets remained afloat (Yang et al. 1999).

2.2.3 Eudragit

Many enteric coating formulations are based on anionic polymers of methacrylic acid and methacrylates (Fig. 5). The polymers are considered unique and versatile since they dissolve at a wide range of pH 5.5–7. In the early 1930s, Röhm & Haas GmbH (Darmstadt, Germany) initiated the systematic research on the first forms of synthetic acrylates and methylacrylates. With the breakthrough development of Eudragit® in 1953, the first pharmaceutical coating drug formulation was introduced into the market. Since then, these coatings have opened a new phase in pharmaceutical research and development (Table 1, Fig. 5).

Eudragit in enteric coatings allows pH-dependent drug release. Eudragit polymers, used in mono or in blend form, have a wide range of pharmaceutical applications: as binding agents in tablets, to increase viscosity, to mask the taste of drugs, and as flow-controlling agents in liquids, suspensions, and emulsions. The polymers can also enhance drug stability and modify drug release characteristics. Overall, these polymers are known to increase drug effectiveness with good storage stability. Their preferred use is in gastrointestinal and colon targeting formulations (Lieberman et al. 1990).

Eudragit S-100 is used for uniform coating of microspheres (Degussa 2001). Composed of polypropylene foam, Eudragit S, ethyl cellulose, and polymethylmethacrylate (PMMA), floating microparticles were prepared by solvent evaporation, and encapsulation efficiency was found to be high. Good floating properties were observed as more than 83% of microparticles were seen to be floating for at least 8 h. In vitro drug release was dependent upon the type of polymer used. At similar drug loading the release rates increased in the following order: PMMA < ethyl

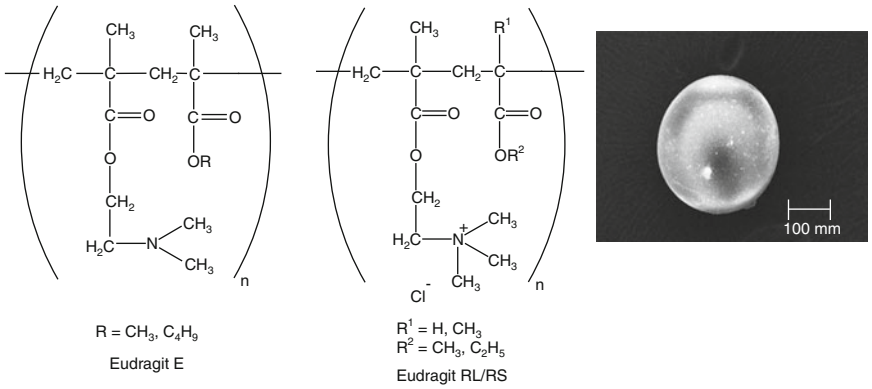


Fig. 5 Chemical structures of Eudragit polymers and scanning electron microscopy photograph of Eudragit S-100-coated core microspheres

cellulose < Eudragit S. This was attributed to the different permeabilities of the drug in the polymers and the drug distribution within the system (Streubel et al. 2002).

2.2.4 Carbopol Polymers

Carbopol® polymers celebrate 50 years of timeless innovation and inspiration. These polymers of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol (Table 1) are produced from primary polymer particles of about 0.2–6.0 μm average diameter (Jian-Hwa 2003). The Carbopol polymers are very mild acids and readily react with alkali to form salts. Aqueous dispersions of Carbopol polymers have an approximate pH range of 2.8–3.2 depending on polymer concentration.

Since the Carbopol polymers are readily water-swellable they find applications in a wide range of pharmaceutical products. Carbopol polymers offer a consistent performance over a wide range of desired parameters (from pH-derived semi-enteric release to near zero-order drug dissolution kinetics) at lower concentrations than competitive systems (Perez-Marcos et al. 1991; de Leeuw et al. 1995). The polymers are used for oral drug delivery systems. Carbopol is used as a standard bioadhesive agent for buccal, intestinal (Lehr et al. 1990, 1992), nasal, vaginal, rectal, and ophthalmic applications (e.g., Pilopine HS® pilocarpine hydrochloride 4% ophthalmic gel has carbopol 940 to impart a high viscosity, Alcon, Freiburg, Germany). Moreover, since Carbopol polymers exhibit high swelling and thickening properties at very low concentrations (less than 1%), they are also used in topical lotions, creams and gels, oral suspensions, and in transdermal gel reservoirs.

2.2.5 Pluronic Block Copolymers

Synthetic copolymers of ethylene oxide and propylene oxide (Pluronic™ block copolymers, BASF, Ludwigshafen, Germany) (Fig. 6) are used as antifoaming

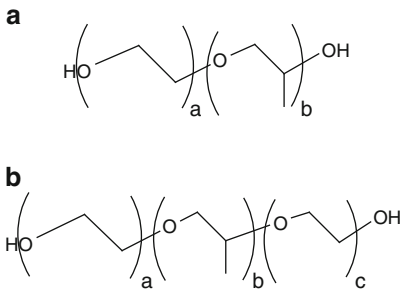


Fig. 6 Chemical structure of Pluronic polymer: (**a**) poly (ethyleneoxide) diblock (**b**) poly(propyleneoxide) triblock copolymer

agents, wetting agents, dispersants, thickeners, and emulsifiers. Biodegradable, biocompatible matrices for drug delivery, including films and microspheres, are formed by blending polymers degrading by hydrolysis such as poly(lactic acid) and Pluronic block copolymers (Park et al. 1994).

API incorporation into the core of the Pluronic micelles can increase solubility, metabolic stability, and circulation time for the agent. Alakhov et al. studied Pluronic-based megestrol acetate formulations in vitro with respect to drug release and in a rat model for bioavailability of after oral administration of 1–7 mg kg^{-1}. An aqueous, micellar formulation comprising a mixture of a hydrophobic (L61) and a hydrophilic (F127) Pluronic copolymer, significantly enhanced the megestrol acetate bioavailability (Alakhov et al. 2004). Moreover, interactions of Pluronic unimers with multidrug-resistant cancer cells can result in sensitization of these cells with respect to various anticancer agents. Animal studies with Pluronic–poly (acrylic acid) (PAA) copolymers have shown that the agents are not absorbed when administered orally (Bromberg 2008). At physiological pH level, the copolymers self-assemble into intra- and intermolecular micelles with hydrophobic cores of dehydrated poly(propylene oxide) and multilayered coronas of hydrophilic PEO and partially ionized PAA segments. These micelles can efficiently solubilize hydrophobic APIs (paclitaxel and steroids), and protect APIs (camptothecin) from the hydrolytic reactions (Bromberg 2008).

2.2.6 Alginates

Alginates (E400–E404) produced by brown seaweeds (mainly Laminaria) are linear unbranched polycarbohydrates containing β-(1→4)-linked D-mannuronic acid (M) and α-(1→4)-linked L-guluronic acid (G) residues (Fig. 7).

Alginates form thermally stable gels in the presence of calcium ions. The formation of gel is at far lower concentrations than any other natural polymer including gelatin. However, the solubility and water-holding capacity of alginate depend on pH (precipitates below pH 3.5), molecular weight, ionic strength and the nature of the ions present.

Alginate beads (Fig. 8) can encapsulate hydrophobic APIs which allows protection of non-stable APIs and offers potential for drug delivery. Due to enhanced

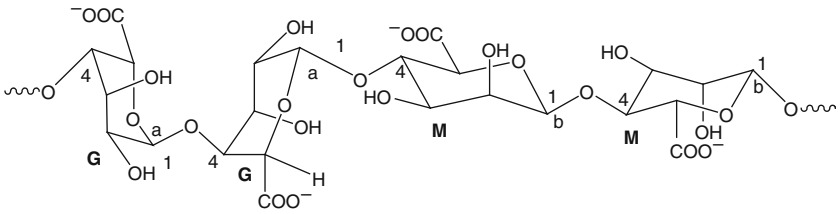


Fig. 7 Chemical structure of alginate

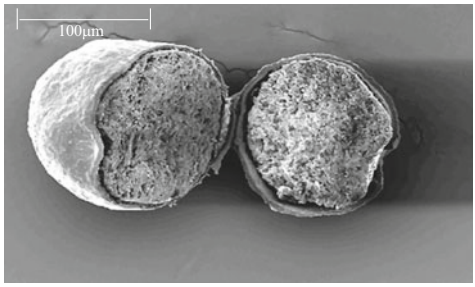


Fig. 8 Alginate beads provide encapsulation protection and delivery options for hydrophobic drugs, which may come in oily phase (modified from http://www.pharmaceuticaltechnology.com)

buoyancy and sustained release properties $CaCO_3$-containing beads appear to be excellent candidates for floating systems (Choi et al. 2002) as shown when incorporating riboflavin and 5-flurouracil (Shishu and Aggarwal 2007). Calcium alginate beads form as alginate undergoes ionotropic gelation by calcium ions and carbon dioxide develops from the reaction of carbonate salts with acid. The evolving gas permeates through the alginate matrix, thereby leaving gas bubbles or pores, and providing buoyancy for the beads.

2.3 Systemically Applied Polymers

Material scientists continuously wish for new materials and ways to manipulate existing ones in order to fulfill unmet needs. Therapeutic agents linked to polymers such as poly(ethylene glycol) (PEG), poly(lactide-*co*-glycolide) (PLGA), and *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymers and polyamines (Table 1) are in clinical trails. Dendritic polymers are instrumental in the development of new trends in targeted drug delivery (Pendri et al. 1995; Wang et al. 2000; Khandare et al. 2006; Betancourt et al. 2007; Duncan and Seymour 2007; Lee et al. 2008; Majoros et al. 2008; Venuganti and Perumal 2008).

Most of the systemic polymers discussed below are used either to form nanoparticles including dendrimers or for prodrug formation of, for example, peptides and proteins for imaging purpose. The polymer architecture and size often affect both

pharmacodynamic and pharmacokinetic properties of such a prodrug (Khandare and Minko 2006). Therefore, it is critical to design a prodrug which would release the API at the site of action to avoid adverse effects.

2.3.1 Poly(lactide-*co*-glycolide) and Related Polymers

Polyglycolide (PGA) was one of the first synthetic biodegradable polymers to be investigated for biomedical application. The highly crystalline polymer (45–55% crystallinity) exhibits a high tensile modulus with very low solubility in the organic solvents. The glass transition temperature of the polymer ranges from 35 to 40°C and the melting point is greater than 200 °C.

Extensive research has been performed to develop a full range of poly(lactide-co-glycolide) polymers (PLGA) (Table 1). Copolymer composition in the range of 25%–75%, poly(L-lactide-*co*-glycolide) forms amorphous polymer. 50/50 poly(DL-lactide-*co*-glycolide) degrades in approximately 1–2 months, 75/25 in 4–5 months, and 85/15 in 5–6 months (Middleton and Tipton 1998). In addition, poly(lactide) (PLA) and PLGA microspheres are some of the most important components of biopolymers used to design and develop biodegradable microspheres containing bioactive agents for therapeutic application (Shive and Anderson 1997).

Biodegradation and biocompatibility of PLA and PLGA devices. Over the past decade, extensive efforts have been made in the development of poly(DL-lactic acid), poly(L-lactic acid) and poly(lactide-*co*-glycolide) copolymer microsphere CRS. PuraSorb®PLG is a semicrystalline bioabsorbable co-polymer of L-lactide and glycolide with a monomer ratio of 80L:20G (Tiainen et al. 2002; FDA 2008c). A co-polymer containing 90% glycolic acid and 10% L-lactic acid was initially used for the development of the multifilament suture Vicryl®. A modified version of the suture, Vicryl Rapid®, which is an irradiated version of Vicryl® to increase the rate of degradation, is currently in the market. Panacryl® is another suture from the co-polymer with a higher lactic acid:glycolic acid ratio in order to decrease the rate of degradation (Field and Stanley 2004).

Microparticles prepared using PLGA are smaller than 10 μm in diameter and are therefore available for uptake by leukocytes, monocytes, macrophages, and other cells of the reticular endothelium system (RES), i.e., liver, spleen, etc. APIs are entrapped into microparticles and nanoparticles by various methods. Efforts have been directed towards understanding plasma protein adsorption of nanoparticles which may facilitate or inhibit phagocytosis by cells of the RES (Bergsma et al. 1993).

2.3.2 PEG Polymers

Poly(ethylene glycol) (PEG) is a linear polyether and is synthesized by an anionic ring opening polymerization of ethylene oxide initiated by nucleophilic attack of a hydroxide ion on the epoxide ring (Table 1). Polyethylene glycol monomethyl

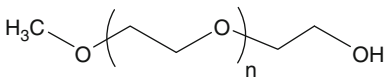


Fig. 9 Chemical structure of mPEG

ether (mPEG-OH)-PEGs are now available in many forms including bis functional derivatives (Fig. 9). The polymer is soluble in both aqueous as well as organic solvents, and is therefore the most preferred candidate in prodrug conjugates. The beginning of PEG chemistry was in 1977 with the findings of Abuchowski, who is considered "the father of PEGylation," and colleagues (Abuchowski et al. 1997). They later foresaw the potential of the conjugation of PEG to proteins and small molecules. Abuchowski founded a company, Enzon Inc., which brought three PEGylated drugs to market (the first one contained PEGylated adenosine deaminase). Over the past 20 years the area of PEGylated proteins has expanded dramatically. Higher molecular weight PEGs (M_w>20,000, especially 40,000) result in increased plasma residence time (Duncan 2003). The systemic toxicity of an anticancer drug can be reduced by tumor-specific targeting of PEGylated conjugates (Minko et al. 2007), and PEGylated adenosine deaminase (Adagen®, Enzon, Bridgewater, NJ) and L-asparaginase (Oncaspar®, Medac, Wedel, Germany) were introduced into the market in the early 1990s. Moreover PEGylated interferon α and G-CSF are approved drugs and PEG-camptothecin (Prothecan) has been subjected to clinical phase II testing (Duncan 2003).

2.3.3 Dextran

Dextrans are natural macromolecules and consist of linear units with covalently linked (1→6′) glycopyranose which are branched at α-(1→4′) position (Fig. 10). The polyglucose biopolymer is characterized by α-(1→6) linkage with hydroxylated cyclohexyl units and is generally produced by enzymes from certain strains of *Leuconostoc* or *Streptococcus*. Dextran contains multiple hydroxyl groups for bioconjugation, and is available in different molecular weight ranges.

Dextran has the most compact structure of all polymers, and is another ideal candidate for prodrug formation due to the following properties: water soluble, nontoxic, highly stable glycosidic bonds.

Dextran is FDA-approved as a plasma expander; it is also being investigated as a carrier for the passive targeting of tumors and inflamed areas according to an EPR effect. A methylprednisolone prodrug has been prepared by dextran conjugation using succinic acid as a spacer (Mehvar et al. 2000) and dextran-conjugated glucocorticoids have been evaluated for colon-specific delivery (McLeod et al. 1993).

2.3.4 HPMA Copolymers

N-(2-Hydroxypropyl)methacrylamide (HPMA) homopolymer was designed in the 1980s as a plasma expander (Kopecek and Bazilova 1973). The hydrophilic HPMA

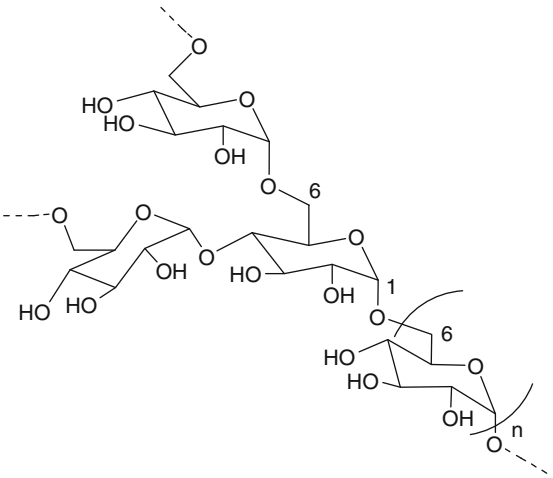


Fig. 10 Chemical structure of dextran

copolymers increase water solubility of APIs and have proven to be biocompatible, non-immunogenic, and nontoxic (Rihova et al. 1989; David et al. 2002). HPMA distribution in the body is well characterized. It has shown great potential as a carrier for targeted delivery of, e.g., antisense oligonucleotides and the controlled release of small molecules (Wang et al. 1998).

The HPMA–doxorubicin conjugates (Fig. 11) were less toxic than the free drug and could accumulate inside solid tumors. The conjugates were synthesized as follows: for subsequent conjugation with a modified antibody, pyridyldisulfanyl groups were incorporated into poly(HPMA) hydrazide using *N*-succinimidyl 3-(2-pyridyldisulfanyl) propanoate. Then the anticancer drug was covalently linked to the remaining hydrazide groups via an acid-labile hydrazone bond (Ulbrich et al. 2003). Finally, human immunoglobulin G was modified with 2-iminothiolane by conjugating it to the HPMA polymer through substituting the 2-pyridylsulfanyl groups of the polymer with –SH groups of the antibody.

2.3.5 Poly(anhydrides)

Poly(anhydrides) (Table 1) have been specifically designed and developed for drug delivery applications. These polymers are aromatic poly(anhydrides) based on monomers of *p*-(carboxyphenoxy)propane and *p*-(carboxyphenoxy)hexane as well as aliphatic poly(anhydrides) based on sebacic acid (Uhrich et al. 1999). Poly (anhydrides) are prepared by melt-condensation polymerization, dicarboxylic acid as a starting material, and a prepolymer of a mixed anhydride formed with acetic anhydride (Uhrich et al. 1999).

Poly(anhydrides) undergo hydrolytic degradation forming water-soluble degradation products which dissolve in an aqueous environment. They also undergo surface erosion due to the high water lability of the anhydride bonds on the surface

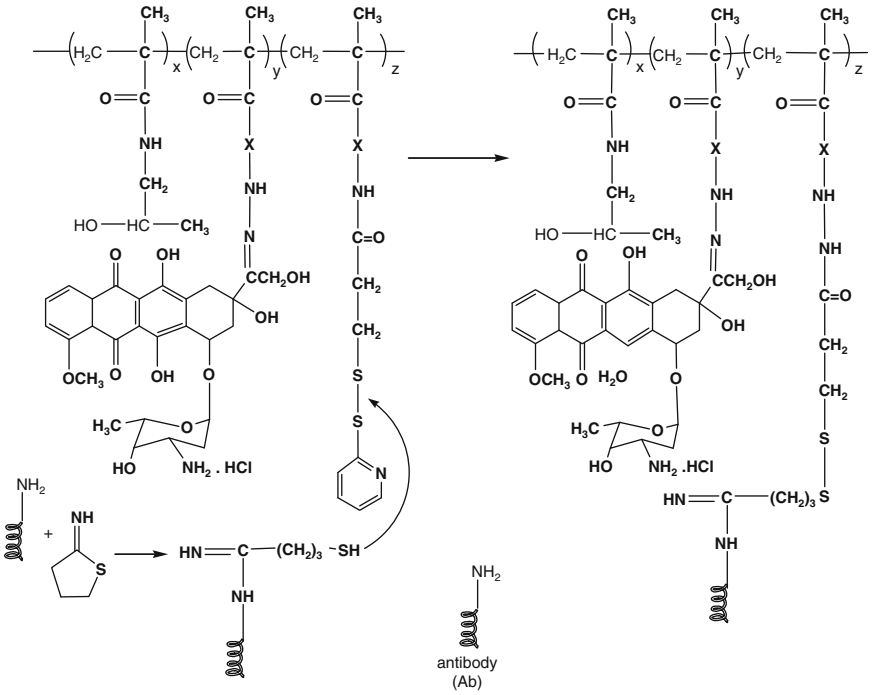


Fig. 11 Poly(*N*-(2-hydroxypropyl) methacrylamide-doxorubicin-antibody bioconjugates. Poly HPMA hydrazides are modified with *N*-succinimidyl 3-(2-pyridyldisulfanyl) propanoate to introduce the pyridyldisulfanyl groups and for subsequent conjugation with modified antibody (Ab) (modified from Ulbrich et al., 2003)

and the hydrophobicity which prevents water penetration into the bulk (Langer and Chasin 1990; Lee and Chu 2008). Due to matrix degradation and erosion, the polymers have been widely used for the incorporation of small molecules and proteins, such as insulin, enzymes, and growth factors (Chasin et al. 1990). An intracranial device of sebacic acid/*p*-(carboxyphenoxy)propane copolymer improves the therapeutic efficacy of a nitrosourea antitumor agent in patients suffering from lethal brain cancer (Brem et al. 1995). Poly(anhydrides) possess satisfactory biocompatibility (Laurencin et al. 1990).

2.4 Current Developments: Dendritic Polymers

In the early 1980s, polymer science research introduced versatile nanosized dendritic polymers, e.g., dendrimers and hyperbranched polymers. The latter are highly branched macromolecules (with 50%–75% branching) with polydispersity index (PDI) typically in the range of 1.5–2.0, but possess a defined chemical structure. Dendrimers, which are named after the Greek word "dendron" for tree, can be

chemically designed and synthesized to possess precise structural characteristics (Newcome et al. 1994; Tomalia and Fréchet 2005). These versatile polymers are synthesized from monomeric units with new branches being added in steps until a uniform tree-like structure is formed.

Dendritic polymers are interesting drug delivery carriers because they are nanosized, chemically defined and multifunctional (Tajarobi et al. 2001; Lee et al. 2005; Majoros et al. 2005).

Dendrimers (Fig. 12) are unique nanosystems because of their monodispersity (PDI ~1.0), nanometer dimension (1–10 nm), low viscosity, multiple functionality at the terminal groups, high solubility, and biocompatibility. Various reports are available in the literature for dendritic conjugation to active agents including methotrexate, camptothecin, and paclitaxel. Additionally, folate residues, antibodies and hormones can be attached on the surface of dendrimers for potential tumor cell specificity and targetability (Stiribara et al. 2002; Haag and Kratz 2006; Zhou et al. 2006).

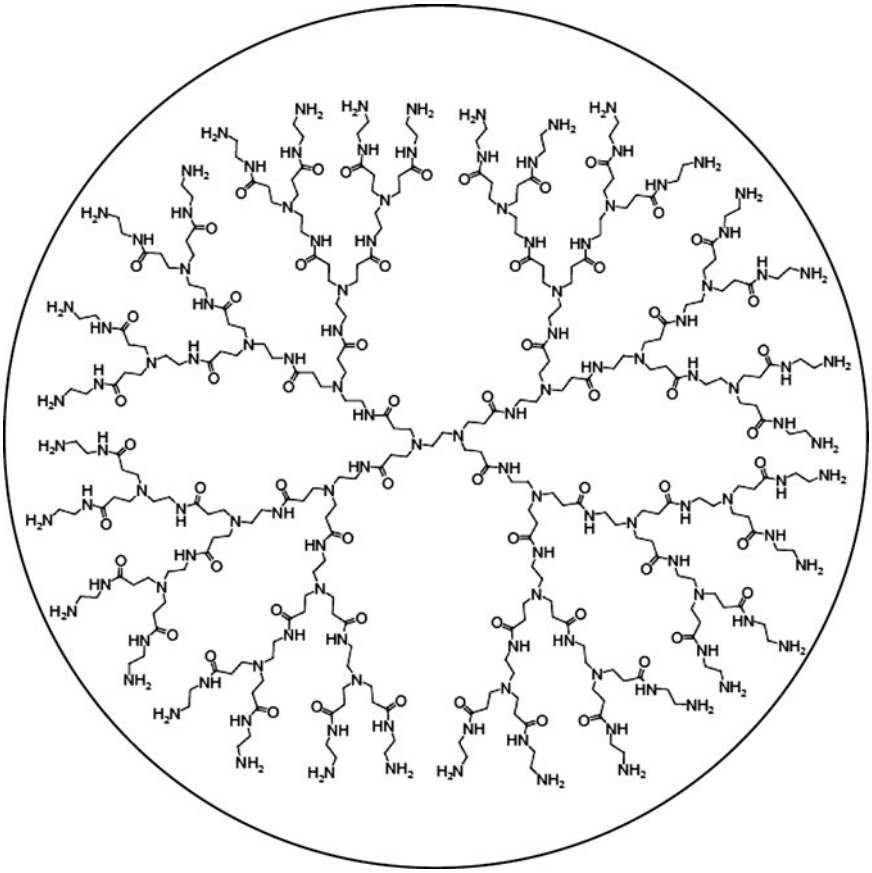


Fig. 12 PAMAM dendrimer (generation 4) used in biological applications

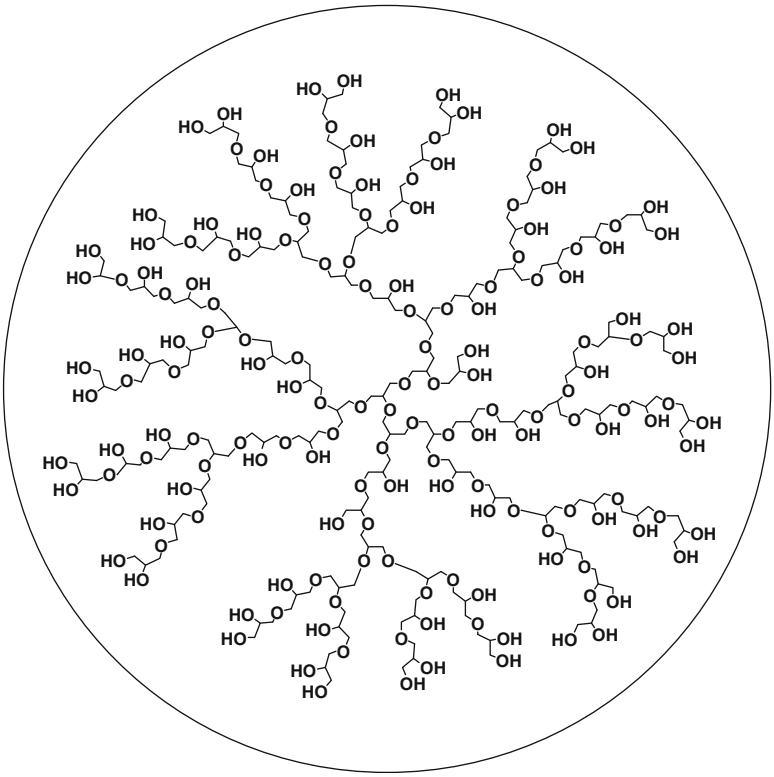


Fig. 13 Dendritic polyglycerols as new highly biocompatible polymeric scaffolds for delivery and multivalency

Dendritic polyglycerols are aliphatic polyethers and possess multiple functional hydroxyl end groups (Fig. 13). They can either be prepared as perfect dendrimer by stepwise synthesis (Haag et al. 2000) or as hyperbranched polymers in kilogram scale (Sunder et al. 1999). Since dendritic polyglycerols are synthesized in a controlled manner to obtain definite molecular weight and narrow molecular polydispersity (~1.7) (Sunder et al. 2000), they have been evaluated for a variety of biomedical applications as delivery vehicles for nonpolar APIs (Türk et al. 2007; Quadir et al. 2008), heparin analogs (Türk et al. 2004), and multivalent selectin ligands (Papp et al. 2008).

3 Specific Aspects of Polymers in Oral Drug Delivery

In the following section we describe specific delivery systems for oral use. For transdermal and pulmonary application systems please refer to the chapters of Guy and Lehr (this volume).

A challenging aspect in CRS development is to provide prolonged zero-order kinetics for the total drug release with no time lag or burst effect (Hamdan et al. 2008). Such release profiles can optimize the dosage frequency in drug therapy. With oral treatment, coated pellets or particles in capsules and compressed tablets are cost-effective; however, the advanced matrix systems, reservoir systems and osmotic pumps systems are currently proving to be the most efficient. Moreover, gastro-enteric-coated forms are designed to protect the API from degradation by gastric pH and enzymes and to prevent nausea and vomiting caused by an API irritating the gastric mucosa. Many APIs also require protective coatings to improve patient compliance by masking unpleasant taste and odor.

It has to be noted, however, that pharmaceutical dosage forms can irritate the gastrointestinal mucosa due to the chemical properties of the polymers in the form of external coating.

3.1 Modified Release Dosage Forms

Imparting the rate and/or site of the drug release by a special formulation design and/or manufacturing method, modified release dosage forms include extended-release, delayed-release, and pulsatile-release. These can be single-unit (e.g., tablet) or multiple-unit (coated pellets or particles in capsules) systems. The latter are more favored because of predictable gastric emptying, no risk of dose dumping, and superior and less variable bioavailability (Roy and Shahiwala 2009).

Extended-release dosage forms allow at least a two-fold reduction in dosing frequency compared to conventional dosage forms administered by the same route. *Delayed release formulations* deliver the loaded API after a lag time, i.e., a period of "no drug release." This type can be used for drug delivery to the colon (Chourasia and Jain 2003), e.g., in the local treatment of colitis ulcerosa and Crohn's disease. Delivering the anti-inflammatory and immunosuppressive agents to the lesional area of the gut enhances efficacy and reduces systemic side-effects. Currently, colonic drug delivery is gaining interest as one of the most important forms of targeted drug delivery.

Pulsatile drug delivery systems (PDDS) are modified-release dosage forms with sequential API release (Haus 2007; Gazzaniga et al. 2008). Pulsatile release formulations can also adjust API plasma levels to clinical needs, delivering a single dose after a programed lag phase, e.g. to suppress asthma attacks in the early morning. Due to circadian fluctuations pulse therapy may also improve testosterone, glucocorticoid, and antihistamine treatment (Dittigen et al. 2000). Thus, delivering drugs in a pulsatile fashion for certain clinical conditions should be highly beneficial.

Gastro-retentive (GR) delivery systems delaying API delivery to the gut are ideal for APIs that are only absorbed in limited areas of the small intestine and thus have a very limited "window of absorption," e.g., due to saturable uptake mechanism.

3.2 Gastro-enteric Coatings

Advantages of enteric coating polymers are good storage stability of the API, pH-dependent drug release, protection of actives sensitive to gastric fluid and subsequent increase in drug effectiveness, and protection of gastric mucosa from aggressive actives. Gastrointestinal and colon targeting are possible. Table 3 summarizes drug delivery sites for absorption of actives at pH 5.5–7.0 referring to Eudragit polymers.

Prozac Weekly™ capsules (Eli Lilly, Bad Homburg, Germany), a delayed-release formulation, contain enteric-coated pellets of fluoxetine hydrochloride (90 mg fluoxetine). Bioavailability following a single 40 mg oral dose achieves peak plasma concentration of fluoxetine 15–55 ng mL^{-1} after 6–8 h (FDA 2006a).

3.3 Matrix Systems

Matrix systems, as the most common controlled delivery system, are tablets and granules. The system is preferred because of its effectiveness as well as ease of manufacturing and thus low cost. It is typically formulated into a once-daily dosage form which contains homogeneously dissolved or dispersed API (Boldhane 2008). A solidifying matrix dissolvable or dispersible in solvents allows for a controlled release. This applies also to APIs with high aqueous solubility that need to be applied in higher doses. With conventional delivery systems, these agents become almost instantaneously available and toxic plasma levels early after intake are soon followed by sub-therapeutic ones, given that the API is eliminated rapidly from the organism (Fig. 1). In contrast, matrix systems can maintain constant plasma levels (Mura et al. 2003). Importantly, matrix systems can also be formulated to avoid interactions of API and food components and the advanced systems can be taken irrespective of the meal pattern.

Delayed release is achievable by the use of an erodible monolith, which delivers the actives in the lower gastrointestinal tract (FDA 2006b). *Controlled release* becomes possible by swelling and eroding of the monolith, and then the API is continuously released throughout the gastrointestinal tract.

Table 3 Drug delivery sites in gastrointestinal tract, polymer grades

Drug delivery site	Polymer dissolution at pH	Eudragit®, grade and form
Duodenum	Above pH 5.5	L30 (powder) D-55 (30% aqueous dispersion)
Jejunum	Above pH 6.0	L100 L (powder) S12,5 (12.5% organic solution)
Ileum	Above pH 7.0	S100 (powder) S12,5 (12.5% organic solution) FS30D (30% aqueous dispersion)
Colon delivery	Above pH 7.0	FS30D (30% aqueous dispersion)

Multilayered matrix tablets are developed which can provide multiple release kinetics of a single API or a combination of two (or more) APIs having identical or different physicochemical properties (Boldhane 2008). Burst release by immediate disintegrating of monolith layer delivers the loading dose required to achieve active plasma concentration. Then a second layer slowly releases the API at a controlled rate as described above. By physical separation a physical/chemical barrier can also avoid the incompatibility between two actives, the excipients and active-excipients interactions. A well-known example of such an interaction is the Millard reaction that occurs during tablet compression.

With the commercially available multiple layer matrix system Cipro XR® (500–1,000 mg daily; Bayer Pharmaceuticals, Leverkusen, Germany) extended ciprofloxacin release from the tablets becomes possible by a barrier layer which separates the core and the dissolution medium. Approximately 35% of the dose is present within an immediate-release component, while the remaining 65% is embedded in a slow-release matrix. In the case of Cipro XR, maximum plasma ciprofloxacin concentrations are attained between 1 and 4 h after dosing, bioavailability is close to availability following ciprofloxacin immediate release when applying identical doses (FDA 2004).

Multilayered matrix tablets can also be used to generate repeat-action products. One layer of the tablet or the outer layer of the compressed coated tablet provides the initial dose by rapid disintegration in the stomach. The inner tablet is formulated with components that are insoluble in gastric media but release API in the intestinal environment. The release profile can be further improved by a degradable barrier layer, a commercially available multilayered matrix system is PAXIL CR® (GlaxoSmithKline, München, Germany) containing the psychotropic agent paroxetine hydrochloride.

3.4 Reservoir Systems

With matrix systems, an unwanted burst effect which leads to higher initial drug delivery and reduction of the effective lifetime of the device may not be eliminated with all APIs, necessitating more advanced systems. In polymeric reservoir systems offering a defined resistance to drug diffusion from the reservoir to sink (Colombo et al. 2000), the driving force is the concentration gradient of the API molecules between the reservoir and sink. Such systems are capable of presenting a linear release pattern. A reservoir tablet system consists of a semi-permeable barrier which is involved in API release from a core site within the tablet. Commonly used methods include coating of tablets or multiparticulates, microencapsulation of drug particles, and press coating of tablets.

There are several advantages and disadvantages associated with reservoir systems. The advantages include linear release of the API which is independent of the solubility of the active agent in the medium as well as pH level and ion strength of the medium. The disadvantages include the complex manufacturing process, rapid

transit of drug levels from the reservoir, low capacity for drug loading and eventual incomplete release of the actives. Complexity of design, manufacturing processes, and the need for specialized equipment may all result in an unbalanced cost–benefit ratio for these systems.

3.5 Osmotic Pump Systems

Osmotic pump systems consist of a tablet sealed by a semi-permeable membrane with an orifice. The elementary osmotic pump generally has a single-layer core containing the API (typically water-soluble) enclosed in a semi-permeable membrane with one or more laser-drilled orifices. This is the one-chamber elementary osmotic pump system.

In the gastrointestinal tract, water is drawn in through the membrane at a controlled rate, gradually dissolving the active ingredient. By the increased pressure, the resulting API solution flows out through the orifice at the same rate that water is flowing in through the membrane. Examples of products utilizing this system are Acutrim (phenylpropanolamine hydrochloride, Norvartis, Nürnberg, Germany), Efidac (pseudoephedrine hydrochloride, Hogil, White Plains, NY, USA), and Volmax (albuterol sulfate, Merck, Darmstadt, Germany; Santus and Baker 1995).

There is also a second type of osmotic pump systems, the *two-chamber osmotic pump system,* also called the *push and pull system,* which is typically used when the API has limited aqueous solubility. In this system, the semi-permeable membrane encloses a two-layer core, one layer containing the active ingredient and the second layer containing a water-swellable osmotic agent (Fig. 14). As water flows into the core through the rate-controlling membrane, the osmotic agent expands which causes the dissolved API to be pushed out through the laser-drilled orifice(s). Examples of products utilizing the push–pull system are Procardia XL (nifedipine, Pfizer, Berlin, Germany) and Minipress XL (prazosin hydrochloride, Pfizer, Berlin, Germany).

The weight of the semi-permeable membrane (increased membrane thickness, as measured by weight) slows release rate of the API. According to the FDA, it is important for an applicant developing a gastrointestinal tract dosage form to determine and carefully control the weight of the semi-permeable membrane during the coating operation. This consideration is important in preapproval inspections (FDA 2008a).

Amongst various pulsatile delivery systems, single-unit pulsatile systems are popular and consist of sub-classes:

- Capsule-based systems (Pulsincap®, Scherer International Corporation, Michigan, US),
- Osmotic systems (Port® System, Therapeutic System Research Laboratory, Ann Arbor, Michigan),

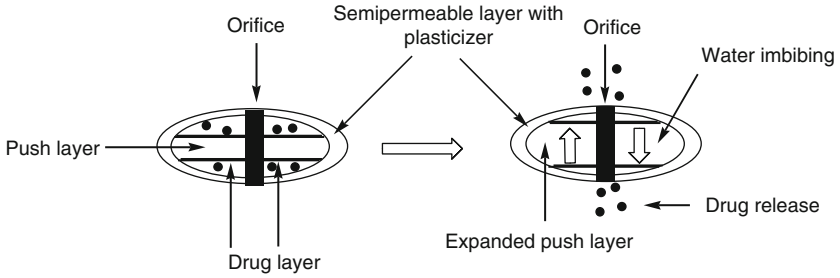


Fig. 14 Multilayered matrix push layered tablets consisting of polymeric plasticizers

- Delivery systems with soluble or erodible membranes (chronotropic) consisting of a core containing drug reservoir coated by a hydrophilic polymer (e.g., HPMC), and
- Reservoir systems with a rupturable coating (e.g., soft gelatin or HPMC).

The length of a swellable hydrogel plug and its point of insertion into the capsule controls the lag time of the Pulsincap® system. Making contact with the dissolution fluid, the hydrogel (e.g., HPMC, PMMA, PVA, PEO) swells and the plug sealing the drug content within the capsule is pushed outside, rapidly releasing the API. The length of the plug and its point of insertion into the capsule controls the lag phase (Arora et al. 2006). Pulsincap® is reported to be well tolerated in human volunteers (Takada 1997; Hebden et al. 1999; Ross et al. 2000).

The osmotic Port® system consists of a capsule coated with a semipermeable membrane. The capsule contains a plug of insoluble yet osmotically active compound and the API. Water entry via the semipermeable membrane increases the pressure within the capsule and after a lag time the plug is expelled. The Port® system is set up for methylphenidate dosing in children with attention deficit hyperactivity syndrome to avoid a second dosing during daytime. Alternatively, pulsatile methylphenidate release (three pulses) becomes possible from dosage units forming tablets. Coating is based on erodible polymers (Arora et al. 2006).

Pulsys® (MiddleBrook Pharmaceuticals, former Advancis Pharmaceutical Corp.) is a once-a-day delivery system delivering three regular pulses of amoxicillin for an optimal bactericidal effect. Preclinical studies have demonstrated its superior efficacy (Arora et al. 2006; Roy and Shahiwala 2009); publication of results of comparative clinical testing in relation to standard therapy is to be awaited. The product is commercially available (Moxatag™ tablet).

The most reliable gastric emptying is possible with pellet formulations as these are distributed freely in the gastrointestinal tract. The pellets are coated with rupturable polymers or polymers changing permeability. In the latter approach, used for diltiazem delivery, delivery rates are controlled by the thickness of the coating. Thickness of the membranes also controls drug delivery rate with rupturable polymers (Arora et al. 2006; Roy and Shahiwala 2009). A controlled and

extended release formulation of verapamil hydrochloride and propranolol hydrochloride is presently marketed; release rate is independent of pH, food and gastrointestinal motility (Innopran® XL tablet, MiddleBrook Pharmaceuticals, Inc.).

References

Abuchowski A, Van Es T, Palczuk NS, Davis FF (1997) Alteration of immunological properties of bovine serum albumin by covalent attachment of polyethylene glycol. J Biol Chem 252:3578–3581

Alakhov V, Pietrzynski G, Patel K, Kabanov A, Bromberg L, Hatton TA (2004) Pluronic block copolymers and Pluronic poly(acrylic acid) microgels in oral delivery of megestrol acetate. J Pharm Pharmacol 56:1233–1241

Arora S, Ali A, Ahuja A, Baboota S, Qureshi J (2006) Pulsatile drug delivery systems: An approach for controlled drug delivery. Ind J Pharm Sci 68:295–300

Bergsma EJ, Rozema FR, Bos RR, de Bruijn WC (1993) Foreign body reactions to resorbable poly (L-lactide) bone plates and screws used for the fixation of unstable zygomatic fractures. J Oral Maxillofac Surg 51:666–670

Betancourt T, Brown B, Brannon-Peppas L (2007) Doxorubicin-loaded PLGA nanoparticles by nanoprecipitation: preparation, characterization and in vitro evaluation. Nanomedicine 2:219–232

Boldhane S (2008) Development and evaluation of oral platform drug delivery technology. In: Shivaji University, vol Ph.D. Thesis pp 12–13

Brazel CS, Peppas NA (2000) Modeling of drug release from swellable polymers. Eur J Pharm Biopharm 49:47–58

Brem H, Piantadosi S, Burger PC, Walker M, Selker R, Vick NA, Black K, Sisti M, Brem S, Mohr G et al (1995) Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. The polymer-brain tumor treatment group. Lancet 345:1008–1012

Bromberg L (2008) Polymeric micelles in oral chemotherapy. J Control Release 128:99–112

Chasin M, Domb A, Ron E, Mathiowitz E, Langer R, Leong K, Laurencin C, Brem H, Grossman S (1990) Polyanhydrides as drug delivery systems. In: Chasin M, Langer R (eds) Biodegradable polymers as drug delivery systems. Marcel Dekker, New York, pp 43–70

Choi BY, Park HJ, Hwang SJ, Park JB (2002) Preparation of alginate beads for floating drug delivery system: effects of CO_2 gas-forming agents. Int J Pharm 239:81–91

Chourasia MK, Jain SK (2003) Pharmaceutical approaches to colon targeted drug delivery systems. J Pharm Sci 6:33–66

Colombo P, Santi P, Bettini R, Prazel CS, Peppas NA (2000) Mechanism-based classification of controlled release devices. In: Wise DL (ed) Handbook of pharmaceutical controlled release technology. CRC Press, New York, p 493

David A, Kopeckova P, Kopecek J, Rubinstein A (2002) The role of galactose, lactose, and galactose valency in the biorecognition of *N*-(2-hydroxypropyl)methacrylamide copolymers by human colon adenocarcinoma cells. Pharm Res 19:1114–1122

de Leeuw BJ, Lueben HL, Pérard D, Verhoef AC, de Boer AG, Junginger HE (1995) The effect of mucoadhesive poly(acrylates) polycarbophil and carbomer on zinc and calcium dependent proteases. Proc Int Symp Control Rel Bioact Mater 22:528–529

Degussa (2001) http://www.degussa-history.com/geschichte/en/inventions/eudragit/in:

Dittigen M, Fricke S, Timpe C, Gercke H, Eichardt A (2000) Method of making a perorally administered solid drug with controlled effective ingredient delivery. US Patent No 6117450, USA

Duncan R (2003) The dawning era of polymer therapeutics. Nat Rev Drug Discov 2:347–360

Duncan R, Seymour L (2007) Hiroshi Maeda–defining the pathway to targeted cancer therapy. J Drug Target 15:456
FDA (2004) http://www.fda.gov/medwatch/SAFETY/2004/jul_PI/CiproXR_PI.pdf
FDA (2006a) http://www.fda.gov/cder/foi/label/2006/018936s076lbl.pdf
FDA (2006b) http://www.fda.gov/medwatch/safety/2006/Paxil%20CR_PI.pdf
FDA (2008) http://www.fda.gov/cber/rules/amendcgmpfinal.htm
FDA (2008a) http://www.fda.gov/cder/dmpq
Field JR, Stanley RM (2004) Suture characteristics following incubation in synovial fluid or phosphate buffered saline. Injury 35:243–248
Ford JE (1994) Fundamental aspects of drug release form hydrophilic matrix tablets. In: Proc Colorcon Controlled Release Symposium, pp 1–26
Gazzaniga A, Palugan L, Foppoli A, Sangalli ME (2008) Oral pulsatile delivery systems based on swellable hydrophilic polymers. Eur J Pharm Biopharm 68:11–18
Guyot M, Fawaz F (2000) Design and in vitro evaluation of adhesive matrix for transdermal delivery of propranolol. Int J Pharm 204:171–182
Haag R, Kratz F (2006) Polymer therapeutics: concepts and applications. Angew Chem Int Ed 45:1170–1179
Haag R, Sunder A, Stumbé JF (2000) An approach to glycerol dendrimers and pseudo-dendritic polyglycerols. J Am Chem Soc 122:2954–2955
Hamdan AM, Moseke C, Blanco L, Barralet JE, Lopez-Carbacos E, Gbureck U (2008) Strontium modified biocements with zero order release kinetics. Biomaterials 29:4691–4697
Haus E (2007) Chronobiology in the endocrine system. Adv Drug Deliv Rev 59:985–1014
Hebden JM, Wilson CG, Spiller RC, Gilchrist PJ, Blackshaw E, Frier ME, Perkins AC (1999) Regional differences in quinine absorption from the undisturbed human colon assessed using a timed release delivery system. Pharm Res 16:1087–1092
Jian-Hwa G (2003) Carbopol® polymers for pharmaceutical drug delivery applications. Drug Del Tech 3:6
Khandare JJ, Minko T (2006) Polymer-drug conjugates: progress in polymeric prodrugs. Progress Polymer Sci 31:359–397
Khandare JJ, Jayant S, Singh A, Chandna P, Wang Y, Vorsa N, Minko T (2006) Dendrimer versus linear conjugate: influence of polymeric architecture on the delivery and anticancer effect of paclitaxel. Bioconjug Chem 17:1464–1472
Kopecek J, Bazilova H (1973) Poly(*N*-(hydroxypropyl)-methacrylamide) radical polymerization and copolymerization. Eur Polym J 9:7–14
Langer R, Chasin M (1990) Biodegradable polymers as drug delivery systems. Marcel Dekker, New York, p 47
Laurencin C, Domb A, Morris C, Brown V, Chasin M, McConnell R, Lange N, Langer R (1990) Poly(anhydride) administration in high doses in vivo: studies of biocompatibility and toxicology. J Biomed Mater Res 24:1463–1481
Lee WC, Chu IM (2008) Preparation and degradation behavior of polyanhydrides nanoparticles. J Biomed Mater Res B Appl Biomater 84:138–146
Lee CC, MacKay JA, Frechet JM, Szoka FC (2005) Designing dendrimers for biological applications. Nat Biotechnol 23:1517–1526
Lee H, Lee K, Park TG (2008) Hyaluronic acid-paclitaxel conjugate micelles: synthesis, characterization, and antitumor activity. Bioconjug Chem 19:1319–1325
Lehr CM, Bouwstra JA, Tukker JJ, Junginger HE (1990) Intestinal transit of bioadhesive microspheres in an in situ loop in the rat: a comparative study with copolymers and blends based on poly(acrylic acid). J Control Release 13:51–62
Lehr CM, Bouwstra JA, Bodde HE, Junginger HE (1992) A surface energy analysis of mucoadhesion: contact angle measurements on polycarbophil and pig intestinal mucosa in physiologically relevant fluids. Pharm Res 9:70–75
Lieberman HA, Lachman L, Schwartz JB (1990) Pharmaceutical dosage forms: tablets. Marcel Dekker, New York, pp 118–233

Majoros IJ, Thomas TP, Mehta CB, Baker JR Jr (2005) Poly(amidoamine) dendrimer-based multifunctional engineered nanodevice for cancer therapy. J Med Chem 48:5892–5899
Majoros IJ, Williams CR, Baker JR Jr (2008) Current dendrimer applications in cancer diagnosis and therapy. Curr Top Med Chem 8:1165–1179
McLeod AD, Friend DR, Tozer TN (1993) Synthesis and chemical stability of glucocorticoid–dextran esters: potential prodrugs for colon-specific delivery. Int J Pharm 92:105–114
Mehvar R, Dann RO, Hoganson DA (2000) Kinetics of hydrolysis of dextran-methylprednisolone succinate, a macromolecular prodrug of methylprednisolone, in rat blood and liver lysosomes. J Control Release 68:53–61
Middleton JC, Tipton AJ (1998) Synthetic biodegradable polymers as orthopedic devices. Biomaterials 21:2335–2346
Minko T, Khandare JJ, Jayant S (2007) Polymeric Drugs. In: Matyjaszewski K, Gnanou Y, Leibler L (eds) Macromolecular engineering: from precise macromolecular synthesis to macroscopic material properties and application. Wiley-VCH, Weinheim, pp 2541–2595
Mura P, Maestrelli F, Cirri M, Gonzalez Rodriguez ML, Rabasco Alvarez AM (2003) Development of enteric-coated pectin-based matrix tablets for colonic delivery of theophylline. J Drug Target 11:365–371
Newcome GR, Moorefield CN, Keith JN, Baker GR, Escamilla GH (1994) Chemistry within a unimolecular micelle precursor: Boron superclusters by site- and depth-specific transformations of dendrimers. Angew Chem Int Ed 33:2413–2420
Papp I, Dernedde J, Enders S, Haag R (2008) Modular synthesis of multivalent glycoarchitectures and their unique selectin binding behavior. Chem Commun 44:5851–5853
Park TG, Cohen S, Langer RS (1994) Controlled drug delivery using polymer/pluronic blends. US Patent No. 5330768 USA
Pendri A, Martinez A, Xia J, Shorr RG, Greenwald RB (1995) Poly(ethylene glycol) fluorescent linkers. Bioconjug Chem 6:596–598
Perez-Marcos B, Gutierrez C, Gomez-Amoza JL, Martinez-Pacheco R, Souto C, Concheiro A (1991) Usefulness of certain varieties of Carbopol in the formulation of hydrophilic furosemide matrices. Int J Pharm 67:113–121
Quadir MA, Radowski MR, Kratz F, Licha K, Hauff P, Haag R (2008) Dendritic multishell architectures for drug and dye transport. J Control Release: 132:289–294
Rihova B, Bilej M, Vetvicka V, Ulbrich K, Strohalm J, Kopecek J, Duncan R (1989) Biocompatibility of *N*-(2-hydroxypropyl) methacrylamide copolymers containing adriamycin. Immunogenicity, and effect on haematopoietic stem cells in bone marrow in vivo and mouse splenocytes and human peripheral blood lymphocytes in vitro. Biomaterials 10:335–342
Ross AC, MacRae RJ, Walther M, Stevens HN (2000) Chronopharmaceutical drug delivery from a pulsatile capsule device based on programmable erosion. J Pharm Pharmacol 52:903–909
Roy P, Shahiwala A (2009) Multiparticulate formulation approach to pulsatile drug delivery: current perspectives. J Control Release 134:74–80
Santus C, Baker (1995) Osmotic drug delivery: a review of the patent literature. J Control Release 35:1–21
Shishu GN, Aggarwal N (2007) Stomach-specific drug delivery of 5-fluorouracil using floating alginate beads. AAPS PharmSciTech 8:48
Shive MS, Anderson JM (1997) Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv Drug Deliv Rev 28:5–24
Smolensky MH, Peppas NA (2007) Chronobiology, drug delivery, and chronotherapeutics. Adv Drug Deliv Rev 59:828–851
Stiribara SE, Frey H, Haag R (2002) Dendritic polymers in biomedical applications: from potential to clinical use in diagnosis and therapy. Angew Chem Int Ed 41:1329–1334
Streubel A, Siepmann J, Bodmeier R (2002) Floating microparticles based on low density foam powder. Int J Pharm 241:279–292
Sunder A, Hanselmann R, Frey H, Mulhaupt R (1999) Controlled synthesis of hyperbranched polyglycerols by ring-opening multibranching polymerization. Macromolecules 32:4240–4246

Sunder A, Mülhaupt R, Haag R, Frey H (2000) Hyperbranched polyether polyols: a modular approach to complex polymer architectures. Adv Mater 12:235–239

Tajarobi F, El-Sayed M, Rege BD, Polli JE, Ghandehari H (2001) Transport of poly amidoamine dendrimers across Madin-Darby canine kidney cells. Int J Pharm 215:263–267

Takada K (1997) Controlled-release preparations. US Patent No 5,637,319, USA

Tiainen J, Veiranto M, Suokas E, Tormala P, Waris T, Ninkovic M, Ashammakhi N (2002) Bioabsorbable ciprofloxacin-containing and plain self-reinforced polylactide-polyglycolide 80/20 screws: pullout strength properties in human cadaver parietal bones. J Craniofac Surg 13:427–433

Tomalia DA, Fréchet JM (2005) Introduction to dendrimers and dendritic polymers. Prog Polym Sci 30:294–324

Türk H, Haag R, Alban S (2004) Dendritic polyglycerol sulfates as new heparin analogues and potent inhibitors of the complement system. Bioconjug Chem 15:162–167

Türk H, Shukla A, Alves Rodrigues PC, Rehage H, Haag R (2007) Water-soluble dendritic core-shell-type architectures based on polyglycerol for solubilization of hydrophobic drugs. Chemistry 13:4187–4196

Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM (1999) Polymeric systems for controlled drug release. Chem Rev 99:3181–3198

Ulbrich K, Etrych T, Chytil P, Jelinkova M, Rihova B (2003) HPMA copolymers with pH-controlled release of doxorubicin: in vitro cytotoxicity and in vivo antitumor activity. J Control Release 87:33–47

Venuganti VV, Perumal OP (2008) Effect of poly(amidoamine) (PAMAM) dendrimer on skin permeation of 5-fluorouracil. Int J Pharm 361:230–238

Wang L, Kristensen J, Ruffner DE (1998) Delivery of antisense oligonucleotides using HPMA polymer: synthesis of thiol polymer and its conjugation to water-soluble molecules. Bioconjug Chem 9:749–757

Wang D, Kopeckova JP, Minko T, Nanayakkara V, Kopecek J (2000) Synthesis of starlike *N*-(2-hydroxypropyl)methacrylamide copolymers: potential drug carriers. Biomacromolecules 1:313–319

Yang L, Eshraghi J, Fassihi R (1999) A new intragastric delivery system for the treatment of *Helicobacter pylori* associated gastric ulcer: in vitro evaluation. J Control Release 57:215–222

Zhou J, Wu J, Hafdi N, Behr J-P, Erbacher P, Peng L (2006) PAMAM dendrimers for efficient siRNA delivery and potent gene silencing. Chem Commun 14:2362–2364

Mucoadhesive Drug Delivery Systems

Juliane Hombach and Andreas Bernkop-Schnürch

Contents

Abstract The uptake of drugs is often limited by the short contact time between the formulation and the absorption membrane and by a fast washout. Using mucoadhesive polymers, however, the residence time of the dosage form on the mucosa can be significantly increased.

In this chapter the composition of the mucus, the different mucoadhesion theories and binding types between mucus and mucoadhesives, mucoadhesion

A. Bernkop-Schnürch (✉)
Institute of Pharmacy, University of Innsbruck, Innrain 52, 6020 Innsbruck, Austria
e-mail: Andreas.Bernkop@uibk.ac.at

M. Schäfer-Korting (ed.), *Drug Delivery*,
Handbook of Experimental Pharmacology 197,
DOI 10.1007/978-3-642-00477-3_9,

tests and factors influencing mucoadhesion are introduced. Various mucoadhesive polymers are also described and an overview of various mucoadhesive delivery systems is provided.

Keywords Mucus · Mucoadhesion · Mucoadhesion tests · Mucoadhesive polymers · Mucoadhesive delivery systems

1 Introduction

Drug absorption is often limited by the residence time of the drug at the site of absorption. In ocular delivery, for example, a drug solution is cleared by the lacrimal fluid within a few minutes after application. Therefore mucoadhesion is an important strategy in order to prolong the mucosal residence time of drug delivery systems. Both systemic and local delivery can be optimized with mucoadhesive dosage forms by retaining in intimate contact with the absorption site or the site of action, which results in high local drug concentrations and a high flux through the absorbing tissue. Moreover the mucoadhesive material itself can also be a therapeutic agent for tissue protection (e.g., gastric ulcers) or lubrication (in the eye or vagina).

2 Mucoadhesion

In pharmaceutical sciences mucoadhesion is defined as the state in which a material and mucus or a mucous membrane are held together for elongated times by interfacial forces (Gu et al. 1988).

2.1 Mucus

In several body cavities the single-layered (e.g., stomach, intestine, bronchi) or multi-layered (e.g., vagina and cornea) epithelia are moistured by the presence of a mucus gel layer. Single-layered epithelia contain goblet cells which secrete mucus onto the epithelial surface whereas multi-layered epithelia contain specialized glands such as salivary glands that secrete mucus onto the epithelial surface. The mucus gel consists of mucin glycoproteins, lipids, inorganic salts and up to 83% water. The mucin glycoproteins are the most important component for the structure, as their cysteine-rich subdomains can form intra- and/or intermolecular disulfide bonds. Because of the presence of sialic acid and sulfate, the mucus is negatively charged. The mucus layer is continuously released but also continuously eroded by

enzymatic and mechanical challenges on the luminal surface. The thickness of this mucus layer differs widely between the various mucosal surfaces from less than 1 μm in the oral cavity (Sonju et al. 1974) to as much as 450 μm in the stomach (Allen et al. 1990).

The mucus has different functions which are more or less developed at the different body sites. First of all it is a diffusion barrier for xenobiotics, especially for drugs. It also protects the mucosa in the stomach from the hydrochloric acid of the lumen. Finally, as with all the organs, the mucus keeps the mucosal membrane moist.

2.2 Mucoadhesion Theories and Binding Types

So far, many attempts have been undertaken to explain the phenomenon of mucoadhesion. As many parameters have an impact on mucoadhesion as described in detail below, various theories of adhesion were suggested but no generally accepted theory was proposed. However, two basic steps are generally accepted. In step I, the contact stage, an intimate contact between the mucoadhesive and the mucus gel layer is formed. In step II, the consolidation stage, the adhesive joint is strengthened and consolidated, providing a prolonged adhesion (Wu 1982).

2.2.1 Chemical Bonds

Bonds between the mucus and mucoadhesive molecules can be formed in different ways (arranged with decreasing strength below):

1. Covalent bonds like disulfide bonds between thiomers and cysteine-rich sub-domains of the mucus layer
2. Ionic bonds such as the interaction between cationic polymers and sialic acid moieties of the mucus
3. Hydrogen bonds occuring in presence of hydrophilic functional groups (e.g., hydroxylic, carboxylic or amino groups)
4. van der Waals bonds between dipoles
5. Hydrophobic bonds based on non-polar groups in an aqueous solution.

2.2.2 Theories of Adhesion

Several theories describe the fundamental mechanisms of adhesion (Ahuja et al. 1997 and Smart 2005).

The *electronic theory* proposes the formation of an electrical double layer at the interface due to electron transfer upon contact of adhering surfaces with differences in their electronical structure.

The *adsorption theory* suggests the attachment of adhesives due to covalent bonds and/or hydrogen bonds and van der Waals forces.

The *wetting theory* is primarily used for liquid systems. It regards the ability of a liquid to spread over a mucosal surface and calculates the contact angle and the energy to separate the two phases.

According to the *diffusion or interpenetration theory* mucoadhesive agents interpenetrate to sufficient depth with mucus glycoproteins which leads to a strong semi-permanent adhesive bond. The depth to which the polymer chains penetrate depends on the diffusion coefficient and the time of contact. The chain flexibility is a crucial parameter favoring a polymeric interpenetration.

In the *fracture theory* the forces required for the detachment of the two involved surfaces after adhesion are described. Normally, however, detachment does not occur at the interface but typically at the weakest point which is the cohesiveness of one of the compounds.

The *mechanical theory* suggests an interlocking of a liquid adhesive into irregularities on a rough surface.

The *mucus dehydration theory* assumes that a dehydration of a mucus gel layer increases its cohesive property and promotes the retention of an adhesive system. It is also possible that glycoproteins of the mucus are carried with the water into the mucoadhesive polymer which leads to interpenetration.

Physical *entanglements* of polymer chains can also explain the mucoadhesive and cohesive qualities.

2.3 Mucoadhesion Tests

To determine the adhesive properties various in vitro methods such as visual, tensile and rheological tests are well-established methods which are described in detail below. Additionally spectroscopic methods can prove chain interpenetration or formation of hydrogen bonds by ^{13}C nuclear magnetic resonance spectroscopy (^{13}C-NMR) (Kerr et al. 1990) or attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy (Jabbari et al. 1993). A novel method called BIACORE® system is based on a chip on which the tested polymer is immobilized and through which mucin particles flow while mucoadhesion is determined based on an optical phenomenon called surface plasmon response (SPR) (Takeuchi et al. 2005).

2.3.1 Visual Tests

The *rotating cylinder* (Fig. 1a) seems to be an appropriate method to evaluate both the duration of binding to the mucosa and the cohesiveness of mucoadhesive polymers. In detail, tablets containing the test polymer are attached to freshly excised mucosa (e.g., porcine intestinal, buccal or nasal mucosa, bovine vaginal

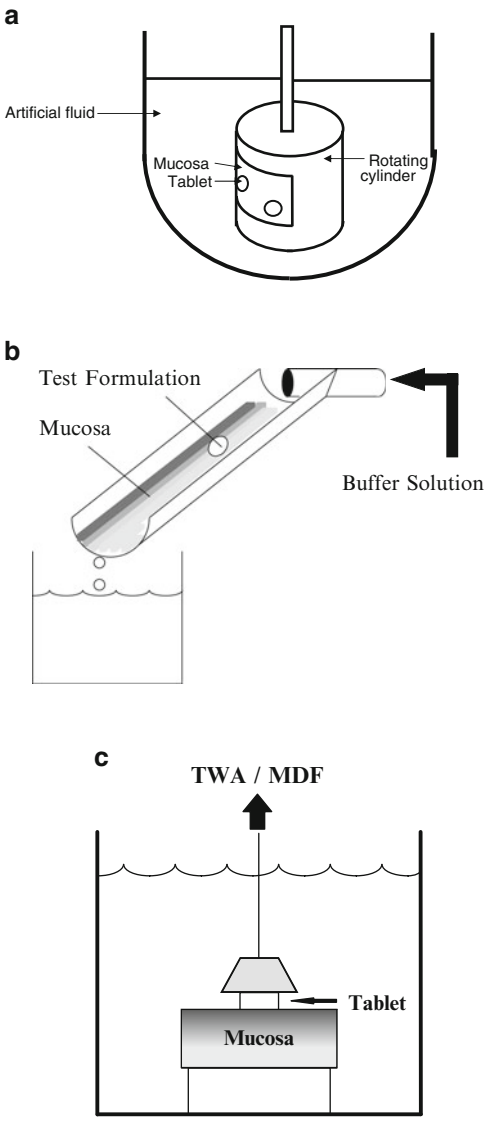


Fig. 1 Set-ups for mucoadhesion tests. (**a**) Rotating cylinder. (**b**) Rinsed channel. (**c**) Tensile studies (MDF, maximum detachment force; TWA, total work of adhesion)

mucosa), which has been spanned on a stainless steel cylinder (apparatus 4-cylinder, USP). Afterwards the cylinder is placed in a dissolution apparatus according to the USP containing an artificial fluid appropriate to the mucosa used at 37°C agitated with 125 rpm. The detachment, disintegration and/or erosion time of test tablets is determined visually.

In the *rinsed channel* method (Fig. 1b) freshly excised mucosa is spread out on a lop-sided channel with the mucus gel layer facing upwards and placed in a

thermostatic chamber at 37°C. After applying the test material on the mucosa, it is flushed with an appropriate artificial fluid at a constant flow rate and the residence time of the mucoadhesive polymer is determined visually or fluorimetrically for fluorescence marked material (Rango Rao and Buri 1989).

2.3.2 Tensile Tests

Tensile studies (Fig. 1c) are one of the most established and used (e.g., Grabovac et al. 2005; Mortazawi and Smart, 1993) in vitro systems for testing mucoadhesion. Flat-faced discs of the test polymer are attached to freshly excised mucosa. After a certain contact time between test disc and mucosa, the mucosa is pulled at a certain rate (mm s^{-1}) from the disc. The total work of adhesion (TWA) representing the area under the force/distance curve and the maximum detachment force (MDF) are determined. Tensile studies can also be carried out by hydrated polymers to minimize the effect of adhesion by hydration (Ch'ng et al. 1985).

2.3.3 Rheological Tests

Due to the chain interpenetration of mucoadhesives with mucin macromolecules, physical entanglements, conformational changes and chemical interactions appear and consequently the rheological behavior changes. An evaluation of the resulting synergistic increase in viscosity can be achieved by mixing the mucoadhesives with mucus and measuring viscosity (Hassan and Gallo 1990) either by classical rotational viscometry at a certain shear rate or by dynamic oscillatory measurements, providing more information about the structure of the polymer−mucin network.

2.3.4 In Vivo Methods

There are few in vivo methods for evaluation of mucoadhesion. Generally the time of adhesion of dosage forms in the body is determined by visual observation or by γ scintigraphy.

Mucoadhesive substances are administered orally and the extent of remaining particles can be evaluated visually after a certain time. The technique can be used to evaluate mucoadhesion on various tissues in animal studies, but is limited to the oral cavity in humans.

In contrast, no tissue limitations seem to exist for γ scintigraphic methods. The most frequently used radionuclides for imaging studies include ^{99m}Tc, ^{111m}In, ^{113m}In and ^{81m}Kr and were already used to study the gastric emptying (Khosla and Davis 1987) and gastrointestinal (GI) transit (Harris et al. 1990) time in humans.

Arbos et al. (2002) describe another method in which fluorescence-labeled nanoparticles are applied to rats, which are sacrificed at certain time points.

The GI tract is removed, divided into various regions and the fluorescence marker is extracted from the different gut regions and quantified by spectrofluorimetry.

2.4 Factors Influencing Mucoadhesion

Many parameters have an impact on mucoadhesion. Both the nature of the polymer and the nature of the environment including the mucus physiology have an influence on the extent of mucoadhesion.

2.4.1 Polymeric Factors

The optimum molecular mass for maximal mucoadhesion is dependent on the type of polymer. In general, low-molecular mass polymers can interpenetrate more easily, whereas entanglements are favored for high-molecular mass polymers.

Furthermore, the higher the polymer concentration, the stronger the mucoadhesion for solid dosage forms (Duchene et al. 1988).

Flexibility of the polymer chains is an important feature for interpenetration and entanglement. Cross-linked polymers show less mobility of the chains, and the chain length which penetrates effectively into the mucus is reduced which decreases mucoadhesion.

Also the three-dimensional structure of the polymer is relevant. Dextrans with a helical conformation are able to shield adhesively-active groups and therefore a much higher molecular mass is needed for the same adhesive strength as a linear polymer.

2.4.2 Environmental Factors

pH influences both the charge of the polymer and the charge of the mucosal surface because of the different dissociation of the functional groups of the polymeric and amino acid backbone, respectively. The degree of hydration of a polymer is dependent on its chemical structure and the pH as described in more detail below.

Higher pressure applied initially for contact, which can increase the depth of interpenetration, and longer initial contact time between the delivery system and the mucus, increase the mucoadhesive strength.

Another important characteristic are the swelling properties being typical for each polymer but also related to the concentration and the presence of water. On the one hand, swelling polymer chains disentangle and simplify interpenetration, but on the other hand, excessive swelling leads to a decrease in the cohesive properties of the polymer.

2.4.3 Mucus Physiology

The natural mucin turnover limits the residence time of the mucoadhesives on the mucus layer. The mucin turnover varies at different mucosal surfaces and in different individuals. The mucus turnover in humans has been estimated to be in the range of 12–24 h (Forstner 1978; Allen et al. 1998). Attention should also be paid to mucus viscosity and thickness. A thicker mucus layer provides more available groups for interactions and a deeper layer for entanglements with polymer chains; however, high mucus viscosity makes entanglements more difficult. Furthermore, diseases like gastric ulcers, bacterial or fungal infections or inflammation can change the mucosal physicochemical properties.

3 Mucoadhesive Polymers and Derivatives

Mucoadhesive polymers can be classified according to their origin (e.g., natural – synthetic), the type of mucosa on which they are mainly applied (e.g., ocular – buccal), their chemical structure (e.g., cellulose derivatives – polyacrylates) or their mechanism of binding (e.g., covalent – non-covalent). They can also be divided according to their surface charge into anionic, cationic, non-ionic and amphiphilic polymers, which is important for the mechanism of adhesion.

Important representatives of the different groups of polymers are listed in Table 1.

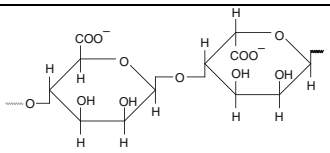
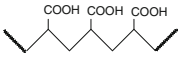
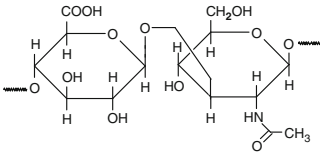
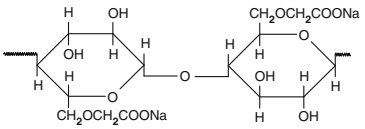
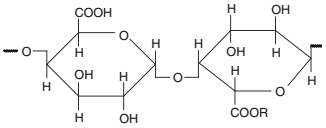
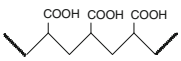
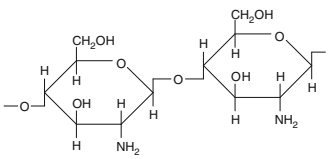
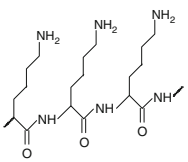
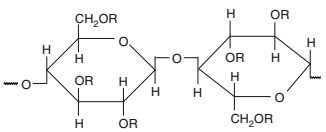
3.1 Anionic Polymers

Mainly carbonic acid groups, and to a small extent, sulfate and sulfonate moieties, are responsible for the adhesion of anionic polymers to the mucus gel layer. Carboxyl groups are able to form hydrogen bonds with hydroxyl groups of the oligosaccharide side chains on mucus proteins. A disadvantage of anionic mucoadhesive polymers, however, is their incompatibility with multivalent cations like Ca^{2+}, Mg^{2+} and Fe^{3+}. In the presence of such cations, these polymers precipitate and/or coagulate (Valenta et al. 1998) leading to a strong reduction in their adhesive properties. Furthermore, the swelling of anionic polymers is pH dependent. The higher the pH, the higher the swelling, which can cause a decrease in cohesive properties of the polymer at higher pH values so the drug delivery system is not mucoadhesive anymore.

3.2 Cationic Polymers

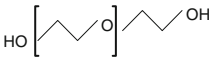
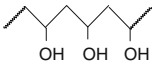
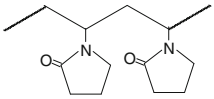
The strong mucoadhesion of cationic polymers is based on ionic interactions between these polymers and anionic substructures such as sialic acid moieties of

Table 1 Mucoadhesive polymers

Polymer	Chemical Structure	Notes
Alginate		Anionic
Carbomer		Anionic, cross-linked with sucrose
Hyaluronic acid		Anionic
Carboxymethylcellulose sodium (NaCMC)		Anionic, 0.3–1.0 carboxymethyl groups per glucose unit
Pectins		Anionic, R = OH or methyl
Polycarbophil		Anionic, cross-linked with divinylglycol
Chitosan		Cationic, primary amino groups can be acetylated to some extent
Polylysine		Cationic
Hydroxypropyl-cellulose		Non-ionic, R = H or hydroxypropyl
Hydroxypropyl-methylcellulose		Non-ionic, R = H or methoxy or hydroxypropyl

(*continued*)

Table 1 (continued)

Polymer	Chemical Structure	Notes
Poly(ethylene oxide)		Non-ionic
Poly(vinyl alcohol)		Non-ionic
Poly(vinyl pyrrolidone)		Non-ionic

the mucus gel layer. Chitosan, for instance, is the most important representative of this group, because it also displays permeation enhancing properties (Artursson et al. 1994; Lueßen et al. 1997) and large amounts are available for a cheap price. In contrast to anionic polymers their swelling is improved at lower pH values.

