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Nanomedicine

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Nanomedicine

Principles of Nanomedicine

Volume 1

Springer

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Preface

This book is comprised of two complementary volumes: *Principles of Nanomedicine* (Volume 1) and *Perspectives of Nanomedicine* (Volume 2). The purpose of this arrangement is to provide a more comprehensive overview of a new and potentially revolutionary branch of healthcare – nanomedicine.

The European Technology Platform on Nanomedicine has defined nanomedicine as:

> the application of nanotechnology to achieve breakthroughs in healthcare. It exploits the improved and often novel physical, chemical and biological properties of materials at the nanometre scale. Nanomedicine has the potential to enable early detection and prevention, and to essentially improve diagnosis, treatment and follow-up of diseases.

Nanomedicine is one of the fastest-growing sub-disciplines of nanotechnology, which itself is described by the US National Nanotechnology Initiative as "science, engineering, and technology conducted at the nanoscale…". One nanometre (1 nm) is one billionth of a metre (10^{-9} m) and the nanoscale is usually referred to as being the range 1 to 100 nm. A further important consideration is that the term nanotechnology is often used for materials or surfaces that have been intentionally altered or manipulated at or around the nanoscale so as to provide useful new properties that come into play at this scale.

It is the ability to consistently measure and manipulate matter at the nanoscale that makes nanotechnology so potentially valuable in medicine. In biology, the basic building blocks of life are themselves nanostructures, and being able to interface nanoscale materials and structures with biological structures at this level gives rise to new opportunities to understand disease mechanisms and to intervene in them by exploiting the often novel characteristics of nanomaterials and nanoscale architecture.

Nanotechnology has the potential to make an impact on nearly all branches of medicine: from earlier and more accurate diagnosis, to the enhanced imaging of smaller and finer structures, to novel medical devices, to highly targeted and more

effective drugs, to regenerative medicine and to more personalised models of medicine. Furthermore, nanomedicine has the potential to address currently unmet medical needs and growing challenges such as diseases associated with an ageing population and unhealthy lifestyles.

Nanomedicine is highly interdisciplinary in nature and brings together fields such as biology, materials science, engineering and information technology. For example, a typical biosensor used in medicine may comprise a biologically-based sensing component, a physical transducer to generate a measurable signal and an IT component to process the data generated.

The first volume, *Principles of Nanomedicine*, provides an overview of the key principles that underpin the development of nanomedicine and describes a range of applications that are emerging within this rapidly-growing new discipline. It consists of chapters that:

- Define nanomedicine and introduce its main sub-disciplines
- Describe "top-down" and "bottom-up" synthetic approaches
- Examine why nanomedicine can be described as both interdisciplinary and revolutionary
- Describe how life is a complex assembly of interacting nanoscale mechanisms and examine how nanobiology is driving new trends in medicine
- Explain the increasing role of nanotechnology in underpinning the diverse and rapidly-growing fields of tissue engineering and regenerative medicine
- Present examples of widely-used simulation and modelling methods that can contribute to the design of nanomaterials utilised in nanomedicine
- Describe a variety of medical nanomaterials including novel and biologically-inspired materials
- Describe multifunctional nanoparticles that can be used for theranostic and imaging applications
- Provide an overview of how nanotechnology can be used to design novel biosensors that provide powerful new diagnostic tools, for example for neurodegenerative diseases, cardiovascular diseases and cancers
- Examine the contribution nanotechnology can make to both improving medical devices and developing revolutionary, new advanced devices
- Examine the potential that remotely-controlled micro- and nano-robots may have for minimally-invasive procedures within the body and in microfluidic diagnostics
- Discuss the current challenges in pharmaceutical science associated with nanotechnology and how nanotechnology can be used in a variety of ways to address issues such as stability, solubility, efficacy, targeting to desired disease sites and overcoming physiological and anatomical barriers
- Explore issues surrounding toxicity and risk in nanomedicine, describe research on developing new risk assessment strategies, outline regulatory approaches and highlight areas for further risk research
- Examine the key ethical principles that underpin the implementation of nanomedicine and other emerging medical and related technologies

Nanomedicine will only fulfil its potential if it can be brought successfully and safely to the market and to the clinic. The second volume, *Perspectives of Nanomedicine*, therefore builds upon those principles outlined in Volume 1 with a series of perspectives on how the commercialisation and clinical implementation of nanomedicine can be facilitated and the challenges that need to be addressed to accomplish these objectives.

Many expectations have been placed on the medical applications of nanotechnology. Chapter 13 examines these expectations, considers the phenomenal pace of research and development, and seeks to differentiate between the hyperbole accorded to some themes of research and the reality of applying the results of this research within a clinical setting.

Nanomedicine cannot expect to have a significant market and clinical impact unless it can be taken up and developed commercially. Chapter 14 explores the business dimensions of developing nanomedical products, including finance and cost issues, business models and strategies, and potentially disruptive effects on existing business models and product markets.

Nanotechnology continues to develop at an astonishing rate and much of this progress has the potential to make a positive impact on medicine. However concerns, such as the potential toxicity of some nanomaterials, remain. Chapter 15 examines the lessons, both positive and negative, that can be learned from this rapid evolution and from on-going research in nanotechnology in order to drive nanomedicine further in the right direction.

Nanotechnology is one of a number of emerging and enabling technologies that are likely to revolutionise the way healthcare is delivered. Chapter 16 explores this intersection of technologies and discusses some of the changes that are likely.

Nanotechnology holds much promise for overcoming challenges in drug design, development and delivery. A number of nano-based pharmaceuticals have already been approved with a great number more in product development pipelines. Chapter 17 explores the need for future regulatory systems that will be able to keep pace with rapid developments in nanopharmaceuticals and that can deliver effective and safe products to the healthcare systems.

Although widely applicable for the diagnosis and treatment of many diseases, one of the fields in which research in nanomedicine has progressed the furthest is in the diagnosis and treatment of cancer. Chapter 18 describes how operating at the nanoscale allows for an impressive level of diversity in approaches and capabilities that enable a range of nanoparticles to address an equally diverse range of targets and provide an integrated, personalized approach to diagnosis and therapy, especially in cancer disease management.

With a rapidly growing number of nanomedical products in research and development, it is useful to review those steps and processes a potential nanomedical product will need to pass through before it reaches the market or clinic. Chapter 19 reviews these processes and other challenges and provides insights into the data and evidence that developers will need to accumulate to achieve the objectives of market entry and clinical adoption.

In these two volumes of the book, each chapter is accompanied by an extensive list of references that will help direct the reader towards additional sources of information.

The editors would sincerely like to thank Springer Science+Business Media LLC, and especially Dr. Kenneth Howell and Ms. Abira Sengupta, for everything that made the publication of this book possible.

Last but not least, welcome to the magic world of nanomedicine. The next new era of nanomedicine is coming!

Bedfordshire, UK — Yi Ge
Zhenjiang, China — Songjun Li
Wuhan, China — Shenqi Wang
Melton Mowbray, UK — Richard Moore

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Volume 1

Chapter 1
Nanomedicine: Revolutionary Interdiscipline

Ferdia Bates

1.1 Introduction

Nanomedicine can be defined as the application of nanotechnology to medicine. One may be forgiven for thinking that, given the fact that it is defined as a subsection of nanotechnology, its boundaries are finite; this assumption is, however, deceptive. The application of the field to medicine has opened up an entirely new horizon of applications and possibilities. Application of this technology to the physiological system has also provided an opportunity to study these biological systems on an altogether more intimate level which has allowed scientists to replicate and refine the established mechanisms in order to apply them in the form of innovative and highly beneficial new technology. A second assumption arising from this definition, especially given the title of this work, is that the cause for referring to nanomedicine as an interdiscipline stems from it being an amalgamation of medicine with an emerging technological field. This, again, is not the case. Delving into this exciting and emerging discipline can be, at times, similar to plunging into a Carroll-esque rabbit-hole with regard to the capabilities of materials that, in their bulk forms, border on the mundane.

Approaching the research and development of nanomedicine chronologically, the technology as a concept can be traced back to the lecture given by the infamous Richard Feynman at Caltech in 1959 titled "There's plenty of room at the bottom". In this talk, Feynman outlined the potential that the harnessing nanoscopic materials could yield [1]. Using the directions given by Feynman, the logical train of thought would lead the inquisitive researcher directly from macro to nano or, from top down. This method of research and development was, and still is, an extremely fruitful method for synthesis and research of nanotechnology; indeed, the products of

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top-down synthesis of nanotechnology are an integral part of modern life. At this point in time, one would be hard pressed not to find an electronic device with did not contain a large amount of nanoscopic circuitry.

This being said, however, for the true revolution, one must look up rather than down. Bottom up synthesis of nanotechnology may not be immediately intuitive for the layman observer but it has proven to be the most fruitful in terms of the yield of technology. Indeed, the nanomedicine that is used in the creation of new medical techniques is predominantly created using the bottom-up techniques and thus, it provides the most justification for the use of the word 'revolution' [2].

Nanomedicine, to reiterate, is far from a finite field. It encompasses a great scope of technology all of which is at the forefront of research and development. For this paper, the sub headings of nanobiotechnology, nanotechnology and nanobiomimetics are used in the definition of nanotechnology as an interdiscipline [3–6]. What follows in a brief discussion and explanation as to why nanomedicine has captured the attention of researcher, physicians and laymen alike.

1.2 Enhancement of Existing Technology: The Top-Down Revolution

When discussing nanotechnology, particularly if one does not have previous experience in the area, the logical thought process would be to start at the macroscopic, or bulk modulus, and to reduce the dimensions until nanoscopic proportions have been achieved. Indeed, this is how much of the nanotechnology currently on the market is synthesised. Lithography, in various forms is one of the most widely spread forms of this top down method, indeed, the computer or mobile phone that is in the vicinity or on the person of the reader is sure to have numerous circuit boards which were printed utilising a lithographic process. Techniques such as photolithography have been an integral part of the fabrication process of microchips in the computing industry for 50 years [7], with the dimensions of the circuits on the chips getting smaller and smaller in accordance with Moore's law, which predicts that computing circuits should double in power or half in size every 18 months [8]. It may surprise the reader as to the degree to which this form of nanotechnology is incorporated into everyday life. There are, in fact, several types of this technique in practice today, all of which work at the nano level. One might very well question the use of the word 'Revolution' in the context of technology of this age however, the advancement and refinement of this technology must also be acknowledged; at this point, techniques such as photolithography have been refined to low nanoscale proportions [9]. Indeed, it is this refinement that directly facilitates the increase in computing power that this generation has become so accustomed to.

Nanomaterials have also been used to replace their bulk counterparts. In this manner, though nanomaterials are being used, they are merely being exploited as smaller versions of their bulk counterparts rather than as an entirely new material such as will be discussed below (see Sect. 1.5.2). Utilising the example of carbon

nanofibres being used to replace carbon fibre as the providers of tensile strength in composite materials or the addition of nanoparticles as strength enhancement agents, a notable and highly useful increase in material strength though it still only serves the same purpose as it did before in the sense that only its existing properties have been enhanced rather than new ones created [10, 11].

The 'top-down' aspect of nanomedicine is perhaps neglected in the face of the more glamorous 'bottom-up' technologies but it nonetheless plays a crucial role in the development of the field albeit, using nanotechnology's role in increasing computer processing power as an example, a back ground one.

1.3 From the Bottom-Up, Introduction of New Technology and the True Revolution

It is important to make the distinction between this section of nanomedicine and the previous one. While the products yielded from top down synthesis are highly innovative, costs for the synthesis process are high and yields are low. Bottom-up synthesis, on the other hand, relies on the chemical synthesis of nanomaterials through various processes. The product achieved is heterogeneous, pure, and greatly more cost effective than its top-down predecessor.

This synthesis procedure has allowed for production of high quality particles and nanostructures on a scale that has allowed the wide spread development of the field [12, 13]. It is also through this synthesis that the smallest particles can be synthesised, those with the most cutting edge functions such those with the ability to transcend the blood brain barrier (see Sect. 1.5.2), a capability that has one of the farthest reaching implications in terms of transmuting new areas of research into curative techniques and thus halting ailments which until now have existed with meagre attenuation from existing clinical treatments, in particular, neurodegenerative conditions.

'Bottom-up' is somewhat of an umbrella term in the sense that it incorporates a great deal of techniques and can refer to both precipitation of a polymer [14] or equally the agglomeration of gold atoms to form nanoparticles [15]. The binding factor between these synthesis processes is that both particles were begot from a mixture of fluids in which they grew to form a colloidal suspension. As was said above, the key advantage to this technique is the ability to produce monodisperse particles of extremely small size.

1.4 Interdisciplinary Medicine, Why the Fuss?

Collaboration and amalgamation of ideas and applications within the various traditional disciplines of medicine has always occurred. An obvious example of this is the prescription of a single medication for several different ailments in a manner

such as to 'harness' side effects observed when characterising the drug during trials; some well-known examples of these being aspirin [16, 17], duloxetine [18, 19] and Viagra [20, 21]. Likewise, the marriage of disciplines to birth an entirely new subspecialty in response to demand is by no means uncommon; Palliative care, Medical Ethics and Biomedical Engineering to name a few. Nanomedicine, however, must be classed as something entirely different for the predominant reason that it uses such a term as interdiscipline in a far more intensive manner.

1.5 Technologies of Nanomedicine

The term 'nanomedicine' was coined to not only to describe the application of several separate technologies to medicine, but also to encompass the hybridisation of these technologies within the application of medicine. The three main subsections of nanomedicine, as shown in Fig. 1.1, can be given as nanotechnology, nanobiotechnology and nanobiomimetics. These three particular groupings were chosen because of their easily definable boundaries. Indeed these three research streams were all well established as individual fields before they were ever combined under the universal banner of nanomedicine. Thus, the term 'nanomedicine' can be used to describe any one of these sections or, more importantly, any one combination of these three sections. The word of "combination" is the key component in

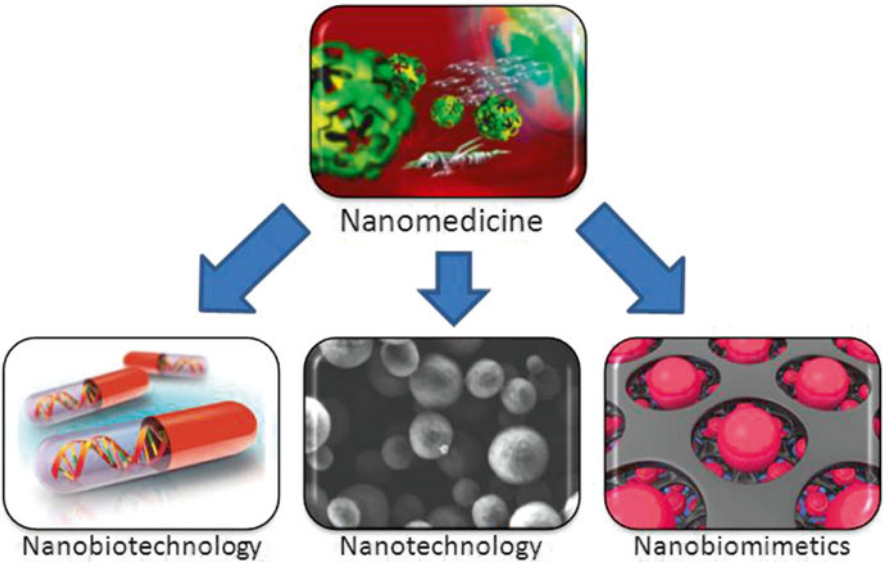

Fig. 1.1 The three main subdivisions of Nanomedicine (Copyright-free images)

the description of nanomedicine particularly when using such implicit adverbs as 'revolutionary'. Indeed, it can be seen from the brief definitions given in Sects. 1.2 and 1.3. The majority of the advantageous treatments and techniques that are described as nanomedicine come from the hybridisation of two separate components. For instance, the delivery of a therapeutic nanoparticle to a site by incorporating a specific biological or non-biological ligand on to its surface; or vice versa, the delivery of a therapeutic compound by attaching it to a magnetic nanoparticle for example.

1.5.1 Nanobiotechnology

To start with nanobiotechnology, it can be defined as the global title given to the intersection of nanotechnology and biology, in other words, it is the exploitation of nanobiological entities for the application to medicine [22]. This can be taken as the oldest form of nanomedicine to be practiced, as demonstrated by the publication dates of the landmark papers cited. One of the first examples for the application of such technologies can be found almost 40 year ago with the discovery of monoclonal antibodies [23]. The degree of integration of this technology into daily modern life may come as a surprise to the layman. Indeed, such common house hold items as the pregnancy test rely on Enzyme-linked Immunosorbent Assay (ELISA) tests which, in turn, rely on enzyme sequences of nanoscopic dimensions; it is to these enzymes that the modern test owes its accuracy [24]. ELISA tests are also used extensively as diagnostic tools within the clinical setting for the determination of disease through the quantification of proteins or antigens with, in the case of the diagnosis of HIV, accuracies up to 98 % [25, 26]. Nanobiotechnology also holds a special prevalence in therapeutics. Techniques such as gene [27] and protein [27, 28] therapy all rely on extracted biological sequences. Intracellular delivery techniques such as viral capitation [29] also owe their existence to nanobiotechnology. Such biological entities also provide the mechanism for many active therapeutic delivery protocols using the phenomenon of antibody-antigen binding by attaching the relevant ligand sequence to the surface of the delivery vessel thus creating a biological recognition element. The age of the field by no means implies that it is a relic of the past; indeed, nanobiotechnology has kept pace with its sister streams of nanomedicine in terms of innovation and development. Prototypical examples of this can be found in the development of nanobodies [30]. Nanobodies are approximately one order of magnitude smaller than their parent molecules, conventional monoclonal antibodies, and thus have greater stability as well as a reduced chance of causing an immunogenic response in vivo. This is advantageous for the therapy because, with this reduction in immunogenicity, comes improved residence time and biodistribution thus improving overall efficacy [31]. Synthesis of these nanobodies is achieved by isolating and replicating the particular site on the antibody where the desired interaction takes place.

1.5.2 Nanotechnology

This brings the discussion neatly onto the second facet of nanomedicine, nanotechnology, which is probably the subsection most commonly associated with nanomedicine. This term is used, in this case, in the more specific sense of all non-biological technologies on the nanoscale. Nanoparticles are the prototype example of this and can be used in a sweeping reference to any nanoscopic rod, tube, cube, sphere etcetera, in the sense they are completely alien to the physiological system and thus have completely unique behaviours; this is opposed to biotechnology discussed above in which existing properties are harnessed and exploited. Such nanomaterials have proven to be highly versatile in both their properties and their applications.

What gives nanotechnology its celebrity is, perhaps, the fact that all nanomaterials are of identical composition to their bulk moduli; though they exhibit properties which are more attributable to quantum mechanics than conventional Newtonian behaviour. This is caused by nanomaterials' extremely high surface area to volume ratio, demonstrated in Fig. 1.2, which increases exponentially with respect to decrease in particle size. With this increase in surface area also comes an increase in surface atoms, which are less rigidly bound atoms that consequently are more disposed to reaction with their environment.

The extent of this decrease in size also allows nanoparticles to reach physiological crannies which were inaccessible to conventional molecules. The 'crannies' of interest for therapeutics, are the blood-brain [32, 33] and blood-testis [34, 35] barrier both of which are notoriously difficult to bypass and thus delivery of treatments to these two vital organs have remained a challenge to modern science. The small size of nanoparticles allows them to navigate the tight junctions which have been impermeable to so many other treatments.

This phenomenon has been harnessed to create some of the most notable innovations in nanomedicine [36].

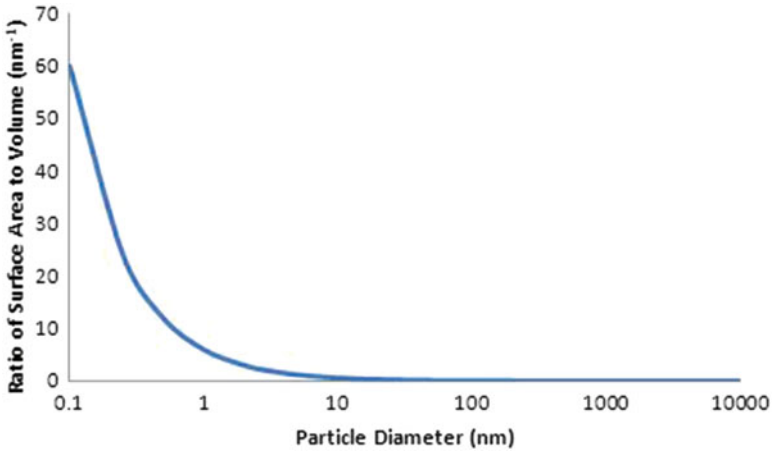

Fig. 1.2 The size effect: the relationship between particle size and surface area to volume ratio for a theoretical spherical particle

1.5.3 The Revolution of Interdiscipline

Active delivery of therapeutics is perhaps one of the most widely known techniques associated with nanomedicine. This is done by attaching a therapeutic to a vector which can then be directed to the targeted site. An example of this is the use of magnets to guide iron oxide nanoparticle-conjugated therapeutics to the target site, most commonly; this is a cancerous tumour. The therapeutic can be, and this is where the revolution begins to manifest, either a conventional chemotherapeutic, a gene or protein as was discussed above, or the particle itself [37–40]. In all cases, increased specificity and efficacy are achieved through the use of nanomaterials.

Conventional therapies can also be enhanced by combining them with nanotechnology; contemporary chemotherapeutics can receive a great decrease in observed side effects through the delivery of the drug to the target site through encapsulation within liposomes. Liposomes are bi-lipid membranes capable of encapsulating several varieties of molecules including hydrophobic, hydrophilic and crystalized drugs as well as therapeutic gene sequences. The liposomes' advantage lies in their ability to be used as triggered release vessels for molecules thus creating a prodrug derivative of the conventional chemical. They are also highly useful for 'disguising' a molecule to improve biodistribution and residence time as well as allowing hydrophobic molecules to disburse in aqueous systems, something highly advantageous in the physiological environment [41–43]. Indeed, in the quest for increased efficacy of treatment, several different components can be combined in order to create a hybrid treatment designed for a very specific target site, thus guarding against non-specific damage to the surrounding tissue. In one example, [44] a magnetisable element has been added to a thermally sensitive liposome containing chemotherapeutics with a temperature dependent trigger; in this strategy, even when the liposome is manually directed to the target site, release will only take place if the thermal parameter, caused by tumour-associated inflammation, is met. Even at this point, the chemotherapeutic still requires the thermal element as well, all of which is designed to defend the patient against the life threatening and debilitating side effects that one was obliged to endure in the past.

To elaborate on the use of nanoparticles themselves as therapeutics, in the case of magnetic particles, they hold a significant advantage over conventional treatment, as do many nanomedications, of being initially inert, or rather, a 'prodrug', this implies that the particle will remain non-interactive until such a time as is appropriate for it to execute its function which, in the case of the magnetic particles, is done via an oscillating magnetic field thus concentrating the effect to the target area and minimising non-specific damage to the surrounding tissue [39]. Of course there are several different nanomaterials that can be used as either detection, delivery or therapeutic agents, some of the most common materials used are heavy atom-elements such as gold [45] and platinum [46] or specific structures of elements such as carbon nanotubes [47]. Though there is an Aladdin's cave of applications of nanotechnology within this field, as this work is not intended to be a review of such, it will not be dwelled upon.

Non-biological nanotechnology has also offered safer solutions to innovative techniques produced by nanobiotechnology. Again, to give but a single example of this, the use of viral delivery vectors in gene therapy, as discussed above, can be quite dangerous given the handling of infectious viruses required to replace the viral payload with the therapeutic; the use of anionic polymer nanoparticles has been proposed as an effective alternative delivery vector [48, 49].

Self-assembly of complex nanostructures has also been permitted through the interdisciplinary collaborations within nanomedicine. Study of DNA, RNA or protein-mediated self-assembly of biological structures has led to another revolutionary line of research, namely, the exploitation of this biological mechanism to facilitate the design and synthesis of complex nanostructures [50]. Again, this innovative new technique stems from the research lines of surface functionalization of nanoparticles, more specifically, the conjugation of a ligand to the surface of a nanoparticle so as to add an antigen-antibody recognition site to the particle. It is a short step to then remove the target site from its host and instead attach it to a different particle thus creating the blue print for the orientation and organisation of the particles and consequently removing the need for costly and time consuming manipulation [51, 52]. Indeed, the potential for this application is truly great; nanoscaffolds for the repair or construction of proteins or nanoparticle arrays for tissue engineering applications [53] are already being proposed and designed.

Thus the unique abilities that the combination of these two already highly innovative subsections should be becoming apparent; the enhancement and optimisation of treatments that has been observed because of such collaborations should, in itself, be ample cause to bestow the title of ‘revolutionary’ on the field of nanomedicine. There is, however, a third subsection which has come, perhaps as the final destination to the train of thought that amalgamated nanobiotechnology with nanotechnology. This is nanobiomimetics.

1.5.4 Nanobiomimetics

Nanobiomimetics can be defined as the design and synthesis of nanomaterials using the structures and mechanisms of biological systems. It is the logical progression from the previous two subsections of nanomedicine described in the previous sections. Biomimetics, in fact, is a discipline that stretches back over half a century. It is however the mimicry of biological structures at the nanoscale that makes this facet noteworthy [54]. Some of nature’s most intriguing mechanisms are confined to the nanoscale, this newfound ability to mimic and refine or improve them holds many significant advantages and thoroughly demonstrates nanomedicine’s worthiness for the title of a ‘revolutionary interdiscipline’, or at the very least acts as a demonstration of how nanomedicine has truly come full circle, the full revolution if you will. To reiterate, the intent of this piece is not to provide a review of the state of the art, but rather to globally demonstrate the innovation of the field. The prototypical example of nanobiomimicry can be taken as the development of aptamers

and molecularly imprinted polymers or 'MIPs'. In the case of MIPs, it must be added that the use of the term 'nano-mimicry' comes from the ability of the polymer to form imprint sites on a scale of singular molecules on the angstrom (0.1 nm) level. These imprints are contained within pores within the structure of the MIP while the polymer itself can be manipulated to form both particles as well as lavers and films on surfaces. Within the molecular imprinting of particles, two distinct strategies can be defined; that of bulk polymerisation, where a polymer 'brick' is allowed to form and is then broken to yield imprinted monoliths, and precipitation polymerisation where nano and macro particles are grown in a solvent suspension. These can be named the top-down and bottom-up synthesis protocols within the field of MIP particles. The applicability of MIPs to the field of nanobiomimetics is due to the increasing volumes of literature being published reporting imprinted nanoparticles via this 'bottom-up' strategy of particle formation [55, 56].

MIPs seek to serve the same purpose as monoclonal antibodies did in the 1970s in the sense that recognition of a molecular target or antigen can be achieved by imprinting a template molecule into the surface of a polymer. This imprint renders the polymer a 'synthetic antibody', or rather, synthetic nanobody, as only the specific binding sites remain [57, 58]. MIPs and nano-MIPs are vastly cheaper than biological ligands as well as having superior stability and extremely high selectivity and specificity [59]. Indeed, this imprinting technique is far from limited to the recognition of biological ligands, MIPs can be imprinted with pharmaceutical compounds for example, thus making them a highly versatile tool in both the synthesis process as well as delivery, distribution and controlled release of a drug [60, 61].

Aptamers can be defined as artificial oligonucleotides or peptide sequences [62] which can be chemically synthesised to form a recognition element for anything from amino acids and proteins to drug molecules or other miscellaneous molecules. The versatility of these ligands is complimented by their inherent lack of immunogenicity in vivo. Having been first reported in the nineties, aptasensors, which are sensors utilising immobilised aptamers as their detection mechanism, have proven to be superior to biological antibodies through superior chemical stability as well as cost effectiveness [63]. Their versatility has also led to their wide spread use as sensors in both laboratory and clinical settings for diagnostic and discriminatory applications [64, 65].

1.6 Conclusions and Future Outlook

Nanomedicine is an innovative and emerging field with roots which can be traced back through several decades. Though nanotechnology is interspersed throughout, and is inseparable from modern life, the true capabilities of nanomedicine are still being realised. Top-down synthesis of nanomaterials, which is expensive and low-yielding, has given way to bottom-up synthesis which has proven to be a vastly more cost effective and efficient way to synthesise products as well as providing results with a much higher degree of precision.

Nanomedicine is an umbrella term used to encompass all applications of nanotechnology to the medical field. The 'bottom-up' nanotechnologies can be divided into three further subcategories, namely nanobiotechnology, nanotechnology and nanobiomimetics. The interdiscipline of nanomedicine comes with the combination of these categories, which are themselves well defined fields, to form innovative novel multidimensional therapies designed to enable the discrimination between the targeted site and the surrounding healthy tissue. What makes these combinational therapies exceptional, and nanomedicine truly a revolutionary interdiscipline, is the unlimited number of dimensions a single treatment can have and thus, a so-called 'intelligent' medicine can be developed which will only execute its purpose once the target site has been reached and identified. The spirit of this interdiscipline and 'combinational' ethos can be found embodied in the discipline of nanobiomimetics whereby the biological systems themselves are studied and replicated in order to achieve a hybrid mechanism which has advantages over both the original biological system and the synthetic alternative.

All of these factors allow for nanomedicine to garner with the title of a 'revolutionary interdiscipline'.

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Chapter 2
Nanobiology in Medicine

Hariprasad Thangavel

2.1 Introduction to Nanobiology

The term 'nano' is a Greek word for 'dwarf', meaning one billionth. It was first used by N. Taniguchi in 1974. A quest for nano began from the noble lecture, 'There is plenty of room at the bottom' presented by the Noble Laureate, Professor Richard Feynman in 1959. In the 1980s, K. Eric Drexler popularized the word 'nanotechnology'. His idea was to build machines on the molecular scale [1]. The principle behind this technology is engineering and manufacturing structures, devices, and systems that have novel properties at the atomic and molecular level. Later, scientists and researchers successfully employed nanotechnology to explore the boundaries of biomedical sciences. They made it possible to use biological processes to construct biocompatible nanostructures. There are several approaches to construct nanostructures and they are top-down (miniaturization) approach, bottom-up (building from atoms and molecules) approach and functional (building materials with desired functionality) approach [2].

Biological studies focused at extremely minuscule to molecular levels are termed as Nanobiology. Most of the fundamental biological functions take place at the level of molecular machineries that have a size range of less than 100 nm. Figure 2.1 demonstrates a good size comparison of biological structures in the nanometric scale [3]. The emergence of nanobiology made opportunities to better understand the functions of these molecular machineries with the help of scanning probe microscopy, modern optical techniques, and micro-manipulating techniques.

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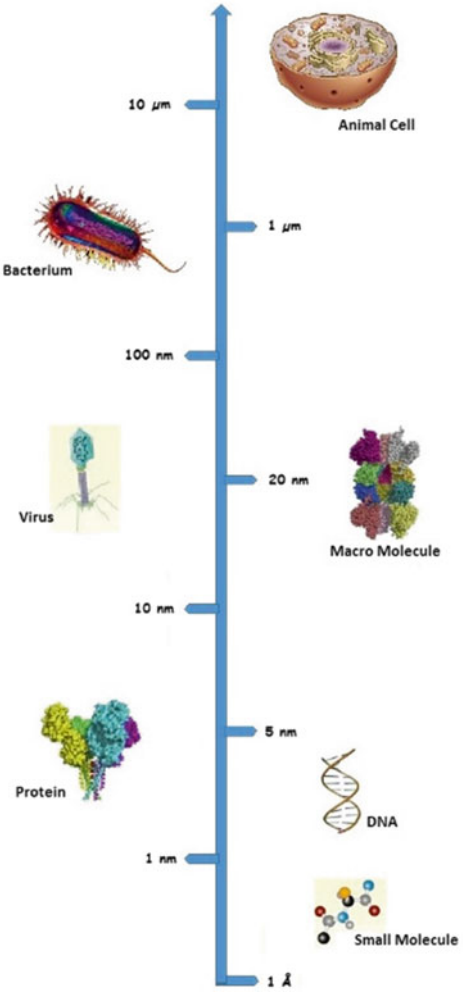

Fig. 2.1 Relative sizes of different biological structures in nanometric scale [3]

Nanobiology research interests can be roughly grouped into two basic categories: nanotechnologies applied to biological systems, and development of biologically-inspired nanotechnologies. The main reason for this categorization is to differentiate between the sources of inspiration. However, nanobiology research are mainly focussed at nanobiological structures and systems, biomimetics, nanomedicine, nanoscale biology, and nanointerfacial biology [4]. The tools, techniques, and technologies derived from nanobiology are applied to medical field directly and indirectly, giving rise to a new ground of medicine termed Nanomedicine. Nanobiology by itself is a blend of various different disciplines not limited to physics, chemistry, biology, computation, and engineering [4]. The different themes of nanobiology can be well understood from Fig. 2.2.

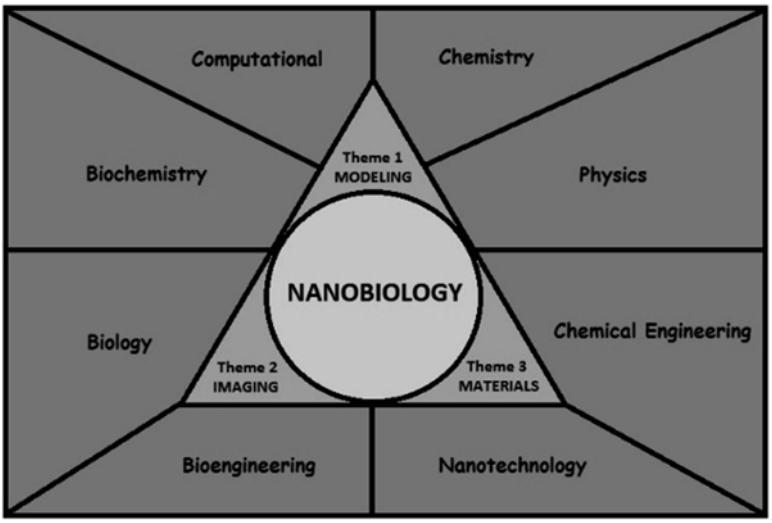

Fig. 2.2 Nanobiology map showing three different themes [4]

Nanobiology can make significant contributions to medicine by achieving success in early detection and diagnostic strategies, and in treatment and prevention of fatal diseases. Based on enhanced efficacy, nanoparticles of polymers, metals or ceramics can conflict life challenging conditions like cancer, AIDS, heart and brain disorders. They can invade bacteria, virus, parasites, etc. Nanoparticles are generally characterized based on their size distribution, shape, surface area, surface reactivity, surface charge, chemical composition, and aggregation state. Factors such as specificity, solubility, stability, biocompatibility, biodegradability, and pharmacokinetics are to be considered predominantly while using nanoparticles in medicine. The purpose of employing nanobiology in medicine is to diagnose diseases early and accurately; and to treat them effectively without any side effects. It has proven potential to cure human cancer by specifically killing the targeted cancer cells leaving the surrounding healthy tissues harmless. Nano-drug delivery system can provide new insight for the treatment of tuberculosis (TB). DNA-based nanotechnology is a new arrival in molecular medicine [5]. Nano-drug therapy sounds promising compared to traditional therapies [6]. It is strongly believed that nanobiology has all the probability to benefit mankind by improving the quality of life, without any doubts.

2.2 Nanobiology and Human Biology

In terms of medical application, the main thing to consider about nanomaterials is their sustainability in the human body. These small materials exhibiting unique surface properties at nanoscale size could be toxic, causing adverse ill effects.

Table 2.1 FDA-approved gold based nanomaterials used in therapeutics [8]

Product/ brand name	Component/ active ingredient	Delivery route	Target	Company	Current status
Verigene	Gold	In vitro diagnostics	Genetics	Nanosphere	FDA-approved
Aurimmune	Colloidal gold nanoparticle coupled to TNF-α and PEG-Thiol (~27 nm)	Intravenous	Solid tumor	Cyt-Immune sciences	Phase-II
Auroshell	Gold coated silica nanoparticles (~150 nm)	Intravenous	Solid tumor	Nanospectra biosciences	Phase-I
Combidex (Ferumoxtran-10)	Iron oxide nanoparticles (17–20 nm)	Intravenous	Tumor imaging	Advance magnetics	NDA filed

The nanomaterials used on purpose should be able to dissolve inside the body without leaving any side effects when they are no longer needed. Nanomaterials have an unknown behavioural property when compared with bulk materials. For example, nanomaterials made of inert element like gold become extremely active at nanometric dimensions [7] and it is one of the most used nanomaterial in medicine. A few FDA-approved gold based nanomaterials which are currently used in therapeutics are listed in Table 2.1. In a research conducted by Han et al., functionalized gold nanoparticles proved to be highly attractive for drug delivery because of their distinctive dimensions, surface tenability, and controlled drug release [9].

The nanoparticles enter human body through four major routes: nasal, oral, dermal and intravenous. Upon entry, they can be distributed throughout the body including brain, heart, lungs, gut, liver, spleen, kidney, and skin. Inside the body, nanoparticles behaviour can be disturbed or altered by factors such as hydrophobic, hydrophilic, lipophobic, lipophilic, active catalysis or passive catalysis. Nanoparticles enter the cell by one of the following four mechanisms: passive diffusion, facilitated diffusion, active transport, and endocytosis. The passive diffusion is achieved by electrochemical or concentration gradient driven mechanism. Positively charged small particles of approximately 20 nm size undergo passive diffusion. Facilitated diffusion is for small particles of size 10–30 nm and these particles get internalized via selective membrane protein channels and concentration gradient driven mechanism. The active transport and endocytosis are meant for larger particles of size ranging from 50 to 500 nm. The former is facilitated by transport protein and are energy dependent, against concentration gradient whereas the later is collective term for energy dependent internalization of substances, forming vesicles.

Several pathways of nanoparticle endocytosis include, phagocytosis, Clathrin-mediated endocytosis, Caveolae-mediated endocytosis, macropinocytosis, other Clathrin- and Caveolae-independent endocytosis, transcytosis (occurs in epithelial cells in blood brain barrier). The possible fates of internalized nanoparticles in the

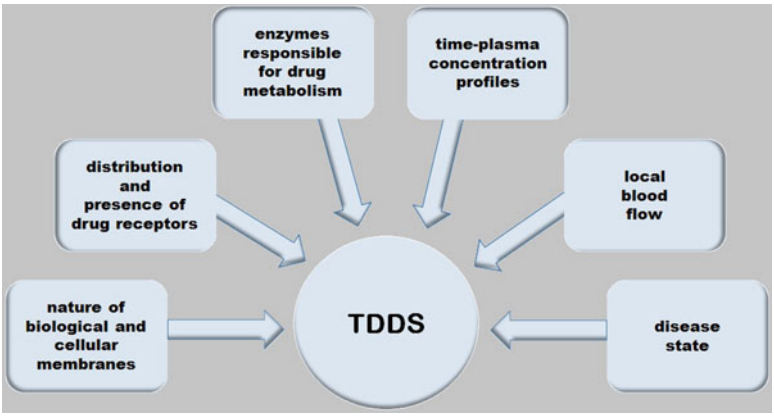

Fig. 2.3 Parameters to be considered for designing effective targeted drug delivery system (TDDS) [8]

cells are: enzymatic degradation or destruction by pH effect (acidic), exocytosis or transcytosis, transportation to intracellular locations escaping from endolysosomal compartment. The internalization of nanoparticles in the cells can cause molecular irregularities and incompatibilities resulting in fatal disorders ranging from interstitial fibrosis in respiratory system to acute coronary syndrome in vascular system. Because of some unresolved complications, not all the nanoparticles are readily used for medical applications. Only very few nanoparticles such as liposomes, dendrimers, organic polymers, quantum dots are tested to be safe in medicine and are found to be promising for in vivo imaging, in vitro diagnostics, and drug delivery. A few important parameters that are to be considered while designing an effective targeted drug delivery system are shown in Fig. 2.3.

2.3 Nanomaterials for Medicine

Materials with structural elements having dimensions in the range of 1–100 nm are termed as nanomaterials. Nanomaterials is a common term applicable for nanoscale materials, nanophase materials, and nanostructured materials. These three materials are different from one another; nanoscale materials, where the material itself fall under nanoscale regime; nanophase materials, these are hybrid materials having nanoscale phase or component; nanostructured materials, here the material structure will have nanoscale size or features [10]. Nanomaterials exhibit unique characteristic properties when compared to their bulk states. These improved properties along with large surface areas made them ideal for use in medical field. Nanomaterials play vital role in medical diagnosis and therapeutics ranging from fluorescent imaging to site-specific targeted drug delivery. More recently, Zhang et al. discussed how the nanomaterials could be designed based on their interactions with biological systems to

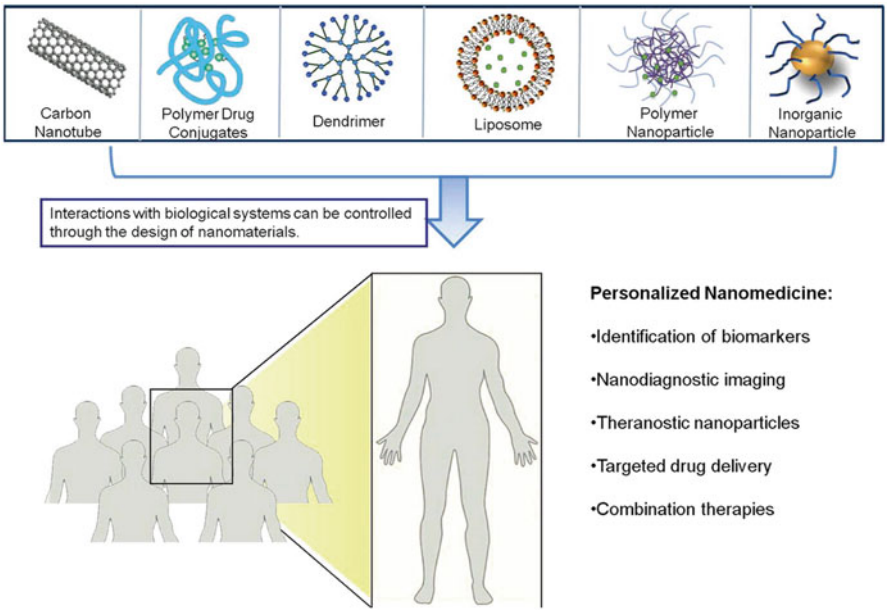

Fig. 2.4 Designing nanomaterials for personalized medicine [11]

meet their role in medical diagnostic, imaging and therapeutics [11]. The graphical view of the above context is depicted in Fig. 2.4. Nanomaterials of biological interest are mainly from two distinct groups: organic nanoparticles such as dendrimer, polymer; and inorganic nanoparticles such as gold, silver, iron, titanium dioxide. The other miscellaneous groups include carbon-based nanoparticles, organic and inorganic hybrids, liposomes, and protein and peptide-based nanoparticles.

Some examples of commonly used nanoparticles in medicine: multifunctional nanoparticles (best suited for intravenous delivery), lipid and polymer nanoparticles (trigger strong immune response), gold and magnetic nanoparticles (promising response in targeted drug delivery), virus based nanoparticles (acts as nanocarriers), dry powder aerosol (inhalable nanoparticles for lung specific treatment), smart nanomaterials (tunable by external stimuli).

Gold nanoparticles can be used for detection purposes. The gold nanoparticle with a DNA linker can result in a large size complex particle. A characteristic colour transition from pink to gray can be seen with naked eye. In medical diagnostics, gold nanoparticles can be used for laboratory analysis. Recently, a study on the development of a gold nanoparticle amplified agglutination system for weak blood group determination has been reported by Wiwanitkit et al. [12, 13]. In fact, gold nanoparticles found its entry in medical filed way back in 1800s. It was used as an important ingredient in many classical Chinese medicinal remedies. Jung et al. treated site-specific coupling of protein G to DNA oligonucleotide with a biolinker for efficient antibody immobilization [14]. The study reported that antibody targeting

on glass slides could be immobilized using this linker system without modifying or spotting antibodies, and the protein G-DNA conjugate brought a simple but effective method to label DNA-functionalized gold nanoparticles with target antibodies. Jung et al. concluded that the DNA-linked protein G construct introduced in this study offered a useful method to manage antibody immobilization in many immunoassay systems [14].

Silver nanoparticles is another commonly used nanomaterial for medical research which is proven to have promising antibacterial properties. Silver nanoparticles changes its colour with size. Silver particle having a size smaller than 10 nm appears to be golden yellow in colour. The colour further changes into red and black when the particle size increase. In 2006, Panacek et al. reported that the size of silver particles have a significant impact on their antibacterial activity. The study revealed that a very low concentration of silver gave antibacterial characteristic and thus the bactericidal property was found to be size-dependent of the silver particles [15]. In another quoted work, Gogoi et al. reported that above a certain concentration, silver nanoparticles were found to reduce the sizes of treated bacteria in addition to their characteristic bactericidal activity [16]. Pal et al. identified that silver nanoparticles underwent a shape-dependent interaction with *Escherichia coli* in their work on determining the antibacterial properties of differently shaped silver nanoparticles against the gram-negative bacteria both in liquid broth and on agar plates [17]. Also, an increase in the antibacterial activities of penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin in the presence of silver nanoparticles was reported by Shahverdi et al. [18]. As like gold nanoparticle, silver nanoparticles can also be used in the DNA linker system.

Superparamagnetic iron oxide nanoparticles are widely used in molecular and cellular imaging [19]. The major advantages of using iron oxide-based nanomaterials are their nontoxic property and biocompatibility. In a study reported by Thorek et al. superparamagnetic iron oxide nanoparticles demonstrated their utility as a novel tool for enhancing magnetic resonance contrast, allowing researchers to monitor physiological and molecular changes, in addition to previously monitored anatomical changes [20]. Hu et al. fabricated magnetic sponge-like hydrogels called ferrosponges by using an *in-situ* synthesis of magnetic iron nanoparticles in the presence of various concentrations of gelatin [21]. By using these unusual magnetic sensitive properties of the ferrosponges, a new drug delivery system can be designed to use in medicine. Titanium dioxide is another inorganic oxide nanoparticle which is recently found to be useful in medical application. Nano TiO_2 is studied to have good antibacterial properties as like silver nanoparticles. An effective organic degradation process was documented by Peralta-Hernandez et al. in their work on investigating the photo catalytic properties of nanostructured TiO_2 – carbon films obtained by means of electrophoretic deposition [22].

Viral nanoparticles (VNPs) and Virus-like particles (VLPs) are naturally occurring bionanomaterials [23]. Both of them are very promising candidates for developing ‘smart’ devices which can be used in several medical applications ranging from tissue-specific imaging to targeted-drug delivery. The major advantages of using viral particles are their exceptional stability and biocompatibility. To add a

few more, they are monodisperse, programmable, multifunctional, and easy to produce on large scale [24]. Cohen et al. investigated the use of aptamer-labeled MS2 bacteriophage capsids for targeted in vitro photodynamic therapy [25]. In their study, they demonstrated a unique virus-based loading strategy for efficient targeted delivery of photoactive compounds for site-specific photodynamic cancer therapy using biologically derived nanomaterials [25]. More recently in a similar study, Zeng et al. affixed folic acid (FA) as targeting moiety on the rigid Cucumber Mosaic Virus capsid and stuffed the interior cavity of CMV with substantial load of doxorubicin (Dox) to design a controlled drug delivery system for cancer therapy [26].

2.4 Nanobiology: Applications in Medicine

Nanobiology can help medicine in many aspects by providing a new range of tools and techniques that can be applied in early detection, disease diagnosis, non invasive treatment, and also in disease prevention. Some of the applications of nanomaterials to medicine include: fluorescent biological labels for imaging, drug and gene delivery platform, detection of pathogens and proteins, probing of DNA structure, tissue engineering and regeneration, tumour destruction via heating, separation and purification of biological molecules, magnetic resonance imaging (MRI) contrast enhancement, and phagokinetic studies [27].

2.4.1 Diagnostic Applications of Nanoparticles

In medicine, nanoimaging and nanovisualization contributes a lot more in recent diagnostics. The use of nanoparticles in imaging can provide new insights to medical diagnostics. Nanomaterials and nanotechnology combined with novel devices have the potential to address emerging challenges in medical field. The application of nanomaterials for imaging and visualization will offer fast, sensitive, and cost effective solutions for the modern clinical laboratory. Rotomskis et al. reported that the properties of nanomaterials such as quantum effect and surface area effect could improve the sensitivity of biological detection and imaging at least by 10–100 folds [28]. Basically, the nanodiagnosis can be classified into naked eye diagnostic system, immunological diagnostic system, and molecular diagnostic system. The protein precipitation systems to qualitatively test the protein in body fluids are the best example for naked eye detection system. The colour changing property of nanomaterials due to aggregation of particles can be applied to diagnostic tests in medicine [29]. Wiwanitkit et al. proposed gold nanoparticle as an alternative tool for the detection of microalbuminuria [12, 13]. Normal urine triggers the precipitation of gold nanoparticle solution resulting in a gray coloured mixture, while urine samples with protein do not. In addition to protein detection, the naked eye detection system using nanoparticles can also be applied to detect small substances such as hormones in body fluid. Gold nanoparticle solution can be used for the detection of human

choriogonadotropin (hCG) in urine, which is a basic diagnostic test to confirm pregnancy in females. The cost of gold nanoparticle is cheaper than the urine strip test. In another study, Bauer et al. aimed at the direct detection of sub-molecular layers of DNA with naked eye, based on the understanding of absorption property of metal nanoparticles [30]. The study focused on the nanolayer coated metallized-PET-chip setup and on the synthesis of DNA nanoparticle conjugates suitable for resonance amplified absorption -point of care tests and the applied usage of those particles in the direct visualization of DNA-DNA binding events [30].

Immunodiagnosis by nanoparticle is the new stage of immunological test in medicine. Over the last decade, the immunological diagnostic systems using nanoparticles achieved considerable momentum in the field of medicine. Immunological diagnostic systems employ nanoparticle labeling. The best example for nano-enabled immunological diagnosis is luminescent quantum dot in immunoassay. Quantum dots are emerging as a new class of biological labels with properties and applied usages that are not available with traditional organic dyes and fluorescent proteins [31]. Zhu et al. reported quantum dots as a new immunoflorescent detection system for *Cryptosporidium parvum* and *Giardia lamblia* [32]. The study concluded that this new fluorescence system exhibited superior photostability, gave 1.5–9 fold higher signal-to-noise ratios than traditional organic dyes in detecting *C. parvum*, and allowed couple-colour detection for *C. parvum* and *G. lamblia* [33]. In addition to quantum dots, superparamagnetic nanoparticles are also used in immunoassays. Kuma et al. reported their development of a liquid phase immunoassay system using magnetic nanoparticles [34]. They developed a highly sensitive immunoassay system using Fe_3O_4 magnetic nanoparticles [34]. Other than these, nanoparticles like silicon di oxide, europium-doped lanthanum fluoride, europium-doped gadolinium oxide, cadmium telluride are also used in nanodiagnosis [35–38]. In another work, Hwang et al. proposed the use of gold nanoparticle-based immunochromatographic test for identification of *Staphylococcus aureus* from clinical specimens [39]. Hwang et al. reported that this detection method was fast, easy to perform, and had a long shelf life at room temperature [39]. More recently, Jiang et al. reported a single step synthesis method to produce water soluble Ag_2S quantum dots for in vivo fluorescence imaging [40]. This work proposed potential cadmium-free (and lead-free) quantum dots for nanodiagnostics and in vivo imaging [40].

Finally, molecular diagnosis is rapidly advancing with the help of nanotechnology. There is a steady progress in the use of electrochemical biosensors for DNA analysis, over the past few years. In 2005, Shen et al. studied polymerase chain reaction (PCR) of nanoparticle-bound primers [41]. They concluded that with either one or two primers respectively bound to the nanoparticle surface, PCR could proceed completely under optimized conditions, having been subjected to certain rules [41]. Kalogianni et al. reported a simple and inexpensive assay that allowed visual detection and demonstration of the PCR-amplified sequences by hybridization within minutes [42]. According to their study, the nanoparticles bound to the target DNA through hybridization, and the hybrids were captured by immobilized streptavidin at the test zone of the strip, producing a characteristic red line [42]. In another quoted study, the use of plasmonics-based nanoprobes that acted as molecular

sentinels for DNA diagnostics were demonstrated. The plasmonics nanoprobe was composed of a metal nanoparticle and a stem-loop DNA molecule tagged with a Raman label, and the nanoprobe utilized the specificity and selectivity of the DNA hairpin probe sequence to detect a specific target DNA sequence of interest [43]. The study completely demonstrated the specificity and selectivity of the plasmonics nanoprobes to detect PCR amplicons of the HIV gene [43].

2.4.2 Therapeutic Applications of Nanoparticles

In recent days, the drug formulation and development becomes easier with the advancement of nanopharmacology. The application of nanotechnology in pharmacology is aimed at finding out novel pharmacological molecular entities; targeted site-specific drug delivery within the body; and providing personalized treatment to reduce side effects and increase drug effectiveness [44]. A graphical view on the beneficial effects of targeted drug delivery system (TDDS) are illustrated in Fig. 2.5 [8].

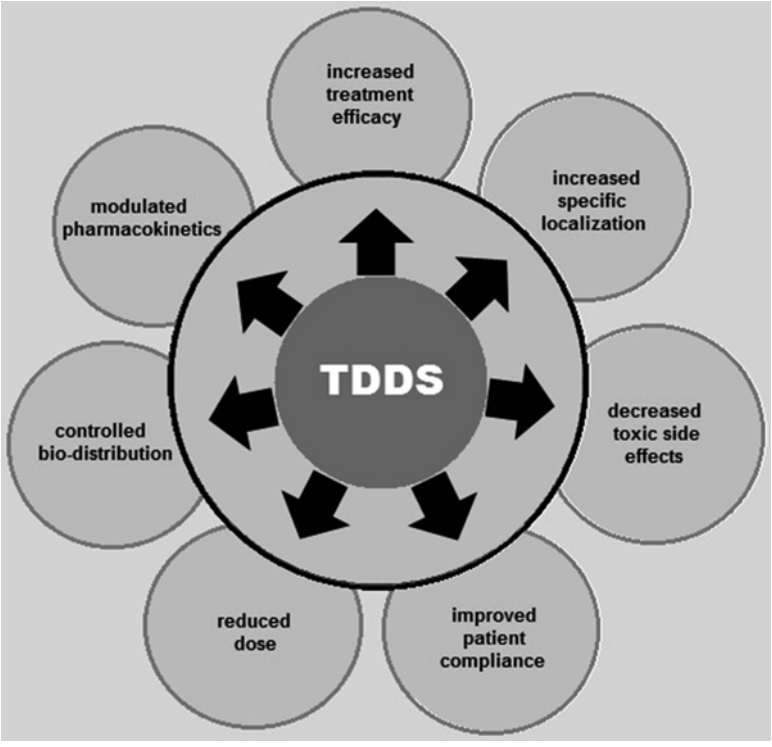

Fig. 2.5 Advantages of TDDS to improve drug efficiency for personalized treatment [8]

Table 2.2 Nanoparticles currently used in therapeutics

Type of nanoparticles	Material used	Application
Polymeric nanoparticles	Biodegradable polymers	Controlled and targeted drug delivery
Quantum dots	CdSe-CdS core-shell	Targeting and imaging agent
Nanopores	Aerogel, which is produced by sol–gel chemistry	Controlled release drug carriers
Nanowires or carbon nanotubes	Metals, semiconductors or carbon	Gene and DNA delivery
Nanoshells coated with gold	Dielectric (typically gold sulphide or silica) core and a metal (gold) shell	Tumor targeting
Liposomes	Phospholipid vesicles	Controlled and targeted drug delivery
Ceramic nanoparticles	Silica, alumina, titania	Drug targeting and biomolecules delivery
Polymeric micelles	Amphiphilic block co-polymers	Systemic and controlled delivery of water-insoluble drugs

Nanoparticles can easily cross any of the biological barriers in human body as their size falls in the nanometer range. A list of nanoparticles and their applications in medical therapeutics are given in Table 2.2. When compared with classical drug administrations, nanosystems provide more advantages with greater therapeutic effect. Nanotherapy has all the potential to deliver treatment for fatal diseases like human cancer, HIV-AIDS, etc., Nanoparticles act as vector in drug delivery systems. The drug can be directly attached (entrapped, coated, or functionalized) to the nanoparticles. After drug discovery, a large number of compounds failed to prove high solubility, stability and bioavailability and are dropped. Nanotechnology-based drug formulation could meet out all the requirements to sustain as a good drug agent [45]. Different nanoparticles such as micelles, liposomes, solid lipid nanoparticles, polymeric nanoparticles, pegylated nanostructures, nanocrystals, nanobodies, cyclodextrin, dendrimers, and metallic nanoparticles are used for drug delivery in medicine. By using these nanoparticles in drug delivery, a novel platform to deliver drug targeted to a specific tissue with a controlled release rate can be developed. Nanoparticles and liposomes (with or without pegylation), dendrimers and micelles are candidate carriers for tumor-specific drug delivery [46]. In medical therapeutics, nanoparticles are applied to surgical medicines, dentistry, gene therapy, stem cell therapy, tissue engineering and regeneration.

Nanosurgery is a new concept in surgical medicine. But, nanosurgery for human beings has rarely been achieved. There are some advents in eye surgery. Femtosecond laser pulses, emitted from lasers working in the near-infrared, based on multiphoton effects allowing both imaging and laser effects to be generated which are in the submicron range and which do not cause collateral damage are available [47]. Kohli et al. reported membrane surgery and nanosurgical cell isolation using high-intensity femtosecond laser pulses [48]. They demonstrated the applicability of using ultra short laser pulses for performing surgery on live mammalian cells [48]. In 2007, another study reported on corneal multiphoton microscopy and intratissue optical

nanosurgery by nanojoule femtosecond near-infrared pulsed lasers [49]. In this study, multiphoton microscopy including multiphoton autofluorescence imaging and second-harmonic generation was used as a new diagnostic tool to perform tissue nonlinear optical tomography with submicron resolution [49]. Nanoneurosurgery is another interesting emerging nanosurgery. In addition, nanocoated tools can also be useful for surgery and orthopaedics. Although bone is a very diverse tissue providing different functions within the body, recently identified nanobiomaterials shown promising solution to orthopaedic problems [50]. Chris Arts et al. reported the use of a bioresorbable nano-crystalline hydroxyapatite paste in acetabular bone impaction grafting [51]. In this work, destructive lever-out tests and in vivo animal tests were performed with various combinations of materials. Chris Arts et al. concluded that TCP-HA granules with a nano-crystalline hydroxyapatite paste could be a valuable addition when TCP-HA ceramic granules are being used for acetabular bone impaction grafting procedures [51].

Nanotechnology has a significant involvement in dentistry materials. He and Swain used a nano-based indentation system to determine the indentation stress-strain response of two kinds of dental ceramics, one kind of dental alloy and healthy enamel [52]. They reported that strong and tough materials with primarily elastic response, such as toughened ceramics, were required to enable dental crown/bridges to have long-term reliability [52]. In another study, Fu et al. studied effects of dental bleaching on micro- and nano- morphological alterations of the enamel surface [53]. The study concluded that the thickness of the enamel smear layer was significantly reduced due to the bleaching process [53]. Hairul Nizam et al. performed a nanoindentation study of human premolars subjected to bleaching agent [54]. According to their study, the exact mechanism by which hydrogen peroxide impacts the dentin and enamel had yet to be completely elucidated; however, it was observed to have an undermining effect on the nanomechanical properties of teeth [54]. Lee et al. reported the changes of optical properties of dental nano-filled resin composites after curing and thermocycling [33]. The objective of their study was to access the colour changes after curing, polishing, and thermocycling of a nano-filled resin composite. Lee et al. concluded that changes in colour and translucency after curing, polishing, and thermocycling varied by the shade group [33].

Gene therapy is the advanced therapeutic concept at present. It involves gene manipulation and transfer. Gene therapy implies local or systemic administration of a nucleic acid construct that can prevent, treat and even cure diseases by changing the expression of genes that are responsible for the pathological condition. This therapy is the hope for treating presently incurable diseases. Viruses are used in most of the clinical experiments today; however, they do have significant drawbacks. Therefore, non-viral vectors based on lipids, hydro-soluble polycations, other non-condensing polymers and nano or microparticles/capsules have been proposed [55]. Both biodegradable and non-biodegradable inorganic particles can be completely fabricated on the nanoscale with the attributes of binding DNA, internalizing across the plasma membrane and finally releasing it in the cytoplasm for final expression of protein [56]. In addition to the classical intravenous injection system,

polymer-based nanoparticle technologies for oral gene therapy is in continuous development [57]. Pan et al. revealed that polyamidoamine dendrimers-modified magnetic nanoparticles might be a good gene delivery system and have potential applied usages in cancer therapy, molecular imaging and diagnosis [58]. In another study, Yamada et al. noted that hepatitis B surface antigen-L (HBsAg-L) particles were able to deliver payloads with high specificity to human hepatocytes. The study indicated that the L particle was a suitable cell- and tissue-specific gene/medicine transfer vector [59].

Stem cell therapy and tissue engineering are few other advanced therapeutic concepts. Stem cell therapy is mainly applied for the treatment of congenital defects and malignancies, whereas tissue engineering is targeted at regenerative medicine. Murugan and Ramakrishna reported that electrospinning was a straightforward, cost-effective technique that could be applied to the fabrication of nano-featured scaffolds suitable for tissue engineering, and it offered usefulness over conventional scaffold methodologies [60]. Chen et al. performed a meta-analysis of applied usage of electrostatic spinning technology in nano-structured polymer scaffold [61]. The study concluded that the nano-structured polymer scaffold could support the cell adhesion, proliferation, site, and differentiation, and this kind of scaffold had a considerable value in the tissue engineering field [61]. In another study, Huang et al. reported their development of nano-sized hydroxyapatite-reinforced composites for tissue engineering scaffolds [62]. In a more recent study, Corradetti et al. illustrated the use of affinity targeted biodegradable nanoparticles to mediate paracrine stimulation as an alternate to withstand the growth and pluripotency of mouse embryonic stem cells [63]. This approach will extremely contribute to the scalable manufacture of stem cells and the clinical delivery of new advanced cellular therapies for regenerative medicine [63].

2.5 Conclusions and Future Outlook

Nanobiology signifies the merger of biological research with nanotechnologies and it is a multidisciplinary field where a wide range of applications such as using bionanomaterials in engineering or engineered nanomaterials in biology and medicine are studied with diverse viewpoints. It confers innumerable welfare measures on humanity like advancement in medicine, nanoparticle coated zidovudine as in AIDS therapy, treating tumour of precise points, storage of hydrogen fuel and drug delivery material. The use of nanomaterials in surgical medicine could help in the development of many nanosurgical tools and nanoprosthetic devices. The concept of nanosurgery is to minimize blood loss, inter- and post-operative complications and to decrease the period of hospitalization for the patient. The advances in nanobiology could help the researchers to create future “SMART MATERIALS” that play an important role in diagnostics and in efficient drug delivery. Nanotechnology will also help in the formation of brand new molecular systems which might perfectly

resemble existing living systems. Though nanobiology has several achievements and promises to add to its credit, it also requires our much appreciated patience. One should always realise that nanobiology can be a promising avenue by which medicine can advance, instead of expecting it to revolutionise medicine.

Moreover, researchers are also working to discover the potential long-term toxicity of nanoparticles, and their metabolic and degradative mechanisms. It is necessary to understand the fate of the drug once delivered to the nucleus and other sensitive organelles. In order to avoid public distrust and address the risks and potential hazards of emerging nanotechnologies: methods and tools should be developed to identify and characterize nanomaterials in biological matrices; international guidance should be developed on the effective exposure control; well-characterized stable benchmark and reference materials should be developed and used for toxicology studies; required data should be collected on human exposure, biomonitoring, and health outcomes that might be related to exposure; education and training should be given to researchers, manufacturers, and users of nanomaterials regarding the safe development and use of nanomaterials; existing regulations should be evaluated and new regulations should be developed if necessary; international guidance should be developed and shared on the best available practices for working with nanomaterials.

The in depth journey through nanobiology will lead one to end up in picobiology. Also, in nature there are many things exist below 1 nm level. Many single molecules are in the picolevel. In volume sense, many substances such as vitamins exist in the picolevel. The science focusing on objects that fall in the range of 1–100 pm is believed to be the next for nanoscience. But, the main question is when we can reach picotechnology.

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Chapter 3
Tissue Engineering *In Vivo* with Nanotechnology

Erik Taylor, Dave A. Stout, George Aninwene II, and Thomas J. Webster

3.1 *In vivo* Nanotechnology Tissue Engineering Studies

The main goal of tissue engineering is to replace diseased and/or damaged tissues with biological substitutes or biocompatible materials that can restore and maintain normal tissue or biological functions. There have been major advances in the areas of cell and organ transplantation, as well as advances in materials science and engineering which have aided in the continuing development of tissue engineering and regenerative medicine [1–7]. But with all of these advancements, there still is a need for better materials and techniques to facilitate tissue growth. One field that can promote this advancement is nanotechnology, which focuses on gathering simple nanosized materials to form complex structures. In a sense, nanotechnology involves utilizing materials which possess at least one physical dimension between 1 and 100 nm to construct structures, devices, and systems that have novel properties [7] – in fact, many biological components, like DNA, involve some aspect of nano-dimensionality [7]. With the knowledge of the nano-dimensionality of nature, it has

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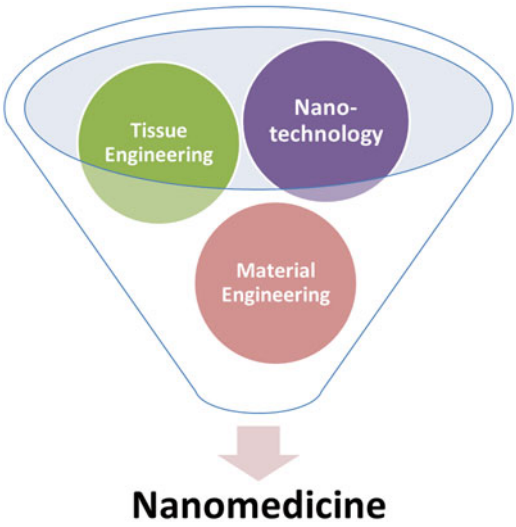

Fig. 3.1 A schematic for the use of bio-inspired tissue engineering techniques with the basic understanding of materials and the use of nanotechnology comprise the field of Nanomedicine. Nanomedicine has logically given rise to high interest in using nanomaterials for tissue engineering applications since these materials have a significant impact towards inhibiting infections, decreasing inflammation, and promoting tissue growth

logically given rise to the interest in using nanomaterials for tissue engineering with these materials having the potential to significantly impact tissue engineering – a new field known as nanomedicine (Fig. 3.1).

Numerous papers, articles, books and research has gone into the utilization, development, analysis, implications and understanding of nanotechnology *in vitro* tissue engineering experiments with the understanding that future research will explore its *in vivo* associations [7–11]. To see where nanotechnology has made an impact from *in vitro* to *in vivo*, we present below some of the most promising tissue engineering *in vivo* experiments. Since nanotechnology has had a large impact *in vivo* on many aspects of medicine, we have delineated studies into particular organs/cells of interest and materials used.

3.1.1 Bone Studies

When looking at natural bone one can see a complex system with intricate hierarchical structures assembled through orderly deposition of nanometer sized hydroxyapatite minerals of low crystallinity within a type I collagenous matrix [12]. Below are *in vivo* experiments divided by the type of nanotechnology material investigated.

3.1.1.1 Nanomaterial-Coating

To mimic the surface of bone, one such practice is to coat implant surfaces. This will allow for the implant to be porous and allow for tissue ingrowth into the implant leading to direct anchorage to host bone – known as biological fixation [13, 14].

One such way of doing this is by soaking the material in an aqueous solution containing major inorganic components present in the body (such as HCO_3^-, Ca^{2+}, HPO_4^{2-}, and Mg^{2+} ions) and then adding, using plasma spray techniques, a material coating [15].

A study using nano-hydroxyapatite (HA) as the coating material, ingrowth chambers implanted into the lateral metaphysics of the distal femur of skeletally mature large coonhound dogs for a period of 8 weeks found that one is capable of promoting bone ingrowth and apposition, affecting the behavior of osteoblasts (bone forming cells) [15]. It was shown that more bone formation was generated in the channel lines with the apatite coating than one with non-coating titanium surfaces [15].

3.1.1.2 Injectable Nanomaterials

Injectable hydrogels have been used to create nanocomposites for tissue engineering as a means to deliver drugs and as a model for extracellular matrices due to their ability to have good biocompatibility, biodegradability and intrinsic cellular properties [16–18].

For example, an *in vivo* project involving 16 male Sprague Dawley® (SD) rats (320–350 g) were used to determine the degradation of an injectable bone regeneration nanocomposite which was comprised of nano-hydroxyapatite/collagen particles in alginate hydrogel carrier [19]. Alginate is a well-known natural polysaccharide compound of 1,4-linked β-D-mannuronate and 1,4-linked α-L-guluronate found in numerous biomedical applications due to its good biocompatibility, low toxicity and inexpensive cost [20–22]. It was shown that after 24 weeks, the injectable bone composite was almost fully degraded and was replaced by connective tissue as well as no fibrous capsule and acute inflammatory reactions were found [19].

Hosseinkhani et al. also showed that using a self-assembled injectable nanohydrogel comprised of a peptide-amphiphile and bone morphogenetic protein-2 (BMP-2) induced bone formation in Fischer male rats, aged 6 weeks [23]. It was demonstrated that where the injection site occurred, they found significant homogenous ectropic bone formation compared to a non-injected site.

3.1.1.3 Nanofiber Materials

Nanofibers are usually defined as fibers with one dimension ≤ 100 nm made of many different materials [24]. In the tissue engineering realm, the appeal of nanofibers is their structural similarity to native extra cellular matrix (ECM) proteins – this network creates a dynamic, three-dimensional nano and microenvironment in which cells are maintained. Signals are transmitted between the cell nucleus and the ECM enabling communication for cell adhesion, migration, growth, differentiation, programmed cell death, modulation of cytokines and growth factor activity, and activation of intracellular signaling [25, 26].

One such *in vivo* example is the use of silk fibroin nanofibers for its attractive properties: biocompatibility, oxygen and water vapor permeability, and biodegradability [27]. Using 30 adult male New Zealand white rabbits, two 8-mm-diameter defects were created, one on each side of the midline where a silk fibroin nanofiber was implanted. After 12 weeks of implantation, *in vivo* examination of the implanted silk fibroin nanofibrous membranes showed a negligible inflammatory response including occasional lymphocytes, eosinophils, foreign-body giant cells, and the subsequent formation of a relatively thick fibrous capsule, thus providing an alternative nanomaterial for bone growth [28]. Also, mimicking the natural nanofibers of the ECM can decrease the inflammation response since inflammatory cells would not recognize it as a foreign material.

3.1.1.4 Nanocomposite Materials

One of the major paradigms in bone tissue engineering is the scaffold, which functions as a structural support and transport vehicle for cells and bioactive molecules necessary for the formation of new bone [29, 30]. The ideal scaffold would need to have many properties that would support adequate bone tissue growth: mechanical properties equal to that of bone, degrade upon bone tissue growth, biocompatibility, pore interconnectivity, porosity, and promote bone tissue ingrowth.

One such way to overcome this obstacle is the use of nano reinforced composites. This would support strength and reinforcement as well as porosity and durability [31]. One such example is the use of ultra-short single walled nanotubes (SWNTs) for reinforcing synthetic polymeric scaffold materials. Reinforced ultra-short SWNT composites were implanted in rabbit femoral condyles and in subcutaneous pockets [32]. Results indicated that the reinforced composites showed favorable hard and soft tissue responses as well at 12-weeks the composites had a threefold greater bone tissue ingrowth and a reduced inflammatory cell density and increased tissue organization compared to their non-reinforced scaffold counterparts [32] – thus providing evidence that nanotechnology can strengthen bone tissue engineering scaffolds to promote bone growth.

Also, when embedding nanofibers into a scaffold one could increase a scaffold's biocompatibility properties and bone formation ability. For example, Osathanon et al. showed using a mouse calvarial defect system that by creating nanofibrous fibrin scaffolds and adding nanocrystalline hydroxyapatite one could promote bone formation around the defected area [33]. Also, they showed when adding micro/nano pore sizes and embedding rhBMP-2 into the nano-based scaffold, one could increase bone formation even more.

Another way to use nanocomposite materials is to use amorphous tricalcium phosphate (TCP) embedded in poly-lactic-co-glycolic acid (PLGA). Amorphous TCP has been shown to be very soluble and reactive due to a high enthalpy of formation [34] and bioactivity [35]. TCP/PLGA nanocomposites were transplanted into New Zealand white rabbits on the nasal and cranial bone and results showed newly formed bone after 4 weeks of implantation which was significantly increased when compared to non-nanocomposite reinforced PLGA scaffolds [36].

3.1.2 Neural Studies

Due to the ever increasing complexity of the central nervous system, many challenges have surfaced (such as vascularization, junction connection, cell interactions and toxicity (Fig. 3.2)) which have hindered the development of nanomaterial tissue engineering applications. Due to this difficult challenge, most studies have been aimed at the regeneration of an injured spinal cord and peripheral nerves.

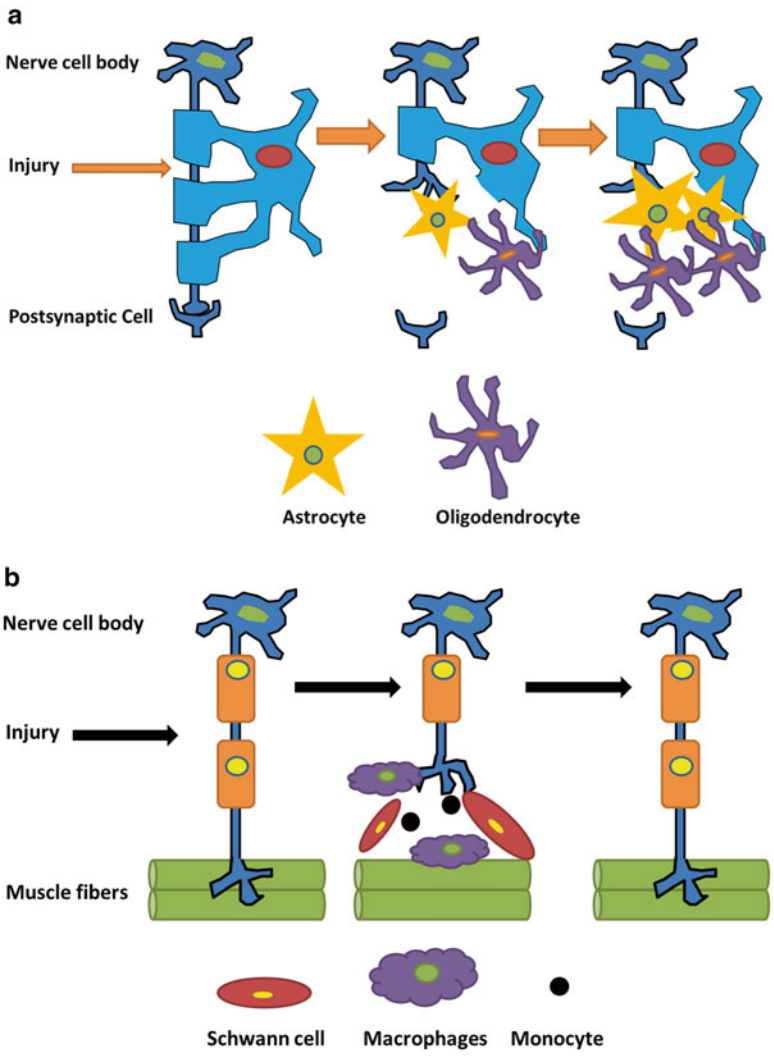

Fig. 3.2 Schematics of injured nerve regeneration in the central and peripheral nervous systems. (**a**) Central nervous system recovery process with glial scar tissue formation and (**b**) peripheral nervous system recovery process involving the activity of Schwann cells, macrophages, and monocytes (Image are adapted and redrawn from previous work) [37, 38]

3.1.2.1 Nanofiber Materials

A leading material for mimicking ECM properties for neural tissue is nanofibers due to their material characteristics and size. Within neural tissue engineering, nanofibers are usually broken up into two sub-categories (self-assembling and electrospun) depending on the materials synthesis. Below are *in vivo* experiments divided by the nanotechnology materials used.

3.1.2.1.1 Self-assembling Nanofibers

A self-assembling nanofiber is composed of a peptide molecule that can instinctively aggregate from an aqueous solution into a stable nanofiber network due to non-covalent interactions in the presence of a salt solution or by changing the pH. These molecules are formed from a hydrophobic tail and a hydrophilic peptide head, which will self-assemble into a cylindrical nanofiber in which the hydrophobic tail will form the core of the fiber and the hydrophilic peptide head will be present at the surface of the fiber [39]. Also, the peptide terminus can be designed to incorporate specific functional ligands that can enhance the surface of the nanofiber scaffold: one can add bone marrow homing proteins [40], insulin growth factors [41] or many other cell enhancing systems [40].

In vivo research has demonstrated the benefits of using self-assembled nanofibers in neural tissue engineering parameters. RADA16-I (reassembly of an ionic self-complementary peptide RADARADARADARADA that forms a well-defined nanofiber scaffold, the 16-residue peptide forms stable β-sheet structure and undergoes molecular self-assembly into nanofibers) has been shown to repair a disordered optic track and return practical vision [42], help in the reconstruction of the lost tissue in acutely injured brains [43] and bridge the injured spinal cord site of rats after transplantation [44].

Upon adding an IKVAV laminin epitope to the peptide-amphiphile self-assembling nanofibers, then injecting these molecules into a mouse spinal cord lesion, reduced astrogliosis resulted – an irregular surge in the number of astrocytes due to various types of injury caused by physical, chemical, and pathological trauma [45] causing neural cell death, and increased Schwann cells at the site of injury [46]. Furthermore, this technique promoted the regeneration of both sensory and motor fibers through the lesion site and indicated greater behavioral improvements via the use of the IKVAV laminin epitope.

3.1.2.1.2 Electrospun Nanofibers

Electrospinning is a process by which an electric field is used to cause a viscous polymer solution to overcome surface tension and create a charged jet that travels through air [47]. The solvents evaporate from the liquid jet as it travels through the air, leaving a charged fiber that can be manipulated by an electric field. This method has been shown to be able to produce nano-fibers can be used in a wide array of

fields, from filters, sensors, textiles, and clothing, to drug delivery systems, medical devices and tissue grafts [48]. It has been shown that electrospun nano-fibers mats that can have similar surface morphology and architecture to cell extracellular membranes found in the body [49], thus they are good candidates for tissue grafts.

In vivo studies have shown the possible uses of electrospun nanofibers in the neural tissue engineering. Electrospun materials made out of chitosan [50], layering systems [51], poly-caprolactone (PCL)/poly-lactic-co-glycolic acid (PLGA) blends [52] and a copolymer of PCL and ethyl ethylene phosphate with encapsulated glial cell-derived neurotrophic factors (GDNF) [52] have all been used in a rat model of sciatic nerve transection with positive results ranging from the promotion of the ingrowth of connective elements to increasing neural cell processes.

Also, Rochkind et al. showed when an electrospun tubular scaffold containing bundles of parallel nanofibers made of a biodegradable dextran sulfate-gelatin co-precipitate seeded with human nasal olfactory mucosa cells or human embryonic spinal cord cells was implanted for the regeneration of a rat spinal cord, the nano-material promoted the partial recovery of function in one to two limbs just 3 months after implantation [53].

3.1.2.1.3 Carbon Based Nanofibers

Despite concern over toxicity, carbon based nanomaterials have attracted wide neural medical attention due to their material characteristics such as conductivity, high strength and biologically inspired size. Webster and colleagues imbedded neural stem cells (Wistar rats, isolated for later implantation from 1 to 3 day old neonate) into carbon nanofibers to see if neural capabilities in rats could be reestablished after an induced stroke.

In vivo preliminary results demonstrated that unfunctionalized carbon nanofibers/nanotubes can: (1) conduct electricity when implanted into damaged, non-conductive, regions of the brain; (2) mimic the nanometer features of key proteins found in neural tissue (such as laminin) to promote neuron functions and decrease glial scar tissue formation; (3) anchor stem cells to promote their differentiation into neurons when implanted into damaged regions of the brain; (4) remain non-toxic and non-migratory when used at low concentrations in the brain; and (5) most importantly, return motor skills to stroke-induced rats at least three times faster than injecting stem cells alone.

Furthermore, it was shown that only the neural stem cells impregnated with carbon nanofibers differentiated into neurons (Figs. 3.3 and 3.4). This may be due to: (1) high electrical conductivity of carbon nanofibers, (2) high surface reactivity of carbon nanofibers which according to *in vitro* studies increased the adsorption of laminin from serum, and/or (3) size similarities to laminin (70 nm cruciform protein). Moreover, this could be because the carbon nanofibers allowed for a favorable substrate for the neural stem cells to adhere to, thus, keeping them in the local environment (i.e., anchoring them) so that they could be stimulated to differentiate into neurons by the conductive carbon nanofibers.

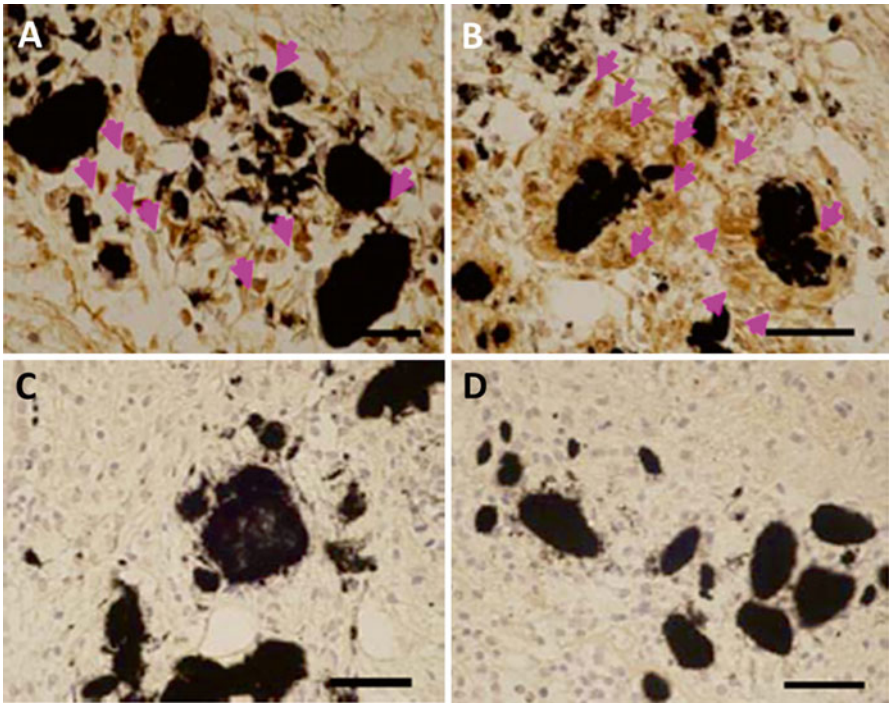

Fig. 3.3 Increased stem cell differentiation into neurons after 3 weeks implantation of hydrophobic carbon nanotubes and stem cells into stroke damaged neural tissue of rats. Immunostaining of stroke damage brain sections where stem cells and carbon nanotubes (*black*) were implanted. (**a**) Nestin = marker for stem cells; (**b**) MAP2 = marker for neurons; (**c**) GFAP = marker for astrocytes; and (**d**) CD11b = marker for meningeal cells. All samples were double stained with BrdU. *Dark brown* (indicated by *pink arrows*) show positively double stained neurons with no scar tissue forming cells (astrocytes and meningeal cells) surrounding carbon nanotubes. Bars = 50 μm. Similar results shown up to 8 weeks [54]

3.1.3 Wound Repair Studies

Wound repair or healing is a process in which the skin, or other organ-tissue, repairs itself after injury [55] both unintentional (falling off a bike and scraping one's hand) or intentional (surgery). In normal skin, the epidermis and dermis exists in an equilibrium state, forming a defensive barrier against the external, or outside, environment. Once the barrier is broken, the physiological process of wound "healing" begins. This process is expressed in four sequential and overlapping phases: (1) hemostasis, (2) inflammatory, (3) proliferative and (4) remodeling [56]. Through the advent of nanotechnology, nanomaterials have been able to help in the wound repair process by affecting all of these overlapping phases as described latter.

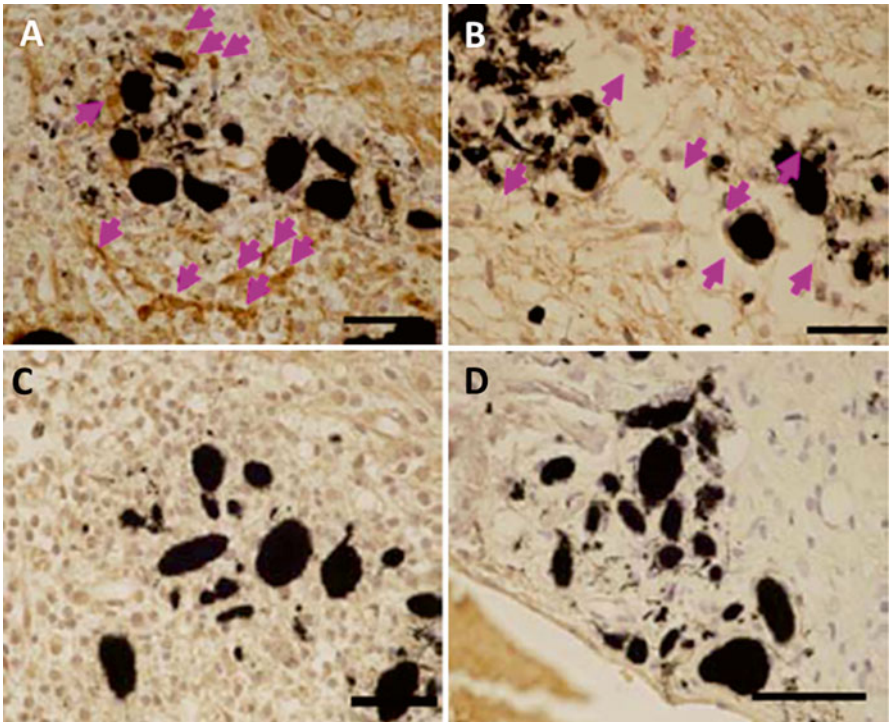

Fig. 3.4 Increased stem cell differentiation into neurons after 3 weeks implantation of hydrophilic carbon nanotubes and stem cells into stroke damaged neural tissue of rats. Immunostaining of stroke damage brain sections where stem cells and carbon nanotubes (*black*) were implanted. (**a**) Nestin = marker for stem cells; (**b**) MAP2 = marker for neurons; (**c**) GFAP = marker for astrocytes; and (**d**) CD11b = marker for meningeal cells. All samples were double stained with BrdU. *Dark brown* (indicated by *pink arrows*) show positively double stained neurons with no scar tissue forming cells surrounding carbon nanotubes. Bars = 50 μm. Similar results shown up to 8 weeks [54]

3.1.3.1 Nanocomposites

Nanocomposites have been used in wound repair to help in the promotion of tissue growth after surgery. An *in vivo* study using a BMP-2 coupled nanosilver-PLGA composite (BMP-2/NS/PLGA) showed that one could help in bone graft therapy. Metallic nanosilver (20–40 nm) combined with PLGA composites and BMP-2 were implanted into 16–18 week old SD rats with infected femoral defects [57]. After 12 weeks, inspection of the bone graft and wound, silver composite grafts were able to heal the femoral defect implanted with BMP-2 coupled with 2 % nanosilver particles, whereas the PLGA BMP-2 failed to heal the defect, thus provided evidence that nanomaterials can help in wound healing.

Nanocomposite *in vivo* studies have also demonstrated wound healing in gastrointestinal tissue. For example, gastrointestinal perforation is one of the major causes of bacterial peritonitis that usually leads to severe sepsis [58]. Postoperative anastomotic breakdown, which is one of the major complications after gastrointestinal operations, and can also cause bacterial peritonitis [59]. Therapeutic treatment by suture repair of a perforated/leaked lesion is crucial for wound repair, but not always foolproof. One way of helping this predicament is the use of nanotechnology. Fujie et al. showed by creating and transplanting a nanocomposite/sheet made of polysaccharides with a thickness of tens of nanometers into a rat's punctured murine cecum after 2 weeks, one could promote the growth fibroblasts on the muscular surface and completely seal the hole [60]. This was also shown with nanosheets composed of Tetracycline(TC) sandwiched between a poly(vinylacetate) (PVAc) layer and polysaccharide nanosheets. It was shown on mice that the growth of fibroblasts around the site of injury, lipocytesor fibroblasts specifically, bridged the tissue-defect site without any associated inflammatory reactions, resulting in an almost complete regeneration of the mucosal defect [61].

3.1.3.2 Nanofiber Materials

Again, nanofiber materials are usually defined as fibers with at least one dimension ≤ 100 nm and can be made of many different materials [24]. In the tissue engineering realm, the appeal of nanofibers is their structural similarity to native extra cellular matrix (ECM) – this network creates a dynamic, three-dimensional microenvironment in which cells are maintained.

In wound healing, nanofibers have aided medicine by creating sutures (a stitch used to hold tissue together) made of nano-systems. One such *in vivo* trial loaded cefotaxime sodium as a model drug into poly(L-lactic acid) (PLLA) using two different electrospinning methods – blend and coaxial electrospinning, which were collected on a grounded rotating edge-sharpened disk collector with a diameter of 280 mm and a maximal angular velocity of 1500 rpm to collect the aligned fiber to obtain fiber diameters between 238 and 965 nm [62]. Finally, the nanofiber sutures were fabricated using a mini type braiding machine, followed by dipping into a chitosan solution for 0.5 h and drying at ambient conditions to obtain a 0.35 ± 0.05 mm material, which is comparable to the diameter to most commercial sutures [62]. Using SD rats, the researchers implanted four different material sutures (blend PLLA-cefotaxime sodium, core-sheath PLLA-cefotaxime sodium, commercial silk and commercial PLLA sutures) and looked at tissue morphology around the wound site. After 3 days of implantation, it was found that conglutination and edema phenomena occurred more frequently in the wound area of the silk and the core-sheath suture groups, whereas the PLLA and the blend suture groups resulted in comparatively smooth anastomosis. These results show that by adding a "nano" approach to wound healing one could promote the process of wound healing.

Another *in vivo* study showed the use of nanofiber sutures to decrease skin dehiscence in rats after 1 week of implantation. This was done by creating materials with

biodegradable poly(ester urethane) urea (PEUU) and poly(lactic-co-glycolic) acid (PLGA), where PLGA was loaded with antibiotic tetracycline hydrochloride and electrospun – where the fiber diameters for the PLGA tetracycline hydrochloride materials were between 100 and 200 nm and the PEUU fibers were around 390 ± 120 nm [63]. Materials were then combined to create a nanofiber composite with a weight percentage of 10 % tetracyline added; known as PEUU-PLGA-tet-10 and implanted into a contaminated abdominal wall from adult female Lewis rats. After 1 week of implantation, the rats were sacrificed and using digital image processes of images of the suture, it was shown that skin dehiscence decreased by 21 % using the PEUU-PLGA-tet-10 versus plain PEUU and abscess formation was not observed in the PEUU-PLGA-tet-10 nanofiber composites, whereas moderate to severe abscess formation was observed in the plain PEUU system.

3.1.3.3 Nanoparticles

Nanoparticles are usually defined as particles with sizes between about 1 and 100 nm that show properties not found in bulk samples [64]. Within the wound healing realm, nanoparticles have shown promising *in vivo* results.

Greenhalgh and Turos studied the use of antibiotic-conjugated polyacrylate nanoparticles on rats for enhanced wound healing on dermal abrasions. The study demonstrated favorable activity of the nanoparticle channeled drug system for systemic or topical application in a murine model. It was also shown that both routes were promising, and when topically applied, the emulsion enhanced wound healing by an average of 3–5 days [65].

Silver nanoparticles have been used on an array of applications ranging from wound dressings [1, 66, 67] to household products like socks and deodorants [68] or paints [69] due to their antimicrobial activity. One such *in vivo* study by Tian et al. showed that silver nanoparticles accelerate wound healing and achieve superior cosmetic outcomes [67]. It was shown in thermal injury mice model, the deep partial-thickness wounds normally healed after 35.4 ± 1.29 days, but in animals treated with silver nanoparticles, healing took place in 26.5 ± 0.93 days. Also, when comparing the appearance of healed wounds it was found that wounds in the silver nanoparticle group showed the most resemblance to normal skin, with less hypertrophic scarring and nearly normal hair growth on the wound surface.

Furthermore, wound healing was also investigated in diabetic mice. In this model, excised wounds treated with silver nanoparticles completely healed in 16 ± 0.41 days after injury, whereas mice in the control group (no nanoparticles) required 18.5 ± 0.65 days [67]. In the nondiabetic littermates, silver nanoparticles still accelerated wound healing relative to the control group.

Using silver nanoparticles and S-nitrosoglutathione (GSNO) to treat burn wounds, Melo et al. also showed promotional wound healing [70]. Shaved dorsum rats were exposed to intense heat (90 °C) and burned, then the nanoparticle compounds were topically administered immediately and up to 28 days after the burn injury, four times a day. Looking at toxicity, results showed no significant difference

in level of urea, creatinine, aminotransferases, and hematological parameters in the control burn groups and treated burn group – indicating no substance induced toxic effects in the kidneys and/or the liver.

3.1.4 Other Tissue Engineering Applications

Due to nanotechnology's vast implications for tissue engineering, *in vivo* experiments with nanomaterials are being conducted on many different organs and cell types. Below are a few examples of *in vivo* experiments where nanotechnology is being utilized.

3.1.4.1 Vascular System

For vascular applications, nanotechnology has fit very well due to the fact that nanomaterials are able to support cellular attachment for endothelial or other cells on the graft surface to avoid platelet adhesion and thrombus formation as well as resist shear stress and the pressure of the blood stream. *In vivo* experiments have shown that one of the suitable purposes of nano-scaffolds for vascular grafts is aligned nanofibers that achieve the structural properties of the endothelium with elongated vascular endothelial cells [71]. For example, aligned Poly-L-Lactide Acid (PLLA) nanofibrous scaffolds used as an artery bypass with or without mesenchymal stem cells allowed the infiltration of vascular cells and matrix remodeling while the grafts with mesenchymal stem cells showed anti-thromogenic properties [71].

3.1.4.2 Cardiac System

Stem cell therapy for heart attack (myocardial infarction) and chronic ischemic heart disease has grown significantly in the past decade, but the benefits of cell therapy have still not been clearly shown. A new approach that has gained attention is the use of nanotechnology, via nanofibers to enable the local intramyocardial release of stem cells, growth factors and/or chemokines [72]. Using male SD rats, Segers et al. were able to modify a SDF-1 chemokine that was resistant to matrix metalloproteinase-2 and exopeptidase cleavage tethered to self-assembling nanofibers RADA16-II which promoted the chemotactic recruitment of stem cells and improved cardiac functions after a myocardial infarction.

Another such example used insulin growth factor IGF-1, a cardiomyocyte growth and differentiation factor, which was bound to biotinylated peptide nanofibers in a sandwich method [41]. *In vivo* results showed that IFG-1 bound to biotinylated peptide nanofibers, when injected into the myocardium, enabled the local delivery of IGF-1 and, in grouping with transplanted cardiomyocytes, reduced apoptosis and increased the growth of the transplanted cells.

3.1.4.3 Visual System

Research has shown that limbal stem cells are a stem cell population responsible for renewing and regenerating the corneal epithelium. Just like the cardiac system, the use of nanotechnology, via nanofibers, can be used as a transportation vessel to enable the local release of limbal stem cells and growth factors [73]. A recent study, using rabbits, implanted an electrospun nanofiber scaffold to transfer corneal epithelial or oral mucosal cell sheets which improved corneal healing and inhibited the local inflammatory reaction [74].

3.2 Nanotechnology for Improved *In Vivo* Antimicrobial Response

Medical device infections can be frequent and costly depending on the device location and the duration of use. Yet the benefits from these devices outweigh this low probability detriment and therefore continue to be used clinically. For example, peripheral or central intravenous catheters (CVCs) resulting in bloodstream infections (BSI) occur in about 4–5 out of every 1,000 CVC devices inserted [75, 76] with an attributed cost per infection estimated at US$34,508–$56,000 [77, 78], and the annual cost of caring for patients with CVC-associated BSIs ranges from $296 million to $2.3 billion [79]. However, CVCs are necessary for the delivery of fluids and medication or for monitoring patient health (such as through the drawing of blood or monitoring of blood pressure).

In addition to transcutaneous extracorporeal devices or other medical devices that are constantly exposed to the nonsterile environment outside the body, implanted tissue replacement devices are also susceptible to infections, resulting in implant failure. For example, prosthetic joint replacements are permanently implanted to alleviate pain, promote mobility, and improve the quality of life, but such implantations also suffer from the risk of infection, which occurs in about 1–1.5 % of all total hip and knee arthroplasties (THAs and TKAs, respectively) in the USA [80]. Although the chance of infection is rare in these procedures, the problem is significant, as periprosthetic implant infections, which are also known as septic failures and cost about US$70,000 per episode, are the most common cause of revision surgery in all TKAs (25 %), the third most common cause in all THAs (15 %), and the most common reason for removal of all TKAs (79 %) and THAs (74 %) [80–82]. Prosthesis device infections are some of the most striking medical device infections due to the widespread use of prosthesis devices, but other implanted medical devices, such as intrauterine devices, mechanical heart valves, pacemakers, tympanostomy tubes, and voice prostheses, can similarly suffer from infection, and could benefit from new techniques to stop infections [83].

Towards the goal of infection prevention, *in vivo* studies are also being conducted to examine the implications of nanotechnology in response to microbial challenges. This growing direction in tissue engineering focuses on improved

antimicrobial response in biomaterials, simulating the immune response of the body which is compromised by the placement of current engineered materials. As many of these *in vivo* studies focus on tissues, this section will also be broken down by the tissue investigated and nanomaterial used.

3.2.1 Wound Repair

3.2.1.1 Antimicrobial Nanofeatured Biomaterials

An overwhelming number of studies focus on improving the wound healing response of materials *in vivo* (Table 3.1). One such method incorporates antimicrobials or antibiotics into nanofeatured tissue engineering devices. In one such work, the authors developed braided drug-loaded nanofibers for suturing and wound repair [62]. Structural applications of electrospun nanofibers were explored for use in sutures. Loading of cefotaxime sodium (CFX-Na) as a model drug into poly(L-lactic acid) (PLLA) was also carried out using two different electrospinning methods, i.e. blend and coaxial electrospinning, which were collected on a grounded rotating edge-sharpened disc collector with a diameter of 280 mm and a maximal angular velocity of 1,500 rpm to collect the aligned fibers to obtain fibers with diameters of between 238 and 965 nm having an average size of 667 nm. Post processing of the fibers using a combination of twisting and hot-stretching at 50 °C with a tension ratio of 50 % to release internal tension and improve the size stability of the materials. Sutures were fabricated using a minitype braiding machine, followed by dipping into a chitosan solution for half an hour, and finally drying at ambient conditions to obtain 0.35 ± 0.05 mm material comparable in diameter to most commercial 2–0 sutures.

Table 3.1 Major players in wound healing (Adapted from [84])

Cell type	Source	Timing and behavior
Keratinocytes	Epidermal wound edges and cut appendage stumps	Migration commences after a brief lag phase
Fibroblasts/ myofibroblasts	Connective tissue wound edges and fibrocytes from circulation	Invasion to form wound granulation tissue commences early, but transformation into myofibroblasts is later
Endothelial cells	Nearby blood vessels	Vasculature near to wound site becomes activated rapidly to allow diapedesis, but sprouting is later
Platelets	Spill from damaged blood vessels	Immediately at the site of tissue damage
Mast cells	Small numbers resident in tissue; others by migration through the pores in blood vessels, (diapedesis) from adjacent vessels	Appear to have an early role in regulating the later inflammatory response by neutrophils
Neutrophils	Diapedesis from adjacent vessels	Earliest of the leukocytes to derive from the blood
Macrophages	Some tissue-resident cells but majority derive from blood-borne monocytes	Secondary influx from the blood after neutrophils have killed the immediate foreign-organism invaders

Antibacterial efficacy against *Staphylococcus aureus* (*S. aureus*; AATCC 6538) and *Escherichia coli* (*E. coli*; AATCC 8099) was carried out using a zone of inhibition study [62]. Zones of inhibition diameters of 28.32–36.29 mm were found with an *S. aureus* challenge using blend sutures and core-sheath sutures respectively, versus 25.33 and 26.12 mm for the same materials challenged with *E. coli*. The minimum inhibitory concentration (MIC) for all strains of CFX-Na-sensitive bacteria was 1.4 and 0.15 μg/ml for *E. coli* and *S. aureus*, respectively [85].

The next goal of the sutures was to determine if the materials could keep a wound closed, the most important function of a suture [62]. For this, four types of sutures were implanted including blended PLAA-CFX-Na sutures, core-sheath PLLA. CFX-Na sutures, commercial silk sutures, and commercial PLLA sutures. Silk is the most commonly used surgical suture material, and can be used in a variety of suturing and legating procedures, but is known to be non-absorbable, meaning the material does not lose tensile strength after 60 days of implantation *in vivo* as caused by material breakdown, causes a foreign body response, a type of chronic immune response to implanted biomaterials, and is more susceptible to infection [86]. Therefore, controlled absorbable sutures are desirable if the process is well controlled, especially if prevention of infection is possible.

For an *in vivo* study, the researchers looked at inflammation, tissue morphology (biocompatibility), and bacterial response [62]. After 3 days of implantation, it was found that conglutination and edema phenomena occurred more frequently in the wound area of the silk and the core-sheath suture groups, whereas the PLLA and the blend suture groups results in relatively smooth anastomosis. These results indicated that the commercial PLLA suture group and the blend suture groups had better performance than the silk group and the core-sheath suture group. This could be explained by the burst release of antibiotics by the blended suture group, reducing inflammation caused by infection, whereas blend sutures released antibiotics more slowly (as observed *in vitro*). Other studies have also shown that PLLA sutures could cause less of an inflammatory reaction compared with the silk sutures [87]. After 4 weeks of implantation, the wounds healed completely with all suture groups.

Previous studies have indicated that low inflammatory reactions should be characterized by an increased ratio of fibroblast cells over inflammatory cells, or by reduced number of macrophages [88]. The biocompatibility study found silk sutures caused a greater foreign body reaction, with macrophages, whereas blend and core-shell structures had better performance in inflammation reduction [62]. This could be explained by fewer lymphocytes around the blend and core-sheath sutures as compared to the commercial silk or PLLA sutures. Secondly, more fibroblasts were found in the blend and core-sheath sutures, which is an indication of favorable tissue recovery.

In the final study by these researchers, the four sutures were evaluated for bacterial contamination [62]. After the wound was re-opened, a muscle sample was taken from the animals, homogenized, and 100 uL was taken and diluted in physiological saline. Then, 100 ul of the diluted suspension was transferred onto an LB plate and subsequently cultured in an incubator at 37 °C for 12 h. The number of bacterial colonies was calculated to obtain the bacterial count, and it was express as the number of bacteria present in 1 g of the musculature. Each experiment was completed with five replicated, and the results were expressed as an average.

Interestingly, this study found that the musculature was asepsis prior to suture implantation, and 3 days after implantation the quantity of bacterial colonies became the largest [62]. As time increased, the number of colonies decreased and after 28 days, few bacteria were observed due to the wound being gradually restored. The authors observed that commercial silk suture group had a serious bacterial infection, while the drug-loaded sutures had preferable performance. Quantification by the authors showed that the actual difference between bacterial colony counts was about 26,000 for the silk, 23,000 for the PLLA groups, 19,000 for the core-sheath, and 17,000 for the blend sutures respectively after 3 days. Although the differences between the sutures became more modest as time proceeded to 7, 14, 21, and finally to 28 days, bacterial contamination was consistently lower. These results were also consistent with the lower inflammatory response observed with the nano-fabricated blend and core-sheath sutures as compared to silk and PLLA commercial products.

3.2.1.2 Antimicrobial Nanoparticles Improve Wound Repair

Nanoparticles are also being used as an injectable or topical to improve tissue responses and wound healing. In one set of *in vivo* studies, polyacrylate nanoparticle emulsions were used for topical wound healing or systemic applications [65]. This study found that the antibiotic-conjugated polyacrylate nanoparticles had activity against MRSA *in vitro* and no cytotoxicity against human dermal cells. The water-based emulsion is capable of solubilizing lipophilic antibiotics for systemic administration, and has shown to protect antibiotics from hydrolytic cleavage by penicillinases, thus improving the activity of penicillin against MRSA. This study demonstrated the favorable activity in murine models of the drug for systemic application by intraperitoneal injection or topical application in a wound abrasion model. *In vivo*, both routes were favorable, showing no signs of inflammation, such as redness or abnormal cytokine release, and when topically applied, the emulsion enhanced wound healing by an average 3–5 days. These nanoparticles might therefore afford promising opportunities for treating both skin and systemic infections. It has also been found that topical delivery of silver nanoparticles promotes wound healing [67]. The beneficial effects of silver nanoparticles on wound healing were investigated in an animal model, and it was found that rapid healing and improved cosmetic appearance occur in a dose-dependent manner. Silver nanoparticles also show antimicrobial properties resulting in a reduction of wound inflammation and modulation of cytokines, a potential explanation of these beneficial outcomes in tissue regeneration.

3.2.1.3 Biomaterials Coupled with Antimicrobial Nanoparticles

Nanoparticles are also being used as a component in biomaterials to improve the antimicrobial response. In one such work, *in vivo* wound healing and antibacterial performances of electrospun nanofiber membranes, materials for wound healing and preventing infection, were fabricated [89]. This study looked at PVA, PCL, PAN,

and other polymers using an electrospinning technique, and the wound-healing response was examined *in vivo* using female SD rats. Either wool protein or silver nanoparticles were incorporated into nanofibers. It was found that wound healing performance was mainly influenced by the porosity, air permeability, and surface wettability of the nanofiber membranes. Interestingly, fiber diameter and antibacterial activity had little effect on wound healing efficiency. Instead, nanofeatured mats were predicted to inhibit exogenous bacterial infiltration via a sieve effect, whereby the multi-layered structure featured nanometer pore sizes smaller than bacteria. Simultaneously, hydrophilic and nano-porous materials effectively removed excess fluids from the wound, which the authors concluded may further reduce the chances of infection. Yet, a limitation to this study is that the authors did not model a contaminated wound site, such as could be caused during accidental injury or surgical intervention.

Waterborne polyurethane-silver nanocomposites have been used to fabricate biomaterials for preventing infection in catheters & subcutaneous wounds [90]. Using polyurethane (PU) with nanosilver (~5 nm size), the material could be loaded up to 30 ppm with a good dispersion, as confirmed by transmission electron microscopy, whereas at higher concentrations the nanosilver aggregated (Fig. 3.5). Important previous observations using nano-gold and nano-silver contributed to the design of this study through determination that the nanoparticle component improved material properties such as microphase separation on the surface of H_{12}MDI-based PU for enhanced the biostability and biocompatibility *in vitro* and *in vivo*, with nanosilver at 30 ppm being more effective [91]. In conjunction with similar materials property improvements also found in this study, the nano-components enhanced fibroblast attachment and endothelial cell response, while allowing a reduction in monocyte and platelet activation, relative to PU alone or nanocomposites with other silver concentrations, and biocompatibility was confirmed in a subcutaneous rat model. The adhesion of *Bacillus subtilis*, *E. coli*, or Ag-resistant *E. coli* on PU-Ag nanocomposites was significantly lower at all concentrations of nanosilver tested, in addition to bacteriostatic ability, whereas PU did not. Commercial catheters were also coated with PU-nanosilver at 30 pmm, and these were inserted into rat jugular veins for evaluation. The results indicated milder inflammation after 3 months, with the wall thickness of the veins being smaller than that with the commercial catheters or the pure PU-coated catheters. Finally, it was concluded that the PU-nanosilver could reduce vein occlusion and improve blood compatibility through an overall better anti-fouling response, and should also be considered as a cardiovascular biomaterial.

3.2.2 Bone Defect and Fracture Repair

A concern about bone tissue infection, termed osteomyelitis, has prompted researchers to incorporate antimicrobial aspects into bone tissue engineering systems. In one such study, the use of BMP-2 coupled with nanosilver-PLGA composite grafts to

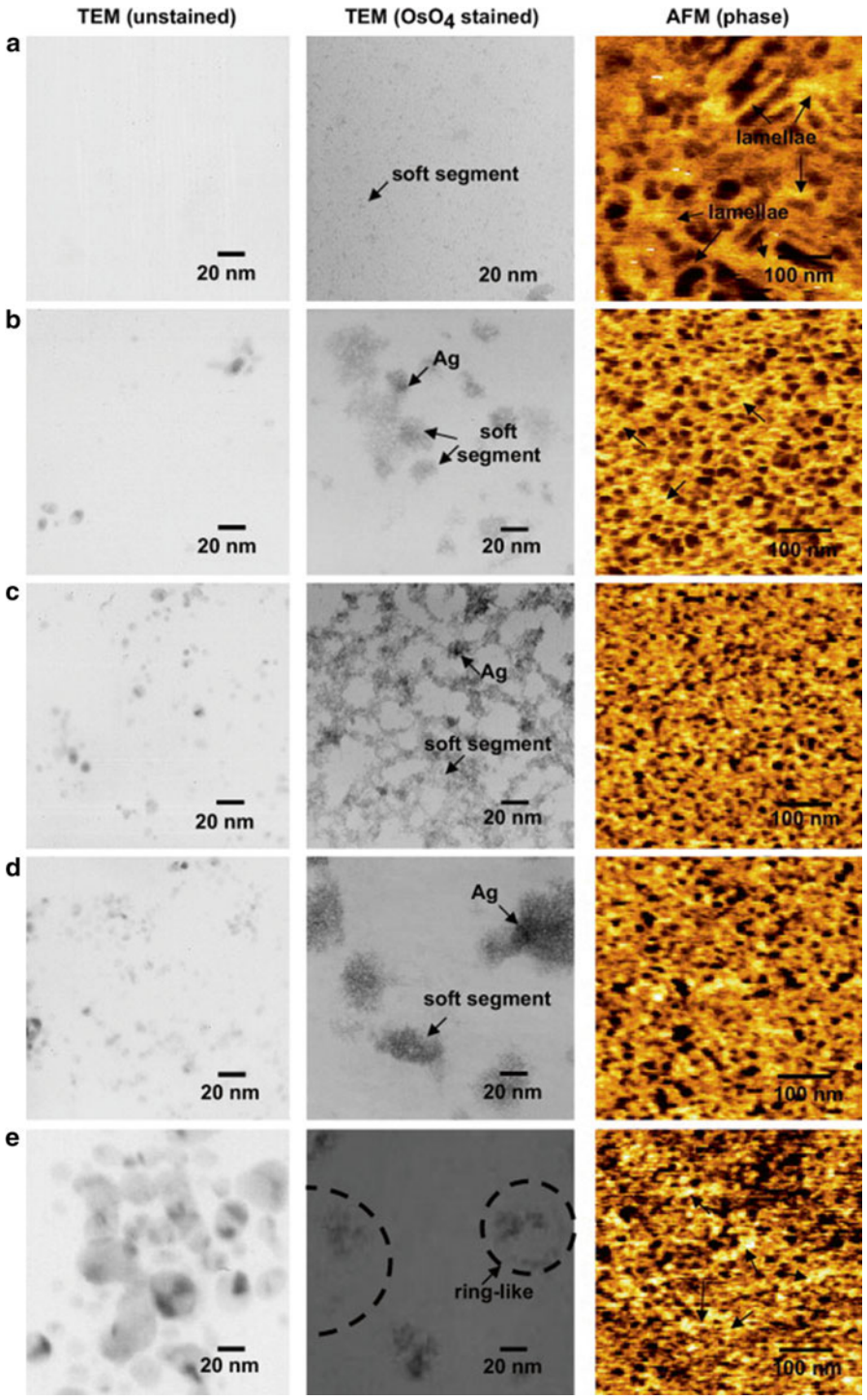

Fig. 3.5 TEM images of unstained or OsO_4 stained samples and AFM phase images for (**a**) the pure PU as well as the PU–Ag nanocomposites containing (**b**) 15 ppm, (**c**) 30 ppm, (**d**) 50 ppm or (**e**) 75 ppm, of nano Ag. *Arrows* in AFM phase images indicate the presence of lamellae [90]

induce bone repair in grossly infected segmental defects used materials for treating infection and healing bone defects [57]. The authors of this study used metallic nanosilver (20–40 nm) combined with PLGA composite grafts. To make grafts, the authors mixed PLGA with various weight percentages of nanosilver (with respect to PLGA), and poured the mixture over a bed of sieved sugar particles (200–300 μm) to generate a paste. This process was used to generate porosity in the structure after removal of the sugar particles via soaking in water. The paste was stacked in a Teflon mold to generate cylindrical grafts, dried for 12 h, lyophilized for 4 h, and washed with distilled water to leach out the sugar, and finally sterilized with 70 % ethanol and further dried. Bone morphogenic protein 2 (BMP-2), an osteoinductive protein used to repair many bone fractures and bone defects, was also injected into some grafts to prepare BMP-2/NS/PLGA bone grafts. The authors used a vancomycin and methicillin resistant clinical strain of *S. aureus*, Mu50, and cultured the microorganisms in the presence of grafts with up to 2 % nanosilver. The authors found that with an *in vitro* challenge of 10^7 CFU/graft, 0.1 % nanosilver resulted in only a slight decrease of growth, a 0.5 % nanosilver graft slowed the emergence of exponential phase by 13 h, and weight percentages of 1 % or higher completely inhibited growth. Using a higher inoculate of 10^8 CFU/graft, 0.1 % and 0.5 % nanosilver had no significant effect, 1 % and 1.5 % slowed the emergence of exponential phase by 10 and 15 h respectively, and 2 % completely inhibited growth. Using this information, the authors decided to use an *in vivo* rat femoral segmental defect model, and with PLGA grafts of 0 %, 1 %, or 2 % nanosilver implanted into SD rats along with an injection of 10^8 CFU (results of this study shown in Fig. 3.6). The authors found that the grafts did not inhibit adherence, proliferation, alkaline phosphatase activity, or mineralization of on growth MC3T3-E1 pre-osteoblasts compared to PLGA controls, or the osteoinductivity of BMP-2. The silver composite grafts were able to heal a femoral defect implanted with the BMP-2 coupled with 2 % nanosilver particles-PLGA were the BMP-2 in PLGA grafts without silver failed to heal the defects.

A very innovative method utilizing nanotechnology to improve antimicrobial response simultaneous to bone tissue engineering is the combination of immune cell regulating functions into a scaffold material. In one study using this concept, multilayer polypeptide nanoscale coatings incorporating IL-12 was used for the prevention of biomedical device-associated infections and fracture repair [92]. This study incorporated IL-12 (an immunoregulatory cytokine) into nanoscale coatings using electrostatic layer-by-layer self-assembly. Specifically, IL-12 activates natural killer cells and macrophages, leading to phagocytosis of bacteria. The coatings were made of albumin, PLL, and PLGA, which was coated onto materials such as quartz, stainless steel, and titanium. When the coating was applied to stainless steel K-wires, the authors found that IL-12 was released *in vitro*, with most of the coating being released within 10 days. Coated wires were prepared and implanted into an open femur fracture model using SD rats to test for the *in vivo* coating stability. For this, six wires were prepared with three implanted and three maintained as controls. An *in vivo* bacterial challenge was also carried out, whereby upon fracturing of the femur, rats were injected with 100 μL suspension of 10^2 CFU/0.1 ml *S. aureus* directly into the site. The fractures were left open for 1 h, and then fixed using an intra-medullary

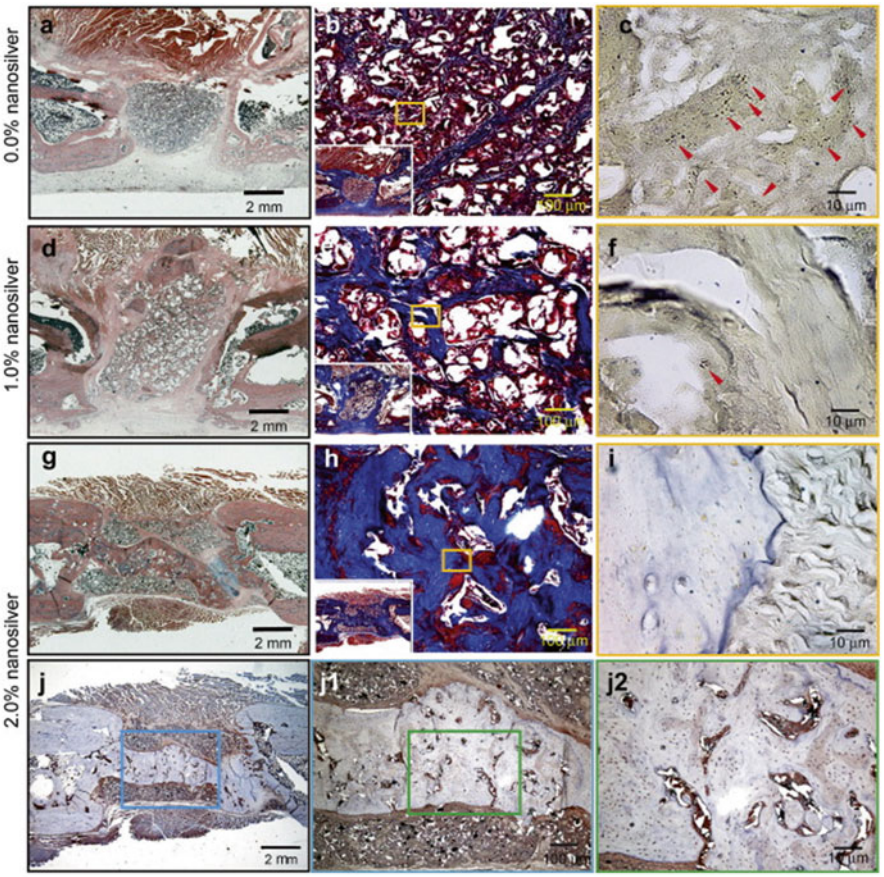

Fig. 3.6 H&E staining (**a**, **d**, and **g**), Masson's trichrome staining (**b**, **e**, and **h**), Taylor modified Brown and Brenn gram stain (**c**, **f**, and **i**) and immunostaining of OCN (**j**, **j1**, and **j2**) of 10^8 CFU *S. aureus* Mu50 contaminated rat femoral segmental defects implanted with 0.0 % (**a**–**c**), 1.0 % (**d**–**f**), and 2.0 % (**g**–**j2**) nanosilver-PLGA bone grafts coupled with 30 μg/ml BMP-2 at 12 weeks post implantation, respectively. Almost no bone regenerated in BMP-2/0.0 %-NS/PLGA (control BMP-2 coupled control PLGA) implanted groups (**a**, and **b**) with obvious continued bacterial contamination (**c**, *red arrows*). Less bone regenerated in the defect area of BMP-2/1.0 %-NS/PLGA implanted groups (**d** and **e**), while only limited bacterial colonies were observed (**f**, *red arrow*). BMP-2/2.0 %-NS/PLGA grafts promoted significantly greater bone formation to form a mineralized bony bridge between the two defect ends (**g**, **h**, and **j**) by eliminating bacteria in the defect area (**i**), Higher magnification figures show active bone regeneration around the mineralized bridge and in the marrow-like cavities in the bridge (**j1**, and **j2**) [57]

stainless steel K-wire. After quantitative colony counts, infections were defined as >2–5 bacterial colonies per plate (or 200–500 CFU per gram of tissue). Using this method, the authors found that IL-12 coated stainless steel K-wires with 10.6 ng dose or higher, as obtained by manipulating the concentration of the IL-12 loading solution and the number of coating nanolayers, had a lower rate of infection (20 % with 10.6 ng IL-12 versus 90 % for control).

3.2.3 Other Tissue Engineering Applications

Understanding of nanotechnology as a tool to improve antimicrobial response is growing as more nanomaterials are being developed and tested. Yet, few *in vivo* studies of nanomaterials have stepped outside of wound repair and bone repair towards improved bacteria inhibition on tissue engineering devices. Some exceptions are gastrointestinal and abdominal tissue repair which have been investigated and will be described below.

3.2.3.1 Gastrointestinal Tissue Repair

Nanotechnology has been used in the production of antibiotic-loaded nanosheets for the treatment of gastrointestinal tissue defects [60]. In a series of studies, ultrathin polymer films (nanosheets) composed of polysaccharides were used as a wound dressing in gastrointestinal tissue defects (See Fig. 3.7 for details about preparation of and tissue repair with nanosheets). To prepare these materials, the authors used a layer-by-layer assembly method using biocompatible and biodegradable chitosan and sodium alginate, whereby single layers, each approximately 29 nm, were prepared via electrostatic interactions by spin-coating on SiO_2 substrates [93]. Because the nanosheets are too thin to lift and use surgically alone, a water soluble polyvinyl alcohol (PVA) with a thickness of about 70 μm was incorporated, which could be dissolved upon placement at the site of injury by the simple addition of saline. These nanosheets showed favorable properties as a tissue engineering device sealing gastrointestinal or other tissues, acting as a physical barrier to bacteria, while attaining equal performance in sealing to sutures, with a less invasive procedure, and without postoperative adhesions [60, 61, 93]. Despite the sealing and healing effects, it was found that bacteria could penetrate through the wound dressing because of the ultrathin structures [60, 61]. To reduce bacterial penetration, tetracycline was incorporated between a polyvinyl-acetate (PVAc) layer and polysaccharide nanosheets [60].

The tetracycline was released for 6 h under physiological conditions. *In vivo* studies showed that overlapping therapy significantly increased mouse survival rate after cecal puncture, suppressing intraperitoneal bacterial count and leukocyte count, therefore showing the antibacterial and anti-inflammatory properties of the wound covering, and providing an efficient way for surgeons to repair tissues in various scenarios.

3.2.3.2 Abdominal Tissue Repair

Abdominal cavity coverage and wound repair is being investigated via elastic, biodegradable polyurethane/poly(lactide-co-glycolide) fibrous sheets with controlled antibiotic release via two-stream electrospinning materials were fabricated [63]. Materials made were a biodegradable poly(ester urethane) urea (PEUU) and a poly(lactide-co-glycolide) (PLGA), where PLGA was loaded with antibiotic

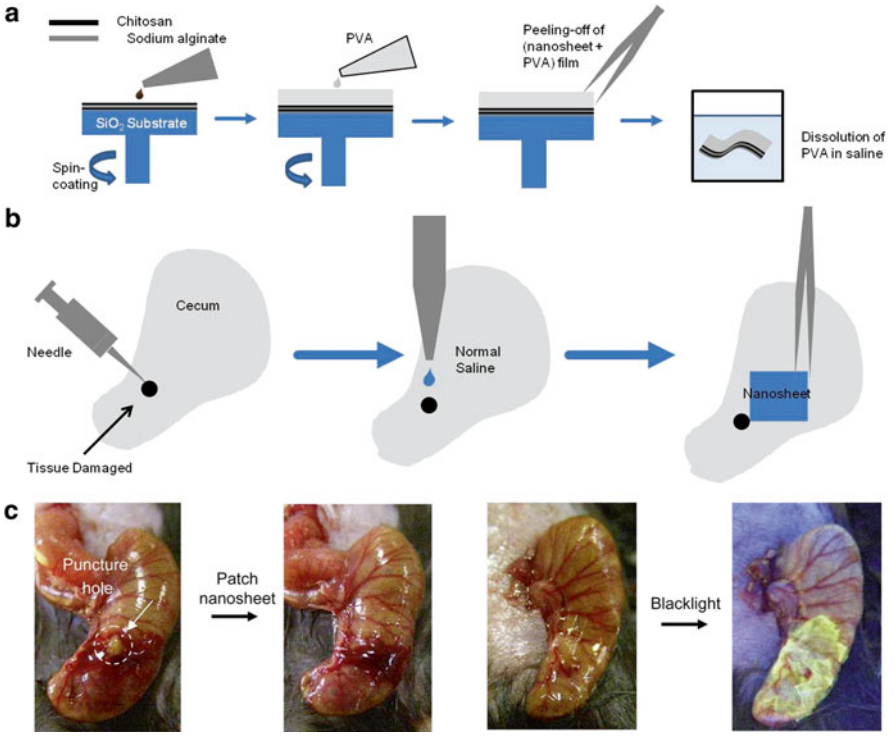

Fig. 3.7 Overview of the methods required for tissue repair using biocompatible and biodegradable nanosheets. (**a**) Nanosheets composed of chitosan and sodium alginate were prepared by layer-by-layer assembly on a SiO_2 substrate, a thicker 70 μm polyvinyl alcohol (PVA) layer allows handling with tweezers, and dissolution of the PVA layer takes place in normal saline. (**b**) After tissue damage caused by a needle puncture into the cecum, the defect site is prepared with normal saline, and the nanosheet is simply applied to the site of damage resulting in PVA dissolution and sealing. (**c**) Macroscopic images of tissue repair after treatment with antibiotic loaded nanosheets containing tetracycline (*TC*), where the TC nanosheets are viewed under a blacklight in the final image (the nanosheet is visible due to fluorescence of TC), thus preventing penetration of bacteria (Image in (**a**) and (**b**) are adapted and redrawn from previous work [60, 93])

tetracycline hydrochloride (PLGA-tet). Using electrospinning, it was possible to create a uniform blend of PEUU and PLGA-tet fibers with nanofeatures. The fiber diameters for the PLGA-tet materials were between 100 and 200 nm, and varied without an obvious trend dependent on the concentration of tetracycline added whereas PEUU fibers were larger with sizes of 390 ± 120 nm. The release profile was controlled, with an initial burst release of 3 h for PLGA or up to 96 h for PEUU-PLGA-tet, followed then by a slower release with 55–90 % remaining after 14 days for the PLGA-tet scaffolds and 0–65 % for the PEUU/PLGA-tet scaffolds remaining after the same period.

The samples were then incubated and growth inhibition of *E. coli* was tested for 24 h after prior incubation for 0, 3, and 7 days in PBS [63]. In this test, the authors

measured the "antibacterial diameters" using a method similar to zone of inhibition and were able to conclude that after incubation for 3 or 7 days, antibacterial activity tended to decrease, where only PEUU-PLGA-tet 10 (the weight percentage of tetracycline added was 10 %) was found to have a significantly larger antibacterial diameter than other composites. Using adult female Lewis rats, fibrous sheets were implanted into a contaminated abdominal wall model. This was achieved by first exposing the abdominal cavity through the creation of a 3 cm long, full-thickness incision approximately 2 cm inferior to the xiphoid process, followed by lavage of the peritoneal cavity, and finally injection of a rat stool slurry (0.25 ml) consisting of 1 g rat stool homogenized in 20 ml of normal saline. Using 7–0 polypropylene with over and over sutures, a 2.5×0.5 cm piece of either PEUU or PEUU/PLGA tet-20 sheet was interposed within the incision space ($n = 5$ for each sample), and closed with 4–0 Vicryl interrupted suture. The animals were sacrificed after 1 week implantation period. The implant was quantitatively assessed using digital image processing of images of the suture line for wound dehiscence. Following opening of the sutures, the implant sites were then qualitatively scored for the extent of abscess formation, or the amount of pus with resulting swelling and inflammation. Abscess formation was also not observed in the PEUU-PLGA tet-20 composite, whereas moderate to severe abscess formation was observed in the PEUU control sample. Using similar methods, it was found that skin dehiscence, or breaking open of the wound, was also decreased by 21 %, using PEUU/PLGA tet-20 versus PEUU.

3.3 Immune Response to Nanomaterials

While there may be several reasons why an implant may fail, inflammation of the implant area is both a major cause, and major indicator of implant failure. According to one study, in their set of 282 cases of failed stainless steel orthopedic implants, 10.6 % exhibited inflammation of the surrounding tissues [94]. Inflammation rates may change depending on the material implanted, location of implantation, and the function of the implanted device; however, inflammation is still a major issue to consider when developing a new implantable material or wound treatment. Implanted materials that cause excess inflammation lead to significant damage of the surrounding tissues and result in the loosening or total failure of the implanted material.

3.3.1 Inflammation Pathway

3.3.1.1 Foreign Body Response Summery

Although the Egyptians recognized inflammation by 1650 BC, it was not until 25 AD that the Roman Cornelius Celsius first defined the process by the observable features of redness, swelling, heat and pain that present with inflamed tissues on the body surface [95]. Inflammation is considered a normal part of healing, and is

closely linked to the activation of the immune system [95]. There are predictable stages to the inflammation/foreign body response that follows any injury or implantation. The first step is nonspecific protein absorption and adhesion of cells, such as monocytes, leukocytes and platelets [19]. This is followed by the formation of giant cells and cytokine release [19]. Neutrophils arrive on the scene very early after any tissue damage, and in an attempt to kill any microbes they may flood the damaged area with free radicals that kill many otherwise-healthy host cells as well as the target infectious agents [84]. This becomes a major issue in chronic wound situations, and may lead to chronic inflammation and scarring. In the case of implanted materials the implanted device may be encapsulated by fibrous tissue [19]. The materials that are considered to be biocompatible reduce the inflammation process by either avoiding the initiation of the foreign body response, by avoiding or reducing the initiation of one or more steps in the foreign body response, or by acceleration the process to reduce the inflammation period.

3.3.1.2 Inflammation in the Central Nervous System

In cases of injury to the central nerves system injury there is a rapid response from the astrocytes in the injured tissue, which may result in the formation of a gial scar, also known as an astrogliosis [96]. The formation of a gial scar may result in the interference of neural repair and the impedance of axon regeneration and extension [96]. Thus, in cases of materials to be implanted or used to treat injuries in the central nerves system it is important to use materials or techniques that will minimize inflammation and thus minimize scar formation.

3.3.1.3 Injectable Scaffolds

A good nerve conduit must be biocompatible and exhibit extremely low immunogenic, cytotoxic, and immunogenic responses. Studies by Chuna et al. have illustrated that synthetic nano-fibers made of self-assembling peptide sequences hold promise in the creation of fibrous biodegradable nerve scaffolds [97]. Studies have shown that a scaffold fibers made of self-assembling natural L-amino acids, RADA16 (Ac-RADARADARADARADA-COHN2), can be functionalized with case specific functional sequences to promote healing and avoid triggering detectable immune response or inflammatory reactions [39].

The peptide sequence RADARADARADARADA (RADA16-I) has also been shown to effectively self-assemble into self-assemble into high-order interwoven nanofiber scaffold hydrogels with extremely high water contents [98]. Meng et al. preformed trials where they created controlled second degree burn wounds on mouse models [98]. Application of this self-assembling liquid dressing to a burn site was able to reduce inflammation, accelerate wound closure, and promote "vigorous" "healthy" healing when used as a wound dressing to treat second degree burns in a rat model.

3.3.1.4 Different Inflammation Cascades

Inflammation after injury or implantation is an important consideration. A biomaterial or treatment must be chosen which will minimize the scarring of the site, yet restore the function of the regenerating tissue. The following section briefly discusses some examples of nanomaterials that have been shown to reduce inflammation *in vivo*.

Using composite biomaterials such as bone-like nano-hydroxyapatite/collagen/poly(lactic acid) (nHAC/PLA) may provide a means for the repair of bone defects that does not require the use of bone allographs, and reduces the amount of inflammation over the course of the healing process [99]. By using materials that can actively support the attachment and re-growth of desired tissues, without triggering immune response, inflammation after an implant surgery can be minimized.

Additionally, it has been shown that silver nanoparticles are able to reduce inflammation through cytokine modulation [70]. Work by Ding et al. illustrated that incorporating nanosilver into scaffolds may be able to inhibit inflammation by reducing the invasion of inflammatory cells after surgery [100]. A study by Melo et al. showed that silver nanoparticles were able to accelerate healing of burn related wounds by modulating local and systemic inflammatory response following burn injury [70]. This indicates that silver nanoparticles in wound repair may be able to reduce post injury inflammation and scar formation, thus leading to accelerated tissue re-growth. As opposed to other implantable materials that just attempt to minimize the activation of the inflammation pathways, it seems that silver nanoparticles actively reduce the inflammatory response mechanisms.

3.3.2 Future of Nanomaterials Inflammation

As new biological nanomaterials are created and implemented, care needs to be taken to ensure that their implantation does not lead to unwanted inflammation at the site of implantation or to systemic inflammation after implantation. Future studied will need to focus on not only suppressing the inflammation following implantation, but also controlling and guiding cellular response, and protein interactions to result is faster, healthy tissue regeneration and shorter recovery times. Broader *in vivo* studies will need to be conducted focusing solely on the inflammation response of a wide array of nanomaterials implanted into a wide array of body systems.

3.4 Conclusions and Future Perspectives

Tissue engineering with nanotechnology is no longer in its infancy; with the growing number *in vivo* studies, we are beginning to understand its benefits. Specifically, nanotechnology offers biomimetic features to scaffolds, such as structural similarities to

ECM, and the ability to form constructs through the process of self-assembly towards improved tissue engineering strategies in bone, neural, wound repair, and through-out the body. More recently, researchers and doctors have switched focus towards the impact of nanotechnology on other important clinical problems, such as infections and excess inflammation caused by tissue engineering with conventional procedures (using micrometer or larger sized materials). Here, it has been found that nanotechnology can provide some very innovative approaches towards simultaneously controlling bacterial growth through loading of drugs (such as nanoparticles), preventing the penetration of (micron-sized) bacteria through nano-featured materials, and controlling the inflammatory response. With the growing applications of nanomedicine throughout the body, future studies must also consider the long-term impacts of implanted nanomaterials. Specifically, toxicity of nanomaterials is still not well understood, and researchers using *in vivo* models will benefit. In summary, the future of *in vivo* nanomedicine is very bright.

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Chapter 4
Nanomaterial Design and Computational Modeling

Zhengzheng Chen, Rong Chen, and Bin Shan

4.1 Introduction

Nanomaterials and nanotechnology may lead to breakthroughs in various fields such as VLSI circuits [1], energy storage solutions [2, 3], environmental protection [4, 5], and biomedical applications [6, 7]. Despite the incredible advances in characterization tools and techniques, there seems to be greater than ever needs to be able to carry out computational simulations with atomistic resolution for nanomaterials. This is in part due to the fact that nanoscale properties are extremely difficult to measure or manipulate, but more importantly, such properties are probably very sensitive to subtle environmental changes and perturbations, making repeated measurements more challenging. This is where computational modeling has much to offer in the booming nanomaterials design and nanomedicine, in that it supplies "virtue experimental methods" to investigate mechanisms of phenomena and even to design artificial structures in order to get desirable properties [8–10]. One good example is the design of nitrogen doped nanotube as chemical sensor which would have potential applications in biomedical fields. It was first proposed based on the gas response sensitivity analysis from first-principles calculations [11] and then a year later, confirmed by experiments, where fabricated CNx nanotubes have rapid sensing capabilities to low concentrations of toxic gases such as ammonia, acetone and OH groups [12]. Another example is the explanation of the presence of the strongest grain-size in nanocrystal metals by molecular dynamics simulations [13]. Computational simulations are able to present detailed insight of phenomena which are very difficult or even impossible to be obtained by experiments. It is clear that

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computational modeling provides invaluable information in both cases and it is computational techniques that give deep insight into the structure-property and structure-functionality relationship in nanomaterials.

Another aspect of the rising interest in nanomaterials design and computational modeling is that with the ever increasing power of CPUs and better designed parallel algorithms, the capability of computer simulation has greatly expanded. Nowadays, modern supercomputers has tera-flop computational power and full quantum mechanical simulations can tackle molecular systems consisting millions of atoms, while state-of-the-art molecular dynamics code can handle billions of atoms. This puts nanomaterials modelling squarely into experimentally accessible regions and the ability of mimicking real experimental systems has made computer simulations a complementary tool for understanding phenomena on the nanoscale.

Over the past few decades, we see a booming interest in computational modeling and the emergence of a number of new theoretical and numerical techniques. In order to tackle nanomaterials systems on different time and size scales, computational modeling has also developed several branches that aim to suitably describe various properties of assorted systems. The number one rule of thumb for computational simulation is that one has to choose the most suitable simulation method for a specific problem. Generally speaking, based on accuracy and size constraints, simulation methods can be categorized as First-Principles (FP) method (accurate, 10–1,000 atoms), tight binding (TB) (approximate electronic structure information, 10^2–10^5 atoms), and molecular dynamics (MD) (empirical potential, $>10^6$ atoms) (Fig. 4.1), which cover a wide area from sophisticated electronic structures to massive bulk properties. In the following sections, the fundamentals of these three simulation techniques are outlined and their applications in nanomaterials modeling are further demonstrated in details.

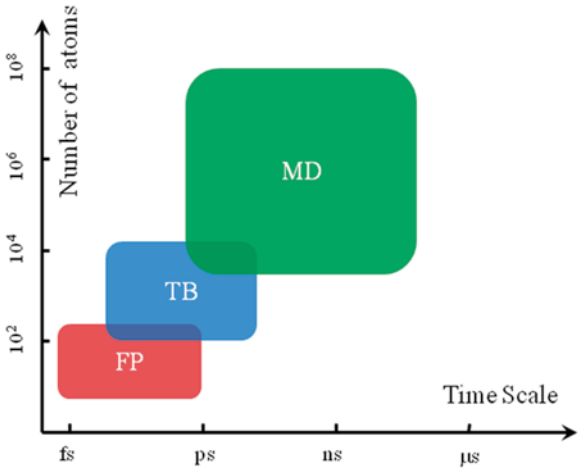

Fig. 4.1 Diagram illustrating suitable system sizes and time scales of FP, TB, and MD methods

4.2 First-Principles Methods

First-Principles methods, as shown in its own name, obtain electronic structure of materials basing on the very foundation of quantum mechanics, Schrödinger equation. Except several basic constants such as Plank constant, atomic mass, Bohr radius and certain approximations required to simplify numerical complexity, such as Born-Oppenheimer approximation, local density approximation, First-Principles methods calculate essential quantities directly without any presetting or empirical parameters. Therefore, this kind of methods has high accuracies, and can be used on most materials. On the other hand, due to the computationally demanding self-consistent solution procedures, FP methods are very time-consuming, and can only treat relatively small systems.

4.2.1 Born-Oppenheimer Approximation

The one single most important approximation employed in most FP calculations is the Born-Oppenheimer approximation, which effectively decouples the electronic and nuclear degree of freedom, and thus greatly simplifying the numerical solutions. By employing Hartree atomic unit, we can express the Hamiltonian of an *N*-ion-*n*-electron system as

$$\hat{H} = -\sum_{I=1}^{N} \frac{\nabla_I^2}{2M_I} - \sum_{i=1}^{n} \frac{\nabla_i^2}{2} + \frac{1}{2} \sum_{I,J,I\neq J}^{N} \frac{Z_I Z_J}{R_{IJ}} + \frac{1}{2} \sum_{i,j,i\neq j}^{n} \frac{1}{r_{ij}} - \sum_{I=1}^{N} \sum_{i=1}^{n} \frac{Z_I}{r_{iI}} \tag{4.1}$$

Capital and lower case letters indicate ions and electrons, respectively. Since ions are ~10^4 heavier than electrons ($M_I \sim 10^4$), it is safe to say that electrons move much faster than ions do.