3.3 Non-Ionic Polymers

In general, non-ionic polymers are less adhesive than both anionic and cationic polymers. Their adhesion is caused by the interpenetration of the polymer and afterwards the entanglement of the polymer chains. Mucoadhesion of non-ionic polymers, however, is neither pH dependent nor influenced by electrolytes.

3.4 Amphiphilic Polymers

Amphiphilic polymers display both anionic and cationic substructures. Therefore mucoadhesion is caused by the formation of hydrogen bonds of carboxylic acid moieties and by ionic interactions with negatively-charged mucosal surfaces. Yet the combination of both properties leads to reduced mucoadhesive properties as compared to the single-charged polymers (Lueßen et al. 1996). On the other hand the cohesiveness of a delivery system can be strongly improved as cationic and anionic moieties within the polymer are stabilized by ionic interactions.

3.5 Polymer Derivatives

A new generation of mucoadhesive polymers are thiolated polymers or so-called thiomers representing polymers to which thiol-bearing side chains are attached

(Bernkop-Schnürch et al. 1999). These novel polymers are supposed to form covalent disulfide bonds with cysteine-rich subdomains of mucus glycoproteins based on thiol/disulfide exchange reactions and/or an oxidation process. Due to the covalent attachment to the mucus the mucoadhesive properties are strongly improved whereas the polymer still also exhibits cohesive properties.

4 Drug Delivery Systems

4.1 Nasal

The nose is not only a region for local drug delivery but also for systemic drug delivery. Advantages of nasal drug delivery are the large surface area of the nose and the thin, porous, highly vascularized nasal epithelium which ensures high absorption and rapid transport of the absorbed substances directly into the systemic blood circulation avoiding drug metabolism in the liver. If drugs are absorbed in the olfactory region they will be absorbed directly into the central nervous system bypassing the tight blood−brain barrier. Furthermore the enzymatic activity of the epithelium is lower than the one of the GI tract and therefore higher bioavailabilities of active pharmaceutical ingredients (API) such as peptides and proteins can be achieved. Nasal delivery, however, also has certain limitations. Only a restricted amount can be administered intranasally as large volumes will disturb the normal function of the nose. Besides hydrophilic drugs, large molecules are not absorbed sufficiently. Moreover a daily production of approximately 1.5–2 L of nasal mucus and cilia beat with a frequency of 20 Hz result in a fast mucociliary clearance of 6 mm min^{-1} (Proctor 1977). A mucoadhesive formulation is required to stay localized in the nasal cavity for an extended time period and increase absorption which otherwise would not occur. This has already been tested with various polymers and APIs such as antibiotics and proteins by several research groups (Ugwoke et al. 2005). Some mucoadhesive polymers such as chitosan and polyacrylic acids also increase epithelial permeability and show enzyme inhibitory activities (Dyer et al. 2002; Lueßen et al. 1994).

4.2 Buccal

Buccal drug delivery can have two different therapeutic aims: either local therapy of the oral mucosa (e.g., antimycotics, antiviral agents, local anesthetics or corticosteroids) or systemic therapy (e.g., proteins, peptides or oligonucleotides). The buccal mucosa has a number of advantages in comparison with other routes of drug administration. It has a rich blood supply that flows directly into the jugular vein and thereby is sparing the drug from first-pass metabolism of the liver and degradation in the GI tract by enzymes (Park and Robinson 1985).

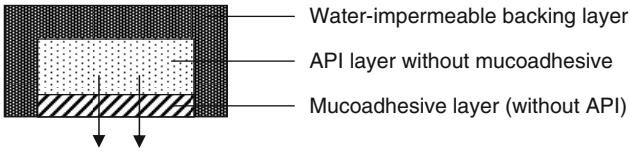


Fig. 2 Example of a patch system for buccal drug delivery

An alternative to conventional dosage forms such as oral gels, oral liquids or lozenges are patch systems. For successful buccal patch delivery systems there is a need for a bioadhesive to retain the drug in the oral cavity and prolong the contact with the mucosa, a vehicle to release the APIs appropriately under the conditions in the mouth, and a strategy to overcome the low permeability of the buccal mucosa. Additionally it is often coated by a water impermeable backing layer to prevent drug release and drug loss in the saliva (when required) and for patient convenience. A schematic example for a patch system is shown in Fig. 2.

4.3 Vaginal

Besides locally acting drugs such as antifungal, antibacterial, antiviral and anti-inflammatory agents, estrogens and spermicides, the vagina also provides a promising site for systemic drug delivery because of its rich blood supply and the large surface area (Vermani and Garg 2000). Besides avoiding the hepatic first-pass metabolism and a reduction in GI and hepatic side-effects, a good permeability to a wide range of compounds including peptides and proteins has been shown (Muranishi et al. 1993) and makes the vaginal route an alternative to the parenteral route for drugs such as bromocriptine, oxytocin, calcitonin, human growth hormone and steroids used for replacement therapy or contraception. However, despite all these advantages, the vaginal route for systemic drug delivery is gender specific, and the epithelial thickness, the vaginal fluid and the cervical mucus (volume, viscosity, pH) vary a lot as they are dependant on age, hormones and the menstrual cycle. More limitations of currently available vaginal delivery systems are leakage and a low residence time which cause poor patient compliance. Robinson and Bologna (1994) overcame this with a mucoadhesive gel based on polycarbophil, that is reported to remain in the vaginal cavity for 3–4 days and is a vehicle for delivery of progesterone or nonoxynol-9, for example.

4.4 Ocular

The ocular bioavailability of conventional ophthalmic formulations such as aqueous solutions and ointments is normally in the range of 2%–10%. The major

problem of ocular drug delivery is the small area for penetration, the presence of the lipophilic corneal epithelium as an absorption barrier, and the short contact time. The contact time is reduced by the drainage of instilled solutions, lacrimation, tear turnover, tear evaporation, biotransformation and protein binding of some APIs (Lee and Robinson 1986). From a 50 µl drop applied to the eye approximately 20–30 µl is lost from overflow and another 2 µl per blink is lost continuously (Maurice and Mishima 1984). Therefore only a low amount of API is available for absorption for only a few seconds. Goblet cells on the conjunctival surface secrete mucin which is spread over the surface of the eye by blinking. Mucoadhesive polymers, as drug carrier systems, can interact with the mucus and prolong the residence time of the medication and thus increase their bioavailability. Mengi and Deshpande (1992), for example, compared poly(acrylic acid) (PAA) (Carbopol 940) and poloxamer hydrogels with eye drops as a delivery system for flurbiprofen in rabbits. However, both gel formulations showed a sustained action and the superior results of the PAA vehicles were assumed by the authors to be due to mucoadhesion. Recently Mansour et al. (2008) developed poloxamer-based in situ gelling formulations of ciprofloxacin hydrochloride which showed controlled release, mucoadhesive properties due to the addition of hydroxypropylmethyl cellulose (HPMC) or hydroxyethyl cellulose (HEC) and improved ocular bioavailability compared with conventional marketed eye drops.

4.5 Oral

APIs and their delivery systems show a high variation in the length of stay in the GI tract due to state-of-feeding and intestinal motility. Intimate contact of a delivery system with the mucosa increases the residence time and therefore higher local drug concentrations improve topical therapy and enhance absorption.

The first segment of the GI tract is the esophagus which lacks surface mucus. Consequently, adhesion appears directly on the epithelium. Bioadhesion to the esophagus occurs commonly when dosage forms are taken in a supine position or without or only with a small amount of water. For esophageal cancer, fungal infections, motility disfunction or gastroesophageal disease, for example, an application of a bioadhesive formulation for local drug delivery can increase the contact time with the epithelium. Alginate demonstrates a high retention time on porcine esophageal tissue, being a potential drug delivery vehicle and providing a barrier to protect the underlaying epithelium from gastric reflux (Batchelor et al. 2004).

Several gastric mucoadhesive systems such as tablets, granules, pellets and particles demonstrated increased retention time in the stomach of rats and dogs (Preda and Leucuta 2003; Hosny and Al-Meshal 1994).

The small intestine is the site of absorption for many drugs. However, high motility activity, relatively short transit time, mucus turnover, and degradation by enzymes all limit drug absorption. Mucoadhesive polymers can lead to a closer and longer contact with the absorbing membrane and therefore increase the absorption.

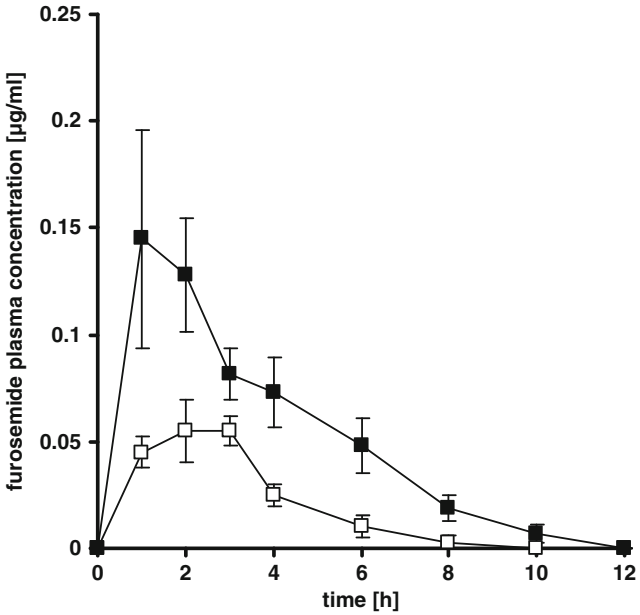


Fig. 3 Plasma levels of furosemide after oral administration of 10 mg as non-adhesive (*open square*) or adhesive (*filled square*) microspheres to fasted volunteers (mean ± S.D., n = 10). Adapted from Akiyama et al. (1998)

When adhesive and non-adhesive microspheres containing furosemide were administered to humans, higher furosemide plasma concentrations and an increased absorption were found with adhesive microspheres compared to non-adhesive microspheres (Fig. 3) (Akiyama et al. 1998).

Colonic mucoadhesion may be more successful than gastric or small intestinal due to a thicker mucus layer, lower disruptive colonic motility and a lower mucus turnover rate. Varum et al. (2008) provide an overview about various colon mucoadhesion studies in animals.

5 Conclusion

The advantages of mucoadhesive drug delivery systems are enormous. Mucoadhesive polymers can prolong the residence time and intimate the contact of the dosage form on the mucosa. The development of multifunctional polymers which also exhibit enzyme-inhibiting, permeation-enhancing and controlled release properties make them interesting for both local and systemic delivery. Although there are already numerous formulations based on mucoadhesive polymers on the market, the number of delivery systems making use of these advantages will certainly increase in future.

References

Ahuja A, Khar RK, Ali J (1997) Mucoadhesive drug delivery systems. Drug Dev Ind Pharm 23:489–515

Akiyama Y, Nagahara N, Nara E, Kitano M, Iwasa S, Yamamoto I, Azuma J, Ogawa Y (1998) Evaluation of oral mucoadhesive microspheres in man on the basis of the pharmacokinetics of furosemide and riboflavin, compounds with limited gastrointestinal absorption sites. J Pharm Pharmacol 50:159–166

Allen A, Cunliffe WJ, Pearson JP, Venables CW (1990) The adherent gastric mucus gel barrier in man and changes in peptic ulceration. J Intern Med 228:83–90

Allen A, Hutton DA, Pearson JP, Sellers LA (1998) Mucus and mucosa. In M O'Connor (Ed.) Ciba Foundation Symposium 1984, Vol. 109, Wiley, New York

Arbos P, Arangoa MA, Campanero MA, Irache JM (2002) Quantification of the bioadhesive properties of protein-coated PVM/MA nanoparticles. Int J Pharm 242:129–136

Artursson P, Lindmark T, Davis SS, Illum L (1994) Effect of chitosan on the permeability of monolayers of intestinal epithelial cells (Caco-2). Pharm Res 11:1358–1361

Batchelor HK, Tang M, Dettmar PW, Hampson FC, Jolliffeb IG, Craig DQM (2004) Feasibility of a bioadhesive drug delivery system targeted to oesophageal tissue. Eur J Pharm Biopharm 57:295–298

Bernkop-Schnürch A, Schwarz V, Steininger S (1999) Polymers with thiol groups: a new generation of mucoadhesive polymers? Pharm Res 16:876–881

Ch'ng HS, Park H, Kelly P, Robinson JR (1985) Bioadhesive polymers as platforms for oral controlled drug delivery II: Synthesis and evaluation of some swelling, water-insoluble bioadhesive polymers. J Pharm Sci 74:399–405

Duchene D, Touchard F, Peppas NA (1988) Pharmaceutical and medical aspects of bioadhesive systems for drug administration. Drug Dev Ind Pharm 14:283–318

Dyer AM, Hinchcliffe M, Watts P, Castile J, Jabbal-Gill I, Nankervis R, Smith A, Illum L (2002) Nasal delivery of insulin using novel chitosan based formulations: a comparative study in two animal models between simple chitosan formulations and chitosan nanoparticles. Pharm Res 19:998–1008

Forstner JF (1978) Intestinal mucins in health and disease. Digestion 17:234–263

Grabovac V, Guggi D, Bernkop-Schnürch A (2005) Comparison of the mucoadhesive properties of various polymers. Adv Drug Deliv Rev 57:1713–1723

Gu JM, Robinson JR, Leung SHS (1988) Binding of acrylic polymers to mucin/epithelial surfaces: structure property relationship. Crit Rev Ther Drug Carr Syst 5:21–67

Harris D, Fell JT, Sharma HL, Taylor DC (1990) GI transit of potential bioadhesive formulations in man: a scintigraphic study. J Control Release 12:45–53

Hassan EE, Gallo JM (1990) A simple rheological method for the in vitro assessment of mucin-polymer bioadhesive bond strength. Pharm Res 7:491–495

Hosny EA, Al-Meshal MA (1994) In-vivo evaluation of a bioadhesive containing indomethacin tablets. Drug Dev Ind Pharm 20:2715–2720

Jabbari E, Wisniewski N, Peppas NA (1993) Evidence of mucoadhesion by chain interpenetration at a poly(acrylic acid)/mucin interface using ATR-FTIR spectroscopy. J Control Release 26:99–108

Kerr LJ, Kellaway IW, Rowlands C, Parr GD (1990) The influence of poly(acrylic) acids on the rheology of glycoprotein gels. Proc Int Symp Contr Rel Bioact Mat 17:122–123

Khosla L, Davis SS (1987) The effect of polycarbophil on the gastric emptying of pellets. J Pharm Pharmacol 39:47–49

Lee VHL, Robinson JR (1986) Topical ocular drug delivery: recent developments and future challenges. J Ocular Pharmacol 2:67–108

Lueßen HL, de Leeuw BJ, Langemeyer MW, de Boer AG, Verhoef JC, Junginger HE (1996) Mucoadhesive polymers in peroral peptide drug delivery VI. Carbomer and chitosan improve the intestinal absorption of the peptide drug buserelin in vivo. Pharm Res 13:1668–1672

Lueßen HL, Lehr CM, Rentel CO, Noach ABJ, de Boer AG, Verhoef JC, Junginger HE (1994) Bioadhesive polymers for the peroral delivery of peptide drugs. J Control Release 29:329–338

Lueßen HL, Rentel CO, Kotzé AF, Lehr CM, de Boer AG, Verhoef JC, Junginger HE (1997) Mucoadhesive polymers in peroral peptide delivery IV. Polycarbophil and chitosan are potent enhancers of peptide transport across intestinal mucosae in vitro. J Control Release 45:15–23

Mansour M, Mansour S, Mortada ND, Abd Elhady SS (2008) Ocular poloxamer-based ciprofloxacin hydrochloride in situ forming gels. Drug Dev Ind Pharm 34:744–752

Maurice DM, Mishima S (1984) Ocular pharmacokinetics. In: Sears MC (ed) Pharmacology of the eye. Springer, Berlin, pp 19–116

Mengi S, Deshpande SG (1992) Development and evaluation of flurbiprofen hydrogels on the breakdown of the blood/aqueous humor barrier. STP Pharma 2:118–124

Mortazawi SA, Smart JD (1993) An in-vitro evaluation of mucosa-adhesion using tensile and shear stresses. J Pharm Pharmacol 45(suppl.):1108

Muranishi S, Yamamoto A, Okada H (1993) Rectal and vaginal absorption of peptides and proteins. Pharm Biotech 4:199–227

Park H, Robinson JR (1985) Physico-chemical properties of water insoluble polymers important to mucin/epithelial adhesion. J Control Release 2:47–57

Preda M, Leucuta SE (2003) Oxprenolol-loaded bioadhesive microspheres: preparation and in vitro/in vivo characterization. J Microencapsul 20:777–789

Proctor DF (1977) The upper airways: I. Nasal physiology and defence of the lungs. Am Rev Respir Disord 115:97–129

Rango Rao KV, Buri P (1989) A novel in situ method to test polymers and coated microparticles for bioadhesion. Int J Pharm 52:265–270

Robinson JR, Bologna WJ (1994) Vaginal and reproductive system treatment using a bioadhesive polymer. J Control Release 28:87–94

Smart SD (2005) The basics and underlying mechanisms of mucoadhesion. Adv Drug Deliv Rev 57:1556–1568

Sonju T, Cristensen TB, Kornstad L, Rolla G (1974) Electron microscopy, carbohydrate analysis and biological activities of the proteins absorbed in two hours to tooth surfaces in vivo. Caries Res 8:113–122

Takeuchi H, Thongborisute J, Matsui Y, Sugihara H, Yamamoto H, Kawashima Y (2005) Novel mucoadhesion tests for polymers and polymer-coated particles to design optimal mucoadhesive drug delivery systems. Adv Drug Deliv Rev 57:1583–1594

Ugwoke MI, Agu RU, Verbeke N, Kinget R (2005) Nasal mucoadhesive drug delivery: background, applications, trends and future perspectives. Adv Drug Deliv Rev 57:1640–1665

Valenta C, Christen B, Bernkop-Schnürch A (1998) Chitosan-EDTA Conjugate: a novel polymer for topical used gels. J Pharm Pharmacol 50:445–452

Varum FJO, McConnell EL, Sousa JJS, Veiga F, Basit AW (2008) Mucoadhesion and the gastrointestinal tract. Crit Rev Ther Drug Carrier Syst 25:207–258

Vermani K, Garg S (2000) The scope and potential of vaginal drug delivery. Pharm Sci Technol Today 3:359–364

Wu S (1982) Formation of adhesive bond. Polymer interface and adhesion. Marcel Dekker Inc, New York, pp 359–447

Intrauterine Drug Delivery for Contraception and Gynaecological Treatment: Novel Approaches

Dirk Wildemeersch

Contents

D. Wildemeersch
Contrel Research Technology Park, University of Gent, 9052 Gent (Zwijnaarde), Belgium
e-mail: d.wildemeersch@skynet.be

M. Schäfer-Korting (ed.), *Drug Delivery*,
Handbook of Experimental Pharmacology 197,
DOI 10.1007/978-3-642-00477-3_10,

Abstract This chapter describes the development of new intrauterine drug delivery products, which are designed to provide improved methods for the prevention and treatment of gynaecological conditions, improvements to birth control methods, and higher levels of safety, user acceptability, compliance, and quality of life for women. The development of frameless intrauterine systems is such an attempt to improve on the performance and acceptability of established intrauterine contraception, potentially solving major problems encountered with conventional IUDs (e.g., expulsion, abnormal or excessive bleeding, and pain). However, the performance of frameless devices depends on proper anchoring of the device, which requires specific technical skills not required for the insertion of conventional IUDs. Moreover, current research paves the way for new developments. The frameless copper and LNG-releasing IUDs/IUSs and framed LNG-IUS are the beginning of a series of innovative developments in this field. New compounds, such as progesterone antagonists and selective progesterone receptor modulators (SPRMs), could be incorporated into polymeric drug delivery platforms for use in the uterus, cervix, or vagina, or subdermally. It is likely that current and new hormone-releasing intrauterine systems could also be useful for bleed-free contraception in HIV-positive (HIV^+ women). It is hoped that this work will contribute to the increase in worldwide use of intrauterine contraception and to non-surgical treatment of frequently occurring women's health problems.

Keywords Intrauterine contraception · Frameless copper IUD · Frameless LNG-IUS · Framed LNG-IUS · GyneFix · FibroPlant · Femilis · Intrauterine treatment

1 Introduction

On July 29, 2005, the International Agency for Cancer Research (IACR) Working Group of the World Health Organization (WHO) concluded that combined estrogen–progestogen oral contraceptives (OCs) and combined estrogen–progestogen menopausal therapy are carcinogenic to humans (Cogliano et al. 2005a, b). This widely publicized statement is based on long-term epidemiologic research, the results of which show a small increase in the risk of breast cancer in current and recent OC users. The research also showed that 10 years after cessation of OC use, the risk appears to be similar to that in never-users. The risks of cervical cancer and hepatocellular carcinoma also increase with duration of use of combined OCs. It is

of enormous public health importance as, worldwide 100 million women – about 10% of all women of reproductive age – currently use combined hormonal contraceptives. On the other hand, however, OCs have many non-contraceptive benefits including decrease in the risk of ovarian, endometrial and colo-rectal cancers. The Working Group concluded that both beneficial and unwanted adverse effects other than cancer have been established for combined hormone therapy but that a rigorous risk/benefit analysis would be of use to put the different effects in perspective, and assess the overall consequences for public health.

Another drawback of OCs, as well as of coitus-related methods, is poor compliance. The efficacy of oral contraceptive pills and barrier methods depends on their correct and consistent use. Imperfect use of contraceptives still results in too many unintended pregnancies. It is estimated that at least 30% of pregnancies are unplanned (National Institute of Clinical Excellence (NICE) 2005). The typical use-related failure rate of the Pill is 5% during the first year of use (Trussell 1998). By contrast, the efficacy of long-acting, reversible contraceptive methods, such as intrauterine devices (IUDs) and intrauterine systems (IUSs),[1] does not depend on daily concordance.

Given the above observations, the search for safe, effective, and convenient contraceptives should not diminish and alternative methods that reduce women's risks should be actively promoted. Long-acting methods should also be developed to maximize the effectiveness of contraception.

For contraceptive purposes, several long-acting methods have been shown to be safe and to minimize the risk of unintended pregnancy. These include:

- Copper intrauterine devices
- Progestogen-only IUSs
- Progestogen-only injectable contraceptives
- Progestogen-only subdermal implants
- Estrogen–progestogen combined vaginal rings.

Intrauterine methods are safe alternatives to OCs. With nearly 160 million users worldwide, the IUD is the second most used contraceptive method after sterilization. In countries where the IUD is commonly used, much of its popularity stems from its efficacy, combined with its long duration of action. Besides, a recent meta-analysis found that copper IUD use might be associated with a decreased risk for endometrial cancer (Beining et al. 2008). There are publications which also suggest that copper IUDs may also have a reduced risk of developing invasive cervical cancer (Lassise et al. 1991; Parazzini et al. 1992). Decreased risk with increased duration of copper IUD use supports a possible protective effect of copper IUD use on the development of invasive cervical cancer. This should be interpreted with caution, however.

Intrauterine contraception is also the most cost-effective reversible method of contraception in use today (World Health Organization 2002). New generation,

[1]The term "intrauterine device" is used for copper-releasing intrauterine contraceptive methods whilst the term "intrauterine system" stands for hormonal-releasing intrauterine methods.

miniature copper and hormone-releasing intrauterine methods, such as those described in this chapter, are available for convenient and effective contraception for all women of reproductive age, including young women who do not yet have children, and for women who need contraception while simultaneously requiring treatment for frequently occurring gynaecologic problems, such as excessive menstrual bleeding.

Contrel Research, a research organization located in Gent, Belgium, was established to manage clinical research and to develop and study innovative drug delivery technologies aimed at improving quality of life through the prevention and treatment of gynaecologic conditions and the development of birth control methods with high levels of safety, user acceptability, and compliance. The development of frameless copper IUDs began in 1985. The frameless copper IUD was approved in the European Union in 1995 and is currently marketed in Europe and China. The frameless levonorgestrel-releasing intrauterine system, under development since 1997, and the framed, T-shaped levonorgestrel IUSs, under development since 2002, are currently in the final stages of clinical testing. The first LNG-IUS (Femilis®, see below) was recently approved for marketing in Mexico.

The purpose of this chapter is to provide an overview of the clinical aspects of these devices and systems which prevent fertilisation and implantation.[2] It is concluded that IUDs and IUSs are particularly attractive as they have the advantage of acting predominently locally, avoiding serious adverse events. They have less impact on menstrual pattern after the first few months. New developments in intrauterine technology are providing smaller frameless devices. They may be ideal for use in younger women because they are small, effective and well tolerated. Unlike OCs, they are genuinely "fit-and-forget." In use, they are much more effective than the Pill in this age group. Moreover they are long acting and reversible. So, the reward is substantial. In the current situation of soaring unintended pregnancies, they should be offered more frequently as first line methods, in combination with condoms if required.

2 Frameless Intrauterine Devices and Systems

2.1 Development of Frameless Intrauterine Devices and Systems

Uterine cavities differ considerably in size and shape, and the uterus is subject to changes in size and volume during the menstrual cycle (Hasson 1984; Kurz 1984). These changes are most pronounced at the time of menses. Therefore, it would be unreasonable to expect one standard-sized IUD/IUS to fit uterine cavities that differ in size and volume from woman to woman and from time to time in the same woman (Fig. 1). Clinical experience has shown that incompatibility between the

[2]IUDs and IUSs act mostly by preventing fertilisation.

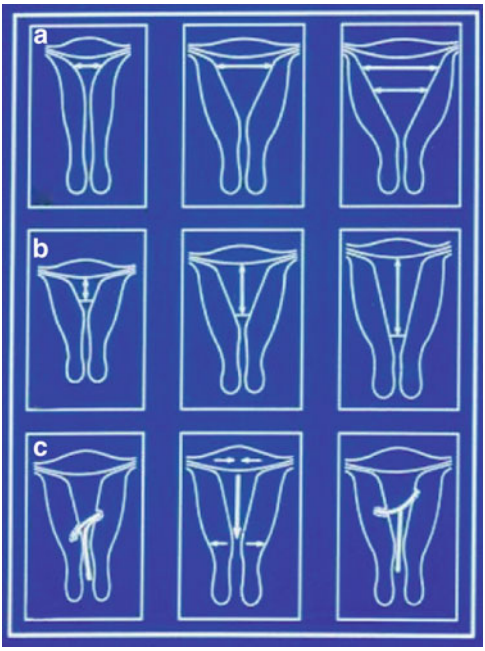


Fig. 1 Different sizes and shapes of uterine cavities. (**a**) Differences in width; (**b**) Differences in length; (**c**) Functional changes and examples of incompatibility

IUD/IUS and the uterine cavity can lead to partial or total expulsion, pain, unintended pregnancy, and abnormal or heavy uterine bleeding leading to removal of the device.

The Lippes Loop, developed in the 1960s, had a high discontinuation rate due to side effects related to its large surface area and size. Thus, it seemed logical that the smaller TCu200, and later the TCu380A or Paragard® (Duramed Pharmaceuticals Inc., USA), would have better acceptability and continuation rates, thanks to their use of copper as a potent anti-fertility agent and T-shaped design causing less distortion of the endometrial cavity (Zipper et al. 1971).

If the width of the uterine cavity is too small, side effects and complications are likely to occur. The crossarms of standard T-shaped IUDs are frequently too long for a large number of uterine cavities, as the average width of most uterine cavities is often smaller than the width of the IUD itself. When the uterine cavity is much longer than the IUD, the device becomes partly or completely lodged in the lower uterine isthmic segment, triggering uterine activity that may promote expulsion and give rise to pain. The most important factor in reducing IUD side effects is the elimination of distortion of the uterine cavity (Howard Tatum, inventor of the T-shaped IUD).

Although incompatibility problems and the effect of the TCu380A IUD on menstrual bleeding are significantly reduced compared to the Lippes Loop, there is still room for improvement, due to the prevalence of bleeding, pain, and expulsion (Xiao 1995). It is for these reasons that frameless copper-releasing GyneFix® IUDs and the frameless FibroPlant® LNG-IUS were developed.

2.2 *Frameless Copper-Releasing Intrauterine Devices*

2.2.1 Description

The frameless standard IUD consists of six copper sleeves or four copper sleeves (small version), each 5 mm long and 2.2 mm in diameter, threaded on a length of polypropylene suture material (Figs. 2 and 3). The sleeves are prevented from sliding off the material by crimping the upper and lower sleeves onto the thread. The proximal end of the thread is provided with a knot, which is inserted and anchored in place in the fundal myometrium using a specially designed inserter (see insertion procedure below). The total, effective surface of copper, including the inner and outer surfaces, is 330 mm^2 for the standard version and 200 mm^2 for the small version. With no plastic body, this implant device is completely flexible.

It should be noted that the frameless IUDs, which consist only of copper sleeves, differ from conventional copper IUDs, which have a copper wire wound around the stem, in terms of their effective copper surface area. Only in the case of sleeves, unattached to a plastic frame, is the nominal and the effective copper surface area the same. When copper wire or copper sleeves fixed to a plastic body are used, that part of the wire or sleeve lying against the plastic body is ineffective and should not be calculated as a part of the effective surface area (Kosonen 1981; Wagner 1999). The effective copper surface area is only 120 mm^2 for the TCu200 IUD and

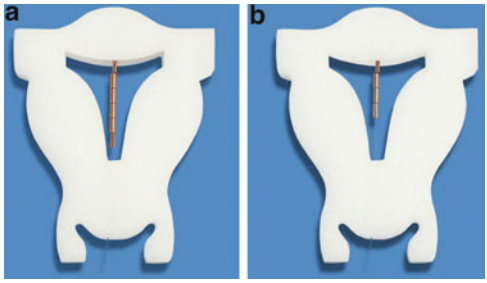


Fig. 2 (**a**) GyneFix® 330 IUD; (**b**) GyneFix® 200 IUD

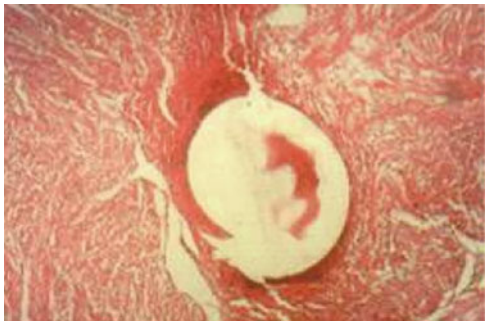


Fig. 3 Histological section at anchor site. In this case there is no or very limited foreign body reaction

252 mm^2 for the TCu380A IUD. The large effective copper surface area of frameless IUD explains its high efficacy (see below).

The safety of the material and the safety of the implant system in terms of the myometrial tissue reaction at the site of the polypropylene anchor was studied in 14 women using a scoring system devised by Sewell (Sewell et al. 1955; Coppens et al. 1989). The interval between the insertion and hysterectomy varied from 1 day to 4 years (Fig. 2). In one-third of the specimens, there was total lack of tissue reaction in the myometrium. In the remaining two-thirds, the reaction was slight to moderate and the diameter of the inflammatory response in the two uteri showing marked reaction did not exceed 1 mm. No tissue reaction was found in two other specimens that were removed 4 years after insertion. No transplanted endometrial tissue could be observed within the adjacent myometrium in any of the cases studied. This study supported both the safety of the material and the safety of the implant system.

2.2.2 Efficacy

The efficacy of the frameless standard IUD has been studied in large-scale, long-term international multicenter randomized and nonrandomized comparative trials in parous and nulliparous women covering 15,000 woman-years of experience. Data from these studies show that the standard IUD is highly effective. Failures ranged from 0.0/100 users to 2.5/100 users (cumulative rates) during the 1–9 years of use.

Efficacy was confirmed in a randomized comparative clinical trial conducted by WHO (1995). The failure rate was slightly lower than that seen with the TCu380A IUD (0.4/100–3.2/100 users) and similar to that seen with the levonorgestrel-releasing IUS (Sturridge and Guillebaud 1996). However, significantly fewer ectopic pregnancies were observed with the frameless IUD than with the TCu380A after 8 years of use (0.1/100 vs. 0.5/100 users, respectively, $p = 0.035$) in a WHO-conducted, randomized, comparative trial (Meirik et al. 2009).

Similar low failure rates were obtained in a multicenter, 3-year study conducted with the small frameless IUD in ~400 women (Cao et al. 2004).

2.2.3 Safety and Side Effects

- **Bleeding**. The most common reason for discontinuing the use of copper IUDs is increased menstrual blood loss (MBL). The magnitude of this increase in MBL is mainly related to the size of the device. With larger types of nonmedicated IUD, such as the Lippes Loop, monthly blood loss is about 70–80 mL, which is approximately double that of normal menses. The amount of excess bleeding is less (50–60 mL) with smaller copper devices, such as the copper T series (Guillebaud et al. 1976).

Clinical trials suggest that MBL with the frameless standard IUD is less than that associated with the TCu380A (Andrade et al. 1987). With the frameless small IUD, studies using the visual assessment technique, using a pictorial chart, have shown no increase in MBL after the first few months. This has been attributed to the very small size of the small frameless version (Wildemeersch and Rowe 2004a). The pictorial chart method does not yield an exact flow in milliliters, but in practice, its sensitivity and specificity are reasonably high and superior to a women's subjective assessment of MBL. For the purpose of evaluating the effect of treatment, the visual assessment technique is highly practical, compared with the quantitative method, because it eliminates the need for women to submit sanitary wear to the laboratory (Janssen et al. 1995).

Figure 4 shows the differences in size among these different IUDs. The surface area of the GyneFix® 200 IUD is one-third of that of the TCu380A and one-sixth of that of the Lippes Loop IUD. The reduced size of the frameless small IUD minimizes the risk of menorrhagia and consequently of anemia. A significant decline in hemoglobin levels already occurs in women with an average MBL of 66 mL over 12 menstrual cycles of IUD use (Guillebaud et al. 1976). It is probable that about 10% of women with IUDs risk secondary anemia, especially those who bleed more than 80 mL per period. It has been suggested that an increased risk of iron deficiency exists, even with a 40-mL blood loss (Jacobs and Butler 1965).

- **Pain**. Because of the small size and flexibility of the frameless IUD, both clinical trials and experience in clinical practice suggest that frameless IUD rarely causes complaints of pain (Wildemeersch 2003; Dou et al. 2001; D'Souza et al. 2003). This is promising for use in nulliparous women with a small uterine cavity for whom standard framed IUDs are generally less suitable. With conventional, framed IUDs, incompatibility between the device and the endometrial cavity causes myometrial distension of the uterus. Depending on the degree of incompatibility, severe cramping pain can lead to abnormal bleeding and partial or complete expulsion of the IUD.

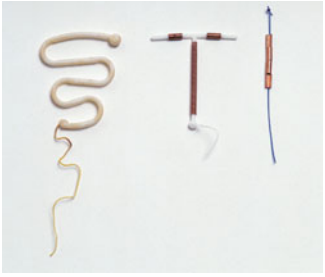


Fig. 4 Three generations of IUDs (from *left* to *right*): Lippes loop (1960), TCu380A (1980), GyneFix 200 (2000)

2.2.4 Insertion, Expulsion and Removal

Since the frameless IUD is a new device, developing proficiency with the insertion procedure may require a number of insertions, depending on the skill of the provider. Doctors, midwives, nurses, or other health care providers may insert the GyneFix®, once they have been properly trained in family planning and have received practical training in IUD insertion in general. Failed insertions are rare if the insertion instructions are followed strictly.

Prior to insertion, a relevant medical history should be obtained to determine conditions that might influence the selection of the IUD as a method of contraception. Physical examination should include a pelvic examination and, if indicated, a "Papanicolaou" smear and appropriate tests for other forms of genital disease. Pregnancy should be ruled out prior to insertion.

IUD providers should be aware that the popularity of the IUD could be much improved if attention is given to pain relief during IUD insertion. If a woman is anxious, the use of local intracervical or local/regional anesthesia should be considered. The use of misoprostol prior to fitting the IUD may also be useful to dilate the cervical canal (Saay et al. 2007).

When the frameless IUD is inserted correctly, spontaneous expulsion occurs in less than 1% of cases over a 5-year period. Extraction studies assessing the force required to retrieve the anchored IUD confirm the reliability of the anchoring concept (Batar and Wildemeersch 2004; Wildemeersch 2004). Long-term multicenter clinical trials using the current GyneFix® insertion instruments have shown low expulsion rates (including failed insertion – see below) in both parous and nulliparous women, ranging from 0.5/100 to 3.0/100 users during the first 3 years of use, compared to expulsion rates with the TCu380A IUD of 2.7/100 to 7.4/100 users (Cao et al. 2004; Wu et al. 2003).

When applied to the frameless IUD, the term "insertion failure" has a broader meaning, which includes failure to implant the knot in the fundal myometrium. Failure to implant the knot means that the device remains in the uterine cavity but is unattached to the uterine wall as intended. This results in the expulsion of the frameless IUD within days or weeks of the attempted insertion.

GyneFix® can be removed from the uterine cavity by exerting traction on the thread.

2.2.5 Perforation

The usually quoted perforation rate of conventional IUDs is 1/1,000 to 3/1,000 insertions. However, the actual incidence of this complication ranges from 0.0/1,000 to 8.7/1,000 insertions and is directly related to the skill of the individual performing the insertion (Tatum and Connell 1989). One major reason for perforation is the failure to establish the size and orientation of the uterus by careful pelvic examination. This is particularly important where there is sharp ante- or retroflexion, or lateral deviation, of the uterus, and where the axis is not straightened prior to

insertion with traction using a tenaculum. Perforation, diagnosed at insertion or later, or translocation of the frameless IUD have not been recorded to date in a large international multicenter clinical trial. However, in post-marketing trials a perforation rate of 1–2/1000 insertions was observed indicating the importance of insertion training.

A recent study by Van Houdenhoven et al. 2006, estimated the incidence of uterine perforations related to the insertion of a LNG-IUS (Mirena®, BayerSchering, Berlin, Germany) at 2.6/1,000 insertions. Total or partial perforation of the myometrium by the hysterometer or the inserter tube increases the risk of partial or total positioning of the IUD/IUS in the abdominal cavity. Insertion in lactating women, even beyond 6 weeks after delivery, was shown to be an important risk factor. An atrophic uterus (caused by long-term use of depot injectable contraceptives) is also a risk factor, since they may cause the fundal myometrium to become thinner.

2.2.6 Special Uses of the Frameless Copper IUD

- **Emergency contraception**. In 1976, copper IUDs were shown to be highly effective for emergency contraception (Lippes et al. 1976). They have three main advantages over oral hormonal emergency contraception: (1) Efficacy is higher for a copper IUD, with pregnancy rates not exceeding 0.1% (Trussell and Ellertson 1995) compared with 1% for progestogen-only emergency contraception (Task Force on Postovulatory Methods of Fertility Regulation 1998). (2) A copper IUD can be inserted at least 5 days after unprotected intercourse, or up to 5 days after the earliest estimated day of ovulation (Webb 1997). In this situation, the copper IUD may act by preventing implantation. When used long-term, the copper ions elicit reactions which usually prevent fertilisation (Mishell 1998). (3) Once inserted, an IUD can provide ongoing contraception for 5 years or more.

  A randomized study compared the frameless IUD with the TCu380S for use in emergency contraception. The results suggest that, although the actual fitting of the frameless GyneFix® IUD may be more painful, it causes less pain during the 30 days thereafter. As a result, requests for removal due to pain are significantly less likely with the frameless IUD at 6 weeks. No pregnancies were reported in this study (D'Souza et al. 2003).

  The high overall continuation rate (>80%) of all emergency IUDs at 6 weeks favors IUD insertion following unprotected intercourse, a finding that is also supported by its superior efficacy in emergency contraception compared to oral hormonal methods. Although the frameless standard IUD was used in this study, and not the more suitable small version, the latter device should be preferred for use in an emergency because of its more acceptable bleeding profile.
- **Immediate postabortal contraception**. Women who have an IUD inserted immediately after having an abortion have fewer pregnancies and repeat

abortions than women who schedule insertion of an IUD for a follow-up visit (Reeves et al. 2007). Thus, the frameless IUD could constitute a useful new option in the prevention of repeat abortions. In limited clinical trials, no expulsion of the IUD occurred following immediate insertion after pregnancy termination at up to 13 weeks gestation (Batár et al. 1998; Gbolade 1999). However, four "early" expulsions, clustered in one center, in a multicenter clinical trial conducted in 212 postabortal women in China during the first 6 months follow-up have been reported (unpublished data). This finding contrasts with expulsion rates following first-trimester abortion from 5/100 to 14/100 users at 2 years with framed IUDs (Lippes Loop, TCu220C, and the Copper 7), as reported by WHO (World Health Organization 1983).

- **Immediate postpartum contraception**. Since 1984, a technique for the insertion and fixation of an anchoring system to suspend an IUD/IUS in the uterine cavity immediately postdelivery was tested in Belgium, Hungary, and China. Several different types of anchor were tested in pilot and multicenter trials. These studies revealed that the immediate postplacental insertion and fixation technique (IPPIF) is safe and is not associated with increased risk of perforation or infection. It was concluded that a frameless, anchored IUD and insertion instrument could be developed into a practical postplacental contraceptive suitable for general use (Wildemeersch et al. 1986; Van Kets et al. 1991, 1993).

2.2.7 Lifespan

Long duration of action is important for IUD users because it is economical and may reduce certain health risks related to frequent removal and replacement. Calculations of the useful lifespan of the GyneFix® 330 IUD, based on weight and surface measurement of removed devices, which were in utero for up to 12 years, show that copper release appears to be constant over a period of up to 12 years and that ~36% of the copper is released throughout that period. Therefore this IUD has a useful life span of at least 10 years.

2.3 The Frameless Levonorgestrel Intrauterine System (LNG-IUS)

2.4 Description

The FibroPlant®-LNG intrauterine system (IUS) is an anchored levonorgestrel (LNG)-releasing device. It is a multicomponent system consisting of a nonresorbable thread with a single knot on its proximal end (Fig. 5). Attached thereto is a 3-cm long and 1.2 mm wide (FibroPlant® 14) or 3.5 cm long and 1.6 mm (FibroPlant® 20) wide fibrous delivery system, releasing 14 μg or 20 μg of LNG per day, respectively. The fiber consists of a LNG-ethylene vinyl acetate (EVA) core and an EVA rate-controlling membrane. Both systems have a lifespan of 5 years.

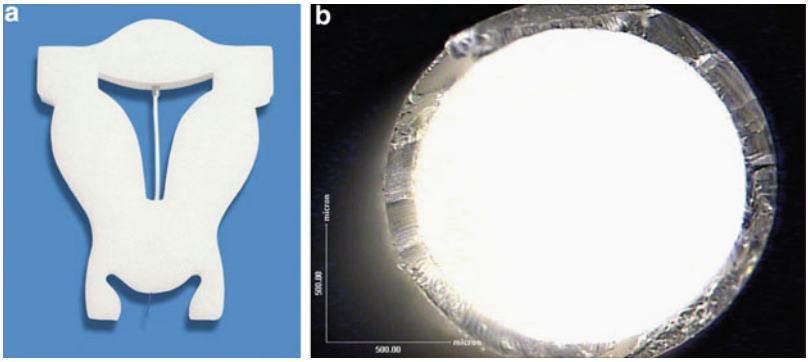


Fig. 5 (**a**) FibroPlant® LNG-IUS. (**b**) Cross-section through fiber showing the drug-containing inner core and the outer rate-controlling membrane

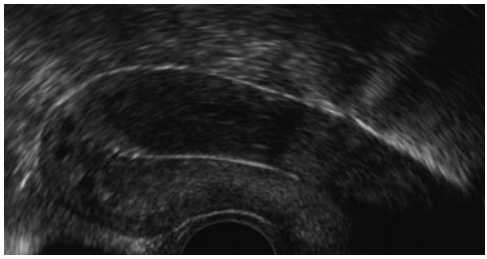


Fig. 6 Vaginal ultrasound of FibroPlant® LNG-IUS

The fiber is fixed to the anchoring thread by means of a stainless steel clip located 1 cm from the anchoring knot. The anchoring knot is implanted into the myometrium of the uterine fundus using the same insertion instrument as the frameless copper releasing IUD, which secures the implant in the uterine cavity.

The frameless LNG-IUS is highly visible on ultrasound (Fig. 6); the metal clip enhances the visibility of the system on X-ray. The visibility of the IUS allows checking its proper location in the uterine cavity at insertion and on follow-up. Since the system has no frame, it is completely flexible, adapting to cavities of every size and shape, in contrast with framed IUSs which sometimes do not fit properly in the uterine cavity.

2.5 Clinical Performance of the Frameless LNG-IUS

The two levonorgestrel-releasing IUSs were evaluated under the following conditions, where endometrial suppression was desired:

- **Hormone replacement therapy**: the acceptability and endometrial safety of continuous parenteral estrogen administration combined with intrauterine levonorgestrel delivery in postmenopausal women (3 year study) was assessed.

- **Menorrhagia**: the impact on menstrual bleeding in women with normal menstruation; in women with idiopathic menorrhagia; and in women with menorrhagia associated with uterine fibromyomas was assessed.
- **Contraception**: contraceptive performance and acceptability up to 5 years in parous and nulliparous women was evaluated.
- **Dysmenorrhea**: the effect of the frameless LNG-IUS was assessed in women suffering from primary and secondary dysmenorrhea.

2.6 Acceptability and Endometrial Safety of the Frameless LNG-IUS in Women Using Estrogen Replacement Therapy (ERT)

To measure the acceptability of the FibroPlant® LNG-IUS, women using oral or parenteral ERT, who had had the IUS in place for a minimum of 3 years, were asked if they would like to continue the combined regimen and if they would accept renewal of the device. At the time of study analysis, 150 postmenopausal women aged 33–78 years were using the low-dose frameless LNG-IUS. The number of women who elected to continue the method was 142 (94.6%). No serious adverse events, such as pelvic inflammatory disease or uterine perforation were observed, nor were any expulsions recorded.

Endometrial safety was evaluated by endometrial biopsy and transvaginal ultrasound (TVU) examination. In a subgroup of 101 consecutive postmenopausal women undergoing endometrial biopsies after 3 years of regimen use, the histologic appearance was that of profound endometrial suppression characterized by glandular atrophy and stromal decidualization. There was a good correlation between the histologic findings and endometrial thickness, which was thin (<4–5 mm) in all women, as measured by TVU (Wildemeersch et al. 2003, 2005a).

2.7 Effect on Menstrual Blood Loss

The effect of the frameless LNG-IUS on MBL in Belgian women with idiopathic menorrhagia, with and without fibroids, and in Brazilian women with normal and menorrhagic menstrual periods was evaluated in four studies (Wildemeersch and Schacht 2001, 2002; Wildemeersch and Rowe 2004b; Andrade et al. 2004). Reduction of MBL was assessed by a visual bleeding assessment scoring (VBAS) technique (Belgian women) and by the quantitative alkaline haematin (QAH) technique (Brazilian women).

For the calculation of the bleeding score a pictorial chart form was used to describe the degree to which the sanitary product was soiled. A score was calculated

by multiplying the number of slightly, moderately and heavily soiled pads and tampons by 1, 5 and 20 for pads and 1, 5 and 10 for tampons, respectively, according to their degree of staining.

A highly significant reduction in MBL (>90%) was observed in all low-dose frameless LNG-IUS LNG users ($n = 98$) with and without menorrhagia. The only exceptions were one woman with a submucous fibroid and another with a large polyp. Amenorrhea occurred in up to 80% of women after 2 years of use; ferritin levels improved significantly in all women tested.

2.8 Contraceptive Efficacy

Three-hundred and four insertions were performed with the frameless LNG-IUS releasing 20 μg of LNG/d, 14.1% of them in nulliparous women. The mean age of all women was 34.7 years (range 15–48). The total observation period was 11,299 woman-months with a follow-up of up to five years. Only one pregnancy occurred after unnoticed expulsion of the LNG-IUS. There were two expulsions and two uterine perforations occurred at insertion during the first year. The devices were easily removed by laparoscopy. The cumulative total use-related discontinuation rate was 23.6 at five years, the majority of them for spotting/bleeding complaints (n = 24), pelvic pain (n = 12), mostly unrelated to the IUS, and some for mood disturbances (n = 5). Sixteen removals were requested for pregnancy wish (Wildemeersch and Andrade 2009, submitted).

2.9 The Effect of the Frameless LNG-IUS in Women Suffering from Primary or Secondary Dysmenorrhea

A noncomparative pilot study (Wildemeersch et al. 2001) was conducted in 18 women aged 16–52 years; eight had primary dysmenorrhea, and ten had secondary dysmenorrhea; four insertions were performed in nulligravid women; twelve women complained of heavy bleeding; three women had significant fibroids (3–6 cm in diameter), and three were suspected of having adenomyosis.

The trial period ranged from 3 to 33 months. All women reported much reduced pain or no pain at all and a considerable reduction in bleeding, which started as soon as 1 month after insertion of the frameless LNG-IUS. The only exception was a woman with multiple fibroids. She reported much reduced bleeding, but her relief of menstrual pain was not as pronounced as that reported by the other women in the study. All women continued to use the method.

3 Framed Levonorgestrel-Releasing Intrauterine Systems

3.1 Development

Since T-shaped IUDs have been used for several decades, health care providers are familiar with their insertion and fitting, so that minimal training is required. The combination of drug-delivery technology with a conventional IUD frame is, therefore, an attractive option for nonspecialist providers (e.g., nurses, midwives, general practitioners) and those who do not insert IUDs on a regular basis.

Although the insertion procedure for T-shaped IUDs is relatively simple, further simplification would be welcome in order to increase the use of intrauterine contraception. The new T-shaped LNG-IUSs, Femilis® and Femilis® Slim, were developed with this objective.

With the simplified insertion procedure, the extremities of the crossarm remain outside the inserter tube and are not folded into it as is done with conventional T-shaped IUDs. This allows the crossarm to unfold immediately upon entry into the uterine cavity. This insertion procedure appears to be simpler than that used with conventional T-shaped copper IUDs, as well as with the Mirena® insertion technique. It is also safer as, due to the unfolding of the crossarm, a narrow protruding element cannot be forced against the fundus of the uterus as could be the case with conventional T-shaped IUDs and the Mirena® LNG-IUS in which the crossarms are folded in the inserter tube during insertion of the IUD/IUS. The risk of perforation is, therefore, minimized (Fig. 7). Since perforation of the uterus is a potentially serious adverse event, any efforts to avoid this complication are obviously welcome.

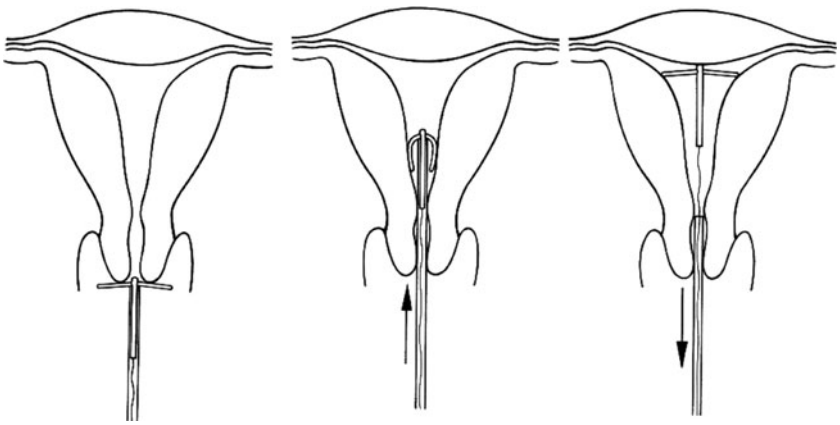


Fig. 7 The simplified insertion procedure of the Femilis® LNG-IUS. Step 1: The loaded inserter is applied against the cervix; Step 2: The Femilis® LNG-IUS is inserted by pushing the loaded inserter into the uterine cavity up to the fundus; Step 3: The inserter tube is removed (while rotating it) and the thread is trimmed

3.2 *The Framed Standard Levonorgestrel-Releasing Intrauterine System*

3.2.1 Description

Femilis® (standard) consists of a 3-cm long and 2.4-mm wide fibrous delivery system (Fig. 8). The inert vector, composed of EVA copolymer, contains 60 mg of LNG and is covered by a rate-controlling membrane, also consisting of EVA. The drug compartment releases about 20 μg of LNG daily. The polyethylene crossarm contains 22% barium sulfate to render it radiopaque. The single tail is made of a 00-gauge polypropylene. The framed LNG-IUS can be used in parous and nulliparous women, whereas the Femilis® Slim should be used only in postmenopausal women with an atrophic uterus.

It is noteworthy that the crossarm of the standard framed LNG-IUS (Femilis®) is 4 mm shorter than that of standard copper-T IUDs and the Mirena® LNG-IUS (Fig. 8). This smaller dimension optimizes compatibility with the uterine cavity of most women, especially nulliparous women (Kurz 1984; Hasson 1984). The short crossarm also avoids contact with the vaginal wall during insertion.

Femilis® Slim (Fig. 8) resembles the standard Femilis® LNG-IUS, but its length is 2.8 cm instead of 3 cm. The drug delivery compartment is only 2.0 mm wide instead of 2.4 mm, and it contains 40 mg of LNG instead of 60 mg. The drug compartment, releasing ~20 μg of LNG daily, is provided with a thin and highly flexible crossarm fixed to the upper part of the drug delivery rod.

The total length of the cross arm is 24 mm, compared to 28 mm for the standard version. The polyethylene crossarm contains 22% barium sulfate to render it radiopaque. The single tail is made of a 00-gauge polypropylene.

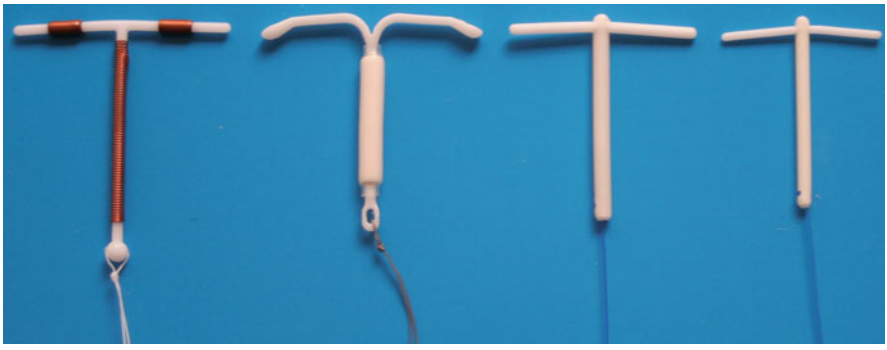


Fig. 8 From *left* to *right*: comparison between ParaGard®, Mirena®, Femilis® and Femilis® Slim

3.3 Clinical Performance of the Framed Standard LNG-IUS

3.3.1 Ease and Safety of Insertion and Contraceptive Performance

In the first contraceptive study with the framed standard LNG-IUS (Wildemeersch 2006), the main focus was on ease and safety of the new insertion technique. The paper, published in 2006 is the first report with the framed LNG-IUS. The push-in technique of insertion was considered simple and safe. Insertion was reported to be easy in virtually all women (97.9%). Pain at insertion was absent in 24.7% and mild in 67.7% of women. With respect to pain, there were no statistical differences between the parous and nulliparous group. It was concluded that the framed LNG-IUS is an effective contraceptive and is easily inserted. The simple and safe insertion procedure could be an advantage for use by nonspecialist providers such as nurses, midwives, general practitioners, and for those not inserting intrauterine devices regularly.

In the second study (Wildemeersch et al. 2009) the main focus was on the contraceptive performance. Two-hundred and eighty insertions were carried out in women with a mean age of 35.7 years (range 17–48), 60% of whom were parous and 40% nulliparous. Twenty-four women with uterine pathology (e.g., fibroids, menorrhagia) were included in the study. The cumulative gross discontinuation life table rates were determined. The total observation period was 8,028 woman-months. The LNG-IUS was easy to insert in 95.7% of the cases, and no perforations occurred. No pregnancies were observed and only one expulsion took place (rate 0.4/100 women at five years). The cumulative total use-related discontinuation rate was 14.7/100 at five years. There were nine removals because of pain, six of which in nulliparous women. Four women requested removal of the IUS for bleeding problems. Fourteen removals were done for 'other' medical reasons among which mood disturbances (five cases) were the most frequent, and twelve for non-medical reasons. Fifteen removals were requested for pregnancy wish. Twelve of these women became pregnant within one year and all had uneventful pregnancies. The framed LNG-IUS was equally well accepted by nulliparous as by parous women. Most women with heavy menstrual bleeding prior to insertion, whether associated with fibroids or not, reported much less bleeding, scanty bleeding of even no bleeding at all. This study suggests that the framed LNG-IUS, which releases 20 μg LNG/d, is a highly effective, well tolerated contraceptive both in parous and nulliparous women.

3.3.2 Retention

The results of the clinical studies suggest that Femilis® LNG-IUS is also well retained by the parous and nulliparous uterus which may be explained by the strong suppressive effect of the hormone, levonorgestrel, on the endometrium, reducing menstrual flow, and the inhibiting effect on uterine peristaltic activity. Previous

studies have suggested that the efficacy of intrauterine-administered levonorgestrel in the treatment of dysmenorrhea could be attributed to the inhibition of prostaglandin synthesis (Lumsden et al. 1983). This may also explain why, in the few cases with downward displacement of the IUS, partial or total expulsion did not occur. Downward displacement does not require replacement of the IUS, unless the patient experiences pain, or the stem of the IUS is visible on speculum examination. With conventional IUDs, such as the TCu380A IUD, expulsion rates of between 2.7 and 7.4/100 users, or higher, are observed during the first and subsequent years (Population Reports 1995). In nulliparous women, however, even higher partial and total expulsion rates – up to 17/100 users during the first year of use – have been reported (Petersen et al. 1990). Downward displacement with copper IUDs reduces the contraceptive efficacy of the IUD significantly in contrast with the LNG-IUS (Kaivola 1990; Anteby et al. 1993). In the Population Council Study, the expulsion rates for the levonorgestrel-releasing IUS (11.7 per 100 users) were higher than those for copper-releasing IUDs (8.3 per 100 users) (Sivin et al. 1990). In the European study the expulsion rates were 5.8 and 6.7 per 100 users, respectively.

Another important factor which may contribute to the low expulsion rate of the Femilis® LNG-IUS is related to the simplicity of the insertion procedure and the immediate unfolding of the crossarm when the IUS is pushed into the uterine cavity. The risk of improper positioning is thereby minimized. The very short time from folding, during the passage through the cervix, and the subsequent unfolding could also contribute to the good retention of the Femilis® LNG-IUS. With conventional IUDs and the Mirena® LNG-IUS, the crossarms are retracted in the inserter tube during insertion. This causes a slower unfolding of the plastic crossarms. When the IUD/IUS is then pushed out of the tube, the crossarms may not unfold completely when pushed halfway into the uterine cavity and, as a consequence, the crossarms may not be properly positioned against the fundus. If the cavity is narrow and the span of the crossarms of the IUD or IUS is significantly greater than the fundal transverse diameter, or if the insertion procedure is not followed strictly, pushing the IUD/IUS out of the tube, instead of retracting the tube as recommended, then a less than optimal positioning may follow and lead to partial or total expulsion due to uterine contraction. The shorter crossarm of the Femilis®, adapting better to small uterine cavities, could, therefore, also be a contributing factor in the proper positioning of the IUS.

3.3.3 Effect on Menstrual Blood Loss

Menstrual blood loss was assessed with the visual assessment technique in 60 women, <48 years of age at study enrollment, who used the Femilis® LNG-IUS for a mean of 18 months (range, 4–31) (Janssen et al. 1995; Wildemeersch and Rowe 2005). Twenty-eight women had normal menstrual periods at baseline (menstrual score <185), and 32 had idiopathic menorrhagia (menstrual score ≥185).

MBL scores dropped significantly during the observation period in all but one woman. The median menstrual score at baseline in women with normal menstrual bleeding was 140 (range, 80–160); the score declined to a median of five (range, 0–150) at follow-up, a decrease of 96%. In the 32 women with menorrhagic bleeding at baseline, menstrual flow dropped from a median score of 232 (range, 185–450) at baseline to three (range, 0–50) at follow-up, a decrease of 99%. Twenty women developed amenorrhea (33%), 10 in the group with normal menstruation and 10 in the group with menorrhagia. Most of the remaining women had oligomenorrhea, requiring minimal sanitary protection. These results are currently being confirmed in a MBL study using the quantitative alkaline haematin technique (unpublished data).

3.3.4 Long-Term Treatment of Non-Atypical and Atypical Endometrial Hyperplasia

Twenty women (16 parous, 4 nulliparous), all negative for hypertension and diabetes, mean age, 54 years (range, 41–67), used the LNG–IUS for a mean of 32 months (range, 14–90 months) (Wildemeersch et al. 2007a). Eight of the women had developed postmenopausal bleeding as a result of unopposed estrogen stimulation, after having taken the estrogen for 6–24 months. One woman presented due to abnormal bleeding during tamoxifen treatment for breast cancer; another woman had abnormal premenopausal bleeding. The histopathologic diagnosis (Kurman classification) was "non-atypical (simple) hyperplasia" in 12 women and "atypical hyperplasia" in eight others (adenomatous hyperplasia with atypia in three of them). In one of the latter patients, an invasive, well-differentiated adenocarcinoma was identified on D&C, but two subsequent endometrial Pipelle samplings did not confirm this finding.

The endometrial progesterone receptor (PR) response was determined during treatment in women with atypical endometrial hyperplasia. All but one patient developed a thin endometrium ($\leq$4 mm in thickness), as assessed by transvaginal ultrasound. One patient presented with a polypoid structure 20 mm in diameter prior to treatment, which diminished gradually to 5 mm by the last follow-up examination, 53 months after insertion of the LNG–IUS. At study initiation, all women presenting with atypical endometrial hyperplasia showed expression of PR in the epithelial cells. The percentage declined significantly over time during treatment. The endometrial histology specimen showed no progression of disease. Profound endometrial suppression with glandular atrophy and/or stromal decidualization was noted in all women. Eight of the 20 women in the study resumed estrogen replacement therapy. All women are continuing to use the method. Successful pregnancy has been observed in two women with complex atypical endometrial hyperplasia treated with a LNG-IUS and ovulation induction after removal of the LNG-IUS (Qi et al. 2008).

3.4 The Framed Slim Levonorgestrel-Releasing Intrauterine System

3.4.1 Clinical Performance of the Framed Slim LNG-IUS

Acceptability and Long-Term Endometrial Safety of the Femilis® Slim LNG-IUS in Postmenopausal Women Using Estrogen Replacement Therapy (Wildemeersch et al. 2005c, 2007b)

The addition of a progestogen to estrogen is necessary as an increased risk of endometrial cancer was detected in users of unopposed estrogen (The North American Menopause Society (NAMS) 2003). Systemic progestogens can cause serious problems, however. The Women's Health Initiative (WHI) study and the Million Women Study (MWS) both showed an increase in breast cancer, cardiovascular disease, and venous thromboembolic events in women using oral postmenopausal hormone replacement therapy (HRT). Since an increase in breast cancer was not observed in long-term users of oral estrogen-only therapy, it has been hypothesized that the increased incidence of breast cancer seen in the two studies may have been caused by the progestogen component of the HRT regimen. An intrauterine system that delivers a progestogen directly to the uterus could theoretically minimize the impact of the hormone on breast and other organ tissues. However, this hypothesis has yet to be confirmed by appropriate studies.

A total of 264 postmenopausal women used the combined regimen consisting of estrogen (oral or parenteral by gel or patch) and an intrauterine LNG-releasing device, either the frameless FibroPlant® LNG-IUS or the T-shaped Femilis® LNG-IUS. Women entered the study only after first using the frameless LNG-IUS and, after expiry, replacing it with the T-shaped LNG-IUS.

A total of 102 postmenopausal women using the framed Slim LNG-IUS (mean age, 57 years, range 47–71; mean duration of regimen use, 70 months, range 25–98), who presented with climacteric symptoms constituted the "long-term" group. Women with prior endometrial hyperplasia were excluded. The majority ($n = 70$) received percutaneous 17β-estradiol, 1.5 mg daily (Oestrogel®), or an equivalent dose by patch or orally, on a continuous basis. The remaining group of women ($n = 32$) used a lower dose on their own initiative.

An endometrium sample was taken with a suction curette. To achieve a representative sample, the biopsies were drawn from all parts of the uterus. Two experienced pathologists and an independent, blinded, pathologist examined the embedded and stained samples according to the diagnostic criteria of Hendrickson (Hendrickson and Kempson 1980) and classified them according to Kurman (1995). The mean endometrial thickness was 3.2 mm (range 3–7 mm). In one woman, the endometrial thickness was 7 mm, but on histology, her endometrium was found to be inactive. All women maintained a thin endometrium during the entire HRT treatment period. The dominant endometrial histological picture was that of epithelial atrophy accompanied by decidualization of the stroma (Kurman classification

2 + 5b; 5b; 2 or 3), which correlates well with the measured endometrial thickness (≤5 mm). In 13 women, only very scanty tissue could be obtained during the last sampling due to profound endometrial atrophy (Kurman classification 1). Weak endometrial proliferative activity was found in two women (Kurman 4a). No endometrial hyperplasia was found.

4 Concluding Remarks

4.1 Long-Acting Contraceptive Methods Should Be Used to Prevent Unintended Pregnancies

User noncompliance with most oral and barrier contraceptive methods is a ubiquitous phenomenon, particularly in younger women. Even in those whose motivation for daily use is good, compliance is often poor (Moreau et al. 2007). This is not likely to change, since the inadequacies of these methods are related to human error as well as lack of education and understanding. The relatively high frequency of side effects of some methods only adds to the problem (Westhoff et al. 2007).

Highly effective and well-tolerated intrauterine methods can solve this problem, because use of intrauterine devices and systems can reduce the number of unintended pregnancies with no intervention by the patient. Besides, intrauterine contraceptive devices are more cost effective than the combined oral contraceptive pill.

Some of the new intrauterine methods described in this chapter, such as the frameless copper IUD and the frameless LNG-IUS, are ideal for use in younger women, because they are small, very effective, well-tolerated, and almost impossible to expel. If a framed IUD/IUS is used, the horizontal crossarm should ideally not be wider than 28 mm (most T-shaped IUDs, including the Mirena® LNG-IUS are 32 mm wide) to avoid incompatibility leading often to cramping and expulsion, complaints that are the most common reasons for discontinuation of the method, as well as attendant bad publicity (Suhonen et al. 2004). A recent review of the clinical experience with the LNG-IUS in nulliparous women concluded that the LNG-IUS is both safe and effective for use in nulliparous women (Prager and Darney 2007). The American College of Obstetricians and Gynaecologists (ACOG) Committee issued a document in 2007 which addresses the major benefits of IUD use in adolescents, a population at particular risk of unintended pregnancy (ACOG Committee Opinion 2007).

Copper IUDs should be offered more frequently to young and adolescent women, even as a first method, in combination with condoms to protect against sexually transmitted infections (STIs), particularly after a first unintended pregnancy. The LNG-IUS, on the other hand, has been shown to offer some protection against pelvic infection (Andersson et al. 1994). The association between oral contraceptive use and the occurrence of cervical ectopia has important implications concerning the susceptibility to cervical infection as it may facilitate penetration of bacteria and viruses into the underlying tissue, including HPV and HIV (Crittchlow

et al. 1995). There is concern that this mechanism may be an important causative factor (even if it is not the only factor) in the occurrence of cervical neoplasia (Beral et al. 1988).

4.2 Long-Term Intrauterine Contraceptive Methods to Replace Irreversible Female Sterilization

Worldwide, tubal sterilization is used by more people than any other method of contraception. All techniques of tubal sterilization in widespread use have low risks of surgical complications. Although tubal sterilization is highly effective, the risk of pregnancy varies by age and method of occlusion. Pregnancies can occur many years after the procedure, and when they do, the risk of ectopic gestation is high (Peterson 2008). Although sterilization is intended to be permanent, expressions of regret and requests for reversal are not uncommon and are much more likely to occur among women sterilized at young ages (Jamieson et al. 2002). In countries where LNG-IUS use is widespread, the incidence of tubal sterilization has decreased substantially (Inki 2007). Health care professionals should realize that the LNG-IUS has much to offer to women who consider their family complete. They should explain that, in addition to offering almost perfect contraceptive protection, the LNG-IUS also provides significant noncontraceptive health benefits, such as reduced menstrual blood loss and dysmenorrhea; regression and cure of endometrial hyperplasia, both non-atypical and atypical; and probably regression of pelvic endometriosis and uterine adenomyosis (Mansour 2007; Bahamondes et al. 2007). Furthermore, the high regret rates within the first year after sterilization should encourage providers to propose the LNG-IUS as the first choice option for women with a complete family who are requesting contraception (Curtis et al. 2006; Grimes and Mishell 2008).

4.3 Safer Contraception

Perhaps it is good to repeat what others have said before: “We need to develop an attitude of zero-tolerance to serious adverse effects of contraceptives.” Hence, the need to develop steroidal drug delivery systems with minimal or no metabolic impact. As a result of technological progress, miniature frameless and framed, long-term intrauterine drug delivery systems can offer women of all ages almost trouble-free contraception with reduced side effects. Despite the minimal absorption of the steroid in the systemic circulation, intrauterine drug delivery systems deserve the status of locally acting methods which are safer than systemically applied hormones. Plasma levels observed with a 20 μg-releasing levonorgestrel intrauterine system are significantly lower than with any other mode of administration of levonorgestrel.

Applied to the uterus, absorption into the systemic circulation is low. An LNG-IUS which has perforated the peritoneal cavity results in 10 times the plasma levels of levonorgestrel seen with a non-perforated LNG-IUS (Haimov-Kochman et al. 2003). This proves that intrauterine delivery of hormones is a safe alternative when compared with other methods of administration of hormones.

Systemic, orally administered hormones, such as the combined Pill, could endanger vascular health, as a recent study suggests. In this study, Rietzschel et al. (2007) express concern about the long-term use of OCs (>10 years). The use of OCs was found to be associated with an unexpected increase in the prevalence of carotid and femoral arteriosclerosis in young, healthy women. There was a 20–30% increased prevalence of plaque formation in the carotid and femoral arteries per 10 years of OC exposure. This study was based on a representative sample (2,524 male/female volunteers, 33–55 years) from the Belgian general population free from overt cardiovascular disease. Vascular echography of the carotid and femoral arteries was systematically performed and atherosclerosis was defined by the presence of carotid or femoral plaque.

4.4 Pain Control with Intracervical Anesthesia for IUD/IUS Insertion

Several measures have been used to reduce patient discomfort during the insertion procedure of an IUD/IUS, including premedication, topical intracervical anesthesia, and loco-regional anesthesia (Thiery 1985; Hollingworth 1996). Vasovagal syncope appears to be less frequent when paracervical block anesthesia (PCB) is used. Although PCB is described as highly effective for minimizing discomfort for IUD retrieval/insertion, many physicians fear PCB because of the risk of inadvertent intravascular injection leading to convulsions and central nervous system depression. However, intracervical anesthesia does not carry this risk and should therefore be available in centers providing IUD/IUS insertion. Prophylactic ibuprofen administration of 600–800 mg may be highly useful and can be combined with intracervical anesthesia (Hubacher et al. 2006).

In some women the use of misoprostol 400 μg, 3 h prior to fitting the IUD/IUS may be useful to dilate the cervical canal. But, it should also be kept in mind that a psychological approach is sometimes better than pharmacological premedication.

4.5 Intrauterine Hormonal, Period-Free, Contraception for All Women

Mirena®, the first levonorgestrel intrauterine system, has been hailed as one of the greatest advances in the field of contraception since the introduction of “the Pill”

(Mansour 2006). Over 10 million women worldwide have chosen the LNG-IUS. Acceptance and continuation of this IUS has been high in women of all age groups, whether they have children or not, due to both its noncontraceptive and contraceptive benefits.

As far as menstrual bleeding is concerned, the majority of women during their reproductive period prefer to menstruate less than once a month or not at all (den Tonkelaar and Oddens 1999). They know that the amenorrhea, which appears under progestogen contraception, is both nonpathological and reversible. Consequently, many women, confident of the birth control provided by these very efficient contraceptive methods, may also wish to space out what they consider useless and unpleasant periods to 3–6 months or even to 1 year or more. The acceptability of contraceptive-induced amenorrhea appears to be similar in the USA and in Europe, with the exception of black women who are less accepting the absence of bleeding than white women (Aubény 2006).

Has the LNG-IUS the potential to revive intrauterine contraception and improve the underuse of the IUD (d'Arcangues 2007)? This could very well be the case, where higher prices of the IUD/IUS could motivate marketing companies to increase their promotional budget especially in Western countries. Ironically, while the Mirena® LNG-IUS was developed with public funds, and was meant to be made available through the public sector in developing countries, poor women in these regions still lack access to this advanced technology for contraception and treatment because of the high cost. A striking fact is the very common occurrence of heavy menstrual bleeding and anemia in less developed countries. Anemia is one of the most widespread, and most neglected, deficiency diseases in these regions. Anemia is common in women during their reproductive years and is particularly severe in those who are pregnant or breast-feeding. The LNG-IUS could be very useful in women prior to pregnancy, to counteract the effect of heavy menstrual bleeding and contribute to healthier infants as higher iron stores reduce the risk of low-birth weight (Palma et al. 2008).

4.6 Intrauterine Hormonal Contraception Can Prevent the Need for Hysterectomy

Those who prescribe the LNG-IUS know that the number of hysterectomies can be reduced substantially. General practitioners and family planning doctors should know that women with heavy bleeding and menorrhagia, whether associated with intramural or subserous fibromyoma(s) or not, can be treated effectively with the LNG-IUS (Magalhães et al. 2007; Kaunitz 2007). It is relatively rare that the LNG-IUS fails and if so, uterine pathology (mostly intracavitary fibroids) should be suspected (Rizkalla et al. 2008). Virtually all studies involving the LNG-IUS uniquely show that it reduces MBL and subsequently improves blood hemoglobin and serum ferritin levels. In the UK and elsewhere, in centers where the LNG-IUS

is promoted by the health care providers, a reduction in the hysterectomy rate of 50% has been seen over the last 10 years (and 75% fewer tubal sterilizations). Studies with FibroPlant® LNG-IUS and Femilis® LNG-IUS suggest that almost 80% of hysterectomies can be avoided. This economical method should, therefore, be used more extensively and promoted in the media so that women know about it. The insertion of an LNG-IUS, is a minor office procedure with only a few risks when compared with invasive surgery for menorrhagia such as transcervical resection of the endometrium (TCRE) and hysterectomy (Hurskainen 2006; National Institute of Clinical Excellence 2005; Milsom 2007). Besides, insertion of the LNG-IUS requires no or limited training in contrast with ablation techniques which require intensive training and therefore have a long learning curve. An additional advantage of the LNG-IUS is that it may also be beneficial in women with endometriosis, adenomyosis, fibroids and endometrial hyperplasia (Bahamondes et al. 2007; Fedele et al. 1997; Varma et al. 2008). Most patients are likely to select a non-invasive treatment. Avoidance of major surgery, combined with no hospitalization and quick recovery, are seen as major advantages of non-invasive management. In women treated with the LNG-IUS, this device would be preferred over hysterectomy by 95% of the patients if the expected success rate were >50% (Boudrez et al. 2004).

4.7 Reducing The Risk of Postmenopausal Heart Disease, Stroke, Dementia and Alzheimer disease

After cessation of ovarian function, when estrogen therapy may be initiated, the LNG-IUS would ensure endometrial protection as a transition to the menopause for as long as hormone therapy is needed (Sitruk-Ware 2007). However, since the publication of the WHI study, many doctors remain confused as to the benefits and risks of hormone replacement therapy. For the reasons discussed earlier, transdermal estrogen and intrauterine progestogen or progesterone administration may be the safest and best accepted route for women with uterus resulting in high patient compliance and providing maximal benefits for postmenopausal women. It is considered "the way forward," as expressed by several experts (e.g., J. Manson, T. Mikkola, F. Naftolin, personal communication), since this regimen could be considered an almost estrogen-only therapy as the absorption of the progestogen in the systemic circulation is very low.

Transdermal estrogen administration has shown to perform significantly better than oral estrogen for menopausal symptom control (Pratapkumar 2006). Recently, new evidence has shown that the age at which HRT is started is of great importance. A window of opportunity could be defined. Women in the age group of 50–59 years who participated in the estrogen-alone arm of the WHI study were asked immediately after the early cessation of the trial to become part of an ancillary study – the

WHI-CACS (Coronary Artery Calcification Study) which looked at the magnitude of coronary calcifications measured by ultra-fast coronary CT. Coronary calcium deposits develop as part of the atherosclerosis process and correlate well with findings of coronary angiography. The results of WHI-CACS are very encouraging, since women who were randomized to the estrogen arm of the WHI had significantly smaller calcification scores than their counterparts in the placebo arm (Manson et al. 2007). The effect was recorded for all degrees of severity, with estrogen users having a 20–30% reduction in the likelihood of being categorized as having a mild to moderate increase in calcification score (less than 100), and a more than 50% reduction in the likelihood of being categorized as an advanced case with a calcification score above 100. This study re-affirms what was actually known for many years, based on animal data and observational studies in women. Estrogen has a wide range of well-documented beneficial metabolic and vascular effects. "It reduces the pace of accumulation of atherosclerosis, and decreases the risk of coronary events, provided that treatment is started *early* in the menopause" (Pines et al. 2007; Vitale et al. 2008; Karim 2008; International Menopause Society (2008); Mikkola 2008).

Also, according to Henderson, a critical window of opportunity may exist which could reduce the incidence of dementia and Alzheimer disease (Henderson 2008). In addition, there appears to be no increased risk of stroke if low dose ERT is started early after menopause in comparison with the overall risk in the WHI trial (Grodstein 2008).

4.8 The Future

New indications will be found for the use of the LNG-IUS in combination with other hormones. For instance, the LNG-IUS in combination with 100 μg per day releasing transdermal estradiol patches has been shown to be highly effective for the management of physical and psychological symptoms of severe premenstrual syndrome (PMS) (Royal College of Obstetricians and Gynaecologists 2007). In addition, the intrauterine route for hormone delivery will probably be used in the future not only for treatment of gynaecological disease but also for its prevention (Fraser 2007).

The frameless and framed IUD/ IUS platforms will be used in the future for the development of other drug delivery systems. Some of these developments are already conceived and proof of concept demonstrated. It is likely that in the near future intrauterine, subdermal and intravaginal drug delivery systems will be developed for the sustained release of progesterone antagonists (PAs) and selective progesterone receptor modulators (SPRMs). These systems may treat or cure conditions, such as fibromyomas and endometriosis/adenomyosis, since they are likely to be more effective than current drug delivery systems, including the LNG-IUS (Maruo et al. 2007). SPRMs could also be used in postmenopausal women (Sitruk-Ware 2008).

In addition, dual compartment delivery systems could be developed that release a PA or a SPRM in combination with a potent microbicide. The combination of locally delivered contraceptive and microbicide compounds could be very useful for bleed-free contraception and for the prevention of HIV transmission in HIV^+ women. Such dual systems might also be useful in HIV^- women to protect them from transmission from their male partner, especially in regions with a high prevalence of HIV.

A dual compartment intrauterine system could also be combined with other viricidal delivery systems, such as a vaginal ring. Due to the high local concentration of antiviral drug, resistance, as well as toxic effects, side effects, and poor compliance leading to treatment discontinuation, may all be avoidable. In addition, the intrauterine delivered hormone or anti-hormone could eliminate menstrual blood loss, which is an important source of viral shedding.

References

ACOG Committee Opinion No 392 (2007) Intrauterine device and adolescents. Obstet Gynecol 110:1493–1495

Andersson K, Odlind V, Rybo G (1994) Levonorgestrel-releasing and copper-releasing (Nova-T) IUD during five years of use: a randomised comparative trial. Contraception 49:56–72

Andrade TL, Andrade MD, Pizzaro Orchard E (1987) Quantitative studies on menstrual blood loss in IUD users. Contraception 36:129–144

Andrade ATL, Souza JP, Andrade GN, Rowe PJ, Wildemeersch D (2004) Assessment of menstrual blood loss in Brazilian users of the frameless copper-releasing IUD with copper surface of 330 mm^2 and users of the levonorgestrel-releasing intrauterine system. Contraception 70:173–177

Anteby E, Revel A, Ben-Chetrit A, Rosen B, Tadmor O, Yagel S (1993) Intrauterine Device Failure: Relation to its Location Within the Uterine cavity. Obstet Gynecol 81:112–114

Aubény E (2006) Will period-free hormonal contraception become the norm of the twenty-first century. Eur J Contracept Reprod Health Care 11:1–5

Bahamondes L, Petta CA, Fernandes A, Monteiro I (2007) Use of the levonorgestrel-releasing intrauterine system in women with endometriosis, chronic pelvic pain and dysmenorrhea. Contraception 75:S134–S139

Batar I, Wildemeersch D (2004) The force required to remove an anchored bioactive substance from the human uterus: results after long-term use. Contraception 69:501–503

Batár I, Wildemeersch D, Vrijens M, Delbarge W, Temmerman M, Gbolade BA (1998) Preventing abortion and repeat abortion with the GyneFix® intrauterine implant system – preliminary results. Adv Contracept 14:91–96

Beining RM, Dennis LK, Smith EM, Dokras A (2008) Meta-analysis of intrauterine device use and risk of endometrial cancer. Ann Epidemiol 18:492–499

Beral V, Hanaford P, Kay C (1988) Oral contraceptive use and malignancies of the genital tract. Lancet 2:1331–1335

Boudrez P, Bongers MY, Mol BWJ (2004) Treatment of dysfunctional uterine bleeding: patient preferences for endometrial ablation, a levonorgestrel-releasing intrauterine device, or hysterectomy. Fertil Steril 82:160–166

Cao X, Zhang W, Zhao X et al (2004) Three-year efficacy and acceptability of the GyneFix® 200 intrauterine system (IUS). Contraception 69:207–211

Cogliano V, Grosse Y, Baan R, Straif K, Secretan B, El Ghissassi F, IARC WHO (2005a) Carcinogenicity of combined oestrogen–progestagen contraceptives and menopausal treatment. Lancet Oncol 6:552–553

Cogliano V, Grosse Y, Baan R, Straif K, Secretan B, El Ghissassi F (2005b) Oral oestrogen–progestagen contraceptives, menopausal treatment and cancer. Lancet Oncol 6:737

Coppens M, Thiery M, Delbarge W, Parewijck W, Van Der Pas H, Van Kets H (1989) The copper-fix IUD: assessment of tissue reaction at the anchor site. Med Sci Res 17:719

Crittchlow CW, Wölner-Hanssen P, Eschenbach DA, Kiviat NB, Koutsky LA, Stevens CE, Holmes KK (1995) Determinants of cervical ectopia and of cervicitis: Age, oral contraception, specific cervical infection, smoking, and douching. Am J Obstet Gynecol 173:534–543

Curtis KM, Mohllajee AP, Peterson HB (2006) Regret following female sterilization at a young age: a systematic review. Contraception 73:205–210

d'Arcangues C (2007) Worldwide use of intrauterine devices for contraception. Contraception 75(6 Suppl):S2–S7

D'Souza RE, Bounds W, Guillebaud J (2003) Randomized comparative emergency contraceptive study: GyneFix versus TCu380A and Nova-T. J Fam Plan RHC 29:23–29

den Tonkelaar I, Oddens BJ (1999) Preferred frequency and charcteristics of menstrual bleeding in relation to reproductive status, oral contraceptive use, and hormone replacement therapy use. Contraception 59:357–362

Dou J, Zhang Y, Zhangh C et al (2001) Clinical comparative study of GyneFix and MLCu375 intrauterine devices. Reprod Contracep 12:181–185

Fedele L, Bianci S, Raffaelli R, Portuese A, Dorta M (1997) Treatment of adenomyosis-associated menorrhagia with a levonorgestrel-releasing intrauterine device. Fertil Steril 68:426–429

Fraser IS (2007) The promise and reality of the intrauterine route for hormone delivery for prevention and therapy of gynecological disease. Contraception 75:S112–S117

Gbolade BA (1999) Immediate insertion of the postabortion version of GyneFix® intrauterine implant system. Contemp Rev in Obstet Gynaecol pp. 29–33

Grimes DA, Mishell DR Jr (2008) Intraterine contraception as an alternative to interval tubal sterilization. Contraception 77:6–9

Grodstein F, Manson JE, Stampfer MJ, Rexrode K (2008) Postmenopausal hormone therapy and stroke. Role of time since menopause and age at initiation of hormone therapy. Arch Intern Med 168:861–866

Guillebaud J, Bonnar J, Morehead J, Mathews A (1976) Menstrual blood loss with intrauterine devices. Lancet 21:387–390

Haimov-Kochman R, Amsalem H, Adoni A, Lavy Y, Spitz IM (2003) Management of a perforated levonorgestrel-medicated intrauterine device–a pharmacokinetic study: case report. Hum Reprod 18:1231–1233

Hasson HM (1984) Clinical studies of the Wing Sound II metrology device. In: Zatuchni GI, Goldsmith A, Sciarra JJ (eds) Intrauterine Contraception: Advances and Future Prospects. Harper & Row, Philadelphia, pp 126–141

Henderson VW (2008). Alzheimer's Disease and Estrogen Revisited. 12th World Congr Menopause, Madrid (Spain), 2008. Abstract Book p. 15

Hendrickson MR, Kempson RL (1980) Surgical pathology of the uterine corpus, vol 12, Major problems in pathology. Saunders WB, Philadelphia, PA, pp 99–158

Hollingworth B (1996) Pain control during insertion of an intrauterine device. Br J Fam Planning 21:102–103

Hubacher D, Reyes V, Lilli S, Zepeda A, Chen PL, Croxatto H (2006) Pain from copper intrauterine device insertion: randomized trial of prophylactic ibuprofen. Am J Obstet Gynecol 195:1272–1277

Hurskainen R (2006) Managing drug-resistant essential menorrhagia without hysterectomy. Best Pract Res Clin Obstet Gynecol 20(5):681–694

Inki P (2007) Long-term use of the levonorgestrel-releasing intrauterine system. Contraception 75:S161–S166

International Menopause Society (2008) HRT in the early menopause: scientific evidence and common perceptions. Summary of the First IMS Global Summit on menopause-related issues. 2008 (www.international menopause society + statements)

Jacobs A, Butler EB (1965) MBL in iron deficiency anemia. Lancet 11:407–409

Jamieson DJ, Kaufman SC, Costello C, Hillis SD, Marchbanks PA, Peterson HB (2002) A comparison of women's regret after vasectomy versus tubal sterilization. Obstet Gynecol 99:1073–1079

Janssen CA, Scholten PC, Heintz APM (1995) A simple visual assessment technique to discriminate between menorrhagia and normal menstrual blood loss. Obstet Gynecol 85:977–982

Kaivola S (1990) Postremoval assessment of the intrauterine position of the IUDs: an analysis of 1012 removals with special reference to pregnancy risk and dislocation. Ann Meet Soc Advancement Contraception, Singapore

Karim R (2008) Relationship between serum levels of sex hormones and progression of subclinical atherosclerosis in postmenopausal women. J Clin Endocrinol Metab 93:131–138

Kaunitz AM (2007) Progestin-releasing intrauterine systems and leiomyoma. Contraception 75:S130–S133

Kosonen A (1981) Factors influencing the dissolution of copper *in utero*. Contracept Deliv Syst 2:77–85

Kurman RJ (1995) Diseases of the Vagina. In: Kurman RJ (ed) Blaustein's pathology of the female genital tract, 4th edn. Springer, Berlin, p 85

Kurz KH (1984) Cavimeter uterine measurements and IUD clinical correlation. In: Zatuchni GI, Goldsmith A, Sciarra JJ (eds) Intrauterine Contraception: Advances and Future Prospects. Harper & Row, Philadelphia, pp 142–162

Lassise DL, Savitz DA, Hamman RF, Barón AE, Brinton LA, Levines RS (1991) Invasive cervical cancer and intrauterine device use. Int J Epidemiol 20:765–770

Lippes J, Malik T, Tatum HJ (1976) The postcoital copper-T. Adv Plan Parent 11:24–29

Lumsden MA, Kelly RW, Baird DT (1983) Primary dysmenorrhea: the importance of both prostaglandins E_2 and $F_{2\alpha}$. Br J Obstet Gynecol 90:1135–1140

Magalhães J, Aldrighi JM, de Lima GR (2007) Uterine volume and menstrual patterns in users of the levonorgestrel-releasing intrauterine system with idiopathic menorrhagia or menorrhagia due to leiomyomas. Contraception 7:193–198

Manson JE et al (2007) For the WHI and WHI-CACS investigators. Estrogen therapy and coronary-artery calcification. N Engl J Med 356:2591–2602

Mansour D (2006). Changing contraceptive needs in different life styles of a Mirena® user. 9th Congr Eur Soc Contraception. Istanbul, Turkey

Mansour D (2007) Copper IUD and LNG IUS compared with tubal occlusion. Contraception 75:S144–S151

Maruo T, Oharaa N, Matsuoa H, Xua Q, Chena W, Sitruk-Ware R, Johansson E (2007) Effects of levonorgestrel-releasing IUS and progesterone receptor modulator PRM CDB-2914 on uterine leiomyomas. Contraception 75:S99–S103

Meirik O, Rowe PJ, Peregoudov A, Piaggio G, Petzold M (2009) For the IUD Research Group at the UNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction. The frameless copper IUD (GyneFix) and the TCu380A IUD: results of an 8-year multicenter randomized comparative trial. Contraception 80:133–41

Mikkola TS (2008). Is there a critical therapeutic window for starting HRT? 12th World Congr Menopause, Madrid (Spain), Abstract Book p. 4

Milsom I (2007) The levonorgestrel-releasing intrauterine system as an alternative to hysterectomy in peri-menopausal women. Contraception 75:S152–S154

Mishell D (1998) Intrauterine devices: mechanisms of action, safety, and efficacy. Contraception 58:45S–53S

Moreau C, Cleland K, Trussell J (2007) Contraceptive discontinuation attributed to method dissatisfaction in the United States. Contraception 76:267–272

National Institute of Clinical Excellence (NICE) (2005). Long-acting reversible contraception? The effective and appropriate use of long-acting reversible contraception. www.nice.org.uk.
National Institute for Health and Clinical Excellence (NICE). Heavy Menstrual Bleeding. http://www.rcog.org.uk/news/rcog-release-variations-treating-heavy-menstrual-bleeding-found
Palma S, Perez-Iglesias R, Prieto D, Pardo R, Llorca J, Delgado-Rodriguez M (2008) Iron but not folic acid supplementation reduces the risk of low birthweight in pregnant women without anaemia: a case control study. J Epidemiol Community Health 62:120–124
Parazzini F, La Vecchia C, Negri E (1992) Use of intrauterine device and risk of invasive cervical cancer. Int J Epidemiol 21:1030–1031
Petersen KR, Brooks L, Jacobson B, Skouby SO (1990) Intrauterine devices in nulliparous women. Proc 1st Congr Eur Soc Contraception, Paris, France
Peterson HD (2008) Sterilization. Obstet Gynecol 111:189–203
Pines A, Sturdee DW, MacLennan AH, Schneider HPG, Burger H, Fenton A (2007) The heart of the WHI study: time for hormone therapy policies to be revised. Climacteric 10:267–269
Population Reports (1995) Johns Hopkin's University, Baltimore. IUDs - An Update. Series B, Number 6
Prager S, Darney PD (2007) The levonorgestrel intrauterine system in nulliparous women. Contraception 75:S12–S15
Pratapkumar AV (2006) A comparison of transdermal and oral HRT for menopausal symptom control. Int J Fertil Womens Med 51:64–69
Qi X, Zhao W, Duan Y, Li Y (2008) Successful pregnancy following insertion of a levonorgestrel-releasing intrauterine system in two infertile patients with complex atypical endometrial hyperplasia. Gynecol Obstet Invest 65:266–268
Reeves MF, Smith KJ, Creinin MD (2007) Contraceptive effectiveness of immediate compared to delayed insertion of intrauterine devices after abortion: a decision analysis. Obstet Gynecol 109:1286–1294
Rietzschel E, de Buyzere M, de Backer D, Bekaert S, Segers P, cassiman P (2007) Asklepios investigators Abstract 3614: anticonceptive drug use and increased carotid and femoral plaque prevalence: population data from asklepios. Circulation 116:II_820
Rizkalla HF, Higgins M, Kelehan P, O'Herlihy C (2008) Pathological findings associated with the presence of a Mirena intrauterine system at hysterectomy. Int J Gynecol Pathol 27:74–78
Royal College of Obstetricians and Gynaecologists (2007) Management of premenstrual syndrome. Green-top guideline No. 48 (http://www.rcog.org.uk/)
Saay I, Aronsson A, Marions L, Stephansson O, Gemzell-Danielsson K (2007) Cervical priming with sublingual misoprostol prior to insertion of an intrauterine device in nulliparous women: a randomized controlled trial. Hum Reprod 22:2647–2652
Sewell WR, Wiland J, Craver BN (1955) A new method of comparing sutures of ovine catgut with sutures of bovine catgut in three species. Surg Gynecol Obstet 4:483–494
Sitruk-Ware R (2007) The levonorgestrel intrauterine system for use in peri- and postmenopausal women. Contraception 75:S155–S160
Sitruk-Ware R (2008) Progesterone receptor modulators: Clinical applications. 12th World Congress on the Menopause, Madrid (Spain), Abstract Book p. 19
Sivin I, Shaaban M, Odlind V, Olsson SE, Diaz S, Pavez M, Alvarez F, Brache V, Diaz J (1990) A randomized trial of the Gyne T 380 and Gyne T 380 Slimline intrauterine copper devices. Contraception 42:379–389
Sturridge F, Guillebaud J (1996) A risk-benefit assessment of the levonorgestrel-releasing intrauterine system. Drug Safety 15:430–440
Suhonen S, Haukkamaa M, Jakobsson T, Rauramo I (2004) Clinical performance of a levonorgestrel-releasing intrauterine system and oral contraceptives in young nulliparous women: a comparative study. Contraception 69:407–412
Task Force on Postovulatory Methods of Fertility Regulation (1998) Randomised controlled trial of levonorgestrel versus the Yuzpe regimen of combined oral contraceptives for emergency contraception. Lancet 352:428–433

Tatum HJ, Connell EB (1989) Intrauterine devices. In: Flilshie M, Guillebaud J (eds) Contraception: science and practice. Butterworths, London, pp 144–171

The North American Menopause Society (NAMS) (2003) Role of progestogen in hormone replacement therapy for postmenopausal women: position statement of The North American Menopause Society. Menopause 10:113–132

Thiery M (1985) Pain relief at insertion and removal of an IUD: a simplified technique for paracervical block. Adv Contracept 1:167–170

Trussell J (1998) Contraceptive efficacy. In: Hatcher RA, Trussell J, Stewart F, Cates W, Stewart GK, Kowal D, Guest F (eds) Contraceptive technology, 17th revised edn. Ardent Media, New York, NY

Trussell J, Ellertson C (1995) Efficacy of emergency contraception. Fertil Control Rev 4:8–11

UNDP, UNFPA and WHO Special Programme for Research, Development and Research Training in Human Reproduction. World Bank: IUD Research Group (1995) The TCu380A IUD and the Frameless "FlexiGard", interim three-year data from an international multicenter trial. Contraception 52:77–83

Van Kets H, Parewijck W, Kleinhout J, Osler M, Zighelboim I, Tatum H (1991) Clinical experience with the Gyne-T postpartum intrauterine device. Fertil Steril 4:197–205

Van Kets H, Parewijck W, Van der Pas H et al (1993) Immediate postplacental insertion and fixation of the CuFix postpartum implant system. Contraception 48:349–357

van Van Houdenhoven K, Kaam KJAF, van Grootheest AC, Salemans TH, Dunselman GA (2006) Uterine perforation in women using a levonorgestrel-releasing intrauterine system. Contraception 73:257–260

Varma R, Soneja H, Bhatia K, Ganesan R, Rollason T, Clark TJ, Gupta JK (2008) The effectiveness of a levonorgestrel-releasing intrauterine system (LNG-IUS) in the treatment of endometrial hyperplasia- a long-term follow-up study. Eur J Obstet Gynecol Reprod Biol 139:169–175

Vitale C, Mercuro G, Cerquetani E, Marazzi G, Patrizi R, Pelliccia F, Volterrani M, Fini M, Collins P, Rosano GM (2008) Time since menopause influences the acute and chronic effect of estrogens on endothelial function. Arterioscler Thromb Vasc Biol 28:348–352

Wagner H (1999) Intrauterine contraception: past, present, and future. In: Rabe T, Runnebaum B (eds) Fertility Control: Update and Trends. Springer, Berlin, pp 151–171

Webb AMC (1997) Intrauterine contraceptive devices and antigestagens as emergency contraception. Eur J Contracept Reprod Health Care 2:243–246

Westhoff CL, Heartwell S, Edwards S, Zieman M, Stuart G, Cwiak C, Davis A, Robilotto T, Cushman L, Kalmuss D (2007) Oral contraceptive discontinuation: do side effects matter? Am J Obstet Gynecol 196(412):e1–e6 discussion 412.e6-7

Wildemeersch D (2004) The force required to remove the frameless 0-suture anchoring system: comparison between pre- and postmenopausal women. Contraception 69:513–515

Wildemeersch D (2006) Ease and safety of insertion, contraceptive performance and effect on menstrual blood loss of a new LNG-IUS, Femilis, in parous and nulliparous women. 9th Congr Eur Soc Contraception. Istanbul, Turkey

Wildemeersch D, Batár I, Affandi B, Andrade ATL, Wu S, Hu J, Cao X (2003) The 'frameless' intrauterine system for long-term, reversible contraception: A review of 15 years of clinical experience. J Obstet Gynaecol Res 29:160–169

Wildemeersch D, Rowe PJ (2004a) Assessment of menstrual blood loss in Belgian users of the frameless copper-releasing IUD with copper surface are of 200 mm and users of a copper-levonorgestrel-releasing intrauterine system. Contraception 70:169–172

Wildemeersch D, Rowe PJ (2004b) Assessment of menstrual blood loss in women with idiopathic menorrhagia using the frameless levonorgestrel-releasing intrauterine system. Contraception 70:165–168

Wildemeersch D, Janssens D, Andrade A (2009) The Femilis® LNG-IUS: Contraceptive performance – an interim analysis. Eur J Contracept Reprod Health Care 14:1–8

Wildemeersch D, Rowe PJ (2005) Assessment of menstrual blood loss in Belgian users of a new T-shaped levonorgestrel-releasing intrauterine system. Contraception 71:470–473
Wildemeersch D, Schacht E (2001) Treatment of menorrhagia with a novel "frameless" intrauterine levonorgestrel-releasing drug delivery system, a pilot study. Eur J Contracept Reprod Health Care 6:93–101
Wildemeersch D, Schacht E (2002) The effect on menstrual blood loss in women with uterine fibroids of a novel 'frameless' intrauterine levonorgestrel-releasing drug delivery system, a pilot study. Eur J Obstet Gynecol Reprod Biol 102:74–79
Wildemeersch D, Defoort P, Thiery M, Tatum H (1986) A new insertion-fixation instrument and technique for immediate postplacental insertion of intrauterine contraceptive devices. Ann Meet Am Fertility Soc, Toronto
Wildemeersch D, Schacht E, Wildemeersch P (2001) Treatment of primary and secondary dysmenorrhea with a novel "frameless" intrauterine levonorgestrel-releasing drug delivery system: a pilot study. Eur J Contracept Reprod Health Care 6:192–198
Wildemeersch D, Schacht E, Wildemeersch P (2003) Performance and acceptability of intrauterine release of levonorgestrel with a miniature delivery system for hormonal substitution therapy, contraception, and treatment in peri- and postmenopausal women. Maturitas 44:237–245
Wildemeersch D, Janssens D, Schacht E, Pylyser K, De Wever N (2005a) Intrauterine levonorgestrel delivered by a frameless system, combined with systemic estrogen: acceptability and endometrial safety after three years of use in peri- and postmenopausal women. Gynecol Endocrinol 20:336–342
Wildemeersch D, Janssens D, Vrijens M, Weyers S (2005b) Ease of insertion, contraceptive efficacy and safety of new T-shaped levonorgestrel-releasing intrauterine systems. Contraception 71:465–469
Wildemeersch D, Janssens D, Weyers S (2005c) Continuous combined parenteral estrogen substitution and intrauterine progestogen delivery: the ideal HST combination? Maturitas 51:207–214
Wildemeersch D, Pylyser K, De Wever N, Dhont M (2007a) Treatment of non-atypical and atypical endometrial hyperplasia with a levonorgestrel-releasing intrauterine system: long-term follow-up. Maturitas 57:210–213
Wildemeersch D, Pylyser K, De Wever N, Pauwels P (2007b) Endometrial safety after 5 years of continuous combined transdermal estrogen and intrauterine levonorgestrel delivery for postmenopausal hormone substitution. Maturitas 57:205–209
World Health Organization (1983) Intrauterine devices: their role in family planning care. Geneva, (WHO offset publication No. 75) p 53
World Health Organization (2002) Prog Reproduct Health Res http://www.who.int/reproductive health/en/
Wu S, Hu J, Wildemeersch D (2003) Performance of the frameless GyneFix and the TCu380A IUDs in a three-year multicenter randomized comparative trial in parous women. Contraception. 61:91–89
Xiao S (1997) The role of scientific research and the scientific institute in the family planning program in China. In: Proc Beijing Intern Sympos Fertility Regulation, 1995. Nat Inst Child Health and Human Develop, Publication No 97-4118
Zipper JA, Tatum HJ, Medel M, Pastene L, Rivera M (1971) Contraception through the use of intrauterine metals. I. Copper as an adjunct to the "T" device. The endouterine copper "T". Am J Obstet Gynecol 109:771–774

Improving Oral Delivery

Franz Gabor, Christian Fillafer, Lukas Neutsch, Gerda Ratzinger, and Michael Wirth

Contents

Abstract It is estimated that 90% of all medicines are oral formulations and their market share is still increasing, due to sound advantages for the patient, the pharmaceutical industry and healthcare systems. Considering biopharmaceutical issues such as physicochemical requirements of the drug and physiological conditions, however, oral delivery is one of the most challenging routes. Recognising solubility, permeability and residence time in the gastrointestinal milieu as key parameters, different characteristics of drugs and their delivery systems such as size, pH, density, diffusion, swelling, adhesion, degradation and permeability can be adjusted to improve oral delivery. Future developments will focus on further improvement in patient compliance as well as the feasibility of administering biotech drugs via the oral route.

F. Gabor (✉)
Department of Pharmaceutical Technology and Biopharmaceutics, Universität Wien, Althanstrasse 14, A-1090, Vienna, Austria

M. Schäfer-Korting (ed.), *Drug Delivery*,
Handbook of Experimental Pharmacology 197,
DOI 10.1007/978-3-642-00477-3_12,

Keywords G/I-physiology · Absorption enhancer · pH-dependent release · Enzyme-mediated release · Floating delivery systems · Hydrogel · Microparticle · Nanoparticle · Biorecognition · Targeting · Osmotic pump · Gastroretentive · Delayed-release

Abbreviations

AAL	*Aleuria aurantia* lectin
API	Active pharmaceutical ingredient
BCS	Bioclassification System
EGF	Epidermal growth factor
EOP	Elementary osmotic pump
EPAS	Evaporative precipitation into aqueous solution
FAE	Follicle associated epithelium
FDA	Food and drug administration
FDDS	Floating drug delivery system
GALT	Gut-associated lymphoid tissue
GI	Gastrointestinal
GRAS	Generally recognised as safe
HBS	Hydrodynamically balanced system
HEC	Hydroxyethyl cellulose
HPMC	Hydroxypropylmethyl cellulose
IBD	Inflammatory bowel diseases
LEA	*Lycopersicum esculentum* agglutinin
MC	Methyl cellulose
OPV	Oral poliovirus vaccine
PEG	Polyethylene glycol
PVP	Polyvinylpyrrolidone
SFL	Spray freezing into liquid
SOTS	Sandwiched osmotic tablet system
UEA	*Ulex europaeus* agglutinin
WGA	Wheat germ agglutinin

1 Introduction

Despite tremendous innovations in the field of drug delivery and the acquisition of detailed knowledge about promising alternative routes of administration, it is estimated that 90% of all medicine usage is in oral form and oral drug delivery systems comprise more than half the drug delivery market. In 2008, the oral drug delivery market is a USD 35 billion industry and is expected to grow as much as

10% per year until at least 2012. Among liquid and semi-solid formulations, the tablet is still the preferred drug product for oral administration, offering sound advantages.

For the patient, peroral administration is the most comfortable mode of therapy. It is painless as compared to injections, convenient compared to enemas, self-manageable without any training and, at least at first sight, easy to use. In practice, however, some basics have to be considered upon application to guarantee adequate oral absorption even of technologically skilled delivery systems: First, the head should be held up to avoid constriction of the pharynx causing reduced patency. Second, the oral dosage form should be co-swallowed with a minimum of 100 ml water of the reel. Otherwise the formulation can adhere in the esophagus, usually in the upper third. When swallowing a 12.5-mm tablet in the supine position without water, orogastral passage was observed in only two volunteers out of 20. Increasing the volume of water to 100 ml led to 92% successful passages (Gallo et al 1996). Upon oesophageal adhesion, repeated sipping of even higher volumes of water did not help to detach the formulation. This is especially important with administration of sticky drugs such as bisphosphonates, tetracyclines, penicillins, non-steroidal anti-inflammatory drugs and theophylline. Third, an upright position of the body or at least 45° inclined is beneficial even for a bedridden patient to guarantee rapid transit into the stomach. In addition, to provide for successful absorption some tablets must not be split into parts (van Santen et al. 2002; Hussar 2000). In the case of enteric-coated and dry-coated tablets, the protective layer would be disrupted and osmotic tablets lose their release characteristics. Accordingly, tablets containing microgram quantities of active pharmaceutical ingredients split into half would suffer from lack of dose uniformity, and fragments of tablets less than 50 mg in initial weight are tricky to handle. Apart from technological problems, toxicological aspects for the nursing staff have to be considered on crushing tablets, especially those containing highly active substances such as hormones and their antagonists, cytostatics, antivirals and immunsuppressives. In addition, sustained release oral dosage forms improve patient comfort as well as compliance by reducing the dosing frequency.

Apart from convenience for the patient, oral drug products are also profitable for the pharmaceutical industry. Nowadays, oral formulations are produced in large quantities with short production times due to skillful automation of the manufacturing process. Adhering to the good manufacturing practice (GMP)-regulations, oral delivery products of high quality are prepared in a reliable and reproducible manner. After covering the costs of development, the ease of preparation results in bulk products being cost-effective and profitable.

Finally, the healthcare system takes financial advantage of simple usage of oral delivery systems without any difficulties. Especially in view of the growing elderly population and consequent rising costs for healthcare, oral delivery products allow for cost-saving therapies without skilled medical intervention.

Consequently, it seems that oral dosage forms are the ideal formulations for therapy. From a biopharmaceutical point of view, however, oral delivery is one of the most challenging routes of administration. As the major task of the

gastrointestinal (GI) tract is absorption of nutrients, it offers a large absorptive area and high blood supply which also supports absorption of drugs. At the same time, however, there are highly efficient life-saving systems and pathways to degrade and inactivate compounds harmful to the body. Thus, improvement of oral absorption by tuning formulation factors is a balancing act considering GI physiology as well as physicochemical properties of the active pharmaceutical ingredient.

1.1 Physicochemical Considerations

Although reports on the peroral uptake of nanoparticles disproved the paradigm that exclusively dissolved matter can be absorbed, it is commonly accepted for the majority of formulations that dissolved molecules of active pharmaceutical ingredients (API) are transported across membranes, considering that passive diffusion strongly prevails over active transport in the case of drug molecules (Florence 1997; Kararli 1989). An ideal absorption process requires a maximum water-solubility for rapid dissolution in the body fluids and for diffusion according to Fick's law. At the same time a minimal lipophilicity is a prerequisite for permeation across the phospholipid bilayer of the cellular membrane. To estimate the lipid solubility of an API, the equilibrium partition of APIs between a hydrophilic and a lipophilic phase followed by calculation of log *P* for non-dissociable APIs or log *D* for acidic or basic API molecules is commonly applied. Due to changing pH during GI transit, only log *D* is meaningful on peroral administration of ionizable APIs. As a rule of thumb, for sufficient absorption of an API its log *P* should range between –1 and 3.4.

In an effort to calculate absorption parameters for drug discovery and early stages of development, the so-called "rule of five" was proposed to predict the drug-like properties of a substance from solubility and permeability parameters. Accordingly, poor absorption or permeation is more likely when there are more than five H-bond donors (–OH and –NH groups), 10 H-bond acceptors, the molecular weight is greater than 500 and the calculated log *P* is greater than 5 (Lipinski et al. 2001). Only drug substances that are substrates for transporters are excluded from this rule.

In view of the fact that nearly a third of the drug substances listed in the United States Pharmacopeia (USP25) are poorly soluble or insoluble in water, chemical and/or technological tricks are necessary to guarantee absorption of drugs (Langguth et al. 2004). Optimising solubility may include using a more soluble salt, e.g. the aqueous solubility of tetracycline is enhanced six-fold by formation of the hydrochloride salt or nine-fold in the case of the phosphate. Attention should be paid to the fact that the presence of excessive chloride ions in gastric acid can depress solubility, in vivo resulting in salting-out of the drug (Thomas and Rubino 1996). A study dealing with the evaluation of seven salt forms of a protein kinase inhibitor revealed that the mesylate monohydrate salt was five times more soluble, more stable, and 2.6 times more bioavailable after oral administration to beagles easier to process than the hydrochloride salt (Engel et al. 2000). Correspondingly,

the solubility of weakly acidic drugs might be enhanced by salt formation with cations. Although the amount of counter ions is usually too low to provoke adverse effects, it should be considered that potassium can be a GI irritant, magnesium can be a laxative and calcium can be a constipant.

When a drug exists in a variety of crystalline forms, they can have different solubilities. Out of different polymorph forms, the metastable one with highest free energy exhibits highest solubility and thus greater absorption than a stable polymorph. For example, the hydrophobic oral hypoglycaemic agent tolbutamide can occur in four polymorphs with different conformations and motility of the toluene and *n*-butyl moieties (Kimura et al. 1999). The metastable polymorph with highest solubility converts itself to another metastable polymorph with nearly identical solubility during dissolution tests within 3 h. According to studies in beagles, the bioavailability of this latter metastable monoclinic polymorph was 2.5 times higher than that of the stable orthorhombic form. As this metastable form of tolbutamide requires at least 60°C and 75% relative humidity for transformation to the less soluble orthorhombic form, no problems are expected during processing, storage or dissolution of the final oral drug product.

Another metastable state of a drug is the amorphous form, which requires less energy for dissolution due to lack of crystalline lattices and thus exhibits higher bioavailability than the crystalline form. Amorphous forms of drugs can even evolve during pharmaceutical processing such as milling, spray drying, lyophilisation, granulation, compression and film coating. To avoid spontaneous crystallisation of amorphous drugs, the storage temperature of the drug formulation should be 50° lower than the glass transition temperature (Yoshioka et al. 1994). In the case of indomethacin with a melting point of about 50°C, co-precipitation with 5% PVP was reported to retain the amorphous state up to 70°C, which allows storage at ambient temperature (Yoshioka et al. 1995).

In addition, co-medication to modify motility or pH, use of tensides or complexation for solubilisation can also improve solubility as a prerequisite for absorption (see Sect. 2.8).

Apart from the physicochemical properties of the drug, the physiology of the GI tract dictates the absorption characteristics of the active pharmaceutical ingredient from the oral delivery system.

1.2 Physiological Conditions in the GI Tract

When a formulation is administered perorally, it meets quite different environmental conditions: a friendly or hostile milieu, a short- or long-term stay, exposure to light or even heavy propulsions. It is obvious that these conditions strongly influence the absorption of the active pharmaceutical ingredient. These environmental conditions are regionally different throughout the GI tract and reflect the anatomy of the stomach and intestine. For some characteristics see Table 1.

Table 1 Some physiological characteristics of the gastrointestinal tract

Section	Length (m^2)	Absorptive area (m^2)	Diameter (cm)	Maximum filling volume (ml)	Fluid volume fasted (ml)[a]	Fluid volume fed (ml)[a]	Fasted pH	Residence time of single oral dosage form	Enzymes	Microorganisms (counts per g content)
Oral cavity	0.15–0.20	–	10	–	–	–	7.0	–	Amylase	–
Oesophagus	0.2–0.3	0.02	2.5	–	–	–	6.0–7.0	3.5 s	–	–
Stomach	0.2	0.1	15	1,500	45 (13–72)	686[b] (534–859)	2.1 (men) 2.8 (women)	Up to 2 h fasted, 2–8 h fed	Pepsin, lipase,	10
Small intestine	7.0	200	–	8,500	105 (45–319)	54 (20–156)	5.5–8.0	3–4 h	Bile, peptidases, lipases, amylase	–
Duodenum	0.2–0.3	0.1	5	500	–	–	5.5–6.5	>5 min	–	10
Jejunum	2.5	60	5	5,000	–	–	6.1–7.1	1–2 h	–	10^5
Ileum	3.5	60	2.5–5.0	3,000	–	–	7.0–8.0	2–3 h	–	10^7
Large intestine	1.5	0.3	–	–	13 (1–44)	11 (2–97)	–	15–48 h	Diverse bacterial enzymes	10^{11}
Caecum	–	0.05	7.0	–	–	–	–	–	–	–
Colon	–	0.25	5.0	3,000	–	–	8.0	–	–	10^{11}
Rectum	0.15	–	2.5	500	–	–	7.0	–	–	–

[a]Mean and extremes in parenthesis; [b]Represents filling volume, not only fluid

Sources: Feldman and Barnett (1991), Rouge et al. (1996), Chawla et al. (2003), Schiller et al. (2005)

1.2.1 Oral Cavity and Oesophagus

Upon administration, the oral dosage form is in contact with saliva, albeit briefly. In man, 0.5–1.5 l saliva is secreted per day, exhibiting a mean pH of 6.4 within the extremes of 5.8 and 7.1. Containing 6 g l^{-1} dry substances, the ptyalin in saliva digests starches and the mucin lubricates solid matter. Except for special buccal bioadhesive or sublingual formulations containing readily absorbable lipophilic drugs, the contact time with the epithelium of the mouth is too short for noteworthy absorption (Smart 2005).

Upon swallowing, the dosage form usually passes the oesophagus rapidly. This transversal and longitudinal highly folded muscular tube 2 cm in diameter joins the pharynx to the cardia of the stomach. Embedded in the submucosa, about 300 glands secrete mucus onto the squamous epithelium, providing moist conditions. Controlled by the medulla oblongata, a normal adult swallows 600–700 times per day, but the frequency is reduced to a tenth during night. According to magnetic marker monitoring of a capsule 16.1 mm in length and 5.5 mm in diameter, the oesophageal transit time lies in the range of less than 10 s and further decreases to about 1.4 s under optimum conditions with increasing volume of co-swallowed water (Weitschies et al. 2005). Whereas an upright body position accelerates the passage compared to a supine one, the oesophageal transit time seems to be independent of the propulsive motor function of the oesophagus. Although the submucosa contains large blood vessels, the short contact time usually excludes any absorption of drugs.

1.2.2 Stomach

After a hopefully unimpeded passage of the cardia, which represents a 5–6 cm long zone of 15–40 mmHg pressure above intragastric one rather than a “spincter”, the drug delivery system enters the stomach. The mucosa of this highly expandable organ contains about 3.5 million gastric pits with four glands each, yielding a density of about 100 gastric pits per square mm of surface. Four types of secreting cells lining these gastric pits secrete mucus as well as up to 1.5 L of gastric juice per day. The gastric juice contains predominantly pepsin, hydrochloric acid, but also gastrin, intrinsic factor, 1.7 g L^{-1} dissolved mucins as well as other compounds. This acidic milieu aids dissolution of basic APIs as a first step towards absorption and efficacy; on the other hand peptides or other acid-labile drugs can be denaturated and/or proteolytically degraded, losing efficacy. At higher magnification, the mucosa consists of a monolayer of columnar epithelial cells 20–40 μm in height which is covered by a viscoelastic mucus layer of mean height 140 μm. The mucus layer with a mean renewal time of 4–5 h establishes a pH gradient ranging from pH 2 in the lumen to neutral pH at the cell surface due to bicarbonate secretion. Thus, the degree of ionisation of basic drugs is converted towards the non-dissociated

form which favors diffusion across the cell membrane. Contrarily, the mucus layer also represents a diffusional barrier against absorption for even small molecules.

Generally, there are two main parameters affecting the gastric fate of a drug delivery system and therefore also absorption: the pH and the gastric retention time (see Table 1). Whereas in fasted humans the gastric pH ranges between 1.3 and 2.1 as determined by the Heidelberg capsule, it is strongly variable after a meal (Dressman 1986). Food such as dairy products can buffer and neutralise gastric acid, but the pH can even become slightly alkaline, provoking, e.g., degradation of enteric coats followed by premature release of acid-sensitive or irritant drug substances.

Being also strongly dependent on the fed or fasted state, gastric emptying determines the arrival of the formulation in the small intestine and thus represents a key parameter for reaching the absorption site. The "interdigestive migrating motor complex" occurs every two hours in the fasted state or after dietary intake as soon as digestible contents leave the stomach. It starts with a lag-phase of no motility for 30–45 min (phase I). Lasting for about 30 min, irregular mixing contractions (phase II) are followed by powerful circular contracting waves, the so-called housekeeper waves, sometimes (hopefully faintly) audible as borborygmi (phase III). These waves protrude all indigestible material including tablets through an open pylorus to the small intestine where the propulsions slowly decline. After 5–15 min of highest activity a resting phase (phase IV) with negligible motor activity is entailed. In the fed state, the "antrum mill," repeated and irregular contractions at a rate of about three per minute mix, grind, repel and empty the stomach of material smaller than 1–2 mm. Thus, in the fasted state, single unit dosage forms larger than 4 mm remain in the stomach until the next housekeeper waves arrive, which might occur within a few minutes up to two hours. In the fed state, these larger monolithic formulations are exposed to the antral mill and they leave the stomach 2–8 h later. This time frame is strongly dependent on the composition of the diet. In general, a high fibre diet entails a longer time of drug absorption (40 min) than a no-fibre diet (30 min) upon administration of solid dosage forms. For comparison, the lag time of a liquid was quite short, amounting to 8–9 min (Walter et al. 1989). In addition, fats and oils prolong the gastric residence time due to inhibition of antral but concurrently increasing pylorus contractions (Houghton et al. 1990). However, uninterrupted food intake leaves no time for housekeeper waves to clean the stomach from large tablets, at least during the night. A problem observed in adipose patients associated with risky adverse reactions is "dose dumping". Once they stop eating for a few hours, all the collected tablets are released at once to the small intestine and provoke symptoms of heavy overdosing. On the contrary, multiple units smaller than 2 mm leave the stomach within 10–40 min in the fasted state, as they are processed like liquids. Upon feeding the residence time of pellets depends on their density. At a density similar to water, the pellets leave the stomach rapidly inside the central liquid stream. At a higher density they settle down to the greater curvature, escape antral milling and are emptied after the meal resulting in gastro-retention for more than 40 min. All in all, drugs are not efficiently absorbed in the stomach.

1.2.3 Small Intestine

Upon passage of the pylorus, the more or less deaggregated dosage form enters the small intestine with a mean diameter of 3–4 cm. Due to folding of the mucosa, the surface area of the epithelium as well as the apical membrane of epithelial cells is larger than a tennis court and favors the absorption of nutrients as well as drug molecules. In spite of similar histology, the absorptive capacity and the secretions are quite different in the duodenum, jejunum and ileum. In man, the absorptive villi in the duodenum are short and broad, in the jejunum they are higher and most numerous, and they become fewer and smaller in the ileum. It was estimated that the mucosal area per centimeter serosal length is about 98 cm in the jejunum, decreasing to about 20 cm in the lower ileum (Kompella and Lee 2001). In addition, the high permeability of the epithelium to small molecules decreases down the small intestine. This underlines the excellent absorptive capacity of the duodenum, which might be counteracted by rapid duodenal transit of seconds up to a few minutes (Schiller et al. 2005).

Moreover, absorption is supported by a high blood perfusion which maintains the concentration gradient of the drug between the intestinal lumen and the plasma. Nearly one third of the cardiac output flows through the gastrointestinal organs with 10% supplying the small intestine at a flow rate of 500 ml min^{-1}. Interestingly, the villi receive 60% of this blood flow (Washington et al. 2002).

There are also different secretions which support or counteract dissolution, integrity and absorption. About 0.7–2.5 l of isotonic pancreatic juice of pH 7.0–7.7 is secreted per day into the duodenum. It contains a wide array of different enzymes including proteases and lipases as well as amylases which can activate prodrugs, degrade protein pharmaceuticals, and increase osmotic pressure by splitting polysaccharides into low molecular weight oligosaccharides.

Additionally, the biliary secretion supports absorption. Up to 600 ml hepatic bile is produced per day, concentrated and pooled in the gallbladder with a volume of 50–65 ml. Concurrent with housekeeper waves or upon ingestion of a meal, bile is released into the duodenum. Containing natural emulsifiers such as bile salts, lecithin and cholesterol, lipids are thereby emulsified in small droplets enabling degradation by lipases. The resulting free fatty acids form mixed micelles with bile acids and most probably accumulate at the cell surface where the fatty acid is absorbed. The bile salts are reabsorbed in the ileum to close the enterohepatic circle. It has been suggested that lipophilic drugs are absorbed by this fat absorption pathway (Florence and Attwood 2006).

To neutralise the acidic chyme and to establish the optimal pH for proteolytic activity, the Brunner's glands located in duodenal crypts secrete bicarbonate as well as mucus. Considering that pH is a logarithmic scale, this change markedly affects the degree of ionisation, the aqueous solubility and therefore the efficiency of absorption. Although different ranges of pH are reported for the anatomical regions of the small intestine, there is a consensus that the duodenal pH is slightly acidic to neutral and the pH remains more or less constant at pH 7.5 until dropping down to pH 5.5 beyond the ileocecal junction (Lui et al. 1986; Hardy et al. 1987).

In addition to this luminal barrier against absorption comprising pH and enzymes, the mucus barrier is another fact to be considered. The Brunner's glands as well as goblet cells secrete granules of mucus which swell upon contact with water. In that way a viscous aqueous layer is formed with a mean thickness of 30–100 μm up to 192 μm adjacent to the absorptive enterocyte. This so-called unstirred water layer acts as a diffusional barrier, sometimes becoming a rate limiting step in absorption (Allen 1984). Interestingly, pH measurements with microelectrodes revealed an acidic microclimate in the range of pH 4.5–6.0 adjacent to the cell surface (Washington et al. 2002). Although weak bases might thus be poorly absorbed, this hypothesis was experimentally disproved.

Another driving force of absorption is the convective water flow. The proximal small intestine absorbs about 8 l water per day at a flow rate of 50 ml min^{-1} (Schiller et al. 2005), probably resulting from both slow passive water transport due to differences in osmotic pressure between blood and luminal contents and due to differences in hydrostatic pressure resulting from contractions, as well as rapid active water transport via aquaporins (King et al. 2004). There is an experimentally confirmed phenomenon that absorption of acidic and basic drugs increased concurrently with increasing water absorption, which contributes to successful absorption in the intestine. This so-called solvent drag might be simply explained by the fact that removal of water concentrates the API and thus promotes diffusion across the mucosal epithelium (Florence and Attwood 2006). As confirmed by magnetic resonance imaging in fasted volunteers, the fluid volume is not homogenously distributed throughout the small intestine, but separated pockets filled with about 12 ml water were detected especially in the distal region. After a meal, this volume decreased from 12 ml to 4 ml and the number concurrently increased (Schiller et al. 2005). This observation raises questions about the impact of this segmentation on impeded absorption of drugs from non-deaggregating dosage forms resulting in intra- and inter-individual fluctuations of plasma levels.

The residence time is another crucial factor determining the extent of absorption. After a meal, the motility pattern in the small intestine comprises both longitudinal contractions of about 2 cm segments, which mix the chyme and expose it to the absorptive surface, as well as peristaltic contractions which propel the food towards the large bowel in a cycle of 1–3 contractions followed by 5–40 s of inactivity. In the fasted state the "interdigestive migrating motor complex" proceeds from the stomach to the small intestine but with decreasing intensity. In contrast to the stomach, the residence time of solutions, pellets and single units was found to be in the same range of about four hours and was not influenced by food (Davis et al. 1987). Compared to tablets, the residence time of microparticles was longer and more reproducible, leading to more predictable plasma levels.

A meal exerts no influence on the residence time of the formulation in the small intestine, unlike the stomach, but it can markedly affect absorption of the dissolved API. Binding of the API to dietary proteins, adsorption to food or competition of the API with dietary compounds for carrier proteins, among other factors, might reduce the absorbed fraction. In addition, the viscous chyme can limit access of the dissolved API to the absorptive epithelium. Conversely, the bioavailability of

drugs with high first-pass effect is enhanced in the presence of food. While the absorptive process itself is not affected, the absorbed fraction increases due to saturation of metabolising brush border and liver enzymes as well as increased prandial blood flow (Melander 1987).

In the small intestine as well as in the large intestine absorption occurs not only to the blood compartment, but also to the lymphatics. The open-ended lymphatic capillaries in the villi are involved in fat absorption; moreover, there is also a local immune system. The gut-associated lymphoid tissue (GALT) consists of single and clustered specialised epithelial cells, the so-called M cells. These oval or rectangular accumulations of M cells are termed Peyer's patches and they are usually located at the mesenteric border of the intestine. The size varies individually from a few millimeters up to extremes of 28 cm length in adults, comprising up to 25% of the GI mucosa. The number of patches increases distally towards the colon. The utility of this route for absorption of particles and vaccination is described below.

In addition, absorption is not uniform throughout the GI tract, but there are local differences in drug absorption. These "absorption windows" are attributed to regional differences in pH, composition and thickness of mucus, surface area and enzyme activity. According to studies in humans, e.g. piretanide is absorbed from the stomach and duodenum (Brockmeier et al. 1986), ciprofloxacin (Harder et al. 1990), captopril (Hu and Amidon 1988) and metoprolol (Jobin et al. 1985) from the small intestine and diltiazem from the large intestine.

1.2.4 Large Intestine

The entrance of dosage forms into the large intestine is controlled by the ileocaecal junction which prevents reflux of large intestinal contents but can also retain larger dosage forms in the small intestine. After administration of a large single-unit dosage form to young and elderly volunteers, a hold-up time of 2–20 h in the large bowel has been reported (Metcalf et al. 1987). Generally, APIs are absorbed to a smaller extent in the large intestine than in the small intestine. The absorptive area is only 0.25% of that of the small intestine, which derives from lack of villi and reduced length, although the gut extends up to 8.5 cm in diameter at the caecum and diminishes to 2.5 cm at the sigmoid segment (Read et al. 1980). For comparison, the absorptive area per cm gut segment is about 1,700 cm^2 in the small intestine and 20 cm^2 in the large intestine. One of the main functions of the colon is the re-absorption of water and electrolytes, which also limits dissolution of drugs. The colon is capable of absorbing nearly 90% of water entering it (Debongie and Philips 1978). Up to 4 L water are absorbed per day at a rate of 2.7 ml min^{-1} to form a semisolid stool containing 60–85% water. The aqueous milieu for absorption is provided by the humidity of the chyme as well as by water pockets with a filling volume of 1–2 ml being irregularly distributed and less abundant in the transverse colon. After a meal, their number increases but their volume remains rather constant. As 90% of orally administered capsules were not in contact with fluid

pockets, the influence of these pockets on the absorption of drugs from solid dosage forms remains to be elucidated (Schiller et al. 2005).

Another function of the colon influencing absorption is mixing and lubrication of the luminal content. Goblet cells located in numerous crypts secrete mucus which reduces friction but also forms a mucus layer covering the absorptive enterocytes. In this way, the mucus layer acts as a diffusional barrier similar to 8% polyacrylamide gels (Smith et al. 1986). The mucus layer is part of a microclimate adjacent to the enterocytes providing a pH of 6.8, in contrast to the varying pH in the bulk phase (McNeil et al. 1987). Colonic motility comprises segmental contractions mainly in the proximal colon which mix the content and increase the contact with the mucosa. Antiperistaltic contractions propel the luminal content towards the ileum and therefore contribute considerably to the increased residence time of APIs and formulations in the colon. Finally, mass movements representing intense and prolonged contractions occurring 3–4 times per day purge the distal colon. Interestingly, coffee with or without caffeine increased motility of the colon within 4 min after consumption in eight responders but six volunteers did not respond (Brown et al. 1990). Altogether, the concerted action of the different motility patterns leads to a high residence time of 24–26 h for children and young adults which is further prolonged in elderly persons up to 110 h (Kirwan and Smith 1974). In healthy volunteers, the colonic residence time of tablets and capsules averaged between 20 and 30 h, offering sufficient time for absorption even at a low rate (Kompella and Lee 2001). In addition, small particles pass more slowly through the colon than large units (Hardy et al. 1985). However, increased dietary fibre decreases the residence time and can abolish any absorptive benefit.

Despite lower blood flow (8–75 ml min^{-1}) compared to the small intestine, colonic delivery opens promising perspectives for peptide and protein delivery because of strongly reduced protease activity, being 20-fold to 60-fold less than in the small intestine (Washington et al. 2002). In addition, there is a neutral luminal pH ranging from 6.4 in the ascending colon via 6.6 in the transverse colon to 7.0 in the descending colon (Evans et al. 1988). The colonic pH, however, is strongly variable and can rise up to 8.0 depending on the protein content of food or drop down in the case of a high fibre diet, therefore influencing drug absorption.

A unique feature of the colon is the manifold bacterial flora of more than 400 different species, of both aerobic bacteria mainly in the proximal colon and anaerobic bacteria in the caecum. Similar to a fermentation chamber, a wide array of reactions such as hydrolysis, aromatisation, reduction, esterification, decarboxylation and deamination are possible, which can influence the amount of API to be absorbed but is also exploited as a strategy for colon-targeted drug delivery (Edwards 1997). Digestion of cellulose by bacterial cellulases yields volatile compounds and these gas bubbles may reduce contact of the API with the mucosa. In addition, short chain fatty acids are formed during fermentation of carbohydrates which can transiently reduce the luminal pH prior to absorption (Lipton et al. 1988).

2 Formulation Parameters to Improve Oral Delivery

In an effort to save costs by accelerated development of drug products, 20 years of research led to the conclusion that the absorption characteristics of an API can be predicted by its solubility as a measure for its physicochemical properties and by its permeability considering the physiological conditions in the alimentary canal. These two parameters form the basis of the Biopharmaceutics Classification System (BCS), comprising four categories which allow prediction of the biopharmaceutical quality of a drug substance (Amidon et al. 1995; Lennernäs and Abrahamsson 2005). Whereas in the case of class I drugs with high solubility and permeability no problems are expected, in the case of class II drugs with low solubility and high permeability, solubility is the rate-limiting step and therefore requires improvement. In turn, in the case of class III drugs with high solubility and low permeability, some effort is necessary to enhance absorption. Finally, in the case of class IV drugs exhibiting both low solubility and low permeability, bioavailability is limited and thus requires both enhancement of solubility and absorption. According to this categorisation, a toolbox of different techniques exists to improve oral delivery by tuning a wide array of parameters as outlined below.

2.1 Size

In course of the last two decades considerable efforts have been made in order to develop approaches which facilitate the production of drug particles with precisely controlled features on the micro- and nanometre scale. For oral dosage forms the interest focused on nanoparticles basically originates from two motives. Firstly, a considerable number of newly developed drug candidates and marketed substances belong to BCS classes II and IV which, as a common characteristic, suffer from insufficient bioavailability due to low solubility. Since nanosizing of a drug can clearly improve the dissolution profile of the compound, it has been established as an essential approach for the handling of this issue (Kesisoglou et al. 2007). Secondly, seminal research reports on the oral absorbability of nanoparticles have catalysed efforts aiming at the development of drug-loaded systems which effectively translocate to systemic circulation. Undisputedly, this route of drug delivery is highly complex. However, since peroral formulations imply high patient compliance, several strategies for the administration of highly potent but sensitive biotech drugs have been investigated (Sood and Panchagnula 2001).

2.1.1 Effects of Nanosizing

Owing to their small size, drug particles in the nanometre range exhibit a quicker and more uniform distribution in the GI tract compared to conventional single-unit dosage forms (Asghar and Chandran 2006). As a consequence of the high surface

area, which enhances interaction of the particles with the epithelial lining and mucus, the application of a nanosized drug is also supposed to lead to prolonged residence times and more uniform bioavailability (Keck and Müller 2006). However, the most distinct effect of nanosizing on a substance's bioavailability is supposed to be due to an alteration of the compound's dissolution behavior. In this regard, the effects on the dissolution velocity elicited by downsizing of a drug crystal into the nanometre range are mainly attributed to an increase of the particle's specific surface area. This can be illustrated when considering a microparticle (radius = 10 μm) which is "broken" into 10^6 nanoparticles (radius = 100 nm). Although bearing an equal total mass, the nanoparticles exhibit a hundred times larger surface area for dissolution. The transfer velocity of solvated molecules from this surface area into bulk solution is determined by the hydrodynamic boundary layer thickness (Borm et al. 2006). For particles with a size of >50 μm the thickness of the layer is fairly constant and typically about 30 μm. In contrast, smaller micro- and nanoparticles are predicted to possess thicknesses comparable to the particle's diameter or radius (Galli 2006). As a consequence of this decreased boundary layer in conjunction with the large surface area, dissolved molecules can more rapidly transfer into bulk solution. The connection of these effects explains the higher dissolution velocity of nanoformulated substances and is illustrated in the Noyes–Whitney equation as extended by Nernst and Brunner (Eq. 1):

$$\frac{\mathrm{d}C_t}{\mathrm{d}t} = \frac{D\,A}{h}(Cs - C_t) \tag{1}$$

where C_S represents the equilibrium solubility of the substance, C_t is the concentration at time t, D is the diffusion coefficient, A is the surface area of the particles and h stands for the hydrodynamic boundary layer thickness (Noyes and Whitney 1897; Brunner 1904; Nernst 1904). Aside from the altered dissolution velocity, nanosized drug crystals are also characterised by increased saturation solubility as a consequence of the higher curvature of the particle surface (Borm et al. 2006; Wu and Nancollas 1998). This relation is given in the Ostwald–Freundlich equation (2) for solid–liquid dispersions:

$$\log\frac{C_r}{C_\infty} = \frac{2\,\sigma\,V}{2.303\,R\,T\,\rho\,r} \tag{2}$$

where C_r stands for the solubility of particles with radius r, C_∞ for the solubility of infinitely large particles, σ for the interfacial tension of the substance, V is the molar volume of the particle material, R the absolute gas constant, T the absolute temperature and ρ the density of the solid.

Below a particle size of about one micron, a notable surface curvature effect is expected which results in an increased dissolution pressure and hence saturation solubility (Müller and Peters 1998). However, since these effects are based on the assumption of spherical particles, it should be highlighted that the practically

observed solubilities for broken crystallites might substantially differ from the predicted ones (Tang et al. 2004; Borm et al. 2006).

Generally, when the dissolution rate of a poorly water-soluble substance is optimised, faster absorption rates are monitored in vivo. Consequently, if an inadequate dissolution rate is the factor limiting a substance's bioavailability, downsizing of the drug particles to the nanometre range has reportedly been a valid approach to enhance performance of the drug (Liversidge and Cundy 1995) and to accelerate its onset of action (Wu et al. 2004; Hanafy et al. 2007). The latter has especially raised interest in the context of the delivery of pain-controlling substances, where therapeutic plasma concentrations should be reached without marked delays. In a study comparing the bioavailability of nanocrystalline naproxen with that of the two marketed products Naprosyn® (suspension) and Anaprox® (tablet), the time to reach maximum plasma drug concentrations was found to be 50% less for the nanosized substance (Merisko-Liversidge et al. 2003). Besides the faster onset of action it has also been reported that the administration of drug nanocrystals can be beneficial for compounds which usually suffer from discrepancies in bioavailability between fed- and fasted-state administration. In particular, poorly water-soluble compounds frequently exhibit an enhanced dissolution rate mediated by components of the food and GI secretion (i.e. bile). By improving the dissolution properties of these substances independent from external factors, nanosizing can standardise the bioavailability and thus the therapeutic response (Wu et al. 2004). This is also an issue for BCS class IV drugs where the additional integration of penetration enhancers in the formulation might prove to be a valuable concept for achieving higher plasma concentrations (Merisko-Liversidge et al. 2003; Wu et al. 2004). When not only fast dissolution but also sustained release of a poorly bioavailable drug is an issue, coating of nanosized drug crystals with mucoadhesive polymers or molecular incorporation of the active agent into polymeric particles with bioadhesive character could enhance performance of the drug delivery system (Takeuchi et al. 2001; Bernkop-Schnürch 2005).

2.1.2 Techniques for the Nanosizing of Drug Particles

Most of the techniques used for nanosizing are based on the processing of an aqueous suspension of drug particles which contains surface active agents in order to stabilise the ultrafine colloids generated. As a rule of thumb particle re-growth due to Ostwald ripening should not be an issue if the processed compound's solubility in water is lower than 1 mg ml^{-1} (Merisko-Liversidge et al. 2003). If dealing with more soluble substances or if the nanocrystals are to be transferred into solid dosage forms, reduction of the water content is deemed necessary. This is achieved by lyophilisation or spray-drying following the diminution process which will enhance the particles' long-term stability as well as re-dispersability.

In the following text a description of the most established processes for the nanosizing of solid drug particles will be given. As illustrated in Table 2, several companies offer formulation development services based on these processes. Clear

Table 2 Overview of the commercially applied technologies for micro- and nanosizing of solid drugs with marketed products (if applicable)

Technology (company)	Process	Marketed product (active agent; company; year of FDA approval; dosage form)
NanoCrystal® Technology (Elan Nanosystems)	Wet-milling	Rapamune® (sirolimus; Wyeth; 2000; tablet) TriCor® (fenofibrate; Abbott; 2001; tablet) Emend® (aprepitant; Merck; 2003; capsule) Megace® ES (megestrol; Bristol-Myers Squibb; 2005; liquid oral suspension)
Insoluble Drug Delivery Platform Technology (IDD®-P) (SkyePharma)	Ultrasonication, homogenisation, milling, microfluidisation	Triglide® (fenofibrate; Sciele Pharma; 2005; tablet)
NANOEDGE® (Baxter)	Precipitation followed by high pressure homogenisation	–
Nanomorph® (Soliqs/Abbott)	Dissolution–precipitation	–
DissoCubes® (SkyePharma)	High-pressure homogenisation	–
Nanopure® (PharmaSol)	High-pressure homogenisation (in water/PEG/glycerol/oils)	–
BioAqueous℠ Solubilization Services (The Dow Chemical Company)	– Precipitation – Template emulsion (Anton et al. 2008) – Spray freezing into liquid (SFL) (Hu et al. 2003) – Evaporative precipitation into aqueous solution (EPAS) (Chen et al. 2002)	– – –

information is not always provided on the method applied for diminution (i.e. in case of IDD®-P), however most of the technologies contain similarities. The alternative concepts included in The BioAqueous℠ Solubilization Services (The Dow Chemical Company) are not described in detail, but for the interested reader literature quotes are provided. Generally, when the rather recent dates of the marketed products' FDA approvals are considered, an increasing number of formulations which include nanosized drug particles can be expected in the future.

Bottom-Up Processes

In order to produce nanosized particles by precipitation, the substance to be formulated must be soluble in a preferably non-toxic water-miscible solvent. In practice, this is a limiting factor for several new chemical entities which are neither

soluble in aqueous media nor in most organic solvents. After mixing the organic solution of the compound with an aqueous solution of stabiliser, the ambient conditions have to be controlled in such a way as to trigger precipitation of the desired crystalline or amorphous drug particles in the nano- or micrometre size range (400–2,000 nm) (Möschwitzer and Müller 2007). At a certain stage of the process, cessation of particle growth is necessary, which is attained by the addition of selected polymers (gelatine, chitosan, poloxamer, etc.). These substances act as growth inhibitors by adsorbing to the particle surface and by increasing the viscosity of the suspension. Finally, the generated drug nanoparticles are stabilised, for instance by spray-drying with excipients (lactose, mannitol, etc.). This step has to be implemented in order to conserve the particle size and amorphous state which determine the dissolution rate and consequently bioavailability of the drug in vivo (Rasenack et al. 2003).

Top-Down Processes

The common feature of top-down approaches for the nanosizing of active agents is the application of an external force in order to induce breakage of the drug particles at crystal imperfections. Depending on the technique applied, the forces for diminution are generated by collisions with milling media, cavitation, high shear forces, interparticle collision or turbulent flow (Möschwitzer and Müller 2007). Common to all techniques is that a certain particle size threshold has to be accepted in practice, which depends on the drug properties as well as the technique-specific practical energy input. This lower limit within practically relevant process times is a result of the downsizing-associated reduction of available crystal lattice imperfections per particle for breakage. As a consequence of this, a further reduction of particle size can only be attained by an exponential increase in the energy input after several processing cycles. Since the arrival at this point is determined by the substance-specific hardness of the drug particles, optimised processing parameters leading to a desired particle size cannot directly be extrapolated to other substances. However, it has frequently been reported that the majority of particles is already notably nanosized in course of a few process cycles. Continued diminution does not necessarily further reduce the mean particle size but narrows the size distribution.