In other words, at every moment that ions move to a new configuration, electrons will instantaneously relax to their new ground state. Therefore, the movements of electrons and ions can be separated. In mathematical terms, this separation is realized by expressing the total wavefunction as follows:

$$\Phi\left(\{\vec{r}_i\};\{\vec{R}_I\}\right) = \phi\left(\{\vec{r}_i\}\right)\Big|_{\{\vec{R}_I\}} \chi\left(\{\vec{R}_I\}\right) \tag{4.2}$$

$\phi\left(\{\vec{r}_i\}\right)\Big|_{\{\vec{R}_I\}}$ is electronic wavefunction with ionic configuration as $\{\vec{R}_I\}$, and $\chi\left(\{\vec{R}_I\}\right)$ is ionic wavefuction, respectively. Furthermore, $\phi\left(\{\vec{r}_i\}\right)\Big|_{\{\vec{R}_I\}}$ satisfies Schrödinger equation:

$$\left\{-\sum_{i=1}^{n} \frac{\nabla_i^2}{2} + \frac{1}{2} \sum_{i,j,i\neq j}^{n} \frac{1}{r_{ij}} - \sum_{I=1}^{N} \sum_{i=1}^{n} \frac{Z_I}{r_{iI}}\right\} \phi\left(\{\vec{r}_i\}\right)\Big|_{\{\vec{R}_I\}} = \varepsilon_{elec}\left(\{\vec{R}_I\}\right) \phi\left(\{\vec{r}_i\}\right)\Big|_{\{\vec{R}_I\}} \tag{4.3}$$

Equations 4.2 and 4.3 are called Born-Oppenheimer approximation. It realizes the separation of movements of electrons and ions, and serves as the very foundation of most modern computational quantum chemistry/physics methods. Combining the above three equations, we can obtain the equation of $\chi\left(\left\{\vec{R}_I\right\}\right)$:

$$\left\{-\sum_{M=1}^{N}\frac{\nabla_I^2}{2M_I}+\frac{1}{2}\sum_{I,J,I\neq J}^{N}\frac{Z_I Z_J}{R_{IJ}}+\varepsilon_{elec}\left(\left\{\vec{R}_I\right\}\right)\right\}\chi\left(\left\{\vec{R}_I\right\}\right)=\varepsilon\chi\left(\left\{\vec{R}_I\right\}\right) \tag{4.4}$$

Equation 4.4 shows that ions move in a potential field ε_{elec}. ε_{elec} is therefore also called "Born-Oppenheimer potential surface". From then on, we focus our discussion on solving the electronic wavefunction $\phi\left(\left\{\vec{r}_i\right\}\right)\Big|_{\left\{\vec{R}_I\right\}}$. More information could be found in Grosso and Parravicini's book [15].

4.2.2 *Density Functional Theory*

Though Born-Oppenheimer approximation simplifies Schrödinger equation in solid systems, Eq. 4.3 is still very difficult to solve. This is mainly because the coupling term $1/r_{ij}$ which makes Eq. 4.3 a non-linear n-body coupled equation. The commonly used technique to overcome the complication of Eq. 4.3 is to transfer this n-body problem to a single body problem. The rigorous demonstration is the density functional theory (DFT) [16, 17]. DFT does not consider concrete electronic orbital configurations, but focuses on the relationship between the total energy and the charge distribution of the system. Further, employing the variation principle with constrained conditions, DFT reformulates Eq. 4.3 into a single-body equation describing the state of a single electron moving in an effective potential field, while all many body interactions are lumped into a so called "exchange-correlation" functional. As Hohenberg and Kohn pointed out, the ground energy of a system can be expressed as a universal functional as its ground charge distribution [16]:

$$E^{\mathrm{HK}}\left[\rho\left(\vec{r}\right);V_{ext}\left(\vec{r}\right)\right]=T\left[\rho\left(\vec{r}\right)\right]+E_{ee}\left[\rho\left(\vec{r}\right)\right]+\int V_{ext}\left(\vec{r}\right)\rho\left(\vec{r}\right)d\vec{r}+E_{II} \tag{4.5}$$

Terms on the right side are kinetic energy, electron-electron interaction, electron-ion interaction, and ion-ion interaction, respectively. Let us ignore the last term by now for it is a constant shift for a given atomic configuration. One can in principle get the minimum of E^{HK} by taking the variation of Eq. 4.5 with respect to ρ for a given external potential V_{ext}. Unfortunately, this is not a practical way at least in the near future since we have no idea about the kinetic functional for an interacting electron-gas system. To overcome this difficulty, Kohn and Sham formulated the KS equation by mapping a non-interacting electron gas system whose charge distribution $\rho_0\left(\vec{r}\right)$ is identical to the ground charge distribution $\rho\left(\vec{r}\right)$ to the real system [17]. The key point of this ansatz is that $\rho_0\left(\vec{r}\right)$ is able to be expresses as the summation of single electron wavefunctions $\varphi_j\left(\vec{r}\right)$:

$$\rho_0(\vec{r}) = \sum_i \varphi_i^*(\vec{r})\varphi_i(\vec{r}) \tag{4.6}$$

and so is $\rho(\vec{r})$. The kinetic energy functional of a non-interacting electron gas $T_0[\rho(\vec{r})]$ can be analytically calculated. We can then take this advantage. Furthermore, Kohn and Sham calculated Hartree interaction $E_H[\rho(\vec{r})]$ instead of $E_{ee}[\rho(\vec{r})]$:

$$E_H[\rho(\vec{r})] = \int \frac{\rho(\vec{r})\rho(\vec{r}')}{|\vec{r}-\vec{r}'|} d\vec{r}d\vec{r}' \tag{4.7}$$

Apparently, $T_0[\rho(\vec{r})] + E_H[\rho(\vec{r})]$ differs from $T[\rho(\vec{r})] + E_{ee}[\rho(\vec{r})]$ in Eq. 4.5. To compensate this difference, one needs an extra term:

$$E_{\text{xc}}[\rho] = T[\rho] + E_{ee}[\rho] - T_0[\rho] - E_H[\rho] \tag{4.8}$$

The importance of $E_{\text{xc}}[\rho]$ is that it contains all the effects from many-body interactions. Combining Eqs. 4.5, 4.6, 4.7, and 4.8, and taking the variation of the total energy with respective to $\varphi_j(\vec{r})$ under the constrain condition:

$$\sum_j \langle \varphi_j | \varphi_j \rangle = N \tag{4.9}$$

we can obtain the equation which ground state wavefunction $\varphi_j(\vec{r})$ satisfies:

$$\left[-\frac{\nabla^2}{2} + V_H(\vec{r}) + V_{ext}(\vec{r}) + V_{xc}(\vec{r}) \right] \varphi_j(\vec{r}) = \varepsilon_j \varphi_j(\vec{r}) \tag{4.10}$$

Explicitly, we define exchange-correlation potential as:

$$V_{xc}(\vec{r}) = \frac{\delta E_{xc}[\rho(\vec{r})]}{\delta \rho(\vec{r})} \tag{4.11}$$

Equation 4.10 is Kohn-Sham (KS) equation. By using KS equation, the ground energy could be rewritten as

$$E_0 = \sum_j \varepsilon_j - \frac{1}{2} \int \frac{\rho(\vec{r})\rho(\vec{r}')}{|\vec{r}-\vec{r}'|} d\vec{r}d\vec{r}' + E_{\text{xc}}[\rho(\vec{r})] - \int V_{\text{xc}}(\vec{r})\rho(\vec{r}) d\vec{r} \tag{4.12}$$

The first term in the right side is called band structure energy, and other three terms are called double counting terms.

One should understand that an exact analytical formula for the exchange-correlation energy $E_{xc}[\rho]$ is generally unavailable for most cases. Its correlation component, however, can be numerically obtained by quantum Monte-Carlo (QMC) method, which has been done by Ceperley and Alder [18]. Several research groups fit their data by different analytic functions and incorporate these functions into KS equation [18–22].

4.2.3 Self-Consistent Field Processes in DFT

Reviewing Eq. 4.10, a paradox may be found: To build up V_H and V_{ext} of Hamiltonian in Eq. 4.10, one has to know $\rho(\vec{r})$ in advance. At the same time, $\rho(\vec{r})$ needs to be solved. In other words, $\varphi_j(\vec{r})$ appears at both side of Eq. 4.10. Therefore, KS equation needs to be solved self consistently. First, an initial guess of $\varphi_j(\vec{r})$ or $\rho(\vec{r})$ is able to be presented. Second, the Hamiltonian is built up and Eq. 4.10 is solved. Third, $\rho(\vec{r})$ is updated according to the output and input of the current step, re-construct Hamiltonian. The above steps are repeated until the convergence criterion is satisfied. Usually, the criterion is chosen as the change of input and output values of total energy at current step. The whole process is called self-consistent field (SCF) calculations. After a set of high-quality eigenfunctions is obtained, the electronic structures could be constructed and analyzed, i.e. charge density, energy band structures, and density of states, etc. Details of SCF are far beyond the scope of this chapter, more details could be found in the books of Martin [23] and Knhanoff [24], respectively.

4.2.4 Examples

Since DFT methods, or more general, First-Principles methods, do not depend on availability of parameters for a given system. This character makes them very suitable to study materials which either is novel or has strong quantum effects [25, 26]. More importantly, as shown in Eqs. 4.6 and 4.10, the ground charge distribution is sensitive to concrete external potentials, arbitrary structural defects and/or alloying elements in principle are able to introduce unique electronic structures and even properties. Therefore, First-Principles methods can be employed to predict or even design new class of materials with desirable properties, which is a very important and vibrant aspect of modern computational materials science.

As an example, the study on the diffusion of an adatom on the Sn-alloying Cu(111) surface is hereby presented [14]. Figure 4.2 shows the potential energy surface (PES) of a Cu adatom on such an alloying surface. Clearly, Sn atoms disturb the profile of PES in two ways: (1) they increase the value of PES at their sites, and (2) they introduce forbidden region around each of them by transferring local valleys to slopes, which are shown as green areas in Fig. 4.2. These two features make

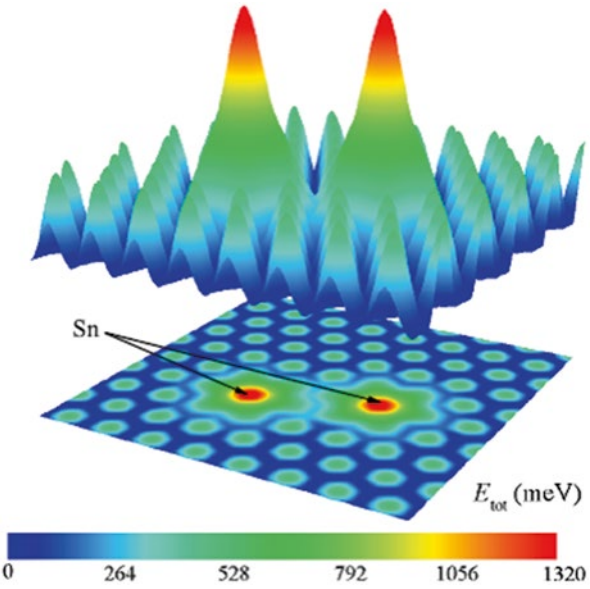

Fig. 4.2 The landscape of total energy of the system with single Cu adatom on the Cu(111) surface alloyed by 2 Sn atoms. The brighter (*darker*) area indicates weak (*strong*) adsorption sites of the Cu adatom. The lowest energy is set to zero [14]

Sn atoms diffusion blockers to a Cu adatom because it is very energetically unfavorable for a Cu adatom approaching sites occupying by Sn atoms. This phenomenon can be attributed to the fact that Sn atoms are larger than Cu atoms and therefore protrude from the surface. Besides the geometrical factor, different electronic configurations between Sn atoms and Cu atoms also contribute to the blocking effect of Sn. Figure 4.3 shows two minimum energy paths (MEP). When a Cu adatom approaches Sn sites, it climbs uphill. Not only local minimums, but also migration barriers rise up. This feature is more apparent along the path which goes through the two Sn atoms (Fig. 4.3b). To explain MEPs, Fig. 4.3c presents local density of states (LDOS) of the surface layer at Fermi energy $D(E_F)$. $D(E_F)$ is higher between the two Sn atoms because of the contribution from *p*-orbitals of Sn, and thus strengthens binding interaction between the surface and the Cu adatom according to Newns-Anderson model. The results of this First-Principles simulation are in good agreement with experimental observation in which alloying with Sn increases the serving lifetime of Cu interconnects by 10 times [27].

4.3 Tight Binding

Tight binding method (TB) is a wide-used semi-empirical computational method. Based on a set of well-chosen basis functions, TB builds up Hamiltonian matrix H of a given system and gets eigenvalues and eigen-wavefunctions by diagonalizing H. Further information of electronic structure, i.e. charge density, band structure, and optimal adsorption spectrum etc. can then be obtained. Different from

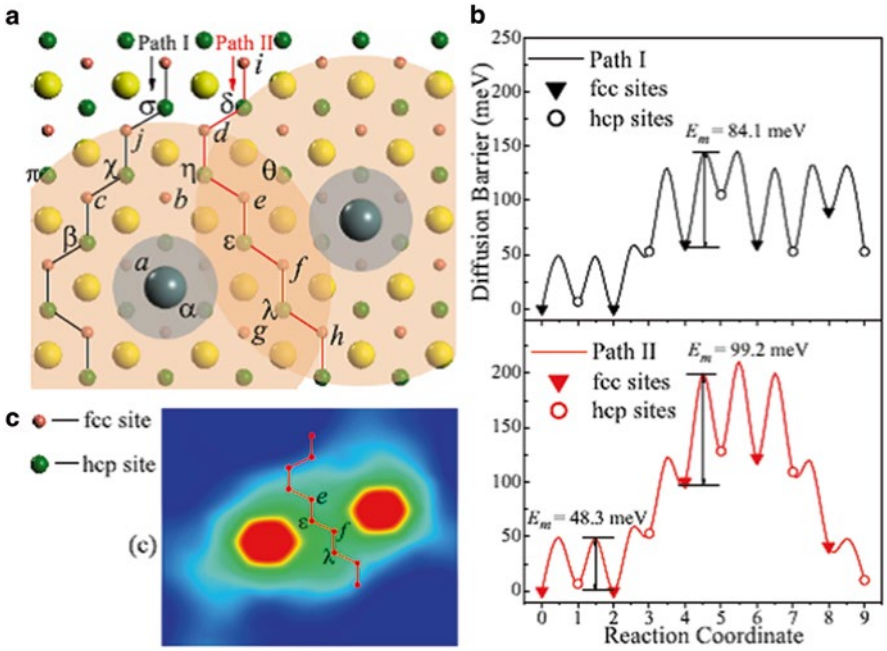

Fig. 4.3 (**a**) Migration paths I and II for a Cu adatom on the Cu (111) surface with two 4th nearest neighbor Sn surface atoms. *Yellow* (*gray*) *circles* denote the Cu (Sn) atoms, while the *green* (*red*) *circles* denote hcp (fcc) sites, denoted by Greek and Latin letters, respectively. (**b**) Migration energy landscape versus reaction coordinates for pathways I and II, respectively. (**c**) LDOS contours at the Fermi level, with bright (*dark*) color indicating high (low) values of LDOS [38]

first-principles methods, elements in H in TB are not directly calculated through SCF. They are expressed as functions of atomic positions with a set of predetermined parameters. With high-quality parameters, TB can accurately simulate systems of $10^3 \sim 10^4$ atoms. This is important since systems with this size could contain complicated atomic structures or functional group. Therefore, TB method is attractive for Nano-material simulations because objects in this area usually have artificial structure and peculiar electronic structures which need to be identified.

4.3.1 Linear Combination of Atomic Orbitals

Theoretical foundation of TB can be viewed by linear combination of atomic orbitals (LCAO) method [28]. Suppose there is a system containing N atoms and there are n_i orbitals belonging to the i-th atom. One eigen-wavefunction can be expressed as

$$\Psi\left(\vec{r}\right)=\sum_{i,\alpha}c_{i\alpha}\phi_{\alpha}\left(\vec{r}-\vec{R}_{i}\right) \tag{4.13}$$

where i and α are indexes of atoms and orbitals, respectively. Accordingly, the energy of the system E is

$$E=\frac{\left\langle\Psi\middle|\hat{H}\middle|\Psi\right\rangle}{\left\langle\Psi|\Psi\right\rangle}=\frac{\sum_{i\alpha,j\beta}c_{i\alpha}^{*}c_{j\beta}\left\langle\phi_{i\alpha}\middle|\hat{H}\middle|\phi_{j\beta}\right\rangle}{\sum_{i\alpha,j\beta}c_{i\alpha}^{*}c_{j\beta}\left\langle\phi_{i\alpha}\middle|\phi_{j\beta}\right\rangle} \tag{4.14}$$

The variation of E with respect to $c_{i\alpha}^{*}$ can be easily calculated:

$$\frac{\delta E}{\delta c_{i\alpha}^{*}}=\frac{1}{\sum_{i\alpha,j\beta}c_{i\alpha}^{*}c_{j\beta}\left\langle\phi_{i\alpha}|\phi_{j\beta}\right\rangle}\left[\sum_{j\beta}c_{j\beta}\left\langle\phi_{i\alpha}\middle|\hat{H}\middle|\phi_{j\beta}\right\rangle-Ec_{j\beta}\left\langle\phi_{i\alpha}\middle|\phi_{j\beta}\right\rangle\right] \tag{4.15}$$

To obtain the lowest value of E, Eq. 4.15 should equal to 0 for any i and α. Therefore, we can straightforward obtain the secular equation of $c_{j\beta}$:

$$\left|H_{i\alpha,j\beta}-ES_{i\alpha,j\beta}\right|=0 \tag{4.16}$$

Equation 4.16 is called generalized eigenvalue equation. $H_{i\alpha,j\beta}$ and $S_{i\alpha,j\beta}$ are elements of Hamiltonian matrix and overlapping matrix expanded by $\{\phi_{i\alpha}\}$, respectively. If $\{\phi_{i\alpha}\}$ is a set of orthogonal functions, Eq. 4.16 is reduced to

$$\left|H_{i\alpha,j\beta}-E\delta_{i\alpha,j\beta}\right|=0 \tag{4.17}$$

which has been familiar with in Sect. 4.2. Eqs. 4.16 and 4.17 are called LCAO method. If $\{\phi_{i\alpha}\}$ are not chosen as actual atomic orbitals, this method is usually named as "tight binding".

4.3.2 Slater-Koster Two-Center Approximation

According to above discussions, the key step in TB method is to construct Hamiltonian matrix H. Appropriate approximations are able to essentially simplify the construction of elements of H. In 1954, Slater and Koster suggested two-center approximation in their classic paper [29], which expresses all Hamiltonian elements with a limited number of two-center integrations. The total number of all these basic integrations is around 30. Therefore, Slater-Koster two-center approximation makes TB method to be practical and is the foundation of modern TB simulating software packages.

Two points of Slater-Koster two-center approximation (SK approximation for short) should be emphasized. First, let us specifically write down the expression of one element of H for a periodic system i.e. crystal. In this case, the basic function is φ_α, the Bloch summation of atomic orbitals ϕ_α:

$$\varphi_{i\alpha}\left(\vec{r}\right)=\frac{1}{\sqrt{N_{\text{cell}}}}\sum_{n=1}^{N_{cell}}\exp\left(i\vec{k}\cdot\vec{R}_i^n\right)\phi_\alpha\left(\vec{r}-\vec{R}_i^n\right) \tag{4.18}$$

The superscript n is the index of cell. $\vec{k}$ is the vector in reciprocal space. After some basic algebra calculations, we can obtain $H_{i\alpha,j\beta}\left(\vec{k}\right)$:

$$H_{i\alpha,j\beta}\left(\vec{k}\right)=\frac{1}{N_{\text{cell}}}\sum_{n,m=1}^{N_{\text{cell}}}\exp\left[i\vec{k}\cdot\left(\vec{R}_i^n-\vec{R}_i^m\right)\right]\times\int d\vec{r}\phi_\alpha^*\left(\vec{r}-\vec{R}_i^n\right)\hat{H}\phi_\alpha\left(\vec{r}-\vec{R}_i^m\right) \tag{4.19}$$

Because $\hat{H}$ is a function of positions of electrons and atoms, Eq. 4.19 can be categorized as three types: (1) on-site integration, in which three integrands, ϕ_α, $\hat{H}$ and ϕ_β are at the same center; (2) two center integration, in which two of the above integrands are at one center, and the other one is at another center; and (3) three center integration, in which each integrand is at its own center. Because of the local nature of ϕ_α and ϕ_β, in most case on-site integration has the largest value while the three center integration is the smallest. Therefore, the contribution to $H_{i\alpha,j\beta}\left(\vec{k}\right)$ is truncated up to two center integrations. Three center integrations and higher order ones are ignored. This truncation essentially decreases the number of terms in $H_{i\alpha,j\beta}\left(\vec{k}\right)$, and is the first point in SK approximation.

Two center integrations are usually understood as "bonding" between two orbitals centering at two atoms. They are explicit functions of the relative position $\vec{R}_{ij}$ between two atoms. Though they could be very different from each other since $\vec{R}_{ij}$ can be any vector, they always can be expressed as a combination of several basic two center terms. This is the second key point in SK approximation. These basic terms could be referred to "bonding terms". These terms are showed as V_{ss}, V_{sp}, V_{pp} and $V_{pp\pi}$, etc. The first two subscripts are the angular momentum numbers of two orbitals, and the third subscript indicates the bonding type, which depends on the relative orientations and symmetries of the two orbitals.

In Figs. 4.4 and 4.5, we show simple examples about how to express two center integrations in terms of bonding terms. One atom is at original point and another atom is at $\vec{R}$, the orientation cosine with respect to xyz axis are l,m and n, respectively. The s-s term is independent from orientation of $\vec{R}$: $\left\langle s\middle|\hat{H}\middle|s\right\rangle=V_{ss\sigma}$ (Fig. 4.4a). The p_y-p_y interaction can be decomposed as $\left\langle p_y\middle|\hat{H}\middle|p_y\right\rangle=m^2V_{pp\sigma}+\left(1-m^2\right)V_{pp\pi}$ (Fig. 4.4b), and the s-p_y term is $\left\langle s\middle|\hat{H}\middle|p_y\right\rangle=mV_{sp\sigma}$, (Fig. 4.5).

Therefore, four basic bonding terms can be used to express all sp-type interactions. That is why SK approximation has made tremendous success. For higher order orbitals, i.e. d, f, and g, etc., one has to use angular momentum theory to calculate two center integrations, which is discussed in detail in a couple of references [30, 31].

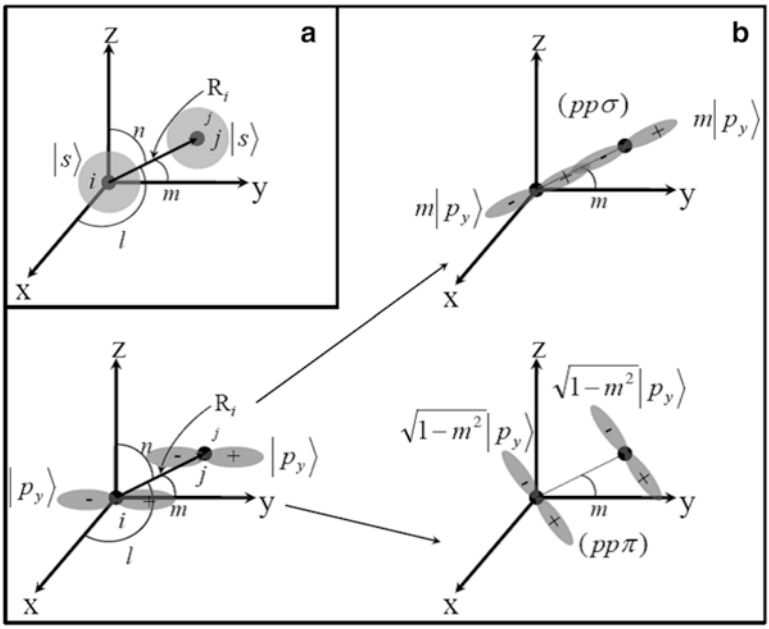

Fig. 4.4 Illustration of two-center approximation of *sp*-type interactions. (**a**) is s-s term and (**b**) is p_y-p_y term

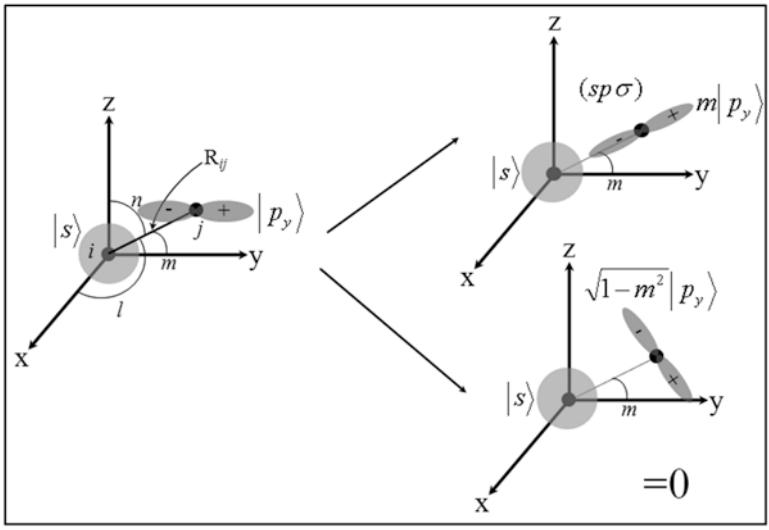

Fig. 4.5 Illustration of two-center approximation of *s*-p_y term

4.3.3 Total Energy in TB

By diagonalizing Hamiltonian matrix, a set of eigenvalues can be obtained. The corresponding energy of the system is then can be expressed as

$$E_{\text{tot}} = 2\sum_{\lambda}\varepsilon_{\lambda} f\left(\varepsilon_{\lambda}\right) \tag{4.20}$$

λ is the band index, the 2 comes from the spin-degeneracy of each band, and $f(\varepsilon_\lambda)$ is Fermi-Dirac distribution at 0 K. However, the TB method might be challenged on its accuracy since the above equation takes into account only on-site terms and "bonding" contributions. E_{tot} should contain Coulomb repulsion of electrons and ions. Therefore, Eq. 4.20 severely overestimates cohesive energy of the system. Practically, Coulomb repulsion is dealt by adding an extra term E_{rep}, we have

$$E_{\text{tot}} = 2\sum_{\lambda}\varepsilon_{\lambda} f\left(\varepsilon_{\lambda}\right) + E_{\text{rep}} = 2\sum_{\lambda}\varepsilon_{\lambda} f\left(\varepsilon_{\lambda}\right) + \frac{1}{2}\sum_{i,j} A\exp\left(-R_{ij}/R_0\right) \tag{4.21}$$

A and R_0 are parameters which are determined by experiments or DFT calculations.

4.3.4 Examples

Compared to full self-consistent first-principles calculations, TB method does have limitation of generality and transferability of parameters. However, its transparent physical picture and its approximate but reasonable way of describing electronic structures make it a very useful tool in analyzing the structure-electronic relationship in nanomaterials. An example of how TB method is used to design a quantum dot based on a single nanotube is illustrated here (Fig. 4.6) [32].

As is well known, a nanotube can be thought of a grapehene sheet rolled into a cylindrical tube. It has a tunable bandgap that is highly dependent on its topological structures, and this very unique electronic property may find many applications in nanoelectronics. For example, by changing the chirality of a single nanotube using topological defects, a variety of metal-semiconductor, metal-metal, and semiconductor-semiconductor junctions can be generated. Quantum dot (QD) can be fabricated on a single wall nanotube (SWNT) by the mechanical deformation. As shown in Fig. 4.6a, kinks on a semi-conductive SWNT create dips on energy band gap. Together with the information of eigenstate wavefunctions, one can conclude that these kinks equivalently behavior like acceptor QDs. This could be an effective and simple way of creating room-temperature quantum dot devices. On the other hand, for a metallic SWNT, the response of electronic structure to the deformation is not very sensitive (Fig. 4.6b).

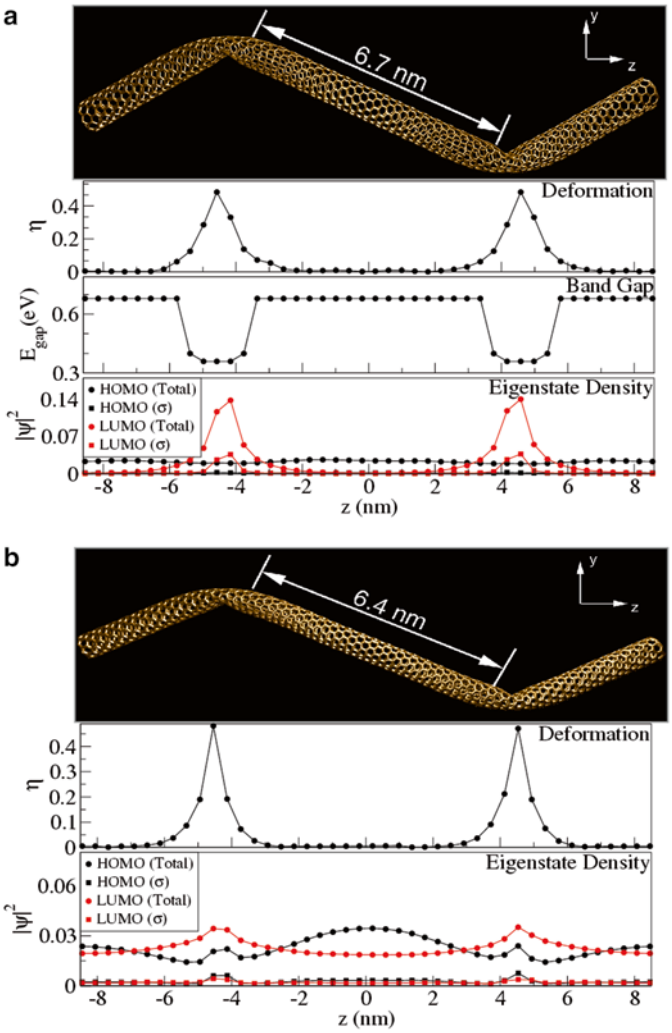

Fig. 4.6 The geometry of tubes used to create nanotube-based quantum wells. The two bending regions are separated by an undeformed segment (**a**) 6.7 nm long in the (10,0) tube and (**b**) 6.4 nm long in the (9,0) SWNT [32]

4.4 Molecular Dynamics

Though molecular dynamics (MD) method perform atomic simulations, it has no quantum mechanics background, which is different from DFT and TB methods. MD treats atoms as classic particles. Movements of atoms are determined by Newton equations. The atomic interaction is presented by empirical potentials, which shows as either analytical functions with parameters or data on grids.

Therefore, MD method does not perform SCF calculations or diagonalization of large matrix, and can be employed on study dynamical evolution of large systems ($10^6 \sim 10^8$ atoms, as shown in Fig. 4.1) in complicated loading conditions, i.e., stress, temperature, and ion bombardment, etc. [33–35]

4.4.1 Empirical Potentials

4.4.1.1 Lennard-Jones Potential

Lennard-Jones potential is a famous type of pair-potential. It describe atomic interactions as

$$V(r) = 4\varepsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right] \tag{4.22}$$

ε and σ are parameters which are determined by fitting important properties, i.e. equilibrium distance, binding energy, etc. In modern MD simulations, Lennard-Jones potential is usually used to describe interactions between gas atoms.

4.4.1.2 Embedded Atomic Method

Embedded atomic method (EAM) presented better simulating results on bulk materials than pair-potential [36]. Besides pair potentials, EAM introduces an addition term. Thus the total energy of the system is expressed as

$$E_{\text{tot}} = \frac{1}{2}\sum_{i,j} V\left(R_{ij}\right) + \sum_{i} F\left[\sum_{j} \rho\left(R_{ij}\right)\right] \tag{4.23}$$

$F[\rho]$ is called "embedded energy", which means the energetic gain when an atom is put into electron gas contributed by other atoms in vicinity. Clearly, EAM has concepts similar to "atomic bonds" and density functional. $V(R)$, $\rho(R)$ and $F[\rho]$ are usually functions with $10 \sim 20$ parameters. One needs fit these parameters to reproduce lattice constant, cohesive energy, vacancy formation energy, surface energy, several elastic constants, and migration energy of a point defect, etc. Recently, Ercolessi and Adams developed the *force-matching* method [37]. This method employs first-principles methods to obtain force acting on each atom in a serial of reference configurations. It then defines a target function as follows

$$F(\mathbf{p}) = F_f(\mathbf{p}) + F_c(\mathbf{p}) = \left(3\sum_{k=1}^{M} N_k\right)^{-1} \sum_{k=1}^{M}\sum_{i=1}^{N_k} \left|\mathbf{f}_{ki}(\mathbf{p}) - \mathbf{f}_{ki}^{\text{ref}}\right|^2 + \sum_{r=1}^{N_c} W_r \left|A_r(\mathbf{p}) - A_r^{\text{ref}}\right|^2 \tag{4.24}$$

M is the number of reference configurations, N_k is the number of atoms in the k-th configuration. **f** is the atomic force, A_r is an abovementioned property of the material, W_r is the assigned weight, and **p** is the set of parameters. By minimizing Eq. 4.24, one can obtain desirable EAM potentials [39]. Different groups have applied the *force-matching* method to get high-qualified potentials for several metals and even binary systems [34, 40], which is an approval to the fidelity of this method.

4.4.2 Integrator of Motion Equations

With a given reliable empirical potential, MD calculates the force on each atom as the negative derivative of potential energy with respect to the position of the atom, then update the velocity and the position of each atom according to Newton equation. Therefore, a mathematical interpretation of MD is to solve a second order ordinary partial equation:

$$\frac{d^2\vec{r}}{dt^2} = f\left(t,\mathbf{r},\mathbf{v}\right) \quad \left(\mathbf{r}_0,\mathbf{v}_0\right) \tag{4.25}$$

Appropriate differential algorithms are essential for MD simulations.

4.4.2.1 Verlet algorithm and Prediction-Correction Algorithm

The Verlet algorithm and prediction-correction algorithm are discussed here since they are widely used and are basis of other advanced algorithms as well. Given position $\mathbf{r}(t)$, velocity $\mathbf{v}(t)$, and force $\mathbf{f}(t)$ at time t, we can get $\mathbf{r}(t+\Delta t)$ at $t+\Delta t$ and $\mathbf{r}(t-\Delta t)$ at $t-\Delta t$ through Taylor expansion:

$$\vec{r}\left(t+\Delta t\right) = \vec{r}\left(t\right) + \vec{v}\left(t\right)\cdot\Delta t + \frac{\vec{f}\left(t\right)}{2m}\cdot\Delta t^2 + \frac{\Delta t^3}{6}\dddot{r} + O\left(\Delta t^4\right) \tag{4.26}$$

$$\vec{r}\left(t-\Delta t\right) = \vec{r}\left(t\right) - \vec{v}\left(t\right)\cdot\Delta t + \frac{\vec{f}\left(t\right)}{2m}\cdot\Delta t^2 - \frac{\Delta t^3}{6}\dddot{r} + O\left(\Delta t^4\right) \tag{4.27}$$

By summing Eqs. 4.26 and 4.27, we have

$$\vec{r}\left(t+\Delta t\right) = 2\vec{r}\left(t\right) - \vec{r}\left(t-\Delta t\right) + \frac{\vec{f}\left(t\right)}{m}\cdot\Delta t^2 + O\left(\Delta t^4\right) \tag{4.28}$$

$$\vec{v}\left(t\right) = \frac{\vec{r}\left(t+\Delta t\right) - \vec{r}\left(t-\Delta t\right)}{2} + O\left(\Delta t^2\right) \tag{4.29}$$

Equations 4.28 and 4.29 are called Verlet algorithm. Since positions and velocities at the time t can be obtained simultaneously, Verlet algorithm can be used to obtain the total energy of the system. Another key feature of Verlet algorithm is the time reversibility, which means if we suddenly flip the velocity of each atom at time $t = n\Delta t$, the system will go back to the initial positions along the same trajectory after n steps. Therefore, the total energy of the system is conserved in Verlet algorithm. Detailed analysis demonstrates that this feature comes from Liouville equation of conserve force systems [41]. Clearly, Verlet algorithm is ideal for micro-canonical ensembles. Main limitation of Verlet algorithm is that fluctuation of energy is large in a short period since velocities has only accuracy to the order of Δt^2, as shown in Eqs. 4.28 and 4.29.

As shown in Eq. 4.25, MD simulation is to solve a second-order ODE. The prediction-correction (PC) algorithm can be performed to get the trajectory of the system. First, the position and the velocity at time $t+\Delta t$ as the linear combination of forces of previous k steps are expressed, and the linear coefficients could be set to equal to the corresponding coefficients of Taylor expansion up to the term of Δt^k, hence the position and velocity are updated as below:

$$\begin{aligned}\vec{r}\left(t+\Delta t\right) &= \vec{r}\left(t\right)+\vec{v}\left(t\right)\cdot\Delta t+\Delta t^2\sum_{i=1}^{k-1}\alpha_i\vec{f}\left[t+\left(1-i\right)\Delta t\right]\\ \vec{v}\left(t+\Delta t\right) &= \frac{\vec{r}\left(t\right)-\vec{r}\left(t-\Delta t\right)}{\Delta t}+\Delta t\sum_{i=1}^{k-1}\alpha_i'\vec{f}\left[t+\left(1-i\right)\Delta t\right]\end{aligned} \tag{4.30}$$

This is called prediction step. Second, one correction step needs to be performed. Calculate $\vec{f}\left(t+\Delta t\right)$ and take it as a new point. Then re-estimate $\vec{r}\left(t+\Delta t\right)$ and $\vec{v}\left(t+\Delta t\right)$:

$$\begin{aligned}\vec{r}\left(t+\Delta t\right) &= \vec{r}\left(t\right)+\vec{v}\left(t\right)\cdot\Delta t+\Delta t^2\sum_{i=1}^{k-1}\beta_i\vec{f}\left[t+\left(2-i\right)\Delta t\right]\\ \vec{v}\left(t+\Delta t\right) &= \frac{\vec{r}\left(t\right)-\vec{r}\left(t-\Delta t\right)}{\Delta t}+\Delta t\sum_{i=1}^{k-1}\beta_i'\vec{f}\left[t+\left(2-i\right)\Delta t\right]\end{aligned} \tag{4.31}$$

Table 4.1 presents PC coefficients with $k=4$. PC algorithm is suitable to complex simulations due to its flexibility. However, total energy of the system is not a conserving quantity in PC algorithm because it is not time-reversible. This is not a severe problem in canonical ensemble simulations since the total energy needs to be manipulated constantly.

4.4.3 Examples

An example of the MD simulation on interaction of a <111>/2 screw dislocation and Cu-precipitate in BCC Fe matrix is presented here [38]. The whole system contains 576,000 atoms. As shown in Fig. 4.7, the diameter of a spherical Cu-precipitate

Table 4.1 PC coefficients with $k=4$

$k=4$		1	2	3
Prediction	α_i	19/24	−10/24	3/24
	α_i'	27/24	−22/24	7/24
Correction	β_i	3/24	10/24	−1/24
	β_i'	7/24	6/24	−1/24

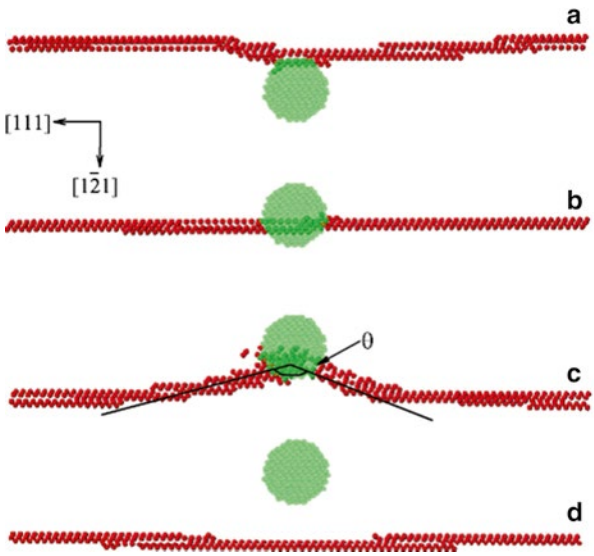

Fig. 4.7 MD snapshots of the dislocation core interacting with the 2.3 nm Cu precipitate as the dislocation glides on the $(10\bar{1})$ plane along the $[1\bar{2}1]$ direction at (**a**) 243 ps, (**b**) 272 ps, (**c**) 312 ps, and (**d**) 320 ps, respectively. *Green* and *red circles* represent Cu and Fe atoms, respectively. [38]

with BCC structure is 2.3 nm. Under an external stress $=700$ MPa, the dislocation can penetrate into the Cu precipitate, as shown in Fig. 4.7a, b. However, the dislocation becomes pinned as it approaches the opposite precipitate-matrix interface, and hence is unable to glide outside the precipitate. Upon increasing the external stress, the dislocation line outside the precipitate continues to glide forward while the short dislocation line segments within the precipitate remains pinned, resulting to a bowing out of the dislocation. The bow-out angle, θ gradually decreases upon increasing the external stress from 180° at $=700$ MPa until it reaches the critical value of $\theta_c=144°$ under 1,000 MPa shear stress, where the dislocation suddenly detaches from the Cu precipitate and the dislocation line renders straight.

Figure 4.8 presents the dislocation core structures during the pinning process as shown in Fig. 4.7. Note that dislocation core in Cu-precipitate spreads along three directions (polarized core), while spreads along six directions in BCC Fe matrix (non-polarized core). When the dislocation reaches to the boundary, it stops moving and transfers its structure from polarized core to non-polarized core (Fig. 4.8b, c).

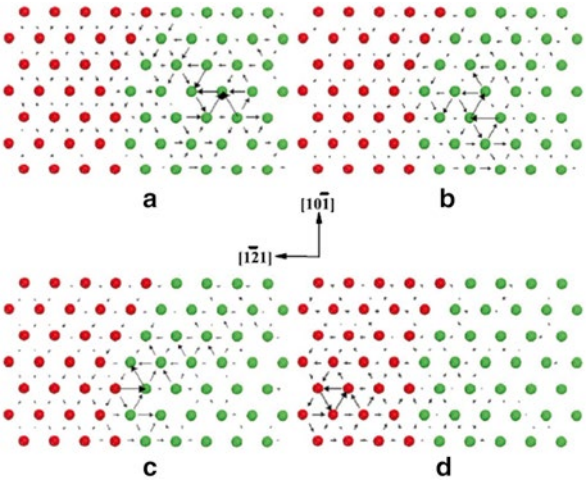

Fig. 4.8 The dislocation core structure during the detachment process in Fig. 4.1 at (**a**) 280 ps, (**b**) 297 ps, (**c**) 303 ps, and (**d**) 315 ps. The *red* and *green spheres* represent Fe and Cu atoms, respectively. The dislocation glides along the $\left[1\bar{2}1\right]$ direction under an external stress of 1,000 MPa [38]

The transferring process corresponds the pinning. And the energy cost during the process is supplied by bowing-out of dislocation core. These results reveal that the dislocation/precipitate detachment process is accompanied with a polarized → non-polarized core transition, which may be responsible for the pinning effect, the above discussion presents a plausible precipitate-size induced strengthening mechanism.

4.5 Conclusions and Future Outlook

Computational simulations with atomistic resolution for nanomaterials has received an increasing interest due to the fact that nanoscale properties are extremely difficult to measure or manipulate, but more importantly, such properties are probably very sensitive to subtle environmental changes and perturbations, making repeated measurements more challenging. In this chapter, the theoretical background of three types of widely-used material simulation methods: first-principles method, tight binding method, and molecular dynamics are introduced, followed by a detailed discussion including further theories with necessary mathematical treatments and examples for each method. A raw picture of applications of atomic simulation methods on material sciences could thus be generated.

Although there are some essential limitations for each kind of simulation methods, virtual material modeling/design has been made massive successes in the past four decades. Correction, extension and even renovation of current simulation methods have been or will be introduced in order to match the rapid development of material sciences. There are reasons to believe that virtual material modeling/design would become more and more important in both sciences and technologies in the near future.

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Chapter 5
Medical Nanomaterials

Steven D. Perrault

5.1 Introduction

Proponents of nanotechnology claim that it will make broad contributions to medical technology over the coming years. But an outsider could ask; why would nanotechnology be so central to a new generation of devices and medicines? What is it about nanometer-scale materials that could provide an improvement on the current state-of-the-art? How can they fulfill current needs within medical practice, and improve how we are able to detect and treat complex diseases such as cancer?

To answer these questions, it's necessary to first understand why new medical technologies are required, and whether it's worth investing money and research into replacing the current technologies. We'll begin this chapter by considering why we need new medical technologies, and what the potential market for nanomedicine might be. In the same context, we can look at what the current standards are for medical technologies. From there, it should start to become clear where nanotechnology can find a home and where it may not be appropriate. We'll then look at how nanomaterials can be systematically organized and described, and the classes of materials that are common in nanomedicine. We can then look deeper at some of these, discuss how they are used in research and how they are being developed towards clinical applicability. This chapter will review some of the most common nanomaterials being developed for medical technologies. More importantly, it will try to provide a framework so that the reader understands why certain directions and materials are being pursued.

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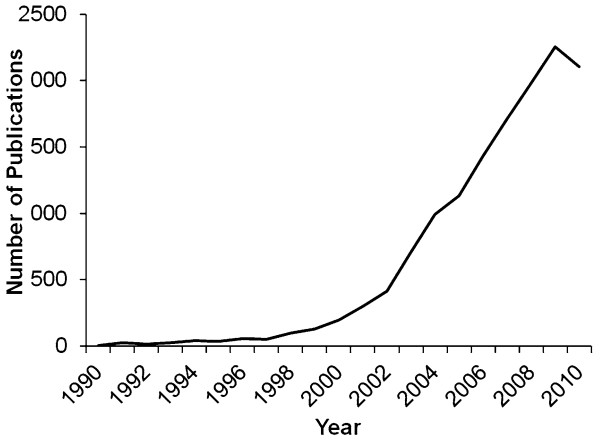

Fig. 5.1 Number of "nanotechnology" publications per year between 1990 and 2010

It's worth saying that at the time of writing this, nanotechnology research is likely at the start of a very long road. A search for "nanotechnology" studies in a journal database shows only a handful of papers published prior to 1990, breaking above 10 per year in 1991, and 100 per year only in 1999 (Fig. 5.1). The field then grew rapidly, reaching 1,000 papers published per year in 2005 and twice that just 4 years later. As of 2012, there is an enormous library of well-characterized nanomaterials available, and a small number that can be purchased commercially. In general, not all aspects of the nanomaterials have been characterised. As our ability to design and assemble more complex nano-scale devices improves, the number of studies, applications and products could grow far beyond what anyone today is imagining. I present some early examples of multi-component medical nano-devices and molecular engineering approaches to their assembly at the end of the chapter.

5.2 The Need for New Medical Technologies

As of 2008, the leading causes of global deaths included a large number of chronic and infectious diseases (e.g. cardiovascular disease, diabetes, cancer, HIV/AIDS, malaria). Because these diseases place such a large burden on individuals and on health care systems, it makes sense to invest in research that aims to reduce the burden. Some of these diseases are entirely preventable diseases, suggesting that more investment is needed in education as well as technology. The HIV epidemic has shown improvement in the past few years thanks in part to preventative programs, as well as to reduced transmission rates from anti-retroviral therapies [1], and there's hope that researchers will discover a vaccine that successfully blocks infection. Other diseases can already be accurately diagnosed and well-managed using current technology, even if they aren't completely treatable (e.g., diabetes). And then there are diseases such as cancer, where we have had very little success at reducing burden.

Ominously, the World Health Organization is predicting a dramatic rise in the global number of cancer cases over the next two decades. This is owing to increased tobacco use in emerging economies, older and larger populations over much of the globe, and decreases in other types of mortality [2].

Cancer is in many ways the most challenging of these, owing to its biological complexity [3, 4]. As our appreciation of its complexity has improved, it has become apparent that many of the molecular diagnostic tools that we need will have to be capable of measuring large panels of molecules simultaneously, rather than detecting a single gene or protein. We need to detect genetic mutations, epigenetic modifications to chromosomes, levels of gene transcription into messenger RNA molecules, levels of translation into proteins, and post-translation modifications. To fulfill these needs we require new technologies that can measure large numbers of genes or proteins in a manner that is relevant to clinical practice, and it is hoped that the properties of nanomaterials can contribute to this goal.

Similarly, we need a new generation of therapeutics designed to impact specific molecular pathways that are known to be central to a disease. Vaccines are one example of biologics that have been around for decades, and which have had an enormously positive impact without any need for nanotechnology. More recently, a number of antibody- and protein-based biologics have been developed to treat forms of cancer [5] and arthritis [6]. As well, gene therapy [7] has shown success against diseases such as severe combined immunodeficiency [8]. Nanotechnology may not be useful for some of these, but for others it may be absolutely necessary. Success with gene therapy has been limited to cells that are relatively easy to access, such as those of the lung, or that can be harvested, transformed and re-implanted. Otherwise, gene therapy will require a vehicle to deliver the genetic "drug" to sites deeper within the body. Therapy using RNA-inhibition faces similar challenges, but a recent and exciting study demonstrated the first success of using it against cancer via an optimized nanoparticle vehicle [9, 10].

In summary, we need new medical technologies that will allow us to put our knowledge to use in diagnosing and treating disease on a molecular and cellular scale. Most current clinical technologies have not kept pace with research, but there are notable exceptions such as the microarray and some biologic therapeutics. Developing the technologies needed to enable an era of personalized medicine will allow clinicians to better predict who many suffer from an illness and allow for prevention, or to match a therapy with a patient and thereby achieve the best possible outcome.

5.3 The Advantage of Nanotechnology

Now that we've started to consider what is needed to exploit our molecular-biomedical understanding of diseases, we can ask how nanotechnology might contribute. Nanotechnology involves the engineering of materials having at least one dimension on a scale of 1–100 nm. These materials provide a number of specific

advantages that can fulfill some duplicate of the requirements of a new generation of medical technologies [11].

To begin with, the scale of the materials used in nanotechnology overlaps with the scale of biomolecules and sub-cellular structures. For example, an immunoglobulin G antibody molecular has a molecular weight of 152,000 Da and a functional diameter of approximately 11 nm [12]. The ribosomes responsible for synthesizing the polypeptide chains of proteins are approximately 25 nm in diameter. This overlap in scale is a first important advantage of nanotechnology, particularly for in vivo applications. Because of this, we can make nanomaterials that are suitable for use in normal physiological environments. For example, a nanoparticle designed for intravenous injection will have a comparable size to the native biomolecules that are normally present in blood. This means that they are unlikely to become stuck and obstruct small vesicles, which was a problem in earlier research that aimed to develop micron-sized particles as drug delivery vehicles [13]. The size of nanomaterials also allows us to engineer a highly-specific interaction between it and target molecules or structures, which is useful for both in vitro and in vivo diagnostic and therapeutic applications [14]. In some cases, the interaction may need to be optimized to increase its strength or the number of molecules involved, which we can achieve by optimizing the molecules on the material's surface. This is possible in part because the size of the material is similar and can be tuned such that it displays the molecules in an appropriate orientation for the target. The size of nanomaterials therefore allows us to produce devices with increased sophistication and a more refined interaction with target biological molecules and structures that are central to disease.

A second major advantage is that nanomaterials provide a platform that can be engineered through a seemingly infinite number of modifications. As mentioned above, this can include addition of biomolecules on the surface of a material to define a binding interaction with some target molecule or structure. In other cases, modifications may take the form of a polymer layer. The addition of poly(ethylene glycol) to the surface of a material is the most common approach for preventing non-specific adsorption of proteins to its surface or for increasing solubility of a poorly soluble drug, and is therefore used on many implantable or injectable devices, as well as on biological therapeutics [15]. Other modifications include addition of a layer of metal or polymer that provides a functional property, such as those described in the next paragraph. The large numbers of modifications that can be made to any nanomaterial mean that its properties can be highly optimized towards a given application, greatly expanding its potential usefulness.

A last major advantage of nanomaterials is that many of them gain useful functional properties due to their size falling within the quantum realm (below 100 nm). Some of these properties are explained by quantum mechanics, such as the interesting optical properties of fluorescent semiconductor nanoparticles (quantum dots) [16]. Recently, Peng et al. [17] has reported efficient photoluminescent behaviour observed on graphene quantum dots (Fig. 5.2). The luminescence can be varied by controlling relevant process parameters [17]. Other materials gain catalytic properties owing to their high surface-to-volume ratio, such as silver nanoparticles which display anti-microbial activity (Fig. 5.3) [18, 19]. These electronic, optical and catalytic functions are perhaps the most exciting properties that nanomaterials have to offer.

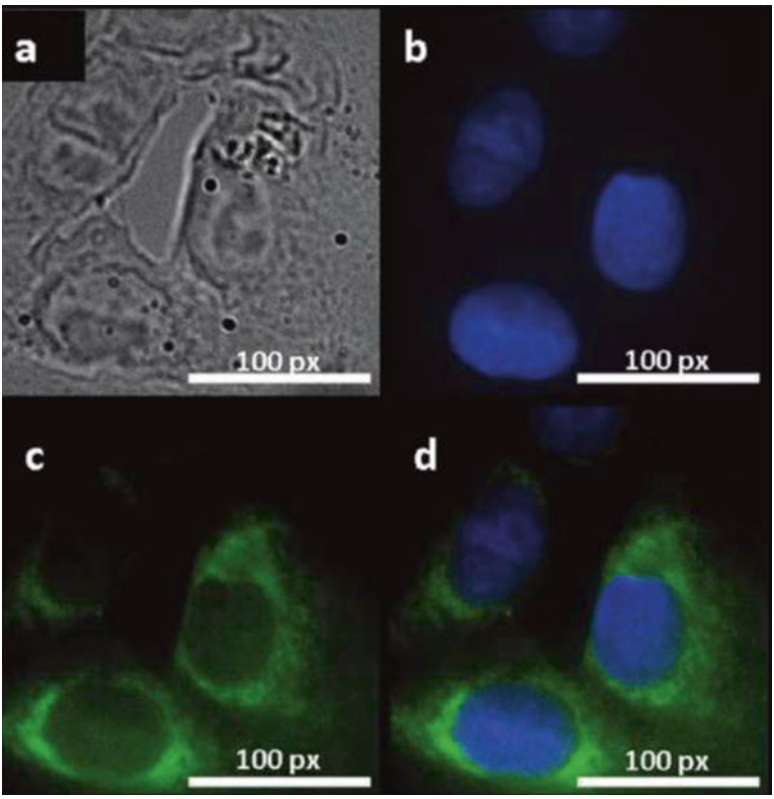

Fig. 5.2 Human breast cancer cells (T47D) exposed to graphene quantum dots. (**a**) Brightfield image of cancer cells. (**b**) Nuclei stained in *blue*, (**c**) *green* fluorescence shows accumulation of quantum dots in the cytoplasm, (**d**) overlay. [17]

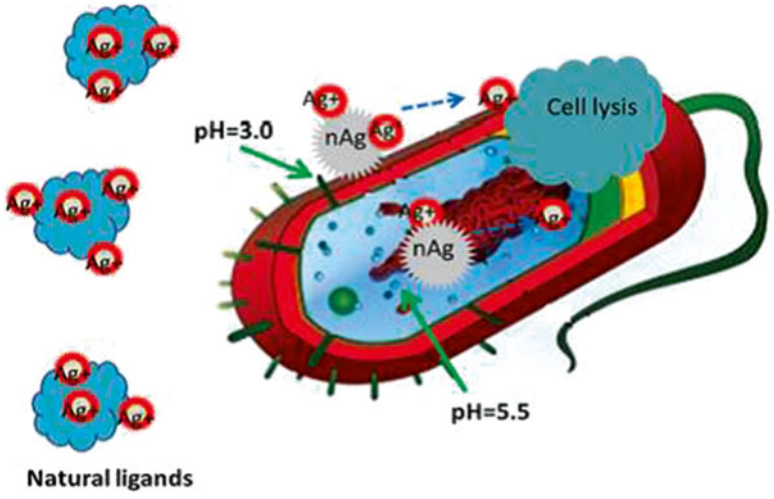

Fig. 5.3 Interaction between silver nanoparticle and the bacterial cell [18]

They provide the potential for a range of diagnostic and therapeutic devices that would otherwise be unimaginable, such as photothermal gold nanorods [20, 21] or nanoshells that can be used for localized ablation of tissue.

Based on these advantages, we can see where nanotechnology can provide gains in medical technology. We can achieve greater control over biomolecular interactions, which will be central to advances in personalized medicine. The material itself provides a platform that can be highly engineered towards a specific goal, such as modifying its in vivo behavior. Finally, we can exploit the electronic, optical and catalytic properties of nanomaterials provide functions to devices that would otherwise be unavailable. We can also see that it may not be useful in every application. If a therapeutic already has favorable kinetics, creating a nanoparticle formulation will unnecessarily increase its complexity and cost. If a diagnostic device is already highly sensitive and meets our needs using current technology, there would be no rationale for developing a nanotechnology-based alternative. Hopefully this has begun to build a framework for what we should expect from nanomedicine.

5.4 The Market for Medical Nanotechnologies

Nanotechnology is a fascinating area of research, and the materials themselves are interesting enough to justify some investment of research time and effort. Beyond this, we've seen that there are clear needs for a new generation of medical devices, and that nanotechnology has specific advantages that will be useful for some of these. There is obvious synergy between these advantages and the goals of personalized medicine, which is to predict, treat, and prevent disease on an individual basis [22, 23]. There is also the opportunity to reformulate many conventional drugs within a nanoparticle vehicle to improve their pharmacokinetics and specific. In almost all cases, we would be aiming to integrate our molecular knowledge of disease with nanomaterials to improve our ability to detect and treat disease.

There is of course already a very large market for medical diagnostics and therapeutics. One report estimated annual spending on in vitro diagnostics in the United States to be $17.6 billion as of 2009 [24]. Much more significant is the market for pharmaceuticals and particularly for oncology therapeutics, which was estimated at $104.1 billion in the United States in 2006 [2], nearly six times the amount spent on all medical diagnostics. Based on this, it's not difficult to believe that medical diagnostics and therapeutics will soon be a trillion-dollar industry, if it isn't already.

How much of this involves nanotechnology? At the moment there are very few marketed diagnostic devices that are reliant on nanotechnology. The most common is likely the lateral flow assay, such as a typical home pregnancy test. This uses antibodies bound to either gold nanoparticles (or alternatively a dye molecule) to determine the presence or absence of a compound. However, each test uses very little material and it is unlikely to amount to a very deep market. The optical properties of gold nanoparticles have also been used to develop a technology platform called Verigene [25], marketed by Nanosphere, which can be adapted for detecting

a wide variety of genetic markers. Nanosphere reported an income of $2-million in 2010, and potentially owns the largest share of the nanotechnology-based diagnostic market. Many other medically-related nanomaterials are also being sold, such as quantum dots (Invitrogen) and gold nanorods (Nanopartz), but for research purposes only.

By far the largest market for medical nanotechnology is currently in nanoparticle-formulated oncology therapeutics [26]. These have been used against cancer for well over a decade now, but account for only $5.6 billion of spending. The formulations currently used in clinics are first generation, and are composed of liposomes, pegylated liposomes, or protein particles [27]. They are relatively low-technology and low-engineering designs in comparison to what is being developed in research labs and even what is currently undergoing clinical trials.

The market for medical technologies is enormous and nanomedicine makes up a very small portion of this. It seems that there are many opportunities for growth. Most of the highest potential nanomaterials and ideas are still at the research stage, and won't begin to make a serious dent in the diagnostic or therapeutic market for another decade. However, we can expect that they will begin to displace older technologies and medicines as we improve our ability to engineer them, exploit their properties and scale-up production.

5.5 Medical Nanomaterials

As mentioned above, there are many different types of nanomaterials, each of which can be modified in a seemingly infinite number of ways. It would therefore be overwhelming to attempt to create a comprehensive description of all the materials that could potentially be used as part of future nanomedical devices. Instead, we'll focus on some general classes and their specific advantages. To make it easier, we'll begin by looking at a recently published nomenclature system that provides a basis for understanding the wide range of available materials.

5.5.1 A Systematic Approach to Understanding Nanomaterials

Until recently there was no unified method available for naming or classifying nanomaterials. A nomenclature presented by Gentleman and Chan in 2009 [28] takes a hierarchical approach, in which a material is systematically classified based on its chemical class, geometry, core chemistry, ligand chemistry, and solubility. We'll make use of their approach to examine what properties define a nanomaterial.

A first major distinction between the many types of nanomaterials is by their different chemical classes. Nanomaterials can consist of either purely organic molecules, as is the case with liposomes, purely inorganic or metallic materials, or some hybrid of the two. This first distinction is important in the development of

materials that may be used in vivo, as organic nanomaterials designed to biodegrade would generally be more biocompatible. At the same time, the interesting and useful optical and electronic properties are restricted to inorganic and metallic materials. For example the fluorescence properties of quantum dots [29] make for an excellent in vivo contrast agent [30], but there are concerns about their toxicity and long-term persistence within the body [31, 32]. Carbon-based fullerene materials such as multiwall nanotubes are an obvious exception to this differentiation based on organic and inorganic material function. Although they are composed purely of carbon, they offer some interesting electronic properties that are more similar to metallic nanomaterials [33].

The second consideration for classifying a nanomaterial is geometry. The size and shape of most nanomaterials is central to many of their important properties. For example, the optical properties of quantum dots are a product of their semiconductor core diameter [29]. The surface resonance plasmon of gold nanoparticles is also dependent on their diameter [34], as well as on their shape. Gold nanorods strongly absorb longer wavelength light than spheroid particles of the same volume, and translate much of that absorption into heat [35–38]. The catalytic properties of metal nanoparticles are dependent on surface area, and therefore on their size [39]. Geometry also determines how nanoparticles behave in vivo. It determines their access to various compartments within the body [13, 40–42], how quickly they will be recognized by the immune system [43], how they are excreted, and how they interact with cells [44, 45]. Geometry is therefore one of the most important parameters for how a nanomaterial is chosen and designed towards a particular medical application.

Next, we can consider both the core chemistry and the outer ligand chemistry. Some materials consist primarily of a single material or crystal structure (described in part by chemical class), but may also include modifications to that primary structure. For example, semiconductor materials are often doped with rare-earth metals in order to optimize their electronic properties [46, 47]. Ligand chemistry defines what is presented to the outside environment. Because of this, engineering the outer ligand can change the solubility of a material. This is particularly important for the many nanomaterials that are highly soluble in non-polar solvents (e.g. quantum dots), but poorly soluble in the aqueous environments in which they would be used in an interaction with biomolecules or cells [48]. The outer ligands also determine how a material interacts with biomolecules and cells, whether there is a specific and defined interaction (e.g., antibody against a viral protein), or whether the purpose of the ligand is to reduce non-specific interactions (e.g., to slow immune recognition). We can see that the core chemistry and outer ligand chemistry are also very important for determining what properties a material will have, and how it will behave within a given environment.

Now that we've seen the major design parameters that define a nanomaterial, we can look more specifically at a few materials more specifically, see how they've been developed over the years and how it is hoped they can contribute to nanomedicine.

5.5.2 An In-Depth Look at Various Nanomaterials

5.5.2.1 From Liposomes to Polymer Nanoparticles for Drug Delivery

Liposomes are generally not considered to fall within the realm of nanotechnology because their dimensions extend far beyond the nano-realm. Although they can be processed to have diameters as small as approximately 50 nm, liposomes of several hundred nanometers to several microns are commonly used for a variety of applications. As well, liposomes are not synthesized and engineered in the same sense as other nanomaterials, and do not offer the same types of interesting quantum properties as conventional nanomaterials. Nevertheless, liposomes are an important topic because they were one of the first particle systems used for drug delivery, a field that is very prominent in nanomedicine.

Liposomes were first scientifically synthesized five decades ago [49]. They are composed primarily of amphipathic phospholipid molecules that spontaneously self-organize into bilayer membranes when in an aqueous environment. The phospholipids can be from a biological source or synthetic, and their mixture within the liposome formulation can be modified to control properties such as membrane fluidity, curvature, charge and stability. Self-assembly of the phospholipids into a bilayer membrane causes encapsulation of a volume of the aqueous buffer as a cavity inside of the membrane, much like cell and organelle membranes that are present in biological systems. Agents that cannot readily diffuse across the membrane can therefore be encapsulated in the inner cavity of the liposome, which can then act as a vehicle for that agent. The liposome design then determines parameters that are important to pharmacokinetics, such as circulation half-life, rather than it being dependent on the agent itself. They are therefore an obvious choice for use as drug delivery vehicles, and have been used extensively to encapsulate and alter the pharmacokinetic behavior of many cancer therapeutics.

Through their size, liposomes are also effective at exploiting an inherent property of tumors. Vascularized tumours typically do not contain well-formed and mature blood vessels comparable to other tissues. Instead, tumour vessels are tortuous in their architecture [50], immature and porous [51, 52]. To improve accumulation of drugs in tumours, we can therefore synthesize particles which have a diameter large enough to remain within the blood vessels of healthy tissue, but small enough to leak from circulation into tumour tissue when passing through their leaky vessels. This property was first discovered by Matsumura and Maeda using dye-labeled albumin protein [53], and was afterwards characterized by Jain using liposomes [40].

Finally, liposomes were one of the first particle systems to have their surface modified with PEG in order to increase circulation half-life [54]. This was a major breakthrough in nanoparticle-based drug delivery because it decreased the fraction of a dose that ended up cleared by the immune system, increasing the fraction that could accumulate within tumours. It was these discoveries that led to the first "nanoparticle" formulations of anti-cancer agents, and to significant reductions in many of the side effects suffered by patients from earlier forms of therapeutics.

Although liposomes display these very useful properties, they are lacking in several areas. Their minimum diameters are approximately 50 nm and they have only moderate stability. Moreover, they do not allow for a great deal of engineering, which limits our ability to rationally design their properties to overcome specific barriers to drug delivery. Polymer-based particles are therefore an excellent alternative, as we are unlimited in the types of polymers that can be used, and how they can be engineered to provide specific, desirable properties. The most common polymer particles being pursued for drug delivery are composed of biodegradable polymers, such as poly(lactic-co-glycolic acid) (PLGA). These offer the same advantages as liposomes in terms of being able to act as vehicles for agents, take advantage of leaky tumour vasculature and avoid immune clearance by PEGylation. It is also possible to rationally design their permeability for the encapsulated agent, thereby allowing for control over release of the agent into the environment [55, 56]. If liposomes can be considered to have contributed to the first generation of nanoparticle drug formulations, polymer nanoparticles are likely to make up much of the second generation, and many polymer-based nanoparticles are now in clinical trials for drug delivery to tumours [27].

5.5.2.2 Gold Nanoparticles as Diagnostic Agents and Therapeutic Vehicles

Colloidal gold or gold nanoparticles have been produced for centuries. Their magnificent interaction with visible light has made them a favorite ink in stained glass and other types of art. The first scientific report of colloidal gold synthesis (and of any metallic nanoparticle) was by Michael Faraday, who published the work in 1857 [57]. Amazingly, his syntheses are still in suspension, and are held at the Royal Institution of Great Britain. More recently, gold nanoparticles have become a favourite nanomaterial for use in a broad range of applications, including as medical diagnostics and therapeutics. This is because they are relatively straight forward to synthesize over a broad range of sizes, it is very easy to modify their surface via adsorption of ligands (e.g., of proteins) or through a thiol-Au bond, and their surface plasmon can be exploited for diagnostic and therapeutic purposes.

5.5.2.2.1 Synthesis Methods

One of the most commonly used methods for synthesis is that published by Turkevich in 1951 [58] and further refined by Frens in 1973 [34]. This uses citrate as a reducing agent and stabilizing surface ligand. Reduction of gold ions in solution to metallic Au^0 results in formation of ordered crystals that grow into stabilized gold nanoparticles. Adsorption of an organic ligand (in this case the oxidized citrate molecules) to their surface stabilizes the particles, preventing them from aggregating and forming their thermodynamically-favored bulk material. The reducing conditions can be altered to control the rate of particle formation, and

through this it is possible to control particle size. Using the Turkevich/Frens method, batches of gold nanoparticles having diameters between 15 and approximately 100 nm can be synthesized, although the quality of larger diameter batches is greatly reduced. An alternative method for synthesis of larger diameter gold nanoparticles makes use of seeded growth. In this approach, ionic gold is reduced in the presence of high quality (i.e. near-spherical and highly monodispersed in size) small diameter "seed" particles, which provide a template onto which the newly reduced Au^0 is added. By separating the reactions that are responsible for initial formation of nanoparticle crystals and for their growth, we could potentially produce batches that are more monodispersed in size and shape. Early attempts at seed-based growth used conditions that failed to suitably favour growth of the existing particle seeds over formation of new particles, resulting in two populations [59]. We were able to overcome this using hydroquinone as a reducing agent [60], which has a long history of use in photographic film processing, where it is used to selectively grow clusters of silver atoms into larger grains. Our method is able to grow very high quality batches of gold nanoparticles having diameters up to several hundred nanometers.

5.5.2.2.2 Diagnostic Applications

Gold nanoparticles display an interesting and useful surface plasmon resonance (a collective oscillation of valance-band electrons) which is different from that of bulk gold material. The interaction of gold nanoparticles with light is dependent on their diameter, or more specifically on the number of surface atoms relative to internal atoms within the crystal structure [61]. Larger particles display red-shifted plasmon maximum relative to smaller-sized particles, and recently developed hollow gold nanoshells display near-infrared surface resonance plasmon absorptions [62]. The plasmon is also highly sensitive to the particle's local external medium, such that changes to ligands on the particle surface or to the solvent results in a measurable shift in the absorption spectra. The dependence of the plasmon on the number of exposed surface atoms means that aggregation of individual nanoparticles into clusters causes a dramatic red-shifting of their surface plasmon resonance. This property provided the basis for development of elegant diagnostic devices in which aggregation of gold nanoparticles and the resulting colour shift is controlled by biomolecules present on the particle surface, and through their specific recognition of target molecules. This phenomenon was first demonstrated by Storhoff, Mirkin and Letsinger [63] for the detection of nucleic acid single-nucleotide polymorphisms, and has now been developed into the Verigene diagnostic device sold by Nanosphere. A second potentially useful property of the surface plasmon is its ability to enhance or quench the emission of a fluorophore [37, 64, 65]. Although still in the research stage, this can be exploited to mask the signal of a fluorescent contrast agent until released by a biological trigger in vivo, such as the presence of an enzyme [66].

5.5.2.2.3 Therapeutic Applications

Gold nanoparticles are a highly versatile material for use in nanomedical applications. Besides the various clever ways in which their properties have been used in diagnostic devices, they have also been used in various forms as therapeutic agents.