Wet-Milling

Wet-milling has been used for the production of nanosized drug particles for several years and is the essential diminution technique of the patented NanoCrystal® Technology (Elan). Basically, a milling chamber which is charged with milling media (pearls), dispersion medium (mostly water), drug particles and stabiliser is subjected to rotary movement as a whole or its contents are stirred by an agitator. The revolving pearls generate high shear forces and interparticle collisions inside the mill, which collectively lead to the diminution of drug crystals to about 80–400 nm. It has to be taken into account that the high inner surface area of the

mill also increases product loss due to adherence of milled drug particles. This can lead to cross-contamination and also is especially unwanted when processing costly substances which, as often is the case with new chemical entities, are only available in small quantities. Impurities abrased from the milling media consisting of stainless steel, glass and ceramics based on yttrium-stabilised zirconium dioxide have been another point of concern (Möschwitzer and Müller 2007). This issue might be overcome by using durable and high-grade milling media such as pearls of highly crosslinked polystyrene resin which only exhibit wear in the range of 0.005% (w/w) based on the drug concentration of the dispersion (Merisko-Liversidge et al. 2003).

If the success of a formulation technology is measured by the number of products in clinical trials and marketed, the NanoCrystal® Technology has to date out-performed its competitors in terms of commercial success (Table 2).

High Pressure Homogenisation

Piston-Gap Homogenisers

High pressure homogenisation has been a standard technique in the food and dairy industry for several decades. In the case of pharmaceutical formulations its application has flourished, as the process is neither limited to emulsions nor to suspensions and can be operated in the lab (0.5 ml) as well as in large batch-scale (2,000 Lh^{-1}) (Keck and Müller 2006; Möschwitzer and Müller 2007; Müller and Peters 1998).

In high pressure homogenisation the particle suspension is forced through the constricted gap of a homogenising valve. Since the volume of suspension flowing over time has to be constant prior to and in the considerably narrower gap (~20 μm) of the valve, the velocity of the fluid instantaneously rises upon entering the passage. Even at room temperature this leads to a drop of the fluid's static pressure below the vaporisation point. As a consequence, the formation of gas bubbles takes place which is frequently termed "cavitation". After having passed the gap, the fluid flow velocity is lowered again leading to implosion of the gas bubbles, which initiates high-energy micro jet-streams. Although it is the major diminution principle attributed to high pressure homogenisation, cavitation is very likely complemented by additional factors such as interparticle collisions and high shear forces (Möschwitzer and Müller 2007).

In the case of solid drug particles, no general rules have been identified for the addition of tensides to the homogenisation process. The amount and type of tenside added has only been reported to be diminution-relevant when processing emulsions (Keck and Müller 2006). This can be explained by the lowering of the surface tension which decreases the energy input required to disintegrate a liquid droplet. In contrast to wet-milling, product loss due to the inner surface area of the equipment as well as wear of particles from machine parts is quite low. Upon application of "hard" homogenisation conditions of 1,500 bar pressure and 20 repeated cycles, only about 0.7 ppm iron were detected in the final suspension by atomic absorption spectroscopy (Krause et al. 2000).

Although high pressure homogenisation of aqueous suspensions is considered a fairly production-friendly process, the use of water as dispersant is not desirable for the subsequent transfer of nanocrystalline drug particles into traditional dosage forms such as tablets or capsules. In most cases the water content of the suspensions has to be reduced prior to further processing, which implies time- and energy-consuming steps such as lyophilisation, spray-drying or fluidised bed drying (Möschwitzer and Müller 2007). In this regard, a more suitable technique licensed under the name Nanopure® (PharmaSol GmbH, Berlin) has been developed, which involves the production of nanocrystalline suspensions in water-reduced (water–glycerol/PEG mixtures) or water-free dispersants (oils, liquid PEG, hot-melted PEGs). Interestingly, diminution of drug particles via high pressure homogenisation can also be attained in these media although the likelihood of cavitation is clearly reduced in systems with low vapor pressures. For this case it is hypothesised that shear forces, interparticulate collisions and turbulent flow mainly contribute to downsizing of the dispersed drug crystals (Keck and Müller 2006). The main advantage of this approach lies in the convenient processing of the suspension after the high pressure homogenisation step. When hot-melted PEGs are used as dispersants, the nanosuspension can either directly be filled into soft or hard-gelatin capsules (solidification in capsule) or can be cooled leading to crystallisation of the PEG matrix and entrapment of the particles. The resulting solid is ground to a powder which can be used for compression into tablets or for the filling of hard-gelatin capsules. This provides additional benefits since the solidified PEG matrix stabilises the nanoparticles and thus enhances the formulation's shelf-life. Moreover, PEG melts at physiological temperature conditions and thereby allows liberation of the monodisperse nanosized drug crystals in the GI tract.

Microfluidisation

This technique relies on the diminution of suspended drug crystals in a Microfluidizer® processor. Basically, an intensifier pump drives the particle suspension through microchannels with fixed geometry until the liquid stream is split in a "Y" fashion and increasingly narrows. As with piston-gap homogenisers the decreasing diameter of the channel leads to an incremental increase in streaming velocity which results in cavitation and high shear rates. Finally the two split streams collide head-on leading to further impacts and diminution. Although very high homogenisation pressures can be attained with this technique, it has been reported that certain substances have to be subjected to a considerable number of processing cycles in order to achieve sufficient particle size reduction (Keck and Müller 2006; Möschwitzer and Müller 2007).

2.1.3 Absorption of Nanoparticles

The uptake of nanosized particles on oral administration has been intensely investigated for the delivery of highly potent protein and peptide drugs, since standard

parenteral application is associated with low patient acceptance especially in the case of chronic therapy (des Rieux et al. 2006). The approaches discussed below are mainly based on drug-loaded polymeric nanoparticles, for which a variety of preparation procedures have been developed.

First reports dealing with the absorption of particulate materials from the GI tract appeared several decades ago. Surprisingly, particles well in the micrometre range ($\leq$150 μm) such as starch granules, pollen, silicate crystals and powdered rabbit hair were found in the subepithelial layers of the mucosa after having been fed to animals (Volkheimer 1974). This observation was attributed to a rather passive mechanical process termed "persorption" which occurs at low frequencies. Since then, more efficient pathways have been identified which could be exploited for the peroral administration of particulates which ultimately translocate to systemic circulation. These routes focus on normal gut epithelial cells and M cells, with the latter being located in the follicle associated epithelium (FAE) which covers underlying singular or associated lymphoid follicles of the gut-associated lymphatic tissue. The sparse M cells exhibit several characteristics which might prove beneficial for the uptake of particles. Compared to normal gut epithelial cells they are reported to be covered with a thinner glycocalyx layer which is supposed to facilitate adherence of particles. Furthermore, M cells have adapted to absorb a wide range of substances and are located in direct proximity to lymphatic tissue (Shakweh et al. 2004). Obviously, this latter fact has generated enormous interest for the development of particulate systems which are able to induce mucosal immunisation (O'Hagan et al. 2006). However, the extrapolation of animal data to humans is difficult due to the different anatomy of the FAE in rodents (10–50% M cells) compared to humans (~5% M cells) (Florence 2005; des Rieux et al. 2006). Consequently, to date it remains elusive whether M cells alone can serve as resorptive sites via which sufficient amounts of nanoparticles are taken up for a systemic effect.

In this regard, normal gut epithelial cells might simply be more preferable due to their abundance in the intestine. By using polymeric nanospheres as a model for an oral drug delivery system, several studies have identified the optimal size for efficient absorption to lie in the range of 50–100 nm (Jani et al. 1990). These results correspond to others obtained from more detailed studies focusing on enterocytes (Jani et al. 1992) and the treatment of inflammatory bowel disease (Lamprecht et al. 2001) which report highest uptakes for particles 100 nm in size. In the latter case high uptake into locally diseased tissue rather than systemic uptake is desired, and was probably reached due to increased uptake of the particles by macrophages and due to entrapment as a consequence of higher mucus secretion in the inflamed tissue. Generally, the role of mucus in the absorption process of nanoparticulate materials is disputed. On the one hand it is argued that uptake is inhibited by entanglement of the particles between the glycoproteins while on the other hand entrapment in the mucus layer supposedly increases the intestinal residence time and contact of the particles with the gut wall.

Obviously, several important problems have yet to be addressed if systemic response with orally administered nanoparticles is to be successfully achieved.

For instance, in the case of insulin several studies have shown conflicting therapeutic effects in animals (Delie and Blanco-Prieto 2005). The outcome of these observations is that, especially for drugs with narrow therapeutic windows, the standardisation of uptake efficiency when formulated as nanoparticles has to be a major topic of research on the way to controlled oral bioavailability (Dearn AR 1997). This must include systematic investigations of the influence of the type of polymer constituting the particle matrix as well as the type and concentration of tenside used for the production procedure (Delie and Blanco-Prieto 2005). Furthermore, the drug loading efficiency of the polymeric carriers is an issue especially for water-soluble compounds such as proteins. Finally, considering the already complex events of particle uptake into the epithelial cell layer, it can be imagined that basolateral export and the subsequent translocation to the bloodstream are underestimated hurdles which still need to be successfully dealt with (Florence 2006).

2.2 pH-Dependent Drug Delivery

Due to distinct changes of the environmental pH in different sections of the GI tract (see Point 1.2 "Physiological Conditions in the GI Tract" of this chapter), a drug dosage system following the oral route is subjected to highly diverse external conditions during its passage. Since the uptake of many agents is known to differ considerably among the consecutive regions of the intestinal mucosa, providing a high concentration of active compound in the section with optimum uptake is of essential importance for gaining adequate bioavailability. For this, the physiological changes in pH may be used as a chemical trigger to specifically release a drug in the region of interest, thereby enabling the protection of the compound prior to the intended area of uptake and the bypassing of adverse effects in these regions. Most pH-dependent delivery systems encompass the prevention of release in the acidic gastric milieu, followed by an unhindered or pronounced release in the area of small intestine or colon featuring a higher pH range. It should be noted that natural differences in gastrointestinal pH among individuals as well as temporary fluctuations caused by dietary factors, disease or co-administration of other drugs might influence the performance of pH-triggered systems and must be taken into account for the development of safe delivery strategies. One general advantage of enteric-coated tablets or multiparticulate systems is their ease of manufacture and relatively low cost compared to other, more complex delivery systems (Friend 2005).

Commonly used approaches for the design of pH-dependent dosage forms are based on polymeric coatings that are applied to a preformed tablet, capsule, pellet or particle, or on the embedding of the API in a pH-sensitive matrix or hydrogel. Alongside the traditional forms of enteric coating, one upcoming field that has received a special boost by pH-triggered systems is the area of colon targeting.

2.2.1 pH-Sensitive Coatings

Today, a variety of polymers with different backbone modifications and side-chain conjugates is available, allowing for a precise control of release at a certain threshold pH. Amongst them, the group of methacrylic acid copolymers, or Eudragit® (trademark of Evonik Röhm, Darmstadt, Germany), is one of the best known and in wide use for the preparation of pH-sensitive coatings or hydrogels. Different types of Eudragit® powders and ready-to-use suspensions are commercially available which represent copolymers of methacrylic acid and methyl methacrylates carrying different functional groups. The ratio of free acid to ester groups determines the extent of swelling and/or dissolution rate of the polymer, and the quantity and type of charged groups at the surface accounts for a prevailing anionic or cationic character (Moustafine et al. 2005). By incorporation of positively charged moieties, such as dimethylamino or quaternary amino groups, the polymer can be considered a polycation (Eudragit® type E, RL, RS). These polycations are insoluble at the neutral pH of the saliva and will, according to the type of charged group, either dissolve completely in the stomach (dimethylamino groups, Eudragit® E) or – in the case of quaternary amino groups – show swelling without complete disintegration (Eudragit® RL and RS). For negatively charged polymers (Eudragit® L, S and FS), the hydration of ionised carboxylate groups occurring at pH 5.5 or higher accounts for insolubility in the acidic gastric fluids with a subsequent release in the alkaline milieu of the lower GI tract. This prevents exposure of the encapsulated compound to harsh conditions in the stomach and enables unhindered delivery to the preferential site of uptake. The fine-tuning of polymer composition, as well as type and extent of derivatisation, allows for a relatively precise controlling of the threshold pH. This provides the possibility for a more accurate determination of the site of dissolution in the small intestine or colon, although limited by the fact that the changes in pH along the lower GI tract are much less pronounced than the step from gastric fluid to duodenum and may be subjected to fluctuations according to dietary status.

The application of different Eudragit® coatings in pharmaceutical dosage forms is widespread with numerous products marketed for local or systemic therapy (e.g. Asacol®, Salofac®, Claversal®, Entocort™ EC, Budenofalk®) (Friend 2005; Fedorak and Bistritz 2005). However, since the pH profile along the GI tract is variable and thus hinders precise control of the site of release, the trend today is toward combined delivery systems that utilise more than one coating or targeting principle. Various systems have been published covering the combination of pH- and time-dependent release, mostly composed of an outer coating layer of Eudragit® grade L, S or FS and an inner core of a swelling polymer such as Eudragit® type RL and RS, ethylcellulose or hydroxypropylmethylcellulose (Vandelli et al. 1996; Gupta et al. 2001; Edsbäcker et al. 2003). Moustafine and coworkers have explored the formation of interpolyelectrolyte complexes between different types of Eudragit® polymers, which possess altered release profiles compared to conventional physical mixtures of the individual polymers (Kumar 2000; Moustafine et al. 2005). Similar ionic complexes can be observed for other polymers that are in use for controlled

release, e.g. chitosan or carboxymethylcellulose, and can be rendered pH-responsive by incorporation of one or more pH-sensitive compounds (Lorenzo-Lamosa et al. 1998).

As mentioned above, more complex delivery systems attract increasing notice today, due to the fact that the combination of various targeting strategies allows for a further fine-tuning of the site of release. Regarding pH-dependent dosage systems, these new developments impact especially on two fields, one being the sector of colon targeting, the other concerning the design of novel pH-sensitive hydrogels.

2.2.2 pH and Colon Targeting

In recent years, increased attention has been paid to the specific targeting of the distal part of the GI tract, not only as a way of improving the treatment of local diseases such as infections, carcinoma or Crohn's disease but also as an interesting possibility for systemic absorption (Yeh et al. 1995). Fragile compounds such as proteins or peptides are subjected to much lower levels of digesting proteolytic enzymes in the colon than the rest of the GI tract, and the long residence time in the colon that accounts for nearly 80% of the total GI transit time represents a large absorption window for the uptake of drugs. The pH-dependent disintegration of polymeric drug carriers or coatings has been evoked as an important principle for the successful realisation of such colon-targeted strategies, although the selectivity of release at the intended area still remains problematic. Due to the lack of distinctive changes in pH in the lower GI tract, there is no effective stimulus that could reliably trigger the disintegration of a target system precisely in the region of the upper colon by pH alone. Additionally, as is known today, the pH in the colon might be subjected to fluctuations and even be more acidic than the pH in the small intestine (Lorenzo-Lamosa et al. 1998; Friend 2005). Thus, many recently developed strategies for colon targeting rely on the combined application of a pH trigger and a second principle that is based on a different mode of operation.

These alternative principles mainly include enzymatic systems, which take advantage of the specific microflora present in the large intestine and use various types of bacterially cleavable polymers that contain azo groups or glycosidic bonds (Brondsted and Kopecek 1992; see Sect. 6 in "Nanoparticle Technologies for Cancer Therapy"). The major drawback of these synthetic or natural polysaccharides such as chitosan, pectin, dextran or chondroitin sulfate is their high solubility in gastric fluid (Chen et al. 2004), hence the combination with a second delivering strategy to avoid disintegration too early is of obvious benefit. To bridge the passage of the upper GI tract, either acid-insoluble pH-dependent or delayed time-dependent coatings can be applied. However, care should be taken regarding time-controlled systems, since the usual transit time required to reach the colon is known to be 4–6 h in fasting condition, but can increase remarkably due to delayed gastric emptying in fed state. Thus, pH-triggered release systems that are less susceptible to variations in dietary status might present the preferred option.

Lorenzo-Lamosa et al. have developed a multiparticulate colonic drug delivery system that is composed of chitosan microcores entrapped within Eudragit® L-100 or S-100 microspheres (Lorenzo-Lamosa et al. 1998). The combination of the colonic biodegradable chitosan matrix with an acid-resistant Eudragit® coating resulted in an unaffected integrity without release during transit through the gastric cavity, followed by an almost zero-order kinetic drug release in small intestine and colon. A connected interplay of pH-triggered dissolution, swelling and bacterial degradation, which is possible due to the formation of an ionic crosslinking between chitosan and the acrylic polymer, is held responsible for the observed release characteristics.

Besides the combination with enzymatic targeting, the principle of pH control together with matrices for delayed release is successfully in use for colon delivery. Entocort™ EC capsules (AstraZeneca, Lund, Sweden) for the treatment of Crohn's disease are composed of an Eudragit® L100 coated shell that contains ethylcellulose pellets carrying the drug (Edsbäcker et al. 2003). Eudragit® coated microgranules, designed to dissolve at pH >6.4, are the working principle of Budenofalk® capsules (Falk Pharma, Freiburg, Germany) (Fedorak and Bistritz 2005). Krishnamachari et al. recently suggested the use of poly(D,L-lactide-*co*-glycolide) (PLGA) microparticles that feature a coating with Eudragit® S-100 for the delivery of budesonide to the site of disease (Krishnamachari et al. 2007).

2.2.3 pH-Responsive Hydrogels

Swellable and biologically degradable hydrogels have emerged as an important tool for enhanced delivery of drugs to defined sections of small intestine or colon, and can be specifically designed to utilise the changes in pH as a stimulus to initiate swelling or dissolution. Again, different targeting strategies can be followed according to the chemical nature of the hydrogel, but the most widespread approach is to design the gel in such a way that swelling in acidic gastric fluid is prevented and the incorporated drug is not released until it reaches neutral or basic regions of the lower GI tract. This selective swelling behavior can be realised by using matrices that contain, e.g., carboxylic groups, which retain a compact network at low pH and increase swelling upon ionisation at higher pH ranges. So far, a huge variety of polymers or copolymers has been assessed for this purpose, most of which are prepared by copolymerisation or by crosslinking of polymer precursors. It should be noted that hydrogels of similar compositions may exhibit different degradation patterns according to the mode of preparation (Yeh et al. 1995). This may be attributed to different network structures resulting from the diverse synthesis methods. Crosslinks between polymers can be provided by covalent bonding, hydrogen bonding or physical entanglement, and can strongly affect the drug release kinetics by either favoring surface erosion or bulk erosion as a degradation pattern. Generally, the higher the crosslinking density, the lower the swelling of the gel in aqueous medium and the slower the drug release (Yeh et al. 1995). Upon reaching the full extent of swelling, the release of the active agent may further be enhanced by disruption of the matrix through chemical hydrolysis or by

incorporation of biodegradable cleavage sites that are now accessible for the degrading enzymes (Akala et al. 1998).

All pH-responsive hydrogels are polyelectrolytes that contain either acidic or basic groups which accept or release protons according to changes in environmental conditions (Qiu and Park 2001). It is important to mention that due to the closeness of adjacent charged groups in the polyelectrolyte matrix the total extent of ionisation may be less than for similar amounts of corresponding monoacid or monobase (Mayo-Pedrosa et al. 2008). The degree of swelling of the hydrogel is mainly determined by electrostatic repulsion between enclosed ionised groups and is much higher than for uncharged polymers. The application of comonomers, such as hydroxyethyl methacrylates and methyl methacrylates, causes a different hydrophobicity of the gel and allows for a further tuning of pH response.

Cationic polymers swell in the acidic pH of the stomach, and can be made of, e.g., chitosan or *N,N′*-dimethylaminoethylmethacrylate (Siegel et al. 1988; Patel and Amiji 1996). Hydrogels containing anionic groups will start to swell or dissolve in the neutral or basic environment of the small intestine or colon, and may be composed of various derivatives of acrylic acid, poly(acrylamide), poly (methacrylic acid), poly(vinylacetaldiethylaminoacetate), or dextran (Akala et al. 1998; Aikawa et al. 1998; Chiu et al. 1999; Qiu and Park 2001). Otherwise inert hydrogel matrices that do not show alterations upon changes in pH can be rendered pH-sensitive by utilisation of appropriate crosslinkers, such as maleic acid crosslinked poly(vinyl alcohol) hydrogels (Peppas and Peppas 1990; Gohil et al. 2006; Basak and Adhikari 2008).

Chitosan is a copolymer of D-glucosamine and *N*-acetylglucosamine that is widely used in drug delivery due to its good biocompatibility, but usually is of limited use for intestinal drug delivery owing to its fast dissolution in the acidic milieu of the stomach (Chen et al. 2004). However, complexes of chitosan derivatives and polyanionic alginate can be used as pH-sensitive hydrogels that withstand the acidic conditions in gastric fluid, provided the chitosan is additionally fixed with an appropriate crosslinker. Since application of glutaraldehyde is restricted for toxicological reasons, Chen et al. have proposed the use of genipin as a naturally derived crosslinking agent of low toxicity with an hydrogel system of alginate and *N,O*-carboxymethyl chitosan (Chen et al. 2004). A sufficient retention of enclosed model drug, followed by subsequent release at neutral pH, could be shown. Recently, the in situ coating of cellulose/PVP pellets with a pH-sensitive hydrogel by photopolymerisation has been described, allowing for specific tuning of pH responsiveness and enabling incorporation of drug in the matrix of the pellet as well as in the coating gel (Mayo-Pedrosa et al. 2008).

2.3 Swelling

Swellable hydrophilic polymers are widely used components of controlled-release systems. They are mainly applied to the production of API-containing matrices or

for coatings. Drug release from a swellable matrix is mainly controlled by the rate of swelling of the polymer and the API diffusion through the gel layer. A broad variety of designs is available which allows for different timed-release schemes ranging from pulsatile delivery to sustained delivery over a prolonged period.

Due to their favorable swelling properties and compressability, the most important representatives of this group of polymers are cellulose ethers such as hydroxypropylmethyl cellulose (HPMC), methyl cellulose (MC), and hydroxyethyl cellulose (HEC). They are well characterised, mostly have "generally recognised as safe" (GRAS) status and are available in a broad range of viscosity and substitution grades. Furthermore, copolymers of methacrylic acid, galactomannans, alginates and many other natural and synthetic polymers can be applied.

Upon water contact, these polymers swell and undergo a transition from the glassy to the more permeable rubbery state resulting in the formation of a gel layer. Initially, water penetration prevails, the swelling front which is the inner boundary of the gel layer moves on and the thickness of the gel layer increases. At the same time, part of the polymer dissolves or erodes at the outer boundary of the gel layer, the so-called erosion front. When water uptake and polymer erosion are balanced, the layer thickness stagnates. Finally, when all the polymer has been hydrated, the gel layer thickness decreases. Moreover, another boundary within the gel layer has been described, the so-called diffusion front which constitutes the border between the already dissolved and the still undissolved drug (Colombo et al. 2000; Gazzaniga et al. 2008). As mentioned above, the gel layer thickness is time-dependent, which is important for the drug release kinetics.

Depending on the rate-limiting step, drug release can be diffusion-controlled, swelling-controlled or chemically controlled. Diffusion-controlled delivery can be modeled by Fick's law with either constant or variable diffusion coefficients. If drug diffusion is faster than polymer swelling, drug release depends on the swelling rate of the polymer network. A prominent example of such swelling-controlled drug release is HPMC. In the case of chemically controlled release, chemical reactions within the delivery matrix are the rate-limiting principle, for example hydrolytic or enzymatic degradation of the polymer or cleavage of conjugated drug from the network (Lin and Metters 2006).

Concerning drug release, another crucial parameter is the mesh size of the hydrogel, which typically ranges between 5 and 100 nm in the swollen state. If an API is smaller than the mesh size, which is the case for most APIs, its diffusion is not delayed. However, for non-porous hydrogels as well as for porous hydrogels with pore sizes similar to the size of the active agent, diffusion is sterically impaired and the diffusion coefficients decrease. By choosing appropriate parameters, the structure and mesh size of swollen hydrogels as well as the degree of gel swelling and degradation can be tuned (Lin and Metters 2006). This allows the design of specifically tailored controlled-delivery devices.

Delayed release that does not depend on pH, ionic strength or temperature is particularly interesting for chronotherapy of chronic diseases which show circadian variations, for example in the case of rheumatoid arthritis or bronchial asthma, which manifest especially in the early morning. Delayed release allows bedtime

administration without exposing the patient to the API during the whole night (Gazzaniga et al. 1994).

The most common delayed-release systems use swellable hydrophilic polymers representing either coated reservoir systems or capsular systems with hydrophilic polymeric plugs (Gazzaniga et al. 1994). Coatings of reservoir systems with hydrophilic polymers are generally applied by press-coating techniques, spray-coating, dip-coating or powder-layering. One example is the Chronotopic™ delivery system which consists of a single- or multiple-unit drug core or gelatin capsule surrounded by a HPMC layer that is responsible for the lag phase (Gazzaniga et al. 1994). It can either be used for delayed release after a programmed lag phase or for time-dependent colon-specific delivery, if an additional outer gastro-resistant film is applied (Sangalli et al. 2001). Double-peak plasma levels were obtained with a reservoir system containing part of the API in the outer layer and part of it in the core, resulting in an immediate initial dose followed by a delayed release. However, even more versatile platforms are available, for example multi-unit devices including a multitude of cores with different release characteristics in one hard-gelatin capsule. By combining differently coated cores even multi-pulse delivery is possible. Another recently introduced controlled-release technology is SyncroDose™ (Penwest Pharmaceuticals, USA), which consists of a drug-containing core coated with an erodible layer of xanthan and locust bean gum mixtures. The lag time of these systems depends on the composition of the coating. Generally, the coating step can be quite tricky. For example, it is rather challenging to obtain uniform HPMC coat layers by double-compression, which are essential for the release characteristics. Thus, alternative methods of compression coating were evaluated, resulting in devices such as the Encore™ system and one-step dry-coated tablets (OSDRC®) (Ozeki et al. 2004; Gazzaniga et al. 1994).

Capsular systems with polymeric plugs are composed of an insoluble shell surrounding the API core, a soluble cap and a plug which consists of a hydrophilic polymeric tablet sealing the open end. After swelling or erosion of the plug, the API is released. Commercialised examples comprise the Pulsincap™ system and the Egalet® system, the latter consisting of a cylinder containing an API core and erodible plugs at both ends (Bar-Shalom et al. 1991; Gazzaniga et al. 1994).

Besides delayed-release sytems, there are numerous further applications of swellable polymers for controlled-release technologies. For instance, they are used for floating gastroretentive delivery systems to entrap low-density components, as well as for expandable systems which have a prolonged gastric residence time due to their size. As for expandable systems, they have to be small enough to be swallowed. In the stomach, they rapidly expand by swelling or unfolding and therefore become too big to pass the pyloric sphincter. After having released their drug load, they have to be emptied from the stomach, preferably by complete erosion. Swellable systems are easier to produce than unfolding systems and might generally have better chances of clinical implementation (Klausner et al. 2003).

Most of the currently used hydrogel delivery systems contain polymers that are not degraded in the GI tract. Nevertheless, enzymatically degradable hydrogels are becoming increasingly important, especially for site-specific delivery to the colon (Friend 2005; Lin and Metters 2006). Moreover, the area of stimuli-sensitive hydrogels is growing. Their swelling or deswelling characteristics and therefore drug release are governed by external factors such as pH, ionic strength or temperature (Qiu and Park 2001).

To conclude, hydrophilic polymers are used for a broad variety of controlled release formulations, from site-specific devices for gastroretentive or colon delivery to time-dependent devices like osmotic pumps. For the future, their use in newly designed delivery systems may be anticipated, as well as a range of new hydrophilic polymers with tailored properties.

2.4 Osmotic Pressure

Oral osmotic pumps have gained increasing interest over the past three decades, because they are widely independent of pH, presence of food and other physiological parameters. Their function is based on an osmotic pressure gradient between the interior of the delivery system and the environment. Osmotic pumps have well-understood properties and can be tailored with a high in vitro–in vivo correlation to obtain either zero-order or patterned release. Today, they are already applied in many therapeutic fields, such as cardiovascular medicine, endocrinology, urology, and diseases of the central nervous system. Their advantages comprise stable drug concentrations in plasma and reduced dosing frequency (Conley et al. 2006).

Osmotically controlled delivery systems consist of a drug-containing osmotically active core surrounded by a semipermeable membrane. In an aqueous environment, water intrudes through the membrane forming a saturated solution of drug and/or additional osmotic agents with a high osmotic pressure. This solution can only be released through predetermined delivery orifices in the membrane. Generally, drug release is governed by the drug solubility, the osmotic pressure gradient, the size of the delivery orifice and the characteristics of the membrane.

For osmotic delivery, a drug should ideally have intermediate solubility (50–300 mg ml^{-1}). Otherwise, solubility-modifying excipients may be added to the drug core. For instance, highly water-soluble drugs can be formulated with sodium chloride in order to reduce their solubility. Drugs with pH-dependent solubility can be co-formulated with acidic or basic components. Interestingly, poorly water-soluble drugs can be released even as a suspension. In this case, the dispersed drug is extruded due to swelling of co-formulated hydrophilic polymers or due to effervescent excipients, such as sodium bicarbonate and citric acid. Yet another approach is the application of poorly soluble drugs in form of cyclodextrin complexes (Verma et al. 2000, 2002).

However, if the osmotic pressure of the saturated drug solution is not sufficient, sugars, water-soluble salts or other osmotically active excipients can be added to

the core. As an alternative, hydrophilic polymers can be included in the core material. They swell upon water influx and thus contribute to the hydrostatic pressure (Thombre et al. 2004).

Another important aspect is the size of the delivery orifice. In many cases, these orifices are created by drilling a hole into the membrane either mechanically or by laser light. Moreover, controlled-porosity osmotic pumps have been developed, too. In that case, the orifices are formed due to the incorporation of leachable materials that are dissolved upon introduction into an aqueous environment (Zentner et al. 1985).

Concerning the membrane, two major prerequisites have to be met: firstly, water penetration has to be possible while the permeation of solutes must be inhibited, and, secondly, the membrane has to withstand the internal pressure. Commonly used materials include cellulose esters such as cellulose acetate, various types of Eudragit®, and ethylcellulose. As the membrane thickness usually amounts to 200–300 μm, sufficient water influx might be an issue. This can be alleviated by adding hydrophilic flux enhancers such as polyethylene glycols (Verma et al. 2000, 2002). Alternatively, asymmetric membranes have been designed which consist of a porous layer providing the mechanical strength and a thin semipermeable layer for diffusion control. They can be used to increase water influx, which might be favorable for drugs with low solubility in order to achieve higher drug release rates (Herbig et al. 1995; Thombre et al. 1999).

Drug release from osmotic pumps usually follows zero-order kinetics, i.e. a constant amount of drug is delivered over time. This delivery regime starts after a certain lag time as soon as the osmotic pressure gradient has been built up and continues until all the osmotically active solid has been dissolved (Verma et al. 2000). In addition, more sophisticated systems have been created which allow for ascending, delayed or pulsed release.

Among the currently available designs of osmotic delivery systems, the best-known example is the elementary osmotic pump (EOP), a single-chamber device, which is mainly used for drugs with intermediate water solubility. The OROS® osmotic technology (Alza Corporation, USA) has been used for a range of marketed products, such as the phenylpropanolamine formulation Acutrim® (Ciba-Geigy) (Shokri et al. 2008). In order to adopt EOP technology for poorly water-soluble drugs, swellable EOPs have been designed, which contain the drug dispersed in a hydrophilic polymer and an osmotic agent. Upon water influx, the internal hydrostatic pressure increases due to polymer swelling and osmotic effects. As a result, the drug is released in dispersed form included in a gel (Shokri et al. 2008).

Besides single-chamber devices, a range of multi-chamber systems has been developed. Push–pull osmotic pumps consist of a bilayer core, which is surrounded by a semipermeable membrane with a small orifice. The upper layer that is near the orifice contains the drug, the lower layer contains an osmotic polymer. Water uptake leads to swelling of the lower parts. The increased internal pressure (“push”) results in the extrusion of the API (“pull”). Similarly, the sandwiched osmotic tablet system (SOTS) contains a trilayer core with a push layer in the middle and two attached API layers (Liu et al. 2000). Besides push–pull systems

with their expanding second chamber, various osmotic pumps with nonexpanding second chambers have been created. Among them are devices where the API solution gets diluted by passing the second chamber. This might be an advantage for certain drugs, if a concentrated solution can induce irritation in the gastrointestinal tract (Verma et al. 2000, 2002).

Recent developments include multiparticulate delayed-release systems consisting of a multitude of API-containing pellets that are coated with semipermeable films, as well as L-OROS® (Alza Corporation) for the delivery of liquid drug formulations or insoluble APIs and OROS-CT® (Alza Corporation) for site-specific delivery to the colon (Verma et al. 2000, 2002).

A variety of release profiles can be obtained by combining osmotic systems with other principles for controlled drug release. For instance, a drug-containing coating can be additionally applied in order to provide an initial dose. Another approach is based on a delayed burst-release of a drug from a capsule coated with a semipermeable layer which is filled with the API and a swellable polymer. After a certain lag time, which can be tuned via the coating, the internal pressure has enormously increased and the capsule "explodes." Among many other approaches for pulsed release, one example is multiparticulate systems that combine osmotically active pellets with different coatings which therefore sequentially release their drug load (Anal 2007).

Examples of marketed products based on osmotic delivery include the nifedipine formulation Procardia® XL (Pfizer), a calcium channel blocker for the treatment of angina, and Covera-HS®, a verapamil formulation. Glucotrol® XL (Pfizer), a glipizide formulation, is used for the treatment of type II diabetes mellitus. Furthermore, there are Cardura® XL (Pfizer), a doxazosin formulation for the treatment of benign prostatic hyperplasia (BPH), Lyrinel® XL (Janssen-Cilag), an oxybutynin formulation for overactive bladder, Concerta® (Janssen-Cilag), a methylphenidate formulation for the treatment of attention deficit hyperactivity disorder (ADHD), Invega®, a paliperidone formulation for the treatment of schizophrenia, and Jurnista® (Janssen-Cilag), a hydromorphone formulation for the treatment of pain (Conley et al. 2006).

To conclude, osmotic pumps are a highly versatile platform for controlling release characteristics. Over the years, the range of drug candidates has been expanded from osmotically active drugs with intermediate water solubility to highly and poorly water-soluble as well as liquid drugs, and a large number of new applications may be expected in the future.

2.5 Density

Besides other physical properties, the density of a dosage form can be exploited for site-specific drug release. It is applied for gastroretentive delivery systems which remain in the stomach for a prolonged time. As a result, bioavailability and therapeutic efficiency of a wide range of substances with local action or absorption

in the stomach and the proximal small intestine can be improved, provided that they are sufficiently stable at acidic pH. Among them are drugs with a small absorption window such as levodopa or furosemide, as well as drugs with limited stability in distal parts of the gut. Sustained release in the stomach can provide less fluctuating plasma levels, e.g. in the levodopa treatment of Parkinson's disease (Crevoisier et al. 1987). Another field of application is the local delivery of drugs for the treatment of gastric ulcers, e.g. misoprostol or antibiotics against *Helicobacter pylori* (Singh and Kim 2000).

The gastric residence time of a pharmaceutical dosage form depends on a variety of physiological factors such as feeding state, quality and quantity of ingested food, age, and state of health. As it also strongly depends on the size and density of the formulation, the most promising approaches for prolonging gastric residence time with a substantial number of human clinical trials are floating and expanding systems, as well as mucoadhesive systems. Floating devices are buoyant in the stomach due to their low overall density compared to the gastric content (1.004–1.010 g cm^{-3}), whereas expanding systems swell or unfold after swallowing which makes them too big to pass the pyloric sphincter (Klausner et al. 2003). Mucoadhesive systems, on the other hand, stick to the gastric mucosa. Although a prolonged gastric residence time can be achieved with such systems, they carry the risk of oesophageal adherence which might result in drug-induced injuries (Talukder and Fassihi 2004; Streubel et al. 2006). Another approach for gastroretention is high-density systems, which owe their weight to heavy inert excipients such as barium sulfate, zinc oxide, titanium dioxide or iron powder (Singh and Kim 2000). They are assumed to sink to the bottom of the stomach and remain entrapped in the folds of the antrum. However, high-density systems have not yet proved efficient in vivo (Bardonnet et al. 2006). In addition, magnetic systems can be applied. In that case, an external magnet placed on the abdomen retains the dosage form which also contains a small magnet in the desired position. The main drawback of this method is the difficulty of exact positioning of the external magnet (Bardonnet et al. 2006).

Floating drug delivery systems (FDDS) and low-density systems can be single unit or multiple unit dosage forms. According to their mechanism of action, they are classified as effervescent and non-effervescent formulations.

Effervescent FDDS become buoyant due to gas generation after a certain lag time upon ingestion. In most cases, carbonates or bicarbonates are used. They liberate carbon dioxide upon contact with gastric contents. A broad variety of designs has been developed. Matrix systems are prepared using swellable hydrophilic polymers such as methylcellulose or chitosan, in combination with carbonates/bicarbonates and tartaric/citric acid. Upon contact with water, the polymer swells, CO_2 is generated and remains entrapped. After a certain lag time, the device starts floating and releases its drug load (Arora et al. 2005). Another approach is to coat the drug-containing cores with an inner layer of effervescent agents, i.e. one sublayer containing sodium bicarbonate and another sublayer containing tartaric acid, and an outer swellable layer of polyvinyl acetate and shellac (Arora et al. 2005; Ichikawa et al. 1991). A similar principle is employed for floating capsules that contain a multitude of granules with different residence times in the stomach

according to their coatings. Increasing the thickness of the swellable membrane layer results in better floating characteristics on the one hand and higher lag time and decreased release rates on the other hand (Arora et al. 2005). Furthermore, a variety of multi-layered tablets has been designed, e.g. a swellable asymmetric triple-layer tablet that contains a separate gas-generating layer (Arora et al. 2005; Yang et al. 1999). For zero-order controlled release in the stomach, a multilayer tablet was composed of two barrier layers and one drug layer. In that way, erosion of the barrier layers accompanies increased API exposure. Yet another interesting approach is the combination of floating and expansion due to the incorporation of rapidly swellable polymers with effervescent materials (Arora et al. 2005). A special case is raft-forming systems which contain hydrophilic polymers that produce a viscous gel layer floating on top of the gastric fluids due to entrapped CO_2 bubbles (Bardonnet et al. 2006). As illustrated above, most designs are based on a combination of effervescent substances with swellable polymers. Nevertheless, ion exchange resins loaded with bicarbonate and surrounded by a semipermeable membrane can be employed as well (Arora et al. 2005). Another approach for gas generation was described in the 1970s. Volatile liquids such as cyclopentene or ether were entrapped in certain chambers where they gasify at body temperature (Arora et al. 2005; Singh and Kim 2000). The majority of effervescent systems, however, rely on carbonates or bicarbonates.

Non-effervescent FDDS are buoyant because of entrapped air or fatty excipients, with hydrodynamically balanced systems (HBS) being one of the most important approaches. They contain swellable hydrophilic polymers, which enclose air bubbles resulting in an overall density that is lower than the gastric contents. Flotation of these devices depends on the type of polymer and on the presence of air inclusions within the matrix. The mixture of drug and swellable polymer can either be filled into capsules or compressed to form tablets. Another prominent approach is the preparation of hollow microparticles, so-called microbubbles, with drug-containing shells. They are prepared by solvent evaporation technique using, e.g., polycarbonate, various Eudragit® types, polypropylene or poly(methyl methacrylate). Other types of floating microbeads include alginate beads that are produced by precipitation of alginate in calcium chloride solution or microspheres prepared by spray-drying. In addition, polypropylene foam powder was proposed as a low-density excipient for single-unit systems as well as for multiparticulate systems (Streubel et al. 2006). Further creative designs include a sheet-like multilayered device with drug-containing layers and barrier films that are sealed together in such a way that small air pockets are entrapped, as well as combinations of several technologies, such as floating devices coated with mucoadhesive polymers (Arora et al. 2005; Singh and Kim 2000).

Marketed products based on FDDS include floating capsules such as Madopar® HBS (Roche), a sustained release formulation of levodopa and benserazide for the treatment of Parkinson's disease, and Valrelease® (Roche), a diazepam formulation. Another example is Cytotec® (Pfizer), a bilayer floating capsule for the local delivery of misoprostol for treatment of gastric ulcers. Furthermore, raft-forming systems are commercially available, such as the antacids Gaviscon®

(GlaxoSmithKline) and Topalkan® (Pierre Fabre), which are used for the treatment of gastroesophageal reflux (Arora et al. 2005; Singh and Kim 2000).

However, some limitations of currently available technologies still remain. A drawback of single unit systems is that they can leave the stomach prematurely. Their "all-or-nothing" emptying process may lead to unreliable and irreproducible therapeutic effects. Multiple unit systems are therefore preferable (Singh and Kim 2000; Bardonnet et al. 2006). According to their mechanism, non-effervescent systems with immediate buoyancy are regarded as safer than effervescent systems that float only after a certain lag time with increased risk of premature emptying from the stomach (Streubel et al. 2006). Generally, efficient floating depends on the presence of enough liquid in the stomach. Thus, patients have to drink water frequently, which could impair patient compliance (Hwang et al. 1998). Keeping in mind also that standard non-disintegrating controlled-release tablets can show significant gastric retention times, gastroretentive technologies still have to prove their additional benefit (Waterman 2007).

Nevertheless, there have been promising results in vitro and in vivo. Because many drug therapies would benefit from reliably prolonged gastric retention, extensive research efforts are still underway. Thus, more successful floating drug delivery systems may be expected in the future.

2.6 Enzyme-Mediated Release in the Colon

The enzyme-mediated liberation of an active agent from its carrier has been a widely studied research topic in drug delivery. Extensive interest in this release-triggering mechanism for oral administration originates from the differential enzyme patterns and activities encountered during GI passage (Table 1). The colon has been a site of special interest due to its high bacterial count and therefore enzyme-rich environment which is dominated by (oxido-)reductive (nitroreductase, hydrogenase, azoreductase, nitric oxide reductase, sulfoxide reductase, etc.) and hydrolytic enzymes (β-glucuronidase, glycosidase, esterase, amidase, sulfatase, etc.) (Bourgeois et al. 2005; Scheline 1973). Since occurrence and high activity of these enzymes are characteristic for the colon, these facts can be exploited for the local liberation of active agents concurrent with low absorption in the upper GI tract. In practice this has been of interest for the therapy of chronic disease states such as colon-involving Crohn's disease, ulcerative colitis (Friend 2005) or colon carcinoma, but also for oral vaccination and the delivery of proteins and peptides. Although it has not been fully resolved to date whether the colon can serve as a location for sufficient protein absorption to result in a systemic effect, its relative lack of protein-degradative enzymes, comparably long residence time of the chyme and high responsiveness to absorption enhancers have driven sustained efforts in this field (Rubinstein et al. 1997; Mackay et al. 1997). Generally, enzyme-degradable drug delivery systems for colon-specific administration are either based on (polymeric) prodrugs or polymers to be used as coating materials for the drug core and as embedding media for drug entrapment into matrix or hydrogel systems (Sinha and Kumria 2003).

2.6.1 Prodrugs

The use of prodrugs for the delivery of therapeutic agents to the colon has mainly been pursued in the context of the local treatment of inflammatory bowel diseases (IBD). Conjugation of the active agent with hydrophilic or high molecular weight molecules is supposed to reduce premature absorption and to increase availability of the drug in the colon (Friend 2005). In order to be able to formulate prodrugs, the active agent molecule has to bear chemically reactive hydroxyl, amino, nitro or carboxyl groups. Using these functionalities, prodrugs with mostly azo and glycoside linkages have been synthesised for colon-specific delivery by conjugation of the drug with artificial polymers, mono-, oligo- or multimers of sugars, amino acids, cyclodextrins (Minami et al. 1998), glucuronic acid (Simpkins et al. 1988), dextran (Larsen et al. 1991), as well as sulfonamides and *p*-aminobenzoic acid (Bourgeois et al. 2005). It has to be emphasised that careful choice of the carrier molecule is an essential developmental step. This has been clearly demonstrated in the case of sulfasalazine for the treatment of Crohn's disease and ulcerative colitis, which consists of sulfapyridine (carrier) azo-linked to mesalazine (active agent). It was found that upon enzyme-mediated cleavage of the azo bond high concentrations of sulfapyridine are absorbed which subsequently lead to side effects (Peppercorn 1984). This has resulted in the development of mesalazine prodrugs incorporating safer carrier molecules such as ipsalazide and balsalazide or olsalazine consisting of two mesalazine molecules as the most elegant example (Chan et al. 1983). Other approaches include linkage of mesalazine to higher molecular weight carriers such as polysulfonaidoethylene (Polyasa®) or polyamidoamine dendrimers (Wiwattanapatapee et al. 2003) with the advantage of fecal excretion of the carrier after drug release in the colon. Generally, in the case of prodrug formation with high molecular weight polymers, the likelihood of absorption of the conjugate in the upper GI tract drastically decreases, leading to more effective colonic delivery. However, care has to be taken when lipophilic APIs are bound to the hydrophilic polymer chains, since sufficient aqueous solubility of the prodrug is a key prerequisite for its enzymatic degradability.

Another approach to the treatment of IBD relies on the β-glycosidic derivatisation of non-polar anti-inflammatory corticosteroids such as dexamethasone, prednisolone or hydrocortisone with carbohydrates, resulting in hydrophilic prodrugs (Friend and Chang 1984). Aside from site-specific delivery to the inflamed tissues, the altered polarity of the resulting compounds decreases absorption in the small intestine, which consequently lowers systemic side effects as well as the therapeutic dose.

2.6.2 Polymeric Coatings and Matrices

The cladding of solid dosage forms with environment-responsive coatings constitutes an essential technique for the development of efficient oral formulations. If preferential release of the active agent is desired in the colon, enzymatically cleavable polymers represent promising coating as well as matrix materials.

The suitability of several classes of polymers and polysaccharides for this purpose has been intensively studied (Friend 2005). In order to attain successful delivery to the colon, the coating material should be characterised by good film-forming properties, low permeability and poor water solubility. However, a certain degree of swellability needs to be present for efficient enzymatic degradation.

The first biodegradable polymers used for colon drug delivery were azo polymers usually consisting of a hydrophilic, a hydrophobic and an azo moiety (Bourgeois et al. 2005). By varying the proportions of the three constituents it has been feasible to design polymers with improved degradation (more hydrophilic) and protection characteristics (more hydrophobic) (Van den Mooter et al. 1992, 1993). However, possible toxicity of the reduced azo aromatic linkers and rather slow degradation rates of the coating material in the colon have constituted drawbacks to this approach. Alternatively, the use of (naturally occurring) polysaccharides might be preferable due to their biodegradability, availability and inexpensiveness and due to the abundance of hydrolysing glycosidases (di-, tri and polysaccharidases) produced by colon-resident bacteria (Havgaard and Brondsted 1996). In order to compensate for the inferior film-forming properties and often high water solubility of these substances, modifications altering the hydrophilicity of the backbone or blending with synthetic polymers have been investigated (Chourasia and Jain 2004). Among the polysaccharides most frequently applied are amylose (Milojevic et al. 1996), guar gum (Tugcu-Demiroz et al. 2004), inulin (Vervoort and Kinget 1996), and chondroitin sulfate. For the latter mucopolysaccharide, the degree of crosslinking with dicyclohexylcarbodiimide can control the drug release characteristics of the formulation (Rubinstein et al. 1992). The polycationic polysaccharide chitosan has also frequently been applied for the development of colon-release forms, but suffers from rather high aqueous solubility at gastric pH levels (Hejazi and Amiji 2003). In order to overcome this and to improve integrity of the chitosan-coated cores in the upper GI tract, acid-resistant coatings with Eudragit L-100R® or L-100S® (see chapter Haag in this book) have been proposed (Lorenzo-Lamosa et al. 1998). Chitosan was also used to formulate beads for multiparticulate hydrogel systems by polyelectrolyte complexation with tripolyphosphate. It was found that the degradation of the hydrogel matrix is dependent on the molecular weight and degree of deacetylation of chitosan (Zhang et al 2002). In the case of pectin, another frequently used polysaccharide, the high solubility in the upper GI tract has also been limited its use as a coating material, but thick-compression coats or a combination with ethylcellulose have been shown to clearly reduce premature drug release from pectin-coated delivery systems (Ahmed 2005). The interested reader is directed to a comprehensive collection of studies dealing with microbially-triggered pectin-containing formulations (Bourgeois et al. 2005).

An alternative and possibly more advanced colon-targeted delivery system (CODES™) contains the synthetic disaccharide lactulose. This system consists of a core with lactulose and drug, an inner acid-soluble coating (Eudragit® E) and an outer enteric coating (Eudragit® L). The latter dissolves in the small intestine, leading to the diffusion of intestinal fluids into the core where the lactulose dissolves. Lactulose diffuses from the core into the colonic lumen and is

subsequently degraded by bacterial enzymes. This leads to a locally acidic environment which in turn results in dissolution of the acid-soluble layer and release of the drug (Katsuma et al. 2002).

In the face of the successful delivery of locally acting therapeutics to the colon by enzymatically triggered release formulations, the exploitation of this site-specific mechanism for the oral delivery of biotech drugs remains an interesting perspective for future investigations.

2.7 Biorecognition

A rather sophisticated way to improve oral delivery is the exploitation of biorecognition mechanisms. They can either be used for bioadhesion in order to achieve prolonged residence times in the GI tract or for highly specific delivery to a certain tissue. Among bioadhesives, a widely studied approach is mucoadhesion of polymers such as chitosan, polyacrylates, and polythiolated polymers due to non-specific interpenetration of polymer chains and mucus. Unfortunately, these classical mucoadhesives have not yet proven successful for peroral delivery in humans (Gabor et al. 2004; Varum et al. 2008). A more site-specific approach is based on cytoadhesion. For this purpose, a drug delivery system is functionalised with a ligand that binds to a specific structure on the surface of a certain cell type. Generally, this is accomplished by covalent coupling of the targeter to the surface of the delivery vehicle. Upon binding at the target site, drug is released. If the target is a receptor that can mediate endocytosis, binding may even result in cytoinvasion – assuming that the delivery system has the right size. A prerequisite, however, is the identification of appropriate target structures, followed by the identification of a suitable ligand that can be coupled to the surface of the delivery system and resists premature degradation in the GI tract. Among a variety of different approaches for targeting to the small intestine, glycotargeting using lectins is one of the most popular.

Lectins are proteins that specifically bind to certain carbohydrate moieties (Sharon and Lis 2004). In the intestine, sugars are present in the mucus and in the glycocalyx of the enterocytes being part of the "sugar code" which represents an important mechanism of biorecognition (Gabius 2000). As known from the literature, the sugar composition of the glycocalyx is a tissue-specific feature that can be exploited for targeting (Gabor et al. 2004).

Targeting to enterocytes has attracted a lot of interest in the last years, especially for enhancing the uptake of nanoparticles aiming at the delivery of drugs that cannot yet be administered perorally because of their poor solubility, absorption or stability in the GI tract. In addition, targeting to M cells is extensively studied for improving oral vaccine delivery. Their main function is the delivery of antigen from the gut lumen to the GALT. Besides targeting to healthy intestinal epithelium, there are approaches for targeting of antibiotics to *H. pylori*, specific delivery of anticancer drugs to colon carcinoma cells and many more.

2.7.1 Targeting to Enterocytes

Based on studies of the binding characteristics of various fluorescein-labelled plant lectins with differing carbohydrate specificities, the glycosylation pattern of enterocytes has been assessed. *N*-Acetyl-glucosamine-binding lectins such as wheat germ agglutinin (WGA) from *Triticum vulgare* and tomato lectin (LEA) from *Lycopersicum esculentum* bind to intestinal epithelial cells to a high degree. WGA interacts with the glycosylated extracellular domains of the epidermal growth factor (EGF) receptor resulting in receptor-mediated endocytosis (Lochner et al. 2003). The intracellular localisation of internalised WGA has been studied using Caco-2 cells, which are a model for human intestinal epithelial cells. The majority of the internalised lectin was found in lysosomes (Wirth et al. 2002). Experiments with Caco-2 monolayers also demonstrated a certain degree of transcytosis for WGA. Cytoadhesion and cytoinvasion using Caco-2 cells were also observed for WGA-functionalised nanoparticles (Weissenboeck et al. 2004; Fillafer et al. 2008). Thus, WGA might be a promising candidate for enhancing peroral delivery of drugs. It mediates not only mucoadhesion and cytoadhesion to enterocytes, but also cytoinvasion and even partial transcytosis. Moreover, it is not degraded by GI enzymes. While some plant lectins are highly toxic, WGA is a dietary lectin that is also found in wheat products. In the concentrations necessary for glycotargeting no toxic effects are expected, although this still has to be confirmed by in vivo studies. Other aspects that still have to be further elucidated include the risk of eliciting an immune response to WGA during its use for drug delivery (Gabor et al. 2004).

Besides lectin-mediated targeting, other approaches comprise targeting to the vitamin B_{12} receptor for endocytosis (Russell-Jones et al. 1999), to peptide transporters (Walter et al. 1996), as well as bile acid-mediated transport (Swaan et al. 1996). Targeting to the transferrin receptor has also been assessed, but this strategy has the critical drawback that the receptor is mainly expressed at the basolateral side of enterocytes.

2.7.2 Targeting to M Cells

M cells are specialised epithelial cells overlying Peyer's patches and mesenteric lymph nodes (Miller et al. 2007). They are able to take up antigen from the gut lumen and process it to the gut-associated parts of the immune system. Therefore, they are the main target for improving the efficacy of oral vaccination.

Oral vaccines have better compliance than parenteral vaccines, they are more cost-effective, do not bear the risk of contracting blood-borne diseases, and can generate both mucosal and systemic immunity (Aziz et al. 2007). Although the overall experience with marketed products has been positive, there are only a few oral vaccines available (Foxwell et al. 2007). Until now, most oral vaccines have been based on live-attenuated organisms (O'Hagan et al. 2006). The best-known

example is the oral poliovirus vaccine (OPV) which was adopted in the 1960s and successfully contributed to the worldwide reduction of polio cases. Nevertheless, live-attenuated OPV still bears the risk of re-acquisition of the neurovirulence and transmission characteristics of wild polioviruses and should therefore be replaced – and in fact is in Europe – by inactivated poliovirus vaccine in order to eradicate polio completely (Bonnet and Dutta 2008). Other examples of available oral vaccines are live-attenuated cholera, typhoid and rotavirus vaccine.

Besides antimicrobial vaccination, oral immunotherapy against type I allergy is an important goal. About one quarter of the population of developed countries suffers from allergies. In this field, the only causal treatment is specific allergen immunotherapy with the goal of shifting the antibody response from a Th2 type immune reaction to a Th1 type resulting in an alleviation of allergic symptoms. To date, it is only available via the subcutaneous and the sublingual routes (Roth-Walter and Jensen-Jarolim 2007).

For mucosal immunisation in the intestine, antigen has to cross the intestinal epithelium and gain access to the gut-associated lymphoid tissue (GALT). This transcytosis occurs mainly at M cells. Their major function is the uptake and transport of antigens from the gut lumen to the GALT which allows the immune system to monitor the gut content. M cells are well suited to this purpose: their apical membrane has a reduced glycocalyx and reduced membrane hydrolytic enzyme activity. Moreover, the number of lysosomes is dramatically reduced which allows transport of intact antigen to the GALT. In the lymphoid follicles, the antigen is captured by antigen-presenting cells followed by the induction of an immune response which finally results in the production of antibodies. Mucosal immunity is mainly due to secretory imunoglobulin A (IgA) which captures the antigen at the mucosa and neutralises virus and endotoxin without causing tissue damage (Brandtzaeg 2007). The gut mucosa contains at least 80% of the body's activated B cells, and about 90% of them produce IgA (Brandtzaeg et al. 1989). Therefore, mucosal vaccines are of special interest in the fight against pathogens that cause mucosal infection or invade through the mucosa (Levine 2003). In addition to mucosal immunity, systemic immunity with long-lasting serum IgG and IgA responses can be induced. The protection ranges from a reduction of symptoms to the complete inhibition of infection.

Recently, the focus in antimicrobial vaccine research has been on subunit and DNA vaccines (Peek et al. 2008). Subunit vaccines use only part of the pathogen, for example a certain protein, as the antigen. They have the advantage that they cannot revert to a virulent form and do not contain contaminants derived from the pathogenic organism. However, oral DNA and subunit vaccines as well as allergens must be protected from the low pH in the stomach and from digestive enzymes.

A range of vaccine delivery systems has been developed over the past years. They include bacterial and viral live vectors, virus-like particles, non-living delivery systems and transgenic plant "edible vaccines". Among non-living systems, liposomes, proteosomes and a range of polymeric particles have been investigated (O'Hagan et al. 2006). Microparticles prepared from the

biocompatible and biodegradable polymer poly(D,L-lactide-*co*-glycolide) are the most extensively studied non-viral delivery platform (Brayden and Baird 2001; Perrie et al. 2007).

Particle uptake into M cells is influenced by their size and surface characteristics. While it is generally accepted that particles below 1 μm are efficiently transcytosed through M cells, there is still some controversy concerning the maximum particle size that can be taken up. Some authors state that the optimum size for transcytosis goes up to 2, 3 or even 5 μm (Roth-Walter and Jensen-Jarolim 2007; O'Hagan et al. 2006; Brayden 2001). Moreover, hydrophobic particles are better absorbed by M cells than hydrophilic ones, negatively or neutrally charged particles better than positively charged ones (des Rieux et al. 2006).

Uptake of antigen from non-targeted systems is usually not sufficient for the induction of mucosal immunity, whereas systems that adhere to M cells are effectively transcytosed. A major challenge is the identification of an M cell-specific target. One of the main drawbacks is the high M cell variability among species and individuals, as well as among different physiological states and ages (des Rieux et al. 2006; Brayden and Baird 2001). Another is the lack of appropriate in vivo and in vitro experimental models (Foxwell et al. 2007). Up to now, various approaches have been studied leading to more or less promising results. The two main strategies are the use of plant lectins for glycotargeting and the mimicry of well-known pathogen–cell interactions (Brayden et al. 2005).

The most widely used plant lectin for M cell targeting is *Ulex europaeus* agglutinin 1 (UEA-1) which specifically binds to α-L-fucose (Foster and Hirst 2005). *Aleuria aurantia* lectin (AAL) is an alternative (Roth-Walter et al. 2004, 2005). UEA-1 and AAL binding have been evaluated in mice. However, humans lack α-L-fucose on the apical surface of M cells.

The other main strategy follows the example of pathogens that use M cells as their entry ports. Bacteria such as *Yersinia*, *Salmonella* and *Shigella* interact with M cells via adhesins. A *Yersinia* adhesin called invasin has been shown to interact with cell surface β_1-integrins (Clark, et al. 1998). In contrast to other polarised epithelia, β_1-integrins are not restricted to the basolateral side in M cells, but are also expressed apically. This has also been exploited for targeting of nanoparticles with the RGD peptide motif (des Rieux et al. 2006). Another adhesin studied for M cell targeting is reovirus adhesin σ1 (Miller et al. 2007). Additionally, the ganglioside GM1, the B subunit of the cholera toxin receptor, was also considered for improving vaccine delivery. Although it is present on enterocytes as well, the anatomical features of M cells make the receptor more accessible and could therefore appear more attractive. In addition, IgA-mediated interaction with M cells might be a further approach (des Rieux et al. 2006).

Although some fundamental challenges on the way to successful M cell targeting still remain, there are encouraging results giving hope that future improvements for the induction of immunity against various pathogens as well as for oral immunotherapy against type I allergy may be expected.

2.7.3 Miscellaneous

Specific targeting has also been proposed for the eradication of *H. pylori* from the stomach. This pathogen, which resides deep in the gastric mucus, causes diseases such as dyspepsia, gastritis and ulcers. It is also associated with a significantly higher risk of gastric cancer and MALT lymphoma. Besides other gastroretentive dosage forms, specifically targeted systems could be applied for even more efficacious delivery of antibiotic drugs. One approach utilises glycotargeting. Interestingly, in that case the sugar is the targeter, and the lectin is the target. The bacterial adhesin BabA2 binds to the fucosylated histo-blood group antigen Lewis b at the surface of gastric cells. Accordingly, fucose-functionalised nanoparticles preferentially adhere to the bacteria. Unfortunately, not all strains of *H. pylori* express BabA2 (Bardonnet et al. 2006).

In addition, cancer-specific cell surface structures might be exploited for targeted delivery. For instance, the β-galactoside specific lectins galectin-1 and -3 are upregulated in many epithelial tumors such as colon cancer and could be used as targets (Minko 2004).

2.8 Absorption Enhancers

2.8.1 General Considerations

Today, several factors are known to negatively influence a drug's bioavailability, including insufficient dissolution, fast degradation of the compound in the gastric or intestinal fluid, poor membrane permeation and an extensive first-pass metabolism. Uptake across the intestinal epithelium has always attracted special attention, representing a critical step in the chain of events that cover the absorption of a drug and its transport to the site of action. When taking a closer look at the physicochemical nature of a specific compound, different properties are associated with reduced membrane permeability, such as a low octanol/water partitioning, the presence of charged functional groups, a large polar surface area or high molecular weight (see Sect. 1.1). A considerable amount of work has been done in order to improve mucosal transport, and can basically be divided into strategies that tend to alter the physicochemical nature of the API itself and strategies that tend to alter the permeability of the membrane. Since the principle of applying chemical modifications to the API itself – that is to say the development of equally active derivatives or prodrugs – is often difficult or even impossible and has to be carried out for each specific agent individually, the strategy of rendering the membrane more permeable would be highly advantageous and could be applied for various substances without further changes.

For the development of formulations that are based on co-administration of one or more absorption-enhancing excipients, several key issues have to be taken into account. Firstly, in order to clarify if an improvement in oral bioavailability by

enhancers is possible, it is important to determine exactly the predominant limiting factor(s) in an API's pharmacokinetic profile. Particular attention should be laid on a thorough understanding of the uptake mechanism, since the principle of absorption enhancement is not limited to influencing membrane permeability, but may also include other strategies such as an impairment of secretory transport. After a thoroughgoing evaluation of a drug's kinetic fate in the human body, the choice of absorption promoter should be based on a detailed understanding of its mechanism of action (Yeh et al. 1995).

Up to now, penetration-enhancing formulations have unfortunately not been able to reach their full potential, due to general problems that apply to the majority of substances independent of their chemical origin. Several difficulties that have to be dealt with are intrinsically associated with the principle of co-administration. For an effective absorption enhancement, the API as well as the excipient have to be present at the site of uptake in sufficient concentrations and at the same time, requiring a synchronised delivery to the intended target area. Detecting differences in intestinal distribution is therefore of obvious importance. If necessary, release may be improved by application of additional delivery strategies, such as enteric coating (Aungst 2000).

The main concern that is common to all types of membrane permeation enhancers is the pervading danger of toxic side effects. The risk of causing severe damage to the mucosal membrane by trying to manipulate its permeability is inherently connected to this approach and gives rise to the controversy about the usefulness of absorption enhancers. It is difficult to accurately determine the severity of damage that is inflicted on the membrane – which may be able to recover – by treatment with a permeation enhancer, but from today's point of view many of the compounds investigated so far seem to be at least as deleterious as helpful. However, some promising results have been published on excipients of different chemical classes that seem to enhance drug absorption without compromising cell viability, even when used in concentrations that effectively improve uptake (Hastewell et al. 1994; Whitehead et al. 2008). For most enhancers studied so far, steep concentration vs. effect profiles in vitro have been shown, along with relatively small safety margins. In many cases, there is no difference between effective and membrane damaging concentrations (Whitehead et al. 2008). It is worth mentioning that most enhancers show a higher cytotoxic potential in cell culture models than in intact intestinal membranes, a fact that is attributed to the presence of repair mechanisms which allow for a certain recovery from trauma (Aungst 2000). However, the potential for recovery is limited and often may not be high enough to sufficiently enlarge the therapeutic concentration window to a safe level.

2.8.2 Strategies of Absorption Enhancement

Achieving beneficial impact on bioavailability by use of absorption enhancers requires thorough knowledge of the drug's pharmacokinetics and a understanding of the working mechanism of the penetration enhancer. Unfortunately, up to now

there is no complete understanding of all the principles that govern enhancer behavior. Besides concepts that directly affect the physiology of the mucosal membrane, additional aspects should be considered in the context of drug absorption, such as the possibility of improving the intestinal dissolution of a poorly water-soluble drug by solubilising excipients. For many absorption enhancers, this feature is known to be the primary cause or at least a supporting factor for the absorption-enhancing potential (Stegemann et al. 2007). Taking a closer look at the intestinal epithelium, an enhancer can principally act as a promoter of the transcellular route or the paracellular route or by interfering with active secretory transport. Concerning membrane-specific effects, one should generally keep in mind that, comparable to an API which might possess sites of preferential uptake in the course of the GI passage, there might be sections with higher and lower response to a specific enhancer. Thus, the effect of absorption enhancement may also be regioselective and has to be coordinated to the distribution profile of the agent (Aungst 2000).

Solubilising Excipients

The trend of today's new chemical entities towards higher molecular weight or increased lipophilicity often entails the problem of limited gastrointestinal solubility and thus may be associated with limited oral bioavailability (Stegemann et al. 2007). Especially for substances in BCS class II (poorly soluble but permeable, see Sect. 2), an enhanced dissolution rate would be expected to result directly in increased uptake after oral administration. The possibilities for influencing drug solubility are numerous and cover modifications to the molecule itself, e.g. by conversion to various salts (see Sect. 1.1), as well as the co-administration of dissolution-promoting excipients. The field of solubilisation enhancement has – apart from using a variety of conventional chemical principles (Strickley 2004) – experienced considerable impact in recent years by applying newly developed techniques such as cyclodextrins or self-emulsifying drug delivery systems, and is still far from being fully explored.

Cyclodextrins are cyclic oligomers of glucose which can be categorised in different groups according to their molecular weight and chemical nature. Regarding oral formulations, the β-cylodextrins and various newly developed derivatives are of major importance (Stegemann et al. 2007). By forming inclusion complexes with lipophilic molecules, cyclodextrins are able to temporarily modify the physicochemical properties of a substance and are able to remarkably improve a compound's solubility and stability (Davis and Brewster 2004). Another advantage of cyclodextrins is their inertness towards biological membranes in living tissue, that is to say they enhance solubility and uptake of the included API but do not get absorbed themselves (Gould and Scott 2005).

Lipid-based drug delivery systems have received increased attention within recent years, due not only to a presumed ability of using the lymphatic pathway of absorption, which covers direct uptake in the lymphatic system, but also due to the

possibility of formulating self-emulsifying drug delivery systems. Such systems are based upon mixtures of several lipophilic, amphiphilic or hydrophilic excipients that together form a thermodynamically stable microemulsion of nanosized droplets when brought into aqueous environment (Porter et al. 2008). Medium-chain glycerides have proven to be suitable candidates for the development of such self-emulsifying systems (Constantinides et al. 1996), and are believed to facilitate transcellular uptake in addition to their use as solubility promoters (Muranushi et al. 1981). Moreover, some excipients contained in self-emulsifying systems may possibly inhibit cytochrome P enzymes and P-glycoprotein-mediated blood-to-lumen transport, e.g. Tween 80 or Cremophor RH40 (Mountfield et al. 2000; Hugger et al. 2002). To date, several formulations employing the principle of self-emulsification have been successfully marketed (e.g. Sandimmun Neoral®, Norvir®).

The Transcellular Route

A major part of the compounds known to enhance absorption are believed to have direct influence on the cellular membrane, thereby facilitating transport via the transcellular route. This group comprises surfactants, medium chain triglycerides, fatty acids, steroidal detergents, acylcarnitines or alkanoylcholines, α-amino acids and others (Aungst 2000). The exact mechanism that renders the cell membrane more permeable is still unknown, but probably includes a variety of different factors such as an increased bilayer fluidity, solubilisation of membrane components and pore-forming effects. So far, most analytical studies have been confined to a detection of intracellular uptake of marker molecules or release of cellular or membrane components into the surrounding medium, both being indicators for an increased membrane permeability without providing further information on the underlying principle. Though the impact on membrane integrity seems to be at least partly reversible for several enhancers, this approach naturally is strongly connected to potential toxic side effects.

The Paracellular Route

In comparison to the transcellular pathway, enhancing transport via the paracellular route requires the – at least transient – opening of the interconnecting tight junctions without affecting the overall membrane integrity, and thus would probably be less dangerous for cell viability. The dimension of the paracellular space is 3–5 nm at maximum, which presumably limits this mode of passage to solutes with a molecular radius of not more than 1.5 nm (~3.5 kDa) (Salama et al. 2006). For permeation enhancers, the trigger that results in opening of tight junctions could not be clearly identified so far, but based on the fact that the zonula occludens is strongly interconnected with the underlying cytoskeleton, a Ca^{2+}-dependent contraction of perijunctional actin fibers is discussed as a possible mechanism (Fasano and Uzzau 1997). Additionally, extracellular Ca^{2+} depletion by chelating agents or other lytic

effects could render the zonula occludens more permeable. An interesting approach in this context describes the utilisation of substances that mimic endogenous modulators of the tight junctions, proposing these molecules as promising candidates for the realisation of a reversible absorption enhancement which should be less damaging. Zonula occludens toxin and its biologically active fragment have been successfully used for this purpose (Fasano and Uzzau 1997).

Secretory Transport Inhibitors

Certain drugs, though lipophilic and thus in principle not hindered in their ability to cross membranes, may nevertheless be limited in oral bioavailability due to active transport from the enterocyte back into the intestinal lumen. Any inhibition of these transport systems, which include, e.g., P-glycoprotein and other members of the family of multidrug resistance-associated proteins, would consequently lead to an increase in net absorptive permeation (Aungst 2000). However, when using this principle attention should be paid to the avoidance of systemic side effects, since these transport proteins are not found exclusively in the intestinal mucosa. In addition to designated inhibitors of these transporters such as cyclosporin (Terwogt et al. 1998), several pharmaceutical excipients that have already been in use for a long time have meanwhile been proven to exert influence on secretory transport, e.g. Tween 80 and Cremophor EL (Nerurkar et al. 1996; Porter et al. 2008; Hugger et al. 2002).

2.8.3 Outlook

For a critical evaluation of absorption enhancers, it is important to mention that development and testing of penetration enhancers is usually carried out using in vitro models such as Caco-2 cells, although the performance of the same enhancers in vivo has been shown to considerably differ in many cases. Often, results obtained in cell culture or Using chamber tests cannot be successfully reproduced in vivo, and it is unclear whether cytotoxicity evaluations performed in vitro are predictive for the situation in vivo (Aungst 2000). Up to now, a comparative evaluation of absorption enhancers has also been hindered by the fact that a multitude of different testing methods has provided highly heterogeneous results. Especially regarding solubilising excipients, novel testing models that take into account, for example, the effect of endogenously derived emulsifiers in the digestive fluids are greatly needed and could further deepen our understanding of absorption enhancement (Porter et al. 2008). Whitehead et al. recently published a study conducted on Caco-2 cell monolayers that included 153 known permeation enhancers, aiming to assess their “therapeutic concentration window” by comparing their enhancing potency with their toxic potential. Taking into account that the results obtained still need to be confirmed by in vivo experiments, the study nevertheless gives a clear indication that effective penetration enhancement is possible without compromising safety (Whitehead et al. 2008).

3 Future Perspectives

In future, the large and growing market share of oral formulations will include developments which might fall into two categories, personalised medicine and formulations for poorly absorbable chemical entities, comprising biotech as well as already established drugs.

Whereas a distinct formulation for each individual person remains a fiction, it is a fact that age strongly influences the required dose and the dosing frequency. Children are often "therapeutic orphans" since pharmaceutical developments as well as clinical studies are conceived for adults. At least in Europe, drug-containing drops or lollipops are not the ultimate solution to meet the demands of paediatric formulations. For the growing elderly population, physiological changes in the gastrointestinal pH, the residence time or the blood flow, and morphological changes such as possible mucosal atrophy or decreasing liver function, as well as sometimes restricted motor function, must be considered when designing a dosage form. Oral formulations meeting these challenges related to age are expected to greatly improve therapeutic success.

Another challenge is to consider circadian rhythms such as those of heart rate, blood pressure, hormone plasma levels, gastric pH, renal function and disease state, e.g. in asthma or allergy. Considering these parameters will require skillful modified release formulations which in turn will reduce side effects and improve efficacy.

Nowadays, the genetic mechanisms behind a growing number of diseases such as cancer or various metabolic diseases are elucidated. Needless to say, there is a huge interest in exploiting this knowledge for novel treatment regimes. However, the main limitation is the lack of appropriate delivery devices. Therapeutic genes as well as proteins and peptides have to be protected from premature degradation and they have to be specifically delivered to their molecular targets. Although there are still fundamental obstacles to successful gene delivery in general, some researchers already dare to hope that one day even gene therapeutics could be administered perorally. To date, despite considerable efforts, the delivery of sufficient amounts of therapeutic proteins and peptides by the oral route remains a challenge. Nature has equipped the gastrointestinal tract with a range of protective mechanisms to limit the entry of bioactive proteins or genetic compounds. Thus, the success of perorally administered biotech-derived therapies will depend on our ability to overcome gastrointestinal barriers without impairing the physiological functionality of the intestine.

References

Ahmed IS (2005) Effect of simulated gastrointestinal conditions on drug release from pectin/ethylcellulose as film coating for drug delivery to the colon. Drug Dev Ind Pharm 31:465–470

Aikawa K, Matsumoto K, Uda H, Tanaka S, Shimamura H, Aramaki Y, Tsuchiya S (1998) Hydrogel formation of the pH response polymer polyvinylacetal diethylaminoacetate (AEA). Int J Pharm 167:97–104

Akala EO, Kopeckova P, Kopecek J (1998) Novel pH-sensitive hydrogels with adjustable swelling kinetics. Biomaterials 19:1037–1047

Allen A (1984) The structure and function of gastrointestinal mucus. In: Boedeker EC (ed) Attachment of organisms to the gut mucosa. CRC, Boca Raton, FL, pp 4–10

Amidon GL, Lennernäs H, Shah VP, Crison JR (1995) A theoretical basis for a biopharmaceutic drug classification: the correlation in-vitro drug product dissolution and in vivo bioavailability. Pharm Res 12:413–420

Anal AK (2007) Time-controlled pulsatile delivery systems for bioactive compounds. Recent Pat Drug Deliv Formul 1:73–79

Anton N, Benoit JP, Saulnier P (2008) Design and production of nanoparticles formulated from nano-emulsion templates – A review. J Control Release 128:185–199

Arora S, Ali J, Ahuja A, Khar RK, Baboota S (2005) Floating drug delivery systems: a review. AAPS PharmSciTech 6:E372–E390

Asghar LFA, Chandran S (2006) Multiparticulate formulation approach to colon specific drug delivery: current perspectives. J Pharm Pharm Sci 9:327–338

Aungst BJ (2000) Intestinal permeation enhancers. J Pharm Sci 89:429–442

Aziz MA, Midha S, Waheed SM, Bhatnagar R (2007) Oral vaccines: new needs, new possibilities. Bio Essays 29:591–604

Bardonnet PL, Faivre V, Pugh WJ, Piffaretti JC, Falson F (2006) Gastroretentive dosage forms: overview and special case of *Helicobacter pylori*. J Control Release 111:1–18

Bar-Shalom D, Bukh M, Kindt Larsen T (1991) Egalet®, a novel controlled-release system. Ann NY Acad Sci 618:578–580

Basak P, Adhikari B (2008) Poly (vinyl alcohol) hydrogels for pH dependent colon targeted drug delivery. J Mater Sci Mater Med. doi:10.1007/s10856-008-3496-0

Bernkop-Schnürch A (2005) Thiomers: a new generation of mucoadhesive polymers. Adv Drug Deliv Rev 57:1569–1582

Bonnet MC, Dutta A (2008) World wide experience with inactivated poliovirus vaccine. Vaccine 26:4978–4983

Borm P, Klaessig FC, Landry TD, Moudgil B, Pauluhn J, Thomas K, Trottier R, Wood S (2006) Research strategies for safety evaluation of nanomaterials, Part V: role of dissolution in biological fate and effects of nanoscale particles. Toxicol Sci 90:23–32

Bourgeois S, Harvey R, Fattal E (2005) Polymer colon drug delivery systems and their application to peptides, proteins, and nucleic acids. Am J Drug Deliv 3:171–204

Brandtzaeg P (2007) Induction of secretory immunity and memory at mucosal surfaces. Vaccine 25:5467–5484

Brandtzaeg P, Halstensen TS, Kett K, Krajci P, Kvale D, Rognum TO, Scott H, Sollid LM (1989) Immunobiology and immunopathology of human gut mucosa: humoral immunity and intraepithelial lymphocytes. Gastroenterol 97:1562–1584

Brayden DJ (2001) Oral vaccination in man using antigens in particles: current status. Eur J Pharm Sci 14:183–189

Brayden DJ, Baird AW (2001) Microparticle vaccine approaches to stimulate mucosal immunisation. Microbes Infect 3:867–876

Brayden DJ, Jepson MA, Baird AW (2005) Keynote review: intestinal Peyer's patch M cells and oral vaccine targeting. Drug Discov Today 10:1145–1157

Brockmeier D, Grigoleit HG, Leonhardt H (1986) The absorption of piretanide from the gastrointestinal tract is site-dependent. Eur J Clin Pharmacol 30:79–82

Brondsted H, Kopecek J (1992) Hydrogels for site-specific drug delivery to the colon: in vitro and in vivo degradation. Pharm Res 9:1540–1545

Brown SR, Cann PA, Read NW (1990) Effect of coffee on distal colon function. Gut 31:450–453

Brunner E (1904) Theorie der Reaktionsgeschwindigkeit in heterogenen Systemen. Z Phys Chem 47:56–102

Chan RP, Pope DJ, Gilbert AP, Sacra PJ, Baron JH, Lennard-Jones JE (1983) Studies of two novel sulfasalazine analogs, ipsalazide and balsalazide. Dig Dis Sci 28:609–615

Chawla G, Gupta P, Koradia V, Bansal AK (2003) Gastroretention – a means to address regional variability in intestinal drug absorption. Pharm Technol 27:50–68
Chen SC, Wu YC, Mi FL, Lin YH, Yu LC, Sung HW (2004) A novel pH-sensitive hydrogel composed of N,O-carboxymethyl chitosan and alginate cross-linked by genipin for protein drug delivery. J Control Release 96:285–300
Chen X, Young TJ, Sarkari M, Williams Iii RO, Johnston KP (2002) Preparation of cyclosporine A nanoparticles by evaporative precipitation into aqueous solution. Int J Pharm 242:3–14
Chiu HC, Hsiue GH, Lee YP, Huang LW (1999) Synthesis and characterization of pH-sensitive dextran hydrogels as a potential colon-specific drug delivery system. J Biomater Sci Polym Ed 10:591–608
Chourasia MK, Jain SK (2004) Polysaccharides for colon targeted drug delivery. Drug Deliv 11:129–148
Clark MA, Hirst BH, Jepson MA (1998) M-cell surface beta 1 integrin expression and invasin-mediated targeting of Yersinia pseudotuberculosis to mouse Peyer's patch M cells. Infect Immun 66:1237–1243
Colombo P, Santi P, Bettini R, Brazel CS (2000) Drug release from swelling-controlled systems. In: Wise DL (ed) Handbook of pharmaceutical controlled release technology. Marcel Dekker, New York, pp 183–209
Conley R, Gupta SL, Sathyan G (2006) Clinical spectrum of the osmotic-controlled release oral delivery system (OROS), an advanced oral delivery form. Curr Med Res Opin 22:1879–1892
Constantinides PP, Welzel G, Smith EH, PL SS, Yiv SH, Owen AB (1996) Water-in-oil micro-emulsions containing medium-chain fatty acids/salts: formulation and intestinal absorption enhancement evaluation. Pharm Res 13:210–215
Crevoisier C, Hoevels B, Zurcher G, Da Prada M (1987) Bioavailability of L-dopa after Madopar HBS administration in healthy volunteers. Eur Neurol 27:36–46
Davis ME, Brewster ME (2004) Cyclodextrin-based pharmaceutics: past, present and future. Nat Rev Drug Discov 3:1023–1035
Davis SS, Khosla R, Wilson CG, Washington N (1987) The gastrointestinal transit of a controlled release pellet formulation of tiaprofenic acid. Int J Pharm 34:253–258
Debongie JC, Philips SF (1978) Capacity of the human colon to absorb fluid. Gastroenterology 74:698–703
Delie F, Blanco-Prieto MJ (2005) Polymeric particulates to improve oral bioavailability of peptide drugs. Molecules 10:65–80
Dearn AR (1997) Atovaquone pharmaceutical compositions. US6018080
des Rieux A, Fievez V, Garinot M, Schneider Y-J, Préat V (2006) Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach. J Control Release 116:1–27
Dressman JB (1986) Comparison of canine and human gastrointestinal physiology. Pharm Res 3:123–131
Edsbäcker S, Bengtsson B, Larsson P, Lundin P, Nilsson A, Ulmius J, Wollmer P (2003) A pharmacoscintigraphic evaluation of oral budesonide given as controlled-release (Entocort) capsules. Aliment Pharmacol Ther 17:525–536
Edwards C (1997) Physiology of the colorectal barrier. Adv Drug Deliv Rev 28:173–190
Engel GL, Farid NA, Faul MM, Richardson LA, Winneroski LL (2000) Salt form selection and characterization of LY333531 mesylate monohydrate. Int J Pharm 198:239–247
Evans DF, Pye G, Bramley R, Clark AG, Dyson TJ, Hardcastle JD (1988) Measurement of gastrointestinal pH profiles in normal, ambulant human subjects. Gut 29:1035–1041
Fasano A, Uzzau S (1997) Modulation of intestinal tight junctions by zonula occludens toxin permits enteral administration of insulin and other macromolecules in an animal model. J Clin Invest 99:1158–1164
Fedorak RN, Bistritz L (2005) Targeted delivery, safety, and efficacy of oral enteric-coated formulations of budesonide. Adv Drug Deliv Rev 57:303–316

Feldman M, Barnett C (1991) Fasting gastric pH and its relationship to true hypochlorhydria in humans. Dig Dis Sci 36:866–869
Fillafer C, Friedl DS, Wirth M, Gabor F (2008) Fluorescent bio-nanoprobes to characterise cytoadhesion and cytoinvasion. Small 4:627–633
Florence AT (1997) The oral absorption of micro- and nanoparticulates: neither exceptional nor unusual. Pharm Res 14:259–266
Florence AT (2005) Nanoparticle uptake by the oral route: Fulfilling its potential? Drug Discov Today Technol 2:75–81
Florence AT (2006) Nanoparticle flow: implications for drug delivery. In: Torchilin VP (ed) Nanoparticles as drug carriers. Imperial College Press, London, pp 9–27
Florence AT, Attwood D (2006) Physicochemical principles of pharmacy, 4th edn. Pharmaceutical Press, London, Chicago
Foster N, Hirst BH (2005) Exploiting receptor biology for oral vaccination with biodegradable particulates. Adv Drug Deliv Rev 57:431–450
Foxwell AR, Cripps AW, Kyd JM (2007) Optimization of oral immunization through receptor-mediated targeting of M cells. Hum Vaccin 3:220–223
Friend DR (2005) New oral delivery systems for treatment of inflammatory bowel disease. Adv Drug Deliv Rev 57:247–265
Friend DR, Chang GW (1984) A colon-specific drug-delivery system based on drug glycosides and the glycosidases of colonic bacteria. J Med Chem 27:261–266
Gabius HJ (2000) Biological information transfer beyond the genetic code: the sugar code. Naturwissenschaften 87:108–121
Gabor F, Bogner E, Weissenböck A, Wirth M (2004) The lectin-cell interaction and its implications to intestinal lectin-mediated drug delivery. Adv Drug Deliv Rev 56:459–480
Galli C (2006) Experimental determination of the diffusion boundary layer width of micron and submicron particles. Int J Pharm 313:114–122
Gallo SH, McClave SA, Makk LJK, Looney SW (1996) Standardization of clinical criteria required for use of the 12.5mm barium tablet in evaluating esophageal lumenal patency. Gastrointest Endosc 44:181–184
Gazzaniga A, Palugan L, Foppoli A, Sangalli ME (2008) Oral pulsatile delivery systems based on swellable hydrophilic polymers. Eur J Pharm Biopharm 68:11–18
Gazzaniga A, Sangalli ME, Giordano F (1994) Oral chronotopic® drug delivery systems: achievement of time and/or site specificity. Eur J Pharm Biopharm 40:246–250
Gohil JM, Bhattacharya A, Ray P (2006) Studies on the cross-linking of poly (vinyl alcohol). J Polym Res 13:161–169
Gould S, Scott RC (2005) 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD): a toxicology review. Food Chem Toxicol 43:1451–1459
Gupta VS, Beckert TE, Price JC (2001) A novel pH- and time-based multi-unit potential colonic drug delivery system. Int J Pharm 213:83–91
Hanafy A, Spahn-Langguth H, Vergnault G, Grenier P, Tubic Grozdanis M, Lenhardt T, Langguth P (2007) Pharmacokinetic evaluation of oral fenofibrate nanosuspensions and SLN in comparison to conventional suspensions of micronized drug. Adv Drug Deliv Rev 59:419–426
Harder S, Fuhr U, Beermann D, Staib AH (1990) Ciprofloxacin absorption in different regions of the human gastrointestinal tract. Investigations with hf-capsule. Br J Clin Pharmacol 30:35–39
Hardy JG, Evans DF, Zaki I, Clark AG, Tonnesen HH, Gamst ON (1987) Evaluation of an enteric-coated naproxen tablet using gamma scintigraphy and pH monitoring. Int J Pharm 37:245–250
Hardy JG, Wilson CG, Wood E (1985) Drug delivery to the proximal colon. J Pharm Pharmacol 37:874–877
Hastewell J, Lynch S, Fox R, Williamson I, Skelton-Stroud P, Mackay M (1994) Enhancement of human calcitonin absorption across the rat colon in vivo. Int J Pharm 101:115–120
Havgaard L, Brondsted H (1996) Current applications of polysaccharides in colon targeting. Crit Rev Ther Dug Carrier Syst 13:185–223

Hejazi R, Amiji M (2003) Chitosan-based gastrointestinal delivery systems. J Control Release 89:151–165
Herbig SM, Cardinal JR, Korsmeyer RW, Smith KL (1995) Asymmetric-membrane tablet coatings for osmotic drug-delivery. J Control Release 35:127–136
Houghton LA, Mangnall YF, Read NW (1990) Effect of incorporating fat into a liquid test meal on the relation between intragastric distribution and gastric emptying in human volunteers. Gut 31:1226–1229
Hu J, Johnston KP, Williams RO (2003) Spray freezing into liquid (SFL) particle engineering technology to enhance dissolution of poorly water soluble drugs: organic solvent versus organic/aqueous co-solvent systems. Eur J Pharm Sci 20:295–303
Hu M, Amidon GL (1988) Passive and carrier mediated intestinal absorption components of captopril. J Pharm Sci 77:1007–1011
Hugger ED, Novak BL, Burton PS, Audus KL, Borchardt RT (2002) A comparison of commonly used polyethoxylated pharmaceutical excipients on their ability to inhibit P-glycoprotein activity in vitro. J Pharm Sci 91:1991–2002
Hussar DA (2000) Tablet-splitting games are bad patient care ... and bad pharmacy practice. Pharm Today 6:5
Hwang SJ, Park H, Park K (1998) Gastric retentive drug-delivery systems. Crit Rev Ther Drug Carrier Syst 15:243–284
Ichikawa M, Watanabe S, Miyake Y (1991) A new multiple-unit oral floating dosage system. I: preparation and in vitro evaluation of floating and sustained-release characteristics. J Pharm Sci 80:1062–1066
Jani P, Halbert GW, Langridge J, Florence AT (1990) Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. J Pharm Pharmacol 42:821–826
Jani PU, Florence AT, McCarthy DE (1992) Further histological evidence of the gastrointestinal absorption of polystyrene nanospheres in the rat. Int J Pharm 84:245–252
Jobin G, Cortot A, Godbillon I, Duval M, Schoeller JP, Hirtz J, Bernier JJ (1985) Investigation of drug absorption from the gastrointestinal tract of man. I. Metoprolol in the stomach, duodenum and jejunum. Br J Clin Pharmacol 19:975–1055
Kararli TT (1989) Gastrointestinal absorption of drugs. Crit Rev Ther Drug Carrier Syst 6:39–86
Katsuma M, Watanabe S, Kawai H, Takemura S, Masuda Y, Fukui M (2002) Studies on lactulose formulations for colon-specific drug delivery. Int J Pharm 249:33–43
Keck CM, Müller RH (2006) Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. Eur J Pharm Biopharm 62:3–16
Kesisoglou F, Panmai S, Wu Y (2007) Nanosizing – oral formulation development and biopharmaceutical evaluation. Adv Drug Deliv Rev 59:631–644
Kimura K, Hirayama F, Uekama K (1999) Characterization of tolbutamide polymorphs (Burger's forms II and IV) and polymorphic transition behavior. Int J Pharm 88:385–391
King LS, Lozono D, Agre P (2004) From structure to disease: the evolving tale of aquaporin biology. Nat Rev Mol Cell Biol 5:687–698
Kirwan WO, Smith AN (1974) Gastrointestinal transit estimated by an isotope capsule. Scand J Gastroenterol 9:763–766
Klausner EA, Lavy E, Friedman M, Hoffman A (2003) Expandable gastroretentive dosage forms. J Control Release 90:143–162
Kompella UB, Lee VHL (2001) Delivery systems for penetration enhancement of peptide and protein drugs: design considerations. Adv Drug Deliv Rev 46:211–245
Krause KP, Kayser O, Mäder K, Gust R, Müller RH (2000) Heavy metal contamination of nanosuspensions produced by high-pressure homogenisation. Int J Pharm 196:169–172
Krishnamachari Y, Madan P, Lin S (2007) Development of pH- and time-dependent oral microparticles to optimize budesonide delivery to ileum and colon. Int J Pharm 338:238–247
Kumar RMN (2000) Nano and microparticles as controlled drug delivery devices. J Pharm Pharm Sci 3:234–258

Lamprecht A, Schaefer U, Lehr CM (2001) Size-dependent bioadhesion of micro- and nanoparticulate carriers to the inflamed colonic mucosa. Pharm Res 18:788–793
Langguth P, Fricker G, Wunderli-Allenspach H (2004) Biopharmazie. Wiley, Weinheim, p 180 Chapter 5
Larsen C, Jensen BH, Olesen HP (1991) Stability of ketoprofen-dextran ester prodrugs in homogeneates of various segments of the pig GI tract. Acta Pharm Nord 3:41–44
Lennernäs H, Abrahamsson B (2005) The use of biopharmaceutic classification of drugs in drug discovery and development: current stauts and future extension. J Pharm Pharmacol 57:273–285
Levine MM (2003) Can needle-free administration of vaccines become the norm in global immunization? Nat Med 9:99–103
Lin CC, Metters AT (2006) Hydrogels in controlled release formulations: network design and mathematical modeling. Adv Drug Deliv Rev 58:1379–1408
Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 46:3–26
Lipton JR, Coder DM, Jacobs LR (1988) Longterm effects of fermentable fibres on rat colonic pH and epithelial cell cycle. J Nutr 118:840–845
Liu L, Ku J, Khang G, Lee B, Rhee JM, Lee HB (2000) Nifedipine controlled delivery by sandwiched osmotic tablet system. J Control Release 68:145–156
Liversidge GG, Cundy KC (1995) Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: I. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. Int J Pharm 125:91–97
Lochner N, Pittner F, Wirth M, Gabor F (2003) Wheat germ agglutinin binds to the epidermal growth factor-receptor of artificial Caco-2 membranes as detected by silver nanoparticle enhanced fluorescence. Pharm Res 20:833–839
Lorenzo-Lamosa ML, Remunan-Lopez C, Vila-Jato JL, Alonso MJ (1998) Design of microencapsulated chitosan microspheres for colonic drug delivery. J Control Release 52:109–118
Lui CY, Amidon GL, Berardi RR (1986) Comparison of gastrointestinal pH in dogs and humans: implications of the use of the beagle dog as a model for oral absorption in humans. J Pharm Sci 75:271–274
Mackay M, Phillips J, Hastewell J (1997) Peptide drug delivery: colonic and rectal absorption. Adv Drug Deliv Rev 28:253–273
Mayo-Pedrosa M, Cachafeiro-Andrade N, Alvarez-Lorenzo C, Martinez-Pacheco R, Concheiro A (2008) In situ photopolymerization-coated pellets for pH-dependent drug delivery. Eur Polymer J 44:2626–2638
McNeil NI, Ling KLE, Wager J (1987) Mucosal surface pH of the large intestine of the rat and of normal and inflamed large intestine in man. Gut 28:707–713
Melander A (1987) Influence of food on the bioavailability of drugs. Clin Pharmacokinet 3:337–341
Merisko-Liversidge E, Liversidge GG, Cooper ER (2003) Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur J Pharm Sci 18:113–120
Metcalf AM, Phillips SF, Zinsmeister AR, Mac-Carthy RL, Beart RW, Wolff BG (1987) Simplified assessment of segmental colonic transit. Gastroenterology 91:40–47
Miller H, Zhang J, KuoLee R, Patel GB, Chen W (2007) Intestinal M cells: the fallible sentinels? World J Gastroenterol 13:1477–1486
Milojevic S, Newton JM, Cummings JH, Gibson GR, Botham RL, Ring SG, Stockham M, Allwood MC (1996) Amylose as a coating for drug delivery to the colon: preparation and in vitro evaluation using 5-aminosalicylic acid pellets. J Control Release 38:75–84
Minami K, Hirayama F, Uekama K (1998) Colon-specific drug delivery based on a cyclodextrin prodrug: release behaviour of biphenylylacetic acid from its cyclodextrin conjugates in rat intestinal tracts after oral administration. J Pharm Sci 87:715–720
Minko T (2004) Drug targeting to the colon with lectins and neoglycoconjugates. Adv Drug Deliv Rev 56:491–509

Möschwitzer J, Müller RH (2007) Drug nanocrystals – the universal formulation approach for poorly soluble drugs. In: Thassu D, Deleers M, Pathak Y (eds) Nanoparticulate Drug Delivery Systems. Informa Healthcare, New York, pp 71–88
Mountfield RJ, Senepin S, Schleimer M, Walter I, Bittner B (2000) Potential inhibitory effects of formulation ingredients on intestinal cytochrome P450. Int J Pharm 211:89–92
Moustafine RI, Kabanova TV, Kemenova VA, Van Den Mooter G (2005) Characteristics of interpolyelectrolyte complexes of Eudragit E100 with Eudragit L100. J Control Release 103:191–198
Muranushi N, Takagi S, Muranishi S, Sezaki H (1981) Effect of fatty acids and monoglycerides on permeability of lipid bilayer. Chem Phys Lipids 28:269–279
Müller RH, Peters K (1998) Nanosuspensions for the formulation of poorly soluble drugs: I. Preparation by a size-reduction technique. Int J Pharm 160:229–237
Nernst W (1904) Theorie der Reaktionsgeschwindigkeit in heterogenen Systemen. Z Phys Chem 47:52–55
Nerurkar MM, Burton PS, Borchardt RT (1996) The use of surfactants to enhance the permeability of peptides through caco-2 cells by inhibition of an apically polarized efflux system. Pharm Res 13:528–534
Noyes AA, Whitney WR (1897) The rate of solution of solid substances in their own solutions. J Am Chem Soc 19:930–934
O'Hagan DT, Singh M, Ulmer JB (2006) Microparticle-based technologies for vaccines. Methods 40:10–19
Ozeki Y, Ando M, Watanabe Y, Danjo K (2004) Evaluation of novel one-step dry-coated tablets as a platform for delayed-release tablets. J Control Release 95:51–60
Patel VR, Amiji MM (1996) Preparation and characterization of freeze-dried chitosan-poly (ethylene oxide) hydrogels for site-specific antibiotic delivery in the stomach. Pharm Res 13:588–593
Peek LJ, Middaugh CR, Berkland C (2008) Nanotechnology in vaccine delivery. Adv Drug Deliv Rev 60:915–928
Peppas LB, Peppas NA (1990) Dynamic and equilibrium swelling behaviour of pH-sensitive hydrogels containing 2-hydroxyethyl methacrylate. Biomaterials 11:635–644
Peppercorn MA (1984) Sulfasalazine: pharmacology, clinical use, toxicity, and related new drug development. Ann Int Med 101:377–386
Perrie Y, Kirby D, Bramwell VW, Mohammed AR (2007) Recent developments in particulate-based vaccines. Recent Pat Drug Deliv Formul 1:117–129
Porter CJH, Pouton CW, Cuine JF, Charman WN (2008) Enhancing intestinal drug solubilisation using lipid-based delivery systems. Adv Drug Deliv Rev 60:673–691
Qiu Y, Park K (2001) Environment-sensitive hydrogels for drug delivery. Adv Drug Deliv Rev 53:321–339
Rasenack N, Hartenhauer H, Müller BW (2003) Microcrystals for dissolution rate enhancement of poorly water-soluble drugs. Int J Pharm 254:137–145
Read NW, Miles CA, Fisher D, Holgate AM, Kime ND, Mitchell MA, Reeve AM, Walker W (1980) Transit of a meal through the stomach, small intestine and colon of normal subjects and its role in the pathogenesis of diarrhea. Gastroenterology 79:1276–1282
Roth-Walter F, Schoell I, Untersmayr E, Fuchs R, Boltz-Nitulescu G, Weissenboeck A, Scheiner O, Gabor F, Jensen-Jarolim E (2004) M cell targeting with Aleuria aurantia lectin as a novel approach for oral allergen immunotherapy. J Allergy Clin Immunol 114:1362–1368
Roth-Walter F, Bohle B, Schoell I, Untersmayr E, Scheiner O, Boltz-Nitulescu G, Gabor F, Brayden DJ, Jensen-Jarolim E (2005a) Targeting antigens to murine and human M-cells with *Aleuria aurantia* lectin-functionalized microparticles. Immunol Lett 100:182–188
Roth-Walter F, Schoell I, Untersmayr E, Ellinger A, Boltz-Nitulescu G, Scheiner O, Gabor F, Jensen-Jarolim E (2005b) Mucosal targeting of allergen-loaded microspheres by *Aleuria aurantia* lectin. Vaccine 23:2703–2710
Roth-Walter F, Jensen-Jarolim E (2007) Oral immunotherapy against type I allergy. J Allergy Clin Immunol 19:21–26

Rouge N, Buri P, Doelker E (1996) Drug absorption sites in the gastrointestinal tract and dosage forms for site-specific delivery. Int J Pharm 136:117–139
Rubinstein A, Nakar D, Sintov A (1992) Chondroitin-sulfate: a potential biodegradable carrier for colon-specific drug delivery. Int J Pharm 84:141–150
Rubinstein A, Tirosh B, Baluom M, Nassar T, David A, Radai R, GlikoKabir I, Friedman M (1997) The rationale for peptide drug delivery to the colon and the potential of polymeric carriers as effective tools. J Control Release 46:59–73
Russell-Jones GJ, Arthur L, Walker H (1999) Vitamin B_{12}-mediated transport of nanoparticles across Caco-2 cells. Int J Pharm 179:247–255
Salama NN, Eddington ND, Fasano A (2006) Tight junction modulation and its relationship to drug delivery. Adv Drug Deliv Rev 58:15–28
Sangalli ME, Maroni A, Zema L, Busetti C, Giordano F, Gazzaniga A (2001) In vitro and in vivo evaluation of an oral system for time and/or site-specific drug delivery. J Control Release 73:103–110
Scheline RR (1973) Metabolism of foreign compounds by gastrointestinal microorganisms. Pharmacol Rev 25:451–523
Schiller C, Fröhlich CP, Giessmann T, Siegmund W, Moennikes H, Hosten N, Weitschies W (2005) Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonace imaging. Aliment Pharmacol Ther 22:971–979
Shakweh M, Ponchel G, Fattal E (2004) Particle uptake by Peyer's patches: a pathway for drug and vaccine delivery. Expert Opin Drug Deliv 1:141–163
Sharon N, Lis H (2004) History of lectins: from hemagglutinins to biological recognition molecules. Glycobiology 14:53R–62R
Shokri J, Ahmadi P, Rashidi P, Shahsavari M, Rajabi-Siahboomi A, Nokhodchi A (2008) Swellable elementary osmotic pump (SEOP): an effective device for delivery of poorly water-soluble drugs. Eur J Pharm Biopharm 68:289–297
Siegel RA, Falamarzian BA, Firestone BA, Moxley BC (1988) pH-controlled release from hydrophobic/polyelectrolyte co-polymer hydrogels. J Control Release 8:179–182
Simpkins JW, Smulkowski M, Dixon R, Tuttle R (1988) Evidence for the delivery of narcotic antagonists to the colon as their glucuronide conjugates. J Phamacol Exp Ther 244:195–205
Singh BN, Kim KH (2000) Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. J Control Release 63:235–259
Sinha VR, Kumria R (2003) Microbially triggered drug delivery to the colon. Eur J Pharm Sci 18:3–18
Smart JD (2005) Buccal drug delivery. Expert Opin Drug Deliv 2:507–517
Smith GW, Wiggins PM, Lee SP, Tasman-Jones C (1986) Diffusion of butyrate through pig colon mucus in vitro. Clin Sci 70:271–276
Sood A, Panchagnula R (2001) Peroral route: an opportunity for protein and peptide drug delivery. Chem Rev 101:3275–3303
Stegemann S, Leveiller F, Franchi D, de Jong H, Lindén H (2007) When poor solubility becomes an issue: from early stage to proof of concept. Eur J Pharm Sci 31:249–261
Streubel A, Siepmann J, Bodmeier R (2006) Drug delivery to the upper small intestine window using gastroretentive technologies. Curr Opin Pharmacol 6:501–508
Strickley RG (2004) Solubilizing excipients in oral and injectable formulations. Pharm Res 21:201–230
Swaan PW, Szoka FC, Oie S (1996) Use of the intestinal and hepatic bile acid transporters for drug delivery. Adv Drug Deliv Rev 20:59–82
Takeuchi H, Yamamoto H, Kawashima Y (2001) Mucoadhesive nanoparticulate systems for peptide drug delivery. Adv Drug Deliv Rev 47:39–54
Talukder R, Fassihi R (2004) Gastroretentive delivery systems: a mini review. Drug Dev Ind Pharm 30:1019–1028

Tang R, Orme CA, Nancollas GH (2004) Dissolution of crystallites: surface energetic control and size effects. Chem Phys Chem 5:688–696
Terwogt MJM, Beijnen JH, ten Bokkel Huinink WW, Rosing H, Schellens JHM (1998) Co-administration of cyclosporin enables oral therapy with paclitaxel. Lancet 352:285
Thomas E, Rubino J (1996) Solubility, melting point and salting-out relationships in a group of secondary amine hydrochloride salts. Int J Pharm 130:179–183
Thombre AG, Appel LE, Chidlaw MB, Daugherity PD, Dumont F, Evans LAF, Sutton SC (2004) Osmotic drug delivery using swellable-core technology. J Control Release 94:75–89
Thombre AG, Cardinal JR, DeNoto AR, Herbig SM, Smith KL (1999) Asymmetric membrane capsules for osmotic drug delivery. I. Development of a manufacturing process. J Control Release 57:55–64
Tugcu-Demiroz F, Acartürk F, Takka S, Konus-Boyunaga O (2004) In-vitro and in-vivo evaluation of mesalazine-guar gum matrix tablets for colonic drug delivery. J Drug Target 12:105–112
Van den Mooter G, Samyn C, Kinget R (1992) Azo polymers for colon-specific drug delivery. Int J Pharm 87:37–46
Van den Mooter G, Samyn C, Kinget R (1993) Azo polymers for colon-specific drug delivery. II: Influence of the type of azo polymer on the degradation by intestinal microflora. Int J Pharm 97:133–139
van Santen E, Barends DM, Frijlink HW (2002) Breaking of scored tablets: a review. Eur J Pharm Biopharm 53:139–145
Vandelli MA, Leo E, Forni F, Bernabei MT (1996) In vitro evaluation of a potential colonic delivery system that releases drug after controllable lag-time. Eur J Pharm Biopharm 43: 148–151
Varum FJ, McConnell EL, Sousa JJ, Veiga F, Basit AW (2008) Mucoadhesion and the gastrointestinal tract. Crit Rev Ther Drug Carrier Syst 25:207–258
Verma RK, Krishna DV, Garg S (2002) Formulation aspects in the development of osmotically controlled oral drug delivery systems. J Control Release 79:7–27
Verma RK, Mishra B, Garg S (2000) Osmotically controlled oral drug delivery. Drug Dev Ind Pharm 26:695–708
Vervoort L, Kinget R (1996) In vitro degradation by colonic bacteria of inulinHP incorporated in Eudragit RS films. Int J Pharm 129:185–190
Volkheimer G (1974) Passage of particles through the wall of the gastrointestinal tract. Environ Health Perspect 9:215–225
Walter E, Kissel T, Amidon GL (1996) The intestinal peptide carrier: a potential transport system for small peptide derived drugs. Adv Drug Deliv Rev 20:33–58
Walter SIE, De Vries JX, Nickel B, Stenzhorn G, Weber E (1989) The influence of different formula diets and different pharmaceutical formulations on the systemic bioavailabilty of paracetamol, gall bladder size and plasma glucose. Int J Clin Pharmacol Exp Ther Toxicol 27:544–550
Washington N, Washington C, Wilson CG (2002) Physiological pharmaceutics: barriers to drug absorption. Taylor & Francis, London
Waterman KC (2007) A critical review of gastric retentive controlled drug delivery. Pharm Dev Technol 12:1–10
Weissenboeck A, Bogner E, Wirth M, Gabor F (2004) Binding and uptake of wheat germ agglutinin-decorated PLGA-nanospheres by Caco-2 monolayers. Pharm Res 21:1919–1925
Weitschies W, Kosch O, Moennikes H, Trahms L (2005) Magnetic marker monitoring: an application of biomagnetic measurement instrumentation and principles for the determination of the gastrointestinal behaviour of magnetically marked solid dosage forms. Adv Drug Deliv Rev 57:1210–1222
Whitehead K, Karr N, Mitragotri S (2008) Safe and effective permeation enhancers for oral drug delivery. Pharm Res 25:1782–1788

Wirth M, Kneuer C, Lehr CM, Gabor F (2002) Lectin-mediated drug delivery: discrimination between cytoadhesion and cytoinvasion and evidence for lysosomal accumulation of wheat germ agglutinin in the Caco-2 model. J Drug Target 10:439–448

Wiwattanapatapee R, Lomlim L, Saramunee K (2003) Dendrimer conjugates for colonic delivery of 5-aminosalicylic acid. J Control Release 88:1–9

Wu W, Nancollas GH (1998) A new understanding of the relationship between solubility and particle size. J Solution Chem 27:521–531

Wu Y, Loper A, Landis E, Hettrick L, Novak L, Lynn K, Chen C, Thompson K, Higgins R, Batra U, Shelukar S, Kwei G, Storey D (2004) The role of biopharmaceutics in the development of a clinical nanoparticle formulation of MK-0869: a Beagle dog model predicts improved bioavailability and diminished food effect on absorption in human. Int J Pharm 285:135–146

Yang L, Eshraghi J, Fassihi R (1999) A new intragastric delivery system for the treatment of *Helicobacter pylori* associated gastric ulcer: in vitro evaluation. J Control Release 57:215–222

Yeh PY, Berenson MM, Samowitz WS, Kopečková P, Kopecek J (1995) Site-specific drug delivery and penetration enhancement in the gastrointestinal tract. J Control Release 36:109–124

Yoshioka M, Hancock BC, Zografi G (1994) Crystallization of indomethacin from the amorphous state below and above its glass transition temperature. J Pharm Sci 83:1700–1705

Yoshioka M, Hancock BC, Zografi G (1995) Inhibition of indomethacin crystallization in poly (vinylpyrrolidone) coprecipitates. J Pharm Sci 84:983–986

Zentner GM, Rork GS, Himmelstein KJ (1985) The controlled porosity osmotic pump. J Control Release 1:269–282

Zhang H, Alsarra IA, Neau SH (2002) An in vitro evaluation of a chitosan-containing multiparticulate system for macromolecule delivery to the colon. Int J Pharm 239:197–205

Transdermal Drug Delivery

Richard H. Guy

Contents

Abstract Transdermal drug delivery is a validated technology contributing significantly to global pharmaceutical care. Since 1980, impressive growth in this field has been observed with many commercial successes; importantly, a new chemical entity was recently developed and approved for transdermal administration without having first been given as an injectable or oral dosage form. The progress achieved has been based on the clearer understanding of skin barrier function, and of the physicochemical, pharmacokinetic and physiological factors which underpin the feasibility of transdermal administration. Novel, non-invasive approaches to enhance and control drug transport across the skin are under intensive investigation, and some technologies, e.g. iontophoresis, have reached true maturity. The "local", subcutaneous delivery of drugs (for example, to underlying muscle and other

R.H. Guy
Department of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath, BA2 7AY, UK
e-mail: r.h.guy@bath.ac.uk

This chapter is a modified version of the article "Transdermal Science and Technology – an Update," published by R.H. Guy in *Drug Delivery System*, **22**: 442–449 (2007).

M. Schäfer-Korting (ed.), *Drug Delivery*,
Handbook of Experimental Pharmacology 197,
DOI 10.1007/978-3-642-00477-3_13, © Springer-Verlag Berlin Heidelberg 2010

tissues) is gaining increasing acceptance, and new opportunities in this under-subscribed area may be envisaged.

Keywords Transdermal drug delivery · Skin · Skin penetration enhancement · Iontophoresis · Minimally invasive technology

1 Introduction

The ultimate objective of drug therapy is a non-invasive device which senses the patient's drug requirement and then delivers the right dose of the active agent at the right rate. Presently, this goal is achieved by the withdrawal of a blood sample, and an analysis of the drug itself, or of a biomarker, followed by drug administration typically via an intravenous injection or infusion.

Passive transdermal delivery meets the non-invasive criterion and is able to provide drug input over extended periods of time at essentially sustained and controlled rates (Fig. 1). But most patches provide only zero-order delivery and cannot offer a monitoring capability. In contrast, iontophoresis and the new generation of so-called "minimally invasive" technologies (such as microneedles and thermal poration methods) are taking a different view of the skin as a platform for information exchange.

This chapter examines the state of the art of transdermal drug delivery and explores the potential for future developments. While discussion concentrates on drugs for systemic effect, the concepts addressed apply just as well to the "local", subcutaneous delivery of anti-inflammatory agents.

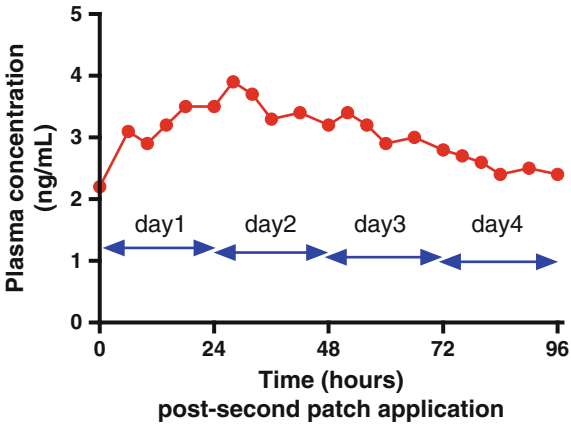


Fig. 1 Steady-state plasma concentrations of oxybutynin following repeated transdermal application over several days (http://www.oxytrol.com/index.asp)

2 The Present State of Transdermal Drug Delivery

2.1 Barrier Function of Skin

Significant advances in understanding the barrier function of the skin have been made in the last 30 years. The architecture of the stratum corneum (SC), the principal resistance to drug transport, has been determined, and the organisation of the SC intercellular lipids has been clearly related to their biophysical properties, and to their role in barrier function (Bouwstra and Ponec 2006).

From this research, straightforward algorithms to predict skin permeability have been derived (Fig. 2) (Potts and Guy 1992), so that the physicochemical feasibility of transdermal drug delivery can be tested in silico before any experiment is performed.

However, despite being able to assess the potential "deliverability" of a particular drug active agent, the pool of potential candidates for transdermal administration has not dramatically expanded. The criteria to be satisfied are identical to those identified before scopolamine (the first approved transdermal drug) was marketed in the early 1980s. Furthermore, even though the physical nature of the skin's barrier is now well-understood, the biology remains a real challenge, in that many chemicals, including some transdermal candidates, provoke irritation or sensitization of the skin. For the moment, circumvention of this problem is not possible, and a drug which is irritating cannot be considered for transdermal delivery. It is absolutely essential, therefore, that any drug/formulation combination being evaluated for transdermal feasibility is tested for irritation and sensitization early on in the development process.

2.2 Transdermally Delivered Drugs

Transdermal administration has several well-known advantages (Delgado-Charro and Guy 2001). Pre-systemic metabolism is avoided, permitting lower daily doses to be administered. Blood or plasma levels of the drug can be kept within the therapeutic window for extended periods of time, prolonging drug action and reducing the dosing frequency needed. Inter- and intra-patient variability are reduced, as a consequence, and patient compliance and acceptability improved. Lastly, input of drug can be stopped by removal of the patch.

$$\log k_p = -2.7 + 0.71 \cdot \log K_{o/w} - 0.0061 \cdot MW$$

$K_{o/w}$ = octanol–water partition coefficient.

MW = molecular weight. k_p units are cm/hr.

Fig. 2 Empirical algorithm for the prediction of a drug's permeability coefficient (k_p) across the skin from an aqueous vehicle (Potts and Guy 1992)

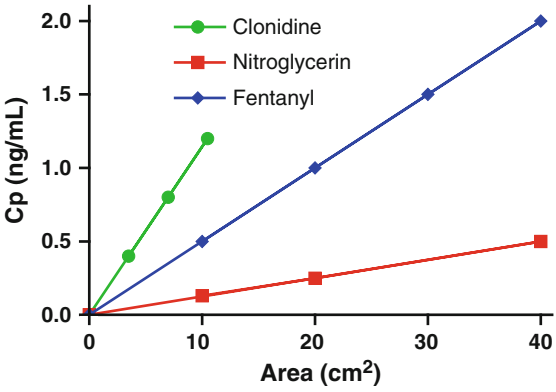


Fig. 3 Linear relationship between systemic, steady-state plasma concentration (Cp) and transdermal delivery system area for clonidine, nitroglycerin and fentanyl (Delgado-Charro and Guy 2001)

On the other hand, transdermal delivery is limited by several factors (Delgado-Charro and Guy 2001). It can only be used for potent drugs whose daily dose is of the order of 10 mg or less. Higher doses can only be accommodated by increasing patch size (Fig. 3). In general, from a practical and aesthetic standpoint, 100 cm^2 is considered to be the maximum acceptable. Transdermal drugs are "small" molecules, of molecular weights (at least, for the moment) no greater than 500 Da; lipophilicity is essential but, because the drug has to exit the SC to be resorbed systemically, some aqueous solubility is also necessary. Self-evidently, transdermal delivery is appropriate for drugs that are poorly (if at all) orally bioavailable, and which have short biological half-lives (implying an appreciable first-pass effect and an inconvenient dosing regimen). Finally, as mentioned already, the drug cannot be locally irritating or sensitising.

Transdermal delivery has been approved for the following drugs: scopolamine (motion sickness), nitroglycerin and isosorbide dinitrate (angina pectoris), clonidine (hypertension), estradiol (hormone replacement therapy), fentanyl (analgesia), nicotine (smoking cessation), testosterone (hypogonadism), norelgestromin + ethinyl estradiol (contraception), oxybutynin (incontinence), selegiline (depression), methylphenidate (attention deficit hyperactivity disorder), buprenorphine (analgesia), rivastigmine (dementia), rotigotine (anti-Parkinsonian) and granisetron (anti-emetic). The spectrum of diseases treated by transdermal administration is remarkable – the sole common link between these drugs is their potency. Rotigotine represents a particularly noteworthy example as it is the first new chemical entity developed ab initio for transdermal delivery. All others were already approved for delivery by other, more conventional routes of administration (usually oral) before their development for the transdermal route.

Oxybutynin (molecular weight is 357.5 Da, log (octanol–water partition coefficient (P)) is ~4.7) may be used to illustrate the characteristics of a transdermally administered drug (Dmochowski et al. 2003). The patch for oxybutynin is a thin, flexible, transparent, acrylic adhesive system which, when applied every 3–4 days (e.g. to the abdomen or upper arm), significantly reduces incontinence episodes. Observed skin irritation is limited. Approximately 4 mg of drug is delivered per day

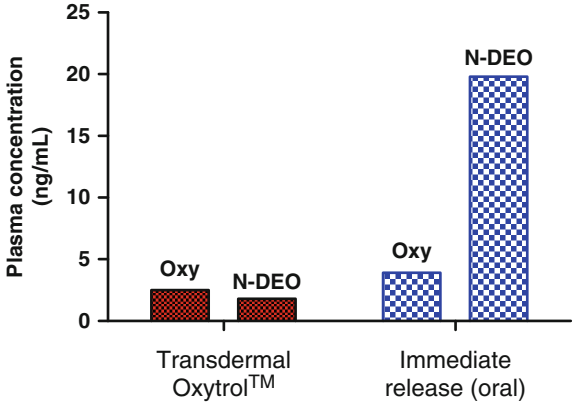


Fig. 4 Average steady-state concentrations of oxybutynin and its major metabolite *N*-desethyl-oxybutynin (N-DEO) in plasma following transdermal delivery (from the commercial Oxytrol™ patch) and oral administration (http://www.oxytrol.com/index.asp)

from a 39 cm^2 patch containing a total oxybutynin loading of 36 mg, and the system maintains relatively constant plasma concentrations (Fig. 1). When given orally, the drug suffers an extensive first-pass effect due to biotransformation via CYP3A4. This problem is totally eliminated by transdermal delivery, as evidenced by the significant reduction in the resulting systemic levels of the drug's principal metabolite (Fig. 4). Furthermore, as a result, the dose of oxybutynin which has to be given transdermally is much less.

2.3 Enhancement of Transdermal Drug Delivery

Passively delivering charged drugs, or compounds with molecular weights above 1,000 Da, is precluded to all intents and purposes by the SC barrier. To circumvent this impasse requires an enhancement technology. The present options can be grouped into three approaches:

- Formulations which include a chemical penetration enhancer;
- Physical action on the drug itself, e.g. iontophoresis; and
- Physical and/or mechanical energy applied to the barrier, such as ultrasound, microneedles, thermal poration.

Reviews of the penetration enhancer literature are abundant (Smith and Maibach 2005) and deliver a consistent message: that is, despite the fact that several marketed patches contain excipients which permit the drug to be transferred across the skin more rapidly, the incorporation of enhancers into transdermal delivery systems leads to an increased incidence of irritation. In fact, there is a direct

correlation between the efficacy of an enhancer to improve drug permeation and its inherent ability to elicit irritation; thus, the better the enhancer, the worse the irritation observed. For the moment, therefore, the enhancer–drug combination, which significantly promotes transport while causing minimal or no irritation, has not been defined (Karande et al. 2005). The regulatory barrier to the incorporation of a declared skin penetration enhancer in a novel formulation is also important. Agencies like the U.S. Food and Drug Administration will, not unreasonably, ask for evidence to demonstrate that a penetration enhancer really acts as such; further, if the excipient is not a generally-regarded-as-safe (GRAS) substance, then information testifying to its safety will also be required. Alternative strategies involving manipulations of a topical formulation include the incorporation of liposomes, or other "vectors", and the use of supersaturation. Despite considerable effort in the former area, no transdermal products which take advantage of this technology have yet been commercialised. With supersaturated vehicles, it has proved possible to improve drug flux without eliciting irritation (Fig. 5); however, there is a major stability problem which, at present, makes the approach practically difficult (Moser et al. 2001). Nevertheless, spray-on formulations are under development (Thomas and Finnin 2004), and these may ultimately demonstrate that the technology is feasible.

Iontophoresis enhances drug transport across the SC, without significant perturbation of the barrier *per se*. In iontophoresis, an electrical potential gradient imposed across the skin drives the movement of (primarily) ionised species through the SC which is normally impermeable to the transport of charged entities (Mudry et al. 2006). Ion electromigration is determined by the current flowing in the iontophoretic circuit and, as a result, the transdermal delivery of a drug is directly proportional to, and controlled by, the charge passed between the electrodes

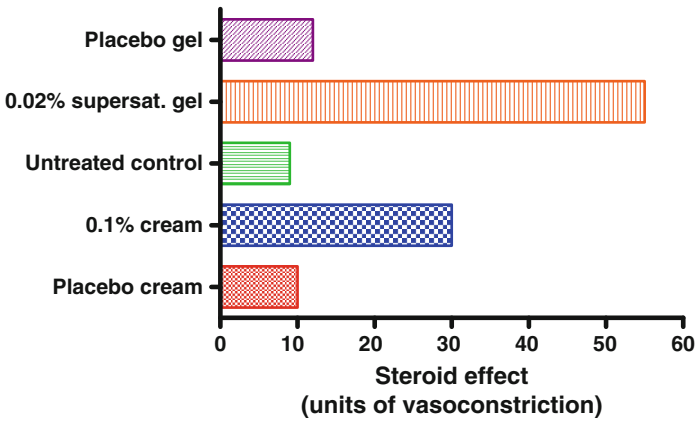


Fig. 5 Vasoconstriction induced by hydrocortisone (measured in arbitrary units) following administration in a marketed cream formulation (0.1%, w/v) and in a supersaturated gel in which the drug was present only at 0.02% (w/v). Three different controls were also evaluated as indicated (A.F. Davis, personal communication)

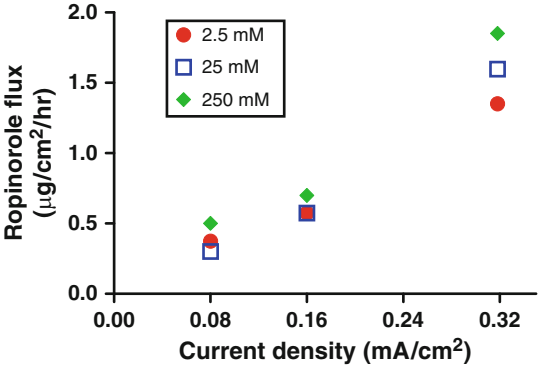


Fig. 6 Iontophoresis of ropinorole across the skin as a function of (**a**) current density, and (**b**) the drug hydrochloride's concentration in the driving (positive) electrode compartment (Luzardo-Alvarez et al. 2001)

contacting the skin (Fig. 6) (Luzardo-Alvarez et al. 2001). However, the ionised drug is only one of several available charge carriers in the system: for example, there may be ions of the same charge in the background electrolyte, and these can compete with the drug to carry charge into the skin; furthermore, ions of opposite charge within the body are able to transport charge in the opposite direction. Iontophoresis efficiency, therefore, is always less than 100% and is often very small. Moreover, electromigration is not the sole mechanism implicated in iontophoresis. Under normal physiological conditions, the skin carries a net negative charge and is therefore preferentially permeable to cations. Because the membrane is charged, the imposition of an electric field across the skin provokes a convective, electro-osmotic flow of solvent in the anode-to-cathode direction, increasing cation transport, retarding that of anions, and providing a means to enhance the transdermal delivery of neutral, water-soluble compounds. Electromigration and electro-osmosis contributions to the overall iontophoretic transport depend on drug size and mobility; the latter becomes increasingly more important with increasing molecular size (Guy et al. 2000). Although the principles of iontophoresis have been understood for more than a century, transdermal devices based on the technology have only recently reached the market (albeit, at least so far, with less than modest commercial success). Integrated delivery systems for lidocaine (inducing local anaesthesia) (Vyteris 2008) and fentanyl (to provide analgesia) (Janssen-Cilag 2009) have received regulatory approval, and an iontophoretic-based device has been introduced for glucose-monitoring in diabetics (Sieg et al. 2005). In the latter example, the electrical current "pulls" glucose across the skin from the extracellular fluid inside the body to the surface, where it is analysed in situ and the result translated into a measurement of glycaemia via a prior calibration. Iontophoresis is therefore a mature technology, feasible both for drug delivery and non-invasive monitoring. Other systems are being developed and applications in fertility treatment and in Parkinson's therapy are anticipated.

The development of technologies with which to "short-circuit" the barrier function of the skin has been the subject of great activity in recent years. These approaches are characterised by the creation of new pathways through the SC and

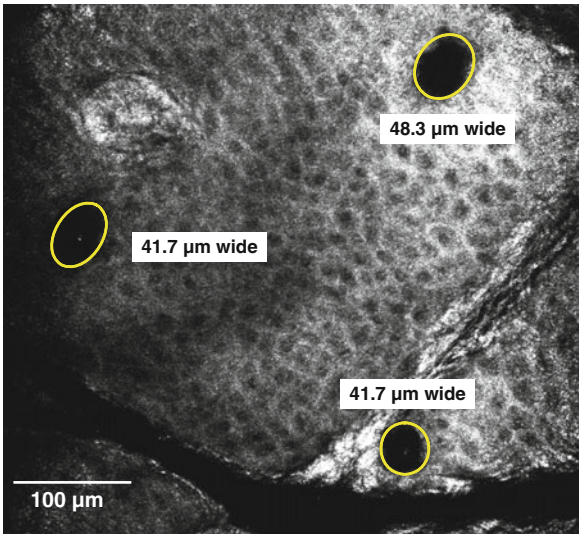


Fig. 7 Confocal microscopy image of human skin in vivo after poration with a microneedle array (MicroCor^{TM}, Corium International, Inc., Redwood City, CA) (G.W. Cleary, personal communication)

into the skin (Guy 2003). The size of these conduits is measured in terms of microns (Fig. 7), making them sufficiently large, therefore, to allow the transport of macromolecules, such as vaccines, proteins and DNA. The methods of skin "poration" include mechanical means, by microneedle arrays or high-velocity particles, or application of physical energy using, for example, ultrasound (sonophoresis) or heat or lasers (thermal poration). The term "minimally invasive" is used to describe these new techniques, reflecting the claim that they provoke little or no physical sensation in vivo. Of these approaches, development is furthest advanced for microneedles, thermal poration and sonophoresis.

Dramatic advances in micromachining and microfabrication have permitted a diverse variety of microneedle arrays to be manufactured (Sivamani et al. 2007). These can be exceptionally small and penetrate only ~100 µm into the skin (and hence cause essentially no sensation). Different materials have been used to make the microneedles which can be solid or hollow. The strategy for their application may be insertion followed by immediate removal and administration of a patch, or the use of biodegradable microneedle arrays intended to permit slower release of an associated drug in situ. Alternatively, the microprojections can be coated with a vaccine, which desorbs upon insertion and then provokes the skin's immune system to amplify the desired pharmacological effect.

The creation of micron-sized portals through the skin using thermal energy may be accomplished by a heated microfilament array or the use of radio-frequency micro-ablation (Altea 2008; Levin et al. 2005). The depth of the pores induced

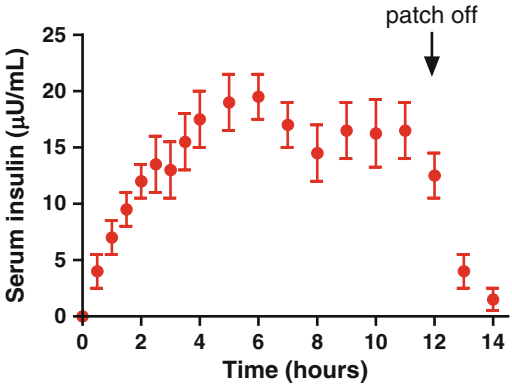


Fig. 8 Serum concentration versus time profile (mean ± SEM; $n = 8$) of insulin in normal healthy subjects following transdermal administration through thermally-porated skin over 12 h (Altea Therapeutics 2008). A minimally effective level is 9–10 μU/mL

ensures access to the viable tissue and that the conduits then become filled quickly with extracellular fluid. In this way, it becomes possible to deliver therapeutic doses of normally skin-impermeable drugs (e.g. the salt of an active species which has much greater stability than the corresponding unionised species) across rather small areas of skin. The thermal ablation of the SC causes the membrane's impedance to fall dramatically, providing an opportunity for feedback control of the energy pulse delivered to the skin. Because of the dimensions of the pores, as with microneedles, the transdermal delivery of proteins such as insulin (Fig. 8), human growth hormone and alpha-interferon has been possible.

Increasing drug transport across the skin by the application of low-frequency (20–100 KHz) ultrasound (Mitragotri and Kost 2004) – so-called sonophoresis – has been pursued for over a decade. While an approved product has yet to reach the market, sonophoresis has been shown to significantly increase the delivery of both small and macro-molecules, and its application to non-invasive glucose monitoring is under active development. Cavitation has been established as the mechanism by which ultrasound creates low resistance pathways across the skin, and the speed with which these conduits appear can be noticeably facilitated by pre-treatment of the SC with a surfactant such as sodium lauryl sulphate. Increased permeability remains for about 12 h, with restoration of the barrier claimed to occur within 1 day post-treatment.

Overall, the minimally invasive technologies described offer several interesting applications, particularly in vaccine and other macromolecule delivery, and in non-invasive glucose monitoring. Despite the high intensity of activity presently underway, the field remains in what may be termed "early-stage" development, with proof-of-principle clearly demonstrated, but long-term confirmation that successful products will result awaiting verification. The key open questions concern the patency of the new pathways created, safety in general associated with the opening of pores in the skin, and economics – that is, will the cost of any technology be competitive with a simple needle and syringe, and will national health systems and insurance companies provide reimbursement for such novel therapies?

2.4 Local and "Subcutaneous" Drug Delivery

Obstacles to the delivery of drugs into the skin for dermatological diseases, and to the immediately underlying tissues to treat local inflammation, are obviously similar to those encountered in transdermal administration. Both objectives have been disappointingly addressed to date with clearly inefficient formulations dominating the market. Real opportunities exist, therefore, to make significant improvements in drug therapy in these areas.

Skin problems represent an ever-increasing fraction of a general practitioner's workload. This can be illustrated by the fact that the incidence of atopic eczema in children has increased from 5% in 1950, to more than 25% in 2000, and is continuing to rise (Cork et al. 2006). Yet, from the majority of topical drug formulations, only a few percent of the applied dose is actually absorbed; that is, the "bioavailability" of topical drugs is very low. There is an obvious need, therefore, to improve this situation by better matching the drug with its vehicle and by ensuring that the utilisation of the active species is improved (as this will undoubtedly lead to more reproducible therapy with fewer, or no, side-effects). As mentioned previously, supersaturation is a means by which a greater fraction of the applied drug dose can be mobilised into the skin, and the creative use of volatile and non-volatile excipients is one approach being explored to take advantage of this metastable state, without difficult-to-resolve stability problems.

Delivery of non-steroidal anti-inflammatory drugs to treat local pain and inflammation in subcutaneous targets beneath the skin (in muscles, joints, tendon, etc.) (Lee and Maibach 2006) is successfully and widely used in Asia. While there has been scepticism in the U.S.A. about the efficacy of such an approach, and only a relatively low level of activity in Europe, there is now a growing interest in the opportunities afforded by this strategy. The key, of course, is to generate convincing evidence that the topical delivery to subcutaneous tissues can be at least as good as oral dosing, in terms of the rate and extent at which the drug reaches the target site. The important advantage of achieving this aim is that an equivalent effect is produced with very low or negligible systemic exposure of the patient to the drug, with a concomitant and significant reduction in unwanted side-effects. Significant growth is this area may be anticipated.

3 Conclusions

Several outstanding contributions of transdermal drug delivery can be identified:

1. Drug administration which is quite different from conventional approaches (for example, oral dosing).
2. Elimination of the "peaks and valleys" in drug plasma levels seen with more conventional routes of delivery.

3. Development of distinct patch technologies and release mechanisms to achieve the desired drug delivery profile.
4. Linear manipulation of the drug dose delivered by varying patch area.
5. Avoidance of the first-pass effect, and modification of drug/metabolite ratios (and reducing, thereby, some important side-effects).
6. Application of the technology to diverse therapeutic areas.
7. Provision of sustained and controlled drug input over periods from 12 h to 7 days.
8. Otherwise difficult-to-formulate drugs have been successfully delivered transdermally.
9. Increased patient compliance.
10. Improved drug utilisation.

It is reasonable to expect that the success of transdermal delivery will be sustained, even though the rate at which new candidates for conventional, passive administration is unlikely to change dramatically. Furthermore, areas of significant growth can be foreseen. For example, while iontophoresis has yet to produce a "home run" in terms of commercial success, the maturity of the technology implies that it is ready to be exploited once the right opportunity is identified. The range of minimally invasive approaches appears likely to impact on macromolecular drug delivery in some way, and a number of interesting "fits" between active and technology are already being examined in depth. Lastly, there seems little doubt that the advances, upon which the transdermal field has grown, will also benefit new approaches to deliver drugs into and beneath the skin where important potential areas of exploitation are clearly evident.

References

Altea Therapeutics (2008) http://www.alteatherapeutics.com

Bouwstra JA, Ponec M (2006) The skin barrier in healthy and diseased state. Biochim Biophys Acta 1758:2080–2095

Cork MJ, Robinson DA, Vasilopoulos Y, Ferguson A, Moustafa M, MacGowan A, Duff GW, Ward SJ, Tazi-Ahnini R (2006) New perspectives on epidermal barrier dysfunction in atopic dermatitis: gene-environment interactions. J Allergy Clin Immunol 118:3–21

Delgado-Charro MB, Guy RH (2001) Transdermal drug delivery. In: Hillery AM, Lloyd AW, Swarbrick J (eds) Drug Delivery and Targeting for Pharmacists and Pharmaceutical Scientists. Harwood Academic Publishers, London, pp 207–236

Dmochowski RR, Sand PK, Zinner NR, Gittelman MC, Davila GW, Sanders SW, Transdermal Oxybutynin Study Group (2003) Comparative efficacy and safety of transdermal oxybutynin and oral tolterodine versus placebo in previously treated patients with urge and mixed urinary incontinence. Urology 62:237–242

Guy RH (2003) "Minimally invasive" technologies for transdermal delivery and clinical chemistry. Challenge in Drug Delivery for the New Millenium. Bulletin Technique Gattefossé 96:47–61

Guy RH, Kalia YN, Delgado-Charro MB, Merino V, Lopez A, Marro D (2000) Iontophoresis: electrorepulsion and electro osmosis. J Control Release 64:129–132

Janssen-Cilag (2009) http://www.janssen-cilag.co.uk/product/detail.jhtml?itemname=ionsys_info

Karande P, Jain A, Ergun K, Kispersky V, Mitragotri S (2005) Design principles of chemical penetration enhancers for transdermal drug delivery. Proc Natl Acad Sci USA 102:4688–4693

Lee CM, Maibach HI (2006) Deep percutaneous penetration into muscles and joints. J Pharm Sci 95:1405–1413

Levin G, Gershonowitz A, Sacks H, Stern M, Sherman A, Rudaev S, Zivin I, Phillip M (2005) Transdermal delivery of human growth hormone through RF-microchannels. Pharm Res 22:550–555

Luzardo-Alvarez A, Delgado-Charro MB, Blanco-Méndez J (2001) Iontophoretic delivery of ropinirole hydrochloride: effect of current density and vehicle formulation. Pharm Res 18:1714–1720

Mitragotri S, Kost J (2004) Low-frequency sonophoresis: a review. Adv Drug Deliv Rev 56: 589–601

Moser K, Kriwet K, Naik A, Kalia YN, Guy RH (2001) Passive skin penetration enhancement and its quantification in vitro. Eur J Pharm Biopharm 52:103–112

Mudry B, Guy RH, Delgado-Charro MB (2006) Iontophoresis in transdermal delivery. In: Touitou E, Barry BW (eds) Enhancement in Drug Delivery. Taylor and Francis, New York, NY, pp 279–302

Potts RO, Guy RH (1992) Predicting skin permeability. Pharm Res 9:663–669

Sieg A, Guy RH, Delgado-Charro MB (2005) Noninvasive and minimally invasive methods for transdermal glucose monitoring. Diabetes Technol Ther 7:174–197

Sivamani RK, Liepmann D, Maibach HI (2007) Microneedles and transdermal applications. Expert Opin Drug Deliv 4:19–25

Smith EW, Maibach HI (2005) Percutaneous penetration enhancers, 2nd edn. CRC Press, Boca Raton, FL

Thomas BJ, Finnin BC (2004) The transdermal revolution. Drug Discov Today 9:697–703

Vyteris (2008) http://www.vyteris.com/home/Our_Products/Lidosite.php

Targeting the Brain – Surmounting or Bypassing the Blood–Brain Barrier

Heidrun Potschka

Contents

Abstract The constituents of the blood–brain barrier, including its efflux transporter system, can efficiently limit brain penetration of potential CNS therapeutics. Effective extrusion from the brain by transporters is a frequent reason for the pharmaceutical industry to exclude novel compounds from further development for CNS therapeutics. Moreover, high transporter expression levels that are present in individual patients or may be generally associated with the pathophysiology seem to be a major cause of therapeutic failure in a variety of CNS diseases including brain tumors, epilepsy, brain HIV infection, and psychiatric disorders. Increasing knowledge of the structure and function of the blood–brain barrier creates a basis for the development of strategies which aim to enhance brain uptake

H. Potschka
Inst. of Pharmacology, Toxicology and Pharmacy, Ludwig- Maximilians-University Munich, Koeniginstr. 16, 80539, Munich, Germany
e-mail: potschka@pharmtox.vetmed.uni-muenchen.de

M. Schäfer-Korting (ed.), *Drug Delivery*,
Handbook of Experimental Pharmacology 197,
DOI 10.1007/978-3-642-00477-3_14,

of beneficial pharmaceutical compounds. The different strategies discussed in this review aim to modulate blood–brain barrier function or to bypass constituents of the blood–brain barrier.

Keywords Blood–brain barrier · Controlled release system · Efflux transporter · Nanoparticles · P-glycoprotein · Pharmacoresistance

1 Introduction

The limited membrane permeability of cells which are constituents of the blood–tissue barrier contributes critically to protection of tissues from putatively harmful xenobiotics. On the other hand the efficient barrier can restrict the brain penetration of central nervous system (CNS) therapeutics. Therefore many promising compounds fail in CNS drug development due to limited access to the target sites in the brain. Moreover the efficacy of marketed drugs can be reduced by a restriction of brain access resulting in a low efficacy or even mere pharmacoresistance. The penetration may be limited by basal barrier function. In some diseases pathophysiology-associated changes can occur at the blood–brain barrier (BBB) which may further restrict brain access. Furthermore drugs may also affect the BBB and may further tighten the barrier. In individual patients the genetics of physiological agents which contribute to BBB structure and function can additionally affect brain access and efficacy of CNS therapeutics. When aiming to optimize brain pharmacokinetics, it is of specific interest to develop strategies to overcome, modulate, or bypass the BBB.

2 Structure and Function of the Blood–Brain Barrier

The BBB critically controls the passage of compounds from the blood to the CNS. The major component of the BBB is a monolayer of brain capillary endothelial cells. The restriction of brain penetration arises from the presence of tight junctions between adjacent endothelial cells and the relative paucity of fenestrae and pinocytotic vesicles. A basal membrane, pericytes, and astrocyte foot processes surround the brain capillary endothelial cells. The close association between brain capillary endothelial cells and surrounding astrocytes seems to be critical for the induction of barrier functions in the endothelial cell layer, including the formation of interendothelial tight junctional complexes.

Due to the BBB, circulating compounds can only gain access to the brain via lipid-mediated transport of small nonpolar molecules through the BBB by passive diffusion or less frequently by catalyzed transport (Pardridge 1999). As a

consequence there is a strong positive correlation between lipophilicity and brain access for the majority of CNS active compounds. However, uptake can be lower than predicted for compounds which are subject to extrusion from the brain by active BBB efflux transporters.

Numerous membrane transporters have been described in BBB endothelial cells which are involved in the influx or efflux of various essential substrates including electrolytes, nucleosides, amino acids, and glucose (Lee et al. 2001), as well as xenobiotics. Efflux transporters at the BBB protect the CNS tissue against changes in the environment by restricting penetration into and facilitating extrusion from brain tissue (Leslie et al. 2005). Because the transporter molecules do not distinguish between harmful xenobiotics and active pharmaceutical ingredients (API) which are used as drugs to treat CNS diseases, the brain efflux transporters can also cause undesirable effects in limiting brain access of drugs which are administered for CNS disease therapy (Loscher and Potschka 2005a). Several brain efflux transporters have been linked to a limited brain penetration of CNS active drugs which restricts drug effectiveness or may even result in mere drug resistance. More than a decade ago, P-glycoprotein (Pgp, ABCB1) was the first drug efflux transporter to be identified in the BBB (Cordon-Cardo et al. 1989; Thiebaut et al. 1987). Since then accumulating data indicate a critical role of different BBB efflux transporters in limiting the brain uptake of a variety of therapeutic agents (Loscher and Potschka 2005b).

The most relevant efflux transporters which have so far been identified at the BBB belong to the class of ABC transporters. ABC transporters comprise two transmembrane domains and two nucleotide-binding domains (Rosenberg et al. 2003), which may also be encoded by two separate polypeptides. For several ABC transporters, for example Pgp, there is more than one substrate-binding site per transporter, allowing for a broad substrate spectrum. Transport of compounds is associated with major conformational changes in the transporter molecule (Martin et al. 2001). ATP binding seems to induce a conformational change which is associated with alterations in affinity and orientation of the substrate binding site (s) such that substrate is released at the extracellular surface of the membrane (Martin et al. 2001). Subsequently, hydrolysis of ATP resets the transporter for the next cycle (Senior et al. 1995).

Depending on structural features of the encoded transporters, *ABC* genes are divided into a number of families (*ABCA, ABCB, ABCC, ABCD, ABCE, ABCF*, and *ABCG*). Due to the fact that the old nomenclature is frequently used in the publications cited in this review, this will be used throughout the chapter. The new nomenclature is given at least once with the first mention of the respective transporter.

Efflux transporters expressed at the BBB include members of the ABCB, ABCC and ABCG family: Pgp (ABCB1), members of the multidrug-resistance associated protein family (MRP/ABCC family), and breast cancer related protein (BCRP/ABCG2).

For the pharmaceutical industry, the question whether a developmental compound is a transporter substrate is of particular interest. Low affinity to BBB efflux

transporters is advantageous for the development of CNS therapeutics, which have to achieve high concentrations in the brain. In contrast, high affinity to BBB efflux transporters is advantageous for the development of drugs, which should act in the periphery in order to avoid CNS side effects.