As mentioned above, gold nanoparticle display a unique surface plasmon resonance that is dependent on the number of surface-exposed atoms relative to those within the particle. For solid spherical gold nanoparticles, the maximum absorption of the plasmon is found in the range of 520–550 nm. However, if the particles are prepared in a manner such that they are hollow, the plasmon shifts into the near-infrared range [62]. Unlike solid spherical gold nanoparticles which heavily scatter light, they efficiently absorb and translate light into heat, giving rise to dramatic photothermal effects in the local environment. Gold nanorods, which are synthesized to produce an elongated aspect ratio, display a plasmon in the 650–800 nm range (aspect ratios of 2.5–3.5) and produce similar photothermal effects [35, 37, 38]. In this case, it is the oscillation of surface electrons along the length of the rod (longitudinal surface plasmon) that gives rise to the photothermal effects. This property of nanoshells and nanorods is being developed as a cancer therapeutic, in which the particles are targeted to tumours by systemic or local administration, and are then optically excited to thermally ablate tissue in a localized manner [67, 68]. Both gold nanorods and nanoshells are now commercially available, and gold nanoshells are being tested in clinical trials for thermal ablation by Nanospectra Biosciences, Inc. Finally, spherical gold nanoparticles have also been used for delivery of conventional molecular therapeutics and neoadjuvants to tumours [69–71]. In this case, the use of gold nanoparticles provides a highly tunable platform, allowing for a rational design approach to the vehicle design and a greater efficiency in tumour accumulation. Engineered gold nanoparticles carrying a potent anti-cancer agent (TNF-α) have been developed as Aurimune nanotherapeutic by CytImmune, and have recently completed Phase I clinical trials.

Gold nanoparticles are likely to remain a very prominent material within nanomedicine, owing to their versatility, biocompatibility and useful optical properties. They are one of the first nanomaterials to be integrated within a saleable diagnostic device, and are likely to contribute to next generation targeted cancer therapies.

5.5.2.3 Multi-component Nano-devices

Nanoparticle-based tumour targeting is one of the most prominent research areas of nanomedicine. It is a decades-old field, and there are already numerous nanoparticle-based formulations of cancer drugs in clinical use [27]. The targeting field has progressed significantly through a rational design, evidence-based approach. It evolved from using large particles that would obstruct small capillaries, to smaller PEGylated particles that could passively exploit the leaky vasculature of tumours [54], to actively targeting particles in which a biomolecule presented on the particle surface can specifically bind to antigens present within the target tissue [30, 72, 73], and

finally to some of the functional nanomaterials that were described above. These advances have overcome some of the primary physiological limitations of drug delivery; the poor pharmacokinetics and tumour accumulation efficiency of many cancer therapeutics. Despite this success, there are additional in vivo barriers to targeting that reduce the effectiveness of therapeutics beyond the point where tumour can be completely eradicated within a patient. These barriers include the permeation of a vehicle or drug through the bulk of a tumour, specificity of targeting for deregulated cells over healthy cells, efficient delivery of the drug into the appropriate compartments within target cells, and the multi-drug resistance pathways that expel toxic drugs out of cells. From this we can see that we may have a long way to go to achieve truly effective drug delivery. Nevertheless, nanomedicine offers perhaps the best means of achieving this, because nanomaterials provide a platform that can be engineered and optimized using a rational approach to overcome these barriers.

In the last few years, researchers have begun to explore the possibility of using multi-component nano-devices, rather than single nanoparticles, to overcome some of the barriers to targeting. The first such example of an multi-component in vivo system was demonstrated by von Maltzahn and Bhatia in 2010. Their approach makes use of a tumour-homing nanoparticle, which can broadcast a homing signal from within the tumour via the native coagulation cascade. This homing signal then attracts a secondary nanoparticle component present in the circulation, increasing its accumulation within the tumour 40-times higher than conventional controls [74, 75].

A second multi-component system demonstrated by myself and Chan in the same year [76] uses in vivo assembly of a two component system to favourably alter tumour accumulation pharmacokinetics of a contrast agent [76]. The first component consists of a PEGylated gold nanoparticle that is systemically administered and passively accumulates in tumour tissue over a 24-h period. The particle size was engineered such that it was small enough to gain access to tumours through their leaky vasculature, but large enough to restrict permeation into the tumour's extracellular matrix [41]. This results in a large accumulation of nanoparticles just outside the tumour vasculature, highly accessible to agents in circulation. The particles were also engineered to present a biomolecule for assembly (biotin) on the periphery of their surface ligands. Contrast agents linked to a secondary assembly component (in this case streptavidin) can then leak from the vasculature and assemble onto the gold nanoparticles within the tumour. In control studies we showed that without assembly, the molecular contrast agent was small enough to rapidly diffuse through a tumour mass, decreasing its overall accumulation and limiting its diagnostic signal-over-noise. Therefore, by using in vivo assembly, we were able to achieve accumulation kinetics that might be comparable to an actively targeting system, but without requiring prior knowledge of antigens presented by the tumour tissue itself.

These studies are the first demonstrations of multi-component systems. In general, they take an approach in which the complexity of the nanoparticle targeting device is increased in order to improve targeting. This multi-component, higher complexity approach may become more prominent in generations of future nanodevices, whether for drug delivery of other nanomedicine applications. There are some non-trivial challenges to nanomaterial design and synthesis that limit the

potential complexity and behaviours that we can achieve, but this author believes that multi-component systems could overcome some of the most important remaining barriers to targeting.

5.6 Summary and Future Outlook

As was mentioned at the start of this chapter, nanomedicine is likely near the start of a long journey. Very few of the most exciting ideas and applications have moved out of research labs and into clinical use, but we are already starting to see a few examples of nanomaterial-based diagnostics and therapeutics. There is clearly a need and a market for new medical devices, and nanomaterials offer some unique advantages that could go a long way towards improving disease detection and treatment. A major limitation to all nanomaterials that are prepared using conventional chemical synthesis is that they don't provide angstrom-level control over features that are central to nanomedicine, such as functionalization with biomolecules. As researchers overcome this and begin to design and assemble more complex devices with improved molecular-scale behaviour, we can expect to see major advances in the types of nanotechnology-based applications, and in our success at making measurable impacts.

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Chapter 6
Multifunctional Nanoparticles for Theranostics and Imaging

Xue Xue and Xing-Jie Liang

Abbreviations

ROS	Reactive oxygen species
SWCNTs	Single-walled carbon nanotubes
DWCNTs	Double-walled carbon nanotubes
MWCNTs	Multi-walled carbon nanotubes
CNTs	Carbon nanotubes
Gd	Gadolinium
MCAO	Middle cerebral artery occlusion
CT	Computed tomography
MRI	Magnetic resonance imaging
GNPs or AuNPs	Gold nanoparticles
PET	Photon emission tomography
IONPs	Iron oxide nanoparticles
SIONPs	Superparamagnetic iron oxide nanoparticles
FR-α	Folate receptor-α
CTCs	Circulating tumor cells
Hb	Hemoglobin
NPs	Nanoparticles

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6.1 Introduction

Nanotechnology is an interdisciplinary and multidisciplinary field, which depends on nanoscale materials, especially contributing to the development of nanomedicine [1]. Medical applications of nanomaterials include drug delivery system, proteins and peptides, nano-specific targeting, imaging and detection [2–5]. The continual identification and development of multifunctional nanoparticles is capable of target-specific delivery of therapeutic and/or imaging agents due to their appropriate features, including larger surface area, physical and chemical properties, structural diversity, etc. [6]. As previous studies have demonstrated, the progress in nanomedicine allows giving personalized treatments to patients with maximal therapeutic agents. In the past decade, nanomedicine has become an alternative and ideal possibility to undergo human healthy issue. Compared to the traditional theranostic strategies, nano-theranostics and imaging system has various advantageous aspects: (1) NPs can easily combine with more than one kind of additional imaging contrast enhancements or therapeutic moieties for simultaneous diagnosis and therapy, and high dosages of imaging agents or drugs can be loaded into NPs with simple physical or chemical conjugation; (2) Multifunctional NPs offer an opportunity to alter the absorbance, distribution, metabolism and excretion of drugs, reducing off-target toxicity and improving the therapeutic index. (3) The self-functionalization of nanoparticles, including pH sensitivity, hydrophobic/hydrophilic properties, electric charge, etc., can help to match the complex biochemical system. (4) With specific targeting moieties or physicochemical optimization of size and surface properties, NPs admit to precise and fast diagnosis through targeting disease sites for drug delivery and imaging. These features provide multifunctional NPs with great potential as innovative diagnostic and therapeutic systems. Furthermore, large mounts of detailed information, including environmental factors, genetic factors, individual differences in personality etc. should be carefully identified for the clinical field [7].

6.2 Design of Multifunctional Nanoparticle for Therapeutics and Diagnostics

Nanotheranostics was coined originally as a term to describe a treatment platform that fusing nanotechnology, therapeutics, and diagnostics based on the test results. The engineering of nanomedicine is required several advantageous therapeutic and diagnostic properties including low toxicity to healthy tissue, enhanced permeation and retention in the circulatory system, specific delivery of drugs to target sites, controlled releasing in pathognostic condition, etc. [8]. Traditional NPs like liposomes were initially developed for drug delivery systems. Recently, many newly nanoscale materials themselves have been broadly used and display distinguished therapeutic response and diagnostic properties in both the research setting as well as in a clinical setting, implying their enormous potential as medicinal candidates.

In 1985, fullerenes and their derivatives were discovered and extensively investigated as their unique physiochemical characters. However, In order to change their extremely high hydrophobicity, which hampers its direct biomedical evaluation and application, the modification of nano-C60 was developed and classified by several approaches: (i) the transfer fullerenes into physiological friendly media have been developed; (ii) chemical modification of the fullerene carbon cage; (iii) incorporation of fullerenes into water soluble micellar supramolecular structures; (iv) solvent exchange and long term stirring of pure C60 in water. These strategies considerably created and influenced various of functional fullerenes with the potential application in biomedicine, especially in the field of photodynamic therapy [9, 10], neuroprotection [11], apoptosis [12–14], reactive oxygen species (ROS) scavenging [15, 16], drug and gene delivery [17–19]. The biological activities of fullerenes are considerably influenced by their chemical modifications and light treatment. Gadolinium metallofullerenes $[Gd@C_{82}(OH)_{22}]_n$ were originally synthesized with the transition metal atom gadolinium (Gd) encapsulated in a C_{82} fullerene cage as a contrast agent for magnetic resonance imaging [20]. Recent research has shown that $[Gd@C_{82}(OH)_{22}]_n$ nanoparticles can also be utilized as a potential chemotherapeutic agent. When combining $[Gd@C_{82}(OH)_{22}]_n$ with conventional anticancer drug, $[Gd@C_{82}(OH)_{22}]_n$ may increase intracellular drug accumulation by nanoparticle-enhanced endocytosis appeared strong effect on circumventing tumor resistance in vivo and in vitro [21]. As an attractive theranostic agent, metallofullerene nanoparticle additionally showed a strong capacity to improve cellular immune response by activating T cells and macrophages, resulting in releasing Th1 cytokines and inducing the maturation of dendritic cells [22]. There were other kinds of vehicles for Gd contrast agent, like spherical, nonporous and monodisperse silica nanoparticles, which also received high quality and local relaxivities [23, 24].

Nanotubes are another members of the fullerene structure family, which are categorized as single-walled nanotubes (SWCNTs), double-walled nanotubes (DWCNTs) and multi-walled nanotubes (MWCNTs). The application of carbon nanotubes (CNTs) was limited because of the toxicity issue to biological systems [25]. Currently, strategies for chemically functionalizing of CNTs have been reported and summarized as, (i) Covalent sidewall chemistry; (ii) Covalent chemistry at defects or open ends; (iii) Non-covalent surfactant encapsulation; (iv) Non-covalent polymer wrapping; (v) Molecular insertion into the CNTs interior [26, 27]. Based on these modifications, carbon nanotubes rank among the major, newly developed nanomaterials are of interest for a board range of biomedical applications. For the past few years, high increased studies are emerged focusing on their biomedicinal properties, including high tensile strength, flexibility, absorptivity, durability, and light weight, have led to the anticipation of a high production volume. Lee et al. investigated the application of amine-modified single-walled carbon nanotubes as a scaffold for stem cell therapy and protection of neuron from injury in a rat stroke model, which associated to improve the tolerance of neurons to ischaemic injury and decreased infarction area caused by transient middle cerebral artery occlusion (MCAO) surgery [28]. This study lays the foundation for further

studies to elucidate the relationship between nanomaterials and pathology action. In addition, carbon nanotubes have strong ability to induce malignant transformation and tumorigenesis [29, 30], decrease ROS-mediated toxicological response [31], and reduce cytotoxicity by binding blood proteins [32], etc.

Additionally, gold nanomaterials, such as spherical NPs, nanorods and gold nanoshells have been developed as multifunctional probes due to their diagnostic effects and unique optical properties [33, 34], including size controllability [35], good biocompatibility [36], surface modification or shape control [37], and photothermal effects [35, 38]. Huo et al. [39] indicated that 50 nm Au@tiopronin NPs can accumulate effectively in tumor xenografts in vivo and have superior penetration and retention behavior (Fig. 6.1). Another study also demonstrated 50 nm gold nanoparticles can easily be taken into cells through endocytosis, which eventually blocked autophagic influx and induced lysosome impairment [40].

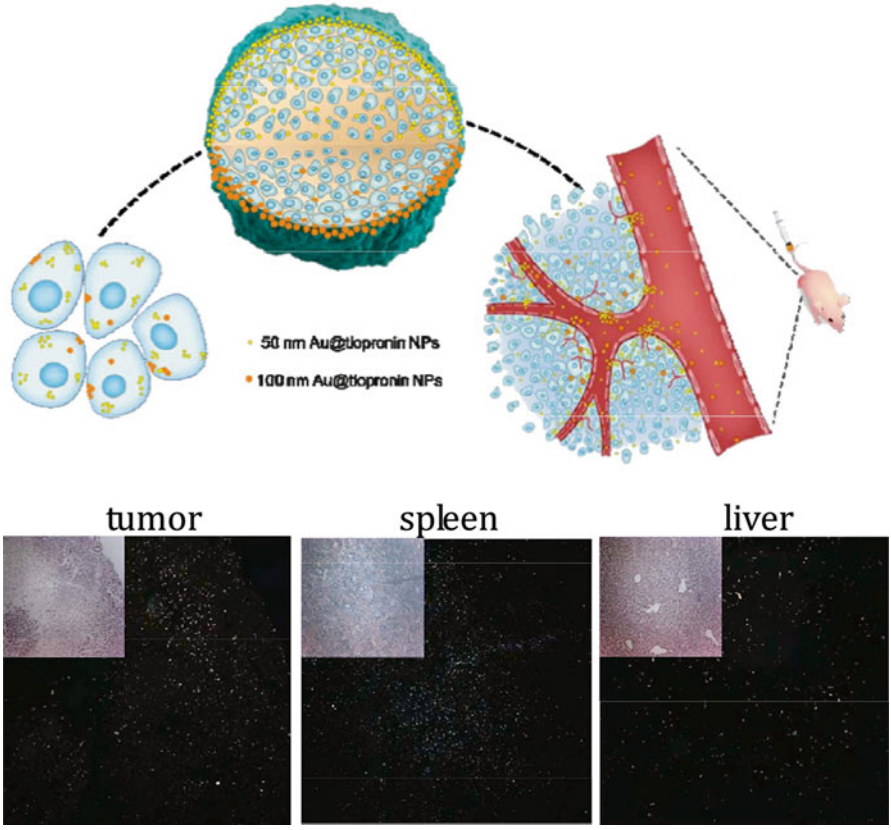

Fig. 6.1 (**a**) Schematic illustration of the localization and penetration behavior of 50 nm and 100 nm Au@tiopronin nanoparticles in monolayer cancer cells *in vitro*, multicellular tumor spheroid *ex vivo*, and xenograft tumor model *in vivo*. (**b**) Au content in the tissues including tumor, spleen, liver at 24 h after i.v. injections of gold nanoparticles (Reproduced with permission [39])

6.3 Designs of Multifunctional Nanoparticle for Medical Imaging

6.3.1 Accumulation and Absorbance

Almost all imaging motifs can be used in pharmaceutical studies to provide anatomic, pharmacokinetic and pharmacodynamic information to address specific properties of complex disease. Currently, nuclear-imaging methods (PET and single photon emission computed tomography, etc.) offer superior sensitivity compared with other modalities, such as ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI). Yet, CT and particularly MRI can provide high-resolution images with great pathological resolution and soft-tissue contrast for diagnostic or prognostic of diseases. Consequently, multimodality imaging is emerging as a powerful combination to provide complementary information for pharmaceutical applications. Small gold NPs (e.g. 5–30 nm), which are considered to provide lower background in blood and tissue and more effectively target biomarkers, can be delivered more easily to cancer cells with different methods, compared to larger NPs [41]. For example, gold nanoparticles provide photothermal ablation therapy due to their unique surface plasmon resonance effects. The radiosensitisation of gold nanoparticles (GNPs) with an average diameter of 5 nm coated with the gadolinium chelating agent dithiolated diethylenetriaminepentaacetic gadolinium (Au@DTDTPA:Gd), which preferentially performed superior accumulation in vitro and in mice bearing tumors, exhibited a chemotherapeutic action [42]. The use of nanostructure based imaging technology requires imaging agents having high accumulating density and specificity for targeting specific cells and tissues. There are also other therapeutic nanoparticles designed based on fluorescent methods. Cabral H. et al. [43] focus on the penetration and efficacy of DACHPt-loaded polymer micellar nanomedicine with different diameters and concluded only the 30 nm micelles could penetrate poorly permeable pancreatic tumors to achieve an antitumor effect in mice (Fig. 6.2).

6.3.2 Distribution

Nanoparticle, which binds with high affinity to specific receptors, could be used to monitor receptor density and distribution in real time. Nanomedical application is designed to track their pharmacokinetics and distribution in real time or monitored at the drug therapeutic target site. To facilitate biodistributional analysis, the versatility of different imaging modalities for nanotheranostics is utilized if the circulation time and the imaged-guided characterization could be advanced in vivo. Various new tools for biomedical research and clinical applications have emerged with recent advances in nanotechnology. In the majority of cases, radionuclides have been used in clinic for improve the understanding of drug delivery process both in animal models and in patients. Types of radionuclide-labeled antibodies, micelles, polymers, liposomes have been subjected to biodistributional analysis for many years.

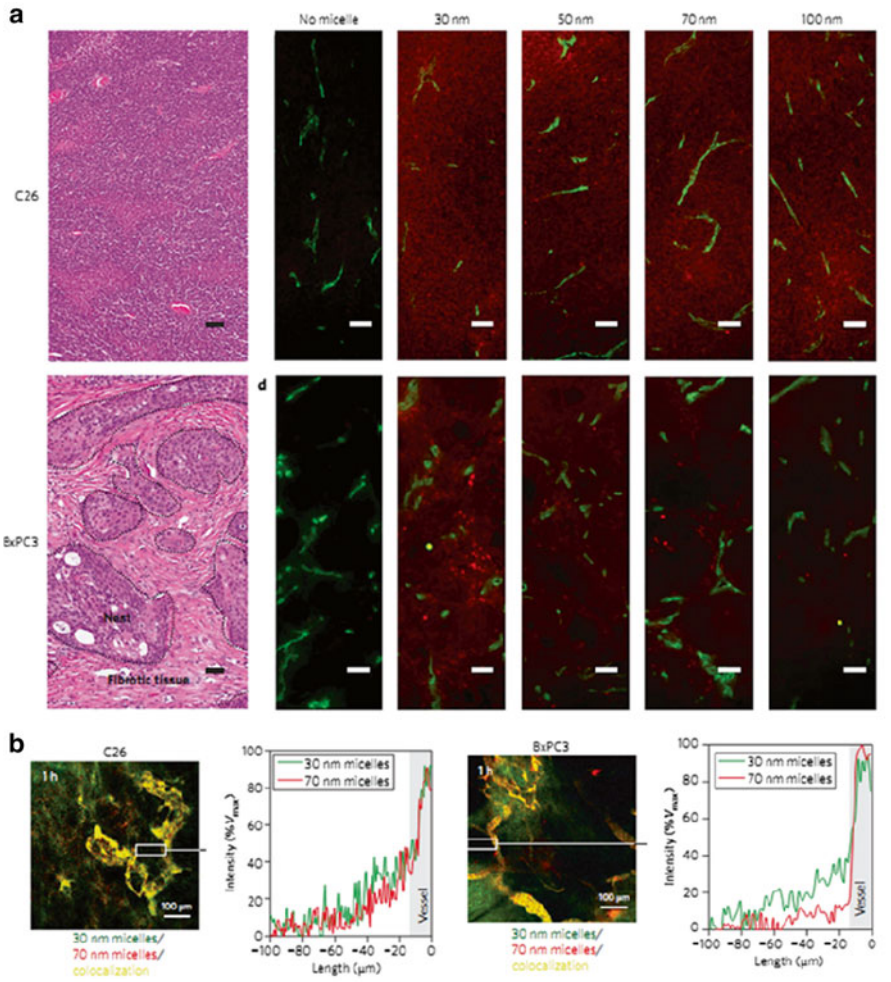

Fig. 6.2 (**a**) Microdistribution of fluorescently labelled DACHPt/m of varying sizes in tumors by histological examination of C26 tumour and BxPC3. (**b**) Real-time microdistribution of DACHPt/m with fluorescently labelled 30 nm (*green*) and 70 nm (*red*) micelles 1 h after injection into C26 and BxPC3 tumours (Reproduced with permission [43])

The high capacity for nanoparticle modification has led to their use as amplifiers for in vivo imaging. Magnetic materials also provide multiple functions in nanotheranostics by the magnetic attraction sensing and the heat generation, as well as serving manipulation by magnetic fields to improve drug delivery or allowing for localized heat therapy [44, 45]. As examples of nanoparticle based products already approved for clinical use, liposomes can encapsulate drug molecules to maintain drug integrity, shield toxicity and minimize side effects to other tissues during drug delivery, while superparamagnetic iron oxide nanoparticles (SIONPs) have

superparamagnetic properties that make them and further evaluation of their efficacy as a powerful magnetic resonance imaging (MRI) contrast agent for imaging and diagnosis of lesions [46]. Torres Martin de Rosales et al. [47] used ^{64}Cu(II)-bis(dithiocarbamate bisphosphonate)-conjugated super paramagnetic iron nanoparticles as dual-modality PET-MRI agent to evaluation the imaging in vivo. The magnetic nanoparticles are ideal for accurate diagnostic use with the sensitive and quantifiable signal of PET and the high soft-tissue resolution of MRI. Namiki et al. [48] also described the utility of lipid-based magnetic nanoparticles as an efficient vector for the RNA interference of cancer. The systemic infusion of LipoMag/fluorescence-labeled siRNA was detected mainly in the magnetic field-irradiated tumor vessels, because EGFR contributed as a target motif. Under a magnetic field, a heavy accumulation of siRNA in tumor lesions was observed only in the LipoMag group (Fig. 6.3). Antitumor effect of the siRNA^{EGFR} was delivered by Lipo/Mag in gastric cancer model.

The ease of functionalizing the surface of nanoparticles with targeting moieties is anther clear advantage in designing molecular probes. Many cancer cells overexpress the folate receptor, and nanoparticles modified with surface folate have been shown to accumulate in tumor cells [49, 50]. Chen et al. covalent conjugated CdHgTe quantum dots and folic acid. These nanoparticles characterized with high targeting affinity and sensitivity were bound to folate transporter-positive cells both in vitro and in vivo, promising candidates for imaging, monitoring and early diagnosis of cancer [51]. The overexpression of folate receptor-α (FR-α) in 90–95 % of epithelial ovarian cancers prompted the investigation of intraoperative tumor-specific fluorescence imaging. In patients with ovarian cancer, intraoperative tumor-specific fluorescence agent folate-FITC imaging for real-time surgical visualization of tumor tissue showed cased the potential applications first-in-human use with ovarian cancer for improved intraoperative staging and more radical cytoreductive surgery [52].

6.3.3 Metabolism and Drug Release

Whereas identifying and monitoring tissues in vivo with various pathologies has received most attention to date, the use of molecular imaging to noninvasively quantify biological changes is potentially far more informative but also more challenging. For this, the imaging probe is a biosensor that not only requires specific targeting but also a reporter system that can be imaged. These probes may be useful for quantifying changes in a biological processes such as enzyme activity, drug metabolism, dynamic analysis, protein-protein interactions, or gene expression [53, 54]. A number of different dynamic analysis have been imaged in vivo using some innovative combinations of fluorescent probes [55]. A hybrid optical probe by incorporating nanoparticle and peptides with artificial tag molecules, which was utilized to detection of prostate cancer related serine protease activity with a high-fidelity and high signal-to-noise-ratio cancer nanoprobe, was applicable for dynamic detection of sensitive cancer biomarker enzymes [56]. Photoacoustic imaging is an emerging

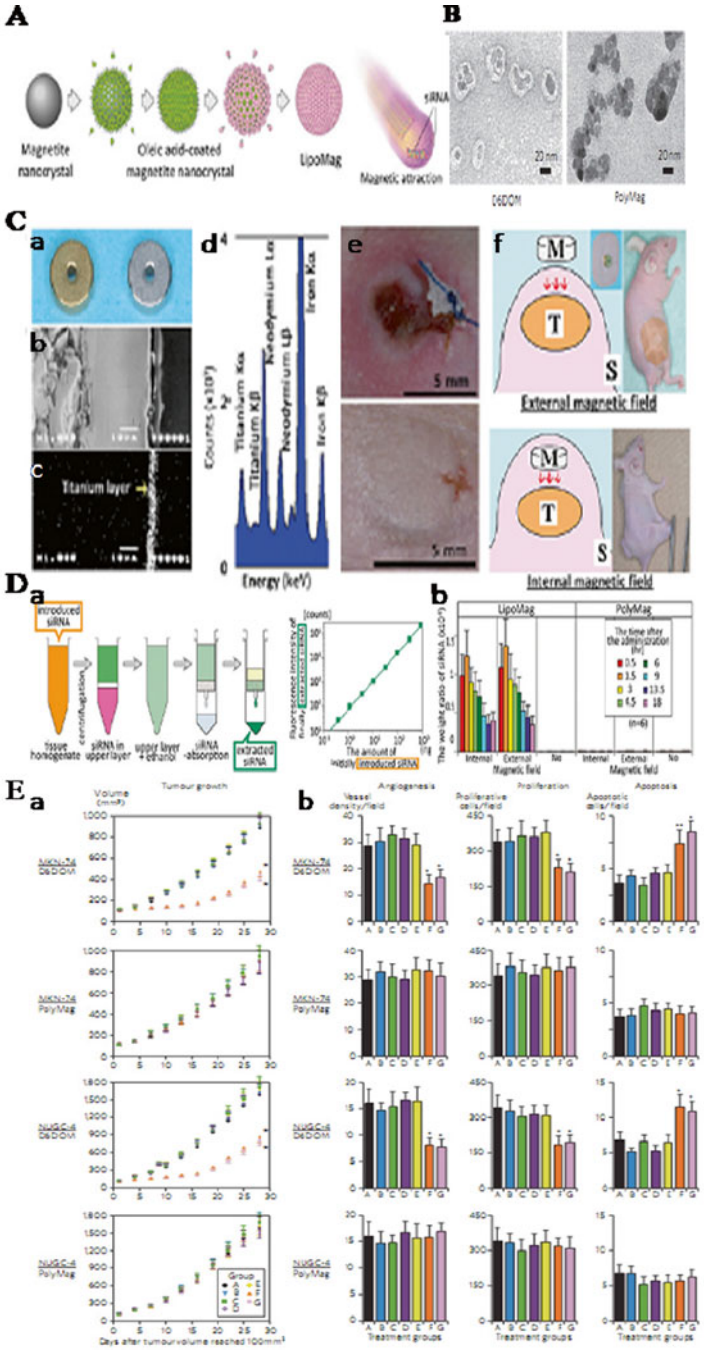

Fig. 6.3 (**a**) Diagram of the Lipo/Mag preparation with oleic acid-coated magnetic nanocrystal cores and lipid shells. (**b**) Transmission electron micrograph of French Press-treated D6DOM40 (*left*) and PolyMag (*right*). (**c**) Metal allergy-free magnets used to the subcutaneous tumor model in

modality that overcomes to a great extent the resolution and depth limitations of optical imaging while maintaining relatively high-contrast. The process of metastasis is the main cause of cancer death [57–59]. Nevertheless, detecting common markers for the development of metastasis, like circulating tumor cells (CTCs), is difficult to trace due to the deficiency of sensitivity diagnosis methods in vivo. To improve detection sensitivity and specificity, gold-plated carbon nanotubes conjugated with folic acid, which were functionalized to target a receptor commonly found in breast cancer cells, were used as a second contrast agent for photoacoustic imaging [60]. It was developed a new platform for in vivo magnetic enrichment and detection of rare targeted magnetic nanoparticles in combination with two-color photoacoustic flow cytometry and accomplished to capture circulating tumor cells under a magnet (Fig. 6.4). Highly sensitive and target specific multiplex detection with infections in static and dynamic conditions (e.g. in blood) that were otherwise previously difficulty to achieve using conventional methods.

6.3.4 Excretion and Clearance

AuNPs excretion can be detected in the kidneys or liver and further research is needed to clarify the properties that affect excretion pathways. A single intravenous injection of AuNPs was rapidly and consistently accumulated in both liver and spleen through 2 months. Significant accumulation in the kidney and testis was starting at 1 month and coincided with a decrease in gold levels in the urine and feces [61]. Regarding overcoming toxicities related to renal clearance of contrast agents, novel gold-silver alloy nanoshells as magnetic resonance imaging contrast agents as an alternative to typical gadolinium (Gd)-based contrast agents. These nanoparticles are very innovative and have the potential to utilize to nephrogenic systemic fibrosis as new candidates for nanotheranostics and imaging [62]. Boros et al. also reported that new Gd derivatives of the $H_{(2)}$ dedpa scaffold greatly improved blood, lung and

Fig. 6.3 (continued) external or internal magnetic field. (*a*) Magnets were coated with titanium nitride (gold-colored, *left*) and regular nickel-coated magnet (silver-colored, *right*), and (*b*) scanning electron micrograph and (*c*, *d*) X-ray analysis of the titanium nitride-coated magnet were shown. (*e*) A titanium-coating can avoid allergic inflammation caused by nickel-coated magnets, which was observed at 28 days after the implantation of titanium nitride-coated magnet. (*f*) External magnetic field for antitumor effect as titanium-nitride coated magnet (*M*) was attached to the tumor lesion (*T*) over the skin (*S*) using adhesive tape (*upper*), and internal magnetic field for tumor lesions as shown a magnet was implanted on tumor tissue under the skin (*lower*). (**d**) (*a*) The distribution of siRNA was quantified by Alexa Fluor-488-labelled siRNA, which were extracted and purified from siRNA in the introduced tissue. (*b*) Weight ratio of siRNA delivered by D6DOM or PolyMag for tumour lesion ($n=6$). In = internal; Ex = external; No = no magnets. (**e**) (*a*) A significant antitumor effect of the D6DOM/siRNA^{EGFR} under a magnetic field was shown in the subcutaneously injected mice. (*b*) Two days after the last treatment, the degrees of angiogenesis, proliferation, and apoptosis were compared by immune-staining of vWF, Ki-67, and ssDNA, respectively. $^{*}P<0.01$, compared with no treatment group (**a**) (Reproduced in part with permission [48])

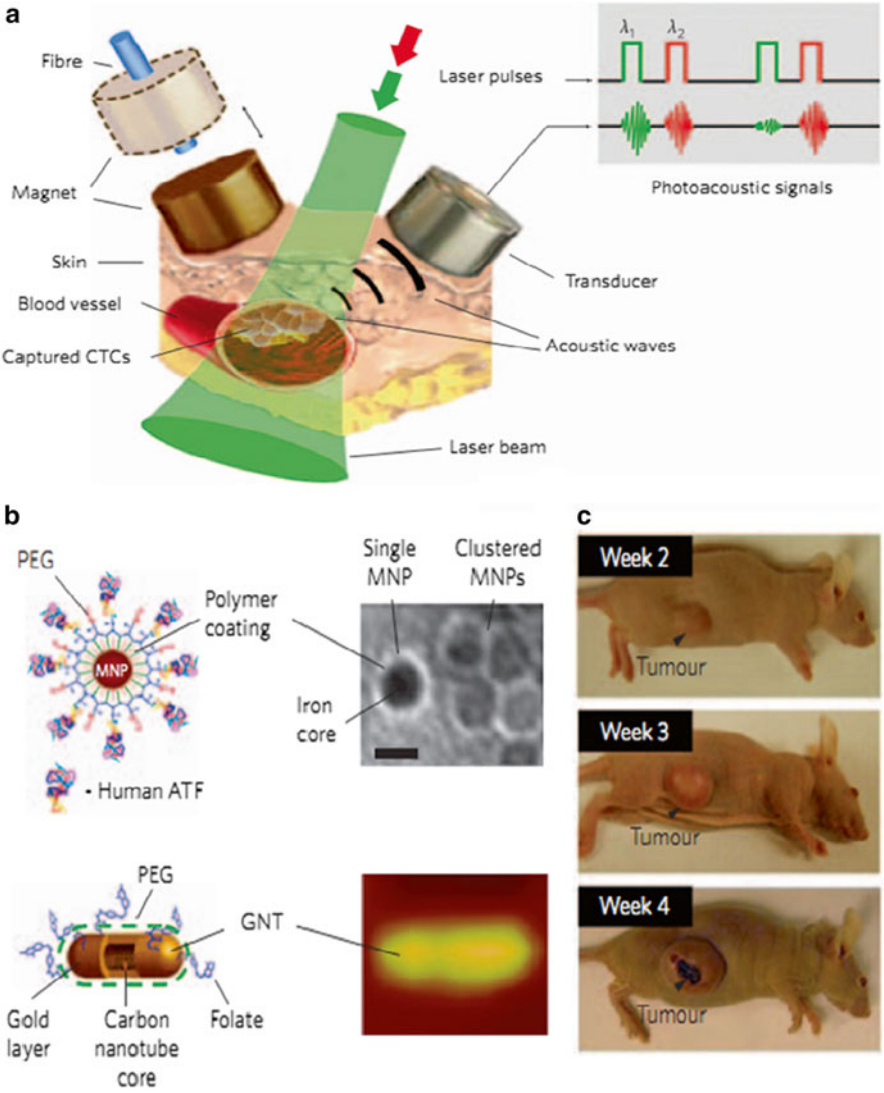

Fig. 6.4 Photoacoustic detection and magnetic enrichment of CTCs. (**a**) Schematic showing the detection setup. CTCs targeted by two-colour nanoparticles can be illuminated by laser pulses at wavelengths of 639 and 900 nm at 10-ms delay. (**b**) The 10 nm magnetic NPs (MNPs) coated with a thin layer (~2 nm) of amphiphilic triblock polymers, polyethylene glycol (PEG) and the amino-terminal fragment of urokinase plasminogen activator (ATF). (**c**) Photoacoustic detection and magnetic enrichment of CTCs in tumour-bearing mice. The size of the primary breast cancer xenografts at different stages of tumour development (Reproduced with permission [60])

kidney clearance compared to previously reported derivatives [63]. Multifunctional mesoporous silica nanospheres with cleavable Gd(III) chelates as MRI contrast agents was quickly cleaved by the blood pool thiols and eliminated through the renal excretion pathway [64]. These multifunctional imaging agents intensively guarantee the nanosafety during the proceed of imaging and theranostics.

Long period real-time imaging is another emergency request, which propose to overcome by nanotechnology. Bourrinet et al. [65] reported renal excretion of radiolabeled iron was retained minimal at day 84 and only 17–22 % injected doses could been excreted in the feces. Incorporation into the body's iron pool upon degradation of iron oxide core is one contributor leading to slow excretion. Briefly, it exists a homeostasis between iron oxide nanoparticles (IONPs) and iron-associated protein (e.g., transferritin, apoferritin, hemoglobin (Hb) molecules), which controls the degradation and clearance of INOPs [66–68]. Moreover, iron from degraded iron oxide nanoparticles could be found in these different forms in mice or rats and contribute to the long-term IONPs biodistribution and clearance kinetics [29, 30, 69, 70].

6.4 Conclusions

In this chapter, we reviewed the design of NPs for both theranoscis and medical imaging. The approach of using multifunctional nanoparticles featuring targets and other auxiliary moieties have exhibited as one of the most promising field to be used as theranostic agents, and trials are underway for the ultimate cure associated to human disease [71]. Based on the new concept of nano-theranostic, clinically approved nanoparticles have consistently shown value in reducing drug toxicity, improving the pharmacokinetics of drugs, even more and more feasible and personalized. It will be finally come in a foreseeable future that nanoscale-based nanotheranastics and imaging will truly help us providing the effective approaches of prevention, therapy and recovery for patients.

6.5 Future Perspective

Nanoparticle scaled agents, which possess unique physicochemical properties that allow integration of multiple functionalities in a single design, have shown tremendous promise in the development of imaging and possess the potential to greatly advance the theranosics and treatment of disease. Multifunctional nanoparticle holds considerable promise as the next generation of medicine that able to contain the early detection of disease, simultaneous monitoring and treatment and targeted therapy with minimal toxicity. The ability of visualization or monitor the absorption, distribution, metabolism and excretion in real time for an intact

organism can reveal physiological and pathological changes that often cannot be studied in isolated cells or tissues. With the advent of highly sensitive and specific imaging technologies, more advanced approaches to image functional changes at the tissue and cellular level are now being explored. To meet the requirements for nanotheragnostic agents, nanoparticles must be required to high stability and controlling function in extreme conditions such as high salt concentrations and wide pH and temperature ranges. Therefore, a flexible and adaptive nanoparticle platform is highly desirable that allows interchangeable therapeutics with high biocompatibility and low toxicity. Future nanoparticle-based theranostics will offer new hope in an attractive way to combat severe or fatal diseases, such as cancer, cardiovascular and neurodegenerative diseases.

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Chapter 7
Medical Nanobiosensors

Eden Morales-Narváez and Arben Merkoçi

Abbreviations

Abs	Antibodies
AD	Alzheimer's disease
AuNPs	Gold nanoparticles
CA125	Cancer antigen 125
CA15-3	Cancer antigen 15-3
CEA	Carcionoembryonic antigen
CJD	Creutzfeldt-Jakob disease
CNTs	Carbon nanotubes
cTnT	Cardiac troponin-T
EGFR	Epidermal growth factor receptor
ELISA	Enzyme linked immunosorbent assay
FRET	Fluorescence resonance energy transfer
HER2	Human epidermal growth factor receptor 2
HRP	Horseradish peroxidase
IUPAC	International Union of Pure and Applied Chemistry
MMP-9	Matrix metalloproteinase 9

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MNPs	Magnetic nanoparticles
MWCNTs	Multi-walled carbon nanotubes
oxLDL	Oxidized low density lipoprotein
PCR	Polymerase chain reaction
PD	Parkinson's disease
PrP	Prion proteins
PSA	Prostate specific antigen
QDs	Quantum dots
SWCNT	Single-walled carbon nanotubes

7.1 Introduction

…the preservation of health is … without doubt the first good and the foundation of all the others goods of this life…

(René Descartes and Discours de la method, 1637)

The concept of diagnosis based on biological samples dated back several thousand years ago documented from the ancient China, Egypt to the Middle Ages of Europe [1]. Nevertheless, it was not until the 1960s when Professor Leland C Clark Jnr., as the father of the biosensor concept, described how to perform reliable and robust measurements of analytes (molecules of interest) presents in the body [2]. Presently, cancer can be diagnosed by screening the levels of the appropriate analytes existing in blood and likewise diabetes is inspected by measuring glucose concentrations. Moreover, the most conventional techniques of diagnostic technologies are the enzyme-linked immunosorbent Assay (ELISA) and the polymerase chain reaction (PCR). Nevertheless, these techniques report different handicaps such as high cost and time required, significant sample preparation, intensive sample handling, and can become troublesome to patients. Accordingly, novel advances in diagnostic technology are highly desired.

Diagnostic technology is an important field for the progress of healthcare and medicine, specifically in early diagnosis and treatment of diseases (which can deeply reduce the expense of patient care related to advanced stages of several diseases). Since molecular diagnostics can profile the pathological state of the patient, nanobiosensors for molecular diagnostics represent a factual and interesting application of the nanotechnology in medicine.

A biosensor is defined by the International Union of Pure and Applied Chemistry (IUPAC) as a "device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles, or whole cells to detect chemical compounds usually by electrical, thermal, or optical signals". Generally, biosensors include biorecognition probes (responsible for the specific detection of the analytes) and a transducer element (that converts a biorecognition event into a suitable signal) [3, 4]. In the twenty-first century, nanotechnology has been revolutionizing many fields including medicine, biology, chemistry, physics, and electronics. In this way, biosensors have been also benefited by nanotechnology, which is an emerging

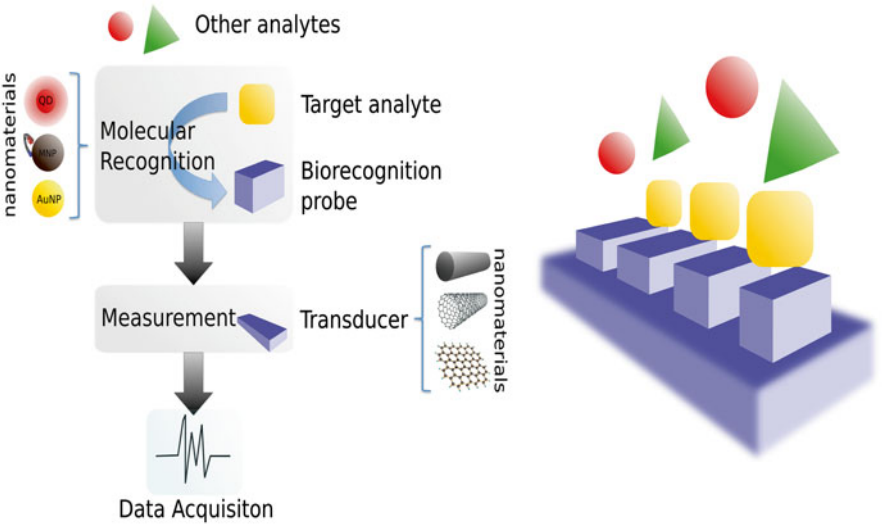

Fig. 7.1 Schematic representation of a nanobiosensor. Normally, a nanobiosensor relies on nanomaterials as transducer elements or reporters of biorecognition events

multidisciplinary field that entails the synthesis and use of materials or systems at the nanoscale (normally 1–100 nm). The rationale behind this technology is that nanomaterials possess optical, electronic, magnetic or structural properties that are unavailable for bulk materials. Since nanomaterials range in the same scale of the diagnostic molecules, when linked to biorecognition probes (such as antibodies, DNA and enzymes), nanostructures allow the control, manipulation and detection of molecules with diagnostic interest, even at the single molecule level. Normally, nanobiosensors are based on nanomaterials or nanostructures as transducer elements or reporters of biorecognition events [5, 6]. Figure 7.1 displays the schematic representation of a nanobiosensor.

This chapter aims at providing a description on the basic principles of the nanobiosensors and a brief survey on nanobiosensing strategies towards medical applications, specifically in the following disease categories: neurodegenerative diseases, cardiovascular diseases and cancer chosen as the most reported application fields.

7.2 Biorecognition Probes

Biorecognition probes, or molecular bioreceptors, are the key in the specificity of biosensors (a non-specific biorecognition event can yield a false result). Biomolecular recognition generally entails different interactions such as hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, pi-pi interactions and electrostatic interactions. In this section the most common biorecognition probes in nanaobiosensors are briefly discussed (see Fig. 7.2a).

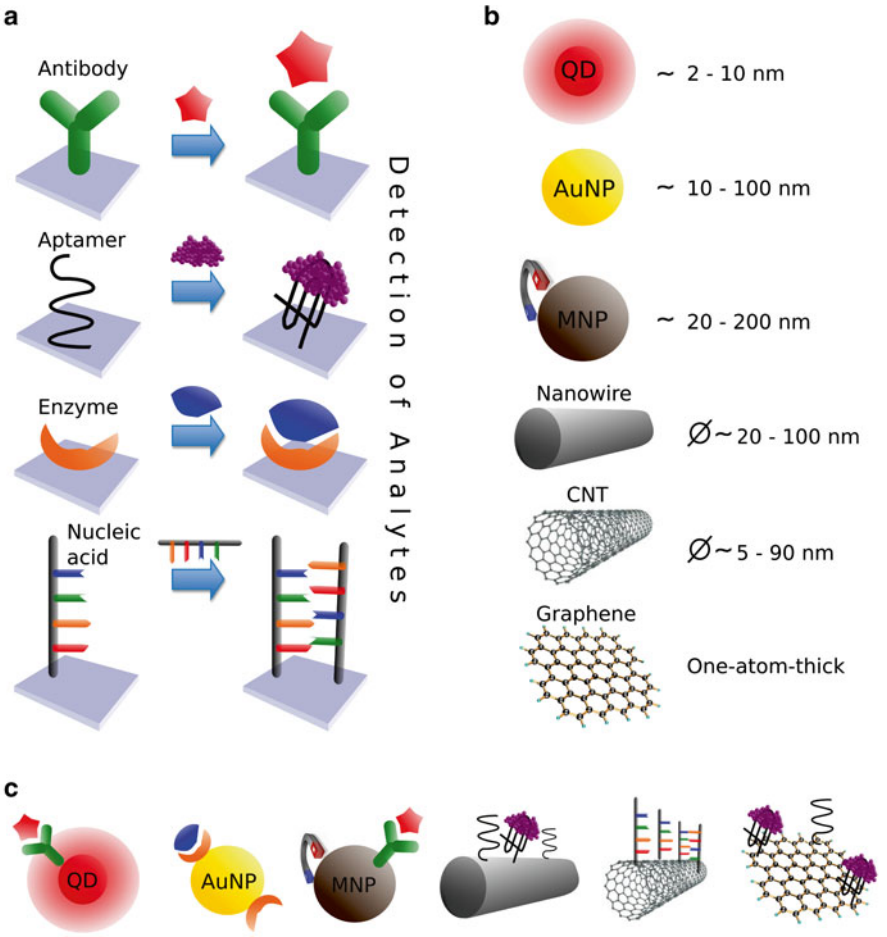

Fig. 7.2 Biorecognition probes and nanomaterials. (**a**) Biorecognition probes. (**b**) Nanomaterials. (**c**) Nanomaterials decorated with biorecognition probes. *QD* Quantum Dot, *AuNP* gold nanoparticle, *MNP* magnetic nanoparticle. Sketches are not at scale

7.2.1 Antibodies

Antibodies are soluble forms of immunoglobulin containing hundreds of individual amino acids arranged in a highly ordered sequence. These polypeptides are produced by immune system cells (B lymphocytes) when exposed to antigenic substances or molecules. Proteins with molecular weights greater than 5,000 Da are generally immunogenic. Antibodies contain in their structure recognition/binding sites for specific molecular structures of the antigen. Since an antibody interacts in a highly specific way with its unique antigen, antibodies are widely employed in biosensors.

7.2.2 Aptamers

Aptamers are novel artificial oligonucleic acid molecules that are selected (in vitro) for high affinity binding to several targets such as proteins, peptides, amino acids, drugs, metal ions and even whole cells [7–9].

7.2.3 Enzymes

Enzymes are protein catalysts of remarkable efficiency involved in chemical reactions fundamental to the life and proliferation of cells. Enzymes also possess specific binding capabilities and were the pioneer molecular recognition elements used in biosensors and continue still used in biosensing applications [10, 11, 126].

7.2.4 Nucleic Acids

Since the interaction between adenosine and thymine and cytosine and guanosine in DNA is complementary, specific probes of nucleic acids offer sensitive and selective detection of target genes in biosensors [12].

7.3 Transduction Modes

In order to detect biorecognition events, biosensors require a transduction mode. Transduction modes are generally classified according to the nature of their signal into the following types: (1) optical detection, (2) electrochemical detection, (3) electrical detection, (4) mass sensitive detection and (5) thermal detection.

7.3.1 Optical Detection

Optical biosensing is based on several types of spectroscopic measurements (such as absorption, dispersion spectrometry, fluorescence, phosphorescence, Raman, refraction, surface enhanced Raman spectroscopy, and surface plasmon resonance) with different spectrochemical parameters acquired (amplitude, energy, polarization, decay time and/or phase). Among these spectrochemical parameters, amplitude is the most commonly measured, as it can generally be correlated with the concentration of the target analyte [13].

7.3.2 Electrochemical Detection

Electrochemical detection entails the measurement of electrochemical parameters (such as current, potential difference or impedance) of either oxidation or reduction reactions. These electrochemical parameters can be correlated to either the concentration of the electroactive probe assayed or its rate of production/consumption [13].

7.3.3 Electrical Detection

Electrical detection is often based on semiconductor technology by replacing the gate of a metal oxide semiconductor field effect transistor with a nanostructure (usually nanowires or graphitic nanomaterials). This nanostructure is capped with biorecognition probes and a electrical signal is triggered by biorecognition events [14, 15].

7.3.4 Mass Sensitive Detection

Mass sensitive detection can be performed by either piezoelectric crystals or microcantilevers. The former relies on small alterations in mass of piezoelectric crystals due to biorecognition events. These events are correlated with the crystals oscillation frequency allowing the indirect measurement of the analyte binding [16]. Microcantilever biosensing principle is based on mechanical stresses produced in a sensor upon molecular binding. Such stress bends the sensor mechanically and can be easily detected [17].

7.3.5 Thermal Detection

Thermal biosensors are often based on exothermic reactions between an enzyme and the proper analyte. The heat released from the reaction can be correlated to the amount of reactants consumed or products formed [18].

7.4 Nanomaterials: The Nanobiosensors Toolbox

Recent advances of the nanotechnology focused on the synthesis of materials with innovative properties have led to the fabrication of several nanomaterials such as nanowires, quantum dots, magnetic nanoparticles, gold nanoparticles, carbon nanotubes and graphene. These nanomaterials linked to biorecognition probes are generally the basic components of nanobiosensors. In order to attach nanomaterials with biorecognition probes, nanomaterials are either electrostatically charged or functionalized with the suitable chemically active group [19–23] (see Fig. 7.2c).

In the following section the most widely used nanomaterials in biosensing are briefly described and they are sketched in Fig. 7.2b.

7.4.1 Zero-dimensional Nanomaterials

7.4.1.1 Quantum Dots (QDs)

QDs are semiconductors nanocrystals composed of periodic groups of II–VI (e.g., CdSe) or III–V (InP) materials. QDs range from 2 to 10 nm in diameter (10–50 atoms). They are robust fluorescence emitters with size-dependent emission wavelengths. For example, small nanocrystals (2 nm) made of CdSe emit in the range between 495 and 515 nm, whereas larger CdSe nanocrystals (5 nm) emit between 605 and 630 nm [24]. QDs are extremely bright (1 QD ≈ 10–20 organic fluorophores) [25]. They have high resistance to photobleaching, narrow spectral linewidths, large stokes shift and even different QDs emitters can be excited using a single wavelength, i.e. they have a wide excitation spectra [26, 27]. Because of their properties QDs are used in biosensing as either fluorescent probes [28, 29] or labels for electrochemical detection (Wang et al. 2011a).

7.4.1.1.1 Gold Nanoparticles (AuNPs)

Synthesis of AuNPs often entails the chemical reduction of gold salt in citrate solution. Their scale is less than about 100 nm. AuNPs have interesting electronic, optical, thermal and catalytic properties [30, 31]. AuNPs enable direct electron transfer between redox proteins and bulk electrode materials and are widely used in electrochemical biosensors, as well as biomolecular labels ([32]; Ambrosi et al. 2009b).

7.4.1.1.2 Magnetic Nanoparticles (MNPs)

MNP are often composed by iron oxide and due to their size (20–200 nm) they can possess superparamagnetic properties. MNP are used as contrast agents for magnetic resonance imaging and for molecular separation in biosensors devices [33–35].

7.4.2 One-Dimensional Nanomaterials

7.4.2.1 Carbon Nanotubes (CNTs)

CNTs consist of sheets (multi-walled carbon nanotubes, MWCNTs) or a single sheet (single-walled carbon nanotubes SWCNTs) of graphite rolled-up into a tube. Their diameters range about from 5 to 90 nm. The lengths of the graphitic tubes are normally in the micrometer scale. CNTs seem a remarkable scheme of excellent mechanical, electrical and electrochemical properties [36, 37] and even can display

metallic, semiconducting and superconducting electron transport [38]. The properties of carbon nanotubes are highly attractive for electrochemical biosensors and also has been used as transducer in bio-field-effect transistors [39, 40].

7.4.2.2 Nanowires

Nanowires are planar semiconductors with a diameter ranging from 20 to 100 nm and length from submicrometer to few micrometer dimensions. They are fabricated with materials including but not limited to silicon, gold, silver, lead, conducting polymer and oxide [41, 42]. They have tunable conducting properties and can be used as transducers of chemical and biological binding events in electrically based sensors such as bio-field-effect transistors [43–45] or even as nanomotors [46].

7.4.3 The Innovative Two-Dimensional Material: Graphene

Graphene is a recently discovered one-atom-thick planar sheet of sp^2 bonded carbon atoms ordered in a two-dimensional honeycomb lattice and is the basic building block for carbon allotropes (e.g., fullerens, CNTs and graphite). Graphene has displayed fascinating properties such as electronic flexibility, high planar surface, superlative mechanical strength, ultrahigh thermal conductivity and novel electronic properties [47]. Owing to its properties, graphene has been employed as transducer in bio-field-effect transistors, electrochemical biosensors, impedance biosensors, electrochemiluminescence, and fluorescence biosensors, as well as biomolecular label [48, 49, 133].

7.5 Nanobiosensing Strategies Toward Medical Applications in Health Priorities: Biomarkers Detection

Biomarkers can be altered genes, RNA products, proteins, or other metabolites that profile biological processes in normal, pathogenic or pathological states and even during pharmacologic or therapy responses of the patient [50–53]. Molecular diagnostics relies on the detection of these biomarkers sourced from biological samples such as serum, urine, saliva and cerebrospinal fluid.

Nanobiosensors can perform a key role in biomarker detection. As innovative devices, nanobiosensors often comprise the following requirements: a tiny amount of sample and assay reagents, fast response, highly sensitive, high accuracy and reproducibility, portability, multiplexing capabilities, user friendly and low cost. This section contains an overview of the powerful advantages of different nanobiosensing strategies for biomarker detection focused on the following disease categories: neurodegenerative diseases, cardiovascular diseases and cancer. Figures 7.3, 7.4 and 7.5 display different biosensors for neurodegenerative diseases, cardiovascular diseases and cancer (respectively). All the exposed applications are summarized in Table 7.1. Since this chapter cannot cover all applications and technical details, the interested reader is referred to some recent literature referenced across the content.

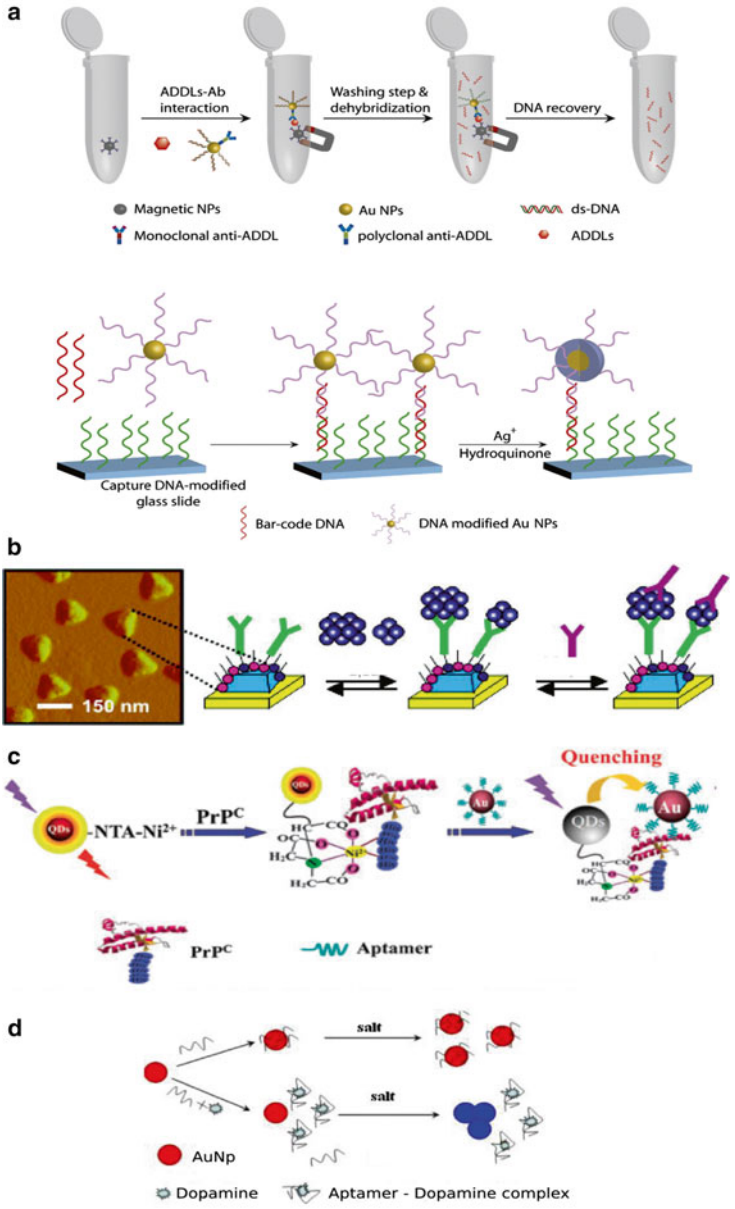

Fig. 7.3 Nanobiosensors for neurodegenerative diseases. (**a**) Bio-bar-code assay for amyloid β (ADDL) detection based on AuNP and MNP (Reprinted with permission from [121]. Copyright 2011, Elsevier). (**b**) Detection of amyloid β by using a nanopatterned optical biosensor based on silver nanoparticles and localized surface plasmon resonance (Modified with permission from Nam et al. 2005. Copyright 2005, American Chemical Society). (**c**) Detection of prion protein (PrP^C) with a long-range resonance energy transfer strategy based on quenching of the light of QDs by AuNPs (Modified with permission from [76]. Copyright 2010, Royal Society of Chemistry). (**d**) Aptamer-based colorimetric biosensing of dopamine using unmodified AuNPs (Modified with permission from [71]. Copyright 2011, Elsevier)

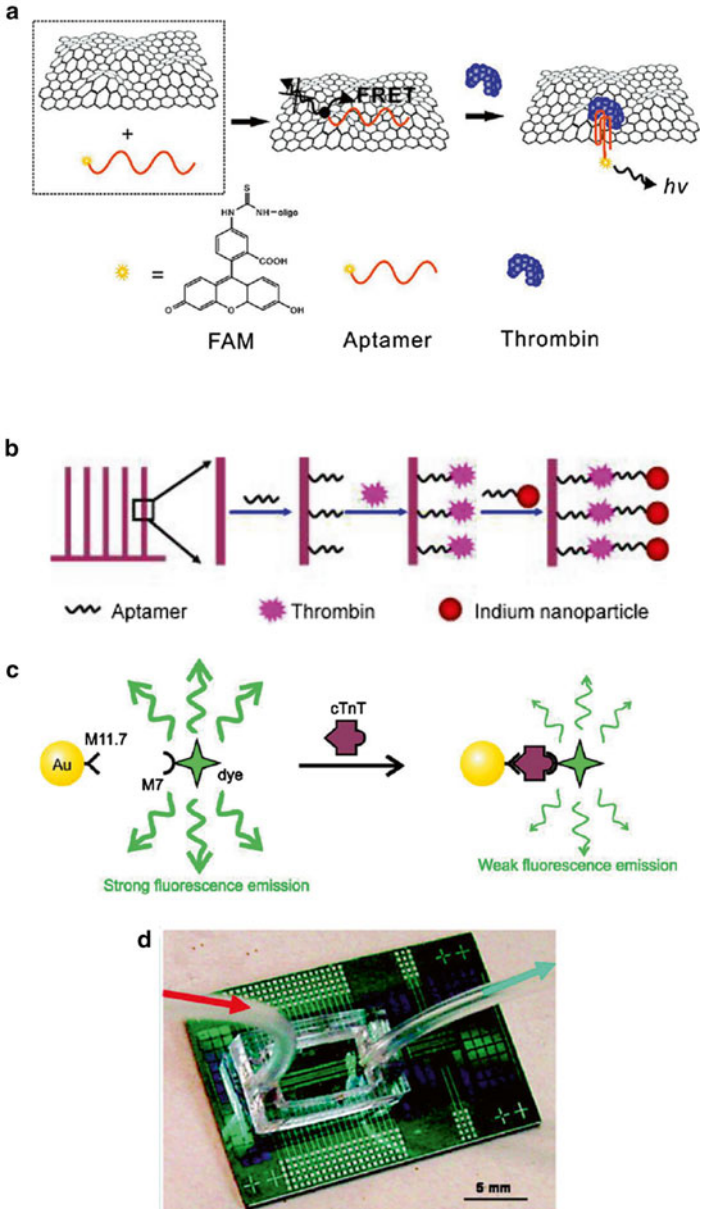

Fig. 7.4 Nanobiosensors for cardiovascular diseases. (**a**) Thrombin detection based on fluorescence resonance energy transfer through graphene as acceptor of organic dyes (FAM) donors connected with specific aptamers (Reprinted with permission from [88]. Copyright 2010, American Chemical Society). (**b**) Aptamer-functionalized indium nanoparticles as thermal probes for thrombin detection on silicon nanopillars using thermal detection (Reprinted with permission from [90]. Copyright 2010, Elsevier). (**c**) Long-range fluorescence quenching by gold nanoparticles in a sandwich immunoassay for cardiac troponin T (M11.7, specific antibody; M7, specific fragment antibody; cTnT, cardiac troponin T) (Reprinted with permission from [93]. Copyright 2009, American Chemical Society). (**d**) Setup for the detection of cardiac troponin T composed of arrays of highly ordered silicon nanowire clusters (Modified with permission from [92]. Copyright 2009, American Chemical Society)

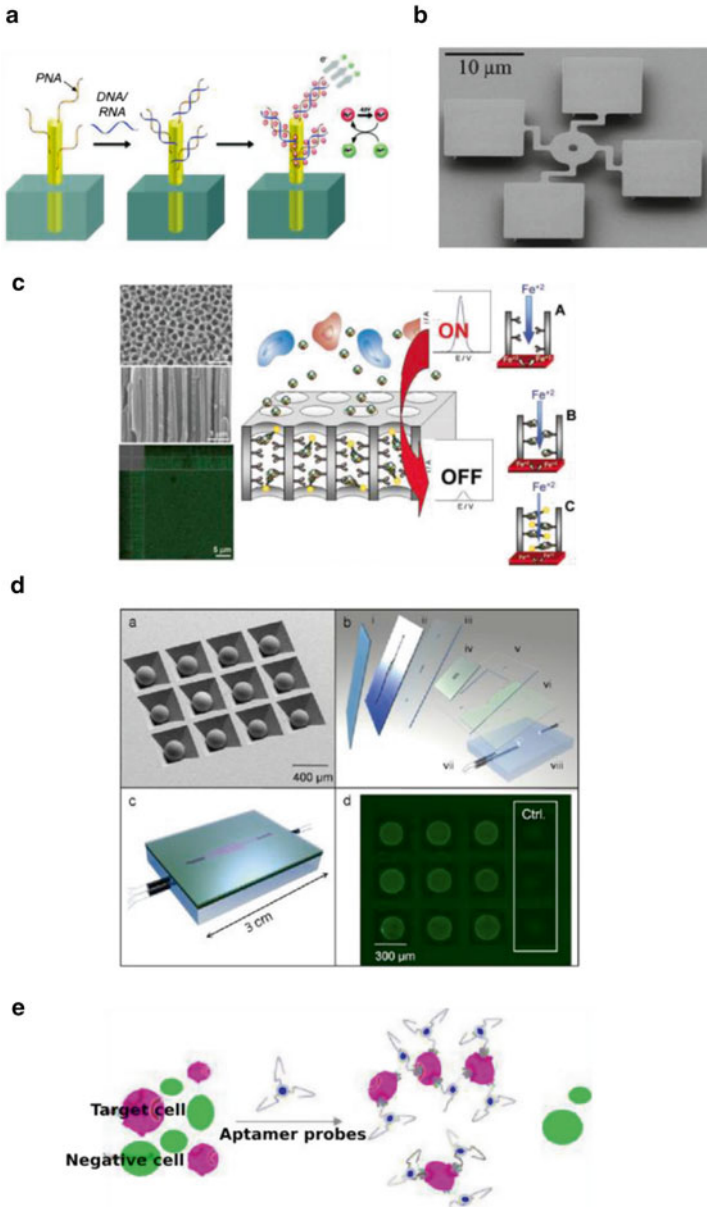

Fig. 7.5 Nanobiosensors for cancer. (**a**) Nanoscale electrode platform for the direct electrocatalytic mRNA (related with prostate cancer) detection using peptide nucleic acid-nanowire sensors (Reprinted with permission from [75]. Copyright 2009, American Chemical Society). (**b**) Trampoline shaped nanomechanical resonator made of silicon for the detection of prostate specific antigen (Reprinted with permission from [2]. Copyright 2009, Royal Society of Chemistry) (**c**) A nanochannel (porous alumina)/gold nanoparticle-based filtering and sensing platform for direct detection of cancer antigen 15-3 using Fe(2/3) as electrochemical signaling indicator (Reprinted with permission from [16]. Copyright 2011, John Wiley and Sons). (**d**) Silicon Nano-bio-chips for multiplexed protein detection: determinations of cancer biomarkers in serum and saliva using quantum dot bioconjugate labels (as fluorescent probes) (Reprinted with permission from [36]. Copyright 2009, Elsevier). (**e**) A sensitive fluorescence anisotropy method for the direct detection of cancer cells in whole blood based on aptamer-conjugated near-infrared fluorescent methylene blue nanoparticles (Modified with permission from [72]. Copyright 2010, Elsevier)

Table 7.1 Nanobiosensors for biomarker detection towards medical applications in different disease categories

Application/detection	Nanomaterials/platform	LOD	Characteristics	Reference
Neurodegenerative diseases				
Amyloid β	Magnetic beads and AuNPs, fluorescent detection (OD)	10 fM	Ultra-high sensitivity. A hopeful beginning towards a diagnosis tool	Khan et al. [57]
	Scanometric detection (OD)	10 aM		
	Nanoscale biosensor based on localized surface plasmon resonance (OD)	7.3 pM	Provides new information relevant to the understanding and possible diagnosis of Alzheimer	Haes et al. [59]
	AuNPs, surface plasmon resonance based sensor (OD)	1 fg/mL	Ultrasensitive detection, focused on early detection	Lee et al. [60]
Amyloid β aggregation inhibitor	Precipitation of AuNP. Optical density of supernatants (OD)	ND	Potential diagnostic tool for diseases involving abnormal protein aggregation as their key pathogenesis processes	Han et al. [62]
Apolipoprotein E	Quantum dots. Fluorescent microarray (OD)	62 pg/mL	High sensitivity. Can be extended to several AD biomarkers	Morales-Narváez et al. [122]
Dopamine	Electrodes modified with CNTs and other features (ECD)	1 nM	High sensitivity and selectivity	Ali et al. [69]
	SWCNTs (ECD)	15 nM	Selective, analyte is detected in the presence of ascorbic acid and uric acid at physiological pH	Alwarappan et al. [70]
	Graphene-modified electrodes (ECD)	ND	Selective detection of dopamine in a large excess of ascorbic acid	Wang et al. [47]
	AuNPs (OD)	0.36 μM	Simple, sensitive and selective aptamer based colorimetric detection	Zheng et al. [73]
	MWCNT as enhancer of electron transfer (ECD)	0.01 mM	Response enhanced by using CNT	Alarcón-Angeles et al. [71]
Prion proteins	Gold-coated magnetic nanoparticles (OD)	N/A	Can provide a useful insight into the affinity of PrP to nanoparticle-functionalized aptamers for diagnosis applications	Kouassi et al. [76]
	Nanomechanical resonator arrays and nanoparticles (MSD)	20 pg/mL	Can be suitably applied to develop ante mortem tests to directly detect prion proteins in body fluids	Varshney et al. [77]
	Long range resonance energy transfer from QDs to the surface of AuNPs (OD)	33 aM	Ultra-sensitive detection. Can be successfully applied in biological media	Hu et al. [78]
	QDs as a highly selective probe (OD)	3 nM	Sensitive, rapid and simple detection	Zhang et al. [79]

Cardiovascular diseases				
Matrix metalloproteinase 9	Peptide decorated MNP (MRI)	N/A	Good candidate for an enzyme reporter probe for in-vivo and for whole-body imaging of protease activity in humans	Schellenberger et al. [85]
Thrombin	Catalytic enlargement of aptamer-functionalized AuNPs (OD)	2 nM	Sensitive detection. One of the first nanobiosensors for thrombin detection	Pavlov et al. [89]
	FRET between graphene (acceptor) and organic dye (donor) (OD)	31 pM	High sensitivity in blood serum samples	Chang et al. [90]
	Aptamer-functionalized indium nanoparticles as thermal probes (TD)	22 nM	Sensitive, selective and low-cost method	Wang et al. [72]
	Label-free colorimetric detection using fribirnogen and AuNPs (OD)	0.04 pM	Highly sensitive, selective, rapid and simple method employed in blood plasma	Chen et al. [92]
Cardiac troponin T	Arrays of highly ordered silicon nanowire clusters (ED)	0.8 fM	Ultra-high sensitivity. Potential point-of-care application	Chua et al. [94]
	FRET between AuNP (acceptor) organic dye (donor) (OD)	0.02 nM	High sensitivity	Mayilo et al. [95]
Oxidized low density lipoprotein	Antibody decorated nanowires in field effect transistors (EC)	N/A	Selectivity	Rouhanizadeh et al. [97]
Myoglobin	Surface electrode modified with AuNPs (ECD)	0.56 nM	The whole procedure takes 30 min. Do not require plasma pretreatment. Small sample amount (1 μL).	Suprun et al. [99]
Cancer				
Probes made of peptide nucleic acid used to detect a gene fusion recently associated with prostate cancer	Nanowires, electrochemical detection	100 fM	One of the first electrochemical sensors to directly detect specific mRNAs in unamplified patient samples	Fang and Kelley. [105]
Prostate specific antigen	Arrays of nanomechanical resonators and nanoparticles (MSD)	1.5 fM	Ultra-high sensitivity in real samples	Waggoner et al. [106]
	Silver enhancement of AuNPs (OD)	15 fM	Diagnostic at earlier timepoint comparing with other procedures	Storhoff et al. [107]

(continued)

Table 7.1 (continued)

Application/detection	Nanomaterials/platform	LOD	Characteristics	Reference
Cancer antigen 15-3	Nanochannels and antibody decorated AuNPs (ECD)	52 U/mL	Simple procedure applied to whole blood that avoids tedious and time consuming labors	De la Escosura-Muñiz and Merkoçi. [108]
	Antibody decorated AuNPs (OD)	15 U/mL	High sensitivity	Ambrosi et al. 2009a
Carcinoembryonic antigen	Nanogold-enwrapped graphene nanocomposites (ECD)	10 pg/mL	Sensitive detection through novel nanocomposites that have a promising potential in clinical diagnosis	Zhong et al. [110]
	Quantum dots capped with silica onto gold substrates (ECD)	50 pg/mL	Satisfactory stability, acceptable veracity, proper for dual-tumor markers detection in clinic serum samples	Qian et al. [112]
Carcinoemrbionic antigen	Antibody decorated QDs and capture antibody capped agarose beads in microfluidic chip (OD)	20 pg/mL	Multiplexing capabilities. High sensitivity in saliva and serum samples	Jokerst et al. [113]
Cancer antigen 125		N/A		
Human epidermal growth factor receptor 2		0.27 ng/mL		
Human epidermal growth factor receptor 2	Antibody decorated graphene encapsulated nanoparticles are patterned as gate of a field effect transistor (ED)	1 pM	High specificity and sensitivity. Suitable for clinical applications	Myung et al. [111]
Epidermal growth factor receptor		100 pM		
Tumor cells	Antibody decorated AuNPs (ECD)	4×10^3 cells/ 0.7 mL	Rapid and simple assay	De la Escosura-Muñiz et al. [116]
Leukemia infected cells	Aptamer decorated near infrared fluorescence nanoparticles (OD)	4×10^3 cells/mL	Quick method, without the need of the complicated separation steps in whole blood samples	Deng et al. [117]
Tumor imaging	Antibody decorated MNP as contrast agent (MRI)	N/A	Could be considered for further research as an MRI contrast agent for the detection of tumors in human	Oghabian et al. [117], Rasaneh et al. [120]

LOD limit of detection, *AuNPs* gold nanoparticles, *QDs* quantum dots, *OD* optical detection, *CNT* carbon nanotube, *MWCNT* multi-walled carbon nanotube, *ECD* Electrochemical detection, *ED* electrical detection, *MSD* mass sensitive detection, *TD* thermal detection, *FRET* fluorescence resonance energy transfer, *MRI* magnetic resonance imaging, *N/A* not available

7.5.1 Neurodegenerative Diseases

Dementia is a meaningful health problem in developed countries with over 25 million people affected worldwide and probably over 75 million people at risk during the next 20 years [54]. Alzheimer's Disease (AD) is the most frequent cause of dementia and results in a progressive loss of cognitive function affecting one in eight people by the time they reach 65 years of age [55, 123]. Diverse sources of evidence suggest that amyloid-β (Aβ) have a causal role in its pathogenesis [56]. Therefore, Aβ is a potential AD biomarker. An overview on recent nanobiosensing approaches for Aβ detection is presented below.