3 The Blood–Brain Barrier as a Limiting Factor in the Treatment of CNS Diseases

As already described, the function of the BBB can critically influence drug efficacy and tolerability. Compounds may be too hydrophilic to penetrate efficaciously by diffusion, whereas transport by brain efflux transporters may function as a limiting factor for brain uptake of lipophilic compounds.

Therapeutic success in many CNS diseases including brain cancer, epilepsy, depression, schizophrenia, and HIV-associated encephalopathy is limited by poor response or complete resistance to drug treatment. Besides other mechanisms, alterations in drug uptake into the brain or into brain parenchymal target cells are considered to be an important reason for therapeutic failure (Loscher and Potschka 2005a; Thuerauf and Fromm 2006). Thereby, disease-associated or therapy-induced changes in efflux transporter expression are thought to critically affect brain pharmacokinetics of a variety of important CNS active drugs.

Many brain tumors are highly resistant to drug treatment and systemic chemotherapy often fails to improve the outcome. A key factor for therapeutic failure of systemic chemotherapy is restricted BBB penetration of potent chemotherapeutic drugs (Nies 2007). Anticancer agents were among the first drugs that were identified to be substrates of BBB efflux transporters, i.e., of Pgp as well as MRPs and BCRP. Several studies indicated that the poor efficacy of systemically administered anticancer drugs is at least partly due to the activity of BBB efflux transporters (Kemper et al. 2004).

In 30%–40% of epilepsy patients antiepileptic drug therapy fails to control seizure activity in an adequate manner. Microdialysis experiments using transporter inhibitors, experiments in knockout mice, as well as in vitro studies have indicated that several antiepileptic drugs are transported by BBB Pgp, and some are also subject to transport by MRPs (Cucullo et al. 2007; Loscher and Potschka 2005b; Marchi et al. 2005; Rizzi et al. 2002; Sills et al. 2002). As antiepileptic drugs generally penetrate well into the brain, they can only be low to medium affinity substrates. Brain penetration of antiepileptic drugs is only restricted when an overexpression of BBB efflux transporters occurs as a consequence of seizure activity. Therefore assays with sufficient sensitivity are required to determine whether antiepileptic drugs are substrates of BBB transporters. In recent years the neglect of this fact resulted in a series of inconsistent data. Thus, further research will be necessary to determine whether all clinically relevant antiepileptic drugs are substrates of BBB efflux transporters, especially of the human isoforms.

Seizure-induced overexpression of BBB efflux transporters in the epileptic brain generally renders a feasible explanation for the multidrug resistance of epilepsy based on limited access of antiepileptic drugs to their target sites. Important support for this concept came from experiments in two different models of drug-resistant epilepsy. Pgp expression in drug-resistant rats significantly exceeded that in drug-responsive rats (Potschka et al. 2004; Volk and Loscher 2005). Recent experimental studies, in which it was demonstrated that drug resistance of seizures can be overcome by transporter inhibitors, rendered further evidence for the multidrug transporter hypothesis of drug-resistant epilepsy (Brandt et al. 2006; Clinckers et al. 2005). When further developing or validating new strategies to overcome drug resistant epilepsy, it must be taken into consideration that it is considered a multifactorial problem, and thus the relative importance of efflux transporter over-expression needs to be elucidated.

Genetic deficiency of Pgp in mice resulted in enhanced brain access for several antidepressants, indicating that these are effluxed into the blood by Pgp (Grauer and Uhr 2004; Uhr and Grauer 2003; Uhr et al. 2003, 2000). Whether this active efflux transport can contribute to therapeutic failure in depression remains to be determined. Due to the lack of models for treatment-resistant psychiatric disorders, it is difficult to test the validity of this hypothesis, which therefore still remains rather speculative. First indirect support for an impact of BBB efflux transporters on therapeutic success in the treatment of psychiatric diseases came from a genetic analysis in schizophrenic patients treated with bromperidol. The MDR1 genotype showed correlation with the therapeutic response to bromperidol (Yasui-Furukori et al. 2006). Recently, Uhr et al. (2008) reported that the MDR1 genotype of depressed patients is a strong predictor for therapeutic success with several antidepressants.

In the treatment of HIV infection, the development of HIV protease inhibitors has resulted in considerable progress. However, a major limitation in their efficacy is the restricted access to the brain which leaves the brain viral reservoir unaffected. Pgp-mediated efflux has been hypothesized to contribute to the limited brain penetration rates of HIV protease inhibitors such as saquinavir, amprenavir, nelfinavir, and indinavir (Banks et al. 2006; Edwards et al. 2002; Kim et al. 1998; Washington et al. 2000). Pgp upregulation at the BBB by the HIV-Tat protein (Hayashi et al. 2005) may further reduce penetration and efficacy of the HIV protease inhibitors in long-term survivors of AIDS. In addition to Pgp, MRP1, MRP2, and MRP4 also accept HIV protease inhibitors as substrates and might therefore be involved in the limitation of their brain access.

Riluzole is the only recognized drug that increases the survival time of patients with amyotrophic lateral sclerosis. Studies in Pgp knockout mice revealed that riluzole and minocycline, a compound which can delay disease onset, are both substrates of Pgp (Milane et al. 2007), so that therapeutic efficacy may be affected by this BBB efflux transporter.

Pgp-mediated extrusion from the brain has a tremendous impact on opiate and opioid analgesic efficacy. Modulation of Pgp function significantly affected the antinociceptive effect of morphine (King et al. 2001; Letrent et al. 1999; Thompson

Table 1 CNS therapeutics as substrates of BBB efflux transporters: examples

Pharmacological group	Examples	Transporters involved
Anticancer drugs	Doxorubicin, daunorubicin, vinblastin, vincristine, paclitaxel, etoposide, topotecan	ABCB1/Pgp, ABCC transporters/MRPs, ABCG2/BCRP
Analgesics	Morphine, methadone, fentanyl	ABCB1/Pgp
HIV protease inhibitors	Amprenavir, indinavir, saquinavir	ABCB1/Pgp, ABCC transporters/MRPs
Antipsychotic agents	Olanzapine, amisulpride	ABCB1/Pgp
Antiepileptic drugs	Phenytoin, carbamazepine, oxcarbazepine, lamotrigine, phenobarbital, felbamate, valproic acid, topiramate	ABCB1/Pgp, ABCC transporters/MRPs
Antidepressants	Amitryptiline, nortryptiline, venlafaxine, paroxetine	ABCB1/Pgp

The transporters listed do not necessarily transport all compounds given, but in some cases transport only single compounds of the pharmacological group

et al. 2000). Thus, Pgp seems to be an important issue in pain control with opioid analgesics, which may influence the onset, magnitude, and duration of the analgesic response (Dagenais et al. 2004). Recently, this assumption was further substantiated as Hamabe et al. (2007) reported a negative correlation between morphine's analgesic effects in a mouse model and the individual Pgp expression rate in the cortex.

Furthermore, Pgp seems to limit the distribution of some antibacterial drugs including fluoroquinolones and erythromycin to the brain (Sasabe et al. 2004; Schinkel 1999). Brain extrusion of these antibiotics may contribute to their limited or lack of efficacy in CNS microbial infections.

To summarize, it has been demonstrated that BBB efflux transporters critically influence CNS effects of numerous APIs (Table 1) and that this influence is of clinical relevance for many of these drugs.

4 Modulation of Blood–Brain Barrier Function

Accumulating knowledge of the impact of the BBB and its efflux transporter system on response to CNS therapy stimulates efforts to develop strategies to target drugs in an optimized manner to the brain tissue (Fig. 1; Table 2). The development of imaging techniques based on positron emission tomography creates the opportunity to study Pgp-mediated transport noninvasively in individual patients and its modulation in vivo (Elsinga et al. 2004; Hendrikse and Vaalburg 2002; Langer et al. 2007; Lee et al. 2006). Further development of these diagnostic techniques will open avenues for selection of patients that may benefit from new strategies aiming to outwit or bypass the BBB.

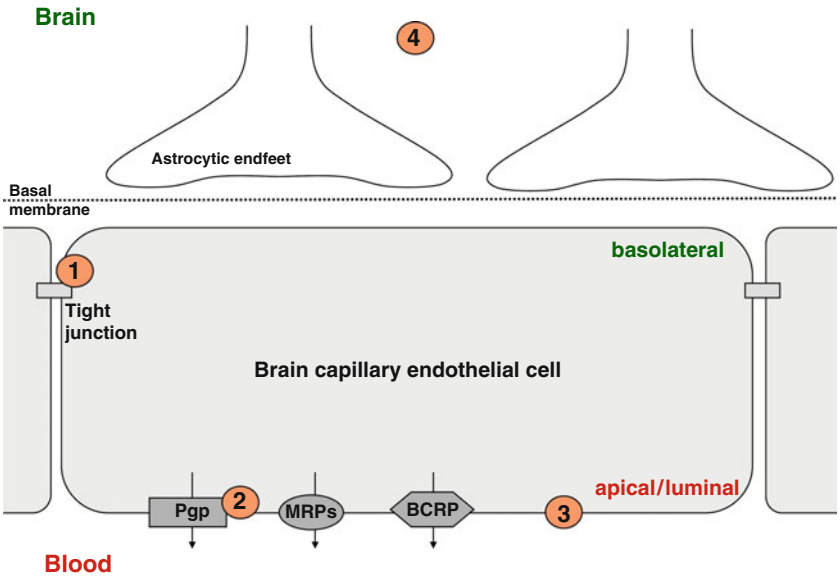


Fig. 1 Strategies to enhance brain penetration of CNS therapeutics. (1) Opening of the BBB can be achieved by disintegrating the tight junctional complex. (2) Modulation of BBB efflux transporter function or inhibition of the induction of efflux transporters results in a more specific enhancement of brain penetration rates. (3) Use of nano-sized carrier systems and drug conjugates allows bypassing of BBB efflux transporter molecules. (4) Direct intracerebral administration (e.g., by implantation of release systems) bypasses the barriers and results in much higher local drug concentrations

4.1 Opening of the BBB

BBB disruption must be transient and reversible to have any role in the delivery of APIs such as anticancer drugs to the brain. A variety of hypertonic solutions has been used to disrupt the BBB (Kroll et al. 1998). With its approval for use in patients, mannitol is most commonly used in both preclinical and clinical studies. Mannitol-mediated BBB opening has been used in combination with anticancer drugs to treat patients with metastatic or primary brain tumors. Some studies have indicated some success and minimal morbidity of the strategy. In a simplified view of the approach, mannitol is considered to result in osmotic shrinkage of endothelial cells thereby inducing tractive forces on the tight junctions which then disintegrate. However, as several structural and functional changes occur in endothelial cells in response to mannitol, the events that result in enhanced permeability may be much more complex. Recently, Farkas et al. (2005) reported that hyperosmotic mannitol induces phosphorylation of beta-catenin. Since beta-catenin is a key component of the junctional complex, its phoshorylation may be important for mannitol-induced reversible opening of the BBB.

In general, osmotic disruption of the BBB is not specific enough to exclude CNS entry of toxic xenobiotics. Furthermore BBB opening and albumin extravasation

Table 2 Strategies to enhance brain penetration

Strategy	Putative relevance for	Experimental evidence	Clinical evidence
Modulation of BBB function			
Opening/weakening of the BBB, e.g., Mannitol Bradykinin analog Alkylglycerol	Brain cancer	+	+
Inhibition of efflux transport	Brain cancer	+	–
	Epilepsy	+	–
	Focal cerebral ischemia	+	–
	Brain HIV	–	–
	Psychiatric diseases	–	–
Prevention of disease- or therapy-associated changes in BBB efflux transporter expression	Brain cancer	–	–
	Epilepsy	+	–
	Focal cerebral ischemia	–	–
Bypassing the BBB			
Nano-sized carrier systems and drug conjugates	Brain cancer	+	+
	Epilepsy	+	–
	Focal cerebral ischemia	–	–
	Brain HIV	–	–
	Psychiatric diseases	–	–
Intracerebral administration	Brain cancer	+	+
	Epilepsy	+	–

may facilitate or even cause epileptogenesis (Ivens et al. 2007; Tomkins et al. 2007; van Vliet et al. 2007). Thus, more specific strategies to target APIs to the brain without disruption of BBB integrity would be advantageous.

Administration of a bradykinin analog (RMP-7) has been suggested as an alternative approach. RMP-7 opens tight junctions by a receptor-mediated mechanism, thereby promoting delivery of the cytostatic carboplatin to glioma implanted in rat brain (Matsukado et al. 1996). However, the bradykinin analog failed to improve carboplatin efficacy in phase II and III clinical trials (Prados et al. 2003).

Further options include the intracarotid administration of alkylglycerol which affects the BBB in a more subtle way. Enhanced drug transport via the paracellular way has been described in rodents (Erdlenbruch et al. 2003a, b). To our knowledge no human data are available so far.

4.2 Inhibition of Efflux Transport

Increasing awareness of the impact of efflux transporters on successful pharmacotherapy of CNS diseases stimulates efforts to develop strategies to modulate

transporter function (Loscher and Potschka 2005a; Thuerauf and Fromm 2006). As Pgp is known to transport a large number of commonly prescribed drugs, efforts to date concentrate especially on this transporter. Mechanisms by which Pgp activity in the BBB can be modulated include direct inhibition by specific inhibitors, functional modulation, and transcriptional modulation (Bauer et al. 2005). Initially, drugs used for other indications and noted to inhibit Pgp in cell culture, such as verapamil, cyclosporin A, and quinidine, have been tested as Pgp modulators (Fox and Bates 2007). Owing to low binding affinity for Pgp, high doses of these early inhibitors were needed and excessive toxicity was observed in patients. Second generation inhibitors were developed as analogs of the initial agents. Valspodar (PSC-833), a non-immunosuppressive derivative of cyclosporin D, exemplifies the development of these agents (Fox and Bates 2007). The compound proved to be better tolerated than inhibitors of the first generation. However, valspodar inhibits CYP enzymes thereby resulting in decreased clearance and increased systemic exposure of co-administered compounds.

Whereas first and second generation Pgp inhibitors were hampered by additional pharmacodynamic effects or by additional effects on drug metabolism (Thomas and Coley 2003), the development of third generation Pgp inhibitors produced selective and more potent modulators, such as tariquidar, laniquidar, zosuquidar, and elacridar (Bates et al. 2002; Thomas and Coley 2003). The three generations of Pgp modulators comprise competitive inhibitors which are substrates by themselves, and noncompetitive inhibitors that induce changes in the conformation which affect transport efficacy.

In view of the complexity of efflux transport, an aim that suggests itself is to develop dual or multipotent inhibitors. Jekerle et al. (2006) recently reported the development of the novel inhibitor, WK-X-34, which modulates both Pgp and BCRP in experimental models. In the clinical setting, co-administration of Pgp inhibitors together with anticancer drugs in oncology has shown some efficacy (Breedveld et al. 2006), although not all studies yielded promising data. Therefore, the continued development of these agents must be awaited in order to establish the true potential of Pgp-mediated reversal of multidrug resistance in the treatment of brain cancer and other CNS diseases. In this context, it is of particular interest that a recent study reported differences in the sensitivity of Pgp located in different cells and blood–tissue barriers (Choo et al. 2006), Pgp localized in the BBB proved to be more resistant to inhibition than Pgp in other tissues (Choo et al. 2006). This resistance can be overcome by a sufficiently high dose of an inhibitor. However, whether this is safely attainable in the clinical situation remains to be determined.

Experimental studies in a rodent glioblastoma model and a rodent melanoma brain metastasis model demonstrated efficacy of the strategy (Fellner et al. 2002; Joo et al. 2008). Brain penetration and efficacy of systemically administered paclitaxel could be enhanced significantly by co-administration of the Pgp inhibitors valspodar or HM30181A (Fellner et al. 2002). Co-administration of Pgp inhibitors also improved the response to antiepileptic drugs and even helped to overcome mere resistance to antiepileptic drugs in several animal models (Brandt

et al. 2006; Clinckers et al. 2005). In these studies the combination proved to be well tolerated. In a rodent model of focal cerebral ischemia, co-administration of a third generation Pgp inhibitor was also substantiated as a promising strategy for neuroprotection (Spudich et al. 2006). Tariquidar enhanced the accumulation and the neuroprotective efficacy of the neuroprotectants FK506 and rifampicin.

In view of the experimental success, it is important to consider that any modulation of transporter function is associated with specific hazards. First, complications with a combination of a Pgp inhibitor or modulator with a CNS active drug may be related to the intended aim. An influence on pharmacokinetics of the therapeutic agent will not only affect the target tissue or target brain region. Enhanced drug concentrations in other brain regions and also in peripheral tissues may promote side effect potentiation. In accordance with this, several trials with combinations of anticancer drugs and Pgp inhibitors had to be closed ahead of schedule due to enhanced chemotherapy-related toxicity (Fox and Bates 2007).

Second, multidrug transporters such as Pgp serve a variety of physiological functions including protection from xenobiotics. Other xenobiotics taken up by the body may be more harmful in the presence of efflux transporter inhibitors due to the influence on their distribution. Furthermore Pgp and MRPs may protect brain parenchymal cells from apoptosis (Gennuso et al. 2004; Pallis et al. 2002), and transporter inhibition may thus promote cell death. Nevertheless, transient inhibition of efflux transporters by short-term administration of inhibitors may be a tolerable strategy to reverse or prevent drug resistance.

With regard to specific brain targeting, evidence exists that modulation of efflux transporter function may indeed enhance brain penetration of CNS therapeutics; however, transporter activity will also be affected in other blood–tissue barriers, haematopoetic cells, and excretory organs.

4.3 Prevention of Disease-Associated or Therapy-Induced Changes of the Blood–Brain Barrier

Expression of efflux transporters is regulated in a highly dynamic manner. This regulatory process can be considered as a mechanism that allows adaptation to changing requirements in detoxification and tissue protection. The regulation of expression has been most intensely studied for Pgp. Knowledge of the regulation of BBB efflux transporter activity is of particular interest because it may prepare the molecular basis for the development of strategies to specifically manipulate BBB function in order to improve pharmacotherapy of CNS diseases. This underlines the specific importance of further research focusing on the different mechanisms of regulation and their interaction.

A variety of xenobiotics including several APIs have been demonstrated to induce expression of multidrug transporters. In the treatment of brain cancer, induction of efflux transporter expression by chemotherapeutic drugs in tumor cells and BBB endothelial cells is a well recognized mechanism that limits drug concentrations at the target tumor cells and contributes to therapeutic failure (Lee and Bendayan 2004; Loscher and Potschka 2005a). The strong induction by anticancer drugs is probably due to their pronounced cytotoxic effects on cells and the induction of a cellular stress response. Orphan nuclear receptors have been recognized as master regulators of drug-induced changes in expression of metabolizing enzymes and of members of the multidrug transporter families (Masuyama et al. 2005). *The orphan nuclear receptor PXR/SXR* (termed pregnane X receptor [PXR] in rodents and steroid and xenobiotic receptor [SXR] in humans) proved to be expressed in rat brain capillaries (Bauer et al. 2004). Its functional relevance for regulation of efflux transporters in the BBB was indicated by the observation that the PXR ligand dexamethasone increased Pgp expression and Pgp-specific transport (Bauer et al. 2004). Thus, PXR/SXR may be a key xenobiotic sensor in brain capillary endothelial cells which mediates induction of Pgp. Targeting these xenosensors by administration of antagonists has been suggested as a means of overcoming therapy-induced resistance mechanisms (Ekins et al. 2007). The ongoing molecular characterization of the binding sites of the receptors has implications for future discovery of molecules that are more selective and potent than currently available antagonists.

Using intestinal and lung carcinoma cell lines it was demonstrated that induction of efflux drug transporters by xenobiotics and especially chemotherapeutics does not necessarily depend on PXR (Huang et al. 2006). Based on the fact that the group also demonstrated that the modes of regulation can be cell-specific, it is currently not clear if these data can be extrapolated to brain capillary endothelial cells. Baker et al. (2005) reported that *epigenetic changes in the MDR1 promoter* occur in response to chemotherapeutic drugs which then enhance the MDR phenotype. Dramatic changes in the temporal and spatial pattern of histone modifications occurred within the 5' hypomethylated region of MDR1, which directly correlated with MDR1 upregulation (Baker et al. 2005). Further research may create a basis for the identification of further targets for prevention of therapy-induced transporter overexpression.

Several CNS pathologies have been associated with changes in efflux transporter expression or function. Epilepsy, which is characterized by recurrent spontaneous seizures, is one of the most common neurological disorders. In animal models of epilepsy a transient increase in Pgp and MRP2 expression was observed in brain capillary endothelial cells, astroglia, and neurons after seizures, which indicates that seizures themselves can induce overexpression of drug transporters (Loscher and Potschka 2005a; Sisodiya 2003). This seizure-induced overexpression proved to be restricted to brain regions involved in seizure initiation and spread. These data are in line with investigations in human epileptogenic tissue dissected from pharmacoresistant patients during epilepsy surgery, which also

indicated high expression rates of efflux transporters (Loscher and Potschka 2005a; Sisodiya 2003). However, definite conclusions from these studies are hampered by the lack of adequate control tissue, because patients who are treated successfully do not generally undergo surgical resection of epileptogenic foci. The cellular mechanisms involved in seizure-induced overexpression of efflux transporters still need to be elucidated. With respect to the excessive glutamate release associated with seizures, it is of particular interest that glutamate proved to upregulate Pgp expression via an NMDA receptor mechanism (Zhu and Liu 2004). Recently, we were able to demonstrate that extracellular glutamate signals through the NMDA receptor and COX-2 in brain capillaries to increase BBB Pgp expression following seizures (Bauer et al. 2008). Consistent with our hypothesis, exposing isolated rodent brain capillaries to glutamate increased Pgp expression and transport activity. These increases were blocked by the NMDA receptor antagonist MK-801 and by the selective COX-2 inhibitor celecoxib. In rats, intracerebral microinjection of glutamate caused locally increased Pgp expression in brain capillaries. Moreover, using a pilocarpine status epilepticus rat model, we achieved an attenuation of seizure-induced increases in capillary Pgp expression by administration of the non-selective COX inhibitor indomethacin. These data suggest that it might be possible to enhance brain uptake of antiepileptic drugs and to overcome transporter-mediated resistance by COX inhibition (Bauer et al. 2008).

In accordance with the seizure-induced molecular changes at the BBB, an upregulation of Pgp has also been described following focal cerebral ischemia (Spudich et al. 2006). As enhanced glutamate release also is a hallmark during ischemic brain damage, this induction may also be related to glutamate release and subsequent activation of inflammatory events, and may be prevented using the same strategies as those already substantiated in an epilepsy model.

Further elucidation of the mechanisms involved in transporter regulation in CNS diseases may open avenues for new strategies to enhance brain penetration of CNS therapeutics. Apart from involved receptors or changes in the promotor region, a variety of mechanisms that contribute to cellular stress responses, including phospholipase C, proteinkinase C, mitogen-activated protein kinase cascades, mobilization of intracellular Ca^{2+}, cytokines, nuclear factor kappa B, and heat shock factor 1, regulate multidrug transporter genes such as *MDR1* (Ho and Piquette-Miller 2006; McRae et al. 2003; Shtil and Azare 2005; Tchenio et al. 2006). Using primary cultured rat brain endothelial cells to examine the effect of oxidative stress on expression of transporters, Felix and Barrand (2002) found a stress-induced increase in Pgp expression and function whereas no such alterations were observed for MRP1. Hartz et al. (2004, 2006) defined a signaling pathway as part of the innate immune response through which Pgp activity is rapidly modulated. Their findings suggested that the inflammatory cytokine tumor necrosis factor (TNF)-alpha reduces Pgp activity via TNF-R1 receptor activation, endothelin-1 release, and endothelin-B receptor signaling. All these findings complete our view of the regulatory mechanisms, and inspire research efforts for prevention of transporter-mediated resistance in CNS diseases.

5 Bypassing the Blood–Brain Barrier

5.1 Nano-Sized Carrier Systems and Drug Conjugates

An alternative approach which avoids compromising the protective function of efflux transporters is to bypass transporter molecules. Different strategies are followed in this regard including nanoparticle encapsulation (Huwyler et al. 1996; Kreuter 2001) or conjugation (Mazel et al. 2001) of transporter substrates.

Nano-sized carrier systems including polymers, emulsions, micelles, liposomes, and nanoparticles may deliver their content to the brain by passive targeting (de Boer and Gaillard 2007). The rate of distribution into the brain is often limited with these systems. The penetration rate will generally depend on the physicochemical features and the physiological conditions. A variety of compounds including several anticancer drugs have been formulated into nano-sized carrier systems. Efficacious delivery has been described in rodents. Whereas free doxorubicin did not induce any relevant effect in a brain tumor model in rats, liposomal doxorubicin increased the survival time (Sharma et al. 1997). Evidence exists that the first dose of doxorubicin thereby promotes subsequent brain uptake of further dosages due to a toxic effect on proliferating endothelial cells and reduction of the angiogenic factor VEGF (Zhou et al. 2002). Polymer-based particles were studied more rarely. They are in most instances formulated by adsorbing the drug onto the particle surface (Kreuter 1995). The particles are then phagocytosed into the cell where the drug is released. Enhanced brain uptake of doxorubicin has been demonstrated when the drug was loaded on the surface of solid poly(butyl cyanoacrylate) particles (Gulyaev et al. 1999). In a rat glioblastoma model long-term remission was achieved in 20% of the animals with the formulation (Steiniger et al. 2004).

In patients, therapeutic drug concentrations were reached in the central tumor mass following administration of liposomal daunorubicin (Zucchetti et al. 1999). In clinical trials the response rate to liposomal doxorubicin or daunorubicin was considered promising by the authors (Hau et al. 2004; Koukourakis et al. 2000a, b; Lippens 1999). However, conclusions are hampered by the fact that the patients received additional radiotherapy or other chemotherapeutic agents. Thus, further trials are necessary to clearly determine the therapeutic potential.

Active drug targeting strategies involve the application of a technology that utilizes endogenous transport mechanisms for site-specific delivery (de Boer and Gaillard 2007). Ligands for targeting may be conjugated to the drug itself or may be attached to the surface of drug-loaded particles. With respect to the BBB, ligand-mediated site-specific delivery involves receptor-mediated transcytosis systems at the BBB to reach extracellular or intracellular targets in the brain. Interestingly, targeting strategies can benefit from pathophysiological mechanisms, when the target is induced during the disease course. Gaillard et al. (2005) have identified a novel carrier protein for targeted delivery which makes use of the diphtheria toxin receptor, which is strongly induced under conditions of neuroinflammation such as

those occurring in many brain diseases, including Alzheimer's disease, Parkinson's disease, multiple sclerosis, ischemia, encephalitis, epilepsy, tumors, etc.

The carrier protein CRM197 is a nontoxic mutant of diphtheria toxin which specifically binds to the diphtheria toxin receptor resulting in receptor-mediated transcytosis and uptake into the brain (Gaillard et al. 2005). This delivery strategy can be applied using CRM197 drug conjugates or CRM197-coated drug-loaded liposomes.

The most widely characterized receptor-mediated transcytosis system for CNS targeting is the transferrin receptor (Pardridge 2002). Drug targeting to this receptor can be achieved either by using endogenous ligands or by using an antibody directed against the receptor (OX-26). The insulin receptor represents another receptor-mediated transcytosis system which has already been used for targeted delivery of drugs to the CNS (Pardridge 2005). An alternate approach is based on LRP1 and LRP2 receptors, which are known multiligand scavengers. Several ligands of these receptors including melanotransferrin, apolipoproteins, and aprotinin have already been used to promote brain targeting (de Boer and Gaillard 2007).

5.2 Intranasal Administration

Nasal drug administration is considered to provide one putative means for targeted CNS drug delivery (Graff and Pollack 2005). Three pathways are generally postulated for a drug administered to the nasal cavity to follow. These routes include direct delivery to the brain, e.g., along nerve sheaths, axonal transport along neurons, and entry into the blood from the nasal mucosa (Graff and Pollack 2005). To what extent a molecule passes along these routes and to what extent it is thus indeed targeted to the CNS critically depends on its chemical features and its formulation (Ugwoke et al. 2001). A recent meta-analysis of all published studies claiming evidence for direct nose-to-brain transport identified only two studies in rats which provide results that can be regarded as an indication for direct transport from the nasal mucosa to the CNS (Merkus and van den Berg 2007). The same analysis did not reveal any pharmacokinetic evidence supporting a claim that nasal administration of drugs in humans will result in an enhanced delivery to their target sites in the brain compared with intravenous administration of the same drug under similar dosing conditions. Thus, it is currently rather questionable whether intranasal administration can be considered as a means for efficacious brain targeting.

5.3 Intracerebral Administration

It is generally possible to achieve much higher concentrations of drug in the brain by direct administration into the cerebrospinal fluid or into the brain parenchyma

(Huynh et al. 2006). Intracerebroventricular or intrathecal drug infusion delivers drugs to the cerebrospinal fluid (CSF) thereby avoiding the BBB and hepatic metabolism. Following delivery to the CSF, APIs still have to pass the ependymal brain–CSF barrier. This is feasible for many small and lipophilic compounds. However, penetration into the parenchyma is limited due to tortuosity, transcapillary loss, cell uptake, and binding (de Boer and Gaillard 2007). Therefore, drugs administered to the CSF have minimal access to the parenchyma by diffusion. As a consequence, intraventricular administration of CNS drugs is considered particularly useful for meningioma treatment, as tumor metastasis is prevented by effects on cells floating in the CSF but is not applicable in glioma therapy (Huynh et al. 2006). The intrathecal administration route fails to result in drug accumulation in parenchymal structures deep within the brain, and is thus applicable rather for treatment of disseminated meningeal or spinal disease (Groothuis et al. 2000).

Implantable controlled release systems can be designed from both degradable and non-degradable polymers (Sawyer et al. 2006). Appropriately designed, polymers can provide reliable sustained release for periods of days to many years. As persistence of the delivery system will limit the clinical use, biodegradable polymers are more common than non-degradable systems. Controlled release systems are already used clinically for treatment of brain tumors (Sawyer et al. 2006). Intracranial implantation of a wafer loaded with carmustine (BCNU) following surgical debulking of the tumor was well tolerated in patients with malignant gliomas and resulted in a modest improvement of patient survival (Brem et al. 1995; Engelhard 2000). A general drawback of controlled release systems for different indications is that the local penetration of the drug is limited due to the restriction of diffusion by the brain parenchyma.

Convection-enhanced delivery was developed to deliver compounds throughout the brain to overcome the diffusion barrier seen with polymeric-controlled release (Bobo et al. 1994). The approach is based on continuous infusion which uses a convective flow to drive the API throughout a larger region of tissue (Huynh et al. 2006). In comparison with bolus injections to the brain parenchyma, the benefits are derived from the greater distribution and continued exposure due to the long infusion time (Sawyer et al. 2006). The technique has been used in chemotherapy, gene therapy, and immune therapy. Clinical use has been established in glioma patients with recurrence of tumors. Patients receive local infusions after surgical resection or infusion directly into the tumor. In clinical trials it has been demonstrated that convection-enhanced delivery is suitable for delivering agents to a large tumor volume. For example, clinical trials with delivery of an immunotoxin into glioblastoma tumors were able to achieve complete regression with minimal systemic toxicity in some patients (Husain et al. 2003).

Alternative approaches for direct delivery include gene therapy involving viral, lipid, polymer-based, and cell-based delivery strategies. For detailed information on these approaches readers are referred to reviews that focus on these techniques (de Boer and Gaillard 2007; Huynh et al. 2006).

6 Conclusions

In recent years awareness of the impact of brain efflux transporters on treatment of CNS diseases has progressively increased. Cumulative knowledge of the structure, function, localization, and substrate specificities of brain efflux transporters has helped to develop and validate strategies to deal with the activity of these transporters in a clinical setting. Several strategies for brain targeting of drugs are already applied clinically, especially for treatment of brain tumors. Recently, particular interest has arisen in the regulation of transporter expression or function in pathophysiological conditions, which may contribute to disease development or progression but may also influence the pharmacotherapeutic outcome. Further research may provide new approaches which prevent a strengthening of BBB function in CNS diseases, and may thereby prevent development of pharmacoresistance.

References

Baker EK, Johnstone RW, Zalcberg JR, El-Osta A (2005) Epigenetic changes to the MDR1 locus in response to chemotherapeutic drugs. Oncogene 24:8061–8075

Banks WA, Ercal N, Price TO (2006) The blood-brain barrier in neuroAIDS. Curr HIV Res 4:259–266

Bates SF, Chen C, Robey R, Kang M, Figg WD, Fojo T (2002) Reversal of multidrug resistance: lessons from clinical oncology. Novartis Found Symp 243:83–96 discussion 96–102, 180–185

Bauer B, Hartz AM, Fricker G, Miller DS (2004) Pregnane X receptor up-regulation of P-glycoprotein expression and transport function at the blood-brain barrier. Mol Pharmacol 66:413–419

Bauer B, Hartz AM, Fricker G, Miller DS (2005) Modulation of p-glycoprotein transport function at the blood-brain barrier. Exp Biol Med (Maywood) 230:118–127

Bauer B, Hartz AM, Pekcec A, Toellner K, Miller DS, Potschka H (2008) Seizure-induced upregulation of P-glycoprotein at the blood-brain barrier through glutamate and COX-2 signaling. Mol Pharmacol 73(5):1444–1453

Bobo RH, Laske DW, Akbasak A, Morrison PF, Dedrick RL, Oldfield EH (1994) Convection-enhanced delivery of macromolecules in the brain. Proc Natl Acad Sci USA 91:2076–2080

Brandt C, Bethmann K, Gastens AM, Loscher W (2006) The multidrug transporter hypothesis of drug resistance in epilepsy: proof-of-principle in a rat model of temporal lobe epilepsy. Neurobiol Dis 24:202–211

Breedveld P, Beijnen JH, Schellens JH (2006) Use of P-glycoprotein and BCRP inhibitors to improve oral bioavailability and CNS penetration of anticancer drugs. Trends Pharmacol Sci 27:17–24

Brem H, Ewend MG, Piantadosi S, Greenhoot J, Burger PC, Sisti M (1995) The safety of interstitial chemotherapy with BCNU-loaded polymer followed by radiation therapy in the treatment of newly diagnosed malignant gliomas: phase I trial. J Neurooncol 26:111–123

Choo EF, Kurnik D, Muszkat M, Ohkubo T, Shay SD, Higginbotham JN, Glaeser H, Kim RB, Wood AJ, Wilkinson GR (2006) Differential in vivo sensitivity to inhibition of P-glycoprotein located in lymphocytes, testes, and the blood-brain barrier. J Pharmacol Exp Ther 317:1012–1018

Clinckers R, Smolders I, Meurs A, Ebinger G, Michotte Y (2005) Quantitative in vivo microdialysis study on the influence of multidrug transporters on the blood-brain barrier passage

of oxcarbazepine: concomitant use of hippocampal monoamines as pharmacodynamic markers for the anticonvulsant activity. J Pharmacol Exp Ther 314:725–731

Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR, Bertino JR (1989) Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. Proc Natl Acad Sci USA 86:695–698

Cucullo L, Hossain M, Rapp E, Manders T, Marchi N, Janigro D (2007) Development of a humanized in vitro blood-brain barrier model to screen for brain penetration of antiepileptic drugs. Epilepsia 48:505–516

Dagenais C, Graff CL, Pollack GM (2004) Variable modulation of opioid brain uptake by P-glycoprotein in mice. Biochem Pharmacol 67:269–276

de Boer AG, Gaillard PJ (2007) Drug targeting to the brain. Annu Rev Pharmacol Toxicol 47:323–355

Edwards JE, Brouwer KR, McNamara PJ (2002) GF120918, a P-glycoprotein modulator, increases the concentration of unbound amprenavir in the central nervous system in rats. Antimicrob Agents Chemother 46:2284–2286

Ekins S, Ecker GF, Chiba P, Swaan PW (2007) Future directions for drug transporter modelling. Xenobiotica 37:1152–1170

Elsinga PH, Hendrikse NH, Bart J, Vaalburg W, van Waarde A (2004) PET Studies on P-glycoprotein function in the blood-brain barrier: how it affects uptake and binding of drugs within the CNS. Curr Pharm Des 10:1493–1503

Engelhard HH (2000) The role of interstitial BCNU chemotherapy in the treatment of malignant glioma. Surg Neurol 53:458–464

Erdlenbruch B, Alipour M, Fricker G, Miller DS, Kugler W, Eibl H, Lakomek M (2003a) Alkylglycerol opening of the blood-brain barrier to small and large fluorescence markers in normal and C6 glioma-bearing rats and isolated rat brain capillaries. Br J Pharmacol 140:1201–1210

Erdlenbruch B, Schinkhof C, Kugler W, Heinemann DE, Herms J, Eibl H, Lakomek M (2003b) Intracarotid administration of short-chain alkylglycerols for increased delivery of methotrexate to the rat brain. Br J Pharmacol 139:685–694

Farkas A, Szatmari E, Orbok A, Wilhelm I, Wejksza K, Nagyoszi P, Hutamekalin P, Bauer H, Bauer HC, Traweger A, Krizbai IA (2005) Hyperosmotic mannitol induces Src kinase-dependent phosphorylation of beta-catenin in cerebral endothelial cells. J Neurosci Res 80:855–861

Felix RA, Barrand MA (2002) P-glycoprotein expression in rat brain endothelial cells: evidence for regulation by transient oxidative stress. J Neurochem 80:64–72

Fellner S, Bauer B, Miller DS, Schaffrik M, Fankhanel M, Spruss T, Bernhardt G, Graeff C, Farber L, Gschaidmeier H, Buschauer A, Fricker G (2002) Transport of paclitaxel (Taxol) across the blood-brain barrier in vitro and in vivo. J Clin Invest 110:1309–1318

Fox E, Bates SE (2007) Tariquidar (XR9576): a P-glycoprotein drug efflux pump inhibitor. Expert Rev Anticancer Ther 7:447–459

Gaillard PJ, Visser CC, de Boer AG (2005) Targeted delivery across the blood-brain barrier. Expert Opin Drug Deliv 2:299–309

Gennuso F, Fernetti C, Tirolo C, Testa N, L'Episcopo F, Caniglia S, Morale MC, Ostrow JD, Pascolo L, Tiribelli C, Marchetti B (2004) Bilirubin protects astrocytes from its own toxicity by inducing up-regulation and translocation of multidrug resistance-associated protein 1 (Mrp1). Proc Natl Acad Sci USA 101:2470–2475

Graff CL, Pollack GM (2005) Nasal drug administration: potential for targeted central nervous system delivery. J Pharm Sci 94:1187–1195

Grauer MT, Uhr M (2004) P-glycoprotein reduces the ability of amitriptyline metabolites to cross the blood brain barrier in mice after a 10-day administration of amitriptyline. J Psychopharmacol 18:66–74

Groothuis DR, Benalcazar H, Allen CV, Wise RM, Dills C, Dobrescu C, Rothholtz V, Levy RM (2000) Comparison of cytosine arabinoside delivery to rat brain by intravenous, intrathecal, intraventricular and intraparenchymal routes of administration. Brain Res 856:281–290

Gulyaev AE, Gelperina SE, Skidan IN, Antropov AS, Kivman GY, Kreuter J (1999) Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles. Pharm Res 16:1564–1569

Hamabe W, Maeda T, Kiguchi N, Yamamoto C, Tokuyama S, Kishioka S (2007) Negative relationship between morphine analgesia and P-glycoprotein expression levels in the brain. J Pharmacol Sci 105:353–360

Hartz AM, Bauer B, Fricker G, Miller DS (2004) Rapid regulation of P-glycoprotein at the blood-brain barrier by endothelin-1. Mol Pharmacol 66:387–394

Hartz AM, Bauer B, Fricker G, Miller DS (2006) Rapid modulation of P-glycoprotein-mediated transport at the blood-brain barrier by tumor necrosis factor-alpha and lipopolysaccharide. Mol Pharmacol 69:462–470

Hau P, Fabel K, Baumgart U, Rummele P, Grauer O, Bock A, Dietmaier C, Dietmaier W, Dietrich J, Dudel C, Hubner F, Jauch T, Drechsel E, Kleiter I, Wismeth C, Zellner A, Brawanski A, Steinbrecher A, Marienhagen J, Bogdahn U (2004) Pegylated liposomal doxorubicin-efficacy in patients with recurrent high-grade glioma. Cancer 100:1199–1207

Hayashi K, Pu H, Tian J, Andras IE, Lee YW, Hennig B, Toborek M (2005) HIV-Tat protein induces P-glycoprotein expression in brain microvascular endothelial cells. J Neurochem 93:1231–1241

Hendrikse NH, Vaalburg W (2002) Imaging of P glycoprotein function in vivo with PET. Novartis Found Symp 243:137–145 discussion 145–8, 180–5

Ho EA, Piquette-Miller M (2006) Regulation of multidrug resistance by pro-inflammatory cytokines. Curr Cancer Drug Targets 6:295–311

Huang R, Murry DJ, Kolwankar D, Hall SD, Foster DR (2006) Vincristine transcriptional regulation of efflux drug transporters in carcinoma cell lines. Biochem Pharmacol 71:1695–1704

Husain SR, Puri RK (2003) Inderleukim-13 receptor-directed cytotoxin for malignant glioma therapy: from bench do bedside. J Neurooncol 65:37–48

Huwyler J, Drewe J, Klusemann C, Fricker G (1996) Evidence for P-glycoprotein-modulated penetration of morphine-6-glucuronide into brain capillary endothelium. Br J Pharmacol 118:1879–1885

Huynh GH, Deen DF, Szoka FC Jr (2006) Barriers to carrier mediated drug and gene delivery to brain tumors. J Control Release 110:236–259

Ivens S, Kaufer D, Flores LP, Bechmann I, Zumsteg D, Tomkins O, Seiffert E, Heinemann U, Friedman A (2007) TGF-beta receptor-mediated albumin uptake into astrocytes is involved in neocortical epileptogenesis. Brain 130:535–547

Jekerle V, Klinkhammer W, Scollard DA, Breitbach K, Reilly RM, Piquette-Miller M, Wiese M (2006) In vitro and in vivo evaluation of WK-X-34, a novel inhibitor of P-glycoprotein and BCRP, using radio imaging techniques. Int J Cancer 119:414–422

Joo KM, Park K, Kong DS, Song SY, Kim MH, Lee GS, Kim MS, Nam DH (2008) Oral paclitaxel chemotherapy for brain tumors: ideal combination treatment of paclitaxel and P-glycoprotein inhibitor. Oncol Rep 19:17–23

Kemper EM, Boogerd W, Thuis I, Beijnen JH, van Tellingen O (2004) Modulation of the blood-brain barrier in oncology: therapeutic opportunities for the treatment of brain tumours? Cancer Treat Rev 30:415–423

Kim RB, Fromm MF, Wandel C, Leake B, Wood AJ, Roden DM, Wilkinson GR (1998) The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. J Clin Invest 101:289–294

King M, Su W, Chang A, Zuckerman A, Pasternak GW (2001) Transport of opioids from the brain to the periphery by P-glycoprotein: peripheral actions of central drugs. Nat Neurosci 4:268–274

Koukourakis MI, Koukouraki S, Fezoulidis I, Kelekis N, Kyrias G, Archimandritis S, Karkavitsas N (2000a) High intratumoural accumulation of stealth liposomal doxorubicin (Caelyx) in glioblastomas and in metastatic brain tumours. Br J Cancer 83:1281–1286

Koukourakis MI, Koukouraki S, Giatromanolaki A, Kakolyris S, Georgoulias V, Velidaki A, Archimandritis S, Karkavitsas NN (2000b) High intratumoral accumulation of stealth liposomal doxorubicin in sarcomas–rationale for combination with radiotherapy. Acta Oncol 39:207–211

Kreuter J (1995) Nanoparticulate systems in drug delivery and targeting. J Drug Target 3:171–173

Kreuter J (2001) Nanoparticulate systems for brain delivery of drugs. Adv Drug Deliv Rev 47:65–81

Kroll RA, Pagel MA, Muldoon LL, Roman-Goldstein S, Fiamengo SA, Neuwelt EA (1998) Improving drug delivery to intracerebral tumor and surrounding brain in a rodent model: a comparison of osmotic versus bradykinin modification of the blood-brain and/or blood-tumor barriers. Neurosurgery 43:879–886 discussion 886–9

Langer O, Bauer M, Hammers A, Karch R, Pataraia E, Koepp MJ, Abrahim A, Luurtsema G, Brunner M, Sunder-Plassmann R, Zimprich F, Joukhadar C, Gentzsch S, Dudczak R, Kletter K, Muller M, Baumgartner C (2007) Pharmacoresistance in epilepsy: a pilot PET study with the P-glycoprotein substrate R-[^{11}C]verapamil. Epilepsia 48:1774–1784

Lee G, Bendayan R (2004) Functional expression and localization of P-glycoprotein in the central nervous system: relevance to the pathogenesis and treatment of neurological disorders. Pharm Res 21:1313–1330

Lee G, Dallas S, Hong M, Bendayan R (2001) Drug transporters in the central nervous system: brain barriers and brain parenchyma considerations. Pharmacol Rev 53:569–596

Lee YJ, Maeda J, Kusuhara H, Okauchi T, Inaji M, Nagai Y, Obayashi S, Nakao R, Suzuki K, Sugiyama Y, Suhara T (2006) In vivo evaluation of P-glycoprotein function at the blood-brain barrier in nonhuman primates using [11C]verapamil. J Pharmacol Exp Ther 316:647–653

Leslie EM, Deeley RG, Cole SP (2005) Multidrug resistance proteins: role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. Toxicol Appl Pharmacol 204:216–237

Letrent SP, Polli JW, Humphreys JE, Pollack GM, Brouwer KR, Brouwer KL (1999) P-glycoprotein-mediated transport of morphine in brain capillary endothelial cells. Biochem Pharmacol 58:951–957

Lippens RJ (1999) Liposomal daunorubicin (DaunoXome) in children with recurrent or progressive brain tumors. Pediatr Hematol Oncol 16:131–139

Loscher W, Potschka H (2005a) Drug resistance in brain diseases and the role of drug efflux transporters. Nat Rev Neurosci 6:591–602

Loscher W, Potschka H (2005b) Role of drug efflux transporters in the brain for drug disposition and treatment of brain diseases. Prog Neurobiol 76:22–76

Marchi N, Guiso G, Rizzi M, Pirker S, Novak K, Czech T, Baumgartner C, Janigro D, Caccia S, Vezzani A (2005) A pilot study on brain-to-plasma partition of 10,11-dyhydro-10-hydroxy-5H-dibenzo(b,f)azepine-5-carboxamide and MDR1 brain expression in epilepsy patients not responding to oxcarbazepine. Epilepsia 46:1613–1619

Martin C, Higgins CF, Callaghan R (2001) The vinblastine binding site adopts high- and low-affinity conformations during a transport cycle of P-glycoprotein. Biochemistry 40:15733–15742

Masuyama H, Suwaki N, Tateishi Y, Nakatsukasa H, Segawa T, Hiramatsu Y (2005) The pregnane X receptor regulates gene expression in a ligand- and promoter-selective fashion. Mol Endocrinol 19:1170–1180

Matsukado K, Inamura T, Nakano S, Fukui M, Bartus RT, Black KL (1996) Enhanced tumor uptake of carboplatin and survival in glioma-bearing rats by intracarotid infusion of bradykinin analog, RMP-7. Neurosurgery 39:125–133 discussion 133–134

Mazel M, Clair P, Rousselle C, Vidal P, Scherrmann JM, Mathieu D, Temsamani J (2001) Doxorubicin-peptide conjugates overcome multidrug resistance. Anticancer Drugs 12:107–116

McRae MP, Brouwer KL, Kashuba AD (2003) Cytokine regulation of P-glycoprotein. Drug Metab Rev 35:19–33

Merkus FW, van den Berg MP (2007) Can nasal drug delivery bypass the blood-brain barrier?: questioning the direct transport theory. Drugs R D 8:133–144

Milane A, Fernandez C, Vautier S, Bensimon G, Meininger V, Farinotti R (2007) Minocycline and riluzole brain disposition: interactions with p-glycoprotein at the blood-brain barrier. J Neurochem 103:164–173

Nies AT (2007) The role of membrane transporters in drug delivery to brain tumors. Cancer Lett 254:11–29

Pallis M, Turzanski J, Higashi Y, Russell N (2002) P-glycoprotein in acute myeloid leukaemia: therapeutic implications of its association with both a multidrug-resistant and an apoptosis-resistant phenotype. Leuk Lymphoma 43:1221–1228

Pardridge WM (1999) Blood-brain barrier biology and methodology. J Neurovirol 5:556–569

Pardridge WM (2002) Blood-brain barrier drug targeting enables neuroprotection in brain ischemia following delayed intravenous administration of neurotrophins. Adv Exp Med Biol 513:397–430

Pardridge WM (2005) Tyrosine hydroxylase replacement in experimental Parkinson's disease with transvascular gene therapy. NeuroRx 2:129–138

Potschka H, Volk HA, Loscher W (2004) Pharmacoresistance and expression of multidrug transporter P-glycoprotein in kindled rats. Neuroreport 15:1657–1661

Prados MD, Schold SJS, Fine HA, Jaeckle K, Hochberg F, Mechtler L, Fetell MR, Phuphanich S, Feun L, Janus TJ, Ford K, Graney W (2003) A randomized, double-blind, placebo-controlled, phase 2 study of RMP-7 in combination with carboplatin administered intravenously for the treatment of recurrent malignant glioma. Neuro Oncol 5:96–103

Rizzi M, Caccia S, Guiso G, Richichi C, Gorter JA, Aronica E, Aliprandi M, Bagnati R, Fanelli R, D'Incalci M, Samanin R, Vezzani A (2002) Limbic seizures induce P-glycoprotein in rodent brain: functional implications for pharmacoresistance. J Neurosci 22:5833–5839

Rosenberg MF, Kamis AB, Callaghan R, Higgins CF, Ford RC (2003) Three-dimensional structures of the mammalian multidrug resistance P-glycoprotein demonstrate major conformational changes in the transmembrane domains upon nucleotide binding. J Biol Chem 278:8294–8299

Sasabe H, Kato Y, Suzuki T, Itose M, Miyamoto G, Sugiyama Y (2004) Differential involvement of multidrug resistance-associated protein 1 and P-glycoprotein in tissue distribution and excretion of grepafloxacin in mice. J Pharmacol Exp Ther 310:648–655

Sawyer AJ, Piepmeier JM, Saltzman WM (2006) New methods for direct delivery of chemotherapy for treating brain tumors. Yale J Biol Med 79:141–152

Schinkel AH (1999) P-Glycoprotein, a gatekeeper in the blood-brain barrier. Adv Drug Deliv Rev 36:179–194

Senior AE, al-Shawi MK, Urbatsch IL (1995) The catalytic cycle of P-glycoprotein. FEBS Lett 377:285–289

Sharma US, Sharma A, Chau RI, Straubinger RM (1997) Liposome-mediated therapy of intracranial brain tumors in a rat model. Pharm Res 14:992–998

Shtil AA, Azare J (2005) Redundancy of biological regulation as the basis of emergence of multidrug resistance. Int Rev Cytol 246:1–29

Sills GJ, Kwan P, Butler E, de Lange EC, van den Berg DJ, Brodie MJ (2002) P-glycoprotein-mediated efflux of antiepileptic drugs: preliminary studies in mdr1a knockout mice. Epilepsy Behav 3:427–432

Sisodiya SM (2003) Mechanisms of antiepileptic drug resistance. Curr Opin Neurol 16:197–201

Spudich A, Kilic E, Xing H, Kilic U, Rentsch KM, Wunderli-Allenspach H, Bassetti CL, Hermann DM (2006) Inhibition of multidrug resistance transporter-1 facilitates neuroprotective therapies after focal cerebral ischemia. Nat Neurosci 9:487–488

Steiniger SC, Kreuter J, Khalansky AS, Skidan IN, Bobruskin AI, Smirnova ZS, Severin SE, Uhl R, Kock M, Geiger KD, Gelperina SE (2004) Chemotherapy of glioblastoma in rats using doxorubicin-loaded nanoparticles. Int J Cancer 109:759–767

Tchenio T, Havard M, Martinez LA, Dautry F (2006) Heat shock-independent induction of multidrug resistance by heat shock factor 1. Mol Cell Biol 26:580–591

Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC (1987) Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc Natl Acad Sci USA 84:7735–7738

Thomas H, Coley HM (2003) Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein. Cancer Control 10:159–165

Thompson SJ, Koszdin K, Bernards CM (2000) Opiate-induced analgesia is increased and prolonged in mice lacking P-glycoprotein. Anesthesiology 92:1392–1399

Thuerauf N, Fromm MF (2006) The role of the transporter P-glycoprotein for disposition and effects of centrally acting drugs and for the pathogenesis of CNS diseases. Eur Arch Psychiatry Clin Neurosci 256:281–286

Tomkins O, Friedman O, Ivens S, Reiffurth C, Major S, Dreier JP, Heinemann U, Friedman A (2007) Blood-brain barrier disruption results in delayed functional and structural alterations in the rat neocortex. Neurobiol Dis 25:367–377

Ugwoke MI, Verbeke N, Kinget R (2001) The biopharmaceutical aspects of nasal mucoadhesive drug delivery. J Pharm Pharmacol 53:3–21

Uhr M, Grauer MT (2003) abcb1ab P-glycoprotein is involved in the uptake of citalopram and trimipramine into the brain of mice. J Psychiatr Res 37:179–185

Uhr M, Grauer MT, Holsboer F (2003) Differential enhancement of antidepressant penetration into the brain in mice with abcb1ab (mdr1ab) P-glycoprotein gene disruption. Biol Psychiatry 54:840–846

Uhr M, Steckler T, Yassouridis A, Holsboer F (2000) Penetration of amitriptyline, but not of fluoxetine, into brain is enhanced in mice with blood-brain barrier deficiency due to mdr1a P-glycoprotein gene disruption. Neuropsychopharmacology 22:380–387

Uhr M, Tontsch A, Namendorf C, Ripke S, Lucae S, Ising M, Dose T, Ebinger M, Rosenhagen M, Kohli M, Kloiber S, Salyakina D, Bettecken T, Specht M, Putz B, Binder EB, Muller-Myhsok B, Holsboer F (2008) Polymorphisms in the drug transporter gene ABCB1 predict antidepressant treatment response in depression. Neuron 57:203–209

van Vliet EA, da Costa Araujo S, Redeker S, van Schaik R, Aronica E, Gorter JA (2007) Blood-brain barrier leakage may lead to progression of temporal lobe epilepsy. Brain 130:521–534

Volk HA, Loscher W (2005) Multidrug resistance in epilepsy: rats with drug-resistant seizures exhibit enhanced brain expression of P-glycoprotein compared with rats with drug-responsive seizures. Brain 128:1358–1368

Washington CB, Wiltshire HR, Man M, Moy T, Harris SR, Worth E, Weigl P, Liang Z, Hall D, Marriott L, Blaschke TF (2000) The disposition of saquinavir in normal and P-glycoprotein deficient mice, rats, and in cultured cells. Drug Metab Dispos 28:1058–1062

Yasui-Furukori N, Saito M, Nakagami T, Kaneda A, Tateishi T, Kaneko S (2006) Association between multidrug resistance 1 (MDR1) gene polymorphisms and therapeutic response to bromperidol in schizophrenic patients: a preliminary study. Prog Neuropsychopharmacol Biol Psychiatry 30:286–291

Zhou R, Mazurchuk R, Straubinger RM (2002) Antivasculature effects of doxorubicin-containing liposomes in an intracranial rat brain tumor model. Cancer Res 62:2561–2566

Zhu HJ, Liu GQ (2004) Glutamate up-regulates P-glycoprotein expression in rat brain microvessel endothelial cells by an NMDA receptor-mediated mechanism. Life Sci 75:1313–1322

Zucchetti M, Boiardi A, Silvani A, Parisi I, Piccolrovazzi S, D'Incalci M (1999) Distribution of daunorubicin and daunorubicinol in human glioma tumors after administration of liposomal daunorubicin. Cancer Chemother Pharmacol 44:173–176

Carriers in the Topical Treatment of Skin Disease

Hans Christian Korting and Monika Schäfer-Korting

Contents

H.C. Korting (✉)
Klinik und Poliklinik für Dermatologie und Allergologie, Ludwig-Maximilians-Universität, Frauenlobstraße 9-11, 80337 München, Germany
e-mail: HansChristian.Korting@med.uni-muenchen.de

M. Schäfer-Korting (✉)
Institut für Pharmazie (Pharmakologie und Toxikologie), Freie Universität Berlin, Königin-Luise-Straße 2-4, 14195 Berlin, Germany
e-mail: msk@zedat.fu-berlin.de

M. Schäfer-Korting (ed.), *Drug Delivery*,
Handbook of Experimental Pharmacology 197,
DOI 10.1007/978-3-642-00477-3_15, © Springer-Verlag Berlin Heidelberg 2010

Abstract Topical drug application is less prone to severe systemic side-effects than systemic application. Starting with the liposomes, various types of nanosized and microsized drug carriers have been developed to increase the notoriously low penetration of active agents into the skin, which limits not only the topical therapy of skin disease but also transdermal therapy.

Today, liposome- and microsponge-based preparations are approved for dermatomycosis, acne and actinic keratosis. Under investigation are drug carriers such as lipid nanoparticles, polymeric particles, dendrimers, and dendritic-core multi-shell nanotransporters. According to the rapidly increasing research in this field, both in academia and industry, a breakthrough appears likely, once stability problems (nanoparticles) and safety concerns (dendrimers) are overcome. Technical approaches and results of in vitro, ex vivo and in vivo testing are described, taking into account pharmacokinetic, efficacy and safety aspects.

Keywords Skin penetration · Cutaneous absorption · Drug carriers · Liposome · Solid lipid nanoparticle · Microemulsion · Polymer particles

Abbreviations

5-FU	5-Fluorouracil
API	Active pharmaceutical ingredient
CAT-1	4-Trimethyl-ammonium-2,2,6,6-tetramethylpiperidine-1-oxyliodide
CMS	Core multi-shell (nanotransporters)
CNT	(Carbo-)nanotubes
CPA	Cyproterone acetate
FITC	Fluorescein 5-isothiocyanate
GRAS	Generally recognised as safe
log*P*	Octanol:water partition coefficient
MCT	Medium chain triglycerides
NLC	Nanostructured lipid carriers
OECD	Organisation for Economic Co-operation and Development
PLA	Polylactic acid
PLGA	Poly(D,L-lactic-*co*-glycolic acid)
PASI	Psoriasis Area and Severity Index
QSPR	Quantitative structure permeability relationship
SCCP	Scientific Committee on Consumer Products
SLN	Solid lipid nanoparticles
TAC	Triamcinolone acetonide

1 Introduction

In the topical treatment of skin diseases, the active pharmaceutical ingredient (API) makes contact with the target site before entering the systemic circulation which – in general – is mandatory for contact with non-target sites. Therefore, systemic side effects are reduced compared to parenteral or oral drug administration. Application of drugs (preparations of API) to the skin surface was and still is used not only for skin diseases but also for antirheumatic therapy, to control gastrointestinal side effects of nonsteroidal anti-inflammatory drugs. Moreover, drug application to the skin surface circumvents hepatic first-pass metabolism and major fluctuations of plasma levels typical of repeated oral administration of rapidly eliminated drugs. Thus transdermal drug application is gaining increasing importance for systemic treatment, e.g. with drugs subject to extensive first-pass elimination such as glyceryl trinitrate and estrogens, as well as for long-term suppression of chronic pain by opioids.

It was, however, only in the 1960s and 1970s that pioneering research opened the field of dermatopharmacology and in particular dermatopharmacokinetics, which allowed for a quantitative insight into drug action on the skin tissue, into API levels at the target site and into the systemic uptake of APIs and toxic agents. Only then did the influence of receptor affinity and drug vehicle for therapeutic efficacy or for suitable surrogate parameters [e.g. skin blanching for the anti-inflammatory potency of glucocorticoids; (McKenzie and Stoughton 1962)], respectively, become obvious. Since surmounting the horny layer (stratum corneum) of the epidermis was revealed as the greatest challenge, detailed investigations followed into its structure and into methods of overcoming or bypassing the horny layer. In fact, early pioneering research proved renal hydrocortisone excretion to be related to the thickness of the horny layer at the target site (Feldmann and Maibach 1969). Later on the shunt pathway of drug penetration via hair follicles was demonstrated (Hueber et al. 1994) – the latter being most relevant when studying percutaneous absorption in furry animals.

2 (Per-)Cutaneous Absorption

When surmounting the horny layer, APIs and toxic agents first enter viable epidermis (Fig. 1). The concentration gradient from the epidermis to the vascularised dermis then allows the agents to gain access to the blood vessels.

2.1 Skin Morphology and Barrier Function

The skin is made up of the epidermis as well as the dermis situated underneath (Fig. 1). Still further down there is the subcutis which is rich in triglycerides. Micrometre-wide hair follicles reaching from the dermis or even from the subcutis

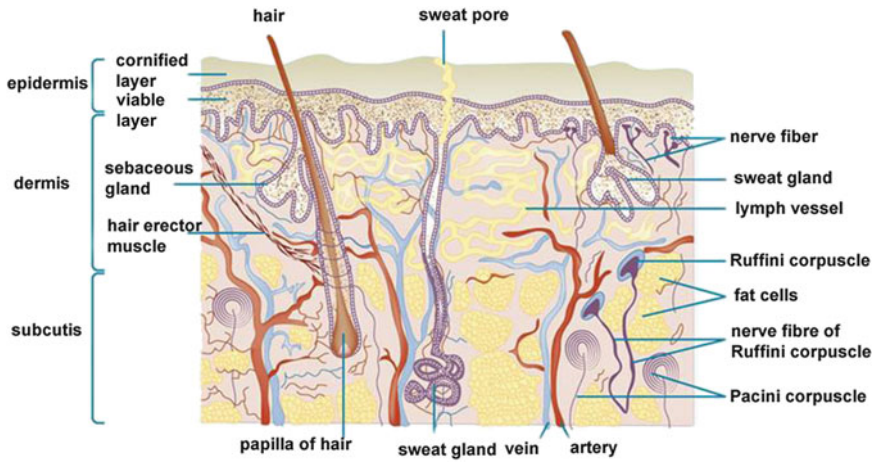


Fig. 1 Morphology of human skin (from Mutschler et al. (2008) with permission)

to the skin surface and thus passing through the epidermis are the most abundant appendages of the skin. Compared to furry animals, hair follicles are low in number in humans and the orifices cover only about 0.1% of the skin surface. In man, gender-related differences are small (Jacobi et al. 2005a).

The vascularised dermis (1–2 mm in thickness) is rich in fibres (collagen, elastin), embedded in ground substance surrounding fibroblasts which are the most numerous cell in the dermis. The most abundant cell of the unvascularised epidermis is the keratinocyte; cellular contacts are formed by desmosomes. Four epidermal layers are detectable by cellular differentiation: the stratum basale which is separated from the dermis by a basal membrane to which keratinocytes make contact by hemidesmosomes, the stratum spinosum, the stratum granulosum and the most superficial stratum corneum which consists of about 15 layers of flat apoptotic keratinocytes, the so-called corneocytes. The thickness of the horny layer amounts to about 10–15 μm, the thickness of the viable epidermis to 50–100 μm.

Other cell types are embedded between the basal keratinocytes, e.g. melanin-producing melanocytes and antigen-presenting Langerhans cells. In inflammatory skin diseases such as dermatitis, psoriasis vulgaris and fungal infections of the skin due to dermatophytes, leucocytes invade the skin. Moreover, in psoriasis the thickness increases due to enhanced proliferation and disturbed differentiation of keratinocytes.

The main penetration barrier which protects humans both from excessive water loss and harm due to toxic agents (and microorganisms) from the environment consists of the horny layer. During differentiation and passage to the surface, keratinocytes degrade phospholipids and synthesise ceramides which are packed into granula and secreted into the intercellular space where the ceramides plus cholesterol, long-chain (predominantly C22 and C24) free fatty acids and cholesteryl sulfate form highly ordered lipid layers serving as a cover. In healthy skin, these lipids are predominantly in the solid state due to an orthorhombic packing, while a hexagonal packing and thus a gel-like structure is characteristic for the most

superficial layers of the stratum corneum (Bouwstra and Honeywell-Nguyen 2002). At the same differentiation stage, nuclei and in particular DNA of keratinocytes are degraded and the water content of superficial skin declines to less than 20%.

Overlapping laterally by about 15% in man, dozens of the cells group together, forming clusters which represent the basic permeation barrier unit. Junctions between the clusters protrude into underlying skin as small furrows of up to several micrometres in size. Hydrophilic channel-like structures (nanopores) of up to 20 nm in diameter are reported to funnel into the inter-corneocyte spaces (for review see Cevc 2004).

In atopic dermatitis, psoriasis and other skin diseases, the penetration barrier is less efficient. The clearly reduced barrier function in atopic dermatitis patients results from a decreased amount of ceramides (especially ceramide 1) and a higher proportion of hexagonal lateral packing of the epidermal lipids (Choi and Maibach 2005; Bouwstra and Ponec 2006). Moreover, there is an irregular pattern of lipid organisation and irregular structure of protein particles of desmosomes. In psoriatic plaques, too, alterations in ceramide composition (again, in particular, decreased ceramide 1 levels) are to be found as well as increased keratinocyte proliferation and incomplete differentiation leading to the formation of a highly irregular horny layer (Choi and Maibach 2005). In fact, the similarities between these diseases are becoming more obvious (Wilsmann-Theis et al. 2008) which is well in accordance with overlaps in their therapeutic management. Even more severely altered organisation of lipid lamellae – plus disturbed protein functions – is seen with lamellar ichthyosis which is due to hereditary defects in the protein or lipid metabolism, respectively (Akiyama and Shimizu 2008).

2.2 Skin Penetration Pathways

In general, agents penetrate and permeate the skin predominantly using the tortuous intercellular pathway between the corneocytes of the horny layer. Therefore both the physicochemical nature of the agent and the anatomical region of application are of relevance. Absorption is rather high from the forehead, less so from post-auricular spaces, even less from belly and arm and least from palms and soles (Feldmann and Maibach 1969; Rougier et al. 1987; Tsai et al. 2003). Fentanyl and sufentanil uptake from foot, thigh, chest and abdomen was remarkably similar, and age and gender effects were not seen (Roy and Flynn 1990).

Moreover, highly lipophilic agents can penetrate the skin via the hair follicle (Ogiso et al. 1996; Münster et al. 2005) and – other than with the horny layer – micrometre size does not exclude penetration into follicular orifices (Hoffman 1998; Lademann et al. 1999; Otberg et al. 2004; Toll et al. 2004). In fact, recently follicular penetration has gained more and more interest – especially against the background of the development of novel drug delivery systems.

Besides acting as a penetration barrier, the horny layer is also a reservoir for topically applied substances. API binding to the keratin of the corneocytes as well

as miscibility with the lipid domains contribute to the reservoir function (Heard et al. 2003). Four days after the application of glucocorticoids under occlusion, skin blanching could be re-induced by renewed occlusion of the treatment area, releasing glucocorticoid from its reservoir (Vickers and Fritsch 1963). From the reservoir, agents can diffuse into viable skin and gain access to the dermal blood vessels; alternatively elimination can occur from the stratum corneum by desquamation of the corneocytes without access to viable skin.

2.3 Assessment of (Per-)Cutaneous Absorption

Based on molecular weight and octanol:water distribution, quantitative structure permeability relationship (QSPR) approaches have been devised to assess the intrinsic permeability of agents (for review see Moss et al. 2002; Geinoz et al. 2004). The procedures have been recently refined (Katritzky et al. 2006; Majumdar et al. 2007; Ottaviani et al. 2007). However, results may be valid only when applied to agents with physicochemical properties close to those of the training set. In fact, percutaneous absorption quantified using human and pig skin ex vivo as well as reconstructed epidermis did not correlate with QSPR data when studying agents widely varying in molecular weight and lipophilicity (Schäfer-Korting et al. 2008a). To unravel vehicle effects on skin absorption, experimental studies are indispensable in any case (Schäfer-Korting et al. 2008b).

Penetration into and permeation of the skin can be quantified ex vivo using human or animal skin, in vitro using reconstructed human skin, as well as in vivo (e.g. man and rat). The experimental approaches described here allow the understanding of the predictability of preclinical and clinical data generated when testing topical drug delivery systems.

Various approaches have been established for the assessment of (per-)cutaneous absorption. For purposes of regulatory toxicology, the OECD has released in vivo guideline 427 based on experiments in the rat (OECD 2004a) and in vitro (ex vivo) guideline 428 (OECD 2004b). The ex vivo techniques make use of Franz diffusion cells for testing (Bronaugh and Stewart 1985). Based on a strictly controlled protocol, this approach has been validated recently using human epidermis, porcine skin and reconstructed human epidermis as test matrix (Schäfer-Korting et al. 2006, 2008a). Concentrations of the agent of interest are followed in an acceptor fluid separated by the skin from the donor vehicle which is applied to the external surface of the skin. To follow drug penetration into the skin, the agent is quantified after removing the skin from the Franz cell (Wagner et al. 2004; Luengo et al. 2006; Stecova et al. 2007; Lombardi Borgia et al. 2008; Schäfer-Korting et al. 2008a). A reasonable agreement of ex vivo and in vivo data generated in man was described (Bronaugh and Franz 1986; Wagner et al. 2002b).

Drug release from topical preparations, e.g. in drug development, is followed using a synthetic membrane instead of skin (Jenning et al. 2000b; Schmook et al.

2001). While release can predict skin penetration promotion by the vehicle, it is the skin which reveals the intrinsic penetrability of the API. In fact, superior skin penetration of ibuprofen over ketoprofen at pH 7.4 became obvious only when using skin ex vivo (from the rat) as test matrix (Takahashi et al. 2002). However, recently efforts have been undertaken to develop matrix systems (e.g. PAMPA-skin) to quantify percutaneous absorption without the need of skin ex vivo or reconstructed epidermis (Ottaviani et al. 2007).

With API applied by the parenteral or oral route, plasma levels reflect the concentrations of the active agent at the target site as soon as a (pseudo) steady state of drug distribution is obtained. This, however, does not hold true with topical therapy. There the API passes the target organ skin *before* entering the circulation. Therefore, in clinical studies for drug absorption from topical dermatics, plasma levels should not be followed – except for studying systemic load and the potential for systemic side effects. Non-invasive alternatives advocated for use in humans are the tape stripping procedure for the quantification of drug levels in the horny layer (Weigmann et al. 1999, 2005; Shah 2001; Wagner et al. 2002a; Löffler et al. 2004; Jacobi et al. 2005b) – which has to be passed by any compound applied to the skin to become active either within the skin or systemically. A transparent and highly flexible adhesive film is attached to the stretched skin with standardised pressure and removed, thereby removing superficial corneocytes (Bashir et al. 2001; Lademann et al. 2005). Since the vehicle influences the amount of stratum corneum removed with each strip, the amount of API has to be related to the amount of stratum corneum obtained (Dreher et al. 2005; Jacobi et al. 2005b). Drug levels in the horny layer are reported to parallel dermal availability (Shah et al. 1998) and the skin blanching response when studying topical glucocorticoids (Pershing et al. 1992; Pelchrzim et al. 2004). Tape stripping, however, might not give meaningful results for APIs targeted to specific strata of viable skin.

Differential stripping is a procedure removing the horny layer by tape stripping in the first step and the contents of the hair follicles by cyanoacrylate stripping in the second (Schaefer and Lademann 2001; Teichmann et al. 2005). Thus differential stripping allows selective access to substances penetrated into the orifices of hair follicles.

2.4 Enhancement of (Per-)Cutaneous Absorption

In many skin diseases (e.g. atopic eczema and psoriasis vulgaris) a partially disturbed barrier can assist xenobiotics to overcome the horny layer (Anigbogu et al. 1996). While this is a major concern when considering nanotechnology safety (Stern and McNeil 2008), adequate skin penetration is still an important challenge in the development of topical dermatics. Conventional drug carrier systems, such as creams and ointments, result in drug uptake of only a few percent, which moreover as a rule is linked to a fairly high variation of uptake rates. Consequently, API levels within the diseased skin may be sub-therapeutic in some patients while inducing unwanted local or systemic side effects in others.

To be well absorbed, a substance should have a

- Molecular mass less than 500 g mol^{-1}
- A low melting point
- Adequate solubility in oil and water and
- An octanol:water partition coefficient (log*P*) of about 1–3.

Saturated or, even better, supersaturated systems favour skin penetration (Moser et al. 2001; Barry 2004).

Moreover, penetration enhancers can improve penetration into the skin. These agents can be grouped (Thong et al. 2007) as follows:

- Solvents and hydrogen bond acceptors (e.g. dimethylsulfoxide, dimethylformamide)
- Fatty acids and alcohols (laurylic acid, oleic acid, ethanol, *n*-octanol and many others) and
- Weak surfactants (e.g. Azone®, terpenes).

Penetration enhancers are found in very high amounts in microemulsions. Special attention should be given to propylene glycol which is not infrequently a component of the vehicles used for reference when testing particulate preparations.

The mechanisms of enhancement are still only partially understood. Based on the chemical structure, there is an interaction with stratum corneum lipids reducing the rigidity of the barrier and favouring the use of the nonpolar pathway or a change of protein conformation (protein denaturation) enabling the use of polar pathways. Some enhancers may also act on both pathways (Thong et al. 2007). Azones result in the formation of an additional fluid phase in mixtures of epidermal lipids (Pilgram et al. 2001). The channel-like structures between keratinocyte clusters can be altered by detergents and fatty acids which favours drug penetration (Barry 2004).

Mixtures of penetration enhancers such as sodium laureth sulfate and phenyl piperazine can be more efficient than the single agents and can allow even macromolecular agents such as heparin, luteinising hormone and oligonucleotides to permeate rat skin (Karande et al. 2004).

In general, changes induced by detergents in particular are linked to skin irritation at large which may result in irritant dermatitis if severe (Fang et al. 2003; Gloor 2004). The risk is enhanced with increasing potency of the penetration enhancers, and if skin occlusion is applied for a further penetration enhancement (for review see Thong et al. 2007).

3 Drug Carriers – Technological Aspects

Novel drug carriers intended for use in skin disease are often designed according to the principles of nanotechnology. To increase loadability with APIs, however, the dimensions of nanocarriers for biomedical use are 100–1,000 nm (Anton et al.

2008) and thus exceed the size of engineered nanomaterials as well as those of incidental components of air pollution, which have at least one order of magnitude less than 100 nm by definition (Stern and McNeil 2008).

According to the structures, nanoparticles can be divided into nanocapsules, exhibiting typical core–shell structures, and homogeneous nanospheres. Correspondingly, microcapsules and microspheres fall into the size range of >0.1 to 1 μm. Biocompatibility is a major prerequisite for pharmaceutical use and it is a challenge to design the formulation of the carrier to fit with the physicochemical properties of the API (e.g. to avoid chemical instability) and the therapeutic needs (e.g. improved skin penetration, prolonged uptake). The design and production of the various types as described below are reviewed in detail elsewhere (Anton et al. 2008). When aiming for drug approval, safety of all components obviously has to be demonstrated according to regulatory purposes.

3.1 Liposomes

According to their particle size, liposomes belong to the nanospheres or microspheres. Small unilamellar vesicles (SUV) have dimensions of 20 up to about 100 nm, large unilamellar vesicles (LUV) are larger than 100 nm, and multilamellar vesicles (MLV) have dimensions exceeding 500 nm. Vesicles are built by amphiphilic molecules such as phospholipids which form bilayers containing water in their cavity and dispersed in an aqueous medium. The polar head groups of the phospholipids (e.g. lecithin) form the interface to the aqueous media. Lipophilic agents are incorporated into the bilayers, the hydrophilic agents loaded are to be found within the water phase inside the vesicles. Hydrophilic agents, however, are often also found outside the liposomes in high amounts.

However, liposomes are metastable systems and their pharmaceutical use is often limited by instability. Instability can be due to leakage of the vesicles, change in vesicle size due to aggregation or fusion, as well as ester hydrolysis and formation of oxidation products. Building up the liposomes from hydrogenated phospholipids (gel-state liposomes), adding epidermal lipids (ceramides, cholesterol) and embedding liposomes into a gel matrix improves vesicle stability over liposomes made up from nonsaturated phospholipids (liquid-state liposomes).

The first liposomal product introduced into the market in 1988 was an econazole preparation for topical therapy of dermatomycosis (Fig. 2; Pevaryl® Lipogel). Recently liposomes loaded with UV-A and UV-B absorbing agents (Daylong® Actinica) have been approved in the European Union as a medical device for protection from actinic keratosis in high risk patients such as organ transplant recipients on long-term use of immunosuppressive agents (Ulrich et al. 2008). Moreover, liposomal diclofenac (Diclac® Lipogel) is authorised for the topical therapy of osteoarthritis (Table 1).

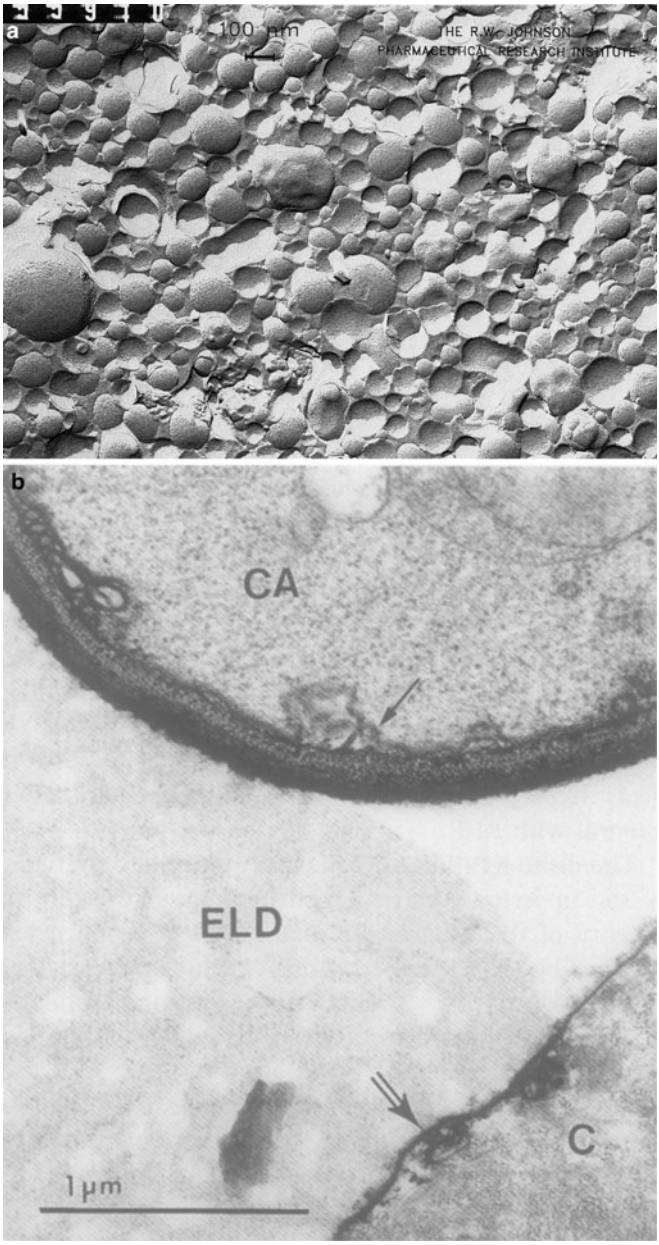


Fig. 2 Econazole-loaded liposomes. (**a**) Electron microscope freeze–fracture photograph of a commercial scale econazole liposomes dispersion (ELD) batch (Pevaryl® Lipogel) six months after production (with permission from Kriftner 1992). (**b**) Interaction of ELD with *Candida albicans* (CA) blastospore and reconstructed epidermis (corneocyte, C). The cell membrane of the CA blastospore is partially detached from CA cell wall (*arrow*). The cell membrane of the corneocyte (C) is impregnated with electron dense material representing applied drug (*double arrow*) (with permission from Korting et al. 1998)

Table 1 Commercially available drug delivery systems for the topical therapy of skin diseases and the transdermal application

API	Vehicle	Commercial product	Company	Indication
Econazole	Liposomes	Pevaryl Lipogel	Cilag, Switzerland	Dermatomycoses
Methoxycinnamates butyl methoxy-dibenzoylmethane	Liposomes	Daylong Actinica[a]	Spirig, Switzerland	Prophylaxis of actinic keratosis
Diclofenac	Liposomes	Diclac Lipogel	Hexal, Germany	Osteoarthritis
Tretinoin	Microsponges	Retin-A Micro	Ortho-Neutrogena, USA	Acne vulgaris
Fluorouracil	Microsponges	Carac	Sanofi Aventis, USA	Actinic keratosis

[a]Medical device

3.2 Niosomes

Evolved from liposomes, niosomes are non-ionic surfactant vesicles made up from polyoxyethylene alkyl ethers, polyoxyethylene alkyl esters or saccharose diesters. Thus niosomes are also related to micelles, yet are of larger size. Niosomes entrap, e.g. tretinoin, with high efficiency (>91%) and protect the labile agent against photodegradation (for review see Date et al. 2006).

3.3 Solid Lipid Nanoparticles and Nanostructured Lipid Carriers

Solid lipid nanoparticles (SLN) are nanospheres or nanoplatelets made up from lipids solid at room and body temperature such as glycerol behenate (Compritol®), glycerol palmitostearate (Precirol®) or tristearin glyceride. The nanodispersion obtained by hot or cold homogenisation under high pressure is stabilised by the addition of surfactants such as poloxamers, polysorbitanes or sucrose esters. SLN nanodispersions can be stable for years. The often rather poor loadability of the SLN due to the limited space for the API within the crystalline lattice is improved when using mixtures of lipids solid and fluid at room temperature (e.g. medium chain triglycerides, MCT); the resulting nanoparticles are named nanostructured lipid carriers (NLC) which in fact stand in between SLN and nanoemulsions (for review see Müller et al. 2007). Detailed physical investigations into NLC made up from Compritol/MCT indicate that the liquid lipid is attached to the solid lipid matrix and can form a nanospoon-like structure (Jores et al. 2005).

In spite of the limited loadability which restricts the amount of drug in relation to the amount of lipid, the encapsulation efficiency can be very high and often exceeds 90% with highly lipophilic agents (cyproterone acetate log*P* 3.28 (Stecova et al.

2007); clobetasol propionate log*P* 3.98 (Hu et al. 2002); prednicarbate log*P* 4.02 (Santos Maia et al. 2002); econazole log*P* 5.32 (Sanna et al. 2007)).

Recently a versatile method, parelectric spectroscopy, has been developed which allows differentiation between API attached to the particle surface and embedded within the particle matrix (for review see Blaschke et al. 2007). The reliability of the results is proven by a comparison with independent methods such as fluorescence anisotropy (solvatochromism) measurements using the lipophilic benzophenoxazone dye Nile red (Lombardi Borgia et al. 2005) and electron spin resonance studies (Braem et al. 2007). A detailed insight into the interaction of carrier and guest molecule is obtainable by changes in the signals emitted by the guest molecule, which, however, limits the applicability to model agents. In general, it is not applicable to APIs.

Drug release can be very rapid (burst release) due to polymorphic transition of the lipid lattice, yet sustained drug release is possible, too, and initial burst release can be followed by sustained release (for review see Schäfer-Korting et al. 2007).

3.4 Micro- and Nanoemulsions

Both micro- and nanoemulsions have droplet sizes smaller than 100 nm in diameter. Microemulsions are transparent dispersions of oil and water stabilised by an interfacial film of surfactant (and co-surfactant) molecules. Microemulsions form spontaneously without high shear equipment. The active agents are solubilised and thus available for rapid penetration into the skin. Increasing thermodynamic activity, the presence of (co-)surfactants acting as penetration enhancers and occlusivity improve skin penetration to variable degrees (Santos et al. 2008).

In contrast to microemulsions, nanoemulsions are metastable systems, the structures depending on the process of preparation: spontaneous emulsification or use of a high shear device. Nanoemulsions appear as water-like fluids, lotions or crystal-clear gels.

3.5 Polymer Particles Including Dendrimers

In contrast to the carriers described above, which are physical aggregates of nano- or microsize dimension, polymer particles are nanosized or microsized macromolecules – which, however, can also form aggregates (Haag and Kratz 2006).

Porous polymeric systems have an open structure; in principle capsules and spheres are possible. The large internal surface of nanospheres and microspheres allows for high loadability; both hydrophilic and lipophilic molecules can be loaded. The spheres can release the loaded material over a prolonged time, with or without burst release. Nanocapsules exhibit a core–shell structure: the core acts as a liquid (mainly oil) reservoir for APIs, the shell is built up from a biodegradable

polymer. Another advantage, besides high drug encapsulation efficiency and protection of instable agents against degradation, consists of low polymer content. Particles are built up from biodegradable synthetic materials such as polylactic acid (PLA), poly(D,L-lactic-*co*-glycolic acid) (PLGA) or polycyanoacrylate (Anton et al. 2008); moreover modified natural products such as chitosan and albumin can be used.

Dendrimers are macromolecules of a randomly branched but well defined dendron-like structure which can reversibly incorporate guest molecules; alternatively the guest molecules can be bound covalently to reactive sites of the dendrimer. These nanoparticles can be designed for specific core–shell structures which actively release the loaded agent by pH-triggered cleavage of the shell (Haag and Kratz 2006). Guest molecules can be loaded into the hydrophilic (e.g. polyglycerol or polyethyleneimine) core or into the lipophilic shell produced by the covalent binding of alkyl ketones. A minimum core size (about 3,000 g mol^{-1}) and a highly branched architecture are required for good encapsulation efficiency (Krämer et al. 2002). For further details see the chapter by R. Haag, this volume.

Microsponges® are spheric delivery systems of a non-collapsible polymer (mainly substituted acrylates or styrene-divinyl benzene) structure with a large porous surface and diameters of 5–300 μm (microbeads: sizes less than 50 μm, macrobeads: sizes about 100–200 μm) and will thus accept a high API load up to their own weight. Loaded into microsponges, labile APIs can be protected from environmental factors (Date et al. 2006). Preparations with fluorouracil for actinic keratosis (Carac™) and tretinoin for acne treatment (Retin-A Micro®) are in clinical use in the USA (Table 1).

3.6 Miscellaneous Nanoparticles

Titanium dioxide and zinc oxide nanoparticles reflect and scatter UV light; titanium dioxide particles of 60–120 nm in size are most efficient. Titanium dioxide particles are considered to be safe (SCCP 2007). Moreover, UV-B and UV-A absorbing agents are active agents of sunscreens which are tested when loaded onto nanoparticles.

(Carbo-)Nanotubes (CNT) are low-weight, high-strength building materials; quantum dots consist of a colloidal core surrounded by one or more surface coatings (shells) that reduce leaching of metals from the core. These nanoparticles have not yet been applied to the skin for biomedical purposes. However quantum dots have a potential for use as diagnostics because of intense photostable fluorescence. Skin penetration of spherical and ellipsoid quantum dots has been evaluated for occupational safety reasons; the particles can penetrate the stratum corneum of porcine skin ex vivo, yet have not been detected in the acceptor medium (Ryman-Rasmussen et al. 2006). A similar behaviour was also seen with nail-shaped quantum dots. If, however, they make contact with viable epidermis, e.g. in diseased skin, an inflammatory response could be initiated, derived from the exposure of human

keratinocytes (Zhang et al. 2008). Due to the current lack of use as drug vehicle, nanotubes and quantum dots will not be considered further here.

4 Current Therapy in the Main Target Indications for API-Loaded Nanoparticulate Delivery Systems

In dermatotherapy, research on new drug entities and drug delivery systems is focussed on frequent diseases often difficult to treat, in particular acne and psoriasis. For severe manifestations, not infrequently highly active APIs which may also induce major unwanted effects have to be prescribed for systemic use.

Acne vulgaris. Almost 85% of young people aged 12–25 years and as many as 8% of adults aged 25–34 years and 3% of those aged 35–44 years suffer from acne. The disease manifests as comedones, when during adolescence androgen induces sebum production while hypercornification of the hair follicle impedes sebum transport to the skin surface. Propionibacteria and micrococci colonising the sebaceous duct degrade sebum and induce the formation of inflammatory mediators with the resulting development of papules and pustules. The therapeutic regimen (Table 2) is dependent on the pathology of the dominant symptom, taking the severity – mild, moderate or severe – into account.

Topical therapy (retinoids, benzoyl peroxide, azelaic acid, antibiotics) is indicated in mild and moderate forms while more severe acne may require combined topical and systemic treatment. Efficacy requires the regular application of anti-acne agents, yet side effects often reduce patient compliance. Oral isotretinoin

Table 2 Anti-acne agents

API	Efficacy	Side effects	Promising drug delivery systems
Retinoids: tretinoin, isotretinoin, adapalene	High in mild to moderate acne: isotretinoin, applied orally also in severe acne forms	Irritation	Liposomes, microsponges, microemulsions, SLN: improved tretinoin skin penetration, fewer side effects
Benzoyl peroxide	Good in mild acne	Irritation, burning sensation	Liposomes, microsponges: improved efficacy and tolerability
Azelaic acid	Sufficient in mild acne	Well tolerated	
Antimicrobials: erythromycin, clindamycin, tetracyclines	Good to high	Induction of drug resistance	Liposomes (increased clindamycin efficiency)
Antiandrogens: cyproterone acetate	High	Antiandrogen effects exclude the use in male patients	Liposomes, SLN: enhanced skin penetration; liposomes: low serum levels, efficacy corresponds to oral dosing

application is the treatment of choice for severe acne, in particular in the male patient; long-lasting remissions are feasible. An increase in liver enzyme blood levels and plasma lipids, as well as severe skin irritation, however, are dose-limiting side effects. Retinoid-induced teratogenic effects are of serious concern in females. In severe acne of females the antiandrogen/progestin cyproterone acetate (CPA) is an option. The drug is applied orally. To avoid the risk of exposure of an unborn child to antiandrogens, CPA is administered in fixed combination with ethinyl estradiol for suppression of conception. The industrial development of equipotent topical application forms failed both with respect to isotretinoin and CPA.

Atopic dermatitis and psoriasis vulgaris. Potent anti-inflammatory agents such as glucocorticoids are still a mainstay for dermatitis of both the atopic and contact-allergic type. The major risk is skin atrophy. Following an initial reversible thinning detectable by skin ultrasound, the process can result in irreversible striae formation with use of potent glucocorticoids over several weeks (Schäfer-Korting et al. 1996). Reconstitution of skin thickness after the application of potent conventional glucocorticoids takes time (Korting et al. 2002) and may not be complete prior to the next exacerbation of dermatitis which requires further glucocorticoid treatment.

Nuclear factor of activated T-cells (NFAT) inhibitors such as tacrolimus and pimecrolimus are moderately potent alternatives for dermatitis, whereas ciclosporin is used for severe psoriasis and dermatitis. However, ciclosporin is active only when applied systemically. In fact the high molecular weight of these agents exceeding 800 g mol^{-1} impairs skin penetration. In recalcitrant psoriasis, oral methotrexate is another option. Methotrexate, however, can induce liver toxicity, and ciclosporin immunosuppression.

5 Clinical and Preclinical Data of Carrier-Loaded API

Progress in drug delivery systems may allow the safer use of these agents by the topical route. While only limited in vivo data have been reported, there is abundant information from ex vivo studies. Due to the major importance of the intercellular pathway in surmounting the horny layer in man, results from studies in human and porcine skin are to be preferred over studies in furry animal skin – both ex vivo and in vivo.

5.1 Liposomes and Niosomes

For improved skin penetration and targeting to the skin but also for transdermal therapy, liposomes have been studied since the early 1980s and have been the subject of detailed reviews, (e.g. Bouwstra and Honeywell-Nguyen 2002; Elsayed et al. 2007). We therefore focus on the most essential and most recent data.

Fundamental studies. Loading to liposomes can increase skin penetration while pretreatment of the skin with empty liposomes often is less efficient. Whereas in the

early days some authors suggested skin penetration of intact vesicles, this is no longer a matter of debate today – except for highly fluidic vesicles. In fact, liposomes tend to fuse at the skin surface. Vesicles have been detected by freeze-fracture electron microscopy up to the ninth tape strip only when applying the rather flexible liquid-state liposomes. Accordingly, rigid gel-state liposomes which are not detectable even in most parts of the superficial stratum corneum result in slower skin penetration compared to liquid-state liposomes. Depending on the phospholipids used to produce the liposomes, however, marked changes in the horny layer structure can be induced, such as the intercellular deposition of liposome ingredients – possibly due to a lysophospholipid content which is known to destroy lipid membranes. In general, application under occlusion reduces skin penetration since occlusion assists the vesicles to fuse at the skin surface (for review see Bouwstra and Honeywell-Nguyen 2002). In contrast, vesicle size and lamellarity appear of minor importance.

Besides improving the permeation of the horny layer, liposomes can target the pilosebaceous unit (for review see Elsayed et al. (2007)). Since the antigen-presenting Langerhans cells of the skin are plentifully located next to the hair follicle, liposomes have recently gained interest for immunisation, too.

Highly flexible liposomes (addition of surfactants for the reduction of interfacial tension) called Transfersomes can increase skin penetration and permeation not only of small molecules (glucocorticoids, ketoprofen) but even of agents of higher molecular weight such as albumin and insulin – thereby, e.g., reducing glucose blood levels in mice and man (Cevc 2004). Although not generally accepted (Bouwstra and Honeywell-Nguyen 2002; Elsayed et al. 2007), squeezing of intact Transfersomes® through the small channel-like structures of the skin is postulated (Cevc 2004). Another approach to improved skin penetration is an increase in vesicle flexibility due to the addition of ethanol; these liposomes are called ethosomes (Dayan and Touitou 2000; Touitou et al. 2000). The enhanced effect on API penetration following ethosomes over an ethanolic solution and liposomes, respectively, can be explained by the synergism of the well-known penetration enhancement by ethanol and the high vesicle membrane fluidity (Dubey et al. 2007; Elsayed et al. 2007). Most interestingly, ethosomes transported the marker agent rhodamine red into deeper skin strata; maximum rhodamin red staining was obtained at a depth of about 130 μm, corresponding to superficial dermis. With conventional liposomes, staining was most pronounced at a depth of 20–60 μm (Dubey et al. 2007). This demonstrates that the carrier system can also modulate API location within the skin tissue.

Liposomes can also act as a drug depot. From multilamellar gel-state liposomes (made up from hydrogenated lecithin/cholesterol) loaded with the hydrophilic spin probe CAT-1 and injected subcutaneously, 60% of the spin probe still remained in intact vesicles after 96 h. In contrast, on injecting CAT-1 solution, the spin probe-related signal declined rapidly due to distribution and bioreduction (Moll et al. 2004).

Anti-acne drugs. With respect to drug delivery systems for acne treatment, the gold-standard tretinoin (Table 2) has been studied most frequently. Various

preparations of liposome-encapsulated tretinoin have been tested in animal and human skin for absorption. Importantly, clinical studies have also been performed in acne patients for clinical efficacy and tolerability. The outcome parameters have been related to those obtained with conventional formulations (for review see Date et al. 2006; Schäfer-Korting et al. 2007). Application to animal and human skin results in higher local retinoid concentrations following liposomal formulations as compared to a gel or a solution. Accumulation in the skin is most pronounced with negatively charged liposomes or when adding hyaluronic acid as drug localiser, while positively charged vesicles appear to favour skin permeation. Clinical effects in acne vulgaris have been investigated in two double-blind studies. Superior tolerability of liposomal tretinoin (equal and reduced drug concentration) has been demonstrated compared to an approved gel, while efficacy was the same. Moreover, also efficacy can be increased if identical tretinoin concentrations are used.

Liposome encapsulation can also improve benzoyl peroxide efficacy in mild acne while reducing irritant reactions, possibly due to targeting of the hair follicle.

Most interestingly, in an open clinical trial in females suffering from moderate to severe acne, topical liposomal CPA has been found equipotent to the oral application of CPA plus ethinyl estradiole. Both preparations were superior to placebo. With the topical application, CPA plasma concentrations are around only 10% of those following oral application (for review see Date et al. 2006). Therefore, liposomes have the potential to overcome the notoriously poor CPA skin penetration which in fact was the reason for the discontinuation of the development of a topical CPA formulation in the early days. Only recently, improved CPA penetration following an alcoholic solution (Iraji et al. 2006) and when loaded to SLN (Stecova et al. 2007) have also been described.

Antifungal drugs. Tinea is a frequent fungal infection; dermatophytes as the relevant pathogens are found on top as well as within the superficial layers of the stratum corneum of glabrous skin. Although azole-type antifungals incorporated in conventional semi-solid vehicles such as creams cure a majority of cases, there is still need for optimisation. A stable econazole liposome formulation embedded into a gel matrix (Table 1; Fig. 2A) has been introduced into the market in several European countries. In an in-vitro model of *Candida albicans*-induced skin infection, econazole liposomal gel appeared superior to the cream of identical strength (Fig. 2B; Korting et al. 1998; Schaller et al. 1999). Moreover, mycological cure rates in tinea pedis were 80% (liposome gel) versus 73% (econazole cream), yet superiority could not be proven statistically (Korting et al. 1997).

Glucocorticoids. Transfersomes enhanced triamcinolone acetonide skin penetration and activity in the standard tests in human volunteers, which are skin blanching and the suppression of UV-induced erythema assay. Interestingly, skin thinning was less severe with Transfersome-loaded triamcinolone acetonide when reducing the glucocorticoid concentration (by 90%) to the equipotent concentration in commercial cream and ointment (Fig. 3; Fesq et al. 2003). The reduced decline in skin thickness following liposomal triamcinolone acetonide, despite remaining

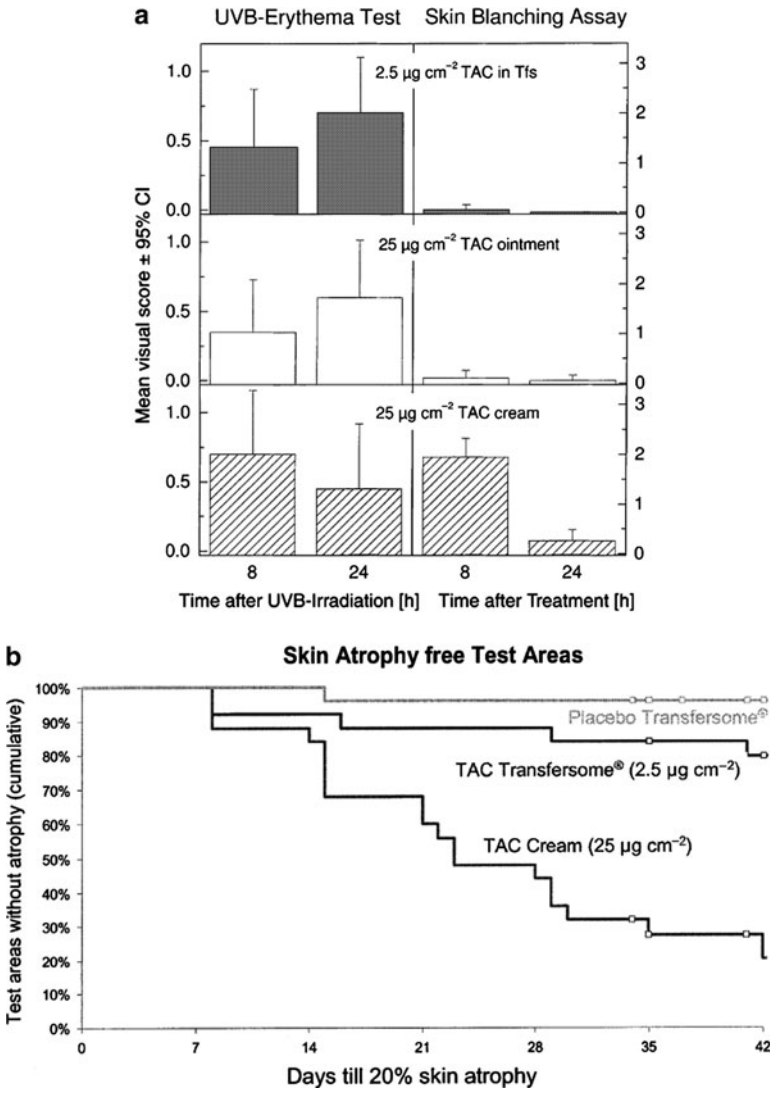


Fig. 3 Triamcinolone acetonide (TCA) loaded transfersomes. (a) Activity in the UVB erythema test and skin blanching assay. (b) Skin thinning effect (with permission from Fesq et al. 2003)

anti-inflammatory effects, opens the door to a safer use of strong conventional glucocorticoids in severe chronic dermatitis in the future, if this result can be confirmed in future studies.

Antipsoriatic agents. From aqueous solution, methotrexate skin penetration is very poor (Weinstein et al. 1989). Liposomes and niosomes may have the potential to permit topical methotrexate therapy, thus replacing the current oral treatment of severe and recalcitrant psoriasis. Methotrexate penetration into the skin

is improved following the application of a solution in 50% propylene glycol (enhancing drug penetration) and elastic liposomes (Trotta et al. 2004). More recent studies on methotrexate-loaded ethosomes (69% entrapment efficiency, stable for at least 120 days) demonstrated enhanced methotrexate permeation across human skin (Dubey et al. 2007). Niosomal methotrexate incorporated into a chitosan gel was tested in human volunteers and psoriatic patients. Efficacy as derived from the decline in the Psoriasis Area and Severity Index (PASI) was superior to methotrexate gel; significant irritation and sensitisation were not seen (Lakshmi et al. 2007).

Antineoplastic agents. Moreover, loading 5-fluorouracil (5-FU) to niosomes (made up from bola-surfactant, Span 80® and cholesterol, loading capacity about 40%) increased the penetration of human stratum corneum membranes eight-fold as compared to an aqueous 5-FU solution and four-fold when adding empty niosomes to the aqueous solution. Moreover, 5-FU cytotoxicity for the human melanoma cell line SKMEL-28, as well as the spontaneously transformed keratinocyte cell line HaCaT, increased (Paolino et al. 2008). This is well in accordance with obviously increased 5-FU efficacy and reduced irritancy when loading to microsponges (Menter et al. 2008).

Transdermal application. As described, liposomes were and still are frequently tested for transdermal use. In a multicentre, randomised, double-blind trial in 397 patients with knee osteoarthritis, Transfersome-loaded ketoprofen was applied daily for six weeks. Placebo and celecoxib 100 mg (suggested daily dose 200 mg) served as reference. Average reduction of pain score following ketoprofen (18.2) and celecoxib (20.3) was similar and was superior to placebo (9.9). Gastrointestinal effects of transfersomal ketoprofen and placebo were comparable (Rother et al. 2007).

5.2 Lipid Nanoparticles

Fundamental studies. Investigations using lipophilic agents such as retinol, prednicarbate, CPA, and the lipophilic marker dye Nile red loaded to Compritol-based SLN proved the agent can penetrate human and pig skin ex vivo more rapidly and to a higher extent than conventional vehicles and a nanoemulsion. In fact drug levels in the skin can exceed those following cream and gel preparations about four-fold for several hours (Jenning et al. 2000a; Santos Maia et al. 2002; Lombardi Borgia et al. 2005; Stecova et al. 2007). Later on, the differences in cutaneous drug concentrations following SLN and conventional formulations decline. The initially enhanced uptake appears to result from a burst release from the solid particles following water evaporation on the skin surface and the change of lipid modification resulting in a more dense packing of the lipid lattice (Jenning et al. 2000b). In fact, adding empty SLN to prednicarbate cream was no more efficient with respect to prednicarbate penetration into human skin ex vivo than the unmodified cream (Santos Maia et al. 2002).

The efficiency of SLN and NLC (mean diameters: 140–180 nm) to improve skin penetration was compared using the dye Nile red which was embedded into the lipid matrices, with NLC preferentially in the liquid lipid phase (Jores et al. 2005; Lombardi Borgia et al. 2005). Loaded to SLN made up from Compritol or Precirol, Nile red uptake increased about four-fold over the uptake following the reference cream. NLC were less efficient both when using MCT and the penetration enhancer oleic acid as liquid lipid (Lombardi Borgia et al. 2005). Scanning electron microscopy demonstrated a rather rapid degradation of SLN applied to the skin surface which appears to facilitate contact of the loaded agent with the skin surface (Küchler et al. 2008). This effect may contribute to improved skin penetration. Another proposed mechanism is skin occlusion which is inversely related with particle size and thus clearly more pronounced with nanoparticles than microparticles (Müller et al. 2002). In contrast, an irritant effect often seen with penetration enhancers can be excluded, since neither Poloxamer 188 used for nanoparticle stabilisation nor the nanoparticle dispersion disturbed phospholipid membranes as studied by force microscopy (Blaschke et al. in press).

The lipid film formed by degraded lipid particles, however, can also retard skin penetration, as observed when studying econazole permeation of porcine epidermis. The antifungal agent was loaded to Precirol/Tween 80-based SLN. Lag time increased and cumulative amount of permeated econazole decreased with increasing lipid content of the particles embedded into a gel matrix (Sanna et al. 2007).

Most importantly, loading to SLN can not only increase skin penetration but can induce epidermal drug targeting, as described for prednicarbate (Santos Maia et al. 2002) and podophyllotoxin (Chen et al. 2006), opening the horizon for more selectivity in drug action and reduced unwanted side effects with respect to the cutis and organ function beyond the skin. When studying the interaction of guest molecule and its carrier, epidermal targeting appeared linked to an attachment of the guest molecule to the carrier surface (Sivaramakrishnan et al. 2004; Stecova et al. 2007). Targeting was not seen with guest molecules incorporated into the lipid matrix (Lombardi Borgia et al. 2005). As described with liposomes, SLN too have the potential to deliver loaded agents to the hair follicle and sebaceous glands (Münster et al. 2005).

Anti-acne drugs. As with liposome loading, local tolerability of tretinoin-loaded SLN can be superior to tretinoin gels as shown by the Draize test in the rabbit (for review see Date et al. 2006). Moreover, the tretinoin isomer isotretinoin was loaded to SLN 30 and 50 nm in diameter made up from Precirol and stabilised by Tween 80 and lecithin. No isotretinoin permeation of rat skin was seen with loaded SLN in spite of a significant accumulation within the tissue – both being in contrast to the retinoid dissolved in 95% ethanol (Liu et al. 2007). This may offer new approaches for the treatment of severe acne, too. Currently long-lasting suppression of severe acne becomes possible only with oral isotretinoin application. Due to the high risk of a teratogenic side effect, however, this is possible in females only under strictly controlled contraceptive measures during the isotretinoin application period and for another 2 months thereafter.

To avoid side effects, topical antiandrogen treatment using lipid nanoparticles may be a future option, given that skin penetration is sufficiently high and systemic absorption low. Penetration of CPA into human skin ex vivo when loaded to SLN, NLC, a nanoemulsion and microparticles was well in accordance with the effects seen when loading the lipophilic dye Nile red: SLN enhanced CPA uptake four-fold, NLC and microparticles were less efficient and following the nanoemulsion CPA amounts in skin did not exceed those following CPA-loaded cream. Permeation of human skin correlated with skin penetration (Stecova et al. 2007).

Besides steroidal antiandrogens such as CPA, there are nonsteroidal agents with high androgen receptor selectivity, e.g. RU 58841. While SLN loading of RU58841 failed, a lipophilic myristate ester prodrug was easily loadable. Interestingly, no prodrug or drug (SLN loaded or non-loaded) has been detected in the acceptor medium following RU58841 myristate application to pig skin ex vivo and despite a rapid release of the active androgen by keratinocytes, fibroblasts, dermal papilla cells and sebocytes. Even more important is the lack of permeability of reconstructed human epidermis (Münster et al. 2005), since this in vitro test matrix is over-predictive compared to human skin (Schreiber et al. 2005). Since Nile red loaded to SLN allowed detection of the dye in high concentrations within the hair follicle and the sebaceous gland up to a depths of about 650 μm (Münster et al. 2005), SLN have the potential to deliver not only anti-acne APIs but also agents for male baldness to the site of disease – which has been claimed also for liposome-encapsulated RU 58841 (Bernard et al. 1997).

Glucocorticoids. Loading the lipophilic prednisolone diester prednicarbate to SLN (Compritol/Poloxamer 188, mean particle size 144 nm), the uptake by human skin ex vivo at 6 h increased about four-fold over the commercial cream, if the steroid was applied as both SLN dispersion or cream containing prednicarbate-loaded SLN. At 24 h, the differences in epidermal prednicarbate concentrations following SLN and conventional formulations had declined. Interestingly, there was prednicarbate targeting to the epidermis (Fig. 4; Santos Maia et al. 2002). This was not seen with, e.g., betamethasone 17-valerate, which was only poorly attached leading to overloaded systems at the relevant concentrations (Sivaramakrishnan et al. 2004).

While prednicarbate-SLN so far were tested in vitro only, a clinical study on clobetasol propionate-loaded SLN in patients with dermatitis demonstrated the expected improved efficacy of pertinent glucocorticoid treatment over the conventional cream (Kalariya et al. 2005). Whether the skin atrophy potential of glucocorticoid-loaded SLN may decline, as observed with transfersomal triamcinolone acetonide (Fesq et al. 2003), currently remains to be demonstrated. In spite of an improved benefit/risk ratio compared to equipotent glucocorticoids, prednicarbate in conventional formulations is not totally devoid of any skin thinning effect (Schäfer-Korting et al. 1993; Korting et al. 2002).

Antiviral systems. Podophyllotoxin is a standard drug for genital warts; their development is induced by human papilloma virus infection of epithelial cells. Since podophyllotoxin can induce severe skin irritation, a well-tolerated alternative by loading the agent to SLN was sought. Podophyllotoxin was loaded to tripalmitin-based SLN stabilised by lecithin and Poloxamer 188 (diameter 36–208 nm) or

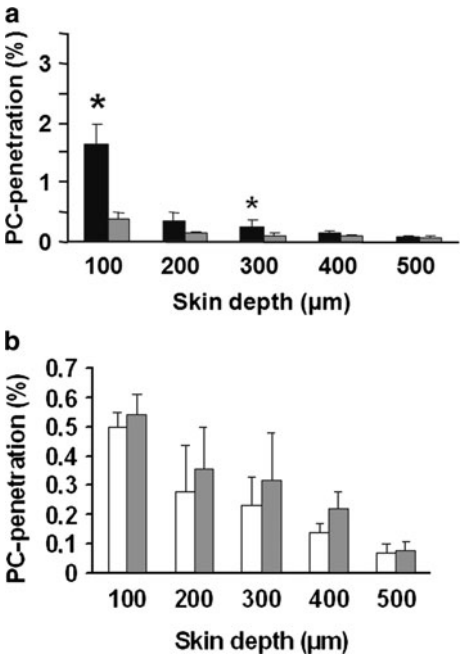


Fig. 4 (**a**) Improved absorption and epidermal targeting (0–100 µm skin depth) of prednicarbate loaded to SLN (*black column*) compared to the respective cream (*grey column*). The preparations were applied to human skin ex-vivo for 6 h. (**b**) Adding empty SLN to prednicarbate cream (*white column*) does not influence prednicarbate skin penetration and distribution compared to PC cream (with permission from Santos Maia et al. 2002)

Tween 80 (bimodal size distribution, peak values of 44 and 194 nm), respectively. With both SLN dispersions, podophyllotoxin permeation of pig skin ex vivo was not observed, which was in contrast with the ethanolic solution serving for reference. Within the skin tissue, however, podophyllotoxin concentrations were similar following the ethanolic solution and Tween-stabilised SLN dispersion; the concentrations were even almost four-fold higher following the lecithin/Poloxamer SLN dispersion. As abundant podophyllotoxin-related fluorescence was detected within the epidermis and close to the hair follicles, the authors considered both transepidermal and follicular uptake to be relevant (Chen et al. 2006). These results may stimulate systematic research on the effects of particle structure as well as the development of delivery systems for the topical treatment for, e.g., herpes virus infections. In particular the topical treatment of herpes labialis still demands improved cure rates, and topical treatment of herpes genitalis is not yet possible.

UV protection. Non-loaded and loaded SLN have been investigated for possible use in the protection of sunburn and skin cancer. Interestingly, cetyl palmitate nanodispersions act both as particulate UV blockers themselves and as sustained release carrier for UV-absorbing 2-hydroxy-4-methoxy benzophenone. Thus UV protection increased three-fold over both individual components (Müller et al. 2002). Recently UV protection was studied testing 3,4,5-trimethoxybenzoylchitin-loaded SLN; efficacy increased if tocopherol was added (Song and Liu 2005).

Transdermal application systems. As described with liposomes, SLN-loading can also retard API release. This was described with flurbiprofen-loaded SLN made up from stearic acid/cholesterol and stabilised by lecithin/Poloxamer 188 at varying

ratios; the entrapment amounted to 72%–93% on average. While burst release was seen with the SLN dispersion, release was sustained when embedding the particles into a polyacrylamide gel. In the acute carageneen-induced rat paw edema model, the intensity of flurbiprofen-related edema suppression ranked in the order: SLN embedded into the gel matrix > SLN dispersion > flurbiprofen solubilised in saline pH 7.4 (Jain et al. 2005).

Rat paw edema – acute carrageneen-induced and long-lasting Freund´s adjuvant-induced – served also for testing of SLN from tristearine glyceride stabilised with lecithin/polyethylene glycol (400) monostearate (mean diameter 123 nm) loaded with triptolide, a diterpenoid triepoxide. With the optimised SLN dispersion, triptolide penetration of rat skin increased 3.4-fold over the solution (propylene glycol/water); a lag phase was not observed. Microemulsions tested in parallel released triptolide even more efficiently (exceeding the solution seven-fold) – but after a lag phase of about 5 h. The rapid penetration following the SLN dispersion is well in accordance with most efficient suppression of acute edema, and the highest uptake from the microemulsion with a very strong influence on long-lasting edema (Mei et al. 2003).

Safety aspects. The lipids used for SLN production often have GRAS (generally recognised as safe) status (Müller et al. 2007), but this describes safety when exposed orally and is less relevant for dermal use. In various cell lines (e.g. HL60) and primary cells (murine peritoneal macrophages, human keratinocytes and fibroblasts) SLN appeared to be rather well tolerated (Müller et al. 1997; Schöler et al. 2001, 2002; Santos Maia et al. 2002; Weyenberg et al. 2007). SLN were reported to be less cytotoxic than polycyanoacrylate and PLGA nanoparticles (Müller et al. 1997). Adding cationic surfactants such as stearylamine or dimethyldioctadecylammonium bromide for stabilisation, however, reduced the viability of macrophages exposed to SLN (Weyenberg et al. 2007).

Application of SLN dispersions to reconstructed human epidermis proved well tolerated (Santos Maia et al. 2002; Küchler et al. 2008), which is well explained by the barrier function of the construct which does not exist with monolayer cultures. Since the barrier of reconstructed human epidermis making contact with the test preparations is less functional than normal human skin (Schäfer-Korting et al. 2008a), tolerability in man should be good. However, it has to be kept in mind that topical dermatics are developed for skin disease and then the barrier can be clearly less functional.

5.3 Microparticles

Besides liposomes, microsponges were studied with anti-acne drugs. Loading tretinoin or benzoyl peroxide to microsponges increased local tolerability of these agents while still influencing activity favourably, including that against *P. acnes* (for review see Date et al. 2006). Recently microsponge delivery systems were

also described for sustained benzoyl peroxide release (Jelvehgari et al. 2006), but the results of ex vivo and in vivo testing are still awaited.

Loading hydroquinone 4% and retinol 0.15% to microsponges for sustained drug release resulted in a well tolerated preparation improving postinflammatory hyperpigmentation and hyperpigmentation in melasma, in an open study of 28 patients (Grimes 2004). Moreover, microparticulate preparations forming a UV-protecting film covering the skin appeared interesting with respect to sunscreens, too (Lademann et al. 2004).

An increasing global health burden is actinic keratosis which may progress to squamous cell carcinoma. Topical 5-FU 5% cream belongs to the current first-line treatment options, but local tolerability is poor. 0.5% 5-FU loaded to microsponges (Table 1) proved to be about equipotent. According to a recent study in 356 patients with actinic keratosis, the 5-FU microsponge-based preparation proved more efficacious than the respective vehicle even when applied for one week only. Therapy was well tolerated, and no patient discontinued treatment because of side effects (Menter et al. 2008).

The skin is also subject to side effects of systemically applied drugs. A dose-limiting effect in, e.g., doxorubicin chemotherapy is palmar–plantar erythema which is triggered by the drug excreted with the sweat, then spread over the skin surface and penetrating the horny layer. The pronounced reservoir in the thick stratum corneum of palms and soles results in the generation of free radicals in high amounts, severely damaging the skin. To avoid this side effect by an efficient barrier cream, microparticles consisting of a mixture of *Hippospongia communis* and silica 10–100 μm in size were loaded with a mixture of antioxidants and incorporated into a cream vehicle. Lasting for 6 h, this formulation formed a homogeneous film on the skin surface even in deep furrows and wrinkles. Openings of hair follicles and sweat glands were covered, too. This barrier may impair doxorubicin penetration and the antioxidant agents loaded to the particles may interfere with free radical production. In contrast, a microparticle-free cream serving for reference did not protect hair follicle orifices (Lademann et al. 2008). This study indicates a new protection strategy against cutaneous side effects of antitumor drugs applied systemically.

Lipid microparticles made up from tristearin glyceride, stabilised with hydrogenated phosphatidylcholine (particle size 10–40 μm) and incorporated into an oil-in-water emulsion, only slightly promoted stratum corneum penetration of the UV-A absorbing agent butyl methoxydibenzoylmethane as compared to an oil-in-water emulsion when applied to human volunteers (Iannuccelli et al. 2008). This is well in accordance with results of previous studies (Stecova et al. 2007; Küchler et al. 2008).

5.4 Microemulsions

The solubility potential of microemulsions promotes skin penetration due to an increase in the concentration of lipophilic APIs and thus an increased concentration

gradient towards the tissue. The main site of solubilisation is the lipophilic moiety of the micellar surfactant film. Moreover, the usually very low interfacial tension allows for excellent contact with the entire application area including, e.g., wrinkles and microscopic gaps. Besides this, isopropyl palmitate and surfactants can induce penetration enhancement due to disruption of the stratum corneum lipid organisation or increasing API solubility within the skin, following incorporation into the skin layers (for review see Kreilgaard 2002; Santos et al. 2008).

Fundamental studies. Methyl nicotinate applied in an isopropyl palmitate microemulsion gel rapidly induced intense erythema. This, however, faded very rapidly whereas liposomes not disrupting the horny layer and forming a depot in the horny layer induced a longer lasting effect. Achieving better contact with the skin, water-in-oil (w/o) microemulsion and liposomes (from hydrogenated lecithin/cholesterol) accelerated benzyl nicotinate penetration into mouse skin as compared to hydrogel. However, efficacy as derived from skin oxygenation due to blood vessel dilation was not influenced (for review see Kreilgaard 2002). In contrast, a microemulsion and a liposomal formulation accelerated butyl nicotinate-induced vasodilation in mouse skin over the hydrogel used for reference. A further improvement was obtained by the synergistic combination of penetration enhancers *N*-lauroyl sarcosine/sorbitan monolaurate added to the microemulsion, since the increased fluidity of stratum corneum lipids facilitated drug penetration (Abramovic et al. 2008). The combined penetration enhancers were reported to be well tolerated in general (Karande et al. 2004). They did, however, induce some skin irritation (Abramovic et al. 2008).

Drugs for skin disease. Besides liposomes and microsponges, microemulsions have been studied with anti-acne drugs (for review see Date et al. 2006). Tretinoin incorporation into a microemulsion can enhance skin levels while reducing permeation of pig skin ex vivo. Moreover, erythema formation in the Draize test in rabbits was less severe. Azelain permeation through mouse skin increased, which was further stimulated when adding the penetration enhancer dimethylsulfoxide.

However, skin irritation due to microemulsions was observed. While enhancing skin penetration of hydrocortisone compared to an amphiphilic cream, skin blanching was counteracted due to skin irritation and thus increased blood flow (Lehmann et al. 2001). Methoxsalen uptake was enhanced up to eight-fold compared to water; efficacy was related to the content of penetration enhancer. Moreover, skin permeation of methotrexate increased up to ten-fold from a lecithin–water–propylene glycol–decanol–benzyl alcohol microemulsion compared to a water–propylene glycol solution (Kreilgaard 2002).

Transdermal application. Aiming at improved therapy for osteoarthritis, microemulsions were developed which enhanced human skin permeation of diclofenac, indomethacin, ketoprofen, celecoxib and rofecoxib three-fold to eight-fold. With celecoxib and rofecoxib, the onset of anti-inflammatory effects was more rapid than conventional vehicles (for review see Kreilgaard 2002; Santos et al. 2008). Loading to microemulsions also enhanced triptolide permeation of rat skin and efficacy in long-lasting rat paw edema (see above). This is well in accordance with the high amount of surfactant and co-surfactant used for production of the microemulsion. Erythema of the treatment site was not detected (Mei et al. 2003).

5.5 Polymer Particles Including Microparticles and Dendrimers

Compared to the investigations into liposomes and lipid nanoparticles, studies with polymer-based particles are rather few in number. Yet these studies, especially when using biostable particles, allow an insight into the processes of skin penetration and thus are of great relevance for the understanding of the function of nanoparticulate drug delivery systems in general.

Biodegradable poly(ε-caprolactone) nanoparticles enhanced the cutaneous uptake of lipophilic agents such as the UV-protection agent methoxycinnamate (Alvarez-Roman et al. 2001) and the dye Nile red (Alvarez-Roman et al. 2004b), whereas flufenamic acid uptake declined when loaded to PLGA nanoparticles (Luengo et al. 2006). In fact, flufenamic acid in general permeates human skin efficiently, even when applied in aqueous solution (Schäfer-Korting et al. 2008a). Loading of biostable poly *n*-butylcyanoacrylate nanocapsules (188 nm in diameter) with indomethacin (77% entrapment efficiency) increased drug release. The nanodispersion promoted indomethacin skin penetration almost ten-fold over the penetration when applying indomethacin Pluronic® gel, and uptake was enhanced almost four-fold when incorporating the nanocapsules into Pluronic gel. Nanoparticles were detected within the horny layer by parallel dye labelling (Miyazaki et al. 2003).

Interestingly, a first comparison of Nile red skin penetration from core multishell (CMS) nanotransporters synthesised using the dendrimer technology (Krämer et al. 2002), forming aggregates of about 40 nm in size, and SLN (average size 180 nm) indicated a clear superiority of the CMS nanotransporters (Küchler et al. 2008). Whether this is due to differences in particle size or results from the material building up the particle still has to be revealed.

The mechanism of penetration enhancement is best evaluated using covalent binding of dyes to biostable particles. Fluorescein 5-isothiocyanate (FITC)-labelled nanoparticles were preferentially localised in the follicular openings. Particle accumulation increased with time and was most pronounced with the smaller particles (Alvarez-Roman et al. 2004a; Luengo et al. 2006). Moreover, a series of studies by the Lademann group revealed that carriers up to 3 μm in size can transport the loaded agent into the open hair follicle. The optimum size is around 300–750 nm (Lademann et al. 1999; Schaefer and Lademann 2001; Toll et al. 2004; Teichmann et al. 2005), since the particles make use of a pump mechanism depending on the movement of the hair and the zigzag structure of the hair surface produced by keratinocyte desquamation. While the spontaneous hair movement in vivo transports particles deep into the follicle, hairs need to be moved by massage in ex vivo studies (Teichmann et al. 2006). Because of the low sebum flow, particles were stored for about 10 days within the hair follicles while free agents were stored for 4 days only (Lademann et al. 2007). Maybe this will allow for specific use in defined fields of dermatotherapy in the future.

Fluorescein-labelled nanoparticles were also enriched in skin furrows, yet penetration of intact particles was not seen (Alvarez-Roman et al. 2004b; Luengo et al.

2006). Therefore, in contrast to transfersomes, stable particles of 20 nm in diameter cannot squeeze through the channel-like structures of the stratum corneum, and loaded APIs have to be released for permeating the horny layer.

Safety aspects. Whereas dendrimers and especially those with amine surfaces can be very toxic, toxicity can be reduced by polyethylene shells surrounding the dendritic core, which should also reduce the aggregation tendency of the nanoparticles (Stern and McNeil 2008). This approach has been followed when designing CMS nanotransporters and in fact toxicity for keratinocytes was remarkably low (Küchler et al. 2008).

6 Miscellaneous Approaches

Iontophoresis and microneedles can improve the penetration of ionised agents into intact skin. These procedures are described elsewhere in this handbook. Stimulating results have been reported with respect to iontophoresis in the treatment of, e.g., acne scars (tretinoin gel) and hypertrophic scars (for review see Patravale and Mandawgade 2008).

7 Conclusion

Loading of APIs to nanoparticles, microparticles and microemulsions for dermal and transdermal use can alter skin penetration. The penetration rate can increase or decrease depending on the nature of the active agent and the preparation. Improved uptake is often linked with higher efficacy. If the concentration of the active pharmaceutical ingredient is adjusted, local tolerability can be improved. Currently only a few drugs based on nanosized or microsized application systems have been approved for topical use and introduced into the market. However, one has to keep in mind the rather slow progress obtained, e.g., in the field of transdermal therapeutic systems, too, which is due to technological challenges and the high standards of drug development set to ensure efficacy and safety.

References

Abramovic Z, Sustarsic U, Teskac K, Sentjurc M, Kristl J (2008) Influence of nanosized delivery systems with benzyl nicotinate and penetration enhancers on skin oxygenation. Int J Pharm 359:220–227

Akiyama M, Shimizu H (2008) An update on molecular aspects of the non-syndromic ichthyoses. Exp Dermatol 17:373–382

Alvarez-Roman R, Barre G, Guy RH, Fessi H (2001) Biodegradable polymer nanocapsules containing a sunscreen agent: preparation and photoprotection. Eur J Pharm Biopharm 52:191–195

Alvarez-Roman R, Naik A, Kalia YN, Guy RH, Fessi H (2004a) Enhancement of topical delivery from biodegradable nanoparticles. Pharm Res 21:1818–1825

Alvarez-Roman R, Naik A, Kalia YN, Guy RH, Fessi H (2004b) Skin penetration and distribution of polymeric nanoparticles. J Control Release 99:53–62

Anigbogu AN, Williams AC, Barry BW (1996) Permeation characteristics of 8-methoxypsoralen through human skin; relevance to clinical treatment. J Pharm Pharmacol 48:357–366

Anton N, Benoit JP, Saulnier P (2008) Design and production of nanoparticles formulated from nano-emulsion templates – a review. J Control Release 128:185–199

Barry BW (2004) Breaching the skin's barrier to drugs. Nature Biotechnol 22:165–167

Bashir SJ, Chew AL, Anigbogu A, Dreher F, Maibach HI (2001) Physical and physiological effects of stratum corneum tape stripping. Skin Res Technol 7:40–48

Bernard E, Dubois JL, Wepierre J (1997) Importance of sebaceous glands in cutaneous penetration of an antiandrogen: target effect of liposomes. J Pharm Sci 86:573–578

Blaschke T, Kankate L, Kramer KD (2007) Structure and dynamics of drug-carrier systems as studied by parelectric spectroscopy. Adv Drug Deliv Rev 59:403–410

Blaschke T, Spangenberg T, Dathe M, Mehnert W, Korting HC, Schäfer-Korting M, Kramer KD (in press) Interaction of drug-carrier systems with targets – a study using atomic force microscopy

Bouwstra JA, Honeywell-Nguyen PL (2002) Skin structure and mode of action of vesicles. Adv Drug Deliv Rev 54(Suppl 1):S41–55

Bouwstra JA, Ponec M (2006) The skin barrier in healthy and diseased state. Biochim Biophys Acta 1758:2080–2095

Braem C, Blaschke T, Panek-Minkin G, Herrmann W, Schlupp P, Paepenmüller T, Müller-Goyman C, Mehnert W, Bittl R, Schäfer-Korting M, Kramer KD (2007) Interaction of drug molecules with carrier systems as studied by parelectric spectroscopy and electron spin resonance. J Control Release 119:128–135

Bronaugh RL, Franz TJ (1986) Vehicle effects on percutaneous absorption: in vivo and in vitro comparisons with human skin. Br J Dermatol 115:1–11

Bronaugh RL, Stewart RF (1985) Methods for in vitro percutaneous absorption studies IV: the flow-through diffusion cell. J Pharm Sci 74:64–67

Cevc G (2004) Lipid vesicles and other colloids as drug carriers on the skin. Adv Drug Deliv Rev 56:675–711

Chen H, Chang X, Du D, Liu W, Liu J, Weng T, Yang Y, Xu H, Yang X (2006) Podophyllotoxin-loaded solid lipid nanoparticles for epidermal targeting. J Control Release 110:296–306

Choi MJ, Maibach HI (2005) Role of ceramides in barrier function of healthy and diseased skin. Am J Clin Dermatol 6:215–223

Date AA, Naik B, Nagarsenker MS (2006) Novel drug delivery systems: potential in improving topical delivery of antiacne agents. Skin Pharmacol Physiol 19:2–16

Dayan N, Touitou E (2000) Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs. liposomes. Biomaterials 21:1879–1885

Dreher F, Modjtahedi BS, Modjtahedi SP, Maibach HI (2005) Quantification of stratum corneum removal by adhesive tape stripping by total protein assay in 96-well microplates. Skin Res Technol 11:97–101

Dubey V, Mishra D, Dutta T, Nahar M, Saraf DK, Jain NK (2007) Dermal and transdermal delivery of an anti-psoriatic agent via ethanolic liposomes. J Control Release 123:148–154

Elsayed MM, Abdallah OY, Naggar VF, Khalafallah NM (2007) Lipid vesicles for skin delivery of drugs: reviewing three decades of research. Int J Pharm 332:1–16

Fang JY, Hwang TL, Fang CL, Chiu HC (2003) In vitro and in vivo evaluations of the efficacy and safety of skin permeation enhancers using flurbiprofen as a model drug. Int J Pharm 255:153–166

Feldmann RJ, Maibach HI (1969) Percutaneous penetration of steroids in man. J Invest Dermatol 52:89–94

Fesq H, Lehmann J, Kontny A, Erdmann I, Theiling K, Rother M, Ring J, Cevc G, Abeck D (2003) Improved risk-benefit ratio for topical triamcinolone acetonide in transfersome in comparison with equipotent cream and ointment: a randomized controlled trial. Br J Dermatol 149:611–619

Geinoz S, Guy RH, Testa B, Carrupt PA (2004) Quantitative structure-permeation relationships (QSPeRs) to predict skin permeation: a critical evaluation. Pharm Res 21:83–92

Gloor M (2004) How do dermatological vehicles influence the horny layer? Skin Pharmacol Physiol 17:267–273

Grimes PE (2004) A microsponge formulation of hydroquinone 4% and retinol 0.15% in the treatment of melasma and postinflammatory hyperpigmentation. Cutis 74:362–368

Haag R, Kratz F (2006) Polymer therapeutics: concepts and applications. Angew Chem (Int Ed) 45:1198–1215

Heard CM, Monk BV, Modley AJ (2003) Binding of primaquine to epidermal membranes and keratin. Int J Pharm 257:237–244

Hoffman RM (1998) Topical liposome targeting of dyes, melanins, genes, and proteins selectively to hair follicles. J Drug Target 5:67–74

Hu FQ, Yuan H, Zhang HH, Fang M (2002) Preparation of solid lipid nanoparticles with clobetasol propionate by a novel solvent diffusion method in aqueous system and physicochemical characterization. Int J Pharm 239:121–128

Hueber F, Schaefer H, Wepierre J (1994) Role of transepidermal and transfollicular routes in percutaneous absorption of steroids: in vitro studies on human skin. Skin Pharmacol 7:237–244

Iannuccelli V, Coppi G, Sergi S, Mezzena M, Scalia S (2008) In vivo and in vitro skin permeation of butyl methoxydibenzoylmethane from lipospheres. Skin Pharmacol Physiol 21:30–38

Iraji F, Momeni A, Naji SM, Siadat AH (2006) The efficacy of topical cyproterone acetate alcohol lotion versus placebo in the treatment of the mild to moderate acne vulgaris: a double blind study. Dermatol Online J 12:26

Jacobi U, Gautier J, Sterry W, Lademann J (2005a) Gender-related differences in the physiology of the stratum corneum. Dermatology 211:312–317

Jacobi U, Weigmann HJ, Ulrich J, Sterry W, Lademann J (2005b) Estimation of the relative stratum corneum amount removed by tape stripping. Skin Res Technol 11:91–96

Jain SK, Chourasia MK, Masuriha R, Soni V, Jain A, Jain NK, Gupta Y (2005) Solid lipid nanoparticles bearing flurbiprofen for transdermal delivery. Drug Deliv 12:207–215

Jelvehgari M, Siahi-Shadbad MR, Azarmi S, Martin GP, Nokhodchi A (2006) The microsponge delivery system of benzoyl peroxide: preparation, characterization and release studies. Int J Pharm 308:124–132

Jenning V, Gysler A, Schäfer-Korting M, Gohla SH (2000a) Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. Eur J Pharm Biopharm 49:211–218

Jenning V, Schäfer-Korting M, Gohla S (2000b) Vitamin A-loaded solid lipid nanoparticles for topical use: drug release properties. J Control Release 66:115–126

Jores K, Haberland A, Wartewig S, Mäder K, Mehnert W (2005) Solid lipid nanoparticles (SLN) and oil-loaded SLN studied by spectrofluorometry and Raman spectroscopy. Pharm Res 22:1887–1897

Kalariya M, Padhi BK, Chougule M, Misra A (2005) Clobetasol propionate solid lipid nanoparticles cream for effective treatment of eczema: formulation and clinical implications. Indian J Exp Biol 43:233–240

Karande P, Jain A, Mitragotri S (2004) Discovery of transdermal penetration enhancers by high-throughput screening. Nature Biotechnol 22:192–197

Katritzky AR, Dobchev DA, Fara DC, Hur E, Tamm K, Kurunczi L, Karelson M, Varnek A, Solov'ev VP (2006) Skin permeation rate as a function of chemical structure. J Med Chem 49:3305–3314

Korting HC, Klövekorn W, Klövekorn G, and the Ecosome Collaborative Study Group (1997) Comparative efficacy and tolerability of econazole liposomal gel 1%, branded econazol convetional cream 1% and generic clotrimazole cream 1% in tinea pedis. Clin Drug Invest 14:286–293

Korting HC, Patzak U, Schaller M, Maibach HI (1998) A model of human cutaneous candidosis based on reconstructed human epidermis for the light and electron microscopic study of pathogenesis and treatment. J Infect 36:259–267

Korting HC, Unholzer A, Schäfer-Korting M, Tausch I, Gassmueller J, Nietsch KH (2002) Different skin thinning potential of equipotent medium-strength glucocorticoids. Skin Pharmacol Appl Skin Physiol 15:85–91

Krämer M, Stumbe JF, Türk H, Krause S, Komp A, Delineau L, Prokhorova S, Kautz H, Haag R (2002) pH-responsive molecular nanocarriers based on dendritic core-shell architectures. Angew Chem (Int Ed) 41:4252–4256

Kreilgaard M (2002) Influence of microemulsions on cutaneous drug delivery. Adv Drug Deliv Rev 54(Suppl 1):S77–98

Kriftner RW (1992) Liposome production: The ethanol injection technique and the development of the first approved liposome dermatic. In: Braun-Falco O, Korting HC, Maibach HI (eds) Griesbach conference liposome dermatics. Springer, Berlin, pp 91–100

Küchler S, Radowski MR, Blaschke T, Dathe M, Plendl J, Haag R, Schäfer-Korting M, Kramer KD (2008) Nanoparticles for skin penetration enhancement – a comparison of a dendritic core-multishell-nanotransporter and solid lipid nanoparticles. Eur J Pharm Biopharm 71:243–250

Lademann J, Richter H, Golz K, Zastrow L, Sterry W, Patzelt A (2008) Influence of microparticles on the homogeneity of distribution of topically applied substances. Skin Pharmacol Physiol 21:274–282

Lademann J, Richter H, Teichmann A, Otberg N, Blume-Peytavi U, Luengo J, Weiss B, Schaefer UF, Lehr CM, Wepf R, Sterry W (2007) Nanoparticles – an efficient carrier for drug delivery into the hair follicles. Eur J Pharm Biopharm 66:159–164

Lademann J, Rudolph A, Jacobi U, Weigmann HJ, Schaefer H, Sterry W, Meinke M (2004) Influence of nonhomogeneous distribution of topically applied UV filters on sun protection factors. J Biomed Opt 9:1358–1362

Lademann J, Weigmann H, Rickmeyer C, Barthelmes H, Schaefer H, Mueller G, Sterry W (1999) Penetration of titanium dioxide microparticles in a sunscreen formulation into the horny layer and the follicular orifice. Skin Pharmacol Appl Skin Physiol 12:247–256

Lademann J, Weigmann HJ, Schanzer S, Richter H, Audring H, Antoniou C, Tsikrikas G, Gers-Barlag H, Sterry W (2005) Optical investigations to avoid the disturbing influences of furrows and wrinkles quantifying penetration of drugs and cosmetics into the skin by tape stripping. J Biomed Opt 10:54015

Lakshmi PK, Devi GS, Bhaskaran S, Sacchidanand S (2007) Niosomal methotrexate gel in the treatment of localized psoriasis: phase I and phase II studies. Indian J Dermatol Venereol Leprol 73:157–161

Lehmann L, Keipert S, Gloor M (2001) Effects of microemulsions on the stratum corneum and hydrocortisone penetration. Eur J Pharm Biopharm 52:129–136

Liu J, Hu W, Chen H, Ni Q, Xu H, Yang X (2007) Isotretinoin-loaded solid lipid nanoparticles with skin targeting for topical delivery. Int J Pharm 328:191–195

Löffler H, Dreher F, Maibach HI (2004) Stratum corneum adhesive tape stripping: influence of anatomical site, application pressure, duration and removal. Br J Dermatol 151:746–752

Lombardi Borgia S, Regehly M, Sivaramakrishnan R, Mehnert W, Korting HC, Danker K, Röder B, Kramer KD, Schäfer-Korting M (2005) Lipid nanoparticles for skin penetration enhancement – correlation to drug localization within the particle matrix as determined by fluorescence and parelectric spectroscopy. J Control Release 110:151–163

Lombardi Borgia S, Schlupp P, Mehnert W, Schäfer-Korting M (2008) In vitro skin absorption and drug release – a comparison of six commercial prednicarbate preparations for topical use. Eur J Pharm Biopharm 68:380–389

Luengo J, Weiss B, Schneider M, Ehlers A, Stracke F, Konig K, Kostka KH, Lehr CM, Schaefer UF (2006) Influence of nanoencapsulation on human skin transport of flufenamic acid. Skin Pharmacol Physiol 19:190–197

Majumdar S, Thomas J, Wasdo S, Sloan KB (2007) The effect of water solubility of solutes on their flux through human skin in vitro. Int J Pharm 329:25–36

McKenzie AW, Stoughton RB (1962) A method for comparing percutaneous absorption of steroids. Arch Dermatol 86:608–610

Mei Z, Chen H, Weng T, Yang Y, Yang X (2003) Solid lipid nanoparticle and microemulsion for topical delivery of triptolide. Eur J Pharm Biopharm 56:189–196

Menter A, Vamvakias G, Jorizzo J (2008) One-week treatment with once-daily fluorouracil cream 0.5% in participants with actinic keratoses. Cutis 81:509–516

Miyazaki S, Takahashi A, Kubo W, Bachynsky J, Loebenberg R (2003) Poly n-butylcyanoacrylate (PNBCA) nanocapsules as a carrier for NSAIDs: in vitro release and in vivo skin penetration. J Pharm Pharm Sci 6:238–245

Moll KP, Stosser R, Herrmann W, Borchert HH, Utsumi H (2004) In vivo ESR studies on subcutaneously injected multilamellar liposomes in living mice. Pharm Res 21:2017–2024

Moser K, Kriwet K, Froehlich C, Naik A, Kalia YN, Guy RH (2001) Permeation enhancement of a highly lipophilic drug using supersaturated systems. J Pharm Sci 90:607–616

Moss GP, Dearden JC, Patel H, Cronin MT (2002) Quantitative structure-permeability relationships (QSPRs) for percutaneous absorption. Toxicol In Vitro 16:299–317

Müller RH, Petersen RD, Hommoss A, Pardeike J (2007) Nanostructured lipid carriers (NLC) in cosmetic dermal products. Adv Drug Deliv Rev 59:522–530

Müller RH, Radtke M, Wissing SA (2002) Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev 54 (Suppl 1):S131–155

Müller RH, Rühl D, Runge S, Schulze-Forster K, Mehnert W (1997) Cytotoxicity of solid lipid nanoparticles as a function of the lipid matrix and the surfactant. Pharm Res 14:458–462

Münster U, Nakamura C, Haberland A, Jores K, Mehnert W, Rummel S, Schaller M, Korting HC, Zouboulis Ch C, Blume-Peytavi U, Schäfer-Korting M (2005) RU 58841-myristate – prodrug development for topical treatment of acne and androgenetic alopecia. Pharmazie 60:8–12

Mutschler E, Geisslinger G, Kroemer HK, Ruth P, Schäfer-Korting M (2008) Mutschler Arzneimittelwirkungen: Lehrbuch der Pharmakologie und Toxikologie. Wissenschaftliche Verlagsgesellschaft, Stuttgart

OECD (2004a) Test Guideline 427: Skin Absorption: in vivo Method. Adopted on 13th April 2004

OECD (2004b) Test Guideline 428: Skin Absorption: in vitro Method. Adopted on 13th April 2004

Ogiso T, Niinaka N, Iwaki M (1996) Mechanism for enhancement effect of lipid disperse system on percutaneous absorption. J Pharm Sci 85:57–64

Otberg N, Richter H, Schaefer H, Blume-Peytavi U, Sterry W, Lademann J (2004) Variations of hair follicle size and distribution in different body sites. J Invest Dermatol 122:14–19

Ottaviani G, Martel S, Carrupt PA (2007) In silico and in vitro filters for the fast estimation of skin permeation and distribution of new chemical entities. J Med Chem 50:742–748

Paolino D, Cosco D, Muzzalupo R, Trapasso E, Picci N, Fresta M (2008) Innovative bola-surfactant niosomes as topical delivery systems of 5-fluorouracil for the treatment of skin cancer. Int J Pharm 353:233–242

Patravale VB, Mandawgade SD (2008) Novel cosmetic delivery systems: an application update. Int J Cosmet Sci 30:19–33