Khan et al. [57] have employed the "bio-bar-code assay", previously developed by Mirkin and co-workers [58], to detect Aβ at femto molar level (10 fM by using fluorescent detection) and atomolar level (10 aM, by using scanometric detection). Plasma samples from control and Alzheimer's patients were assayed. This technic is based on oligonucleotide-modified gold nanoparticles. A complex: magnetic bead – capture antibody – Aβ – detection antibody – AuNP – DNA strands is performed. And finally, each analyte binding is reported by the presence of thousands of DNA strands; i.e. the more complexes are created, the more DNA released (see Fig. 7.3a). Despite the results were hard to reproduce, is a hopeful beginning to a clinical diagnostic tool. Haes and colleagues [59] have reported an optical nanobiosensing platform based on localized surface plasmon resonance spectroscopy, so as to monitor the interaction Aβ/specific antibodies. Clinical samples, extracted from cerebrospinal fluid, were assayed in this sensor based on nanopatterned surfaces achieving a picomolar sensitivity (7.3 pM) (see Fig. 7.3b). This technology provides new information relevant to the understanding and possible diagnosis of AD. Lee et al. [60] have developed a surface plasmon resonance based biosensor [61] for Aβ detection. The procedure enhances the surface plasmon resonance signal by using antibody decorated AuNP. This approach is focused on ultrasensitive detection towards early detection achieving a detection limit of 1 fg/mL. Han et al. [62] have proposed a screen for Aβ aggregation inhibitor by using Aβ-conjugated AuNPs. Aβ aggregation of AD patients serum was visualized through Aβ aggregation-induced AuNP precipitation. This approach is a potential diagnostic tool for diseases involving abnormal protein aggregation as their key pathogenesis processes. The authors of this chapter have recently studied a microarray for another potential Alzheimer Disease's biomarker screening; i.e., Apolipoprotein E. This microarray is reported by QDs nanocrystals, exploiting their advantageous photonics properties, a limit of detection up to 62 pg mL^{-1} can be achieved [63].

Parkinson's disease (PD) is a common chronic neurodegenerative disorder. The classical clinical features are progressive tremor, rigidity and bradykinesia [64] Dopamine (DA) is a neurotransmitter with a variety of functions in the central nervous system. It affects the brain's control of learning, feeding and neurocognition. Disorders in DA levels have been associated with Parkinson's disease, among others psychiatric disorders such as schizophrenia, and depression [65–66]. Thus, DA has an active research as biomarker and is a promising analyte toward molecular diagnosis of Parkinson's disease.

Since DA is electrochemically active, electrochemical detection of DA by oxidative methods is a preferred procedure. Nevertheless, DA is always subsisting with ascorbic acid in real samples and the products of DA oxidation can react with ascorbic and regenerate dopamine again impacting the accuracy of detection gravely. Different approaches have been reported to avoid this shortcoming. Ali et al. [67] demonstrated that DA can be electrochemically detected by altering the electrode surface with a thin layer of an in situ polymerized poly(anilineboronic acid)/CNT composite and a thin layer of the highly permselective Nafion film. The detection limit is of ~1 nM. Alwarappan and co-workers [68] explored the performance of electrochemically pretreated SWCNTs for the electrochemical detection of DA in the presence of ascorbic acid and uric acid at physiological pH. This approach showed a successful selective response with a detection limit of about 15 nM. A MWCNT as enhancer of electron transfer combined with β-Cyclodextrin (β-CD) as molecular receptor is also reported as a DA electrochemical sensor system. The proposed molecular host–guest recognition based sensor shows a electrochemical sensitivity for amperometric detection of DA over the range 0.01–0.08 mM [69].

Wang et al. [70] proposed a graphene-modified electrode that was applied in the selective detection of DA with a linear range from 5 to 200 **μ**M in a large excess of ascorbic acid. Zheng and co-workers [73] explored the interaction of DA-binding aptamer and DA by using unmodified citrate-coated AuNPs as colorimetric signal readout, where AuNP aggregation is induced in the presence of the analyte and the stability of the solution is preserved in the absence of the analyte (see Fig. 7.3d). A selective nanobiosensor was achieved with a detection limit of 0.36 **μ**M. The sensitivity and selectivity of these approaches enable their potential use in diagnosis of PD.

Creutzfeldt-Jakob disease (CJD) is a neurodegenerative disease characterized by rapidly progressive dementia, myoclonus, ataxia and visual disturbances, extrapyramidal and pyramidal involvement, as well as a kinetic mutism [74]. Prion proteins (PrP) once transformed from their normal cellular counterparts (PrP^{c}) into infectious form (PrP^{res}) are transmissible infectious particles, destitute of nucleic acid, that are believed to be responsible for causing the fatal CJD in humans [75]. Since disorders in PrP levels are expected to be present in samples derived from CJD patients, there is an enormous interest in PrP screening technologies; some nanobiosening approaches are presented below.

Kouassi et al. [76] have developed an assay for PrP assessment based on aptamer-mediated magnetic and gold-coated magnetic nanoparticles. Analyte detection was reported by using Fourier transform infrared spectroscopy. The proposed assay can provide a useful insight into the affinity of PrP to nanoparticle-functionalized aptamers for diagnosis applications. Varshney and co-workers [77] have reported PrP detection in serum by exploiting micromechanical resonator arrays. Secondary antibodies and nanoparticles were used as mass amplifiers to detect the presence of small amounts of PrP onto mechanical resonators. This device showed a limit of detection of about 20 pg/mL and the authors are currently working so as to enhance the detection limit. Hu et al. [78], have explored an ultra-sensitive detection strategy for PrP based on the long range resonance energy transfer from QDs to the surface of AuNPs. Energy transfer from QDs to the surface of AuNPs occurs with high

efficiency and the fluorescent signal of QDs was quenched as a consequence of the molecular recognition between PrP (bound with high specificity to QDs) and an aptamer specific for the PrP (conjugated to AuNPs) (see Fig. 7.3c). This procedure achieves a very low detection limit of 33 aM and might be successfully applied in biological media. Zhang and co-workers [79] have reported the use of QDs as a highly selective probe for PrP detection. When the suitable treated QDs were mixed with PrP, the concentration of QDs in supernatant decreased due to the precipitation resulting on a reduced fluorescence intensity of the supernatant. This phenomenon was used for quantitative detection with a detection limit of 3 nM. The reported method shows a sensitive, rapid and simple performance.

7.5.2 Cardiovascular Diseases

Cardiovascular diseases is the cause of nearly half of all deaths in the Western world [80] and is also a major cause of death, morbidity, and disability in Asia and Africa [81, 129–131]. Cardiovascular diseases include hypertension with or without renal disease, stroke, atherosclerosis, other diseases of arteries, arterioles, and capillaries, and diseases of veins and lymphatics. In addition, there are different forms of heart disease such as rheumatic fever/rheumatic heart disease, hypertensive heart disease, heart and renal disease, ischemic heart disease, diseases of pulmonary circulation and so on [82]

Matrix metalloproteinase 9 (MMP-9), also known as gelatinase B, is an enzyme important in inflammation, atherosclerosis and tumor progression processes; furthermore MMP-9 is a potential biomarker involved in cardiovascular diseases [83, 84]. Schellenberger and colleagues [85] have developed a technology for MMP-9 screening. They have reported a nanobiosensor based on peptide decorated MNPs that is proper for in vivo imaging of MMP-9 activity by magnetic resonance imaging. This system has a great potential as reporter probes for assessing enzyme activity of proteases by in vivo magnetic resonance imaging and can help towards diagnosis and monitoring applications [86].

Thrombin (also known as factor IIa) is the last enzyme protease involved in the coagulation cascade and it converts fibrinogen to insoluble fibrin, causing blood clotting [87]. Therefore, thrombin plays a central role in cardiovascular diseases [88]. Thrombin detection is under active research; for example, currently, an inquiry on the Web of Knowledge displays more than 50 approaches based on nanotechnology related with thrombin detection. About 30 of them exploit the use of AuNPs and aptamers are the most common biorecognition probes in these sensors.

As far as is known, one of the first nanobiosensors for thrombin detection was reported by Pavlov and colleagues [89]. They developed an amplified optical detection of thrombin onto solid phase by the catalytic enlargement of thrombin aptamer-functionalized AuNPs. This system exhibited a detection limit of ca. 2 nM. Chang and co-workers [90], have proposed a biosensor based on fluorescence (or Förster) resonance energy transfer (FRET) [91] between graphene sheets as acceptors and fluorescent dyes as donors. Fluorescence of dye labeled aptamer is quenched when aptamer attach to graphene due to energy transference between dyes and graphene.

Dyes emission is recovered by biorecognition events, i.e. when thrombin binds with aptamers to perform quadruplex-thrombin complexes (FRET is not present). Fluorescence emission is proportional to thrombin concentration (see Fig. 7.4a). The procedure was applied to blood serum samples achieving a high sensitivity with a detection limit of ca. 31 pM. Wang et al. [47] have reported an innovative thermal biosensor for thrombin detection in serum samples by using aptamer-functionalized indium nanoparticles as thermal probes onto silicon nanopillars (see Fig. 7.4b). This device is characterized by a sensitive (detection limit of ca. 22 nM), selective and low-cost method. Chen and co-workers [92], have published a label-free colorimetric thrombin detection by using fibrinogen decorated AuNPs. Mixing of thrombin into the fibrinogen decorated AuNPs solutions in the attendance of excess fibrinogen yields the agglutination of the capped AuNPs. The absorbance of the supernatant of the assayed solution is inversely proportional to the thrombin concentration. This procedure is highly sensitive, selective, rapid and simple and can be suitably employed in blood plasma with a detection limit of 0.04 pM.

Human cardiac troponin-T (cTnT) is a key protein biomarker related specifically to myocardial damage [93]. Since cTnT prevails in elevated concentrations in the bloodstream of a patient suffering from heart attack, this biomarker can be employed as a helpful linker between a patient suffering from unstable angina or a more dangerous case of myocardial infarction [94]. Mayilo and colleagues [95] have developed a biosensing platform based on FRET effect with AuNP as acceptors of organic dyes donors. The fluorescence of the antibody fragment – organic dye conjugated is quenched by performing an immunocomplex with an antibody decorated AuNP. The fluorescence quenching is proportional to the detected cTnT (see Fig. 7.4c). This platform accomplished a detection limit of 0.02 nM in serum. Chua and co-workers [94] have designed a label-free electrical detection of cTnT by using complementary metal-oxide semiconductor-compatible silicon nanowire sensor arrays. Nanowires are decorated with specific antibodies against cTnT and biorecognition events are reported by electrical signals. Setup is displayed in Fig. 7.4d. This system achieved a limit of detection as low as ca. 0.85 fM fg/mL in undiluted serum and it possesses portability characteristics for point-of-care application.

Oxidized low density lipoprotein (oxLDL) is recognized as a biomarker for acute myocardial infarction in patients with coronary artery disease. Specifically, oxLDL at elevated levels can forecast acute heart attack or coronary syndromes [96, 128]. Rouhanizadeh and colleagues [97] have reported a biosensor based on electrical detection by using antibody decorated nanowires in field effect transistors. This biosensor enables to differentiate the LDL cholesterol between the reduced (native LDL) and the oxidized state (oxLDL). Acute myocardial infarction can also be diagnosed preventively by measuring myoglobin protein levels [98]. Suprun et al. [99] have designed a electrochemical nanobiosensor for cardiac myoglobin screening based on direct electron transfer between Fe(III)-heme and electrode surface modified with antibody decorated AuNPs. Notably, 1 μL of undiluted plasma of healthy donors and patients with acute myocardial infarction was analyzed with a limit of detection as low as 0.56 nM. Since the procedure takes 30 min, it can be used for rapid diagnosis.

7.5.3 Cancer

Cancer is the predominant cause of death in economically developed countries and the second major cause of death in developing countries [100]. Cancer is a pathology mainly characterized by the chaotic growth of cells with an altered cell cycle control. Since the name of a specific cancer depends upon the tissue or body cells in which it originated, there are many different types of cancers and the most frequent are: breast, lung, prostate, skin, cervical, colon and ovarian cancer among others.

Cancer results in complex molecular alterations. These alterations can be unveiled by using technologies that assess changes in the content or sequence of DNA, its transcription into messenger RNA or microRNA, the production of proteins or the synthesis of several metabolic products [101]. Nevertheless, validation of accurate cancer biomarkers has been slow and is under active research [101, 102]. Table 7.2 shows a list of some potential cancer biomarkers. More details about cancer markers can be founded in the literature [103, 104,127, 129,134, 135].

Biosensors for cancer detection are highly attractive and they are under vigorous development. An overview of some recent nanobiosensing approaches is provided below (Table 7.2).

Fang et al. (2009) [105] have developed an electrochemical sensor to directly detect specific mRNAs in unamplified patient samples (without PCR amplification). The sensor relies on peptide nucleic acid decorated nanowires. Probes made of peptide nucleic acid have been used to detect a gene fusion recently associated with prostate cancer (see Fig. 7.5a). This system exhibits a sensitivity of 100 fM. Waggoner and colleagues [106] have designed a nanobiosensing platform based on arrays of nanomechanical resonators for PSA detection. The surfaces of the proposed trampoline-like devices (see Fig. 7.5b) are capped with specific antibodies and the mass of bound analyte is detected as a reduction in the resonant frequency. Antibody decorated nanoparticles are used in order to enhance sensitivity. Real samples were assayed with a detection limit of 1.5 fM. Storhoff et al. [107] have applied a procedure for the detection of prostate cancer recurrence by using AuNP in monitoring PSA levels of clinical samples. This strategy employs functionalized AuNPs as a probe of PSA captured onto antibody capped glass slides. PSA amount was quantified through silver enhancement of AuNPs [108]. Since this method show a detection limit of 15 fM, they achieved a diagnostic of prostate cancer recurrence in

Table 7.2 Potential cancer biomarkers and their applications

Biomarker	Abbreviation	Type of cancer	Application
Human epidermal growth factor receptor 2	HER2	Breast	Predictive
Epidermal growth factor receptor	EGFR	Breast	Predictive
Cancer antigen 15-3	CA15-3	Breast	Monitoring
Carcionoembryonic antigen	CEA	Colon	Monitoring
Cancer antigen 125	CA125	Ovarian	Monitoring
Prostate specific antigen	PSA	Prostate	Monitoring

clinical samples at earlier timepoint comparing with other procedures. In this regard, the device designed by Waggoner et al., probably might exhibit a similar clinical performance.

De la Escosura-Muñiz and Merkoçi [109] have designed a nanochannel/nanoparticle-based filtering and sensing platform for direct electrochemical detection of CA15-3 in blood. A membrane with nanochannels capped with specific antibodies enable both the filtering of the whole blood assayed without previous treatment and the specific detection of the target analyte. Captured analytes are reported by adding antibody decorated AuNPs as electrochemical reporters (see Fig. 7.5c). This process avoids tedious and time consuming labors and has shown a limit of detection of 52 U/mL. The same group have developed an optical ELISA for the analysis of the same antigen useful for the follow-up of the medical treatment of breast cancer. AuNPs were used as carriers of the signaling antibody anti-CA15-3 – HRP (horseradish peroxidase) in order to achieve an amplification of the optical signal. The developed assay resulted in higher sensitivity and shorter assay time when compared to classical ELISA procedures while working between 0 and 60 U CA15-3/mL (Ambrosi et al. 2009a).

Zhong et al. [110] have reported the detection of CEA through nanogold-enwrapped graphene nanocomposites as enhancer probes of electrochemical immunodetection. An immunocomplex capture antibody – CEA is reported by antibody decorated nanocomposites yielding an amplified signal with detection limit as low as 10 pg/mL. Myung et al. [111] have performed a graphene-encapsulated nanoparticle-based biosensor for the selective detection of HER2 and EGFR. Antibody decorated graphene encapsulated nanoparticles are patterned as gate of a field effect transistor where biorecognition events are detected electrically. This biosensor has shown high specificity and sensitivity (1 pM for HER2 and 100 pM for EGFR). These novel procedures have a promising potential in clinical diagnosis.

Qian and colleagues [112] have proposed a simultaneous detection of CEA and IgG by using QDs coated silica. Two different types of QDs (CdSe and PbS) are capped with two different types of detection antibodies respectively. Capture antibodies against the two target analytes are deposited onto gold substrates so as to perform immunocomplexes. After assay steps, since the selected QDs have a different electrochemical response, the target analytes are measured by voltammetry with a detection limit of 50 pg/mL. Jokerst and colleagues [113] have developed a microfluidic biosensor [114] for the multiplexed screening of CEA, CA125 and HER2 based on detection antibody decorated QDs and capture antibody capped agarose beads. QDs are used as fluorescent probes of specific biorecognition events performed onto an array of localized agarose beads (see Fig. 7.5d). Notably, ELISA method and the employment of organic dyes as fluorescent probes in the same platform were compared with this approach. The best limit of detection was achieved by taking advantage of the powerful optical properties of the QDs. For example, real samples of saliva and serum were assayed with a detection limit of 0.02 ng/mL CEA

and 0.27 ng/mL HER2. These systems could be applied to multiple tumor markers screening in clinic samples.

Since cancer cells can quickly infect their surrounding cells and the disease can spread subsequently, the detection of a tiny amount of infected cells is vital towards early diagnosis [115]. De la Escosura-Muñiz et al. [116], have designed a rapid and simple tumor cell detection device based on the specific binding between cell surface proteins and antibody decorated AuNPs. AuNPs are employed as electrochemical probes (based on the enhancement of hydrogen catalysis) achieving a detection limit of ca. 4×10^3 cells/0.7 mL. Deng and colleagues [117] have developed a system based on aptamer decorated near infrared fluorescence methylene blue nanoparticles for leukemia infected cells detection (see Fig. 7.5e). Cancer cells have been detected quickly without the need of the complicated separation steps in whole blood samples by using the fluorescence properties of the nanoparticles. They have been able to detect from 4×10^3 to 7×10^4 cells/mL in a linear range. Gold nanoparticles have also been used, through a simple electrochemical approach, for cancer cell monitoring by Maltez-da Costa et al. [118]. This platform has achieved a limit of detection around 8.3×10^3 cells/mL. Oghabian and co-workers [119] and Rasaneh et al. [120] have proposed strategies based on antibody decorated MNP as contrast agent for tumor screening by magnetic resonance imaging. Antibodies against HER2 were used for the specific detection of tumor mice cells. These strategies could be considered for further research as an MRI contrast agent for the detection of tumors in human.

7.6 Conclusions and Future Perspectives

We have described the basic principles of the nanobiosenors and we have discussed several nanobiosensing approaches for biomarker detection towards medical applications in different disease categories. Nanobiosensors offer powerful capabilities to diagnostic technology. They can enable to reduce cost, sample amount and assay time. These novel sensors can exhibit high selectivity and unprecedented sensitivity. Despite these advantages, nanobiosensors possess some potential weaknesses; for example, some of the nanomaterials production technologies are still expensive and the inherent toxicity of these materials overall while being applied for in-vivo analysis is little known yet. The exposed nanobiosensors are successfully applied as research devices and they are far from being applied in the public domain; nevertheless, close consensus with regulatory agencies (such as the European Medicines Agency or the U.S. Food and Drugs Administration) to develop comprehensive standards for nanobiosensors and procedures will ensure the operative and realistic transition of nanobiosensors to common medical devices.

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Chapter 8
Nanotechnology in Advanced Medical Devices

Sabeeh Habib-Ullah, Dan Fei, and Yi Ge

Abbreviations

AFM	Atomic Force Microscopy
AMD	Advanced Medical Device
CCMV	Cowpea Chlorotic Mottle Virus
CT	Computed tomography
EBID	Electron Beam Induced Deposition
ECM	Extracellular Matrix
ESF	European Science Foundation
HSE	Health and Safety Executive
LOC	Lab on a Chip
MD	Medical Device
MEMs	Microelectromechnical System
MNP	Magnetic Nanoparticles
MPA	Mercaptopropionic Acid (MPA)
MRI	Magnetic Resonance Imaging
NEMs	Nanoelectromechnical Systems
NMs	Nanomaterials
NP	Nanoparticles
OSHA	Occupational Safety and Health Act

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PAMAM	Poly(amidoamine)
PB	Prussian Blue
PEG	Polyethylene Glycol
PMMA	Polymethyl Methacrylate
POC	Point of Care
QD	Quantum Dots
REACH	Registration Evaluation, Authorisation and restriction of Chemicals
SPION	Superparamagnetic Iron Oxide Nanoparticles
SPR	Surface Plasmon Resonance
SWCNT	Single Walled Carbon Nanotube
USPION	Ultra-small Superparamagnetic Iron Oxide Nanoparticles
VNP	Viral Nanoparticles
WHO	World Health Organisation

8.1 Introduction

Nano refers to materials and systems being in the proportion of a billionth of a metre, i.e. 10^{-9} m and it originates from the Greek word 'nano' meaning dwarf. The term of 'nano' was first formally introduced by Tokyo Science University Professor Norio Taniguchi in a paper on ionsputter machining 1974 [5]. Generally these systems are agreed to be between 1–100 nm.

The potential of nanoscience was first expounded by Richard P. Feynman in 1959 [4], in his famous speech "Plenty of Room at the Bottom". Nanotechnology is now one of the world's leading industrial, academic, and political drivers. In a short time its emergence as an innovative and multidisciplinary platform has secured funding and investment on an unheard of scale [1]. Nanotechnology is projected to be worth $2.6 trillion in manufactured goods by 2014 [2]. Moreover the market impact of nano-based applications is estimated to be $300 billion within the next 12 years for the United States alone [3].

Nanomaterials (NMs) and nanotechnology hold the potential to initiate a new industrial revolution. The characteristics that make these materials commercially suitable are their size, aspect ratio and possibility to functionalise their surfaces. Most experts agree that this new phase of technology could have an impact on every aspect of life. Their uses range from drug delivery, food hygiene, to astrobiology and ocular implants.

Recently NMs have caused a flurry of activity in the medical field. A large effort has been made to use the technology itself as an analytical tool, as well as using nano-sized material to enhance the current paradigm. This was codified in 2004 [6], the definition for nanomedicine that the Medical Standing Committee of the European Science Foundation (ESF) compiled is:

> the science and technology of diagnosing, treating, and preventing disease and traumatic injury, of relieving pain, and of preserving and improving human health, using molecular tools and molecular knowledge of the human body

Further, the ESF demarcated five main disciplines of nanomedicine:

1. Analytical tools
2. Nanoimaging
3. Nanomaterials and nanodevices
4. Novel therapeutics and drug delivery systems
5. Clinical, regulatory, and toxicological issues

These five categories are generating a considerable amount of research [7]. However, it is difficult to interpret which of these fields are classed under medical devices, because they can be a range of articles. The World Health Organisation (WHO) has developed its own definition for medical devices [8]:

"Medical device" means any instrument, apparatus, implement, machine, appliance, implant, *in vitro* reagent or calibrator, software, material or other similar or related article, intended by the manufacturer to be used, alone or in combination, for human beings for one or more of the specific purposes of:

- diagnosis, prevention, monitoring, treatment or alleviation of disease
- diagnosis, monitoring, treatment, alleviation of or compensation for an injury

This clearly illustrates the breadth of products that could be considered as medical devices (MDs). Tools such as tongue depressors, injections and even spoons can be considered as MDs. Advanced MDs (AMDs) would clearly be a step above these mundane tools, clearly the use of NMs could potentially cause a device to be labelled in that bracket [9]. Instead of looking at a broad range of devices, it is beneficial to focus on the exceptional that are making a noticeable impact in the current climate of AMDs.

Nanoimaging and diagnostics are showing potential to revolutionise the gold standard techniques of today. Imaging systems can be grouped by the energy used to construct pictorial information, the type of information that is acquired or the spatial resolution that is obtained. Macroscopic imaging equipment that provide data for anatomical and physiological systems are regularly used in clinics across the world, the most common being computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound. Whereas the emergence of molecular imaging techniques, that can be attributed to nanotechnology, are beginning to be used in clinical and pre-clinical settings [10]. This advancement has opened the possibilities of actually imaging biological functions [11].

Figure 8.1 shows the possibilities of nanoimaging and diagnostics; its potential to measure biological processes [12] is similar to a biopsy, but better. This can be achieved without surgery and possible long term monitoring of specific conditions. Applications comprise early detection and diagnosis of state and stage of disease, assessing response to treatment, and studying biological processes in real time [13]. Compared to CT scans, MRI scans and others of this ilk, they are advantageous in providing information for evidence based medicine.

Similar to this are biosensors and nanobiosensors [14, 15], which are comprised of two elements, a detection device and a transducer. The first provides sensitivity and specificity, while the latter gives an indication of the presence of the desired substrate [16]. These devices are being primed to make point of care (POC)

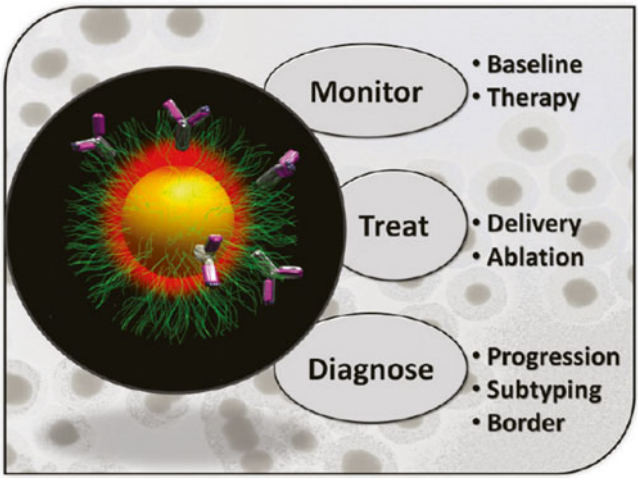

Fig. 8.1 The potential and consequences of nanoimaging and diagnostics [12]

technology feasible, the purpose of which is to deliver real time information on the state of the body. Hence barriers to overcome are to produce something easily fabricated and inexpensive with recognition capabilities, focusing on increasing sensitivity, specificity and enhanced response time [17].

There is already evidence of this on the market with glucose sensors for diabetics. However with nanotechnology in the ascendency, a fast paced diagnostic tool that can run hundreds of tests in less than a minute is conceivable. This lab on a chip (LOC) will utilise the miniaturisation capabilities of nanotechnology to revolutionise patient care. The idea is to produce a small chip that has multiple detection points and transducers to produce a signal, which can then be converted into meaningful information [18]. A key example is the work of Sung et al., in 2010 they demonstrated a microfluidic approach to analyse the pharmacokinetic-pharmacodynamic profile of an anti-cancer drug [19]. The results indicated that their approach was a better alternative to assess drug profiles on cell cultures. Although the work was completed on a micro level, the idea is to exemplify the direction the field is heading towards.

The impact of technology on surgery is often discussed but not truly examined Surgery is also being advanced through the rise of nanotechnology. It can range from the simplicity of the removal of a mole to the complexity of an open heart quadruple bypass. However, surgeons can now use an array of nano-driven materials and devices to aid their work. This extends from advances in intraoperative imaging, tissue healing and wound care to the procedures of nanosurgery involving nanoneedles, nanotweezers and precision lasers [20]. This is leading to more accurate and effective clinical practice combined with surgical precision to produce better invasive procedures.

For completeness, it would also be beneficial to examine the risk to reward ratio. There is scope within nanotechnology for misuse and exploitation. Therefore any regulatory and ethical quandaries should come under consideration in the subsequent discussion.

8.1.1 Nanoimaging

Imaging is a very important aspect within industry. Its uses vary from the x-rays in airports, X-raycrystallography, and MRI to determine chemical compositions. It also plays a critical part in medicine and can be classed as a MD. The WHO clearly states that devices can be used to investigate, diagnose, prevent and alleviate injuries, diseases or a physiological process [8]. The "advanced" can clearly be attributed to the introduction of nanotechnology. This can range from quantum dots, to high-tech processes such as Atomic Force Microscopy (AFM), or a combination of current machinery with a nanotechnology component; such as contrast agents combined with MRI. These tools can be used in prevention, monitoring and treatment of diseases. There is a lag time of information received and can hinder clinician decisions. However, the arrival of new technologies provides real time data along with a unheard of depth of information leading to a more complete and informed diagnosis.

8.1.1.1 Quantum Dots

Quantum dots (QDs) are nanoparticles (NPs) of a few hundred atoms. They absorb light at a wide range of wavelengths, but radiate near monochromatic light of a wavelength that depends on the size of the crystals. Of particular usefulness is the amount of control exerted over QD characteristics; temperature, duration and ligand molecules used during production of QDs can govern size and shape [21]. Hence, the radiation absorbed and light emitted can also be controlled [22]. Furthermore because the materials employed are inorganic, they are more durable in the body than their organic counterparts, they are highly observable using electron microscopy [23].

There are some toxicity issues with QDs, but functionalising them with multiple moieties has led to some favourable outcomes. In 2002, Akerman et al. used polyethylene glycol (PEG) as a surface cap for cadmium selenide QDs [24]. They examined their penetration of tumours in immune-deficient mice and compared it to peptide tagging (Fig. 8.2). Their results demonstrated that QD uptake potential and fluorescent characteristics were enhanced by PEGylation.

Furthermore endocytosis uptake of QDs and labelling of cell surface proteins with QDs conjugated to antibodies were investigated and demonstrated [25]. This procedure has since been improved to show that cells can be imaged and observed in real time. This technology has progressed towards a pilot study of QDs in non-human primates. The use of phospholipid micelle-encapsulated CdSe/CdS/ZnS QDs were observed over a 90 day period. Our results show that acute toxicity of these quantum dots in vivo can be minimal. Blood and biochemical markers remained within normal ranges, but chemical analysis revealed that majority of the initial dose of cadmium remained in the liver, spleen and kidneys. This suggests that the breakdown and clearance warrants further investigation [26]. Furthermore, this technology has been used to investigate specific processes that range from binding of QDs conjugated to the nerve growth factor to membrane specific receptors and intracellular uptake, tracking of membrane protein at the single molecule level, and recognition of ligand bound QDs by T cell receptors [27].

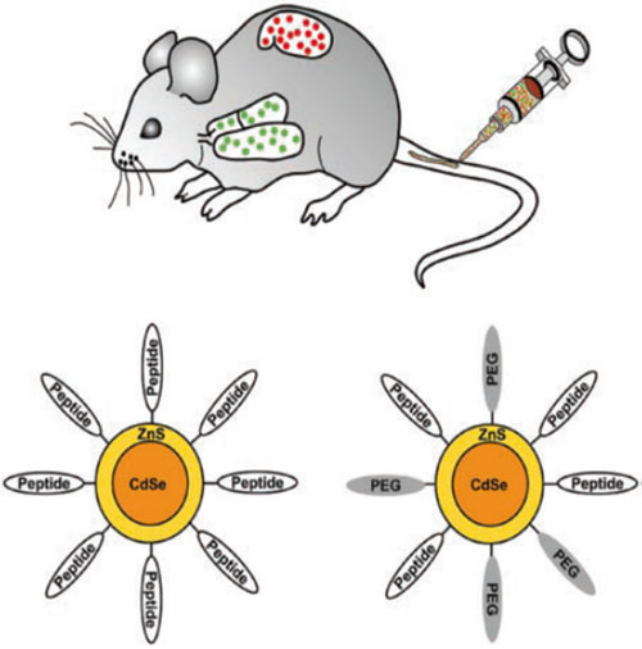

Fig. 8.2 Delivery of QDs into specific tissues of mice. (*Upper*) Design of peptide-coated QDs. (*Lower*) QDs was coated with either peptide only or with peptides and PEG, increasing solubility and bioavailability [24]

8.1.1.2 Magnetic Resonance Imaging

Magnetic resonance imagings (MRIs) are the most widely used diagnostic tools across the world. It is the instrument of choice due to its range, non-invasiveness and minimal side effects. It can be used to give high resolution images of soft tissues; specifically for tumours, brain, central nervous system and cardiac function [28]. MRIs exploit the difference in relaxation times of protons. When protons are exposed to a magnetic field, they become excited and then return to their equilibrium state at different rates T_1 and T_2. It is this information that is used to construct images to help diagnosis and treatment of patients.

Viral nanoparticles (VNPs) are virus-based NP preparations that can be used as a scaffold with a variety of properties. VNPs can be found in the form of bacteriophages and plant or animal viruses. Additionally they are biocompatible and biodegradable; there are organic and non-organic forms. However there is potential to combine these formulations to produce a more functional NP. Yet there have been no explorations of their toxicity, either as singular (organic or non-organic) or in a conjugated format [29].

In 2005, Allen et al. investigated Cowpea chlorotic mottle virus (CCMV) as MRI contrast agents [30]. It has 180 metal binding sites and *in vivo* CCMV binds calcium ions at specific sites; however gadolinium ions (Gd^{3+}) can also do this. Gadolinium is more useful because it increases proton relaxation during MRI, which gives sharper signals. The unusually high proton relaxation values of the Gd^{3+}-CCMV complex can be attributed to the VNP size and number of ions bound. These findings are encouraging, but at the moment VNPs remain proof of concepts until further investigations are completed.

Like VNPs magnetic nanoparticles (MNPs) have gained attention due to their properties. Their magnetic characteristics enable tracking through MRI. MNPs include metallic, bimetallic, Ultra-small and super-paramagnetic iron oxide nanoparticles (USPIONs and SPIONs) [31]. The USPIONs and SPIONs are preferable because of their lack of cytotoxicity, and surface being amenable to functionalization [32]. Hence, there is greater penetration in biological systems, and increased proton relaxation times present sharper signals for better imaging.

New vehicles are being developed that encapsulate multiple imaging molecules onto the MNP for use in integrated imaging systems. These agents can assist investigators to produce images across varied techniques. This signifies that one contrast agent could be used for MRI as well as CT and positron emission tomography scans, offering clinicians the ability to gain a selection of information efficiently [10]. It has been reported that gold NPs coated with gadolinium chelates produces MR contrast [33]. Such MRI active inorganic can be used for imaging of the vasculature, liver and other organs, as well as molecular imaging and cell tracking.

Of course these are not the only particles used for MRI contrast agents. Nanotechnology is developing so quickly that multimodal NPs are being developed not only for MRIs but other imaging techniques. Table 8.1 clearly shows that NPs of different types and sizes can target different organs, and all of them are in the preclinical stages of trials.

8.1.1.3 Atomic Force Microscopy

AFMs are a multipurpose instrument, they can be employed for imaging, determining and manipulating materials at the nanoscale. It can be used to work out surface structure, using forces and interactions such hydrogen bonding, Van der waal, and electrostatic forces. It consists of a small cantilever (of nano-dimensions) that produces a signal when deflected. This is extremely useful in biological imaging, mapping interactions at the cell surface, using high resolution images.

AFM has been used for a multitude of purposes. It can be classed as an AMD due to the fact that it is an analytical tool that is used for monitoring/imaging. Most recently it has proved an effective tool in diagnosis the root causes of protein folding diseases such as Parkinson's, Alzheimer's, and Huntington's diseases. The use of AFM and other nanoimaging techniques has been instrumental in understanding the structure and aggregation of key proteins [35]. Its ability to work on

Table 8.1 A comparison of different nanoparticles, their functions and the cancers they effect [34]

Cancer site	Nanoparticle	Clinical application	Current status
Breast	Iron oxide nanoparticles + Herceptin	Detection of small tumors on MRI	Preclinical
	Iron oxide nanoparticles + uMUC- tumor antigen	MRI and monitoring tumor response to chemotherapy via antigen expression and change in size	Preclinical
	Dendrimer	Contrast agent for micro-MR lymphangiography	Preclinical
	Iron oxide nanoparticle	Detection of sentinel lymph node	Clinical
Colon	Iron oxide particles	MRI of CRC and métastasés	N/A
	QDs	Visualization of cancer using fiber optics	Preclinical
Prostate	Iron oxide nanoparticles	Detection of metastasis with high resolution MRI	Preclinical
	Dendrimer + Prostate specific antibody	Targeting of antigen expressing cells	Preclinical
Brain	Iron oxide nanoparticles	Dual function particles to help define tumor margins accurately intra-operatively	Preclinical
Pancreas	Iron oxide particles	Enhances normal pancreatic tissue on MRI enabling easy visualization of PDAC	Preclinical

singular molecules and isolating its interactions has led to progress in developing understanding and therapies for such diseases. Neurodegeneration in Alzheimer's disease has been linked to β-amyloid (Aβ) peptide build up. AFM has shown that Aβ interacts with the plasma membrane by changing the structure, which leads to the disruption of ionic homeostasis [36]. This was further investigated to conclude that amyloid proteins disrupt distinct regions of the bilayer membranes, which may represent a common mechanism of membrane disruption for protein folding diseases [37].

Nanotechnology has provided the means to make breakthrough machinery more efficient. AFM imaging is generally slow; it takes between 1 and 2 min per frame, making it difficult to record biological processes that generally occur in seconds. Collagen (it is one of the main components of the extracellular matrix) imaging was improved by an inversions feedback/feedforward process. Furthermore it reduced positioning errors as well as increased scan time at high frequencies [38].

In 2006, Voitchovsky and colleagues were able to examine Purple membranes formed by bacteriorhodopsin, it is a combination of crystals and lipids [39]. Using AFM the group were able to observe stiffness and lipid mobility. This work was extended by using AFM to actually study the dynamics of the purple membrane. Yamashita and co-workers were able to film the dynamics of the membrane using high speed AFM, determining how the crystals within it were assembled [40].

8.1.1.4 Optical Tweezers

Optical tweezers are another method of imaging, they can control objects with light. They offer a spatial resolution of 1 nm, a force resolution of a piconewton, and a time resolution of a millisecond. This makes them ideally suited to examine biological processes ranging from the size of a single cell to the minuteness of isolating a single molecule [41].

They were first introduced by AT&T Bell Laboratories. An optical tweezer uses force exerted by the electric field of a focused beam of light. In response to this, a small object develops a dipole, which allows it to be focused at a particular point. The focal point is formed on the optical axis, and the radiation pressure interacts with the electric force, allowing the isolation of a nanosized object. The object of interest can then be examined.

Ermilov and colleagues used optical tweezers to study the interaction of plasma membranes with their own cytoskeletons [42]. Their results showed that combining this technique with fluorescence imaging allowed them to create a profile of forces and stresses acting on the structures of interest. Pine and Chow went further by not only immobilising a neuron but isolating it as well [43]. They found they could move these neurons at a speed of 200 μm/s without actually causing damage to it. The duo also managed to cage these neurons for cell culture. The implication of this study is vast, this technique could be potentially used to grow neurons and graft them for victims of spinal injuries.

Combining this technique with confocal microscopy has allowed the multi-planar imaging of intercellular immune synapses [44]. This collective technology enabled the characterization of complex behaviour of highly dynamic clusters of T cell receptors at the T cell/antigen-presenting cell synapse. This work also identified the presence of receptor rich molecules.

The ability of such techniques to manipulate and measure forces has found several applications such as understanding the dynamics of biological molecules, cell-cell interactions and the micro-rheology of both cells and fluids [45]. A prime example is using optical tweezers to measure the interaction between NPs and prostate cancer cells [46]. The study revealed that the functionalised NP and cell binding was improved by the presence of folic acid. The works presented here show how nano-technology has made a positive impact on medical devices and their capabilities.

8.1.2 Nanobiosensors

The biosensors market has become very lucrative, it is estimated that annual global investment in this technology for research and development was thought to be $300 million. The introduction and fruition of nanotechnology has produced a huge amount of investigation and innovation; over 6,000 articles and 1,100 patents were issued and pending between 1998 and 2004 [16]. This indicates that effort within this field is commercially worthwhile and is rapidly progressing. It is important to note that the addition of NMs is what changes a biosensor to a nanobiosensor.

The uses of biosensors have increased rapidly and have become applicable across a range of fields. Most commonly they are used for clinical, environmental and food purposes, to name but a few [47]. As mentioned before there are two main parts to a biosensor, a recognition element that combines with a transducer (Fig. 8.3). The signal produced is then amplified against some baseline to produce a significant reading. The most dominants types of biosensors can be categorised into three classes, electrochemical, optical and followed by piezoelectric, the latter being the least common. Each of these different categories is differentiated by their transducer technology, as opposed to the molecular recognition element, which can be the same for each of them.

The structure of biosensors has an obvious effect on their functions. The bio-recognition element is based on the high affinity of receptor and analyte, such as an enzyme, a strand of nucleic acid or even an antibody [48].

8.1.2.1 Electrochemical Biosensors

This particular type of biosensor is the most common. It has been used for a variety of reasons. Of interest is the fact that they have been used to detect emerging infectious diseases, however despite the abundance of technology this aspect is still underdeveloped. Environmental detection has been based on monitoring toxic effects in cells, genes and possible endocrine disruptions. This technology is also being heavily mooted to make an impact in POC diagnosis potentially providing information for early detection of disease [17]. Where electrochemical sensors differ is in how signalling is achieved in the transducer element. It can be based on a measured voltage (potentiometric), a current (amperometric), or the transport of charge (conductometric) [16].

8.1.2.2 Optical Biosensors

These particular sensors have been developing rapidly, even more so considering the impact of nanotechnology. Optical sensors employ optical fibres orplanar waveguides to direct light. This is then directed to the sample and interactions produce a signal which can then be compared to its baseline. The signals produced include absorbance, fluorescence, chemiluminescence, surface plasmon resonance to probe refractive index, or changes in light reflectivity and can be read at the sensing film [16, 49].

The advantages of optical biosensors are their rapidity, the insusceptibility of the signal to interference and the potential for greater data, as a result of the changes in the electromagnetic spectrum. Optical methods can be employed to use multiple wavelengths on a sample without interfering with one another. This arrangement can lead to direct or indirect detection [49]. Deposition techniques can be used to produce optical biosensors. Screen printing and ink-jet printing are extremely fast and precise, producing great quantities of low-cost and reproducible biosensors [50].

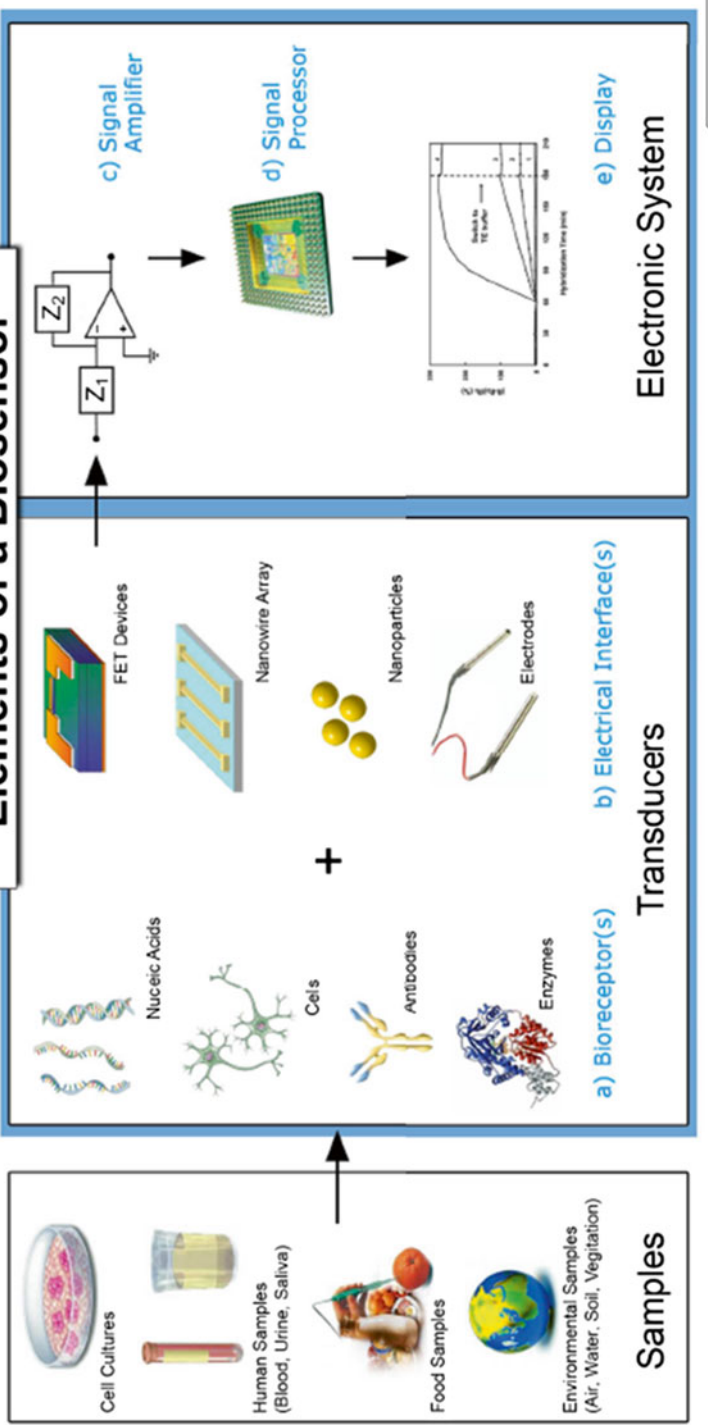

Fig. 8.3 Elements and components of biosensors [48]

8.1.2.3 Piezoelectric Biosensors

As the name suggests this particular biosensor is based on the piezoelectric phenomenon. This was first investigated in 1880 by Jacques and Pierre Curie, who observed that mechanical stress applied to the surfaces of various crystals, caused a correspondingly proportional electrical potential across the crystal, the converse was also true [51]. A multitude of crystalline materials (tourmaline, lithium niobate, aluminium nitride to name but a few) exhibit the piezoelectric effect, however the characteristics of quartz make it the most common crystal employed [52]. These crystals have been used as microbalances owing to their small size, high sensitivity, simplicity of construction and operation, light weight, and the low power required [92]. Similar to the other classes of biosensor, this particular methodology has been employed across a wide range of settings, including environmental and clinical.

8.1.2.4 Nanotechnological Impact

Due to the ubiquitous properties of NMs their use to construct these AMDs is no surprise. In 2003, Davis and co-workers reported on the investigations carried out in their laboratory, which culminated in the construction of a glucose biosensor [53]. The novelty of this sensor was that its construction took place on a single-walled carbon nanotube (SWCNT). The results showed that SWCNTs are highly biocompatible, and their unique structure has added benefits, including having a large surface area that makes it ideal for biological loading. This functionalised surface can exchange electrons and this mechanism can be employed in the fabrication of SWCNT-based biosensing devices.

QDs have also been used in biosensors. Cadmium Telluride was employed because its surface area was conducive to functionalization [54]. Furthermore its conductive properties allowed facilitated the oxidation of the thiocholine-acetylcholinesterase complex, thereby increasing sensitivity. Considering that QDs are so widely used and investigated as biological probes and contrast agents, this is a novel way of using them.

In relation to this green and orange Cadmium Telluride QDs were used as part of a fluorescent biosensor [55]. They were employed to observe proton flux driven by ATP synthesis in viruses. The key findings showed that different fluorescence occurred simultaneously and independently, further they were pH-dependant and showed no interference. An added benefit was that the fabrication of these QDs was inexpensive and convenient, making this commercially viable.

Gold NPs have many uses, in biosensors they are employed because their functions are heavily linked to their structural conformation. Depending on their shape, the surface plasmon resonance (SPR) is affected [56]. This is the oscillation of conduction electrons resonating with the wavelength of light used for excitation. By changing of the gold NPs shape from spheres to rods, the aspect ratio changes, thereby affecting the altering the SPR from the visible region to that of near infrared.

Gold NPs provide better biocompatibility, it also significantly increases the receptor area, thus improving sensitivity. In 2008, Li et al. conjugated gold NPs with

3-mercaptopropionic acid (MPA), poly(amidoamine) (PAMAM) dendrimer to obtain films on which Prussian blue (PB) was electrochemically deposited [57]. The purpose of this was to examine the response to the reduction of hydrogen peroxide. The investigation found that the sensitivity and limit of detection was enhanced, pH range and electrochemical stability and response were also improved. Furthermore, gold NP assemblies were used to trap proteins [58]. Using localised surface plasmon resonance nanotransducers delivers new leverages in hot spot-based nanosensing. The intensity of this electromagnetic hot-spot can be fine-tuned to gain picomolar sensitivity.

Recently, silicon nanowires were found to be able to detect microRNA, at a limit of <100 1 fM [59]. It was found that optimising the surface functionalisation and fabrication protocol, a theoretical limit of detection of 1 fM could be achieved.

It is clear that NMs have enhanced the field of biosensors to new heights. Never before have academics been able to explore biological and cellular processes at such close quarter. Moreover, the addition of NMs can actually enhance specificity, sensitivity and reproducibility. The miniaturisation of this technology is leading to POC and LOC diagnostics.

8.1.2.5 Progress to Lab On a Chip (LOC)

It is imperative to demonstrate the impact that nanotechnology has on the progression from a biosensor to a LOC. In 2009 Lakshmi et al. presented N-phenylethylenediamine methacrylamide (NPEDMA), which contains both aniline and a methacrylamide group, capable of independent polymerisation via free radical or electrochemical methods [60]. This work was then furthered by the group by developing an electrochemical sensor for catechol and dopamine using NPEDMA and a molecularly imprinted polymer (MIP) [61]. The MIP employed in this case is a tyronisase mimicking polymer. It has two copper binding sites and is more stable than its natural counterpart.

MIPs can be considered as polymeric NPs that are able to recognize biological and chemical molecules. The synthesis is based on the formation of a moulding complex between an analyte (template) and a functional monomer. The template is removed leaving impressions of precise recognition sites matching in characteristics to the analyte, intermolecular interactions determine the molecular recognition [62]. Hence it is particularly desirable to use a MIP in conjunction with NPEDMA.

Electropolymerisation of the NPEDMA resulted in the formation of a polyaniline (PANI) layer, a conducting polymer, on a gold electrode surface. The catechol specific MIP was then photochemically grafted on to this layer, in effect creating a network of molecular wires, to conduct a signal for molecule capture. This was a novel method of producing a biosensor with recognition and transducer capabilities.

This approach was then further modified again to examine what impact PANI nanostructures had on the catechol sensor. Berti et al. sputtered gold on an alumina membrane and used it as a mould for poly-NPEDMA [63]. The tryonisase-mimicking MIP was then attached and resulted in the improvement of catechol detection, the lower limit was determined to be 29 nm, a thousand fold increase.

This emphasises the capability of nanostructures to improve diagnostic performances of sensors.

This approach was expanded upon by Akbulut and colleagues. In 2011 they used polychemical grafting to coat NPEDMA to polystyrene microtitre plates [64]. The purpose of this was to create a library of different polymers that could be attached to the plates. The idea of was to assess the feasibility of this to identify a small catalogue of biological and organic compounds. The analytes were a herbicide-atrazine, organic dyes (eosin, bernthsen and meldola), and bovine serum albumin. The results showed that this method of screening is suitable for a myriad of applications and is cost effective, reproducible and easily manufactured.

This work was furthered improved upon by synthesising a polymer to improve and enhance the characteristics shown by NPEDMA [65]. The new polymer is called N-(N_0,N_0-diethyldithiocarbamoylethylamidoethyl) aniline (NDDEAEA). This structure is similar to NPEDMA in that it is based on PANI, but has the ability to be functionalised twice.

This could have far reaching implications over some future applications. In effect, this case study has developed two new polymers with a myriad of different characteristics, the first of which has already been explored to some extent. NPEDMA and NDDEAEA seem to offer potential not only in analytical technology but in other fields. The impact of nanotechnology is vast on AMDs, as demonstrated by this series of work. A polymer and MIP were constructed to form a biosensor, this was essentially modified and optimised to form precursor to a lab on a chip with enhanced limits of detection. This work is only one step short of forming an LOC, consider the key components, there is a molecular circuit linked to a possibility of a library of polymers that can be manipulated to detect any analyte.

The possibilities are endless; furthermore the construct is reproducible and inexpensive. An example of this, is use of nanolithography to create nanoarrays of gold [66]. To demonstrate its multiplex analyses, horseradish peroxidase and anti-horseradish peroxidase antibody was used as a model for a recognition system. The enzyme-linked immunosorbent assay performed had a detection limit 100 pg/mL. It was found that these chips could be stored for 50 days when stored at 4 °C without any significant loss of activity.

8.1.3 Surgery and Clinical Applications

Surgery and clinical applications have been on an upward curve since the exploration of nanotechnology. This was also predicted by Richard P. Feynmann in ‘Plenty of room at the bottom’ talks about the impact of nanotechnology on surgery and its applications:

> It would be very interesting in surgery … if you could swallow the surgeon. You could put the little mechanical surgeon inside the blood vessel and he goes into the heart and looks around (of course the information has to be fed out). He finds out which valve is the faulty one and slices it out. Other small machines may be permanently incorporated into the body to assist some inadequately functioning organ.

It is clear that some of the advances in AMDs are leading to the fulfilment of this vision. This involves the applications of nanotechnology in the surgery. The idea of molecular machines, high precision tools, and nanoimaging are leading to the beginnings of a new field, nanosurgery.

Moreover, giant strides are being made in clinical applications of nanotechnology. This consists of tissue engineering, regenerative medicine and implantation of these AMDs.

8.1.3.1 Nanosurgery

Surgery has always been a macro-scale project, involving cutting, controlling and splicing of organs, muscles and bones. However, as technology has progressed, so have the requirements of this particular field in medicine, leading to more precision and less invasive measures. In the latter twentieth century, the emphasis was on miniaturisation, small incisions, laparoscopic procedures by fibre-optic visualisation, vascular surgery by catheters and microsurgery under microscopes to refine protocols and diminish trauma [67].

One of the major tools that advocate nanosurgery is the use of high precision lasers. A landmark work by Shen and colleagues demonstrated the selectivity and suitability of a femtosecond laser for the ablation of subcellular structures. They focused laser pulses beneath the cell membrane to ablate cellular material. Shen and colleagues were able to selectively remove regions of the cytoskeleton and individual mitochondria without affecting nearby structures or compromising cell viability. Using this approach demonstrated that mitochondria are structurally independent functional units [68].

This work has led to a plethora of development in the use of this 'nanoscissors' technique. Most recently, experiments conducted on human metaphase chromosomes and fixed cell nuclei show it is possible to induce sub-100 nm effects with near-infrared femtosecond laser pulses [69]. Another study used a multimodal imaging system with nanosurgery capabilities, for the selective ablation of sub-cellular components in cancer cells [70]. The work resulted in precise destruction of structures in the cancer cells while leaving them fully functional.

As mentioned before AFM is an instrument that is used in nanomedicine and is highly precise. A study was done to modify the tips of AFM for using electron beam induced deposition (EBIM). This technique was used to make a 'nanoscalpel', 'nanotome' and 'nanoneedle' [71].

Figure 8.4a–c shows the structures of the nanoscalpel being constructing. This was fabricated using an electron beam of low velocity depositing carbon atoms along the tip. This extended along the tip in a self-supporting structure that can then form a blade in the nano scale.

Nanotomes use a similar technique. The process that follows is identical to nanoscalpels, but there are two blades deposited and extended. The difference is that a single filament is extended between the blades, giving it a similar functionality to a vegetable peeler.

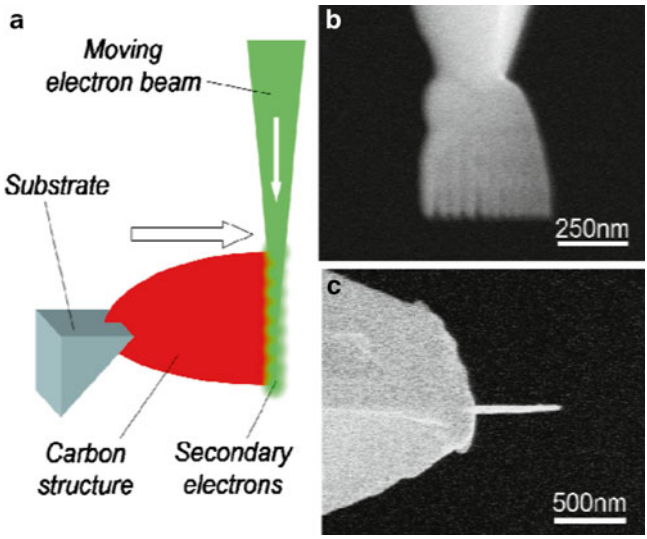

Fig. 8.4 (**a**) Fabrication of nanotool structures using electron beam induced deposition. (**b**, **c**) Nanoscalpel blades imaged from the side (**b**) and from the top (**c**) using SEM [71]

Nanoneedle is processed in a similar way to a scalpel, but the deposition is lateral as well. This allows the needle to be thickened and provide more support, as the thin structure limits the force that can be applied to the nanoneedle. The nanoneedle has several uses, it can be used for profiling structures, and if the needle is thickened then indentations can be made and studied. Beard and colleagues showed that this particular tip could also be used to remove a cluster of proteins from a cell [71]. While the nanoscalpel function can be used for cellular dissections, making controlled incisions. The nanotome can be used to peel back layers of a cell, which can then be imaged.

These nano-surgical tools provide a new route to the manipulation and dissection of cellular structures. They can be used to ablate and manipulate subcellular structures. Although in their infancy, these methods could be developed and used to complete complex surgeries at the nanoscale. Cellular level surgery has been proposed using a nano robot based on AFM technology [72]. It has multiple functions including imaging, characterizing mechanical properties, and tracking. Furthermore, the technique of tip functionalisation facilitates the robot ability for precisely delivering a drug. Therefore, the nano robot can be used for conducting complicated nano surgery on samples such as live cells and bacteria. Additionally, the software in this nano robot provides a "videolized" visual feedback for simultaneously monitoring the operation of nano surgery and observation of the surgery results.

8.1.3.2 Implantable Devices

Nanotechnology is in the process of modernising implantable devices. AMDs that were of macro proportions are now being miniaturised and made more efficient. This is partly due to increased knowledge in the field of biomimetics, the process of

using the way in which nature successfully produces something to create a material or device [20].

Implantable devices can cover many aspects and can deal with sensory issues as well as regulatory issues such as glucose sensors, retinal implants, prostheses with nerve control to name but a few. The ideal scenario is to reach a stage of miniaturisation and biocompatibility that can then produce devices capable of autonomous power, self-diagnosis, remote control and external transmission of data [73].

The auditory nerve contains near to 30,000 axons which cochlear implants stimulate [74]. From 1972 near a 100,000 people have been fitted with the device, however the work being developed is focusing on the nanoscale as the ear contains such structures [75]. The research that produced in this field is focused on developing smaller implants that can aid the work of axons to produce and relay an audible signal for the brain to process.

The optical nerve contains near a million fibres, and must deal with complex data and its processing [74]. In 2007, Alteheld and colleagues compiled a review to report on the developments of visual implants [76]. Electrical stimulation of the retina of blind subjects resulted in ambulatory vision (allowed movements without collisions and stumbling) and some character recognition. This was done by developing a wireless intraocular prosthesis, and having external feedback. Most recently a trial was carried out on 20 patients with varying degrees of blindness, to determine the electric charges needed to stimulate visual perception [77]. In 15 patients this was concluded to be in the range of 20–768 nC. This work illustrates the progress being made within this field, from exacting some visualisation to working out the exact range of charge needed to induce a visual event.

Another key AMD that is being developed for clinical use, is an implantable drug reservoir. This can be used in many scenarios but primarily for diabetics. This concept is to have a system in the body that has sensor that monitors a particular metabolite and releases the needed drug. Microelectromechnical systems (MEMs) and nanoelectromechanical system (NEMs) are ideal for this purpose. Some of these systems include microneedle-based transdermal devices, and micropump-based devices. Most notably the latter has been used for insulin delivery, glucose injection for diabetes, and administration of neurotransmitters to neurons [74].

It is apparent that progress within implantable devices is being made rapidly. This can be attributed to the technological advances and ease of access to information. The range of work being done is broad, focusing on producing an applicable product or technique, as well as optimising it.

8.1.3.3 Tissue Regeneration and Prosthetics

The goal of tissue engineering and prosthetics are very similar. The idea is to provide a platform for the body to either encourage repair or an opportunity to assimilate a new material (the surface of a joint replacement) and possibly to graft new material to a damaged organ. With an ageing population, these options are becoming necessary, especially when you consider the damage done by lifestyles and everyday use of organs and joints.

Considering the lifetime associated with orthopaedic implants and the rigours it goes through, nanomaterials are a viable alternative for current materials. The lifetime of implants are severely hampered by the eventual loosening between the joint and prosthesis [78]. This implies the bonding cement, polymethyl methacrylate (PMMA) that is widely used, is inefficient. Using nano-manipulated titanium showed an improvement in adhesion and promotion of cell growth. By introducing sub-micron features on titanium compared to flat surfaces, led an increase of endothelial cell adhesion density by 200 %. Whereas nanometre surface features promoted a 50 % increase in endothelial cell adhesion density compared to flat titanium surfaces. Using aligned patterns of such features on titanium, results highlighted that both endothelial and bone cells preferentially adhered onto sub-micron and nanometre enhanced surface features when compared to flat regions [79]. The use of titanium oxide nanotubes in dental implants were shown to be arrayed like collagen fibres. Furthermore the nanotubes increased roughness and surface area providing superior performances in multiple areas. It had greater hydrophilicity, greater cell adhesion and growth for osteoblasts and a better bioactivity and compatibility than current materials [80].

Tissue engineering is similar to this aspect in that it wants to promote the body to repair regenerate new cells. This can be done by introducing an extracellular matrix (ECM) that the body recognises as 'self'. Signals are transmitted between the cell and the ECM would facilitate communication for "cell adhesion, migration, growth, differentiation, programmed cell death, modulation of cytokine and growth factor activity, and activations of intracellular signalling" [74].

For example, hybrid biomaterials are being employed to better emulate natural ECM. These materials can be used to encourage healing while reducing the formation of scarring [93]. RGD peptides (R: arginine; G: glycine; D: aspartic acid) have been found to promote cell adhesion, RGD have also been amalgamated into man made ECMs to promote cell proliferation [81].

Some of the topics mentioned in this section are varied; however they do fall under the category of medical devices under the definition of the WHO. The connection may not be obvious but the "replacement, modification, or support of the anatomy or of a physiological process" combined with the monitoring and diagnosis of using a technology, renders that a medical device. The added caveat of the device being of nano-dimensions or functionality not only drives this branch of medicine but provides the epithet of 'advanced'.

8.1.4 Hindrances and Effects to be Considered

So far the focus has been on the positive impacts of nanotechnology and AMDs. However there are some minor and/or serious hindrances and effects that pertain to the success, ethical considerations, regulatory issues and the inherent toxicity of some NMs need to be considered before making an informed decisions.

8.1.4.1 Scientific Hindrances

The work discussed here are some of the more important breakthroughs within AMDs. However this does not even begin to compare to the volume and standard of work begun. In 2007, the amount of papers being published that were nano-related numbered near 15,000, this influx of publications is also reflected within the movements in intellectual property. In 2003, 90,000 patents were submitted for consideration. This illustrates the sheer amount of activity this field has generated.

However, the issues surrounding the use of this technology are manifold. A prime example of this is the use of NPs as imaging agents that are introduced within the body. Usually only 5 % of NPs administered to the body remain after 12 h, while 80 % of the initial dose are eliminated from the body. Further to this it is very difficult to ascertain how swiftly these particles would go to the intended target, NPs would be in systemic circulation for an extended period of time once they are released in a subject [82]. QDs are especially perilous as their outer shells can be worn away and can cause the production of free radicals and DNA nicking [83]

Another issue within progression and realisation of AMDs are the kind of studies that are being done. There is a distinct lack of comparability between investigations which can be attributed to one cause: the drive of getting published. Researchers and their funding are heavily linked to their work being heralded in journals of repute. Consequently, the techniques they use are always new, protocols and models are novel and inventive. Therefore, promising work is sometimes not explored and improved upon by other scientists, which leaves avenues incomplete.

Another issue with publications is that the lack of "no effect" studies is hindering the body of knowledge accrued [84]. This would provide researcher with protocols and methods of investigations that are a waste of resources. Moreover, having access to such data would provide a more complete picture, this would be crucial in designing possible resolutions for projects. In light of this the editors of three scientific journals have agreed to also publish the results of "no-effect studies" [85]. This would be instrumental in the use of resources of academia, governments and industries to translate scientific research into consumable products such as AMDs.

8.1.4.2 Regulatory Hindrances

As has already been discussed NMs size heavily contributes to their unique properties. However, this same feature that allow NMs to interact with biological entities but can also have deleterious effects on cellular mechanisms, and cell viability itself. It must also be considered that as of 2010, NMs have been used or present within over 800 consumer products [86]. There is a growing concern among cynics and advocates of NMs about their toxic potential, and there are calls from some quarters to have a global moratorium on all research and release of products, until regulations are in place to ensure protection from hazards [87].

Toxicity issues range from the points of entry to the range of effects themselves as shown in Table 8.1. Size and biopersistence play a large role in the extent of damage incurred. NMs can enter the body upon contact of the skin, inhalation and ingestion. This can cause a variety of issues ranging from free radical causing DNA damage, cell death and the formations of cancer [85].

In 2007, the European health and safety executive (HSE) initiated the Registration, Evaluation, Authorisation and restriction of Chemicals (REACH). Previous to this regulation, it was supposed that NMs act identically to their bulk equivalents, whereas studies show this is far from the truth. The necessity to weigh the impact of NMs, and also set some guidelines in how they should be approached commercially is key to the propagation of nanotechnology in AMDs. This however is not enough, and to remedy this, a motion has been passed by the European parliament to reassess the regulation of handling of NMs. Guidance has also been set for companies and they are recommended to adopt the precautionary principle [88].

In the US the Occupational Safety and Health Act (OSHA) of 1970 protects employees from illness at work. The slow response of OSHA standards-setting compared to the swiftly developing nanotechnology sector has left many staffs unprotected [89].

However in March 2008, the FDA and nano-health Alliance organised a workshop to identify the most pressing barriers to the success of nanotechnology. They classified the followings as the most important hurdles:

- Determination of the distribution of nanoparticulate carriers in the body following systemic administration through any route;
- Development of imaging modalities for visualizing the biodistribution over time;
- Understanding mass transport across compartmental boundaries in the body;
- Development of new mathematical and computer models that will lead to predicting risk and benefit parameters;
- Establishment of standards or reference materials and consensus testing protocols that can provide benchmarks for the development of novel classes of materials; and
- Realization of an analytical toolkit for nanopharmaceutical manufacturing, accompanied by a specification sheet of toxicological, safety, and biodistribution properties obtained through standardized, validated methods" [74].

These initiatives that are being initialised by various taskforces are an indication of intent. The progress of nanotechnology is held in high regard by industry and academia and its success must be ensured.

8.2 Summary and Outlook

AMDs are part of a wave of health related technology that has been pervading the medical field for at least three decades. However, this aspect of medicine is being driven to new heights by the dawn of nanotechnology. The research presented here is not definitive; the purpose of this work was to highlight the present climate of AMDs and the impact of NMs, NPs and novel techniques.

AMDs range from imaging agents, to techniques such as optical tweezers. The purpose of which is to reach a resolution within cellular activity that actually identifies the structure, function and even movement of specific cell mechanisms. This is typified by the capabilities of AFM and optical tweezers. The issue with the research examined, is that none of these techniques are making it to the clinical setting.

However, the potential within the diagnostic techniques covered is vast. The most common future is the combination of QDs and MRI contrast agents. They have the potential to be combined into theranostics, devices/particles that not only give information about disease but deliver drugs to them as well. Ideally these particles would be loaded with therapeutic agents and functionalised with target and imaging ligands, thereby providing an intermediary that has bio-penetration, medication and surveillance capabilities.

Similarly biosensors have been revolutionised by the introduction of nanotechnology. Generally they all have the same type of technology; a recognition element and the transducer technology. They can be defined by their transducer element, and the three most common were discussed. The issue between them of course lies between the interface between the recognition and transducer, the more integrated it is, the better the sensor. The dynamic range, specificity and sensitivity have been improved beyond compare by NMs. It is yet to be seen whether a particular type of architecture will translate on to the commercial market, what is certain is the possibilities on the horizon [90]. Of course the discovery of NMs and conducting polymers has made this easier and is bringing POC and LOC technologies within grasp, as the case study of MIPs has conveyed.

Surgical applications of AMDs have also been accelerated by the nano-revolution. For example, as shown in Fig. 4, the tips of AFM could be modified using EBIM to create ‘nano scalpels’ and ‘nano needles’. This technique has allowed surgery at the subcellular level. This could be combined with implantable devices and tissue grafts to make near flawless additions to the human body with minimal damage. However most of the literature only deals with in vivo and in vitro. This technology will only move forward if more robust studies can push this into the clinical arena.

The future looks quite interesting in the way of coupling nanosurgery with wireless robotic surgery such as the da vinci robot. In 2010, over 300,000 surgeries were completed using robotic techniques. It is motivating that miniaturisation is being heralded as one of key implications to drive this technology forward [91]. Using this with nanotools and combining them with gold standard clinical practice would drive this even further.

It has been demonstrated that nanotechnology has impacted the development and evolution of AMDs. The use of NMs can promote an ordinary MD to the advanced category. There are some barriers to overcome, but it is globally understood that this technology needs to be nurtured correctly for it to come to fruition. It has provided platforms for interdisciplinary collaborations and is commercially lucrative for all parties involved. Most importantly this nanotechnology and AMDs will change the face of global healthcare.

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Chapter 9
Wireless Actuation of Micro/Nanorobots for Medical Applications

Soichiro Tottori, Li Zhang, and Bradley J. Nelson

9.1 Introduction

People have envisioned tiny robots that can explore a human body, find and treat diseases since Richard Feynman's famous speech, "There's plenty of room at the bottom," in which the idea of a "swallowable surgeon" was proposed in the 1950s [1]. Even though we are at a state of infancy to achieve this vision, recent intense progress on nanotechnology and micro/nanorobotics has accelerated the pace toward the goal [2–6]. A number of research efforts have been recently published regarding the development from the basic principles and fabrication methods to practical applications [7–10]. Not limited in vivo applications, the integration of micro/nanorobots to lab-on-a-chip systems can also be foreseen because of the nature of their size and liquid operating environments [11, 12]. This interdisciplinary research of micro/nanorobot-based medical treatments or diagnosis has been investigated from many different aspects, such as locomotion, functionalization, imaging, biocompatibility, interface, etc (Fig. 9.1). In this chapter we focus on wireless actuation of micro/nanorobots, which plays an important role in locomotion and part of functionalization. The chapter starts from wireless locomotion by means of magnetic fields, bacteria, and chemical reaction, followed by wireless actuation of robotic tools that function to manipulate targets.

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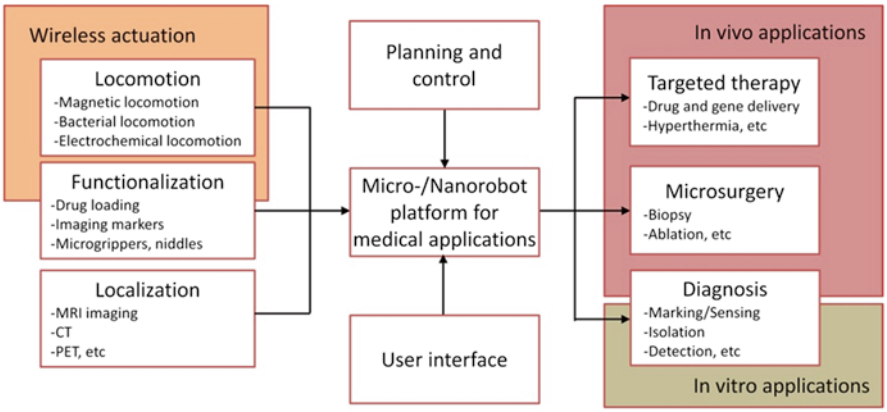

Fig. 9.1 A road map of micro/nanorobots for medical applications

9.2 Magnetic Actuation

Safety issues with respect to transmitting energy through body tissues and biocompatibility of the wirelessly driven device must be taken into account for in vivo applications. As MRI systems have been used extensively in clinical practice, it is accepted that the limited strength and frequency of these magnetic fields are safe. Therefore, magnetic actuation is one promising approach for powering and manipulating medical micro/nanorobots in vivo. Moreover, the human body is magnetically transparent, which implies that interference of magnetic fields by human bodies is practically negligible.

In general, magnetic actuation can be categorized into two types: force-driven and torque-driven. Magnetic attraction forces are generated by gradient fields, whereas magnetic torques are generated by misalignment of magnetizations of the devices and magnetic fields. Using the average magnetization **M** of the magnetic body in Amps per meter (A m^{-1}), the magnetic force and torque are described, respectively, as:

$$\mathbf{F} = V\left(\mathbf{M} \bullet \nabla\right)\mathbf{B} \tag{9.1}$$

and

$$\mathbf{T} = V\mathbf{M} \times \mathbf{B} \tag{9.2}$$

where V is the volume of the body, and **B** is the flux density of an external field in Tesla (T).

9.2.1 *Gradient Field for Concentration and Steering of Magnetic Therapeutic Carriers*

As described in Eq. 9.1, magnetic carriers are attracted to the direction of the high flux density of gradient fields. Magnetic nanoparticles are widely used to magnetically-tag therapeutic carriers [13, 14]. The approach of using gradient fields to move magnetic therapeutic carriers can be further divided, depending on the applied magnetic systems, into the following three groups: concentration using permanent magnets placed outside the body, direct concentration at implants, and steering of magnetic carriers in flows using the MRI system.

9.2.1.1 Concentrating Magnetic Carriers at Targeted Sites by External Magnets

Concentration of magnetic carriers using permanent magnets or electromagnets is probably the most straightforward method of using magnetic-field-based manipulation. Figure 9.2a shows that a strong magnet is placed on the surface or incised region of a patient body, and the magnetic therapeutic carriers flowing in the circulatory system are trapped in a local region by magnetic attraction [13, 16]. This approach has been investigated for treatment of tumors located at various sites in vivo, such as livers [17, 18], brains [19, 20] and lungs [15].

In Dames et al. [15], Rudolph and coworkers demonstrated that magnetic aerosols that contain superparamagnetic iron oxide nanoparticles (SPIONs) can be collected by external electromagnets in mouse lungs. The single SPIONs themselves do not have sufficient magnetization to be attracted by a gradient field, however, the magnetic aerosols (~3.5 μm) containing approximately 2,930 SPIONs can be guided in a high magnetic flux gradient (larger than 100 T m^{-1}). Figure 9.2b shows that SPIONs were collected in the right lung, as the magnet's tip was located directly above the right lung lobe, surgically exposed by thoracotomy. Figure 9.2c and d are histological images of right and left lungs, respectively, in which the brown areas show the accumulated SPIONs. The magnetic aerosol can also contain several different drugs, biodegradable nanoparticles, liposomes, nanocrystals and so on. The major challenge for scaling up from mouse to human size is generation of large magnetic gradient fields to a relatively far location from the tip of the electromagnet core.

9.2.1.2 Concentrating Magnetic Therapeutic Carriers Directly at Implants

An alternative approach to concentrate magnetic particles in vivo is proposed by using magnetic implants with an externally applied uniform field. Externally applied uniform fields induce magnetization of magnetic implants and flowing magnetic therapeutic carriers simultaneously. Gradient fields are generated surrounding the

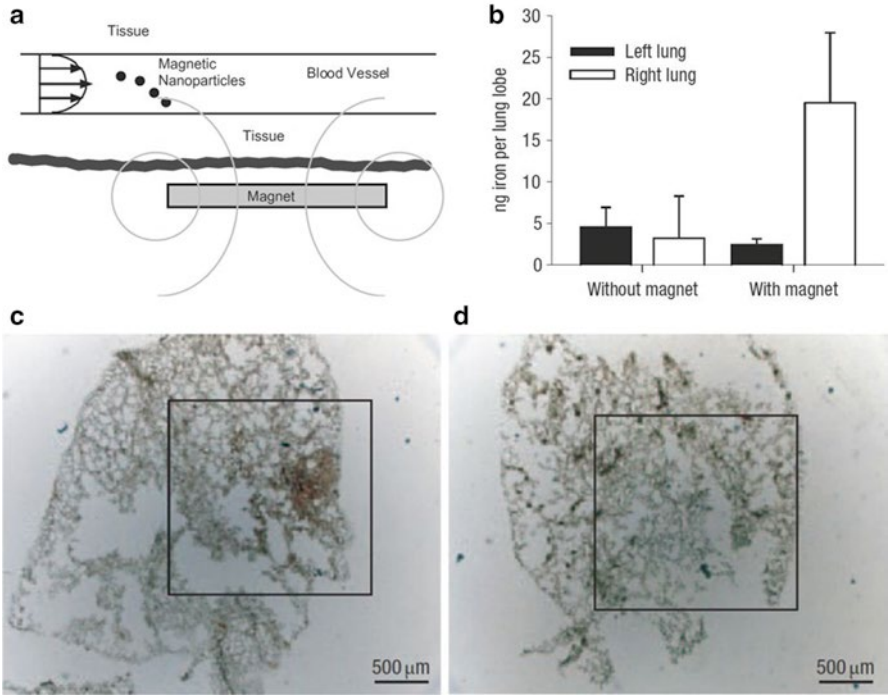

Fig. 9.2 Concentration of magnetic therapeutic carriers. (**a**) Schematic illustration of capturing magnetic therapeutic carriers: a magnet is place outside or an incised region in order to collect magnetic therapeutic carriers flowing in the circulatory system ((**a**) Reprinted with permission from Pankhurst et al. [14]. Copyright 2003 IOP Publishing Ltd.) (**b**) The bar graph shows the amount of SPION in the *left* (without a magnetic field) and *right* (with a magnetic field) lungs (thoracotomy, 400 breathing cycles, ~300 μl SPION solution, 12.5 mg ml^{-1} Fe, $n = 3 \pm$ s.d.). (**c–d**) Lung histology after nanomagnetosol application. (**c**: right lung, **d**: left lung) ((**b–d**) Reprinted with permission from Dames et al. [15], Copyright 2007 Macmillan Publishing Ltd: Nature Nanotechnology)

ferromagnetic implants because the implants possess much higher magnetic permeabilities than that of air and bodily tissues. In comparison to the method described in the last section, i.e. direct use of gradient fields generated by external magnets, the method using magnetic implants can provide relatively strong gradient fields, because the source of gradient is installed at the targeted site inside the body [21]. Based on this concept, targeting of magnetic therapeutic carriers at the implanted sites has been demonstrated to deliver drugs or cells [22–26].

In Polyak et al. [26], Levy and co-workers demonstrated targeting delivery of magnetic nanoparticle-loaded endothelial cells to the stent surface, as illustrated in Fig. 9.3a. They applied the magnetic stents made from 304-grade stainless steel instead of conventional non-magnetic medical-grade stainless-steel 316 L. The bovine aortic endothelial cells (BAECs) functionalized with magnetic nanoparticles were accumulated onto the magnetic stents in the uniform field. In vivo acute rat

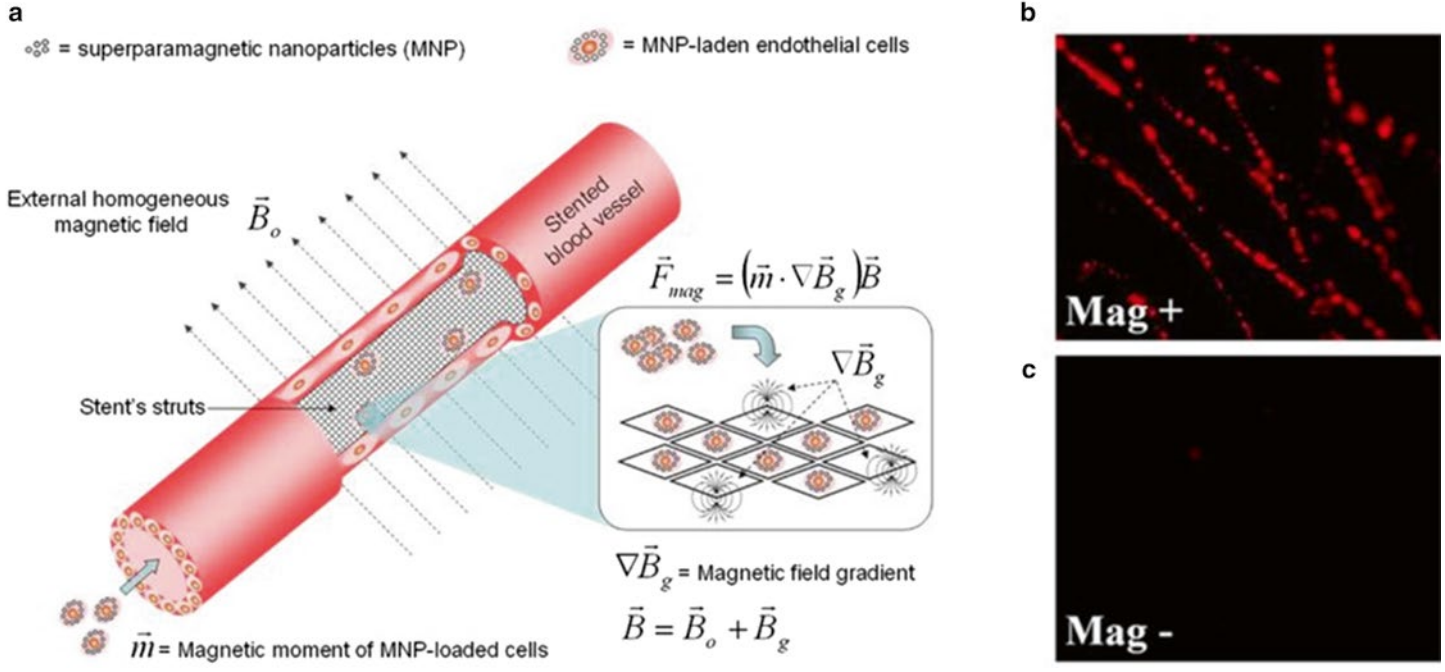

Fig. 9.3 Localizing magnetic therapeutic carriers around magnetic implants using uniform magnetic field. (**a**) Schematic illustration of MNP-laden endothelial cells attracted to the magnetic stent in the presence of a uniform magnetic field. (**b**, **c**) In vivo experiment of MNP-loaded BAECs capturing on the implanted stents. BAECs preloaded with fluorescent MNPs were tranthoracically injected into the left ventricular cavity. The uniform magnetic field of 100 mT was applied for 5 min. (**b**) Fluorescent image of the MNP-laden BAECs accumulated on the stent implanted in the rat carotid artery. (**c**) Control experimental results without magnetic fields ((**a**–**c**) Reprinted with permission from Polyak et al. [26]. Copyright 2008 National Academy of Science U.S.A.)

carotid stenting studies were performed by transthoracic injection of MNPs-loaded (MNP: magnetic nanoparticle) BAECs into the left ventricular cavity of the rat. Figure 9.3b and c show the fluorescent images of MNPs collected onto the 304-grade stainless steel stents with and without the uniform magnetic field, respectively. Clearly, the MNPs were collected on the surface of the stent in the presence of magnetic fields.

9.2.1.3 Steering Magnetic Therapeutic Carriers in Flows by MRI Systems

Using MRI systems to steer magnetic therapeutic carriers flowing in the circulatory systems is one of the emerging areas of magnetic control [27]. Localization of magnetic therapeutic carriers using permanent magnets is limited to the organs that are close to the skin because magnetic gradient fields decay rapidly as the distance between the target and the externally placed magnets increases. The gradient field provided by a MRI system is appealing for steering magnetic therapeutic carriers deep inside the body because of its gradient linearity, high-strength and real-time imaging feedback.

The MRI-system-based steering of magnetic therapeutic carriers in bifurcations in vitro and in vivo has been reported recently [28–31]. For example, in Pouponneau et al. [30] Martel and co-workers demonstrated in vivo guidance of magnetic therapeutic carriers for the treatment of hepatocellular carcinoma (HCC) via trans-arterial chemoembolization in the hepatic artery using rabbits. Figure 9.4a and b show that the magnetic therapeutic carriers: biodegradable polymer particles containing anti-tumor drug and magnetic nanoparticles were steered in the hepatic arterial bifurcation in the presence of a gradient field. In Fig. 9.4c, the result shows that the deposition of magnetic therapeutic carriers in the targeted and untargeted lobes were increased and decreased by MRI steering, respectively. In Fig. 9.4d, the magnetic therapeutic carriers, visualized as dark spots in MR images, were collected in the left lobe. The histological image in Fig. 9.4e shows that magnetic therapeutic carriers were deposited within a branch of the hepatic artery.

9.2.2 Magnetic-Torque-Driven Propulsion at Low Reynolds Number

Locomotion of micro/nanorobots in a fluidic environment at low Reynolds number is challenging, since viscosity dominates over inertia [32, 33]. A scallop-like motion, one of the typical examples of reciprocal motions, cannot get net forward displacement but just moves forward and backward repeatedly at the same place in a low Reynolds number regime because the flow is time-reversible. In nature, microorganisms utilize various non-reciprocal locomotion strategies. For example, spermatozoa propel themselves by propagating a wave motion on their flexible tails, and *E. coli* bacteria swim by rotating their helical flagella.

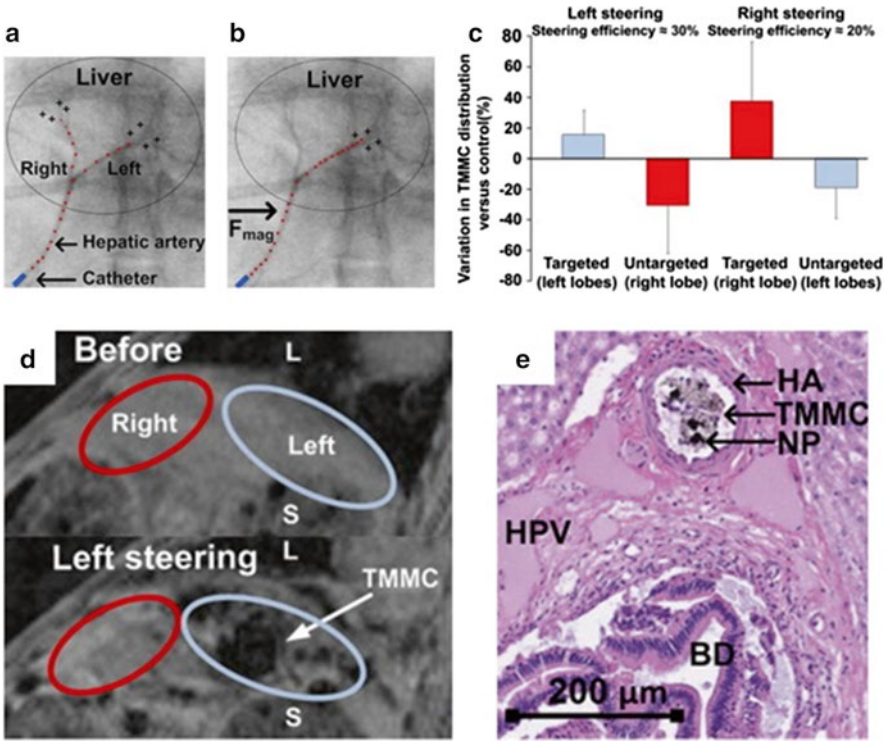

Fig. 9.4 Steering magnetic therapeutic carriers for liver chemoembolization using an upgraded MRI system. (**a**, **b**) Fluoroscopy images of the rabbit hepatic artery with superposed images of the magnetic therapeutic carriers without (**a**) and with (**b**) the MRI control. (**a**) The microparticles are released from the catheter in the artery and distributed to both lobes. (**b**) MRI steering of magnetic therapeutic carriers to left lobe to preserve the right lobe from the chemoembolization. (**c**) Variation in TMMC (therapeutic magnetic microcarriers) distribution versus control in the liver lobes with left and right steering based on Co analysis. Mean ± SD. (**d**) T2*-weighted MR images of the rabbit liver before and after the operation. The red and blue lines indicate the right and left lobe, respectively. "S" indicates the stomach and "L" the lung. (**e**) Histological image of liver parenchyma and the blood vessels dyed with hematoxylin and eosin. HA: branch of hepatic artery, HPV: hepatic portal vein, BD: bile duct, NP FeCo nanoparticles ((**a**–**e**) Reprinted with permission from Pouponneau et al. [30]. Copyright 2011 Elsevier Ltd)

Inspired by the above mentioned microorganisms, many microscopic artificial swimmers have been developed [34–44]. For example, artificial microswimmers with traveling wave-induced propulsion were presented by Dreyfus and co-workers in Dreyfus et al. [34]. Figure 9.5a and b show the illustration and optical microscope image of the microscopic swimmer consisting of a chain of colloidal magnetic particles connected by DNA linkages, respectively. Oscillating magnetic fields induce a wave motion into the flexible body, resulting in a net forward movement. Inspired by helical bacterial flagella, artificial bacterial flagella (ABFs) consisting of ferromagnetic Ni heads and rolled-up helical tails were reported by our group elsewhere

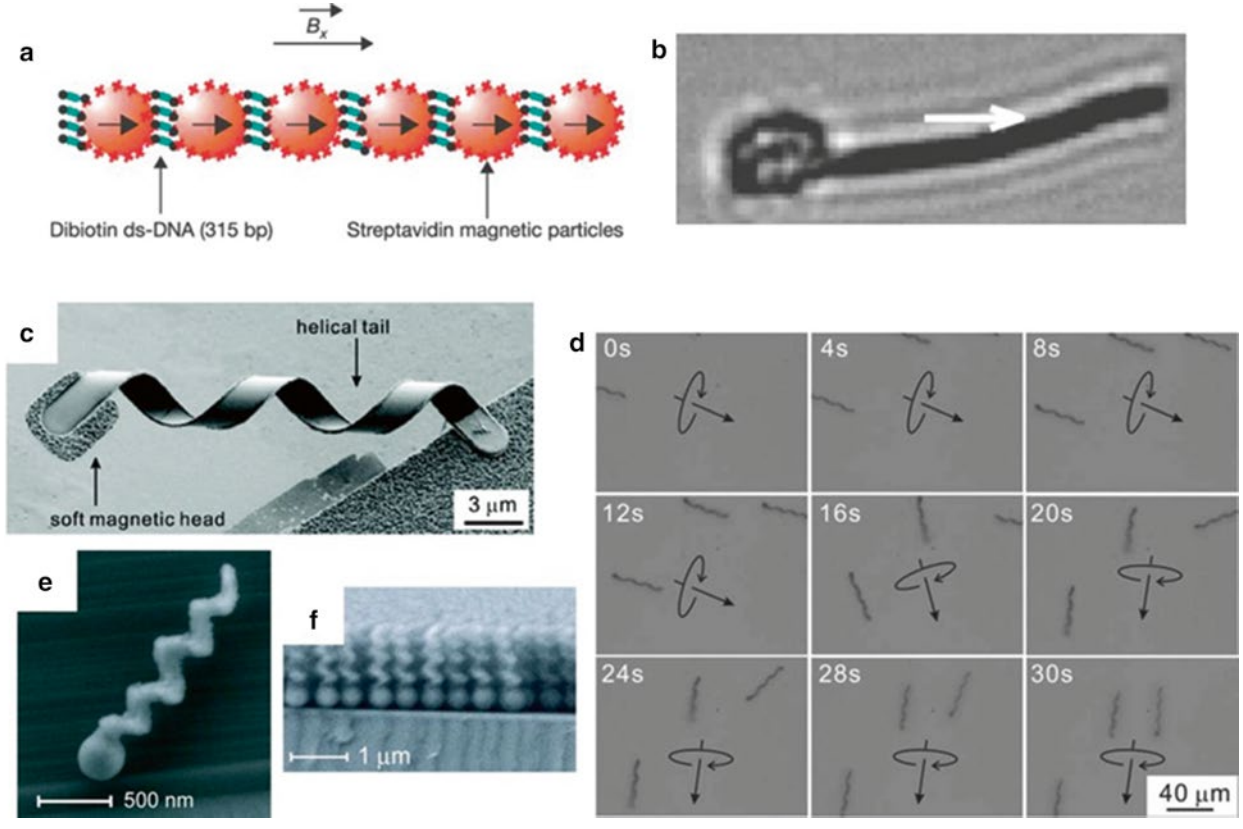

Fig. 9.5 Propulsion of non-reciprocal motions created by a oscillating or a rotating field. (**a**) Schematic representation of a flexible magnetic filament. (**b**) Optical microscopy image of the microscopic artificial swimmer. The white arrow indicates the direction of an external field ((**a**, **b**) Reproduced with permission from Dreyfus et al. [34]. Copyright 2005 Nature Publishing Group). (**c**) Helical microswimmer comprised of the magnetic head and the rolled-up helical tail. (**d**) Steering of the three ABFs as an entity ((**c**, **d**) Reproduced with permission from Zhang et al. [43]. Copyright 2009 American Chemical Society). (**e**) The microscopic helical swimmer fabricated with GLAD technique. (**f**) A wafer section with a nanostructured film after GLAD of SiO_2 helices ((**e**, **f**) Reproduced with permission from Ghosh and Fischer [37]. Copyright 2009 American Chemical Society)

[42, 43]. Figure 9.5c shows the SEM image of an as-fabricated ABF with a diameter of 2.8 μm and a length of approximate 25 μm. The top-down fabrication process of the ABFs is based on the self-rolling technique: curling generated by the stress releasing of multilayered nanoribbons [45, 46]. By applying a rotating magnetic field, the ABFs were rotated in sync with the external field rotation, resulting in a forward displacement. Swimming of the multi-agent ABFs was also demonstrated, in which the group was controlled as a single entity, as shown in Fig. 9.5d. In Ghosh and Fischer [37], Fischer and Ghosh reported an alternative fabrication approach of helical swimmers using glancing angle deposition (GLAD) of chiral structures, as shown in Fig. 9.5e. GLAD method provides porous thin films with engineered structures, such as straight pillars, zigzags structures, and helices [47]. The SEM image in Fig. 9.5f shows as-fabricated vertical array of SiO_2 helical nanostructures on self-assembled silica beads with a diameter of 200–300 nm, which had an ultra-high density of – 10^9 helices per cm^2. The helical nanostructures were released by sonication and subsequently were coated with a 30-nm thick Co thin film by evaporation. In order to generate a corkscrew motion, the helical devices were also actuated in a weak strength (6 mT) rotating magnetic field. The maximum swimming speed of approximately 40 μm/s (20 body-lengths per second) was reported. The helical swimming microrobots can also be utilized to manipulate microobjects with or without a physical contact [41, 44, 48, 49]. Once functionalized by liposomes loaded with chemical or biological substances, these helical devices have the potential to perform targeted delivery of energy and controlled drug releasing in vivo. Previously SiO_2 helical devices coated with a fluorophore were demonstrated as a proof of concept [37].

9.2.3 *Scaling Effects of Micro/Nanorobots Driven by Magnetic Force and Torque*

Stokes flow is the flow at very low Reynolds number ($Re << 1$), which indicates the ratio of inertia and viscous forces. For instance, the drag force and torque of a sphere in Stokes flow are given by

$$\mathbf{F}_{\mathbf{drag}} = 3\pi\mu d\mathbf{U} \tag{9.3}$$

and

$$\mathbf{T}_{\mathbf{drag}} = \pi\mu d^3\Omega \tag{9.4}$$

where d is the diameter of the sphere, $\mathbf{U}$ is the translational velocity, and $\mathbf{\Omega}$ is the angular velocity of the rotating sphere. Comparing with Eqs. 9.1 and 9.2, in which both magnetic force and torque are directly proportional to the volume of the magnetic body (for a sphere, i.e. $\pi d^3/6$), the maximum velocity and angular velocity by applying a gradient field and a uniform rotating field can be computed. The

translational velocity **U** of a sphere induced by a gradient pulling is proportional to the term of d^2, whereas the angular velocity **Ω** by a uniform rotating field is independent of d. Therefore, when the robots are scaled-down, with the same applied field, the translational velocity will reduced rapidly, whereas the angular velocity can be maintained as a constant. This calculations also implies that if a rotational motion can be converted to a translational motion with a converting ration of less than d^2, the corkscrew locomotion based on rotational motions can be more efficient than that based on direct translational pulling using a gradient field [50].

The propelling motion of helical swimmers is described with the force f, torque τ, angular velocity ω, and translational velocity v as Purcell [33],

$$\begin{bmatrix} f \\ \tau \end{bmatrix} = \begin{bmatrix} A & B \\ B & C \end{bmatrix} \begin{bmatrix} v \\ \varpi \end{bmatrix} \tag{9.5}$$

where the parameters A, B and C are given by geometrical parameters of a helix and the viscosity of environmental fluid [50, 51]. Since the parameters A, B and C are directly proportional to the terms L, L^2 and L^3 (here, L is the characteristic length of the helix) respectively, the velocity v is computed to be proportional to the characteristic length L. In comparison to the fact that the velocity induced by direct pulling is proportional to the second power of its characteristic length, the helical propulsion is more feasible for small scale locomotion due to the scaling effect.

9.3 Bacterial Actuation

Bacteria, as natural "microrobots," can perform robotic tasks with high intelligence. In nature, bacteria can respond to various environmental cues, for example, magnetic field (magnetotaxis), light (phototaxis), electric field (galvanotaxis), chemical concentration (chemotaxis), and heat (thermotaxis). Here we show two examples of micromanipulation using bacteria based on their magnetotaxis and phototaxis.

Magnetotactic bacteria (MTBs), which possess flagella and magnetosome, swim in the direction of a magnetic field [52]. In Martel et al. [53], Martel and coworkers demonstrated that the magnetic steering of MTBs, and explained that their motion can be potentially trackable by a clinical MRI system. Figure 9.6a and b show controlled swimming of a MTB in the presence of an external magnetic field with and without a cargo [54]. Martel's group also demonstrated micromanipulation using a swarm of MTBs, by which large thrust force can be generated for pick-and-place microobjects [56].

Using phototactic bacteria enables light control of micro/nanorobotic components [55, 57]. In Weibel et al. [55], Whitesides and co-workers demonstrated pick-and-place manipulation of micro cargos by phototaxis of bacteria and photoreactive chemical linkages. Figure 9.6c shows phototaxis of *Chlamydomonas reinhardtii* (*CR*) under LED lights. In order to transport microscale cargo, chemical

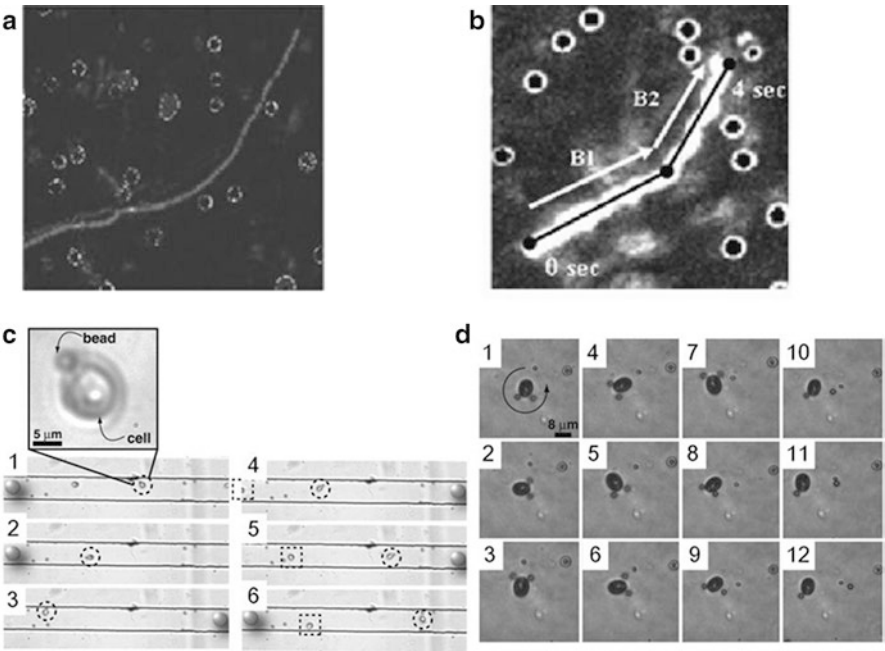

Fig. 9.6 Bacterial actuation. (**a**) Movement path of the MTB without cargos steered by an external field. (**b**) The single MTB pushed the 3-μm-diameter microbead with a control of external field. The white arrows B1 and B2 represent the direction of the field, and the black line indicates the path of microbead transported by the MTB. Images have edges of 36.0 μm ((**a**, **b**) Reproduced with permission from Martel et al. [54]. Copyright 2006 American Institute of Physics). (**c**) A sequence of images showing how the cell in C can be steered by using positive phototaxis. Illuminated LEDs are indicated by the presence of a cartoon of the LED. (**d**) A series of chronological images showing the photochemical release of a bead from a cell with two PS beads (3 μm diameter) attached by exposing UV light (λ = 365 nm, 80 W) for 20 s. The arrow in frame 1 indicates the direction in which the cell is rotating. The time that had elapsed between the frames was 2 s. In (d9), the bead was released from the cell and slowly diffused away ((**c**, **d**) Adapted with permission from Weibel et al. [55]. Copyright 2005 National Academy of Science, U.S.A.)

links were formed between cargos and *CR* bodies. The attached-cargos can be photochemically released by exposing a UV light of a specific wavelength. Figure 9.6d shows the optical microscope image of photochemical releasing of the attached microbead from *CR* by illuminating the UV light.

9.4 Chemical-Fuel-Driven Actuation

Like biomotors, such as myosins, kinesins, and dyneins, utilize ATP as chemical fuels, manmade micro/nanomotors that propel themselves by employing chemical fuels have been developed for about a decade. The first bimetal nanomotors were

demonstrated by two groups independently, one from Pennsylvania State University [58] and the other from University of Toronto [59] in 2004. A number of excellent reviews published recently regarding catalytic nanomotors from their fabrication, consideration of materials, and functionalization to their practical applications [60–63]. Here, we introduce the basic principle and some recently reported biomedical applications of these chemical-fuel-driven micro/nanomotors.

9.4.1 Principle and Fabrication

To date self-propelled micro/nanomotors exhibit locomotion in two types: bubble-induced propulsion and self-electrophoretic motion, as shown in Fig. 9.7a. The bubble-induced propulsion is based on the oxygen bubbles generated in decomposition of hydrogen peroxide. The tubular micro/nanomotors with asymmetric open ends, with conical channel, lead the bubbles emitted from the largely-opened ends. The conical microengines can be fabricated with self-rolling technique or template-assisted electroplating. The self-electrophoretic motion occurs with multisegment nanorods integrated with Au-Pt bimetals. The hydrogen peroxide is oxidized at the platinum segment, and the generated electrons travel to the gold segment, where reduction reaction occurs. Because of this asymmetrical redox reaction, the nanorods with Au-Pt bisegments move toward the direction of platinum segments. Ni segments are often integrated for steering by an external magnetic field. The multi-segmented nanorods are synthesized into the templates with cylindrical nanopores using template-assisted electrodeposition.

9.4.2 Applications

The chemical-fuel-driven micro/nanomotors can reach extremely high speeds, up to approximately 3 mm/s [67], thus the thrust force is sufficient to transport microobjects. Figure 9.7b shows transport and assembly of magnetic microplates using a microtubular motor [64]. Controlled manipulation of animal cells, i.e. CAD cells (cathecolaminergic cells), was also demonstrated, as shown in Fig. 9.7c [65]. To realize stable and selective pick-and-place manipulation, ferromagnetic layers can be integrated into the motor and the cargo for magnetic attraction [68, 69]. Releasing of the magnetic cargo is possible by turning the magnetic motor with a high speed, which is attributed to the different drag force applied on the motor and the cargo. Alternatively, UV lights can trigger photochemical releasing of the streptavidin-coated polystyrene cargo from the catalytic nanomotor [70], which is the same concept with the photochemical release of cargos from *CR* bacteria in Weibel et al. [55].

Surface-functionalized micro/nanomotors are applied for isolation of targeted biological samples, such as circulating tumor cells [71], nucleic acid [66], proteins [72], and bacteria [73], from raw complex biological media [11]. Figure 9.7d1

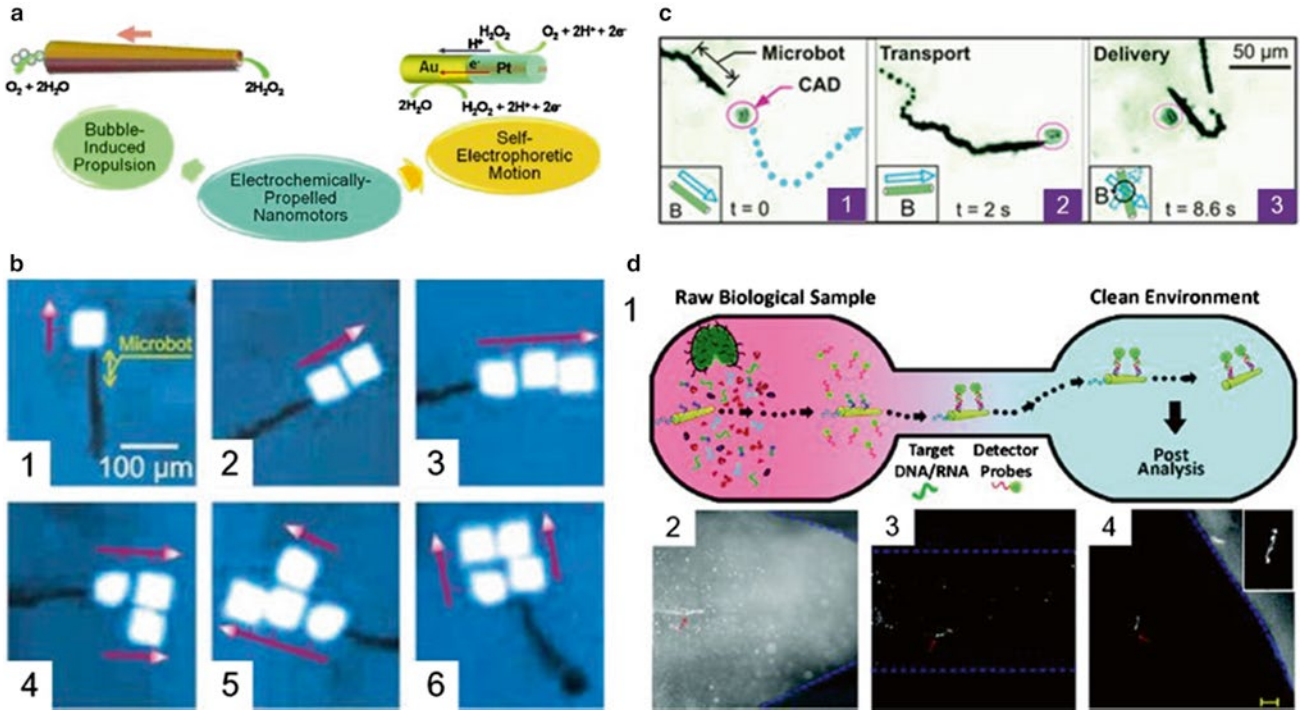

Fig. 9.7 Electrochemically-powered nanomotors. (**a**) Two types of electrochemically-powered nanomotors and their motion mechanism: bubble-induced propulsion by conical tubes and self-electrophoretic motion by bisegment rods ((**a**) Reproduced with permission from Campuzano et al. [11]. Copyright 2011 The Royal Society of Chemistry). (**b**) The catalytic microrockets assembling four magnetic plates in different configurations ((**b**) Adapted with permission from Solovev et al. [64]. Copyright 2010 John Wiley and Sons). (**c**) Controlled manipulation of CAD cells by a catalytic microrocket ((**c**) Adapted with permission from Sanchez et al. [65]. Copyright 2011 The Royal Society of Chemistry). (**d1**) Schematic illustration of isolating nucleic acid by a modified microrocket from a raw sample (*left*) to a clean/separate location (*right*). (**d2–d4**) Optical microscopy images of the modified microrocket (*red arrow*) captured the targeted DNA (**d2**) and transported it across a 6-mm-long channel (**d3**) to the clean well (**d4**) ((d) Adapted with permission from Kagan et al. [66]. Copyright 2011 American Chemical Society)

shows the schematic illustration of nucleic acid isolation by using functionalized motor from a raw biological sample to a clean microwell [66]. The outer surface of the microtubular motor was coated with a gold layer and modified with a binary self-assembled monolayer (SAM) of specific thiolated capture probe (SHCP) and a short-chain 6-mercapto-1-hexanol (MCH). The functionalized micromotors were then driven in the mixture of hydrogen peroxide and a biological sample that contains the nucleic acid (synthetic 30-mer DNA or bacterial 16S rRNA) tagged with fluorescent nanoparticles for optical visualization. Figure 9.7d2–d4 shows the optical microscopy images of capturing and transporting the targeted nucleic acids to the clean well using a micromotor.

Chemical-fuel-driven motors can also be applied for sensing based on the velocity change of their motion: the speed increases in high concentration of Ag ions in the hydrogen peroxide fuel [74]. The detection of nucleic acid was as well demonstrated by combining Ag nanoparticle-tagged detector probe [75].

To date in vivo application of chemical-fuel powered nanomotors is rare unless the current chemical fuels (mainly H_2O_2) can be replaced with biocompatible chemicals or chemicals which are inherently contained in the human body. Previously, as a proof-of-concept, bioelectrochemical motor driven by oxygen and glucose were reported [76].

9.5 Wirelessly-Actuated Robotic Tools

One of the next steps from wireless locomotion is wireless manipulation, such as holding, pinching, penetrating, etc, using nano-/microrobots. Variety of fabrication methods have been investigated and applied to create such functional wireless micro/nanorobots [77]. In this section, we explain some examples of recently reported fabrication methods and the wirelessly-actuated microrobotic tools developed with these method.

The microholders with comparable sizes enable pick-and-place manipulation of cargos without dropping off during transportation. In Tottori et al. [41], the crawlike structures were implemented at one end of the helical micromachines. The SEM image of as-fabricated functionalized micormachine is shown in Fig. 9.8a. The structures were fabricated by means of three-dimensional lithography, in which the focused UV laser is scanned in the thin layers of photocurable polymer. In comparison to the conventional two-dimensional lithography, three-dimensional lithography provides almost arbitrary shapes with a submicron resolution. The combination of electron beam evaporation of ferromagnetic thin films allows magnetic wireless actuation of the devices. The functionalized helical micromachines were capable of transport polystyrene microbeads not only horizontally but also against gravity. The process is divided into the following four stages: approaching, loading, transporting, and releasing, as illustrated in Fig. 9.8b. A polystyrene microparticle with a diameter of 6 μm was transported in three dimensions above the Si substrate with two different heights, as shown in Fig. 9.8c. The loading and releasing of the microparticle were realized by pushing it forward and swimming backward, respectively.

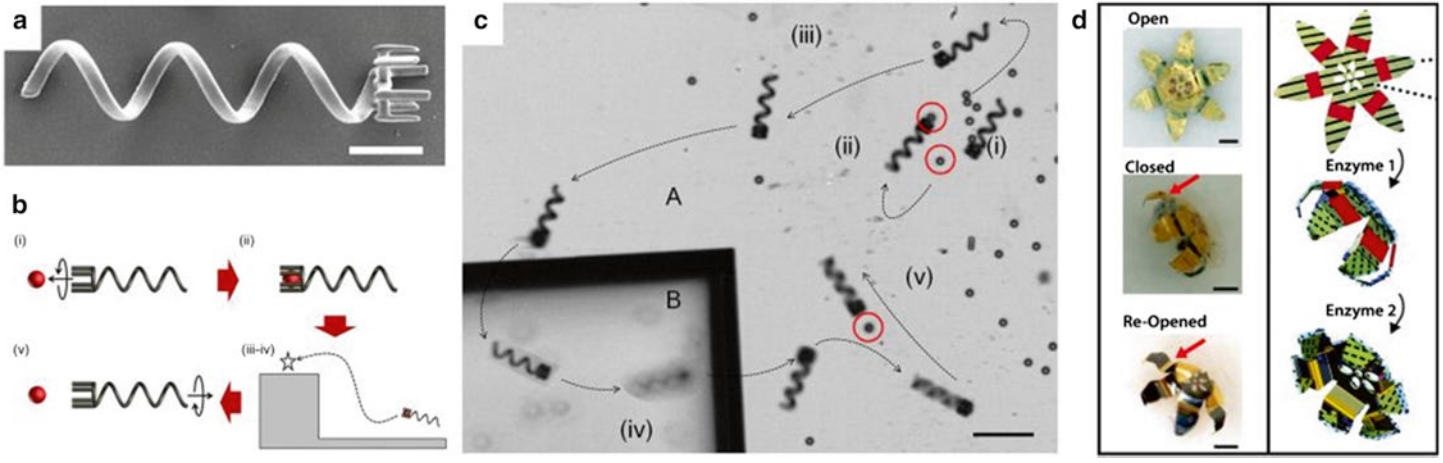

Fig. 9.8 Wirelessly-actuated robotic tools. (**a**) SEM image of a helical micromachine with a hand-like microholder. The scale bar is 10 μm. (**b**) Schematic illustration of cargo transport by the magnetic helical micromachine with a hand-like microholder. (**c**) Time lapse image of transporting 6-μm-diameter microbead in 3D. The scale bar is 50 μm ((**a**–**c**) Adapted with permission from Tottori et al. [41]. Copyright 2012 John Wiley and Sons). (**d**) Biochemically activated microgrippers. The scale bars are 200 μm ((**d**) Adapted with permission from Bassik et al. [78]. Copyright 2010 American Chemical Society)

For microsurgery applications, microtools capable of gripping may be highly useful. Gripping normally requires open-and-close motion of the devices. Based on the development of a micro-origami technique, reconfigurable microgrippers, capable of closing and re-opening wirelessly by external cues, have been fabricated with multilayer flexible joints possessing internal stress [79, 80]. By resolving or altering mechanical property of the reactive layers selectively, the microgrippers can close from a planar shape. Re-opening is also feasible by dissolve the other side of layer with different chemical etchant. A more elegant approach is to engineer the internal or surface stress of the films by biological stimuli. Such kind of autonomous microgrippers are realized recently, e.g. enzyme-responsive microgrippers [78], as shown in Fig. 9.8d. Proteases and glucosidases induce closing and re-opening, respectively. Remote motion control of the microgrippers integrated with a magnetic film can be done by using external magnets. To improve the positioning precision of miniaturized robotic tools, such as microgrippers, an electromagnetic control system is required. A good example of such an electromagnetic setup is OctoMag, which is capable of performing 5-DOF wireless micromanipulation of magnetic device in a large workspace [81].

9.6 Conclusions and Future Outlook

This chapter addressed the recent development of micro/nanorobots with focus on tetherless actuations for locomotion and/or manipulation. Precise motion control and functionalization of the micro/nanorobots by suitable wireless actuation methods enhances the quality of targeted medical treatments, i.e. high concentrating drug delivery and/or microsurgery with minimal side effects. The micro/nanorobots can also be integrated with in vitro biomedical applications, which may provide an alternative processing procedure in comparison to that of a conventional approach. Further investigations on not only wireless actuation technology but also imaging, biocompatibility, etc, are required in the future for practical medical applications.

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Chapter 10
Pharmaceutical Nanotechnology: Overcoming Drug Delivery Challenges in Contemporary Medicine

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10.1 Challenges in Delivery of Contemporary Therapeutics

Drug discovery process has been in forefront utilizing recent advances in molecular biology, -together with medicinal chemistry, protein structure based screening, and computational analysis, as part of rational approach to discovering drug molecules that will address unmet clinical needs. For example, proteins identified from structural biology platform can serve as targets for discovering new drug molecules. The discovery of antisense oligonucleotides (ASN), plasmid DNA (pDNA), peptides and protein therapeutics has also shown a greater potential in treating several complex diseases. A recent development in drug discovery is RNA interference (RNAi) which uses small stretches of double stranded RNA with 21–23 nucleotides in length, to inhibit the expression of a gene of interest bearing its complementary sequence [1]. Small interfering RNA (siRNA) can induce RNAi in human cells. This RNAi technology has many advantages over other posttranscriptional gene silencing methods, such as gene knockouts and antisense technologies [2]. In addition, only a few molecules of siRNA need to enter a cell to inactivate a gene at almost any stage of development. MicroRNA (miRNA), advancement from siRNA, is a new class of drugs still in the investigative stage based on nucleic acid chemistry. miRNA with 19–25 nucleotides in length, interfere pathways that involve in disease process [3]. In general, all these recent drugs have shown a great potential in the clinical management of several complex diseases like cancer, metabolic diseases, auto-immune diseases, cardiovascular diseases, eye diseases, neurodegenerative disorders and other illness [1].

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Despite the diversity and size of therapeutic libraries are continually increasing, delivering them to the disease sites has been hampered by physico-chemical attributes of drugs and physiological barriers of the body. For example, many small and macromolecular drugs (ASN, pDNA, peptides, proteins, siRNA and miRNA) often fail to reach cellular targets because of several chemical and anatomical barriers that limit their entry into the cells [4, 5]. Therefore, the outcome of therapy with that contemporary therapeutics is often unpredictable, ranging from beneficial effects to lack of efficacy to serious adverse effects. These challenges have been discussed in the following sections with an attempt to apply nanotechnology-based concepts in designing of drug delivery systems (DDS) that overcome barriers in drug delivery.

10.1.1 Chemical Challenges

Physico-chemical properties impact on both pharmacokinetics and pharmacodynamics of the drug in vivo, and must be considered when selecting a suitable delivery method. The chemical challenges faced by small and macromolecular drugs (ASN, pDNA, peptides, proteins, siRNA and miRNA) are many folds, which mainly include:

(i) Molecular size
(ii) Charge
(iii) Hydrophobicity
(iv) In vivo stability
(v) Substrate to efflux transporters

Size, Charge and Hydrophobicity The chemical properties that mainly affect drug permeability through anatomical barriers are molecular size and solubility. High molecular size and increased hydrophobicity are the predominant problems particularly associated with combinatorial synthesis and high throughput screening methods [6]. These methods allow for identification of lead molecules faster based on their best fit into receptors, but shift the molecules towards high molecular size and increased hydrophobicity, resulting in poor aqueous solubility [6]. One estimate shows that around 40 % of the newly discovered molecules are poorly aqueous soluble, thus need a suitable delivery method to achieve pharmacologically relevant concentrations in the body [7, 8]. Oral route for drug delivery remains popular due to ease of administration and patient compliance. However, oral absorption can be hindered by poor aqueous solubility of therapeutics in GI fluids. The rate of dissolution, which is a prerequisite for oral absorption, depends on the drug solubility in the GI fluids. In addition, drug molecule must possess adequate lipophilicity (logP) in order to efficiently permeate across intestinal epithelial cells [9]. This is one of the reasons for hydrophilic macromolecules such as proteins, peptides, and nucleic acid constructs do not show any oral bioavailability and resulting in limited clinical success [10].

Drug transport mechanisms involving in intestinal epithelium are transcellular and paracellular transport [11]. Transcellular mechanism involving in transport of drug molecules across the cell membrane, which occurs by (1) passive diffusion, (2) facilitated diffusion, (3) active transport, and (4) transcytosis. Lipophilic drug molecules can diffuse freely across the epithelial membrane barrier while hydrophilic and charged molecules need specialized transport carriers to facilitate cellular uptake. Transcytosis process involving endocytosis and exocytosis mechanisms is mainly for macromolecular (proteins, peptides) drugs. Recent studies show that orally given nanoparticles can pass through the epithelial membranes in GI tract through the endocytosis process [12, 13], and this can be a potential route for transport of macromolecular therapeutics.

Paracellular route, on the other hand, involves diffusion of hydrophilic drugs between the cells of epithelial or endothelial membrane through sieving mechanism. The formation of tight junction between the epithelial and endothelial cells strictly limits the paracellular drug transport. Molecular cut-off for the paracellular transport is approximately 400–500 Da [14]. Molecular mass less than the cell junction can easily pass through paracellular route regardless of polarity, for example, water and ions. It has been observed that the diffusion of drugs with molecular size <300 Da is not significantly affected by the physicochemical properties of the drug, and which will mostly pass through aqueous channels of the membrane. However, the rate of permeation is highly dependent on molecular size for compounds with MW > 300 Da. The Lipinski rule of five suggest that an upper limit of 500 Da as being the limit for orally administered drugs [15]. Numerous studies are focused on identifying the nature of cellular tight junctions and the signaling molecules involved in preserving the barrier function in order to find right approach to promote oral drug absorption.

Increased hydrophobicity of a molecule also causes greater protein binding. Protein binding is both help and hindrance to the disposition of drugs in the body [16]. Elimination and metabolism may be delayed because of highly protein bind. Therefore, protein binding affects both the duration and intensity of drug action in the body.

Stability In vivo stability is also a critical chemical property of the drug that affects drug levels in the body. For example, the extent of drug ionization, stability in the acidic environment of the stomach or stability in the presence of gut enzymes, as well as presence of food and gastric emptying can reduce oral bioavailability of many small and macromolecular drugs. On the other hand, drugs are subjected to metabolism in the body by different sequential and competitive chemical mechanisms involving oxidation, reduction, hydrolysis (phase I reactions) and glucouronidation, sulfation, acetylation and methylation (phase II reactions). Cytochorme P450 enzymes which catalyze oxidation reaction are mainly responsible for first-pass biotransformation of majority of the drugs in the body, thus limiting oral absorption and systemic availability of the drugs [17]. Cytochrome P450 abundantly present in the intestinal epithelium and liver tissue, and metabolizes several chemically unrelated drugs from major therapeutic classes [17].

Besides this, macromolecular drugs such as proteins and peptides, ASN, pDNA, siRNA and miRNA have poor biological stability and a short half-life resulting in unpredictable pharmacokinetics and pharmacodynamics. Proteins and peptidal drugs are highly prone to enzymatic cleavage in the blood circulation and tissues, whereas nucleic acid therapeutics are highly susceptible to degradation by intra- and extra-cellular nucleases, leading to degradation and a short biological half-life [5, 18]. DDS have the potential to overcome the challenges of degradation and short biological half-life, and can provide safe and efficient delivery of macromolecular therapeutics.

Expression of Membrane-Bound Drug Efflux Pumps If the drug molecules are substrates to efflux pumps, their transport through cellular membranes is severely restricted [20–21]. The ATP-binding cassette (ABC) efflux pumps are transmembrane proteins present at various organ sites within the body, and use ATP as a source of energy to actively transport drug molecules across the lipid cell membranes [23]. Among the ABC family of efflux pumps, P-glycoprotein (P-gp) is highly expressed in epithelial cells of the small intestine, which is the primary site of absorption for the majority of the orally given drugs [24]. Efflux pumps also present on the luminal side of the endothelial cells of BBB, and restrict entry of hydrophobic molecules into the brain [25, 26]. The multi-drug resistance in many cancers is linked to the ABC efflux transporters which express on cell membranes and produce intracellular drug levels below the effective concentrations necessary for cytotoxicity [19]. All these efflux transporters preset a broad overlap in substrate specificities and act as a formidable barrier to drug absorption and availability at target sites [24].

DDS can be employed to overcome most of these chemical challenges. For example, paclitaxel is a potent anticancer drug, is poorly absorbed after oral administration and its bioavailability is <6 % [27]. The obvious reason for its low bioavailability are high molecular weight, poor aqueous solubility, the affinity to drug efflux pumps, and rapid metabolism by cytochrome P450 enzymes in the gut [24]. Nanoemulsions and self-emulsifying DDS have been employed recently for the successful oral and parenteral delivery of paclitaxel [20–22, 28, 29]. Similarly, to protect RNA based therapeutics from enzymatic cleavage, several DDS have been proposed and they are at different stages of preclinical and clinical development.

10.1.2 Remote Disease Targets

Anatomical and physiological barriers involved in the body restrict the direct entry of small and macromolecular drugs into the target extracellular or intracellular tissue locations [4, 30] resulting in sub-optimal doses at target site and reduced efficacy. However, cytotoxic drugs and RNA therapeutics have their target sites inside the cells, therefore need to be delivered intracellular in sufficient doses to produce therapeutic effect. The first limiting anatomical barrier for orally administered drugs is epithelial lining of gut walls, where from drugs will permeate through either by transcellular or paracellular transport. This transport is in turn dictated by the chemical properties of drugs as alluded above. Therefore, altering the chemical properties

by making the drugs in salt form, encapsulating in DDS based on cyclodextrins, lipid or polymeric carriers, or using permeability enhancers could promote bioavailability of drugs [20–22, 29]. Cytochrome P450 and efflux transporters present in the enterocytes of intestinal walls also forms as another limiting barrier to drug permeability [24]. Use of cytochrome P450 and efflux pump inhibitors can promote oral drug absorption. For example, pre-treatment with curcumin results in inhibition of P-gp and cytochrome P450 expression in the GI tract, leading to increased oral bioavailability and efficacy of drugs [20–22, 31].

For the drug molecules given intravenously, the limiting anatomical barrier is that of vascular endothelium and basement membrane. In addition, blood serum proteins, proteolytic enzymes, RNases etc. limit the effective drug delivery to the target sites [4, 30]. CNS disease are likely to rise to 14 % by 2020 mainly due to the ageing population, however, many newly discovered small and macromolecular therapeutics do not cross into the brain after systemic delivery [32]. Because brain is protected by blood-brain-barrier (BBB), which is composed of very tight endothelial cell junction and presence of several efflux transporters, resulting in formation of dynamic formidable barrier to drug transport [33]. However, once at the BBB, hydrophobic drugs with the size below <500 Da generally do transport through lipid cell membranes by passive diffusion, but if they are substrates to drug efflux pumps, they will be pumped out from the brain. Hydrophilic molecules also cannot transport efficiently as there is very limited paracellular transport present in the tight junctions of the BBB [20–22].

Cancer mass is another complex anatomical barrier in drug delivery. For example, solid tumor microenvironment is heterogeneous and structurally complex and presents a challenging barrier in drug delivery. The cytotoxic drugs which are intended to kill a large proportion of tumor cells in a solid tumor, must uniformly distribute through the vascular network, pass through capillary walls, and traverse the tumor tissue [34]. Nevertheless, the drug distribution in tumors is not uniform, and only a fraction of tumor cells is exposed to lethal doses of cytotoxic agents [34]. Tumor microenvironment is composed of tumor cells with varying proliferation rate and stromal cells (fibroblasts and inflammatory cells) that are surrounded in an extracellular matrix and nourished by a vascular network, and regions of hypoxia and acidity [34–37]. Each of these components may different from one site to another in the same tumor mass, and all of these factors effects tumor cell sensitivity to drug treatment [34]. In addition, stromal components in tumors contribute to an increase in interstitial fluid pressure, which limit the penetration of macromolecular drugs [38]. Furthermore, the three-dimensional nature of solid tumor tissue itself affects the sensitivity of constituent cells to chemo and radiation treatments [34, 39]. For instance, the tumor cells grown as spheroids in cell culture or tumors grown in animals, are more resistant to cisplatin and alkylating agents than the corresponding cell dispersions [40].

In addition, certain intracellular infections, like leishmaniasis and listeria, where macrophages are directly involved in the disease are not accessible to drug delivery, thus necessitating specific drug delivery strategies [41]. To overcome all these challenges, it is highly important to develop DDS that render protection to the drug from biodegradation in the body, while allowing their transport through the anatomic and physiological barriers to increase their bioavailability at the target tissue.

10.2 Nanotechnology Solutions

The science of nanotechnology has begun just in the last decade, but in this short time, it has been successfully applied in several fields ranging from electronics to engineering to medicine. Recent understanding of cellular barriers and molecular profile of diseases, and controlled manipulations of material at the nanometer length scale, nanotechnology offers great potential in the disease prevention, diagnosis, and treatment [30, 31]. Nanotechnology has also allowed for challenging innovations in drug delivery, which are in the process of transforming the delivery of drugs. Nanosystems fabricated using controlled manipulation of material are exploited for carrying the drug in a controlled manner from the site of administration to the target site in the body. They are colloidal carriers with dimensions <1,000 nm and can traverse through the small capillaries into a targeted organ down to target cell and intracellular compartments, which represent the most challenging barrier in drug targeting. The critical attributes of any nanoparticle DDS are to (1) protect a labile drug molecule from both in vitro and in vivo degradation, (2) maintain the effective pharmacokinetic and biodistribution pattern, (3) promote drug diffusion through the epithelium, and/or (4) enhance intracellular distribution. However, the specificity, sensitivity and simplicity are very important for any nanosystem to be clinically successful as a DDS. Several types of nanoparticle DDS have been evaluated for their potential drug delivery applications are in various stages of clinical development, these are discussed in the next sections.

10.2.1 Enhancing Solubility and Permeability

Solubility and permeability are two of the most critical biopharmaceutical characteristics impacting the successful delivery of drug molecules through anatomical membranes in the body. If the drug molecule is not a substrate to efflux transporters and metabolizing enzymes, then the solubility (hydrophilic and hydrophobic) plays a major role in determining oral intestinal permeability. Biopharmaceutical Classification System (BCS) is proposed based on the solubility and permeability properties of the drugs [42] which classifies drugs into one of four classes. Class I drugs are highly soluble and permeable in the GI tract, therefore, bioavailability is not an issue with Class I drugs. Class II drugs are poorly aqueous soluble but highly lipophilic. They are well permeable across the GI tract due to high lipophilicity, but the bioavailability is likely to dissolution rate limited due to low aqueous solubility. Class III drugs are highly soluble but have low permeability due to their low lipophilicity. In both Class II and Class III examples, DDS plays a critical role in overcoming poor solubility and permeability. On the other hand, Class IV drugs show low solubility and low permeability, and exhibit poor and variable bioavailability. Methods to enhance both solubility and permeability should be adopted for these drugs.

To improve solubility and permeability, several methods have been employed over the years. Such as preparation of prodrugs, use of chemical or physical permeability enhancers to transient openings of the tight junctions, or direct administration to the target site. However, formulation efforts can best exemplify in improving poor solubility and permeation profiles of both small and macromolecular drugs. Nanoparticle DDs like, liposomes, nanoemulsions, nanosuspensions, solid-lipid nanoparticles (SLN), micelles or polymeric nanoparticles are highly useful over the current methods to deliver the highly hydrophilic or highly lipophilic molecules across the intestines and BBB. For example, drug nanocrystal suspensions (nanosuspension) allow for increased dissolution velocity and saturation solubility of poorly aqueous soluble drugs, which is accompanied of an increase in oral bioavailability [43]. In addition, nanocrystals can be delivered intravenously for controlled drug release, and their surface can be tailored for both passive and active targeting. On the other hand, lipid-based systems like nanoemulsions and SLN could allow for the delivery of lipophilic drugs, by incorporating them in the lipid core of the formulation. These DDS can enable direct transfer of drug to the intestinal membranes and excluding the dissolution of drugs in aqueous fluids in GI tract. In once such study, we have formulated highly lipophilic paclitaxel into deoxycholic acid modified nanoemulsion, which showed increased oral bioavailability compared to paclitaxel solution [20–22, 44]. In another example, saquinavir, an anti-HIV protease inhibitor incorporated in nanoemulsion, showed enhanced oral absorption [45].

Recent studies show that nanoemulsions made using oils rich in omega-3 and omega-6 polyunsaturated fatty acids (PUFA) can promote drug delivery to the brain [45]. This is some extent attributed to the presence of PUFA transporters on the abluminal membrane side of the endothelial cells of BBB [46]. Tissue and cell permeability also altered by surface modification of the nanoparticles with targeting ligands which can facilitate the nanoparticle uptake along with its payload into the cells. These aspects have been discussed in the next sections.

10.2.2 Targeted Delivery to Disease Sites

Targeted delivery exploiting the structural changes and cellular markers of a given pathophysiology can potentially reduce the toxicity and increase the efficacy of drugs. This is highly important in case of diseases like cancer, where dose-limiting toxicities and drug resistance constitute major barriers to drug success. General targeting mechanisms consists of passive and active targeting [30].

Upon parenteral delivery, passive targeting depends on the size of the DDS and the disease vascular pathophysiology in order to preferentially accumulate the drug at the site of interest and avoid distribution to normal tissue [30]. For example, nanosystems escape from the blood circulation and accumulate in sites where the blood capillaries have open fenestrations as in the sinus endothelium of the liver [47] or when the integrity of the vascular endothelial membrane is perturbed by inflammation due to infections, rheumatoid arthritis or infarction [48] or by tumors [49]. In the liver, the size of capillary fenestrae can be as large as 150 nm [50] and

liposomal nanocarriers showed extravasation to hepatic parenchyma [47]. Nanosystems in the size range of 50–200 in size can extravasate and accumulate inside the tumor tissue and inflammatory sites [51, 52]. Therefore, the nanomedicine in the size range is expected to provide therapeutic benefits for treating these diseases. In case of solid tumors, passive targeting involves in the transport of nanosystems through a newly formed leaky tumor microvasculature into the tumor interstium and cells (Fig. 10.1). This phenomenon has named as "enhanced permeability and retention" (EPR) effect, first discovered in murine tumors for macromolecules accumulation by Maeda and Matsumura [53]. EPR effect is observed in many human solid tumors with the exception of hypovascular tumors (prostate or pancreatic cancer) [54, 55]. This effect will be optimal if nanosystems can escape reticulo-endothelial system (RES) and show longer circulation half-life in the blood. Poly(ethylene glycol) (PEG) grafting on nanosystems will evade RES uptake, allow for prolonged circulation in the blood and enhance tumor accumulation through EPR. Besides, the RES uptake of non-PEG grafted nanosystems also offers an opportunity for passive targeting against intracellular infections such as leishmaniasis, candiasis, and listeria which reside in macrophages [41].

The specificity of passive targeting can be remarkably improved when the targeting ligands are used with nanosystems, which selectively bind to cellular markers overexpressed on the disease site [56] termed as active targeting (Fig. 10.1). For example, folic acid-nanoparticles can be used to target tumor cells that over express folate receptors, such particles internalize via folate receptor mediated endocytosis [57]. In another example, arginine-glycine-aspartic acid (RGD) sequence containing peptides can be conjugated to nanoparticle to target $\alpha_5\beta_5$ or $\alpha_5\beta_3$ integrin receptors over express on endothelial cells of the newly formed angiogenic blood vessels and also on tumor cells. Furthermore, the targeting ligands anchored to nanosystems will allow for carrying of many drug molecules compared to direct conjugation of targeting ligands with drug molecules.

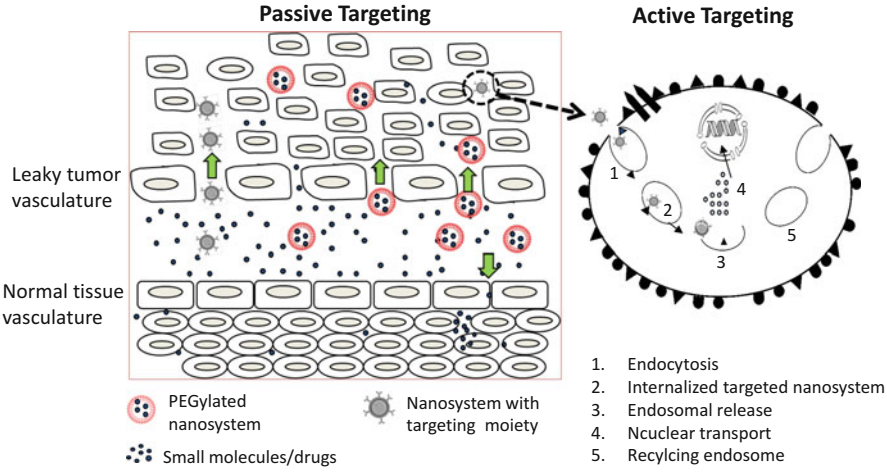

Fig. 10.1 Schematic illustration of passive and active targeting strategies in tumor drug delivery

10.2.3 Intracellular and Subcellular Delivery

The nanosystems once in the disease vicinity, they need to enter the cells and transfer the payload to sub-cellular organelles. There are two mechanisms playing a role in intracellular and subcellular delivery are non-specific or specific uptake of nanosystems by cells [30, 58]. In case of non-specific uptake, cells surround the nanosystems and forms a vesicle in the cell called an endosome. The endosomes then fuse with the highly acidic organelles called lysosome, which are rich in degrading enzymes. Endosomes usually travels in a specific direction and join at the nuclear membrane. Specific uptake on the other hand, involves receptor mediated endocytosis, where the actively targeted nanosystem binds to the cell-surface receptor, resulting in internalization of the entire nanoparticle-receptor complex and vesicular transport through the endosomes. The receptor can be re-cycled back to the cell surface following dissociation of complex. After the cellular internalization, stability of the payload in the cytosol and delivery to specific organelles, such as mitochondria, nucleus etc, is also essential for therapeutic activity. However, many drugs do not survive in the lysosomal environment. For example, 99 % of the internalized gene molecules undergoes degradation in endosomes. Thus buffering the endosomes for safe release of its contents helps in efficient gene delivery. Towards this, polycationic nanosystems have been explored, which causes endosomes to swell and burst, leading to the safe release of trapped content [59]. In another strategy, instead of trafficking drug carrier to the lysosome, the endosomal contents were released into the cytoplasm, thus bypassing the lysosomal degradation of the drug molecules [60, 61]. For example, a cyclic RGD functionalized polyplex micelles were taken into the cellular perinuclear space selectively through caveolae mediated endocytosis, thus escaping the lysosomal degradation of its active content [61].