Pelchrzim R, Weigmann HJ, Schaefer H, Hagemeister T, Linscheid M, Shah VP, Sterry W, Lademann J (2004) Determination of the formation of the stratum corneum reservoir for two different corticosteroid formulations using tape stripping combined with UV/VIS spectroscopy. J Dtsch Dermatol Ges 2:914–919

Pershing LK, Silver BS, Krueger GG, Shah VP, Skelley JP (1992) Feasibility of measuring the bioavailability of topical betamethasone dipropionate in commercial formulations using drug content in skin and a skin blanching bioassay. Pharm Res 9:45–51

Pilgram GS, van der Meulen J, Gooris GS, Koerten HK, Bouwstra JA (2001) The influence of two azones and sebaceous lipids on the lateral organization of lipids isolated from human stratum corneum. Biochim Biophys Acta 1511:244–254

Rother M, Lavins BJ, Kneer W, Lehnhardt K, Seidel EJ, Mazgareanu S (2007) Efficacy and safety of epicutaneous ketoprofen in Transfersome (IDEA-033) versus oral celecoxib and placebo in osteoarthritis of the knee: multicentre randomised controlled trial. Ann Rheum Dis 66:1178–1183

Rougier A, Lotte C, Maibach HI (1987) In vivo percutaneous penetration of some organic compounds related to anatomic site in humans: predictive assessment by the stripping method. J Pharm Sci 76:451–454

Roy SD, Flynn GL (1990) Transdermal delivery of narcotic analgesics: pH, anatomical, and subject influences on cutaneous permeability of fentanyl and sufentanil. Pharm Res 7:842–847

Ryman-Rasmussen JP, Riviere JE, Monteiro-Riviere NA (2006) Penetration of intact skin by quantum dots with diverse physicochemical properties. Toxicol Sci 91:159–165

Sanna V, Gavini E, Cossu M, Rassu G, Giunchedi P (2007) Solid lipid nanoparticles (SLN) as carriers for the topical delivery of econazole nitrate: in-vitro characterization, ex-vivo and in-vivo studies. J Pharm Pharmacol 59:1057–1064

Santos Maia C, Mehnert W, Schaller M, Korting HC, Gysler A, Haberland A, Schäfer-Korting M (2002) Drug targeting by solid lipid nanoparticles for dermal use. J Drug Targeting 10:489–495

Santos P, Watkinson AC, Hadgraft J, Lane ME (2008) Application of microemulsions in dermal and transdermal drug delivery. Skin Pharmacol Physiol 21:246–259

SCCP (2007) The Scientific Committee on Cosmetic Products (SCCP) Opinion on safety of nanomaterials in cosmetic products. http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_123.pdf

Schaefer H, Lademann J (2001) The role of follicular penetration. A differential view. Skin Pharmacol Appl Skin Physiol 14(Suppl 1):23–27

Schäfer-Korting M, Bock U, Diembeck W, Düsing HJ, Gamer A, Haltner-Ukomadu E, Hoffmann C, Kaca M, Kamp H, Kersen S, Kietzmann M, Korting HC, Krächter HU, Lehr CM, Liebsch M, Mehling A, Müller-Goymann C, Netzlaff F, Niedorf F, Rübbelke MK, Schäfer U, Schmidt E, Schreiber S, Spielmann H, Vuia A, Weimer M (2008a) The use of reconstructed human epidermis for skin absorption testing: results of the validation study. Altern Lab Anim 36:161–187

Schäfer-Korting M, Bock U, Gamer A, Haberland A, Haltner-Ukomadu E, Kaca M, Kamp H, Kietzmann M, Korting HC, Krächter HU, Lehr CM, Liebsch M, Mehling A, Netzlaff F, Niedorf F, Rübbelke MK, Schäfer U, Schmidt E, Schreiber S, Schröder KR, Spielmann H, Vuia A (2006) Reconstructed human epidermis for skin absorption testing: results of the German prevalidation study. Altern Lab Anim 34:283–294

Schäfer-Korting M, Korting HC, Kerscher MJ, Lenhard S (1993) Prednicarbate activity and benefit/risk ratio in relation to other topical glucocorticoids. Clin Pharmacol Ther 54:448–456

Schäfer-Korting M, Mahmoud A, Lombardi Borgia S, Brüggener B, Kleuser B, Schreiber S, Mehnert W (2008b) Reconstructed epidermis and full-thickness skin for absorption testing: Influences of vehicles on steroid permeation. Altern Lab Anim 36:441–452

Schäfer-Korting M, Mehnert W, Korting HC (2007) Lipid nanoparticles for improved topical application of drugs for skin diseases. Adv Drug Deliv Rev 59:427–443

Schäfer-Korting M, Schmid MH, Korting HC (1996) Topical glucocorticoids with improved risk-benefit ratio. Rationale of a new concept. Drug Saf 14:375–385

Schaller M, Preidel H, Januschke E, Korting HC (1999) Light and electron microscopic findings in a model of human cutaneous candidosis based on reconstructed human epidermis following the topical application of different econazole formulations. J Drug Target 6:361–372

Schmook FP, Meingassner JG, Billich A (2001) Comparison of human skin or epidermis models with human and animal skin in in-vitro percutaneous absorption. Int J Pharm 215:51–56

Schöler N, Hahn H, Müller RH, Liesenfeld O (2002) Effect of lipid matrix and size of solid lipid nanoparticles (SLN) on the viability and cytokine production of macrophages. Int J Pharm 231:167–176

Schöler N, Olbrich C, Tabatt K, Müller RH, Hahn H, Liesenfeld O (2001) Surfactant, but not the size of solid lipid nanoparticles (SLN) influences viability and cytokine production of macrophages. Int J Pharm 221:57–67

Schreiber S, Mahmoud A, Vuia A, Rübbelke MK, Schmidt E, Schaller M, Kandarova H, Haberland A, Schäfer UF, Bock U, Korting HC, Liebsch M, Schäfer-Korting M (2005) Reconstructed epidermis versus human and animal skin in skin absorption studies. Toxicol In Vitro 19:813–822

Shah VP (2001) Progress in methodologies for evaluating bioequivalence of topical formulations. Am J Clin Dermatol 2:275–280

Shah VP, Flynn GL, Yacobi A, Maibach HI, Bon C, Fleischer NM, Franz TJ, Kaplan SA, Kawamoto J, Lesko LJ, Marty JP, Pershing LK, Schaefer H, Sequeira JA, Shrivastava SP, Wilkin J, Williams RL (1998) Bioequivalence of topical dermatological dosage forms – methods of evaluation of bioequivalence. Pharm Res 15:167–171

Sivaramakrishnan R, Nakamura C, Mehnert W, Korting HC, Kramer KD, Schäfer-Korting M (2004) Glucocorticoid entrapment into lipid carriers – characterisation by parelectric spectroscopy and influence on dermal uptake. J Control Release 97:493–502

Song C, Liu S (2005) A new healthy sunscreen system for human: solid lipid nanoparticles as carrier for 3, 4, 5-trimethoxybenzoylchitin and the improvement by adding vitamin E. Int J Biol Macromol 36:116–119

Stecova J, Mehnert W, Blaschke T, Kleuser B, Sivaramakrishnan R, Zouboulis CC, Seltmann H, Korting HC, Kramer KD, Schäfer-Korting M (2007) Cyproterone acetate loading to lipid nanoparticles for topical acne treatment: particle characterisation and skin uptake. Pharm Res 24:991–1000

Stern ST, McNeil SE (2008) Nanotechnology safety concerns revisited. Toxicol Sci 101:4–21

Takahashi A, Suzuki S, Kawasaki N, Kubo W, Miyazaki S, Loebenberg R, Bachynsky J, Attwood D (2002) Percutaneous absorption of non-steroidal anti-inflammatory drugs from in situ gelling xyloglucan formulations in rats. Int J Pharm 246:179–186

Teichmann A, Jacobi U, Ossadnik M, Richter H, Koch S, Sterry W, Lademann J (2005) Differential stripping: determination of the amount of topically applied substances penetrated into the hair follicles. J Invest Dermatol 125:264–269

Teichmann A, Ossadnik M, Richter H, Sterry W, Lademann J (2006) Semiquantitative determination of the penetration of a fluorescent hydrogel formulation into the hair follicle with and without follicular closure by microparticles by means of differential stripping. Skin Pharmacol Physiol 19:101–105

Thong HY, Zhai H, Maibach HI (2007) Percutaneous penetration enhancers: an overview. Skin Pharmacol Physiol 20:272–282

Toll R, Jacobi U, Richter H, Lademann J, Schaefer H, Blume-Peytavi U (2004) Penetration profile of microspheres in follicular targeting of terminal hair follicles. J Invest Dermatol 123:168–176

Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M (2000) Ethosomes – novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Control Release 65:403–418

Trotta M, Peira E, Carlotti ME, Gallarate M (2004) Deformable liposomes for dermal administration of methotrexate. Int J Pharm 270:119–125

Tsai JC, Lin CY, Sheu HM, Lo YL, Huang YH (2003) Noninvasive characterization of regional variation in drug transport into human stratum corneum in vivo. Pharm Res 20:632–638

Ulrich C, Degen A, Patel MJ, Stockfleth E (2008) Sunscreens in organ transplant patients. Nephrol Dial Transplant 23:2712

Vickers CF, Fritsch WC (1963) A hazard of plastic film therapy. Arch Dermatol 87:633–636

Wagner H, Kostka KH, Adelhardt W, Schaefer UF (2004) Effects of various vehicles on the penetration of flufenamic acid into human skin. Eur J Pharm Biopharm 58:121–129

Wagner H, Kostka KH, Lehr CM, Schaefer UF (2002a) Correlation between stratum corneum/water-partition coefficient and amounts of flufenamic acid penetrated into the stratum corneum. J Pharm Sci 91:1915–1921

Wagner H, Kostka KH, Lehr CM, Schaefer UF (2002b) Human skin penetration of flufenamic acid: in vivo/in vitro correlation (deeper skin layers) for skin samples from the same subject. J Invest Dermatol 118:540–544

Weigmann H, Lademann J, Meffert H, Schaefer H, Sterry W (1999) Determination of the horny layer profile by tape stripping in combination with optical spectroscopy in the visible range as a prerequisite to quantify percutaneous absorption. Skin Pharmacol Appl Skin Physiol 12:34–45

Weigmann HJ, Jacobi U, Antoniou C, Tsikrikas GN, Wendel V, Rapp C, Gers-Barlag H, Sterry W, Lademann J (2005) Determination of penetration profiles of topically applied substances by means of tape stripping and optical spectroscopy: UV filter substance in sunscreens. J Biomed Opt 10:14009

Weinstein GD, McCullough JL, Olsen E (1989) Topical methotrexate therapy for psoriasis. Arch Dermatol 125:227–230

Weyenberg W, Filev P, Van den Plas D, Vandervoort J, De Smet K, Sollie P, Ludwig A (2007) Cytotoxicity of submicron emulsions and solid lipid nanoparticles for dermal application. Int J Pharm 337:291–298

Wilsmann-Theis D, Hagemann T, Jordan J, Bieber T, Novak N (2008) Facing psoriasis and atopic dermatitis: are there more similarities or more differences? Eur J Dermatol 18:172–180

Zhang LW, Yu WW, Colvin VL, Monteiro-Riviere NA (2008) Biological interactions of quantum dot nanoparticles in skin and in human epidermal keratinocytes. Toxicol Appl Pharmacol 228:200–211

Medical Devices for the Treatment of Eye Diseases

Tsutomu Yasukawa and Yuichiro Ogura

Contents

Abstract Development of intraocular drug delivery systems (DDSs) is urgently required for the treatment of eye diseases, especially in the posterior segment of the eye (the vitreous cavity, retina, and choroid), most of which are refractory to conventional pharmacologic approaches; eye drops and systemically administered drugs cannot achieve therapeutic drug concentrations in the posterior segment of

T. Yasukawa (✉)
Department of Ophthalmology and Visual Science, Nagoya City University Graduate School of Medical Sciences, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya Aichi 467-8601, Japan
e-mail: yasukawa@med.nagoya-cu.ac.jp

M. Schäfer-Korting (ed.), *Drug Delivery*,
Handbook of Experimental Pharmacology 197,
DOI 10.1007/978-3-642-00477-3_16, © Springer-Verlag Berlin Heidelberg 2010

the eye. Repeated intravitreal injections of anti-angiogenic agents are effective in the treatment of age-related macular degeneration, but there remain risks of serious side effects such as endophthalmitis associated with repeated injections. Intraocular DDSs may address these problems. Intraocular sustained drug release from implantable or injectable devices has been investigated to treat vitreoretinal diseases. A reservoir-type nonbiodegradable implant was first launched in the market in 1996 for the treatment of cytomegalovirus retinitis secondary to the acquired immunodeficiency syndrome, followed by clinical trials for a variety of potent devices to treat other challenging eye diseases. An injectable rod-shaped insert releasing a steroid is presently being assessed in a phase III trial to treat macular edema secondary to diabetic retinopathy or retinal vein occlusion. Thus various types of intraocular DDSs will be commercially available to treat vision-threatening intraocular diseases in the near future.

Keywords Age-related macular degeneration · Biodegradable polymer · Controlled release · Intraocular drug delivery system · Microsphere

List of Abbreviations

AIDS	Acquired immune deficiency syndrome
AMD	Age-related macular degeneration
API	Active pharmaceutical ingredient
CMV	Cytomegalovirus
CNV	Choroidal neovascularization
DDS	Drug delivery system
ECT	Encapsulated cell technology
EVA	Ethylene vinyl acetate
HAART	Highly active antiretroviral therapy
PLGA	Poly(lactic-*co*-glycolic acid)
PVA	Poly(vinyl alcohol)
PVR	Proliferative vitreoretinopathy
RPE	Retinal pigment epithelium
VEGF	Vascular endothelial growth factor

1 Introduction

The eye is a photosensory organ with specific structures similar to those of a camera. The eye should preserve not only the transparency of ocular media but also its configuration, in order to stabilize refractory structures (e.g., cornea and

lens). For this aim, the most effective shape is a balloon-like configuration with little vasculature, few cellular components, and transparent fluid inside. Aqueous humor, the intraocular circulating fluid, is well conditioned with sufficient nutrients for nonvascular structures and a strictly limited amount of macromolecules such as proteins and lipids for homeostasis and transparency. This forms the *blood–aqueous barrier,* composed of ciliary nonpigmented epithelium and iridal vascular endothelium with tight junctions. The outer and inner *blood–retinal barriers*, which are formed by the retinal pigment epithelium (RPE) and retinal vascular endothelium, respectively, regulate retinal homeostasis (Maurice and Mishima 1984; Geroski and Edelhauser 2001; Ambati and Adamis 2002; Yasukawa et al. 2004, 2006, 2007). In this way the intraocular space is separated from the systemic blood circulation (Fig. 1) and therefore systemically administered drugs cannot easily reach the intraocular space (Pharmacological Therapy for Macular Degeneration Study Group 1997; Ip and Gorin 1996). Therefore, high drug dosages must be used to achieve efficacy, frequently causing side effects in other healthy tissues. On the other hand, the eye is covered with collagenous layers (e.g., cornea and sclera) and epithelial and endothelial barriers (e.g., cornea and RPE). These barriers, continuous tear production, frontward flow of aqueous humor, and choroidal circulation limit the penetration of topically administered drugs (e.g., eye drops and ointments) (Lang 1995; Kim et al. 2007) (Fig. 1). Consequently, eye drops must be instilled frequently or at high concentrations to achieve therapeutic concentrations even in the anterior segment of the eye. It is much harder to deliver drugs to the posterior segment because of the longer

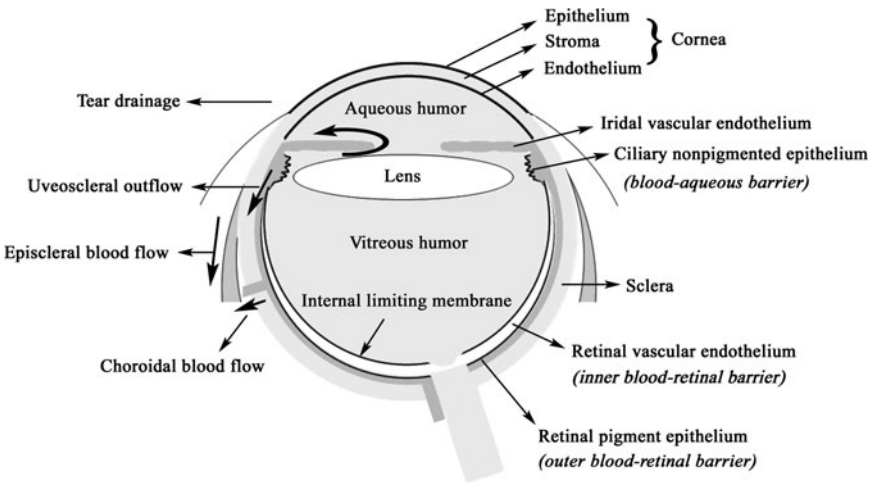


Fig. 1 Barriers which limit drug delivery into the posterior segment of the eye. The cornea, tear drainage, episcleral blood flow, and intraocular counterdirectional convection limit the delivery of locally administered drugs into the posterior segment of the eye. Systemically administered drugs cannot reach the vitreous cavity and the retina easily because of the blood–retinal barriers

diffusion distance and counterdirectional intraocular convection from the ciliary body to Schlemm's canal.

Considering these problems, most recent therapies for vitreoretinal diseases, for example exudative (wet) age-related macular degeneration (AMD) and macular edema, have been administered by periocular or intraocular injection (Ip et al. 2004; Gillies et al. 2006; Rosenfeld et al. 2006; Brown et al. 2006; Augustin and Schmidt-Erfurth 2006). In the vitreous cavity, however, the half-life of most drugs with a low molecular weight is as short as a few hours, even large molecules such as antibodies have a half-life of a few days (Bakri et al. 2007a, b). Therefore, repeated intravitreal injections may be required, potentially associated with adverse effects such as cataract formation, vitreous hemorrhage, endophthalmitis, and retinal detachment (Cantrill et al. 1989; Cocherau-Massin et al. 1991; Heinemann 1989; Ussery et al. 1988).

Drug delivery systems (DDSs) may overcome the aforementioned problems of pharmaceutical approaches. Regarding DDSs to the anterior segment of the eye, some inserts used instead of eye drops are already commercially available: Ocusert® Pilo (controlled-release pilocarpine, Alza Co., Palo Alto, CA), Mydriasert® (IOLTech, La Rochelle, France), and Lacrisert® (hydroxypropyl cellulose ocular insert, Merck & CO., Inc., Whitehouse Station, NJ) (Fig. 2). Ocusert® Pilo inserts, which became commercially available in 1974, contain a core reservoir consisting of pilocarpine and alginic acid. The core is surrounded by

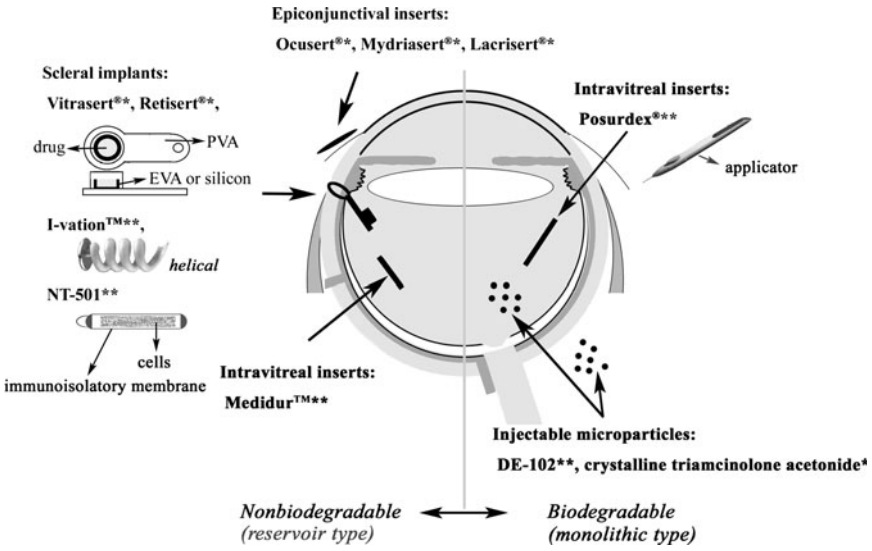


Fig. 2 Controlled release systems. Ocusert®, Mydriasert®, Vitrasert®, and Retisert® are clinically available, nonbiodegradable (reservoir-type) devices. Lacrisert® is a biodegradable (monolithic-type) insert in the conjunctival sac. *Commercially available medical devices. **Products under clinical trials

a hydrophobic ethylene vinyl acetate (EVA) copolymer membrane that controls diffusion of pilocarpine, an active pharmaceutical ingredient (API), from the insert into the eye. Pilocarpine can decrease intraocular pressure in patients with glaucoma. While commonly used eye drops should be administered four times a day, the insert releases pilocarpine for a week after it is placed in contact with the conjunctival surface. Mydriasert® is an insoluble-matrix retropalpebral ophthalmic insert containing phenylephrine and tropicamide as APIs to obtain sustained mydriasis during surgery or fundus examination. Lacrisert® is biodegradable and inserted daily into the conjunctival sac instead of eye drops in the treatment of dry eye. These types of inserts could be easily removed if adverse effects develop. However, these inserts do not provide striking benefits over conventional use of eye drops. On the other hand, it must be beneficial but more complicated and hard to develop DDSs to the posterior segment of the eye. The implants should be placed into the sub-Tenon's, intrascleral, or intravitreal space. In this case, any mechanical effects of the remaining matrix and any pharmacologic effects of the drug used should be considered.

In this section, we review medical devices for the treatment of diseases in the posterior segment of the eye which are clinically available, under clinical trial, or being tested preclinically.

2 Targeted Vitreoretinal Diseases

Vision loss easily impairs quality of life. In industrialized nations, AMD, diabetic retinopathy, and retinitis pigmentosa as well as glaucoma are main causes of legal blindness, while cataract and corneal diseases are still sight-threatening in developing countries. Retinal disorders are one of the most challenging diseases in the body, involving AMD, diabetic retinopathy, retinitis pigmentosa, and macular edema secondary to diabetic retinopathy, uveitis, pseudophakia, and retinal vein occlusion. Some eyes with glaucoma are refractory to any medications and surgical approaches. In such cases, retinal ganglion cells are damaged, resulting in progressive visual field defect. Thus, most hard-to-treat eye diseases are associated with alteration of retinal functions. Because the retina is a neurosensory organ with binocular harmonized function, the retina and, in particular, its center, called the *macula*, require homeostasis in (1) the structure and the location, (2) transparency, and (3) neurophysiology. AMD and macular dystrophy often lead to irreversible impairment of neurophysiologic functions at the macula. Macular edema impairs not only the structure but also the transparency and physiologic functions of the retina. To date, advances in surgery enable even the peeling of the internal limiting membrane, the innermost thin layer of the retina, by the use of special instruments, resulting in recent highly improved visual outcomes in eyes with macular holes. However, most other macular diseases are still refractory to any type of treatment.

Many vitreoretinal disorders are refractory to the effects of current medications because of the aforementioned difficulties in pharmacologic approaches. Therefore,

a DDS may be necessary in the treatment of a variety of intraocular diseases. Potentially, an intraocular controlled (sustained) release system can be used for diseases in which repeated local administration of drugs is likely to be effective, such as wet AMD, macular edema, cytomegalovirus (CMV) retinitis, and uveitis. In addition, in diseases that initially require vitreoretinal surgery and in which complications or recurrence may occur postoperatively, intraocular controlled release devices implanted adjunctively during surgery may improve recovery (e.g., proliferative vitreoretinopathy (PVR), choroidal neovascularization (CNV), and diabetic retinopathy). Moreover, a controlled release system may be necessary to treat chronic diseases with no satisfactory therapies, such as geographic atrophy (dry AMD), macular edema, and retinitis pigmentosa.

2.1 Cytomegalovirus (CMV) Retinitis

CMV retinitis used to be an object of public concern, because it occurred in approximately 25% of patients with acquired immune deficiency syndrome (AIDS) and was a leading cause of blindness in terminally ill patients (Gross et al. 1990). This background urgently required the development of a new treatment modality. Early diagnosis and efficient management are necessary to preserve vision. Systemic administration of ganciclovir or foscarnet first proved effective in slowing the progression of CMV retinitis. However, these drugs frequently have serious side effects: ganciclovir causes myelosuppression and foscarnet kidney dysfunction, which may necessitate discontinuance of the therapy (Henderly et al 1987; Holland et al. 1986; Jabs et al. 1987). Therefore, intravitreal administration of ganciclovir was then carried out but needed to be repeated to maintain intraocular drug concentration in the therapeutic range, potentially associated with inherent risks of retinal detachment, cataract, vitreous hemorrhage, and endophthalmitis (Cantrill et al. 1989; Cocherau-Massin et al. 1991; Heinemann 1989; Ussery et al. 1988). A pressing need to preserve the quality of life in patients with AIDS focused on the development of new intraocular DDSs, leading to the first commercial use of a controlled release system for ganciclovir with a nonbiodegradable polymer device (Vitrasert®) (Sanborn et al. 1992). This proved to be effective and biocompatible clinically, although it required surgery to remove the exhausted unit or to repeat implantation (Morley et al. 1995; Sanborn et al. 1992; Smith et al. 1992). More recently, highly active antiretroviral therapy (HAART), a combination of reverse-transcriptase-inhibiting nucleosides and human immunodeficiency virus type 1-specific protease inhibitors, has been established to normalize immunity in patients with AIDS and promote the regression of several opportunistic infections including CMV retinitis (Autran et al. 1997; Mitchell et al. 1999; Vrabec et al. 1998). Thus HAART has significantly reduced the need for an intraocular device for patients with AIDS. Yet, a number of scientists that gained fundamental knowledge in the field of intraocular DDSs, and now continue to develop new devices to treat a variety of other challenging vitreoretinal diseases.

2.2 Noninfectious Uveitis

The uvea is the intraocular tissue with melanin granules, involving the iris, the ciliary body, and the choroid. *Uveitis* is an ocular autoimmune or inflammatory disease occurring in the uvea and adjacent tissues such as the sclera and the retina. Uveitis has acute or chronic features affecting local or diffuse areas of the eye, and it has the potential for recurrence. The disease often requires long-term medication with steroids, immunosuppressive agents, antibiotics, or their combination in order to suppress inflammation or prevent recurrence in specific cases. Persistent inflammation in the anterior segment sometimes results in posterior synechia of the iris limiting the dilation of the iris, secondary glaucoma with or without peripheral anterior synechia of the iris, and cataract formation, while longstanding inflammation in the posterior segment sometimes leads to hazy vitreous humor, macular edema, exudative or ischemic retinal vascular disorders, and other retinal dysfunctions. The necessity of long-term medication led to the development of a controlled release system for fluocinolone acetonide with a nonbiodegradable polymer device (Retisert®), which was approved by the US FDA in 2005 (Jaffe et al. 2000).

2.3 Macular Edema

Macular edema, if persistent, often leads to irreversible loss of visual acuity, accompanying a variety of diseases including diabetic retinopathy, uveitis, and retinal vein occlusion (Fig. 3). This disease is characterized by leakage of serum from retinal capillaries and subsequent swelling of the macula, the center of the retina.

Diabetic macular edema is one of major causes of legal blindness in patients with diabetes mellitus. There are approximately 500,000 cases requiring treatment in the USA every year. Permeability of retinal vasculature is enhanced by vascular endothelial growth factor (VEGF) secreted from retinal cellular components in response to occlusion of microcapillaries and subsequent ischemia. Also macular edema is affected by a variety of general conditions: degree of diabetic nephropathy as well as retinopathy, hypertension, anemia, and hyperlipidemia. Diabetic macular edema is classified into two types: focal and diffuse. Focal macular edema is often caused by microaneurysms with a compromise of the blood–retinal barrier integrity, which is treatable by laser photocoagulation of the microaneurysms. On the other hand, diffuse macular edema is caused by dilation of retinal capillaries around the macular area. Diffuse macular edema is currently treated by grid laser treatment, intravitreal or sub-Tenon's injection of a steroid, triamcinolone acetonide (Ip et al. 2004; Gillies et al. 2006), or vitrectomy. More recently, some nonbiodegradable or biodegradable devices with various corticosteroids for intraocular use (Medidur®, I-vation®, and Posurdex®) as well as intraocular injection of anti-VEGF agents (Lucentis®, and Avastin®) are under clinical trial in the treatment of diffuse diabetic macular edema.

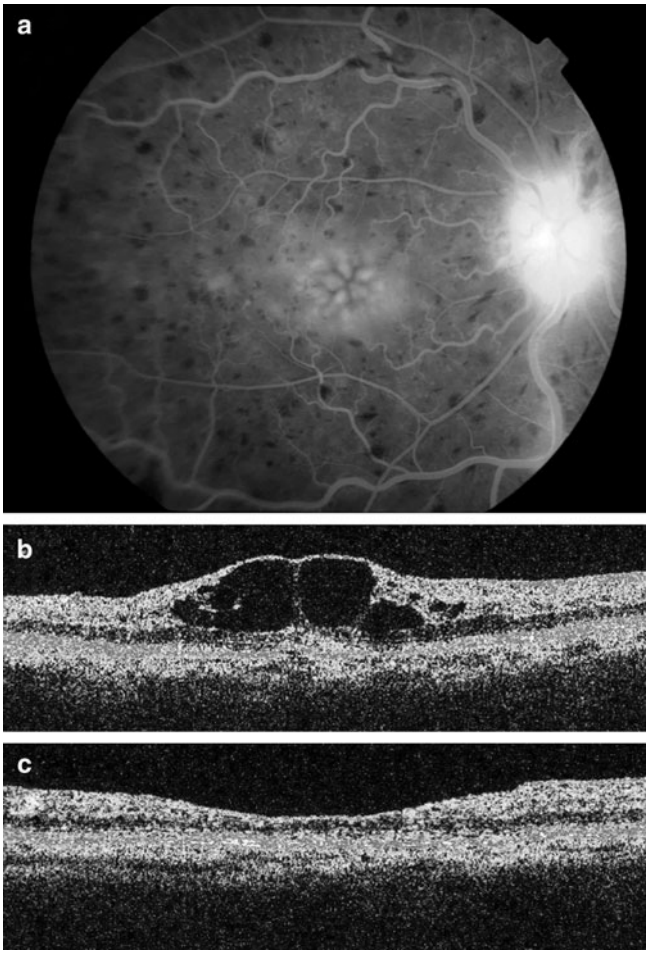


Fig. 3 Macular edema secondary to central retinal vein occlusion. (**a**) Fluorescein angiography revealed cystoid macular edema. (**b**) Optical coherence tomography showed a retinal section with cystic spaces at the macula. Visual acuity is 20/100. (**c**) Intravitreal injection of crystalline triamcinolone acetonide (4 mg) reduced the macular edema and improved visual acuity to 20/50. However, macular edema often recurs and requires repeated administration of drug, laser photocoagulation, or vitrectomy

2.4 Retinitis Pigmentosa

Retinitis pigmentosa is a clinically and genetically heterogeneous group of eye diseases with primary retinal and RPE degeneration resulting from a variety of genetic alterations in physiologic functions of photoreceptors and underlying RPE cells as a sensor of visible light. Gene mutations involve autosomal dominant and recessive, X-linked, and mitochondrial modes. Most causal genes may be associated with the retinoid cycle, involving e.g., *rpe65, abca4, vmd2*. Generally, an

electroretinogram is negative or non-recordable before the onset of visual field defect. Many cases have progressive visual field defect, in some cases accompanied by loss of visual acuity. To date, there have been no successful treatment modalities to improve genetic alteration or even delay the progression of symptoms. However, a recent report on gene therapy has shed hopeful light on the treatment. Intravitreal injection of *rpe65*-encoding adenovirus vector delayed the worsening of visual function in eyes with Leber's congenital amaurosis (Maguire et al. 2008). This result suggests that long-term supply of defect or altered proteins derived from gene mutations may rescue eyes with retinitis pigmentosa. Intracellular delivery of proteins as well as gene therapies using viral or nonviral vectors, siRNAs, and so on may have great potential to treat retinitis pigmentosa.

2.5 Age-Related Macular Degeneration (AMD)

AMD is a leading cause of legal blindness in people over 50 years of age in most developed countries (Klein et al. 1992; Ryan et al. 1994). Fifteen million Americans and more in other western countries are affected by AMD. About 14–24% of US citizens aged 65–74 years and 35% of people over 75 years of age have the disease. There are approximately 200,000 new cases of AMD each year in USA, and the annual incidence is expected to grow as the population ages. AMD is classified into two types: choroidal neovascularization (CNV; exudative or wet AMD) and geographic atrophy (dry AMD). Geographic atrophy represents the death of photoreceptors and underlying RPE cells and the atrophy of underlying choriocapillaries, resulting in gradual impairment of central vision with no successful treatment. In contrast, CNV often results in acute or subacute irreversible visual loss (Ryan et al. 1994) (Fig. 4). CNV has been treated by surgical removal, macular translocation with scleral retraction sutures or 360° retinotomy, laser photocoagulation, photodynamic therapy, transpupillary thermotherapy, or radiation (American Academy of Ophthalmology 2000; Bressler 1997; Chakravarthy et al. 1993; Kaplan 1996; Macular Photocoagulation Study Group 1986, 1990, 1994a, b).

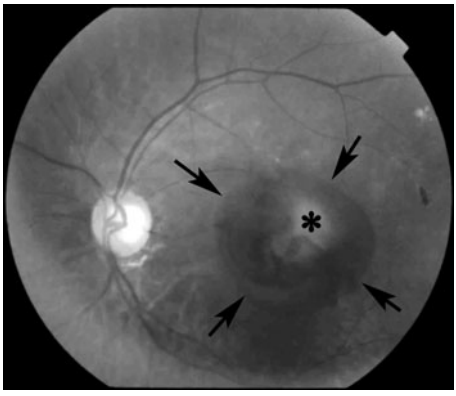


Fig. 4 Exudative form of age-related macular degeneration. Choroidal neovascularization (*asterisk*) invaded into the subretinal space with fibrin and hemorrhage (*arrows*). Even with the newest treatments (anti-VEGF therapies), the improvement of already-decreased vision is limited.

Despite a variety of treatments, recovery of vision in eyes with CNV is still highly limited. In addition, the recurrence of CNV is a serious problem. Macular translocation with 360° retinotomy often dramatically improves visual acuity (Eckardt et al. 1999; Pertile and Glaes 2002). However, severe complications such as postoperative PVR and diplopia have yet to be minimized.

Recently, many clinicians, investigators, and pharmaceutical companies have been very interested in the development of pharmacologic therapies for AMD, because photodynamic therapy with a photosensitizer, topical steroid therapy, and more recently the use of anti-VEGF agents appeared relatively effective exudative AMD (Blumenkranz et al. 2002; Rosenfeld et al. 2006; Brown et al. 2006; Augustin and Schmidt-Erfurth 2006; Adamis and Shima 2005). A number of clinical trials are now completing, ongoing, or prepared. Recent successful cases have been performed by intravitreal or sub-Tenon's injections because of the past history of the failures of pharmacologic approaches by systemic use of interferon alpha and thalidomide in the treatment of exudative AMD (Ip and Gorin 1996; Maguire et al. 2001; Pharmacologic Therapy for Macular Degeneration Study Group 1997). However, more recent new agents have been or are planned to be tested, not only by intravitreal or sub-Tenon's injection but also by systemic administration or even by eye drops. Thus, recent major trends in the development of new compounds tend to make many subsequent innovators blind to the impaired access of pharmacologic agents to the posterior segment of the eye as a critical issue. The development of an intraocular controlled-release system or drug targeting is desired as eagerly as the development of a new potent anti-angiogenic agent.

2.6 Proliferative Vitreoretinopathy (PVR)

PVR, the major cause of failure of retinal detachment surgery, involves intraocular pathologic wound healing: the formation of fibrous membranes composed of RPE cells, glial cells, macrophages, and fibroblasts on or beneath the retina (Jerdan et al. 1989; Machemer and Laqua 1975; Retinal Society Terminology Committee 1983). RPE cells that scatter into the vitreous cavity or subretinal space after retinal detachment produce extracellular matrix proteins, proinflammatory cytokines, and chemokines. This results in breakdown of the blood–retinal barrier, recruitment of other inflammatory cells, and myofibroblastic transdifferentiation of RPE cells (Scheiffarth et al. 1988). Contractile forces generated within the fibrous tissue formed ultimately lead to tractional retinal detachment and/or macular pucker, which threaten vision. RPE cells and fibroblasts are usually the predominant cell types in the epiretinal membranes of PVR. A variety of cytokines may play a role in the pathogenesis of PVR, including transforming growth factor-beta (Gonzalez-Avila et al. 1995), platelet-derived growth factor (Milenkovic et al. 2003), basic fibroblast growth factor (Hueber et al. 1996–1997; La Heij et al. 2002), tumor necrosis factor-alpha (Armstrong et al. 1998), epidermal growth factor (Milenkovic et al. 2003), interleukin-1beta (El-Ghrably et al. 2001), interleukin 6

(El-Ghrably et al. 2001; La Heij et al. 2002), interleukin 8 (El-Ghrably et al. 2001), interferon gamma (El-Ghrably et al. 2001), hepatocyte growth factor (Hinton et al. 2002), connective tissue growth factor (Hinton et al. 2002), and VEGF (Armstrong et al. 1998). Thus many substances can be candidates in the treatment of PVR. On the other hand, a single treatment targeting one of these substances may be insufficient to treat PVR. Because surgical approaches are necessary in many cases to mechanically reduce the retinal traction, some devices for intraocular controlled release of drugs can be applied intraoperatively as an adjunct to decrease the incidence of PVR.

3 Nonbiodegradable Devices

The development of intraocular controlled release systems has been enthusiastically investigated since the late 1980s because of the urgent necessity to establish a new treatment modality alternative to repeated intravitreal injections of ganciclovir in the treatment of CMV retinitis in patients with AIDS. These efforts led to the first commercial product, Vitrasert®, which was a non-biodegradable implant containing ganciclovir (Fig. 2) (Sanborn et al. 1992). This reservoir-type implant is made up from non-biodegradable polymers such as poly(vinyl alcohol) (PVA), EVA, and silicon laminate, and contains an API in the inner space. This type exhibits the most stable and long-standing release profile of an API, as compared with other types of implants, because it can store a large amount of an API and regulate drug release merely by total surface area and thickness of PVA, a permeable polymer (Fig. 5a) (Okabe et al. 2003; Yasukawa et al 2004, 2006). EVA or silicon laminate, a nonpermeable polymer, is used to limit the practical surface area for drug permeability, while PVA is used to construct the framework of the device and regulate the rate of drug permeability. On the other hand, the disadvantages of this type involve the relatively large size of the device requiring a large incision for implantation, which may increase the risk of vitreous hemorrhage, subsequent epiretinal membrane and retinal detachment, and the potential need for removal surgery to exchange the implant or treat possible complications such as retinal detachment and API-induced adverse effects. In fact, the same type of implant, Retisert®, which releases fluocinolone acetonide for the treatment of chronic non-infectious uveitis, has a critically high incidence of steroid-induced cataract and glaucoma. The investigators must consider not only sustained effects but also possible adverse effects of APIs and nonbiodegradable devices in designing controlled release systems.

3.1 Vitrasert®: Nonbiodegradable Implant with Ganciclovir

Vitrasert® (Bausch & Lomb, Rochester, NY) is a reservoir-type implant that delivers ganciclovir, an antiviral drug, intraocularly in patients with AIDS-related CMV retinitis, approved in 1996 by FDA. Ganciclovir is a synthetic nucleoside

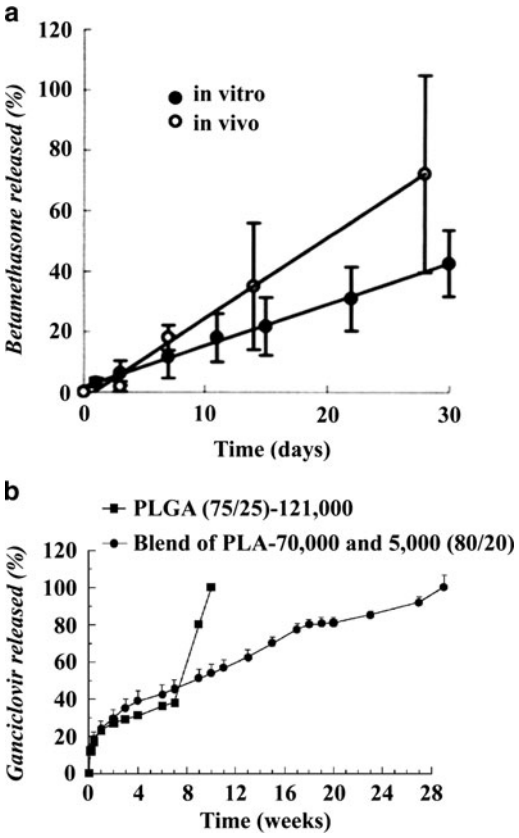


Fig. 5 The release profiles of APIs from a non-biodegradable implant (**a**) and a biodegradable implant (**b**). A non-biodegradable implant is advantageous for stable and sustained release, while a biodegradable one requires no removal or exchange surgery. While a biodegradable implant (*closed squares* in **b**) generally exhibits a triphasic release pattern of APIs, a blend-type biodegradable implant (*closed circles* in **b**) can improve stability and period of API release. Reproduced from Okabe et al. (2003) and Yasukawa et al. (2000)

analog of 2′-deoxyguanosine that inhibits replication of viruses including CMV, herpes simplex virus-1 and -2, Epstein–Barr virus, and varicella zoster virus. This intravitreal implant contains a ganciclovir tablet composed of 4.5 mg of ganciclovir and 0.25% of magnesium stearate as an inactive ingredient, coated with PVA and EVA. PVA and EVA are non-degradable polymers used for controlling the rate of drug release. Hydrophobic EVA was used to limit the surface area available for release of the hydrophilic drug. Ganciclovir is slowly released over a 5–8 month period of time across the water-permeable PVA membrane following the entrance of fluid into the device. Ganciclovir is partially dissolved with imbibed water up to the saturated concentration and continuously diffuses across the PVA membrane. The reservoir implant yields zero-order release kinetics, while the inner solution remains saturated with ganciclovir. Vitrasert® requires surgical implantation. A 5.5 mm sclerotomy is made circumferentially 4 mm posterior to the limbus. After trimming prolapsed vitreous humor, the ganciclovir implant is placed into the vitreous cavity through the sclerotomy and fixed with anchoring sutures. Then the sclerotomy and the conjunctiva are sutured. Caution is needed in handling the device so as not to damage the PVA membrane. After the implantation, most

patients will experience an immediate decrease in visual acuity for the following 2–4 weeks. This temporal visual impairment may result from the surgical procedure itself. The implanted device will require the removal procedure if re-implantation is necessary or if any complications such as retinal detachment occur.

3.2 *Retisert®: Nonbiodegradable Implant with Fluocinolone Acetonide*

Retisert® (Bausch & Lomb, Rochester, NY) is a reservoir-type implant with the same shape as Vitrasert®, designed for the treatment of chronic non-infectious uveitis (Jaffe et al. 2000) (Fig. 6a). The implant consists of a tablet containing 0.59 mg of the active ingredient, fluocinolone acetonide, and inactive components such as microcrystalline cellulose, PVA, and magnesium stearate. The implant is coated with PVA and silicon laminate to yield sustained release of a corticosteroid, fluocinolone acetonide, at a steady rate of 0.3–0.6 μg/day to the posterior segment of the eye for a period of 30 months. The device is 5 mm long, 2 mm wide, and 1.5 mm thick, smaller than Vitrasert®. Fluocinolone acetonide reduces inflammation and lowers intravitreal VEGF levels in the eye. The recurrence rate of uveitis is reduced to 7–14% in the 34-week period post-implantation, in contrast to 40–54% in the control group. The implant also decreases the necessity to use systemic and topical administrations of steroid. The implantation procedure is same as that for Vitrasert®. Caution should be exercised in handling the implant in order to avoid damage to the PVA membrane, which may result in an unexpectedly increased rate of drug release from the implant. Intraocular sustained release of steroid, however, ironically results in a marked rate of steroid-induced intraocular complications. Within 34 weeks post-implantation, approximately 60% of patients will need medications to treat steroid-induced glaucoma. Moreover, within two years after implantation, approximately 32% of patients will require glaucoma (filtering) surgery (Callanan 2007). Also within a post-implantation period of two years, most phakic eyes will develop cataract formation and require cataract surgery. Nevertheless,

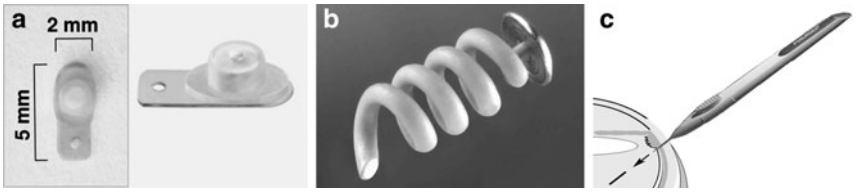


Fig. 6 A variety of medical devices for DDSs to the posterior segment of the eye. (**a**) Retisert® (Bausch & Lomb) contains a tablet with fluocinolone acetonide encapsulated with nonbiodegradable polymers. (**b**) I-vation™ (SurModics) is a helical nonbiodegradable implant with triamcinolone acetonide, implantable through a small incision. (**c**) Posurdex® (Allergan) is an injectable rod with dexamethasone placed with a special applicator

because long-standing intraocular inflammation itself can be associated with incidence of secondary glaucoma and cataract, the sustained and excellent efficacy of the implant to treat chronic uveitis is highly appreciable.

3.3 *I-vation™: Nonbiodegradable Implant with Triamcinolone Acetonide*

A unique nonbiodegradable implant, I-vation™ (SurModics, Irvine, CA), is currently in a phase I clinical trial for the treatment of diabetic macular edema. This device contains and releases 0.925 mg of a corticosteroid, triamcinolone acetonide, slowly to the posterior segment of the eye. The shape of the device is a unique scaffold designed for minimally invasive implantation, different from those of Vitrasert® and Retisert® which require more invasive surgery for implantation (Fig. 6b). The implant's small diameter enables implantation through a sclerotomy with a 25-gauge needle. The unique helical design maximizes the surface area available for drug release as well as enabling anchoring of the implant against the sclera. The thin cap attached is placed under the subconjunctival space. The triamcinolone acetonide is coated with a blend of polybutyl methacrylate and polyEVA. The safety and biocompatibility of I-vation™ have been derived from preclinical studies for up to 9 months post-implantation. SurModics is sponsoring a Phase I safety study called STRIDE (Sustained Triamcinolone Release for Inhibition of Diabetic Macular Edema) for the treatment of diabetic macular edema. Early clinical results show I-vation™ to be safe and well tolerated. Average macular thickness in eyes with diabetic macular edema decreased to 230 μm at month 6, as compared with preoperative thickness of 376 μm. The study subjects will be followed for three years. The release profile of drugs can be customized by varying ratios, thickness, and total surface area of constituent polymers in the coating. It remains to be seen whether the sclera tolerates anchoring of the device for long periods and after removal of the device.

3.4 *Medidur®: Nonbiodegradable Insert with Fluocinolone Acetonide*

Medidur® (Alimera Sciences Inc., Alpharetta, GA; pSivida Inc., Watertown, MA) is an injectable nonbiodegradable intravitreal implant for the treatment of diabetic macular edema. The Medidur® insert is only 3.5 mm in length and 0.37 mm in diameter, designed to release a constant amount fluocinolone acetonide to the posterior segment of the eye over a period between 18 and 36 months. The insert is a reservoir-type implant like other nonbiodegradable implants. In contrast to them, however, it is designed for sutureless insertion in an office setting by use of

25-gauge transconjunctival injector system without the necessity for surgical incisions in the conjunctiva and the sclera and subsequent suturing. The insert releases 0.2 μg or 0.5 μg of fluocinolone acetonide to the vitreous cavity per day. A phase III trial is under way for the treatment of diabetic macular edema. It should be ascertained whether the insert that is suspended without any fixation in the vitreous cavity will have any adverse effects and, if necessary, can easily be surgically removed.

4 Biodegradable Devices

Compared with non-biodegradable implants, biodegradable implants have the following merits: no need of removal surgery, and flexibility in shape. They can be processed into a variety of configurations such as microparticles, rods, discs, tablets, and implants (Fig. 2) (Yasukawa et al. 2004, 2006, 2007). Recently, Posurdex®, a rod that is injectable using a special injector with a 22-gauge needle, is under phase III clinical trial in the treatment of macular edema secondary to retinal vein occlusion or diabetic macular edema. This implant is composed of the homogenous mixture of dexamethasone as an API and poly(lactic-*co*-glycolic acid) (PLGA), a biodegradable polymer, categorized as the monolithic type. In general, this type has three phases of release of an API: (1) the first burst derived from APIs deposited on the surface of the implant; (2) the diffusion phase driven by osmotic pressure and polymer biodegradation; and (3) the final burst originating from sudden disintegration of matrix of the implant (Fig. 5b) (Yasukawa et al. 2004). Therefore, if the API possesses toxic effects at high concentrations, investigators should take the first and the final bursts into consideration. In contrast to reservoir-type implants, API release profiles for monolithic-type devices should be affected by a variety of factors involving types and molecular weight of polymers, mixing rate of polymers and APIs, and total surface area of the device. Yasukawa and Kunou et al. demonstrated that a blend of two kinds of polymers with different molecular weights resulted in reduction of the final burst and more stable and long-standing release of APIs (Fig. 5b) (Yasukawa et al. 2000; Kunou et al. 2000). Thus the release profile of APIs from biodegradable implants may become as stable as non-biodegradable ones, while the duration of API release may be shorter due to the limited amount of APIs contained.

4.1 Posurdex®: Biodegradable Insert with Dexamethasone

Posurdex® (Allergan Inc., Irvine, CA) is a biodegradable polymer matrix prepared with PLGA that releases dexamethasone over approximately five weeks and phase III clinical trials have been evaluating its usefulness in persistent macular edema associated with diabetic retinopathy, retinal vein occlusions, uveitis, and post-cataract surgery. The prototype of this insert was implanted through a 20-gauge scleral incision into the vitreous cavity in the operation area. A phase II clinical trial

showed that patients who had been implanted with the insert containing 0.7 mg of dexamethasone had the greatest improvement in vision, and most of these patients exhibited a three-line increase in an eye chart to measure visual acuity. Adverse effects such as elevated intraocular pressure or cataract formation were not observed in the treated group. Thereafter, a novel insertion system employing a disposable applicator with a 22-gauge needle was developed for Posurdex® (Fig. 6c). The insert is injected in the office setting using this applicator. The clinical trials are currently in phase III to investigate efficacy and safety in larger numbers of patients with diabetic macular edema.

Surodex® (Oculex Pharmaceuticals, Sunnyvale, CA) is a PLGA pellet with 0.06 mg of dexamethasone to provide sustained release of dexamethasone over 7–10 days after insertion into the anterior chamber. Surodex® achieves higher intraocular drug levels than with conventional dexamethasone eye drops, effectively reducing post-cataract surgery inflammation (Tan et al. 1999). This insert is approved for use in cataract surgery in Singapore.

4.2 Injectable Microspheres

While it is advantageous that microspheres are injectable, in the 1990s intravitreally injected microspheres had been assumed to impair the transparency of intraocular media. Thereafter, intravitreal injection of crystalline triamcinolone acetonide began to be carried out in the treatment of macular edema and exudative AMD (Ip et al. 2004; Augustin and Schmidt-Erfurth 2006; Gillies et al. 2006). Triamcinolone acetonide is hydrophobic and mostly suspended in the vehicle. Nevertheless, crystalline triamcinolone acetonide mostly sinks down into the inferior part of the vitreous cavity, where it does not impair the ocular media and visual functions. This successful use of drug suspensions will provide great opportunities for intraocular DDSs using microparticles. The ongoing clinical trial of Posurdex® clearly indicates that biodegradable polymers are biocompatible. In the near future, many types of biodegradable implants and microparticles will proceed to clinical trial. In Japan, sub-Tenon's injection of microspheres with betamethasone (DE-102, Santen Pharmaceuticals, Ikoma, Japan) is currently in phase II/III clinical trial for the treatment of diabetic macular edema. Also, microspheres with pegaptanib, an aptamer with affinity to VEGF (Macugen; Pfizer Inc., New York, NY), have been investigated in the laboratory. These microspheres, when injected intravitreally in rabbits, slowly release pegaptanib into the vitreous cavity for four months.

5 Triamcinolone Acetonide Crystal Suspensions

Crystal suspensions of triamcinolone acetonide have made great impact in the treatment of vitreoretinal diseases; intravitreal and sub-Tenon's administrations of triamcinolone acetonide have been widely used in the treatment of macular

edema, exudative AMD, and uveitis (Ip et al. 2004; Gillies et al. 2006; Augustin and Schmidt-Erfurth 2006) (Fig. 3c). Because of its hydrophobicity, injected crystals gradually dissolve, resulting in sustained release of triamcinolone acetonide. Intravitreal injection of 4 mg or sub-Tenon's injection of 20 mg of triamcinolone acetonide provides 3-month efficacy in the chorioretinal tissue. While intravitreal injection is more effective than sub-Tenon's injection, it has more frequent risk of steroid-induced glaucoma and cataract progression. Nevertheless, both complications are treatable in most cases. Thus the crystal suspensions are considered in a sense as DDSs without any polymer matrix as a base. Future candidates for devices to release steroids may need to have a release profile superior to that of intravitreally injected crystalline triamcinolone acetonide.

6 NT-501: Encapsulated Cell Technology (ECT)

ECT is a novel technology developed by Neurotech Pharmaceuticals, Inc. (Lincoln, RI, USA) to allow sustained delivery of cell-derived factors to the posterior segment of the eye. ECT implants consist of cells, which have been genetically modified to secrete therapeutic factors, and envelopes of semi-permeable hollow fiber membrane (Fig. 2). The permeability of the hollow fiber membrane enables long-term cell survival by allowing influx of oxygen and nutrients and preventing direct contact of encapsulated cells with cellular and molecular elements of the immune system. Encapsulated cells continuously produce the therapeutic protein, which diffuses out of the implant at the target site. Protein delivery in the vitreous cavity for as long as 18 months has been achieved by an ECT device, NT-501, containing human cells genetically engineered to secrete ciliary neurotrophic factor. NT-501 is fixed with suturing at the pars plana as for Vitrasert® and Retisert®. A phase I clinical trial was completed in early 2006 in ten patients diagnosed with retinitis pigmentosa at the National Eye Institute in the USA. ECT-mediated delivery of ciliary neurotrophic factor was found to be safe over a six-month treatment period. In addition, unexpectedly, visual acuities tended to improve a variable extend from baseline. NT-501 is currently in a phase II/III clinical trial for the treatment of retinitis pigmentosa and in a phase II clinical trial for the treatment of dry AMD. ECT devices can potentially release antibodies and cytokines, too.

7 Conclusion

In the treatment of exudative AMD, repeated intraocular injections of anti-VEGF agents recently exhibited remarkable inhibitory effects in contrast to other existing treatment modalities such as photodynamic therapy, laser photocoagulation, and surgery. A great number of drug candidates are now in clinical or preclinical trials.

However, some trials are by systemic use or even by eye drops, not taking specificity in ocular pharmacokinetics into consideration. Some scientists have forgotten or did not recognize the previous unsuccessful history of interferon and thalidomide. Other therapies by a single compound may be less effective than anti-VEGF therapies, because VEGF must be a key regulator in many steps of inflammation and angiogenesis and thus its blockage can inhibit inflammatory reaction and angiogenesis directly and rapidly. Controlled release systems may be necessary to further improve efficacy and to reduce the incidence of adverse effects as compared with repeated intravitreal injections of anti-VEGF agents. In the 1980s, AIDS-associated cytomegalovirus retinitis accelerated advances in intraocular drug delivery systems, having realized the clinical use of non-biodegradable implants. Today, a variety of biodegradable implants and microparticles are also available for clinical use. Now, most challenging vitreoretinal diseases are targeted.

References

Adamis AP, Shima DT (2005) The role of vascular endothelial growth factor in ocular health and disease. Retina 25:111–118

Ambati J, Adamis AP (2002) Transscleral drug delivery to the retina and choroid. Prog Retin Eye Res 21:145–151

American Academy of Ophthalmology (2000) Photodynamic therapy with verteporfin for age-related macular degeneration. Ophthalmology 107:2314–2317

Armstrong D, Augustin AJ, Spengler R, Al-Jada A, Nickola T, Grus F, Koch F (1998) Detection of vascular endothelial growth factor and tumor necrosis factor alpha in epiretinal membranes of proliferative diabetic retinopathy, proliferative vitreoretinopathy and macular pucker. Ophthalmologica 212:410–414

Augustin AJ, Schmidt-Erfurth U (2006) Verteporfin therapy combined with intravitreal triamcinolone in all types of choroidal neovascularization due to age-related macular degeneration. Ophthalmology 113:14–22

Autran B, Carcelain G, Li TS, Blanc C, Mathez D, Tubiana R, Katlama C, Debré P, Leibowitch J (1997) Positive effects of combined antiretroviral therapy on $CD4^+$ T cell homeostasis and function in advanced HIV disease. Science 277:112–116

Bakri SJ, Snyder MR, Reid JM, Pulido JS, Ezzat MK, Singh RJ (2007a) Pharmacokinetics of intravitreal ranibizumab (Lucentis). Ophthalmology 114:2179–2182

Bakri SJ, Snyder MR, Reid JM, Pulido JS, Singh RJ (2007b) Pharmacokinetics of intravitreal bevacizumab (Avastin). Ophthalmology 114:855–859

Blumenkranz MS, Bressler NM, Bressler SB, Donati G, Fish GE, Haynes LA, Lewis H, Miller JW, Monés JM, Potter MJ, Pournaras C, Reaves A, Rosenfeld PJ, Schachat AP, Schmidt-Erfurth U, Sickenburg M, Singerman LJ, Slakter JS, Strong A, Vannier S; Treatment of Age-Related Macular Degeneration with Photodynamic Therapy (TAP) Study Group (2002) Verteporfin therapy for subfoveal choroidal neovascularization in age-related macular degeneration: 3-year results of an open-label extension of 2 randomized clinical trials–TAP Report no. 5. Arch Ophthalmol 120:1307–1314

Brown DM, Kaiser PK, Michels M, Soubrane G, Heier JS, Kim RY, Sy JP, Schneider S; ANCHOR Study Group (2006) Ranibizumab versus verteporfin for neovascular age-related macular degeneration. N Engl J Med 355:1432–1444

Callanan DG (2007) Novel intravitreal fluocinolone acetonide implant in the treatment of chronic noninfectious posterior uveitis. Expert Rev Ophthalmol 2:33–44

Cantrill WH, Henry K, Melroe NH, Knobloch WH, Ramsay RC, Balfour HH (1989) Treatment of cytomegalovirus retinitis with intravitreal ganciclovir: long-term results. Ophthalmology 96:367–374

Chakravarthy U, Houston RF, Archer DB (1993) Treatment of age-related subfoveal neovascular membranes by teletherapy. Br J Ophthalmol 77:265–273

Cochereau-Massin I, Lehoang P, Lautier-Frau M, Zazoun L, Marcel P, Robinet M, Matheron S, Katlama C, Gharakhanian S, Rozenbaum W, et al. (1991) Efficacy and tolerance of intravitreal ganciclovir in cytomegalovirus retinitis in acquired immune deficiency syndrome. Ophthalmology 98:1348–1355

Eckardt C, Eckardt U, Conrad HG (1999) Macular rotation with and without counter-rotation of the globe in patients with age-related macular degeneration. Graefes Arch Clin Exp Ophthalmol 237:313–325

El-Ghrably IA, Dua HS, Orr GM, Fischer D, Tighe PJ (2001) Intravitreal invading cells contribute to vitreal cytokine milieu in proliferative vitreoretinopathy. Br J Ophthalmol 85:461–470

Geroski DH, Edelhauser HF (2001) Transscleral drug delivery for posterior segment disease. Adv Drug Deliv Rev 52:37–48

Gillies MC, Sutter FKP, Simpson JM, Larsson J, Ali H, Zhu M (2006) Intravitreal triamcinolone for refractory diabetic macular edema. 2-year results of a double-masked, placebo-controlled, randomized clinical trial. Ophthalmology 113:1533–1538

Gonzalez-Avila G, Lozano D, Manjarrez ME, Ruiz VM, Terán L, Vadillo-Ortega F, Selman M (1995) Influence on collagen metabolism of vitreous from eyes with proliferative vitreoretinopathy. Ophthalmology 102:1400–1405

Gross JB, Bozzete SA, Mathews WC, Spector SA, Abramson IS, McCutchan JA, Mendez T, Munguia D, Freeman WR (1990) Longitudinal study of cytomegalovirus retinitis in acquired immune deficiency syndrome. Ophthalmology 97:681–686

Heinemann MH (1989) Long-term intravitreal ganciclovir therapy for cytomegalovirus retinopathy. Arch Ophthalmol 107:1767–1772

Henderly DE, Freeman WR, Causey DM, Rao NA (1987) Cytomegalovirus retinitis and response to therapy with ganciclovir. Ophthalmology 94:425–434

Hinton DR, He S, Jin ML, Barron E, Ryan SJ (2002) Novel growth factors involved in the pathogenesis of proliferative vitreoretinopathy. Eye 16:422–428

Holland GN, Sakamoto MJ, Hardy D, Sidikaro Y, Kreiger AE, Frenkel LM (1986) Treatment of cytomegalovirus retinopathy in patients with acquired immunodeficiency syndrome. Use of the experimental drug 9-[2-hydroxyl-1-(hydroxy-methyl)ethoxymethyl] guanine. Arch Ophthalmol 104:1794–1800

Hueber A, Wiedemann P, Esser P, Heimann K (1996–1997) Basic fibroblast growth factor mRNA, bFGF peptide and FGF receptor in epiretinal membranes of intraocular proliferative disorders (PVR and PDR). Int Ophthalmol 20:345–350

Ip M, Gorin MB (1996) Recurrence of a choroidal neovascular membrane in a patient with punctate inner choroidopathy treated with daily doses of thalidomide. Am J Ophthalmol 122:594–595

Ip MS, Gottlieb JL, Kahana A, Scott IU, Altaweel MM, Blodi BA, Gangnon RE, Puliafito CA (2004) Intravitreal triamcinolone for the treatment of macular edema associated with central retinal vein occlusion. Arch Ophthalmol 122:1131–1136

Jabs DA, Newman C, De Bustros S, Polk BF (1987) Treatment of cytomegalovirus retinitis with ganciclovir. Ophthalmology 94:824–830

Jaffe GJ, Ben-nun J, Guo H, Dunn JP, Ashton P (2000) Fluocinolone acetonide sustained drug delivery device to treat severe uveitis. Ophthalmology 107:2024–2033

Jerdan JA, Pepose JS, Michels RG, Hayashi H, de Bustros S, Sebag M, Glaser BM (1989) Proliferative vitreoretinopathy membranes. An immunohistochemical study. Ophthalmology 96:801–810

Kaplan H (1996) Submacular surgery for choroidal neovascularization. Br J Ophthalmol 80:101

Kim SH, Galban CJ, Lutz RJ, Dedrick RL, Csaky KG, Lizak MJ, Wang NS, Tansey G, Robinson MR (2007) Assessment of subconjunctival and intrascleral drug delivery to the posterior segment using dynamic contrast-enhanced magnetic resonance imaging. Invest Ophthalmol Vis Sci 48:808–814

Klein R, Klein BEK, Linton KLP (1992) Prevalence of age-related maculopathy: the beaver dam eye study. Ophthalmology 99:933–943

Kunou N, Ogura Y, Yasukawa T, Kimura H, Miyamoto H, Honda Y, Ikada Y (2000) Long-term sustained release of ganciclovir from biodegradable scleral implant for the treatment of cytomegalovirus retinitis. J Control Release 68:263–271

La Heij EC, van de Waarenburg MP, Blaauwgeers HG, Kessels AG, Liem AT, Theunissen C, Steinbusch H, Hendrikse F (2002) Basic fibroblast growth factor, glutamine synthetase, and interleukin-6 in vitreous fluid from eyes with retinal detachment complicated by proliferative vitreoretinopathy. Am J Ophthalmol 134:367–375

Lang LC (1995) Ocular drug delivery: conventional ocular formulations. Adv Drug Deliv Rev 16:39–43

Machemer R, Laqua H (1975) Pigment epithelium proliferation in retinal detachment (massive periretinal proliferation). Am J Ophthalmol 80:1–23

Macular Photocoagulation Study Group (1986) Recurrent choroidal neovascularization after argon laser photocoagulation for neovascular maculopathy. Arch Ophthalmol 104:503–512

Macular Photocoagulation Study Group (1990) Persistent and recurrent neovascularization after krypton laser photocoagulation for neovascular lesions of age-related macular degeneration. Arch Ophthalmol 108:825–831

Macular Photocoagulation Study Group (1994a) Visual outcome after laser photocoagulation for subfoveal choroidal neovascularization secondary to age-related macular degeneration: the influence of initial lesion size and initial visual acuity. Arch Ophthalmol 112:480–488

Macular Photocoagulation Study Group (1994b) Laser photocoagulation for juxtafoveal choroidal neovascularization: 5-year results from randomized clinical trials. Arch Ophthalmol 112: 500–509

Maguire MG, Fine SL, Maguire AM, D'Amato RJ, Singerman LJ (2001) Results of the age-related macular degeneration and thalidomide study (AMDATS). Invest Ophthalmol Vis Sci 42:S233

Maguire AM, Simonelli F, Pierce EA, Pugh EN Jr, Mingozzi F, Bennicelli J, Banfi S, Marshall KA, Testa F, Surace EM, Rossi S, Lyubarsky A, Arruda VR, Konkle B, Stone E, Sun J, Jacobs J, Dell'Osso L, Hertle R, Ma JX, Redmond TM, Zhu X, Hauck B, Zelenaia O, Shindler KS, Maguire MG, Wright JF, Volpe NJ, McDonnell JW, Auricchio A, High KA, Bennett J (2008) Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med 358:2240–2248

Maurice DM, Mishima S (1984) Ocular pharmacokinetics. In: Sears ML (ed) Pharmacology of the eye. Springer, New York

Milenkovic I, Weick M, Wiedemann P, Reichenbach A, Bringmann A (2003) P2Y receptor-mediated stimulation of Muller glial cell DNA synthesis: dependence on EGF and PDGF receptor transactivation. Invest Ophthalmol Vis Sci 44:1211–1220

Mitchell SM, Membrey WL, Youle MS, Obi A, Worrell S, Gazzard BG (1999) Cytomegalovirus retinitis after the initiation of highly active antiretroviral therapy: a 2 year prospective study. Br J Ophthalmol 83:652–655

Morley MG, Duker JS, Ashton P, Robinson MR (1995) Replacing ganciclovir implants. Ophthalmology 102:388–392

Okabe K, Kimura H, Okabe J, Kato A, Kunou N, Ogura Y (2003) Intraocular tissue distribution of betamethasone after intrascleral administration using a nonbiodegradable sustained drug delivery device. Invest Ophthalmol Vis Sci 44:2702–2707

Pertile G, Glaes C (2002) Macular translocation with 360-degree retinotomy for management of age-related macular degeneration with subfoveal choroidal neovascularization. Am J Ophthalmol 134:560–565

Pharmacological Therapy for Macular Degeneration Study Group (1997) Interferon alfa-2a is ineffective for patients with choroidal neovascularization secondary to age-related macular degeneration. Results of a prospective randomized placebo-controlled clinical trial. Arch Ophthalmol 115:865–872

Retinal Society Terminology Committee (1983) The classification of retinal detachment with proliferative vitreoretinopathy. Ophthalmology 90:121–125

Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY, Kim RY; MARINA Study Group (2006) Ranibizumab for neovascular age-related macular degeneration. N Engl J Med 355:1419–1431

Ryan SJ, Stout JT, Dugel PU (1994) Subretinal neovascularization. In: Ryan SJ (ed) Retina. Mosby, St. Louis

Sanborn GE, Anald R, Torti RE, Nightingale SD, Cal SX, Yates B, Ashton P, Smith T (1992) Sustained-release ganciclovir therapy for treatment of cytomegalovirus retinitis. Use of an intravitreal device. Arch Ophthalmol 110:188–195

Scheiffarth OF, Kampik A, Gunther H, von der Mark K (1988) Proteins of the extracellular matrix in vitreoretinal membranes. Graefes Arch Clin Exp Ophthalmol 226:357–361

Smith TJ, Pearson PA, Blandford DL, Brown JD, Goins KA, Hollins JL, Schmeisser ET, Glavinos P, Baldwin LB, Ashton P (1992) Intravitreal sustained-release ganciclovir. Arch Ophthalmol 110:255–258

Tan DTH, Chee SP, Lim L, Lim ASM (1999) Randomized clinical trial of a new dexamethasone delivery system (Surodex) for treatment of post-cataract surgery inflammation. Ophthalmology 106:223–231

Ussery FM, Gibson SR, Conklin RH, Piot DF, Stool EW, Conklin AJ (1988) Intravitreal ganciclovir in the treatment of AIDS-associated cytomegalovirus retinitis. Ophthalmology 95:640–648

Vrabec TR, Baldassano VF, Whitcup SM (1998) Discontinuation of maintenance therapy in patients with quiescent cytomegalovirus retinitis and elevated $CD4^+$ counts. Ophthalmology 105:1259–1264

Yasukawa T, Kimura H, Kunou N, Miyamoto H, Honda Y, Ogura Y, Ikada Y (2000) Biodegradable scleral implant for intravitreal controlled release of ganciclovir. Graefes Arch Clin Exp Ophthalmol 238:186–190

Yasukawa T, Ogura Y, Kimura H, Sakurai E, Tabata Y (2006) Drug delivery from ocular implants. Expert Opin Drug Deliv 3:261–273

Yasukawa T, Ogura Y, Tabata Y, Kimura H, Wiedemann P, Honda Y (2004) Drug delivery systems for vitreoretinal diseases. Prog Retin Eye Res 23:253–281

Yasukawa T, Tabata Y, Kimura H, Kunou N, Ogura Y (2007) Development of drug-delivery systems to the posterior segments of the eye. Expert Rev Ophthalmol 2:197–211

Retraction Note

Biosensing and Drug Delivery at the Microscale

Novel Devices for Controlled and Responsive Drug Delivery

Andrea A. Robitzki and Randy Kurz

M. Schäfer-Korting (ed.), *Drug Delivery*,
Handbook of Experimental Pharmacology 197,
DOI 10.1007/978-3-642-00477-3_3,

The book chapter "Biosensing and Drug Delivery at the Microscale: Novel Devices for a Controlled and Responsive Drug Delivery" published in the *Handbook of Experimental Pharmacology* vol. 197: *Drug Delivery* has been retracted by the authors because its current version is based on a preliminary manuscript with incorrect quotations, references, and partly identical wording wiht other original manuscripts.
The authors declare that they have done everything to correct this error to prove their intention to satisfy all parties concerned.

The authors deeply regret the errors.

A.A. Robitzki (✉)
University of Leipzig, Center for Biotechnology and Biomedicine, Deutscher Platz 5, 04103 Leipzig, Germany
e-mail: andrea.robitzki@bbz.uni-leipzig.de