Cellular uptake could be enhanced using of arginine rich cell penetrating peptides (CPP's) [62]. For example, HIV-1 Tat peptide was used to promote non-specific intracellular delivery of various therapeutics following systemic administration [63]. A number of cationic CPP's like penetratin also have been identified to promote intracellular drug delivery. In addition to intracellular delivery, use of delocalized cationic amphiphiles or mitochondriotropic nanosystems can promote mitochondrial drug delivery [64, 65].

10.2.4 Enabling Non-invasive Delivery

Non-invasive delivery is an alternate to systemic delivery of drugs, and mainly includes drug delivery via intranasal, pulmonary, transdermal, buccal/sublingual, oral and trans-ocular routes [66, 67]. Patient compliance has been found to be much higher when drugs given by non-invasive routes and therefore they are considered to be a preferred route of drug delivery. However, the preferred route of administration for a given drug selected based on several factors, such as biopharmaceutical properties (solubility, permeability and stability) of a drug molecule, disease state, onset of action, dose frequency and adverse effects. For example, sumatriptan and

zolmitriptan administered via intranasal route provide rapid-onset of relief from migraine related pain in minutes compared to oral tablet in hours. Similarly, potent peptidal drugs like calcitonin, desmopressin allows therapeutic blood levels that are not achieved with oral route of administration. In another example, selegiline and fentanyl transdermal products eliminate GI related adverse effects. In addition, non-invasive insulin products for inhalation and buccal administration improve patient compliance by reducing multiple daily injections.

In general, oral route is much convenient for high doses of administration. However, macromolecular drugs are not stable in the GI fluids, where intranasal, buccal/sublingual or pulmonary offers a non-invasive route of choice. These routes also favor treatments that need faster absorption of drug and where a rapid systemic exposure is well tolerated. Transdermal delivery is useful in chronically administered treatments (chronic pain, depression, Parkinson's, dementia, attention deficit-hyperactivity disorder and hormonal therapies), where sustained plasma profiles and low C_{max} to C_{min} ratio are required.

10.3 Illustrative Examples of Nanotechnology Products

Nanotechnology based concepts have been extensively applied in engineering of nanosystems for delivery of contemporary therapeutics in a controlled manner from the site of administration to the target disease in the body. The history of nanosystems reaches back to 1950s when the first polymer-drug conjugate was reported with N-vinyl pyrrolidine conjugated to glycyl-L-leucine-mescaline [68]. However, the most relevant nanosystems were conceptualized only after the first report of liposomal preparations in 1964 [69] and their subsequent use as vehicle for drug delivery application [70]. Soon after, synthesis of albumin nanoparticle was reported in early 1970s [71] with a subsequent early attempt of exploiting them as the first protein based DDS [72]. The pharmacological effects of polymer-based nanoparticles were studied [73] and their application as DDS envisioned around the same time [74]. As alluded earlier, ground breaking discovery of EPR effect in tumors by Matsumura and Maeda further emphasized on relevance of the size of delivery vehicle [53]. These seminal works drew tremendous attention on nanosystems for a sustained and controlled delivery of drugs. It was realized that for an optimized delivery system, the size of the payload vehicle should be between 10 and 100 nm. Kidneys easily clear off particles smaller than 10 nm while the particles larger than 100 nm are removed by the RES [73]. Since then, several different types of nanosystems have been researched and much focus has specifically been on tailoring the size, physical properties and surface functionality of the delivery systems for varying therapeutic applications. The collective research input on the nanotechnology based improvement of DDS has enabled several products in to the market in the past two decades (Table 10.1).

Sandimmune® and Taxol® are US Food and Drug Administration (FDA) approved dosage forms of cyclosporine and paclitaxel respectively, formulated

Table 10.1 Nanotechnology-based products in clinical application

Nanotechnology platform	Trade name	Active agent	Indication(s)	Approval year
Liposomes	Abelcet	Amphotericin B	Fungal infection	1995
	AmBisome	Amphotericin B	Fungal infection	1997
	Amphotec	Amphotericin B	Fungal infection	1996
	Daunoxome	Daunorubicin	Antineoplastic	1996
	DepoCyt	Cytarabin	Lymphomatous meningitis	1999
	Doxil/Caelyx	Doxorubicin	Antineoplastic	1995
	Myocet	Doxorubicin	Antineoplastic	2000
	OncoTCS	Vincristine	Non-Hodgkin's lymphoma	2004
Micelles	Estrasorb	Estradiol	Vasomotor symptoms	2003
Nanocrystal	Emend	Aprepitant	Antiemetic	2003
	Tricor	Fenofibrate	Hypercholesterolemia and hypertriglyceridemia	2004
	Triglide	Fenofibrate	Hypercholesterolemia and hypertriglyceridemia	2005
	Megace ES	Magesterol acetate	Anorexia, cachexia or an unexplained significant weight loss in AIDS patients	2005
	Rapamune	Sirolimus	Immunosuppressant	2000
Nanoemulsion	Tocosol	Paclitaxel	Nonsuperficial urothelial cancer	2003
Nanoparticle	Abraxane	Paclitaxel	Metastatic breast cancer	2005
Nanotube	Somatuline depot	Lanreotide	Acromegaly	2007
Superparamagnetic iron oxide	Feraheme injection	Ferumoxytol	Treatment of iron deficiency anemia in patients with chronic kidney disease	2009
	Feridex	Ferumoxide	MRI contrast agent	1996
	GastroMARK	Ferumoxsil	Imaging of abdominal structures	1996

using Cremophor®EL as solubilizing nonionic surfactant. However, due to hypersensitivity reactions associated with these products, Cremophor®-free formulations based on nanosystems have been developed and commercialized. Genexol^{PM} is one such example of Cremophor-free polymeric micelles formulated paclitaxel where poly-(ethylene glycol) is used as a nonimmunogenic carrier while biodegradable poly-(D,L-Lactic acid) forms the drug solubilizing hydrophobic core [75, 76]. Several such DDS including liposomes, nanoemulsions, polymeric nanoparticles, micelles and nanocrystals (Fig. 10.2) have been developed, granted regulatory approval and have been marketed since then. The following section will focus on each of such DDS with illustrative examples of commercialized products.

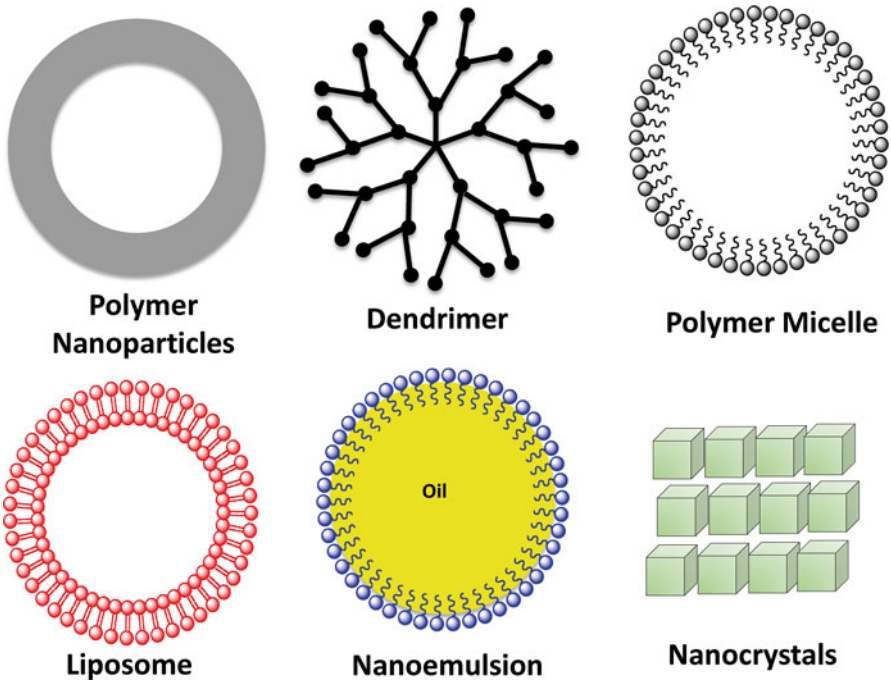

Fig. 10.2 Different types of pharmaceutical nanosystems used in drug and gene delivery

10.3.1 Lipid-Based Nanosystems

Lipid based carriers are extremely popular since they facilitate a controlled administration of both small and macromolecular drugs at therapeutically relevant doses. Liposomes and nanoemulsions are two most commonly used lipid based nanosystems for drug delivery application.

Liposomes Liposomes are vesicles formed of a lipid bi-layer, first developed by Alec Bangham in 1961, and their lipid bi-layer membrane is similar to that of cellular membranes. The lipid bi-layer of liposomes is composed of phospholipids with a hydrophilic head and a hydrophobic long-chain tail [77]. The hydrophilic core of the liposomes facilitates in compartmentalizing water-soluble drugs into the aqueous core while the hydrophobic bi-lipid membrane has been exploited to load water-insoluble drugs. Initial attempts using liposomes as nanosystems focused largely on improving their circulation time in the blood and targeting efficiency. PEG-modification of liposomes, first reported in 1990 [78] has by far been the most promising approach to achieve longer circulation of the liposomes in the blood. There has been a plethora of literature since then on the application of PEG-modified liposomes to achieve a selective delivery of drugs post-administration [79–81]. However, several other surface modifications of liposomes such as poly[N-(2-hydroxypropyl) methacrylamide] [81] poly-N-vinylpyrrolidones [82] polyvinyl

alcohol [83] and amino acid-based polymer–lipid conjugates [84] have been explored. Many studies showed that the opsonization of the liposomes might be dependent on the hydrophobicity of the surface, charge of the lipid and the molecular weight of the modifying polymer [85]. Antibody [86], folate [87] and peptide [88] mediated surface receptor targeting has been primarily enabled directing the liposome based drug delivery to the target organ.

The first liposome based formulation, PEG-liposome encapsulated doxorubicin was approved in 1995 (Doxil™, Orthobiotech) initially for the treatment of HIV-related Kaposi Sarcoma [89, 90] and later for ovarian cancer and myeloma. Doxil has remarkably reduced the cardiotoxicity by lowering cardiac exposure to free doxorubicin [77, 91]. Besides, it also increased half-life and tumor accumulation compared to free doxorubicin [92]. Furthermore, antibody modification of Doxil has shown a much higher tumor accumulation and enhances the cytoxicity of the doxorubicin [93]. In a study conducted on 53 patients suffering from advanced Kaposi's sarcoma, 19 patients showed partial and 1 patient showed complete response on administration of Doxil™ once every 3 weeks [94]. The success of liposomal doxorubicin has led to several liposomal-based drug formulations that are either approved for clinical application or are undergoing different phases of clinical trial. Some of the key drugs that have been exploited for liposomal formulation are shown in Table 10.1.

Nanoemulsions Nanoemulsions are heterogeneous system of two immiscible liquids; typically oil-in-water (o/w) or water-in-oil (w/o) with a droplet size in the range of 50–200 nm. These kinetically stabilized nano-sized droplets have several advantages over macroemulsions such as higher surface area and hence more free energy, higher stability with lower creaming effects, coalescence, flocculation and sedimentation [95]. The formation of nanoemulsions however requires an external shear force to decrease the droplet size to desired range and their productions methods are broadly classified as high-energy and low-energy methods. The high-energy methods could include laboratory or industrial scale high-pressure homogenization, microfluidization or laboratory scale ultrasonication [96]. However, these methods may not be conducive for applications involving thermolabile drugs, nucleic acids and proteins. Low-energy methods such as spontaneous emulsification, the solvent-diffusion method and the phase-inversion temperature (PIT) method are used for such payloads [95, 97]. The nanoemulsions serve as an excellent vehicle for solubilizing lipophilic drugs into the oil phase or hydrophilic drugs in the aqueous phase. The application of nanoemulsions as DDS has been envisaged only in the past decade and several attempts have been realized to increase their stability, circulation time and achieve a targeting efficiency [20–22, 98, 99]).

For example, propofol was first formulated in Cremophor® EL by Imperial Chemical Industries as ICI35868, and went into clinical use. However, due to the toxicity of Cremophor®, it was withdrawn from the market, reformulated in oil-in-water emulsion and launched by the trade name Diprivan® (ICI, now AstraZeneca). Apart from propofol as active pharmaceutical ingredient, the formulation contains generally regarded as safe grade excipients (GRAS) such as soyabean oil, glycerol, egg lecithin and disodium edetate [100]. Diprivan® is used as a short acting,

intravenous sedative used in intensive care medicine. It is known to have low toxicity, controlled sedation effect, rapid onset, a short duration of action and quick recovery despite prolonged usage [100, 101] TOCOSOL is another Cremophor® EL-free nanoemulsion formulation of paclitaxel that was approved by FDA in 2003 for the treatment of nonsuperficial urothelial cancer. Dexamethasone (Limethason®, Mitsubishi Pharmaceuticals), alprostadil palmitate (Liple®, Mitsubishi Pharmaceuticals), flurbiprofen axetil (Ropion®, Kaken Pharmaceuticals) and Vit A, D, E, K (Vitalipid®, Fresenius Kabi) are some other examples of therapeutically relevant compounds that have been formulated in nanoemulsions for clinical applications. Recently, NanoBio Corporation has formulated an emulsion-based antiviral drug NB 001 that shows potent activity against HSV-1 virus and antifungal drug NB 002 for the treatment of distal subungual onychomycosis (DSO). Both these formulations are currently in phase II/III trails.

10.3.2 Polymer-Based Nanosystems

Polymeric nanoparticles clearly are the most studied system for drug delivery applications. Different polymeric materials, natural, semi-synthetic and synthetic, have been exploited as polymer-drug conjugate or polymer-based nanoparticle for drug encapsulation to facilitate therapeutic applications. It is important to realize that while polymer-drug conjugate is a system which involves a single polymer chain conjugate to the drug, polymer-based nanoparticles are actually made up of several polymer chains which encapsulate the drug of interest.

Polymer-drug Conjugate Polymer-drug conjugates preparation date back to early 1950 [68] and the field has rapidly evolved since then [102]. Most drug molecules suffer from permeability through biological membranes, short half-life, non-specific distribution and dose dependent toxicities. Polymer conjugates on the contrary not only tremendously improves the in vivo circulation time of the drug but also facilitates passive delivery of these conjugates through leaky vasculature in diseases like cancer and inflammation [103]. They however also suffer from certain drawbacks such as polymer dependent toxicity, immunogenicity, rapid drug release, conjugate instability and poor drug loading. Several endeavors have been taken to overcome some of these shortcomings with much success. Besides, many bio-inspired polymers such as proteins (albumin, antibodies etc.) have also been looked upon as promising candidates for drug delivery applications.

The first polymer conjugate to undergo clinical trial was SMANCS where anti-tumor protein neocarzinostatin was (NCS) was covalently conjugated to two styrene maleic anhydride (SMA) [53]. SMANCS was approved subsequently in Japan in 1994 to treat advanced and recurrent hepatocellular carcinoma [104]. PEG-conjugate were the first candidate to get US FDA approval when PEG-L-asparaginase conjugate (Oncaspar) was accepted to treat acute lymphoblastic leukaemia [105]. Several other PEG -onjugates of drugs such as Neulasta (PEG-G-CSF; neutropaenia associated with cancer chemotherapy), PEG-asys (PEG-IFNα2a; Hepatitis B and C),

PEG-Intron (PEG-IFNα2b; Hepatitis C) have been approved to clinical treatment while several others are under various preclinical development. Besides, several other polymers (or their derivatives) conjugates (products names) such as polyglutamate (CT-2103, CT-2106), dextran (DOX-OXD, DE-310), N-(2-hydroxypropyl) methacrylamide (PK1, PK2, MAG-CPT, AP-5280, AP-4346) are being looked upon as promising candidates in their preclinical trial stages.

Though first protein nanoparticle based drug conjugation was reported in as early as 1974 [72] the first approved conjugate was realized only in 2005 when paclitaxel bound to albumin (Abraxane, AstraZeneca) was approved by FDA for treatment of metastatic breast cancer [106]. It is a non-targeted formulation with particle size around 130 nm, which is localized into the tumor partly through EPR effect and partly through albumin-binding protein. Clinical studies have demonstrated that Abraxane increases the therapeutic response, reduces the rate of disease progression and improves the survival rate among the patients. Antibodies have also been explored for drug conjugation and some examples of products from this class of nanovector includes Gemtuzumab (Mylotarg), Tositumomab and ibritumomab tiuxetan (Zevalin) [107, 108].

Micellar Delivery Systems Micelles are submicroscopic structures formed in an aqueous phase by amphiphilic surfactants or polymers that have a polar and a non-polar group. The typical size of these structures for delivery application ranges from 10 to 100 nm. These structures have a hydrophobic core, which facilitates the solubility of a lipophilic therapeutic agent and a hydrophilic corona that is exploited for surface functionalization to improve their tumor accumulation. These properties render them an attractive choice as carriers for drug delivery applications. Conventional surfactants however have a very high critical micellar concentration, and therefore are prone to disintegration on dilution in the blood stream [109]. Alternatively, polymeric micelles are usually prepared by self-assembly of a copolymer having hydrophobic moiety forming the biodegradable core while hydrophilic component for the surface. These polymers form micelles in aqueous media but at a much lower concentration compared to conventional surfactants [110]. Such polymeric micelles have been extensively researched for drug encapsulation, enhanced tumor targeting and longer in vivo circulation to aid an improved delivery system. Various approaches have been utilized to prepare polymeric micelles of desired properties using block copolymer, their lipid [111] or cyclodextrin [112] derivatives, diblock copolymers [113], triblock copolymers [114], pluronic polymer [115] and graft polymers [116].

Genexol-PM, a cremophor-free PLA-PEG copolymer-based micellar formulation completed its preclinical Phase I trial in 2004 [75]. Currently, the formulation is in its Phase II trial for the treatment of the patients suffering from taxane-pretreated recurrent breast cancer. SP1049C is another doxorubicin encapsulated pluronic polymer micelle based formulation that is under Phase II preclinical trial for the treatment of advanced level inoperable adenocarcinoma of esophagus [117]. NK911 is yet another example of a micellar formulation of PEG and doxorubicin conjugated poly (aspartic acid) which is under preclinical development [118].

Dendrimer Delivery System Dendrimers are roughly spherical nanoparticles made of several monomers, which branch out radially from the center. The advantages associated with dendrimers such as their controlled size, multiple valency, water solubility, modifiable functionality and an internal core render them a promising choice as drug carriers. They are therefore applied as delivery vehicles in several administration routes such as intra-venous, ocular, dermal and oral [119]. Their biocompatibility and immunogenicity has been studied in vitro as well as in vivo and similar to cationic macromolecules like liposomes and micelles, cationic surface groups render dendrimers cytotoxic to cells [120, 121]. Surface functionalization of dendrimers with PEG [122] or fetal calf serum [123] however has shown to reduce the cytotoxicity effects. The drug could be loaded on the dendrimers mainly by physical interaction or by covalent attachment. Physical adsorption of drug could suffer from poor drug loading and less control on drug release kinetics. Alternatively, the pro-drug approach is far more viable where the drug is chemically attached to the dendrimer directly or using a linker giving a much better pharmacokinetic and pharmacodynamic profile [124].

The field of dendrimer-based DDS has evolved greatly in the last decade and several dendrimer-drug conjugates are in their preclinical testing. One of the key examples is conjugation of PEO modified 2,2-bis (hydroxymethyl) propionic acid based biodegradable dendrimer to doxorubicin, which shows 9-fold higher tumor accumulation and 10-fold less cytotoxicity than free drug. The intra-venous administration of prodrug to doxorubicin-nonresponsive tumor showed a rapid tumor regression in a single dose [125]. Poly(glycerol-succinic acid) dendrimer (PGLSA)-camptothecin prodrug similarly has shown an enhanced solubility, cellular uptake and retention [126]. Since these initial success reports, several drugs such as artemether, cisplatin, diclofenac, mefenamic acid, dimethoxycurcumin, diflunisal, etoposide, ibuprofen, 5-florouracil, indomethacin and many more have been conjugated to dendrimer and are undergoing preclinical/clinical trials [127].

10.3.3 Nano-sized Drug Crystals

Poor aqueous solubility is one of the key problems with many small drug molecules, which affects their delivery and therapeutic applications. It is a well-established fact that with size reduction to nanometer scale, the properties of a material is governed by quantum laws and entirely different from its macro/micro size counterpart. A drug nanocrystal is therefore drug particle with its size in the nano-range i.e. 10–100 nm, and a suspension of such nanocrystals is popularly known as nanosuspension [219]. The suspension of these nanocrystals can be achieved in aqueous solutions as well as non-aqueous medium (liquid PEG, oil) with help of stabilizers like amphiphilic surfactants (poloxamers, PVP, phospholipids, polysorbate 80) or polymeric (hydroxypropyl methyl cellulose) materials. The hallmark of drug nanocrystals is that these crystals are pure drug particles with no carrier system. Similar to typical nanoparticle preparation, drug nanocrystals could be prepared by a

"bottom-up approach" (molecular level to nanocrystals) such as precipitation method or "top-down approach" (macro/micro level to nanocrystals) such as pearl milling (technology owned by Elan Nanosystems), high-pressure homogenization in water (technology owned by Skyepharma as well as Baxter) and in non-aqueous medium (technology owned by Pharmasol). Sometimes, a combination of the two approaches is used for nanocrystal production e.g. Nanoedge® (Baxter) that uses precipitation followed by homogenization. The major advantages of nanocrystallized drug are increased rate of drug dissolution and saturation solubility, improved oral bioavailability, reduced dose variations and general applicability to all routes of administration.

Rapamune® was the first nanocrystalline drug to obtain FDA approval in 2000 and was licensed to Wyeth Pharmaceuticals. It was produced by pearl milling method developed by Elan Nanosystems and contains rapamycin as the active drug. The formulation is marketed in two forms as tablets and oral suspensions. Soon after, Emend® was approved in 2003, which contains Aprepitant and is marketed by Merck. The production process has been developed by Elan Nanosystems and it is used for the treatment of emesis. Tricor® (drug Fenofibrate), Megace ES® (drug Megestrol acetate) and Theralux® (drug Thymectacin) are three other drugs which have been developed by Elan Nanosystems and have been licensed to Abbott, Par Pharmaceuticals and Celmed respectively. Several other products have however been introduced by other companies which include Semapimod® (Guanylhydrazone, Cytokine Pharmasciences), Paxceed® (Paclitaxel, Angiotech) and Nucryst® (Silver, Nucryst Pharmaceuticals).

10.4 Multifunctional Nanotechnology

As detailed in previous sections, biological system presents several barriers to effective drug delivery. It is therefore germane to develop drug delivery strategies to circumvent these barriers. This could be achieved by making the right choice of material as delivery vehicle, surface modification to increase targeting and intracellular availability of the drug and improving the functionality of the delivery system to achieve the diagnostic applications [128]. The nanosystem with these multifunctional abilities (Fig. 10.3) offer new possibilities in diagnosis, treatment and disease monitoring. The following sections will provide an in depth discussion on these aspects of drug delivery systems.

10.4.1 Choice of Materials for Nanotechnology

The material property of the delivery system is essentially the most important factor that governs the biocompatibility of formulation, stability and bioavailability of the drug and its clearance from the body. It is also equally important to

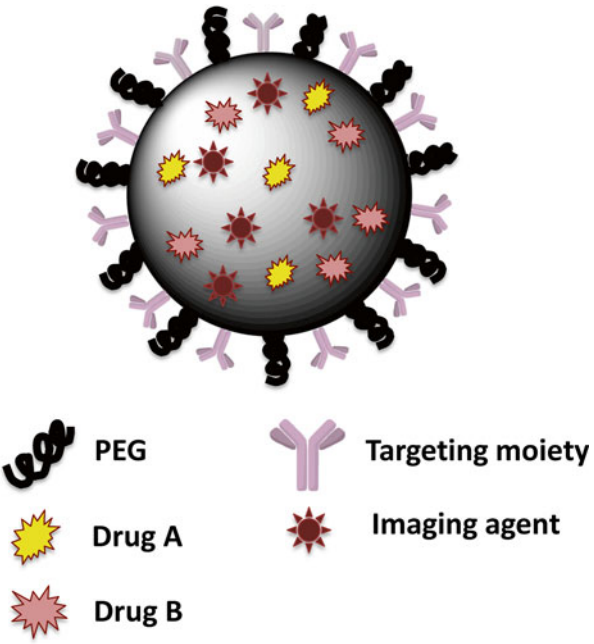

Fig. 10.3 A conceptual model of multifunctional nanomedicine with targeting ability, imaging capability, and drug/gene delivery in a single platform

understand the microenvironment of the target where the drug has to be delivered to achieve an effective therapeutic concentration. Design of nanosystems governed by microenvironment of the disease site results into a class of delivery systems that are popularly known as stimuli-responsive DDS. The delivery payload, route of administration and material safety profile, would also govern the components of such delivery vehicles.

pH Responsive Delivery Systems The physiological profile of an infected, cancerous or inflammatory body tissues differ drastically compared to the normal tissue. It has also been noted that various cellular compartments maintain their own characteristic pH levels; such as a lysosomal pH is around 4.5 where as in a mitochondria, the pH is around 8. These physiological differences result into a transmembrane pH gradient within the cellular compartments in a cell as well as among the cells. Such subtle differences in physiological environment could be actively exploited to design a pH responsive delivery system, which would be stable at physiological pH of 7.4 but actively degrade to release the drug under other conditions [129]. For example, a tumor is composed of rapidly dividing and metabolizing cells that are always short of the desired food and oxygen supply and thus rely on glycolytic pathways for harvesting energy to sustain [130]. The lack of oxygen in the tissue results in development of acidic condition within the tumor cells that could be exploited to achieve the delivery of a desired payload. The physicochemical properties of the delivery vehicles in response to difference in pH therefore are important characteristic, which has been actively focused in the past two decades.

Poly(β-amino ester) (PbAE) is a biodegradable cationic polymer which has been used for pH stimuli responsive delivery of drug. The polymer rapidly degrades under acidic environment with pH levels below 6.5 to release its payload into the cells. Significantly enhanced accumulation of drugs in the tumors has been demonstrated using PbAE polymer, leveraging pH stimuli-responsive delivery compared to a non-responsive polymer based delivery [131–133]. It has also been shown that the pH sensitivity of the polymeric delivery systems can be tailored by altering the length of the hydrophobic carbon chain length [134]. The pH responsiveness of poly (alkyl acrylic acid) polymer can be controlled by the choice of the monomer as well as the ratio of carboxylated to non-carboxylated alkylacrylate monomer. This polymeric system has been used for enhanced and effective in vitro transfection of lipoplex formulations. In yet another study, pullulan acetate-sulfadimethoxine polymer conjugate has been utilized to develop pH responsive, self-assembled hydrogels for an enhanced delivery of doxorubicin [135].

Polymers have also been directly conjugated to the target drug using pH responsive spacers, which would degrade under the low pH environment inside the tumors or lysosomes/endosomes to release the drug. In one such attempt, poly (vinylpyrrolidone-co-dimethyl maleicanhydride) (PVD) was conjugated to doxorubicin and its pH responsive controlled release increased the accumulation of the drug in to the tumor site [136]. Similarly, copolymer N-(2-hydroxypropyl) methacrylamide (HPMA) [137] and linear PEG based nanosystems are other candidates which have shown promise in delivery of drug to the tumor targets [138, 139]. Hydrolytically labile hydrazone linkage has been used for the drug release by enzymatic action in the lysosomes/endosomes from the polymeric or protein-based conjugate [138]. Serum albumin conjugates of anticancer drugs such as chlorambucil and anthracyclines have shown an enhanced antiproliferative activity compared to free drug [140]. Polyacetals are other pH labile candidates, which have been exploited for developing polymer based pH-responsive DDS [141].

Liposomes have similarly been suitably modified to achieve pH stimuli and controlled drug delivery. The intact pH-sensitive liposomes are internalized into the cells by endocytosis and fuse to the endosomes to deliver its contents inside the cytoplasm [77]. The desired modification of the liposomes is mainly achieved by using new lipid candidates, which provides acid sensitivity to liposomes or by conjugation of pH sensitive polymers on liposome surface to render them prone to pH sensitive degradation. Mildly acidic amphiphiles have been used to design such phosphotidylethanolamine based liposomes where at physiological pH, these amphiphiles act as stabilizers [142] but get protonated under acidic conditions causing a destabilization of the liposome and facilitating the delivery of the payload [143]. These delivery systems have been successfully researched to show in vitro delivery of antitumor drugs, toxins, DNA, antisense oligonucleotides and antigens [144]. Other lipids such as cholesteryl hemisuccinate (CHEMS), poly(organophosphazenes) and dioleoyl phosphatidyl ethanolamine (DOPE) have also been used for pH-sensitive liposomal formulations [145–147].

Micelles are yet another class of nanocarriers which have been extensively investigated to develop pH-responsive delivery. One approach to realize this aim has

been the employment of titratable amines or carboxylic groups on the copolymer surface such that the micelle formation relies on the protonation of these groups [148, 149]. In certain cases, water-soluble block copolymers exist in different forms depending on the pH of their aqueous solution and thus have been manipulated for drug delivery applications [150]. Besides, several other water-soluble copolymers have been extensively used to develop long circulating, pH responsive micelles. Some of the common examples include block copolymers based on poly[4- vinyl-benzoic acid (VBA) and 2-N-(morpholino)ethyl methacrylate (MEMA), poly (acrylic acid)-b-polystyrene-b-poly(4-vinyl pyridine) (PAA-b-PS-b-P4VP), Poly[2 (dimethylamino)ethylmethacrylate]-block-poly[2-(N-morpholino)ethyl methacrylate] (DEA-MEMA), poly(L-lactide)-b poly(2-ethyl-2-oxazoline)-b-poly(L-lactide) (PLLA-PEOz-PLLA) ABA triblock copolymers and diblock copolymers (PEOz-PLLA) etc., have been used for such applications [30].

Dendrimers are relatively new class of materials that are being investigated to develop pH-responsive delivery systems. One promising report has been the use of dendrimer composed of 2,2-bis(hydroxymethyl)propanoic acid monomer which has been conjugated to doxorubicin to produce a pH responsive delivery [151]. In another recent attempt, the terminal ends of core-forming PEO dendrimers have been modified with hydrophobic groups using acid-sensitive acetal groups. The hydrophobic groups are cleaved off the dendrimer in acidic environment resulting in the release of the drug [152].

Thermo-responsive Delivery Systems The cancerous cells are known to be highly fragile and sensitive to heat-induced damage (compared to normal cells) largely due to their rapid dividing nature. Incorporation of components that facilitate heat induction in presence of external stimuli such as magnetic field has therefore been looked upon as attractive choices to pursue. These facts have led to the development of hyperthermia as an adjunct to the radiation and chemotherapy for treatment of cancer cells. Several recent research efforts have shown that loading of superparamagnetic iron oxide particles to a delivery system leads to hyperthermia induced cell death at tumor sites [153, 154]. Use of drug delivery vehicle to localize these magnetic particles in the tumor sites ensure that only cancerous cells are subjected to elevated temperatures without affecting the normal cells. The tumor ablation by hyperthermia coupled with incorporation of an antitumor drug in the formulation leads to enhanced efficacy and accumulation of the drug [155, 156].

The thermo-sensitive polymers display a low critical solution temperature (LCST) in aqueous solution, below which they are water-soluble but become insoluble above it. This interesting property makes them an exciting choice as thermo-responsive DDS. One such example has been the accumulation of rhodamine– poly(N-isopropyl acrylamide-co-acrylamide) conjugate at the tumor site using targeted hyperthermia [156]. Certain amphiphilic polymers exhibit thermo-sensitivity where they have a temperature sensitive hydrophilic component and a hydrophobic component. Poly (N-isopropylacrylamide) (NIPAAm) and its other copolymers have been the most researched thermo-sensitive amphiphilic polymers [157]. In an interesting report, gold nanoparticles coated cross-linked Pluronic®

(poloxamer) micelles that showed a thermo-sensitive reversible swelling-shrinking behavior caused by hydrophobic interactions of copolymer chains in the micells [158]. Several other illustrations of such polymer based thermo-responsive nanocarriers have been accounted in details in literature for further reading [27].

Fabrication of temperature-sensitive liposomes has been an area of tremendous interest to the researchers due to the simple known fact that the membranes of different phospholipids are known to undergo phase-transition from gel-to-liquid crystalline and lamellar-to-hexagonal transition and are release small water-soluble components during such transitions. One popular example is use of dipalmitoylphosphatidylcholine as primary lipid for liposome formation. It shows a leaky behavior at gel-to-liquid transition at 41 °C and this transition can be tailored by adding distearoylphosphatidylcholine as a co-lipid [159]. Polymers have also been employed to design thermo-sensitive liposomes that also show LCST. These polymer chains exhibit a coil-to-globule transition with a change in temperature and thus impart temperature-regulated functionality to the liposomes [160]. Such polymers stabilize the liposomes in their hydrated form below the LCST but their dehydrated form destabilizes the liposomal structural integrity resulting in delivery of the drug [161]. Several reports exploit the modification of liposomes with NIPAAm copolymers for the fabrication of thermo-responsive substitutes [160, 162].

Redox-Responsive Delivery Systems Nucleic acid based therapeutics has acquired considerable interest lately and numerous attempts have been made to deliver ASN, pDNA, siRNA and miRNA, peptides and proteins for treatment of many genetic diseases. However, successful delivery of these biomolecules to the target cells is an important challenge considering the fact that these agents are highly prone to degradation. A stimuli-responsive system will be of tremendous application as DDS for these biomolecules to ascertain their structural integrity and therefore the therapeutic functionality. It has been established that there is a redox potential difference between the reducing extracellular space and the oxidizing intracellular compartment, which can be potentially exploited to guide the DDS into the cells [163]. Redox-sensitive delivery systems largely rely on components containing disulfide linkage that are taken up in the cell by endocytosis and the disulfide linkage is disrupted in the lysosomes to facilitate payload delivery [164]. The glutathione pathway plays a key role in reduction of the disulfide linkage in the reducing intracellular environment by maintaining an elevated level of reducing glutathione. Besides, the disulfide crosslinking also ensures more stable and robust structural integrity of the nanosystem that decreases the chances of early release of the payload.

One of the strategies to exploit the redox stimuli has been the use of polyaspartamide that uses positively charged groups in the polymer to electrostatically entrap DNA while the thiol groups on the polymer chain form the disulfide linkage resulting in formation of thiopolyplexes [165]. Thiolated gelatin particles have also been shown to form gelatin thiopolyplexes and have been used as potential redox-responsive nanosystem for pDNA delivery [166, 167]. Thiolated polyethylene imine has been directly conjugate to DNA to form polyplexes [168, 169] or have been used with a crosslinking agent [170] to successfully delivery DNA into the

cells with high transfection efficiency. In yet another report, glutathione sensitive polymer coated chitosan particles were used for designing of nanosystems stabilized by disulfide bond to provide gene delivery [171]. FDA has recently approved redox-responsive anti-DC33 antibody conjugate (Mylotarg®) for the treatment of acute myeloid leukemia [172].

Disulfide bond based redox-responsive liposomes have also been explored to enhance liposomal stability and delivery efficiency. Such liposomes are formed by a standard phospholipid along with a small chain lipid of which the hydrophobic and hydrophilic ends are linked by disulfide bond. These liposomes show tremendous structural stability until they reach the reducing environment inside the cells where the disulfide bond cleavage results in destabilization and delivery of the gene [173]. Thiocholesterol lipid based liposomes have been shown to successfully delivery gene into the cell in the reducing environment of the cells [174]. Mitomycin C conjugate with a cleavable disulfide bond incorporated into liposomes has shown lesser toxicity and better therapeutic potential than the free drug [175].

10.4.2 Surface Modification to Increase Availability at Tissue and Cell Levels

A careful designing of the nanosystems will enable them to deliver the drugs successfully to the target disease through active or passive targeting. However, to do so successfully, the DDS should be available in the blood stream for longer period of time by avoiding recognition by the components of immune system, circumventing the process of opsonization and preventing subsequent clearance by the RES. The longevity of nanosystem in the circulation not only allows their deposition at the target site through EPR effect but also improves targeting ligand to interact to its receptor. Suitable surface modifications of the nanocarriers for a prolonged and sustained presence in the body have therefore garnered tremendous interest.

Water-soluble polymers have been most commonly used to improve the retention time of the nanosystem in the blood and PEG is found to be most efficient in this regard. The PEG coating on the nanosystem surface provides a steric hindrance that prevents the interaction and binding of blood proteins to nanoparticle surface. The fact that RES recognition of a foreign object in the body largely depends on the binding on these plasma proteins to the surface, the sterically stabilized nanocarriers successfully escape body clearance [176]. This property to evade the immune system is popularly known as the "stealth" effect of the polymer. PEG is an excellent choice as surface protection moiety due to its high solubility in aqueous medium, flexibility of chain length, low immunogenicity and low toxicity. Besides, it does not interfere with the biological performance of the drug loaded in the delivery vehicle. PEG therefore by far is the most studied surface modifying agent to improve the residence time of the pharmaceutically relevant nanosystems. It has also been observed that while the particles modified with brush-like PEG effectively escape the immune response, surfaces modified with mushroom-like PEG molecules seem

to activate the immune system against the particles [177, 178]. Literature serves several derivatives of PEG that have actively been used to enable the surface functionalization of the delivery vehicles [179].

Besides PEG alone, copolymers of PEG have also been explored for surface modification of drug delivery constructs. Block copolymer of PEG-polylactide glycolide (PLGA) forms a hydrophobic core of PLGA and a hydrophilic shell of PEG that shows a longer residence time in the blood circulation [180]. Such polymeric preformed particles of PLGA could also be functionalized by PEG derivatives to prevent recognition by the immune system and therefore an enhanced retention time in the body. For example, the PLGA particles functionalized with polylysine-PEG copolymers shows a considerably reduced opsonization [181] while PEG modified poly (cyanoacrylate) particles provided longer-circulation as well as permeation into the brain tissue [182]. In a similar attempt, surface modification of polystyrene nanosystems by hydrophobically-modified dextran and PEG-dextran was studied to show that the stability of construct could be tailored by the density and also the nature of the surface modifying polymer [183]. Lipid derivatives of PEG have similarly been used to prepare PEG modified liposomes for enhanced circulation and improved performance of the delivery system [184].

Even though use of PEG has largely dominated the surface modification of DDS to increase retention time, several other alternatives have also been explored. The pre-requisite for a substitute of PEG has to be a water-soluble, biocompatible and non-immunogenic material. Polyoxomers, polyoxamines, polysorbate 80 and many more polymers have been used to modify the surface of nanoparticles to improve the bioavailability inside body. Lipid derivatives of poly (acryl amide) and poly (vinyl pyrrolidone) as well as other amphiphilic polymers such as poly (acryloyl morpholine) (PAcM), phospholipid (PE)-modified poly(2-methyl-2-oxazoline) or poly(2-ethyl-2-oxazoline), phosphatidyl poly glycerols, and polyvinyl alcohol have been successfully employed for surface modification of the liposomes.

10.4.3 Image-Guided Therapy

Imaging is an indispensable component of therapy and has been routinely used in hospitals and clinics for diagnosis of diseases and defects in the body. Conventional methods such as computerized axial tomography (CAT), magnetic resonance imaging (MRI), X-Ray imaging etc. have been employed in medical science for past several decades. Therefore, it was only fitting that with the advent of nanotechnology and more specifically nano-pharmaceutics, the concept of "molecular imaging" has been envisioned. Ability to image a DDS has therefore been an integral aspect of drug delivery application since it provides a visual feature to locate the site and extend of a disease in the body. Besides, it also enables a real-time assessment of the site of localization of a delivery vehicle in the body, its extent of sequestration in a particular organ and more specifically within a cell in question. For instance, presence of an imaging modality in a delivery vehicle customized to target a metastatic

tumor could be essentially tracked to the end site of its localization providing a direct visual evidence of the efficiency of a targeted or non-targeted system as well as the location of the tumor in the patient. Owing to the versatility of such a delivery system, extensive endeavors have been exercised to develop multifunctional nano-system (Fig. 10.3) comprising of targeting ligands, therapeutic agent(s) as well as imaging agents. To this date, several organic and inorganic imaging agents have been explored including liposomes [185], dye-conjugated silica [186], quantum dots [187], gold nanoparticle and nano-shells [188] magnetic nanoparticles [189] and many other contrast enhancing agents. Along with advances in conventional techniques like CAT and MRI scan, many new molecular imaging approaches such as radioactivity-based imaging (gamma scintigraphy, positron emission tomography (PET), single-photon emission computed tomography (SPECT)), surface enhanced raman scattering (SERS), optical coherence tomography (OCT), near-infrared fluorescence imaging etc., are been actively researched.

Radiolabelled probes are the most commonly used imaging agents in the drug delivery systems. Gamma scintigraphy provides a 2-dimension imaging ability while SPECT and PET enable a 3-D scanning. These techniques have their own advantages and disadvantages [190]. However, radioactivity based imaging systems are plagued by difficulties such as handling radioactive material, regulations concerned with their administration, their residence and clearance time from the body. Alternatively, improvement in MRI by the use of magnetic nanoparticles [191] or contrast enhancing agents [192] in the delivery system has been explored with vigor because of the non-invasive nature of the technique. Complexes of gadolinium, manganese, ferrofluids as well as superparamagnetic iron oxide are some of the most commonly applied contrast enhancing agents in MRI scans. Other popular imaging modalities include application of fluorescent dyes and quantum dots [193], SERS agents such as gold and silver nanoparticles [194].

10.4.4 Combination Therapeutics

Reports of multiples drug resistance (MDR) against antibacterial, antiviral, antifungal and anticancer drugs have become regularity in the previous decade. Numerous research endeavors have been applied to understand the origin of MDR and design therapeutic agents against them. However, the more we strive to overcome the medical enigmas by new drug discovery, the more complex the problem of MDR becomes. The gravity of the situation can be envisaged by a fact that the probability of MDR tuberculosis infection in acquired immunodeficiency syndrome (AIDS) patient is many folds more than a normal person. The inception of drug resistance has triggered the use of combination of drugs targeting a disease causing organism/process. The components of combination therapy may impact different independent targets, complement each other effect on the same target or bind independent of each other to give a combined effect for containment of the

disease. Such combination therapy has successfully been realized in the treatment of cancer, diabetes, bacterial and viral infections and asthma.

Co-administration of paclitaxel and ceramide using nanosystems has been proven to be extremely effective against MDR ovarian cancer [131, 132] as well as brain tumor cells [195] compared to the effect of individual drugs. Similarly, the use of a combination of paclitaxel and curcumin [28, 196] as well as doxorubicin and curcumin [197] enables to overcome the MDR in cancer cells. Several commercialized drugs such as Vytorin®, Caduet®, Lotrel®, Glucovance®, Avandamet®, Truvada®, Kaletra®, Rebetron®, Bactrim® and Advair® are actually a combination of two drugs [198]. Celetor Pharmaceuticals have developed CombiPlex® technology to launch combination chemotherapies for treatment of cancer. The technology uses high throughput screening, mathematical algorithm for synergy analysis and advanced nanosystems to predict right drug combination for therapy. This platform is meant to design chemotherapies so as to maintain an optimized ratio of the drugs in the body for enhanced efficacy. Their formulation CPX-1 is a fixed ratio combination of irinotecan and floxuridine that has shown positive results in its Phase-1 trial and is currently under Phase-2 trial for treatment against colorectal cancer [199]. CPX-351 similarly is a combination of cytarabine and daunorubicin and is under Phase-1 trial for the treatment of acute myeloid leukemia [200].

10.5 Regulatory Issues in Nano-pharmaceuticals

10.5.1 Approval of Pharmaceutical Products in the US

Despite the advances in nanomaterial application in disease diagnosis and drug delivery, significant amount of work still to be done in terms of characterizing nanomedicine safety and long term effects on biological system. Currently, all nanomedicine go through the FDA's traditional regulatory pathway within the Center for Drug Evaluation and Research (CDER) or Center for Devices and Radiological Health (CDRH). This pathway includes the following general requirements prior to approval.

(i) CDER reviews applications for new drugs.
(ii) Prior to clinical testing, laboratory and animal testing is performed to determine pharmacokinetic and pharmacodynamic attributes of the drug to determine a likely safety and toxicology profile in humans.
(iii) Clinical trials are performed in stages to determine if the drug is safe in healthy, then sick patients, and whether it provides a significant health benefit.
(iv) A team of FDA physicians, chemists, toxicologists, pharmacologists, and other pertinent scientists evaluates clinical data, and if safety and efficacy are established, the drug is approved for marketing.

Prior to the initiation of clinical trials, pre-clinical testing and manufacturing are regulated by several levels of regulation or guidance. These are FDA internally generated guidance documents, codified regulations listed in Title 21 Code of Federal Regulations (CFR) and International Conference on Harmonization (ICH) guidelines. Guidance documents are not codified law, but represent the Agency's current thinking on a particular subject. They do not create or confer any rights for, or on any person and do not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both [201].

Title 21 is the portion of the CFR that governs food and drugs within the United States for the FDA. It is divided into three chapters: Chapter I – Food and Drug Administration, Chapter II – Drug Enforcement Administration, and Chapter III – Office of National Drug Control Policy.

Most of the Chapter I regulations are based on the Federal Food, Drug, and Cosmetic Act. Notable sections in Chapter I are:

(a) 11 Electronic records and electronic signature related
(b) 50 Protection of human subjects in clinical trials
(c) 54 Financial Disclosure by Clinical Investigators [33]
(d) 56 Institutional Review Boards that oversee clinical trials
(e) 58 Good Laboratory Practices (GLP) for nonclinical studies

The 200 and 300 series sections are regulations pertaining to pharmaceuticals:

(a) 202–203 Drug advertising and marketing
(b) 210 cGMP's for pharmaceuticals
(c) 310 Requirements for new drugs
(d) 328 Specific requirements for over-the-counter (OTC) drugs

The 600 series covers biological products (e.g. vaccines, blood):

(a) 601 Licensing under section 351 of the Public Health Service Act
(b) 606 cGMP's for human blood and blood products

The 700 series includes the limited regulations on cosmetics:

(a) 701 Labeling requirements

The 800 series are for medical devices:

(a) 803 Medical Device Reporting
(b) 814 Premarket Approval of Medical Devices [104]
(c) 820 Quality system regulations (analogous to cGMP, but structured like ISO) [128]
(d) 860 Listing of specific approved devices and how they are classified

ICH guidelines are the result of The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use and are unique in bringing together the regulatory authorities and pharmaceutical industry of Europe, Japan and the US to discuss scientific and technical aspects of drug

registration. Since its inception in 1990, ICH has evolved, through its ICH Global Cooperation Group, to respond to the global face of drug development, so that the benefits of international harmonization for better global health can be realized worldwide [202]. The FDA has adopted ICH guidance within four main categories as described below.

1. *ICH – Efficacy*
   (a) Clinical Safety E1–E2F
   (b) Clinical Study Reports E3
   (c) Dose-response Studies E4
   (d) Ethic factors E5
   (e) Good Clinical Practice E6
   (f) Clinical Trials E7–E11
   (g) Clinical Evaluation by therapeutic Category E12
   (h) Clinical Evaluation E14
   (i) Pharmacogenomics E15–E16
2. *ICH – Joint Safety/Efficacy (Multidisciplinary)*
   (a) MedDRA Terminology M1
   (b) Electronic Standards M2
   (c) Nonclinical Safety Studies M3
   (d) Common Technical Document M4
   (e) Data Elements and Standards for Drug Dictionaries M5
   (f) Gene Therapy M6
   (g) Genotoxic Impurities M7
   (h) Electronic Common Technical Document (eCTD) M8
3. *ICH – Quality*
   (a) Stability Q1A–Q1F
   (b) Analytical Validation Q2
   (c) Impurities Q3A–Q3D
   (d) Pharmacopoeias Q4–Q4B
   (e) Quality of Biotechnological Products Q5A–Q5E
   (f) Specifications Q6A–Q6B
   (g) Good Manufacturing Practice Q7
   (h) Pharmaceutical Development Q8
   (i) Quality Risk Management Q9
   (j) Pharmaceutical Quality System Q10
   (k) Development and Manufacture of Drug substance Q11
4. *ICH – Safety*
   (a) Carcinogenicity Studies S1A–S1C
   (b) Genotoxicity Studies S2
   (c) Toxicokinetics and Pharmacokinetics S3A–S3B
   (d) Toxicity Testing S4

(e) Reproductive Toxicology S5
(f) Biotechnology Products S6
(g) Pharmacology Studies S7A–S7B
(h) Immunotoxicology Studies S8
(i) Nonclinical Evaluation for Anticancer Pharmaceuticals S9
(j) Photo-safety Evaluations S10

The most relevant FDA regulatory document associate with nanomedicine manufacturing is the 'Liposome Drug Products' guidance document proposed in August of 2002 [203] This document currently guides development of liposomal based drugs, which generally fall into the definition of nanomedicine based on particle size. The guidance provides recommendations for drug development applicants on chemistry, manufacturing and controls (CMC), human pharmacokinetics and bioavailability; and labeling documentation for liposome drug products submitted in new drug applications (NDAs). The guidance recommendations are segmented as follows.

1. *Chemistry, Manufacturing, and Controls*

   (a) Description and composition
   (b) Physiochemical Properties
   (c) Description of Manufacturing Processes and Controls
   (d) Control of excipients: Lipid Components
   (e) Control of Drug Product Specifications
   (f) Stability
   (g) Changes in Manufacturing

2. *Human Pharmacokinetics and Bioavailability*

   (a) Bioanalytical Methods
   (b) In Vivo Integrity (Stability) Considerations
   (c) Protein Binding
   (d) In Vitro Stability
   (e) Pharmacokinetics and Bioavailability

3. *Labeling*

   (a) Product Name
   (b) Cautionary Notes and Warnings
   (c) Dosage Administration

Nanomedicine platforms have a number of common issues that are related to regulatory oversight. Some of these include functional qualities such as significantly different chemical properties than corresponding small or large molecules, different PK/PD/ADMET properties, delivery, targeting, release, stabilization, and bioavailability. Characterization, in terms of physiochemical attributes and general CMC issues (stability, sterility, etc.), are also common to many of the nanomedicine platforms, but differ greatly from the traditional small/large molecule drug [204].

While nanomedicine are becoming more prevalent in the areas of cancer, AIDS, and brain disorders, there are concerns that the unique properties of nanoparticles, such as size, shape, affinity, and surface chemistry may not fit the traditional safety and quality evaluation protocol proposed under current regulations.

The FDA and European Medicines Agency (EMA) have begun to address the lack of a more comprehensive regulatory framework for nanomedicine through the establishment of international scientific workshops such as the EMA 1st International Workshop on Nanomedicine in September of 2010 [205]. The FDA has also recognized the need for specific nanomedicine guidance, and is working toward that goal. In August 2006, the FDA established a Nanotechnology Task Force to determine the regulatory framework needed to develop safe and effective FDA-regulated products that use nanotechnology materials. The resulting Nanotechnology Task Force Report recommended that the FDA pursue the development of nanotechnology guidance for manufacturers and researchers, and that because of the emerging and uncertain nature of nanotechnology and the potential for multiple medical applications, there was a requirement for transparent, consistent and predictable regulatory pathways.

Current FDA recommendations, until specific guidance documents are developed, are to follow current FDA guidance including all normal testing procedures, normal drug stability testing, and those associated with CMC, in vivo, and in vitro analysis. Though understanding specific technical and scientific aspects of the drug product, tests should be designed accordingly. All parts of the drug product should be tested for stability, both individually and formulated. It will be critical for nanomedicine drug companies to communicate and develop acceptable procedures in concert with the FDA as early in the product development process as possible [204].

10.5.2 Preclinical and Clinical Development

There are more than twenty FDA approved products that contain nanomaterials (Table 10.1). To date, all of these products have been approved through the traditional regulatory pathway. As previously described nanomedicines are becoming more prevalent in the areas of cancer, AIDS, and brain disorders. There are currently hundreds of nanotechnology companies and research facilities trying to benefit from the emerging nanomedicine marketplace. Within the life sciences industry sector, funding has been primarily focused on those companies that apply nanotechnologies to ‘conventional’ therapeutics (i.e. drugs as either chemicals or biologics) to increase or extend their application; for example, targeted drug delivery systems (Nemucore Medical Innovations, BioDelivery Sciences International, CytImmune Sciences Inc., NanoBioMagnetics Inc., Nanobiotix, Nanotherapeutics Inc.), diagnostics (Nanosphere Inc., Oxonica Ltd) and medical imaging systems (Life Technologies Inc.- Qdots). These products and applications have a relatively

well-defined route to commercialization (subject to the regulatory hurdles facing nanotechnologies in general) [206].

Most notable of the nanomedicine products are the combinatorial drugs that combine targeting, drug delivery, stability, protection, and imaging. Figure 10.3 illustrates a typical combinatorial nanomedicine unit. The multifunctional nanoparticle is by nature a complex mixture of hydrophobic/hydrophilic molecules, inorganic components, peptides, and/or small molecule organic drug molecules. Many issues, regarding in vivo and in vitro assays need to be developed to segregate different properties of a multifunctional drug product. Some of these are:

(a) Synergies or interactions between the nanoparticle components
(b) Biocompatibility
(c) Long-term/chronic exposure assays/data
(d) General toxicology assays and analytics
(e) Animal models
(f) Molecular weight
(g) Particle size
(h) Charge distribution
(i) Purity
(j) Contaminants
(k) Stability – individual components and formulated
(l) Consistency in manufacturing
(m) PK/PD/ADMET assays/profiles
(n) Aseptic processing/sterilization
(o) Immunogenicity

10.5.3 Knowledge Management, Manufacturing and Scale-Up

Process development and manufacturing of nanomedicine is at its early stages of development and thus is also in its seminal stages of preparing to respond to the guidance of the FDA. With FDA's push to move from quality by testing to quality-by-design (QbD) (Fig. 10.4) for nanomedicine community to succeed in this new environment it is imperative to develop robust documented, process knowledge for the fabrication of nanomedicine. Acquisition and development of process knowledge will enable practitioners to bring novel therapies to the clinic with unique multifunctional capabilities. Articulation of the key variables (equipment, materials, idiosyncratic protocols etc.) at an early stage (i.e. the discovery lab) involved in production process will lead to a better understanding of how to translate good lab scale synthesis into scale processes for future clinical translation and assist manufacturing partners to produce material according to FDA's QbD principles.

QbD first implemented in pilot capacity by the FDA in 2005 has been formally adopted as a way to harmonize the development lifecycle of biopharmaceuticals and

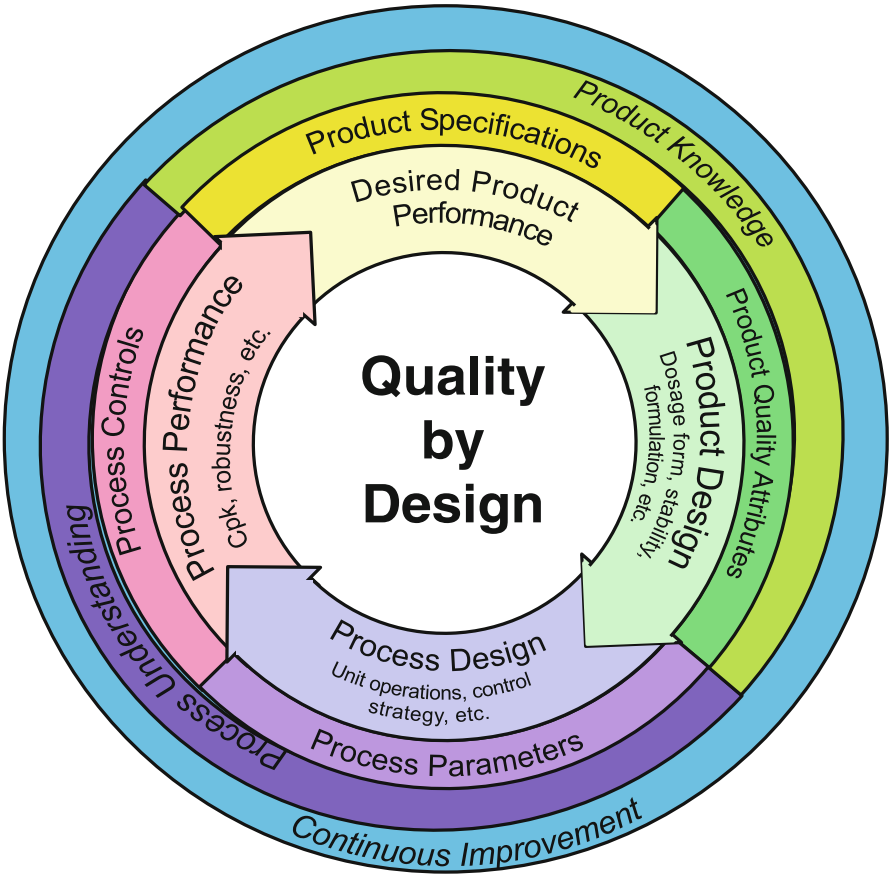

Fig. 10.4 The United States Food and Drug Administration recommended quality-by-design (QdB) approach to link product knowledge with process knowledge and create a continuous improvement product development environment

move away from sampling to find product defects to an environment were "in control" validated processes drive a data rich environment where variations within specification are acceptable. QbD is based on the underlying principle that quality, safety and efficacy must be designed into a product and that quality specifically cannot be tested or inspected into a product. Officially defined as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management" [207]. We consider QbD to be essential for the development of manufacturing processes for nanomedicine. QbD creates a continuous knowledge cycle, an important concept for advancing beyond the seminal steps for identifying innovative means to scale production of complex nanomedicine products [208]. The FDA

has come out with guidance that covers Pharmaceutical Development, Quality Risk Management, and Quality System with a predisposition that the future state of biopharmaceutical manufacturing, of which nanomedicine will be a part, will be an environment governed by QbD [207, 209]. Table 10.2 illustrates the differences in approach and the information requirements of QbD over traditional biopharmaceutical manufacturing which is dependent upon inspection, testing, locked processes and reproducibility [207].

It is important that nanomedicine manufacturers understand that QbD is knowledge rich environment dependent upon user definition of critical quality attributes (CQA), such that the physical, chemical, or biological property or characteristic of the intended nanomedicine should be within a proper range or distribution to ensure product quality. Linking CQA to process inputs (raw materials, chemicals, biologics etc), and process parameters (temperature, pressure, pH, etc) is performed in the early stage experimentation defined as the "design space" which is defined as the range of input variables or parameters for a single operation or it can span multiple operations. Early articulation of the design space, CQA and process inputs can provide a very flexible operational environment with the desired attributes for scale-up manufacturing.

Importance of Knowledge Management in Nanomedicine Nanomedicine holds the promise to cure complex diseases like cancer and save lives [213]. Today, academic scientists lead the development of the complex multifunctional nanomedicine, but for all their promise, there is a striking lag in clinical translation. This lag rests on the fact that nanomedicine investigators under appreciate the value of target product profiles (TPP), a key component of QbD, for ensuring that processes used in the laboratory are compatible with commercial scale-up processes and regulatory guidance [210]. A solution to this problem is at very early stage, put information into the hands of investigators to guide efforts towards nanomedicine that will have a chance to make it to the clinic. Innovation in informatics is another essential area and is complementary to the NIH's proposed investment to create National Center for Advancing Translational Sciences [211].

Nanomedicine translation faces substantial challenges related to managing the complex data streams emerging from the work at the bench, from process development work, and from preclinical studies all with important attributes required to drafting a TPP. The critical information developed during these activities is required to navigate a complex regulatory environment. Without effective data capture solutions and subsequent translation of large quantities of data into shared information, it will be "challenging" to coordinate the bench level process with scale-up process development, risk management and regulatory compliance. We are currently developing a software package, Fig. 10.5, designed to assist academics in overcoming this translational bottleneck for nanomedicine by consolidating existing drug development best practices into a single package for use as a guide to further advance nanomedicine development.

"Nanolytics", developed by Nemucore Medical Innovations, Inc. (NMI) is a knowledge management system for information pertinent to development of TPP, processes development plans, validation plans and risk management assessment

Table 10.2 Quality-by-design (QdB) approach in manufacturing

Aspect	Traditional approach	Enhanced QbD approach	Informatics requirements
Overall pharmaceutical development	a. Mainly empirical	a. Systematic, relating mechanistic understanding of input material attributes and process parameters to drug product CQAs	a. Knowledge management across entire life cycle
	b. Developmental research often conducted one variable at a time	b. Multivariate experiments to understand product and process	b. Process traceability and change management from development through manufacturing
		c. Establishment of design space	c. One-point access to all phases and all levels of data
		d. Process Analytical Technology (PAT) tools utilized	
Manufacturing process	a. Fixed	a. Adjustable within design space	a. Full documentation of process analysis and verification decisions
	b. Validation primarily based on initial full-scale batches	b. Lifecycle approach to validation and, ideally, continuous process verification	b. Integration of these decisions with PAT tool configuration setups
	c. Focus on optimization and reproducibility	c. Focus on control strategy and robustness	
		d. Use of statistical process control methods	
Process controls	a. In-process tests primarily for go/no go decisions	a. PAT tools utilized with appropriate feed forward and feedback controls	a. Web-based "Digital Dashboard" providing remote process monitoring
	b. Off-line analysis	b. Process operations tracked and trended to support continual improvement efforts post-approval	b. Record of all significant parameter variances and trends
Product specifications	a. Primary means of control	a. Part of the overall quality control strategy	a. All lifecycle documents (from URS though PBR) interlinked, with traceability of changes
	b. Based on batch data available at time of registration	b. Based on desired product performance with relevant supportive data	
Control strategy	a. Drug product quality controlled primarily by intermediate and end product testing	a. Drug product quality ensured by risk-based control strategy for well understood product and process	a. Inventories and risk assessments of all systems, equipment and processes
		b. Quality controls shifted upstream, with the possibility of real-time release or reduced end-product testing	b. Decisions on parameter prioritization and acceptable variances
			c. Integration with PAT records, for refinement analysis
Lifecycle management	a. Reactive (i.e., problem solving and corrective action)	a. Preventive action	a. Change management integrated across all lifecycle phases
		b. Continual improvement facilitated	

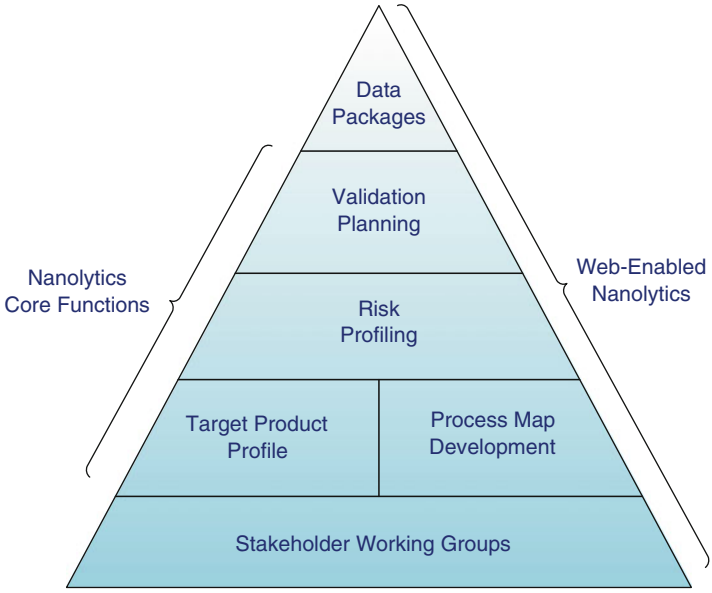

Fig. 10.5 "*Nanolytics*": Conceptual framework for combination of informatics with processing technology for optimization of nano-pharmaceutical formulations

needed to support effective nanomedicine translation. Nanolytics allows academic investigators early in research to contextualize how a nanomedicine could move to the clinic. Unlike either small molecule or biologic development the creation of nanomedicine, which are complex molecular entities, is very process and design intensive. A manual process already demonstrated value of an informatics approach to identify barriers (use of equipment not compatible with scale-up) and risks (regulatory, material, etc.) to translating these nanomedicine to the clinic. Nanolytics software consists of three suites: a TPP Suite, a Process Suite and Validation Suite. These suites and the knowledge they will manage should mitigate cost and reduce time of development of scale-up processes, lower barriers to clinical development for nanomedicine and leverage research costs more effectively [211, 212]. Nanolytics allows for the input of key information based on initial research and outputs documentation on how to achieve for the pilot scale production of the target nanomedicine. As always is the case, better information, begets a more realistic product development plans. This development of information "outside" of the typical areas of focus of a nanomedicine researcher will reduce risk and clarify efforts in translating nanomedicine from bench to bedside.

Significance to Nanomanufacturing Practices Developing manufacturing capability in the past has been capital intensive and typically relegated to a commercial

responsibility. But with many of the advances happening in nanomedicine there is a discreet need to lower the barrier to access manufacturing capabilities on a molecule agnostic platform. In an effort to create such an environment we have begun the process to establish the first in the nation FlexFactory™ nanomedicine manufacturing facility compliant with QbD principles. FlexFactory™ was developed by Xcellerex, Inc, (Marlborough, MA) to transition from single molecule manufacturing footprint to a modular, single use backbone which is agnostic to molecule. FlexFactory™ provides the ideal manufacturing environment for nanomedicine as the controlled environmental units (CEMs) are able to maintain a single unit operation of a manufacturing process with the ability to grow with the progress of the molecule from preclinical thru commercial launch. The innovation of the FlexFactory™, briefly, is if a unit operation needs to change for the development of a new nanomedicine manufacturing process the modular CEMs can be opened a new unit operation installed, the new step and the new process validated allowing for the production of a nanomedicine that conforms to different CQA. While there are other modular platforms that can be used in a similar manner they often have to be pieced together. The FlexFactory™ system has withstood numerous FDA audits, inspections, and license applications for a variety of biologics. The sophistication required for biologic therapeutic manufacturing is suspected to be similar to the complexity required for multifunctional nanomedicines. This level of complexity and novelty of scaling nanomedicine production is why we have taken a two-step approach to aggregate knowledge using Nanolytics and the molecule agnostic manufacturing platform FlexFactory™, Fig. 10.6.

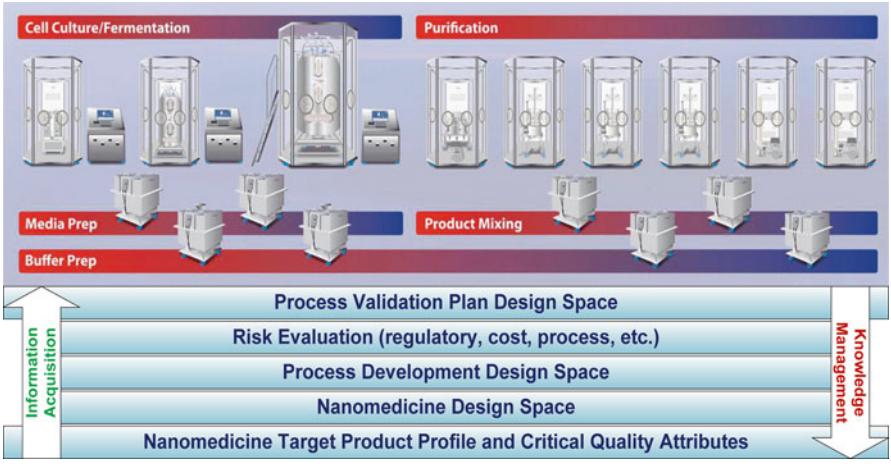

Fig. 10.6 NMI FlexFactory™ footprint shown to illustrate that data captured in Nanolytics serves as foundation for manufacturing Information and knowledge about product characteristics, process, and systems drive manufacturing design to optimize manufacturing of nanomedicine

10.6 Conclusions and Future Outlook

With greater understanding of chemical and physiological barriers associated in drug delivery and advances in nanomedicine design, there is an opportunity to efficient delivery of small and macromolecular drugs to complex diseases. Along these lines, the nanosystems have been engineered with specific attributes such as biocompatibility, suitable size and charge, longevity in blood circulation, targeting ability and image guided therapeutics, which can deliver the drug/imaging agent to the specific site of interest, based on active and passive targeting mechanisms. These systems cannot only improve the drug delivery to the target disease, but also the resolution of detection at cellular and sub-cellular levels.

To fully realize the potential of nanosystems for delivery of contemporary therapeutics in clinical setting, it is imperative that researchers also address the material safety, scale-up and quality control issues. Scale-up and quality control becomes extremely challenging especially when dealing with nanosystem designed to carry multiple drugs, imaging agents and targeting moieties. Furthermore, in vivo fate of nanomedicine engineered using novel nanomaterials are need to be fully assessed before being used in clinical application.

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Chapter 11
Harmful or Helpful, the Toxicity and Safety of Nano-sized Medicine

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11.1 Introduction

11.1.1 Chapter Overview

Engineered nanomaterials bring well known benefits to manufacturing and material properties, whether it is lower cost, novel functionality or improved dispersal within a system. However, there is a wide body of research that also suggests that there are potential issues of increased toxicity with at least some nano-scale materials. This chapter summarises the recent research into the toxicity of engineered nanomaterials, the current understanding of the different regulatory approaches that are applicable to the healthcare industry in particular, and highlights areas of concern that require addressing within the near future.

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11.1.2 Paradox of Nanomaterials as Toxins and Therapeutic Agents

Engineered nanomaterials have been suggested by the European Commission as comprising materials with external dimensions or having internal structures or surface structures that measure between 1 and 100 nm [1]; although this recommended definition does not concur with previous recommendations from a European Scientific Committee [2, 3]. The recommended definition encompasses all kinds of nanomaterials, irrespective of their origin, and determines that materials that contain particles (in an unbound state or as an aggregate or agglomerate) where 50 % or more of the number size distribution of the particles has one or more dimensions in the stated size range [1]. The EC definition does not identify nanomaterials as hazardous (i.e. intrinsic adverse properties of an agent [4]); however materials that do not conform to the definition may still exhibit nano-related effects. Conversely the European Medicines Agency (EMA) describes nanotechnology as "*the use of tiny structures – less than 1,000 nm across – that are designed to have specific properties*" [5] suggesting that there is further disagreement between different sectors.

Nanomaterials are considered to represent a major advance in engineering, enabling functional coatings with unique properties and reducing costs for manufacturers amongst other benefits [6]. Particularly in the field of medicine, nano-scale drugs have enabled advances in cancer diagnostics, imaging and therapy [7–11]. However, as is common for all emerging technologies, there are concerns that the associated benefits from the development and commercial use of a technology will overtake the consideration of the potential inherent adverse effects of a substance (hazard) and exposure to susceptible organisms (receptors [4, 12]), and legislation must follow development.

As the use of engineered nanomaterials increases in manufacturing and industrial contexts so too does the investigation into their beneficial use within medicine; there are strong comparisons to be drawn between the study of risks associated with engineered nanomaterials and those of nanomedicines. In the published literature to date, however, there has been little discussion as to the toxicity of nanomedicines. Many researchers have reported that toxicity issues related to nanomaterials used in nanomedicine are often ignored (e.g. Kagan et al. [13], Moghimi et al. [14] and Linkov et al. [15]) but there is a widely held belief that, with the development of novel materials, there is also a moral requirement for the consideration of the toxicological and environmental effects of those materials. Whilst the majority of this chapter will draw upon the published literature on the toxicity and risk of engineered nanomaterials, the concerns and issues raised should be remembered to be highly comparable with the situation facing nanomedicines though differences will be indicated where possible.

11.2 Toxicology of Engineered Nanomaterials: Size Really Does Matter

11.2.1 Engineering Benefits of Nanomaterials

The global market for nanotechnology is expected to reach US$ 1 trillion by 2015 and to employ approximately two million workers [16, 17]. The high level of industrial and commercial interest in nanotechnology can be considered to be a result of the opportunities presented by the changes in properties and functionality that occurs at the nano-scale [18]. These altered properties may result in, for example, improved performance, providing large changes in composite properties as a result of relatively small additions of materials, and can even allow entirely new functional properties to be incorporated into products, thereby impacting on manufacturing capabilities, methods and costs [19–21]. However, with increased commercial use, there is also an increased possibility of unknown and/or underappreciated adverse human health and environmental impacts occurring, particularly when the mechanisms and interactions with the biological environments (both human and the wider environmental receptors) are under-explored [12].

For many, the benefits represented by nano-scale manufacturing are that nano-scale or nano-structured materials have novel properties when compared to bulk forms of a material [20]. In many cases these properties do not correspond to those of the bulk material or even those of the constituent atoms or molecules. Instead, the altered properties can be considered as unique to the nano-scale and are related to the different proportions or states of surface atoms, absence of grain boundaries or even distortion of the atomic or electronic structure of the material [22].

Bulk materials are typically composed of collections of grains, each grain atomically bonded to its neighbour but possessing a unique crystallographic orientation. Only rarely are these bulk materials composed of a single grain. In these cases they are referred to as single crystals and, due to their rarity and difficulty in fabrication, tend to attract a considerable price premium. As the atomic structure and bonding are disrupted across the grain boundaries, the chemical and mechanical properties of the material become altered from that of a single crystal material. As the size of the polycrystalline body as a whole approaches that of the individual grains, changes in material properties arise due to the changes in the proportion of material near to the grain boundaries. At this stage the crystal structure is still identical to that of the bulk material and, for a given mass of material, there will be a higher proportion of surface atoms resulting in an increase in specific reactivity. Decreasing the size of the material still further into the nano-region results not only in a continued change in the ratio of surface atoms to bulk atoms and associated changes in specific reactivity, but also in a deformation of the crystal lattice as the atomic bonds are distorted as a consequence of trying to minimise the surface area relative to the bulk volume of the crystal. This has two effects: firstly the reactivity of the surface atoms is altered as atomic bonds become easier to break; and secondly the inter-atomic separations of individual atoms are altered, changing the crystal structure and giving rise to changes in physical, thermal, electronic and optical behaviour.

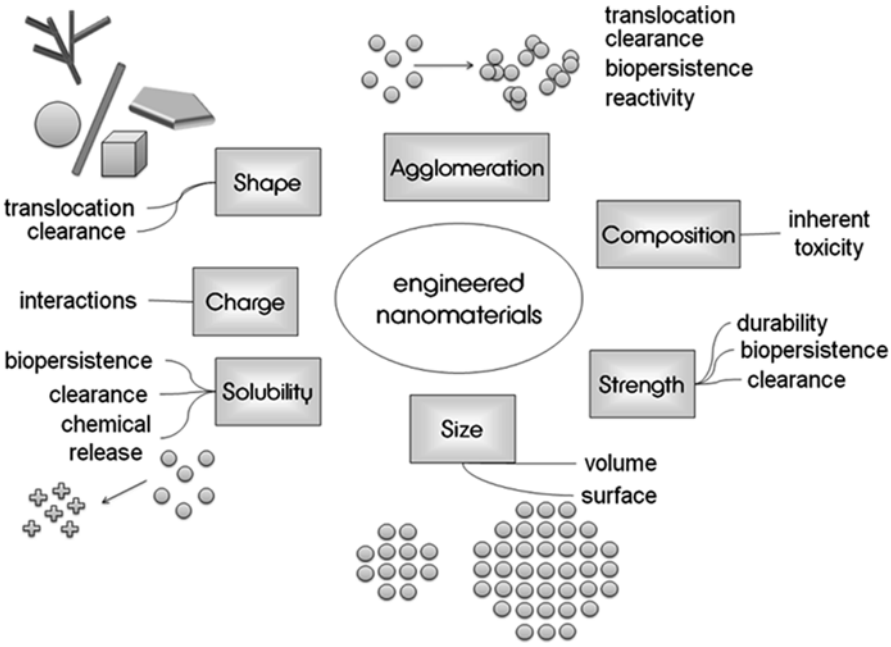

Fig. 11.1 Properties of engineered nanomaterials (ENM) and their environmental effects [48]

These changes will result in altered physiochemical properties of a nanomaterial when compared to the bulk material (Fig. 11.1) and will vary depending on the size of the nanomaterial. While such changes may be relatively innocuous and have no immediate effect for many nanomedicines, there may be far reaching consequences that arise at later stages within the life cycle of the material. Such changes may be simply an increase in strength that makes it harder to process or break down nanomedicines, changes in reactivity such that dissolution or reaction rates are altered, but may potentially extend to changes in fundamental properties that interfere with the fundamental diagnosis. Such variations will also impact the mechanical properties of the materials; whilst this may not be a clinical issue for many nanomedicines, it may cause changes in material strength, reducing degradation within the environment. Once a nanomaterial is dissolved the inherent properties of the chemicals within the particle are considered to be the toxic agent and the particle itself is no longer a concern [23].

11.2.2 Nanotoxicity

There are examples where toxicity is dependent on the size of the particle, and where quantum effects and functional properties (i.e. the optical, magnetic or electrical properties) can dominate material behaviour [16]. Studies have shown that nanoparticle toxicity can be altered by the size of the nanoparticle [24], although

this may not be a linear relationship [25]. Additionally, the surface chemistry or addition of a functionalised coating of the nanoparticle has been shown to relate to changes in toxicity [26–28]. The solubility of nanoparticles influences their toxicity, with insoluble nanoparticles showing an increased measure of toxicity as the particle size decreases [29] and highly soluble nanoparticles also showing, in some cases, more toxicity to cells than less soluble nanoparticles [27]. However, given the number of variables and lack of standardised test procedures or "standard" nanoparticles to benchmark experiments, it is not currently possible to predict with any accuracy how particular physical or chemical properties of nanoparticles will influence toxicity. There are many toxicity tests that use established cell lines rather than whole body models, which may not provide relevant exposure conditions to enable conclusions to be drawn. Whilst in vitro tests may give an indication of the mechanism of toxicity and can be considered as more ethically justifiable (considering the drive towards refinement, replacement and reduction of animal models within toxicological assessment), it is possible that nanoparticles may result in unpredictably higher in vivo toxicity by causing greater damage than bulk forms due to interactions with biological fluids (e.g. blood, extracellular fluid) or may be altered within a biological environment either through the formation of agglomerates or coating with protein shells.

It has been suggested that the increased surface area to volume ratio shown by nanoparticles may be the overriding attribute that influences the biological reactivity of such compounds. Indeed this change does result in an increased proportion of atoms at the surface of the particle and thereby increases the potential number of reactive groups for a given mass or dose of material [22]. However, surface modification may also result in the presence of specific surface groups that alter the reactivity or change the lipophilicity of the particle.

A number of common toxicological pathways have been suggested for nanomaterials and nanoparticles (see Table 11.1). These mechanisms are a result of: surface properties such as electron donor/acceptor active groups or redox cycling via metal ion coatings (e.g. Fe^{3+}) or organic compounds (e.g. quinones) resulting in direct or indirect free radical generation; dissolution of the particle by the media which may be retarded or passivated by a coating (either intentional or formed as a result of interaction with the biological media); or UV radiation leading to free radical generation. Whilst such observations seem to imply a common underlying process, relevant to a number of different toxicological mechanisms, involving free radical generation

Table 11.1 Generic toxicological mechanisms identified with engineered nanomaterials (ENM) and examples of ENMs that have been shown to result in such end points

Toxicological mechanisms	Examples (from literature)
Oxidative stress and free radical production	Metal oxides, carbon nanotubes
Inflammatory reactions	Metal oxides, carbon nanotubes
Cell membrane damage (lysis/death)	Metal oxides, carbon nanomaterials
Fibrogenesis (asbestos-like damage)	High aspect ratio particles (e.g. rods), carbon nanotubes
Blood platelet and clotting interference	Silicon oxide

that leads to an increase in reactive oxygen species (ROS) [30, 31], so as to result in increased oxidative stress, this has yet to be demonstrated as the primary mechanism across the wide variety of nanomedicines or nanomaterials that are now available.

11.2.3 Specific Issues Related to Nanomedicines

The recommended definition of nanomaterials [1] identifies that special circumstances may occur for pharmaceuticals and that the definition 'should not prejudice the use of the term 'nano' when defining certain pharmaceuticals and medical devices'. The current EMA definition considers that the structures are less than 1,000 nm across and designed to have specific properties [5] – a concept that has not yet been considered by the other recommendations. The EMA specifically recognises that the Committee for Medicinal Products for Human Use (CHMP) has recommended approval of medicines based on nanotechnology, including medicines composed of liposomes (containing active substances such as doxorubicin) and nano-scale particles of an active substance (such as paclitaxel and aprepitant) [5].

Size is a major factor when considering the translocation, reactivity and fate of nano-scale materials within a body and this will directly influence their toxicity [14]. Many "non-nano" medicines are given as small molecular drugs within a carrier membrane or matrix. Once released from their carrier (i.e. the surrounding membrane is dissolved within the stomach), the molecules will stay in the blood stream until the majority are removed via first-pass clearance in the kidneys. This will reduce the active therapeutic dose but also increase the possibility of nephrotoxicity or parenteral exposure.

Conversely nanomedicine forms are larger than normal molecular-based drugs, which suggests that this comparison would run counter to the issues previously discussed. The larger particle size may mean that the nanomedicines do not undergo first-pass clearance from the bloodstream to the same extent, as size influences elimination from the body. For instance, if a nanoparticle is greater than 20 nm in diameter, it will avoid first pass elimination, thereby resulting in a prolongation of systemic exposure. Particles smaller than 150 nm will also avoid sequestration by sinusoidal fenestrae in the spleen and the liver (which are approximately 100–150 nm diameter [32]), again prolonging systemic exposure. Therefore, if the objective is to ensure maximum exposure and reduce elimination from the body, the "ideal" diameter for a nanomedicine particle is between 20 and 150 nm in diameter. The increased exposure, increasing the time in the blood stream, will enable a greater proportion of the dose to reach the target organ when compared to comparable molecular drug forms. The resultant increased availability of the nanomedicine would mean that a reduced dose would be required to achieve the same therapeutic outcome [33]. However, the reduced elimination will also expose a greater number of potential target tissues to the nanomedicine and increase the possibility of translocation within the body. This also means that, whilst current nanotoxicological studies on chemical exposure concentrates on the difficulties related

to reduced particle size, nanomedicine requires consideration of the increased particle size (or presence of a particle) compared to a molecule.

The size of the nanomedicine may also determine the therapeutic targeting. Passive targeting is possible with nanomaterials where the diameter is less than 300 nm. The preferential targeting of tumours by nanomedicines is increased by enhanced permeability of the angiogenic tumour vessels and increased retention of the circulating nanomedicine within the body [34]. Active targeting requires surface modifications for specific ligands (e.g. peptides, antibodies, aptamers or small molecules) – enabling a further reduction in the dose whist ensuring that the necessary therapeutic concentration is maintained at the target site. The improved specificity would be anticipated to lead to an improved therapeutic index and reduce the potential for adverse side effects (i.e. effectively reducing the potential for toxicity of the nanomaterial [34]).

The benefits of nanomaterials in nanomedicine also directly reflect their toxicity. Whilst nanomaterials are developed for their surface properties (versus bulk materials), and the surface is in direct contact with the body tissue, it is rational that this is an indicator of the potential severity of toxicity [16]. Therefore the shape and size of nanomaterials which determined their unique beneficial effects will also influence their toxicological effects. These characteristics will also influence the translocation of nanomaterials throughout the body – a mechanism dictated by shape, size and surface coating. As toxicity tests are conducted on healthy animals and preclinical tests on nanomedicines are conducted on "healthy" subjects (volunteers) rather than on subjects suffering from disease states, the actual effects elicited in diseased tissues cannot be truly characterised before clinical application in humans suffering from the particular conditions for which the medicine is intended. This suggests that additional investigations may be required, not only to consider how physical and chemical characteristics dictate the translocation and distribution of nanomaterials around the body but also to consider how this and the effects of the nanomedicine may alter when administered to a diseased subject.

11.2.4 Toxicity Testing of Nano-scale Particles

Alongside the development of suitable approaches to characterise the toxicity of nanomaterials in general, there needs to be separate consideration as to whether, when nanovesicles are used as drug carriers, the toxicity of the incorporated drug is altered (either reduced or increased). In medicinal testing, the toxicity of the whole formulation is appropriate rather than considering the separate components (i.e. even at phase 1 testing) and this does not (at least initially) need to distinguish between the specific toxicities of the individual components.

Nanomedicine products may encompass a wide variety of structures, which are not directly considered by existing hazard characterisation approaches. In general the majority of nanomedicines currently under trial are either nano-sized carriers where the external surface (the carrier) is exposed to the body until exposure to a

stimulus causes the drug to be released – i.e. delivered to the target tissue (e.g. liposomes), nanosuspensions used to improve drug solubility, or metal nanoparticles used for bioimaging of, for example, tumours [35].

Currently, uncertainties relating to the risks posed by nanoparticles (including nanomedicines) are difficult to address because of the absence of knowledge on the range of potential interactions of these at a physiological and/or molecular level, and due to the possible consequences for human health [36]. In such circumstances, use of expert judgment is essential as is common in a range of risk assessment approaches [36, 37]. Whilst drawing on such expert knowledge may provide some insights when these are not otherwise objectively measurable and, may be crucial to identifying important variables, estimating uncertainties in parameters and weighing the strengths of competing models and mechanisms [4], it does require a degree of understanding of the specific issues associated with the particular scenario under consideration. In terms of assessing the potential hazards and risks associated with nanomedicines, the associated unknowns and significant knowledge gaps mean that application of expert judgement may be unsubstantiated and potentially difficult to justify.

11.3 EC/UK Regulation

In Europe and its Member States, the regulatory assessment of medicines is covered under specific legislation, however the generic chemicals either imported or manufactured for use within this process are considered separately [12, 38]. To date, in chemical regulations, the hazard assessment of nanomaterials and nanoparticles has not been well distinguished from that of larger particles or bulk materials, and the risk assessment process has followed suit, despite indications that the behaviour of nanomaterials may be different [12]. Indeed, whilst there is now a suggested definition and guidance on testing needs from the European Commission [1], this has yet to be adopted in Member State legislative approaches and the significance with regard to nanomedicines remains uncertain. Additionally, there is the further complication that the prime current distinction between chemicals for regulatory purposes is based by their chemical formulae (CAS number), which does not allow for particle size or shape to be adequately considered. This is further complicated as some materials that are declared or marketed as “nano” are not, whilst some that are not explicitly labelled as “nano” are [6].

In Europe, regulations distinguish between medicinal products and medical devices. A medicinal product is defined as substance or combination of substances presented as having properties for treating or preventing disease in human beings (Directive 2001/83/EC) and can be considered to cover the majority of nanomedicines; these are subject to more stringent regulation. The regulation of medical devices (used for medical purposes in patients, in diagnosis, therapy or surgery) is less developed and – in large part – relies on self-regulation [39]. Nanomedicines are considered under these same regulations [39].

Pharmaceutical products are regulated by country-specific organisations. The Medicines and Healthcare products Regulatory Agency (MHRA; UK) adopts legislation from the European Community, where the legislation for medicinal products for veterinary and human use is established under the EU medicines regulatory regime (Regulation 726/2004 and Directives 2004/27/EC [human] and 2004/28/EC [veterinary]) and controlled by the EMA.

Within the EU, any new medicine (including nanomedicines) requires a national or EU Marketing Authorisation to be granted before marketing, with failure to obtain this authorisation penalised as set out in Regulation (EC) No. 658/2007 which considers the safety, quality and efficacy of the medicines. The MHRA and expert advisory bodies within the UK control new medicine production to ensure that they meet the required standards on safety and effectiveness throughout the lifetime of the product (known as pharmacovigilance). Within this process, international standards set by the World Health Organisation (WHO; resolution WHA22.50; WHA28.65) provide quality assurance for the global market and reduce the need for repetition of toxicity testing. However, for nanomedicines where the standard toxicity testing regimen may not be able to deal with the specific issues, there remains an outstanding need for additional research and guidance. It is noted that the current regulatory framework has no specific provisions for nanomaterials [37], but that the EMA is actively considering potential changes to legislation [40].

In any event, pharmaceuticals must pass through extensive preclinical assessment before entering into clinical trials (Phases 1–3; Harman 2004) (Fig. 11.2).

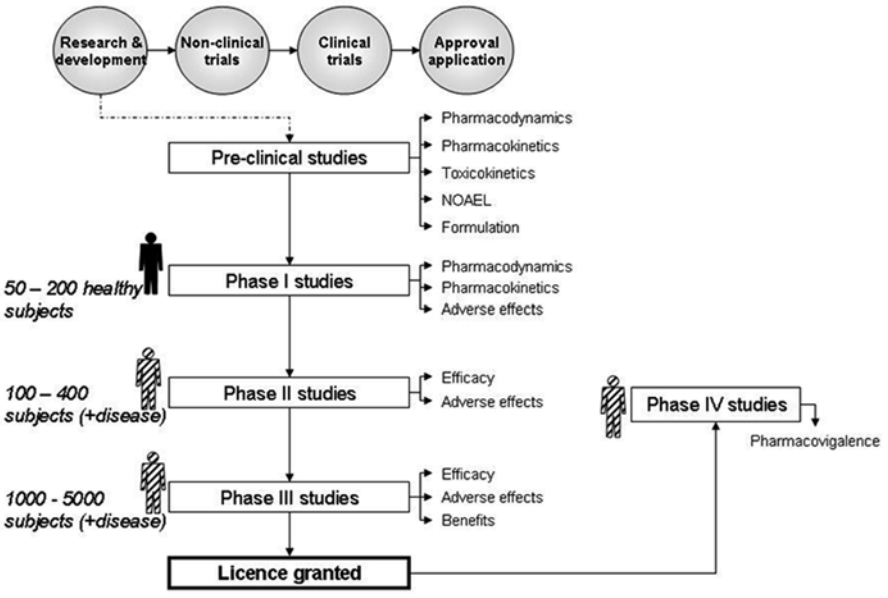

Fig. 11.2 Risk assessment in pharmaceutical development. Phase IV studies (pharmacovigilance) ensures the continuous monitoring of unexpected events over a set period of time

Within this process, guidelines must be followed that control the conduct of the supporting studies, including Good Laboratory Practice (GLP; OECD ENV/MC/CHEM(98)/17, 1998), Good Manufacturing Practice (GMP; EU Directive 2004/27/EC) and Good Clinical Practice (GCP; EU Directive 2005/28/EC).

Preclinical safety studies are intended to define both pharmacological and toxicological effects, both prior to and throughout clinical development, using both in vitro and in vivo studies to aid characterisation [39]. However, pharmaceuticals that are similar, both structurally and pharmacologically, to an available product may need less extensive toxicity testing and, since nanomaterials are not explicitly recognised within EC legislation, it may be difficult to adequately distinguish novel nanoscale medicines (e.g. where there are changes in size or shape) from previously tested medicines. The existing preclinical tests require use of high multiple doses on two animal species, extensive histopathology (on most organs), functional tests (including cardiac, neurologic, respiratory, reproductive, immune systems) and, potentially, extended treatment periods (up to 2 years or more for carcinogenicity). However existing designs do not take into account the particular issues surrounding nanoscale particles [12, 19, 38, 41, 42], which include:

- Dosimetry – many tests use concentration to determine dose levels, however this is not appropriate for use with nanoscale particles. Instead surface area:volume ratio, particle number and particle size distribution are suggested to enable a measurement of potential reactive surface available; and
- Detection of nanomaterials – nanomaterials, individually or in small aggregates, will not be detectable by light microscopy. Whilst chemical labelling will help to show the location of nanoparticles within histology samples, this will also change the properties of the material. Electron microscopy techniques may be used to image accumulation of particles, but this will be difficult to isolate. Additionally, it is necessary to confirm the chemical structure of the nanomaterials using techniques such as energy dispersive X-ray (EDX) and X-ray photoelectron spectroscopy (XPS).

In vitro assays of various types can be used to evaluate and characterise biological activity related to clinical activity, i.e. pharmacodynamic behaviour. Cell lines or primary cell cultures may also be used to examine, for example, the direct effects on cellular phenotype and look for proliferative responses [43]. Mammalian cell lines or tissue cultures can – at least to some extent – be used to predict specific aspects of in vivo activity and to assess quantitatively the relative sensitivity of various species (including humans). Studies can be used to determine receptor occupancy, receptor affinity and pharmacological effects, to assist in the selection of appropriate animal species for further in vivo pharmacological and toxicological tests. In vivo studies assessing the pharmacological activity (including defining mechanisms) are often used to support the rationale of the proposed use of the product in clinical studies.

Data on the pharmacokinetics and bioavailability of the product in the animal species will help to inform decisions as to the appropriate amount and volume that should be administered in studies to test animals. These latter investigations will provide further information on both pharmacological and toxicological dose–

response relationships, and include the definition of what constitutes a toxic dose and what represents the no observed adverse effect level (NOAEL) [4].

Subsequent clinical trials – informed by the preclinical data – allow safety and pharmacokinetic data on humans to be collected as well as – in later phases – its clinical efficacy. Thus it is possible to compare the biological properties of the product as predicted from studies in animal models with the data gained from humans. Unlike the situation in many other fields, risk assessment of medicines therefore involves not only the extrapolation of data across species from studies in animals in relation to the potential toxic effects in humans but also the direct evaluation of human toxicity and pharmacological effect data. In such circumstances, safety assessments are not based on the application of a standard (default) uncertainty factor to the NOAEL from animal studies but the findings from such studies remain important in assessing the adequacy of the safety assessment arising from the clinical trials.

The use of nanoparticles and nanotechnology in medicines are numerous [34, 35]. Commercial applications have been identified as focusing on drug delivery and targeting, as well as the bioavailability of medicines but novel applications are likely to make use of the increased functionality that the nanoscale offers. Of particular interest is the increased surface area to volume ratio of nanoparticles, which in turn increases the particle surface energy [22] and which may make such particles more biologically reactive [44]. This increased biological activity may be beneficial or harmful. However, the same properties that make a nanoparticle of medical interest also mean that it is harder to predict, based on current understanding, the behaviour of the nanoparticle and, therefore, its toxicity profile. There are currently no specific regulatory requirements to test nanoparticles for health, safety and environmental impacts separate to those for bulk materials [5, 23]. Yet, it may be argued that, in any event the regulatory requirements on the testing of pharmaceuticals are already extremely robust and designed to support cautionary developments (as opposed to precautionary [45]) and these are therefore applicable to the manufacture of nanoscale medicines.

11.4 Conclusions

Despite strenuous research efforts to date, the development of methods that permit the detailed characterisation of the potential hazards of nanomaterials (including nanomedicines) is still in its infancy [46]. The assessment of risks associated with the use of such materials – in terms of human or environmental health – remains fraught with uncertainties and knowledge gaps – some of which have yet to be even fully delineated [47]. Specifically these knowledge gaps prevent a full understanding of the potential hazards that may be associated with nanomedicines and are further complicated by contradictory guidance on labelling or for distinguishing between different morphologies of particles. Whilst nanomedicine involves the direct intentional exposure, as well as potentially indirect exposure, of humans to

engineered nanomaterials, there are still a number of distinct essential knowledge gaps to be addressed before this novel field is understood, including:

- What are the specific nanomaterial physicochemical properties that need to be characterised for a nanomedicine to inform the potential fate and transport of nanomaterials within the body?
- How can the limited data that are currently available on nanomedicine's toxicity, exposure and environmental fate and transport, be used to inform experimental procedures?
- How do the specific physical and chemical characteristics of nanomaterials contribute to toxicity specifically in relation to nanomedicine applications?
- How may specific delivery mechanisms influence nanomedicine toxicity?

Additionally, there is also the possibility that unexpected interactions between nanomedicines and co-administered traditional pharmaceuticals (or indeed other exogenous substances present in the body) will result in particular concerns given the wide range of chemicals to which we are exposed in everyday life and anticipating the possible interactions, changes in chemical behaviour or in tissue behaviour that could arise.

It is clear that nanomedicines are one of many novel applications of nanomaterials in today's society and offer many potential clinical benefits. However, the implications and uncertainties that are now emerging as to the potential toxicity of non-medical nanomaterials should be considered in terms of their significance to the development of nanomedical applications, in particular to inform the development of appropriate hazard and risk assessment methodologies and risk management techniques that are suited to medical applications and to inform approaches to the communication of evidence and understanding of the balance between benefit and risk that may associate with nanomedical materials.

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Chapter 12
Ethical Implications of Nanomedicine: Implications of Imagining New Futures for Medicine

Donald Bruce

12.1 A Historical Introduction

Nanomedicine is seen as one of the most exciting prospects amongst all the potential application of emerging nanosciences and technologies. Sophisticated and exquisitely finely focused instrumentation is providing new understandings of processes of the body and mechanisms of disease. These in turn should open up increasing possibilities for more precise diagnosis and monitoring, and for therapeutic or prophylactic interventions, at the scale of genes, proteins and cells of our bodies. Many of the anticipated benefits of nanomedicine remain as future prospects, and at times there here has been a regrettable tendency to exaggerate. Yet nanotechnologies are beginning to emerge from their initial discovery and exploratory phase. In 6 years since 2008, successive annual *Clinam* European conferences on clinical nanomedicine have reflected a growth in nanomedical techniques, products and clinical trials [1]. This is evidence that this field of nanotechnology is beginning to show at least the first fruits of its promise.

As the scope and influence of nanotechnological applications in medicine increases, there is a corresponding responsibility to consider their ethical and social implications. Ten years ago concerns were expressed at the gap between the rapid development and ethical assessment [2]. But since then numerous studies have explored these questions. A series of European Commission funded projects included a scoping review in 2005 from the Nano-2-Life European Network of Excellence [3, 4], and reports from the NanoBioRaise [5] and NanoMed Round Table [6], Observatory Nano [7] and Deepen [8] projects, and an opinion on nanomedicine of the official European Commission's European Group on Ethics [9].

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Early concerns over potential risks had been raised in a report from the ETC Group in 2003 [10]. A seminal scientific study by the Royal Society and Royal Academy of Engineering in 2004 highlighted how little was known scientifically about the behaviour of nano-sized particles regarding health and the environment. While this was not deemed sufficient to justify what was seen as an over-precautionary call by ETC for a general moratorium, the academies' report was notable for pointing to the need for substantial research into the risk aspects, and also for including ethics in its considerations [11].

The Nano-2-Life and NanoBio-Raise reports noted a gap of legitimation and accountability between researchers and developers and a European general public which was largely unaware of what nanotechnology was [4, 5, 12]. This gap of engagement has begun to be addressed a number of national studies, for example in the UK, Germany and Switzerland and Netherlands, as well as the above EC research projects [13–15].

These activities were energised in part by concerns of governments and the EU, that nanotechnologies might arouse public suspicions and NGO opposition comparable to that experienced with genetically modified food. It was widely acknowledged that nanotechnologies should be opened to public debate as a matter for so-called upstream engagement. For example, stakeholder groups were invited members of a UK Government forum on nanotechnologies which ran for several years [16].

But in general, nanotechnologies have not so far aroused major controversies in Europe. There have been critical reports from NGO groups in particular sectors like food and cosmetics, and the use of nanosilver as an anti-bacterial agent in hospitals [17, 18]. More worrying were a few organised protests of militant groups in France which disrupted a national programme of public debates, and a series of deeply disturbing letter bomb attacks in Mexico, which seriously injured scientists [19]. Such militant opposition does not seem to represent general public attitudes. From the various studies mentioned above, the prospects for nanomedicine so far seem to have met with a broad approval among the wider European population, but with some concerns and at times some useful insights.

For example, a public engagement study for the UK Engineering and Physical Sciences Research Council (EPSRC) [20] asked people about specific nanomedical research priorities. General support was expressed for most applications of nanomedicine, but tempered by concerns about long terms risks and what may happen when the medical research goals enter the domain of large commercial corporations. But its participants also brought important human insights that nanotechnological solutions might sometimes be too sophisticated. Nanosilver surface coatings in hospitals might be less useful than simply doing more ordinary cleaning. Implanted theranostic devices could become too 'smart' if they did not allow control by either the patient or a doctor.

Because the technologies are themselves evolving, ethical reflection is inevitably work in progress. This chapter surveys some of the main ethical and social issues which have emerged to date. Eight themes will be considered: cross-cutting issues, diagnostics, remote monitoring, targeted drug delivery, theranostics, regenerative

medicine, risk and uncertainty, and the relationship of nanomedicine to notions of human enhancement. Three types of questions may arise, running as threads through these themes. In some cases there may be ethical issues with the techniques themselves. There will be ethical and social implications from their uptake by individuals and societies, including possible unintended consequences. Thirdly some more fundamental questions are asked about the values, goals and presuppositions that accompany the technical drivers of nanomedicine.

12.2 Generic and Cross-Cutting Ethical Issues

The first generic question often asked is whether applying nanotechnologies to medicine raises any 'new' ethical issues. The EC European Group on Ethics observed that, notwithstanding the revolution it promised, nanomedicine did not raise issues in biomedical ethics that had not been encountered and considered before [8]. While there was no dramatic new question for the European Commission legislative bodies to face, it did not mean that there are no ethical issues to consider. Nanotechnologies tend to be 'enabling technologies' which provide novel means to existing medical objectives, like rapid point-of-care diagnostics or targeting drug delivery to diseased cells. As is often the case, such new technologies may raise old problems in new ways, or amplify existing issues, or shed new light on them. As the Royal Society report noted, the important thing is to address them, whether new or not [10].

A second generic question relates to implicit values in nanomedicine. The 'enabling' concept does not represent the full story. Nanomedical innovations are not just neutral devices or tools, with no ethical significance. They are considered to be 'value-laden'. This means that the diagnostics, devices and drugs which are being enabled by nanotechnologies all, to some extent, embody certain values, visions and tacit assumptions about future medicine and healthcare. The innovators could be said to be co-producing values and artefacts at the same time. This is indeed inevitably the case for early stage innovation. But it is the task of ethical reflection, social analysis and public engagement to check that the values of technical experts are ones which cohere with ethical views in the wider society [21, 22]. Often, underlying changes of value are difficult to see at the time. The NanoMedRound Table report drew attention to the perspective that nanotechnologies are enabling technologies within a wider progressive reshaping of medicine through technologies in general, whose values and goals need to be duly examined [6].

It is important to consider how these changes relate to our understandings of the human person, of society, and of the role and limits of medicine. By nature, nanomedicine seeks to measure and intervene in the body at the most reduced scales. In doing so, it brings together disciplines with different contexts and concepts – physical and materials sciences, engineering, biotechnology, neurosciences, informatics and medicine. Applying an inherently reductionist focus from the analytical sciences to complex systems in the human body may create some conceptual tensions to the broader practices of medicine. The focus on biological functions at the

smallest levels needs also to be duly related to our wider understandings of human beings, derived from culture, religion and ethics, and to concepts like human dignity, personhood, divine image, autonomy, and so on. What is the moral status of human beings, considered in relation to normal adult life, to the earliest (embryonic and/or foetal) and last weeks and months of life, and to people being subjects in medical research, or of novel or experimental medical treatments?

To take one example, at the end of life, no guarantees can be made of life quality into extreme old age, yet breakthroughs inspired or enabled by nanomedicine may mean that many more people will in future survive into a final, frail stage of life. How should we handle the ethical decisions arising from longer average life spans? Is human dignity only associated with a certain quality of life? Or is it something which is intrinsic to a human being, wholly independent of one's state of bodily health or capability? What is it that makes life worth living – is it only the possession of certain faculties, or is it something fundamental in being human, regardless of our frailty? These are old questions, but ones which nanomedicine may amplify in particular ways.

Again, as technologies develop at the brain-machine interface, these renew long-standing discussions about the relationship between one's identity, the mind/brain and the body. In pursuit of nanomedical solutions, how far should we develop devices which promote direct brain-machine interactions, or apply external or internal controls to the brain? Is our human responsibility or autonomy modified if we have a neurotransplant? Should technologies devised and permitted in a strict medical context then be applied without limit to non-medical interventions?

The Nano-2-Life review noted that, whilst welcoming the many new possibilities to treat disease and alleviate suffering, medicine should not be reduced to engineering solutions. In applying techniques derived from nanosciences, it is important not to lose sight of the wider values of medicine and health care which see the patient as more than mal-operating functions. Materials scientists and bioengineers may make very good devices to help, but they may not be the best doctors to treat the whole person, or to help people face the point when a condition is beyond even the best human ingenuity to treat [7].

A third type of cross-cutting ethical issues relates to social justice. In a world of much inequity, how far should we promote advanced nanomedical technologies, if the likelihood is that they will favour only a few who can afford them, or only those countries with the relevant innovation and regulatory infrastructure? For optimal cases, nano-based therapies may be cheap, or may achieve a net saving in long term healthcare costs. But for some applications, the additional expense of sophisticated therapies may place further financial strain on health care systems. These in turn pose further dilemmas for those responsible for apportioning stretched resources. If nano- and other technologies find medical treatments for hitherto intractable problems, medicine is likely to become constrained less by untreatable conditions, and more by the lack of resources to treat everyone. The situation is more acute, when considered in wider global terms. So much could be done to alleviate suffering on a very wide scale by much more basic health provisions than nanotechnology. There is thus also an opportunity cost, both in ethics and resources, in concentrating on developing high-tech medicine. Do we have the appropriate balance?

12.3 Diagnostics, Information and Predictive Medicine

One of the most far-reaching impacts of nanomedicine is likely to be in the area of diagnostics. It is foreseeable that nano-enabled 'lab-on-a-chip' devices may be able to perform a rapid genome analysis on a simple blood sample, for example. If the equipment could become affordable, what has hitherto been an expensive specialist analysis could become routinely used in a family doctor's surgery. Similarly, nanoscale methods and devices could act as 'biomarkers' to monitor chemical changes in the body's metabolism that would be early indicators of developing a disease conditions, long before the physiological appearance of the normal symptoms. Such rapid point-of-care diagnostics could greatly extend the scope of information available to doctors and patients about health status, both in the range of conditions, and the range of people.

This is seen as part of a major shift from the evidential medicine based on observable symptoms, to a predictive, pre-symptomatic, 'information based' medicine, in which the early indications of an incipient disease state could be picked up early, perhaps years before the symptoms were observed. The hope is that it should then be possible to address the condition long before it takes serious hold in the body. This may delay or reduce the onset, or even prevent it altogether.

A goal of this pre-symptomatic approach to medicine is to move beyond addressing clinical symptoms, or families known to be at risk of a particular disease, to be able to pick up indications in people who would consider themselves healthy. A core assumption in such knowledge-based medicine is that information is taken to be a universal good. In many cases having more specific and relevant information about the condition of a patient will indeed be of much benefit. But is this always the case? When examined more closely, the value of the information is highly context-dependent.

Three questions arise:

(a) How useful is information about our present and future health status?
(b) What preventive interventions are justified within the body, based on what level of diagnostic and especially pre-symptomatic information, and on what levels of probability?
(c) To whom should my health status information be known, other than myself?

Consider four types of preliminary indication of a medical condition that might be obtained using nano-enabled diagnostics. The first two are situations where the knowledge gives a clear and unambiguous diagnosis.

1. As the doctor, if you have an indication, you know what to do, but you don't usually know early enough. For example in atherosclerosis, the first indication may be a heart attack 'out of the blue'. If doctors and patients only knew that the condition was developing, actions could be taken that might delay the condition, or make it much less serious, or in the best cases could prevent it from developing altogether. Here, there is a strong ethical case for pre-symptomatic information, for knowing in advance.

2. In the second case, the disease will certainly develop, but there is nothing that can be done to prevent it. It is relatively uncommon to know with such certainty, and usually it is on the basis of genetic information. Huntington's disease is the classic case, where a late-onset highly distressing, terminal degenerative disease can be detected by a simple genetic test. If the defect is found, the outcome is unavoidable. The offer to test members of families in which the disease is known to run leads to two typical reactions. Some choose to have the test – to remove the uncertainty and know one way or the other – to have prior warning of what will indeed happen, or to have the relief of knowing that they and their children will be free of the disease. Others choose not to have test, not wishing to know any earlier in their lives whether so devastating and unpreventable degradation is about to happen to them.

   The other two situations are probabilistic. The diagnostic information only indicates an increased *propensity* to developing a certain condition, but it is not certain that it will develop significantly. Perhaps it is not clear that the level is sufficient for the condition to take hold, or the test or biomarker shows a positive indication of only one of several factors all of which need to come into play before the disease really develops. Some of these other factors may be known, but others may as yet be unknown. What the doctor can tell a patient is that they have a greater than average *probability* of developing the particular condition, perhaps a lot higher in some cases, but not that they will necessarily get it. Again there are two types.
3. The condition might never develop, but there is nothing that can be done, if it did. This is a probabilistic version of case 2. The worth of having the information is even more problematic.
4. In case 1, the doctor or patient can do something about it, but the condition may never actually develop. This presents a real challenge as to what to do. It will depend on the nature of what can be done – the degree of invasiveness to one's body, the restriction or disruption to one's daily life, activities and expectations, the risks involved. If the actions were a simply change of diet or getting more exercise, this might not pose much of a problem. If a much more invasive and profound intervention is involved, like a mastectomy or prostate removal, or if the procedure or treatment itself carries a significant risk, the patient is left with a dilemma. Moreover, if the indication now entails starting to take pharmaceutical drugs for the rest of one's life, there are likely to be side-effects of the drugs when taken on a long term basis.

These examples are given to illustrate the complex range of contexts into which the information provided by nano-diagnostics would be received by doctors, patients and their families and carers. Pre-symptomatic information may be very beneficial in many situations, but not in all. In case 1, the information may well be life-saving, whereas in case 2 it may be an advanced warning of one's death. In cases 3 and 4, the information is only probabilistic. It is not a foregone conclusion that such information is necessarily a benefit. The predicted condition may still never happen. People are likely to vary in their attitude. For some, the knowledge of the probability

would represent prudent foreknowledge and some actions they could take, just in case. But for others it would just be more stress to one's life, and they would rather get on with living and face the situation if it arises.

Advanced knowledge of a future disease or condition also carries the personal problem of admitting to oneself that one is now an 'ill' person. Does a person in their 30's, who feels perfectly healthy and otherwise in the prime of life, now want to start lifelong preventive medicine, for a condition that might normally only appear in their 70's? And if it is not certain that it would develop, at what percent probability and what degree of seriousness of outcome, does one judge that it is indeed worth beginning such a course of action?

None of these are new issues in medicine. But what is different is their *scope*. Hitherto such questions were typically faced by families which carried a serious genetic disease, for which a test existed, but they were not often experienced in the wider population. The new situation that nanomedical diagnostics is likely to open up is that the range of testable medical conditions, and the availability of testing within the population, will be very much wider. The sorts of dilemmas indicated in the examples above are likely to become much more commonplace.

In considering the tests enabled by nanodiagnostics, careful consideration needs to be given to who wants the information, and under what circumstances? A recurring theme in literature, from Greek myths, through Macbeth to Harry Potter, is that having advanced information is something humans do not typically handle well. A considerable re-education may be needed of what people might in future expect from a visit to the doctor. A point-of-care genome analysis may tell her what antibiotic to prescribe for my persistent cough, but when does she tell me that my genome also shows, say, that I have my higher than average risk of colon cancer?

In such cases, at what point should people be told, and what should they be told? In general it is a doctor's duty to tell the patient material information for their health. But how does the doctor allow for the fact that I might prefer not to know, given that even to reveal to me that there *is* information is likely to prompt me to want to know what it's about, or else to worry about what it might be? Important factors will include such things as explaining to the patient about the extent and expectations from a test, and discussing how far they wish a preliminary result to be investigated further. Regulatory bodies have basic principles and guidelines on matters such as consent and confidentiality, see for example in [23], but these may need to be kept under review in the light of advances in nanodiagnostics in the next few years.

There is also an increased relevance to some long-standing ethical questions: to whom the information should be available, other than the patient and the doctor, and who has the right to interpret its implications outside my immediate health context? Insurance companies, employers, the police, or state databases may each consider they have legitimate claims to my data, under certain circumstances. There were serious concerns in the past over insurance obligations. Insurance companies feared people having tests and then taking out large insurance protections based on the result. Some people in families at risk did not wish to have a genetic test, however, not for fear of finding they have a susceptible gene, but for fear of what an insurance company might do with the result. In the UK there is an ongoing moratorium protecting

people who undergo genetic tests from having to disclose the information to insurers, except in the limited cases [24]. The availability of tests has not grown as much as anticipated [25], but nanodiagnostics may change this picture in future.

A further issue is sheer volume of information which may become available, and how it could be handed and interpreted. Already the internet has made far more medical information available to patients, both good and bad, about diseases and treatments. How well can either doctors or patients cope with all the new specific information that may emerge about my health and its implications from nanomedicine? This is likely to change the balance of the doctor-patient relationship. Some people would no doubt welcome being able to take greater charge of their health in a more informed way, but others will prefer to leave most of it to the professionals. This is also dependent on the type of healthcare system and culture which one is in. The British National Health Service and the private healthcare systems of the USA represent two very different situations, for example.

Another practical implication is that a greatly increased degree of personal counselling is likely to be needed, to help families respond to the dilemmas which the additional knowledge may bring. Significant contact time between professional and patient may be needed, first to understand, to let the implications sink in, and then to begin to decide amongst possible options. Experience from genetic counselling suggests that this may require several meetings over a relatively short period of time. Counselling services for rare genetic conditions have needed relatively modest resources. If the dilemmas of pre-symptomatic medicine become commonplace, much greater emphasis will inevitably need to be put on counselling.

This may seem a long way from nanoscale biomarkers as means of monitoring the condition of certain key health parameters. But if the technologies are as successful as expected then, the impacts of success need also to be considered wisely. The complexities discussed above indicate that it would be naive to embrace nanodiagnostics as part of the ‘knowledge economy’, without assessing what knowledge is beneficial and what is not, and under what circumstances it should be given, when, and to whom. Information, as such, is blind to human circumstances. It is up to humans not to be driven only by the logic of data, but to take account also of wider values and considerations. To reveal what would normally be hidden can certainly have important advantages for some areas of medicine, but it may on occasions disrupt more natural patterns of human knowledge. The religions and literature of many cultures suggest that in a person’s life there may a proper time to know, and a proper time not to know, about a future event, like a terminal disease.

12.4 Remote or Personal Monitoring of Health Status

A development closely related to diagnostics is to combine in a nanoscale device both the means to monitor a health parameter in a patient, and a way to transmit the information to another location. Such ‘smart’ nano-scale implants in the body may

allow someone to go home sooner after an operation, if the healthcare professionals at the hospital can continue to follow the patient's recovery remotely. If key biomarkers fall or rise beyond prescribed warning levels, indicating that something is going wrong, this can act as an alert for action to be taken. This might be a local treatment, or bringing the patient back into hospital.

This concept is has considerable attractions. It would both reduce the time the patient is away from home and loved ones, and also free up much needed bed space in the hospital more quickly. In more remote and rural areas, like the Scottish islands, the ability to monitor a patient remotely could be of great benefit if he is 3 h drive away from a main hospital, provided sufficient local infrastructure was also on hand to respond at need. This could be extended to very elderly or chronically ill people, or those with a known susceptibility, like heart or stroke patients at risk of a recurrence. Again, changes in critical parameters could forewarn of the need to take preventative measures.

One downside is that this represents a degree of surveillance by a third party. One's whereabouts, and to some degree, something of one's private activities will be known and followed. Even with benevolent intentions, and restricted to particular professionals, family members and carers, some people may not welcome a sense of their privacy being 'snooped on'. On the other hand, it can provide a sense of security that someone is on hand to help if something starts to go wrong. A weekly video or phone link to a nurse to go through the week's readings can become a welcome reassurance, and a point of regular contact, for people living alone with a long term medical condition.

A second implication is the degree of responsibility that is shifted to the home, the patient and the carer. This may be something welcomed. If one has an elderly parent who is living on their own, it could ease some of the stress to know that particular health functions were being monitored. If I am the patient, it might be reassuring for me to keep a regular check on my critical measurements, which become part of the way of life of living with my condition. On the other hand, it can be a considerable additional stress to keep taking or checking a measurement, day in day out, and interpreting what it means if things are not going well.

Looking further into the future, if monitoring health parameters by implants or particles becomes commonplace, there may be pressure to use them for other than medical reasons. Elite sports and military use are examples where monitoring is already done, which are accepted within their special contexts. One could imagine equivalent arguments being put forward for certain occupations, like a pilot or bus driver where many other lives are in one's hands. Technically, it would be a relatively short step to a more general surveillance of performance in a work context, or for an insurer to want to monitor one's risk levels. But this would cross a significant ethical line where the primary beneficiary of people's private health information is no longer the individual him/herself, but various third parties. This may represent another clash between the value of human persons and a merely functional logic to use the capacities which some new nanotechnology may enable. At this point I would argue we lose the precedence of human values at our peril.

12.5 Implants and Targeted Delivery

The flag ship concept of nanomedicine is the notion of targeted drug delivery. Typically a pharmaceutical product is encapsulated in a nanoscale carrier, to which has been attached an array of ligands variously designed to carry the particle through hostile media in the body, to recognise specific diseased cells of the body, or toxins or other malicious entities, and, on encountering them, to release the active ingredient to do its job. It should do this without affecting healthy cells nearby, or other organs of the body. In particular, it is intended to overcome the problem of the introducing chemicals systemically. At present, in order to have a sufficient concentration of the active chemical in the affected organs, it has to be introduced into the whole body, and chemicals powerful enough to attach a cancer cell, say, may impact harmfully in many unintended elsewhere in the body, causing significant side effects. A growing number of targeted pharmaceuticals are in use, and many more are likely to follow.

There is a strong ethical case in favour of nanomedical methods which have a reasonable prospect of addressing the problems of systemic drug delivery. The primary ethical questions are about risk, long term implants, and overclaiming. There are issues of risk in relation to the nanocarrier and its behaviour and ultimate fate in the body. Because of their size, nanoparticles have the potential to pass through barriers of the body and end up in strange places. While the understanding of nanoparticle risks remains relatively poorly developed, a precautionary approach is appropriate. Given the present uncertainties of the technique, targeted delivery is better focused on the more serious or intractable medical conditions, until a body of substantial experience has been accumulated of these therapies in practice. Ongoing and long term monitoring needs to go hand in hand with more fundamental risk studies on different materials and formulations. Carriers that the body will naturally degrade or 'functionalised' particles have been seen as more favourable than pure inorganic materials which would remain largely inert.

This leads to a more general question about implanting nanoscale devices into the human body. This might be to monitor the progression of disease, to deliver therapies in situ, to provide scaffolds for replacing damaged or failing tissues, or to provide external monitoring of our health. How far should we make nano-technically enabled interventions in the body? Should this remain something done exceptionally, under particular conditions, as with macro interventions like hip replacements and stents? Or should we expect this eventually become the normal pattern, in widespread use? The technology may be beguiling. What other important factors need to be taken into consideration, and what takes precedence if conflicts between values arise?

The last point in this section is a tendency for some promoters of targeted delivery to overclaim about their products. For example, video simulations create an almost military scene of unmanned capsules zooming through blood vessels, seeking out their targets, and delivering their payload by precision impact on the affected cells. The military analogy is perhaps unfortunate, but the medical equivalents of collateral damage and wrong targeting of supposedly precision bombing, are relevant issues.

Targeted drug delivery should be a step change improvement compared with system delivery of a pharmaceutical, but risks of unintended consequences remain, say if the intended therapeutic molecule hits the wrong target, or if it has side effects which perhaps the researchers did not look for.

The tendency to exaggerate can sometimes present an ethical problem in itself. Hopes for the benefits of for high tech therapies are raised amongst vulnerable patients, or hard pressed policy makers. At earlier stages in innovation, the prospects claimed by the researcher or a company about a 'breakthrough' under idealised research conditions, with a view to attracting further funding, can raise misleading expectations. In practice, applications will only be realised after a critical review of their feasibility, clinical reliability, economics, and safety, and unfortunately many fail at one hurdle or another. Once approved for clinical use, a technique may work well in some patients but prove less effective in others. Such is the nature of innovation. A degree of modesty is therefore called for, both out of respect for the vulnerability of the human patients and recognition of the finite understanding of the method. At this nano-medical interface, the emphasis needs to be that of the doctor treating a human person, rather than the impersonal logic of technique, however good it may be.

12.6 Theranostics

A special case of implants is the so-called 'theranostic' device (*thera*peutic – diag*nostic*) which would combine a measurement and monitoring role with some kind of therapeutic delivery. The delivery is activated in response to a critical change in level of a parameter which has just been measured. The attraction is not needing to wait before activating the therapeutic response, perhaps to maintain some function like blood sugar levels within a tolerated range, if these were about to drift outside the range. The advantage is that the remedial action is rapid and does not depend on the patient or perhaps a nurse to have to step in, notice the change and activate the response.

It depends, however, on having established a close numerical correlation between what is measured and the degree of therapeutic response. This has to have been established in advance with considerable precision and reliability in order to programme the device accordingly. One problem is applicability and reliability. Can one indeed produce an algorithm so robust, or so flexible, that it applies infallibly to all patients who would present with this condition? It may be possible to tune the device initially for the particular patient, but will the settings continue to be valid as the patient's metabolism changes, with different activities, at different times of day, times in a woman's monthly cycle, etc. To the extent that the device responds automatically, there must be a very high resilience to misleading data. How reliable in engineering terms is the equipment, for example as materials degrade with age, or pump flows become restricted? If modifications became required from time to time, would this be possible?

Conceptually, theranostics relies on the assumption that a necessarily limited set of measurements in a device is sufficient on its own to represent accurately a complex physiological change and to deliver the correct response without human intervention. Such an assumption requires a great deal of trust in the reliability of the design concept, the programming and the engineering. To produce a 'black box' of sufficient flexibility and reliability would seem to be a tall order. Indeed, some companies in this area are reluctant to make devices too automated, in recognition of factors like the variability in patients.

In the UK public consultation on potential research fields in nanomedicine, theranostics received a lower priority than several other nanomedical applications. People expressed concern that scientist might make such a device to be *too* smart. In envisaging theranostic devices implanted in their bodies, people felt they or a doctor should keep some control over the implant and its operation. A degree of human judgement was necessary rather than depending on algorithms and programming. It is another case where human values are needed to modulate engineering logic.

12.7 Regenerative Medicine

One of the most intriguing prospects of nanotechnology is to be able to construct material objects 'atom-by-atom' to any shape or form desired. While its more exaggerated claims have been rightly criticised, one potentially useful application is in regenerative medicine. The isolation of human embryonic stem cells and induced pluripotent cells have opened up many new possibilities to replace lost or damaged cells in vital organs of the body. One aspect is the possibility to regrow nerve cells, for example in spinal cord following an accident, or in the retina in certain cases of blindness. This requires an appropriate tissue scaffold to be grown starting at the nanoscale and building upwards. A number of nanomaterials are being investigated.

While the basic ethical rationale is very good, such techniques run into the serious ethical problems, if they entail the use of cells originally derived via the destruction of human embryos. It is not the place here to rehearse the arguments for and against human embryonic stem cells, but, suffice to say, in some countries and for some individual patients, such technologies would be ruled out unless they could be achieved based on non-embryonic sources of the cells. Fortunately some of the best prospects lie in encouraging the body's own stem cells to regrow, so the ethical problem may be avoidable, but it is important to aware of the issue. Other potential ethical questions would arise if it becomes possible to regrow brain tissue, and in attempts to construct organs by this method outside the body.

12.8 Risk and Uncertainty

The risks associated with the use of nanoparticles and nanoscale implants in the human body have been much discussed. These include the transport of particles to unintended parts of the body, side-effects on the body's metabolism, and the long

term use of implants. Given that there will always be risk associated with these types of interventions, there is an ethical dimension to how one sets a tolerable level risk, and against what criteria. Once scientific data become available, the numerical probabilities and consequences remain as numbers on a page, until it is decided what levels constitute acceptable or unacceptable risks. These are ultimately ethical judgements. To make such judgements will require not only much good research but also much careful engagement with different publics, patients' groups and their carers.

Risk may be calculated with a good deal of reliability in areas of established engineering experience. Nanotechnologies, however, typically go beyond well trodden paths. A second dimension is thus how one handles the inevitable uncertainty associated with novel procedures involving tiny devices in the body. How precautionary should we be? From fields such as genetically modified food, two version of the precaution principle emerged – hard and soft. Hard precaution is the inclination not to proceed if a significant case for a risk of harm can be made. Soft precaution argues that one should proceed unless there is reasonable evidence that there is a risk of significant harm, but which at this point cannot be sufficiently evaluated. In principle both are resolvable by further evidence, one way or the other. In practice, however, some uncertainties are likely to remain intractable, or would take a very long time to assess. In the meantime, patients are longing for treatments to their conditions.

The key question will be at what point it is considered that enough is now known to proceed, or it is concluded that the intended process would entail unacceptable risk. Some general principles are that, in areas of uncertainty, the initial focus should be on applications with a high degree of medical benefit, the more serious diseases, on situations where there is a degree of reversibility or recovery from adverse effects, and where there is an ability to track the fate of nanoparticles or implants. But a strict ethical principle of 'do no harm' may not be achievable. This brings us to a final and fundamental point about the way we handle risk.

Since risk is inherent to the human condition, we should resist undue demands for certainty and safety, or a culture of blame for techniques which fail. There is a profound difference between negligence, given what you knew but did not act on, and not knowing something which no one had reason to know at the time. Hindsight can be very destructive in this respect. The question is, set against all the other risks of human living and the particular condition of the patient, how much or little risk is tolerable? And, having decided, everyone involved should recognise that no one can guarantee success.

12.9 Human Enhancement

One cannot end a chapter on the ethics of nanomedicine without briefly considering the issue known as human enhancement. All the examples considered so far address using nanotechnologies for explicitly medical goals. A topic of increasing debate in the last 10 years has been whether we should use these, or other technological methods, to enhance the human body beyond its present capacities. Should nanotechnology only 'make humans better', in the sense of treating diseases and injuries, or should we

use it to 'make better humans', by using technology to improve the basic specification of the human body and brain directly?

The first significant study, the 2002 US report NBIC report on 'converging technologies for improving human performances', was optimistic about this latter prospect [26]. A European expert group urged submitting enhancement goals to wider social scrutiny, if our humanity is not to be redefined by a techno-logic driven primarily by technical and economic feasibility [27]. More recent reports are from the UK academies [28] and the Dutch Rathenau Instituut [29] as well as an increasing academic literature and several European Commission studies, some broadly approving, and some more critical ([30–34]). It must be said that only a few technologies exist today. Most of what is discussed in these reports remains as future prospects whose practical feasibility is very uncertain, and some have criticised the field for indulging in too much speculative ethics [35]. The implications are sufficiently far reaching to take the question seriously, however, and we summarise some of the key issues.

The first is the basic ethical issue of whether we should seek to make serious changes to human body and its metabolism. This depends on one's view of the human being and of human technological intervention. Traditional presuppositions hold that there are moral or societal bounds which should act as a restrain on what may otherwise be feasible technically. These limits are drawn from the insights of the religious and cultural traditions, philosophy and theology, the arts and humanities, and the social sciences. Christian thinking for example grounds human nature in God's creation of human beings 'in God's image', although two recent studies considered that this did not mean that enhancements are necessarily prohibited, as such [36, 37]. Traditional views are challenged by transhumanist belief that humans are destined to go beyond our current biological limitations.

It may be helpful to think of this question in terms of three general views. One view sees the human body and its capacities as something 'given' which it is not to be majorly changed. The transhumanist philosophy regards the human body as evolutionary, in principle open to be changed without limit. An intermediate position recognises that humans could be changed in degree but not without limits, not change whatever we regard as our human nature. In summary these are: change nothing, change anything, or draw lines as to limit what may be changed.

The second ethical question is what is meant by the idea human enhancement, and whether enhancements really do enhance? The assumption is made that if I do something that improves some functional capability in my body or brain, it is an enhancement. But on what basis are we to judge whether actually it constitutes an enhancement, beyond a purely subjective view? A focus on improving human performance, for example, seems too limited a criterion, compared with more holistic concepts of the human person. The assumption that to be that little bit faster, stronger, smarter, more retentive, more musical, we are somehow happier and better as humans seems too vulnerable to things going wrong. A better question would be in what sense are we better as human beings by having a particular capacity enhanced? It might indeed be appealing to do certain things better than one could naturally, but would it make the difference between a good life and a poor one? Beyond a certain

basic point of physical survival and necessity, what matters most to humans are their relationships. Wider issues such as love, friendship, creativity and spirituality seem to matter more than functional abilities.

A few examples serve to illustrate that enhancements may not be as straightforward as the term suggests at first sight. Suppose retinal implants would provide a true recovery of sight to some blind people, and this is extended to offer vision into the infrared region for the normal sighted. It is said that this would have considerable safety advantages for night driving, for example. On the other hand, would one use the new capacity to drive faster, and not more safely? And why not simply use some form of spectacles to achieve the same end? Secondly, cognitive enhancing drugs have become used by students concentrating for exams, but the value only exists as long as only a few have the advantage. If all students used it, there would be no competitive advantage, but no one would then dare stop using it. Thus all would become locked into a pointless 'enhancement', and one which would not reflect their true ability. A third example is in the field of sport. There are plenty of examples where the over-amplification of critical functions can be pursued out of proportion to the rest of the body, resulting in significant overall damage. The same harmful imbalances have been observed in genetic engineering of animals for faster growth rate, both by selective breeding and molecular intervention [38]. We may need to consider rather carefully before calling an intervention an enhancement.

A third issue is reliability, risk and regulation. Whereas medical devices and pharmaceuticals are subject to strict and complex testing and regulation, there is little or no regulation of enhancements. There is no comparable system to test and guarantee that an implant or a chemical enhancement both does 'what it says on the packet' and is safe and reliable, and is not a false product of the modern equivalent of the quack doctor. Recent experience of unscrupulous and invalidated stem cell treatments underlines the importance of having a system of regulation and validation.

Safety testing also raises a problem. Riskier medical procedures are justified only for the more serious medical conditions. For enhancement technologies there is no comparable balancing good of saving life or preventing serious suffering. This marks an important distinction between nanotechnologies to address medical conditions and those intended to enhance healthy human beings. In academic debate, some have criticised this distinction, because it assumes ideas of a 'human nature' and what is 'normal' to humans, which are merely human constructs of our times, but which do not have any ultimate grounding philosophically. As observed above, this depends somewhat on one's world view.

In contrast to this, one of the findings of a recent public engagement study was that people do seem to have an implicit sense of normalcy in human capacities, even if it would be hard to pin it down to any sort of definition [39]. In assessing a range of potential human enhancement applications, people often made a distinction: technologies to bring people who are ill or disabled in some way 'up' to the norm, were broadly accepted, but using the same technologies to take healthy people beyond the norm, were viewed sceptically or even objected to. Some special situations were perhaps acceptable, for example, for rescue workers in a disaster to take a drug to do without sleep. But to do this in everyday life was seen as abnormal.

To a first approximation the medical – enhancement distinction seems valid. There are indeed situations where the distinction is blurred, which should be considered on their own merits, rather than invalidating the distinction. As Holm has pointed out, we do not think yellow and red are not valid as colours just because they can blur to form orange [40]. Thus whereas nanomedicine would be generally favoured, enhancements were viewed much more critically.

A last main group of issues of human enhancement are its many social implications. Amongst these are three of particular note. The first is a general point that the various implications of enhancement technologies are too important to be treated just as matters of personal preference, but should be regulated at a societal level in most cases. The second is a deep concern that in practice human enhancement is promoted primarily by and for those who are already in high levels of economic and social advantage. Enhancements would inevitably be available mostly for the rich and priviledged, thereby enhancing their advantages still further. Advocates of enhancements point out that any new technology tends to create new winners and losers, and that one should not object to enhancement on that basis. Many things once considered luxuries are now cheap and widely available, like televisions, mobile phones and computers. But suppose enhancement technologies really did prove to be as good as some claim, this would give those who could afford to use them 'hard-wired' advantages. While those less well-off were waiting for the prices to come down, the rich would get ever further ahead, in what would become a 'nano-divide'. Some argue that to pursue personal human enhancements, without regard for those who miss out, people might be enhanced functionally but diminished in humanity [36].

The final social issue is whether, faced with the issues like poverty, poor health, climate change, and global food security, the idea of human enhancement is largely a distraction for the well-off, and which misses the point. It might be argued that what is wrong with the human condition is not a lack of strength, longevity, intelligence, beauty, athleticism, art, science or even education. It lies in deeper moral and spiritual shortcomings of humanity, individually and collectively, as the world's ongoing conflicts show. However much we 'enhanced' ourselves physically, these inherent human failings would remain because these would seem to lie beyond technical fixes.

12.10 Conclusions and Postscript

This chapter has considered a range of ethical and social issues associated with likely advances in nanotechnologies, primarily as applied to medicine, but also possible enhancements of the human body. The technical logic of nano-enabled medicine always needs moderating with ethical reflection to apply human values to achieve wise solutions. In many cases a good ethical case can be made for the considerable medical benefits, but enthusiasm for new technical solutions should not lose sight of the wider perspective of human values, and the long experience of the

practice of medicine. Nanomedicine needs to maintain a human face. Human enhancement, on the other hand, does not have the ethical benefit of making an ill person better. It depends for its appeal on a more elusive idea of making 'better' humans. It remains to be seen whether it would actually offer significant improvements to the human condition that would outweigh social concerns, risks and practical problems. It also begs the wider question whether the deepest needs of the human condition cannot be met by technology.

Important social and conceptual changes are likely to accompany the application of nanomedicine, especially in pre-symptomatic diagnostic information giving advanced knowledge about our future health status. We think of ourselves as relatively well or relatively ill. But if in the long term, nanotechnologies might eventually make much hitherto unsuspected data about our bodies accessible to us, what now is a well person? If read-outs of genes, chemicals or other parameters will represent almost any body function, may we find that we are all to some extent 'ill', or at least probably ill?

In many cases, such knowledge will be welcome and valuable, and in some circumstances even be life saving. But a sense of proportion is also needed about 'knowledge-based medicine' and our health status. In his witty Victorian English tale, *Three Men in a Boat*, Jerome K. Jerome recounts going to the British Museum library to look up an ailment in a medical encyclopaedia. But out of curiosity he reads on and finds that he seems to show the symptoms for half of the diseases in the book. 'I went into that reading room a happy healthy man. I crawled out a decrepit wreck.' He went to his doctor with a full list of his supposed diseases, and is given a prescription. He goes to the pharmacy and discovers the prescription is for a daily diet of beef steak, a pint of beer, good exercise and early to bed … 'and don't stuff your head with things you don't understand!' [41].

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Chapter 13
Nanomedicine: A Hyper-expectation and Dawning Realisation?

Ferdia Bates

13.1 Introduction

At this point in time, it could be assumed that to some extent nanotechnology, defined as the manipulation of material and the development of structures at a scale of between 1 and 100 nm, has to some extent permeated the lives of the all within the first world through at least one of its numerous applications. Perhaps the most poignant of these applications is that within the medical field where nanomedicine affects a person's most intimate possession, their health. This may be in something as seemingly mundane as the titanium oxide nanoparticles contained within sunscreens or as striking as increasingly effective cancer treatments, prodrugs and nanopharmaceutics. Whatever the impression that nanomedicine leaves, it is unequivocally present within society with increasing levels of sophistication.

The degree to which we rely on nanotechnology already or expect to rely on it in the future is a moot point. For years we have been conditioned to expect enormous things from science and technology, and thus far this expectation has been satisfied. Technological advancement has allowed a single generation to witness drastic changes in society the likes of which have never before been seen. Within the twentieth century, the fundamental ideas and values of this global society have undergone a radical upheaval which has occurred in hand with technological advancement. The perception of fantastical and seemingly endless innovation and technological growth is due in part to the human trait of linear thinking. Isaac Newton once demonstrated this, and his modesty, in the highly quotable line:

> If I have seen further it is only by standing on the shoulders of giants

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This single sentence can be taken as one of the most accurate paradigms for human thought in the sense that a mind can only imagine future technology based on the extrapolation of the state-of-the-art in the present thus the analogy of seeing ahead from a static, albeit lofty, point. The idea of fantastical technological advancement depicted by a current state-of-the-art can be demonstrated very effectively by the evolution of the popular conception of robotics with respect to time; this can be seen in the advancement of technology projected in yesteryear and the actual realisation of robotics in the present day. These predictions now seem comical to this current generation who can see the juxtaposition of the prediction beside the advancement actually achieved in the same time period (Fig. 13.1). In Fig. 13.1, the right depicts two modern examples of function and convenience while the left shows the now-anachronistic 'future' technology where the humanoid robot is set against a back drop of a flying-saucer spaceship on some non-disclosed foreign planet. The automaton in image is inspired by 'Robby the Robot', star of the films 'Forbidden Planet' (1956) and 'The Invisible Boy' (1957), who is perhaps one of the most recognisable examples of what a robot was thought to be in the 1950s, along with B9 from the series 'Lost in Space' (1965–1968), both of which – or 'whom' – were designed by the renowned Robert Kinoshita. These robots displayed personalities and wit with a level of cognition that still soars above current computing power [1, 2]. As the function of these celebrity robots was, at the core, to help and convenience their human masters, one could argue that this modern age has indeed, to some

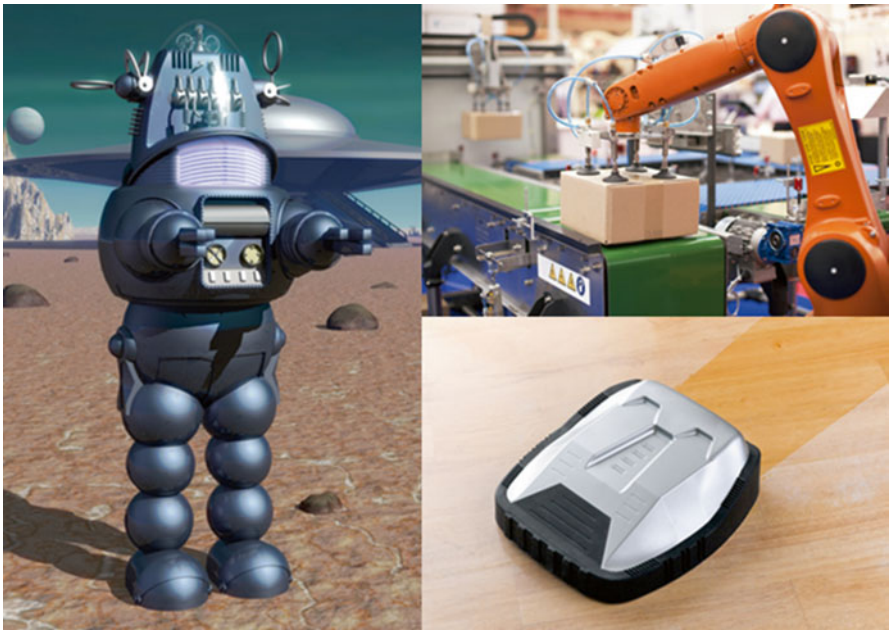

Fig. 13.1 The contrast between past predictions and present realisations of robotics technology (Copyright free images)

extent, seen the realisation of such predictions and yet there is the contrasting reality that one would still not expect anything close to the comic cornucopia displayed by those celebrity-bots from a modern day robotic vacuum.

The consolation for a conversationally barren non-humanoid vacuum cleaner is the gargantuan supply of 'unforeseen' novel technology and innovation which these predictions didn't even take account of such as personal computers, the internet, laptops, mobile phones and smart phones – a marriage of all the aforementioned technologies and something that has affected society in an unprecedented manner.

This is a good example of a paradigm shift idea, the manner in which the future is thought to be as given by the projections of the time in contrast to how that future actually transpires to be when such a time arrives with all the novel innovations it brings with it. In this way, though hyper-expectation is often less of a matter of over-exaggeration and more of misdirection, dawning realisation can be expected to bring realisation of new unforeseen technologies as well as the cul-de-sacs of optimistic speculations and attenuators associated with implementation of an ideal. This trait is ingrained in the collective mind of each generation and is the ultimate dictation as to how one can perceive the future; it is a trait compounded by the increasingly large generational gaps which technological progress has brought.

The acceleration of technological advancement with respect to time can be identified as far back as the industrial revolution, though progress as striking as was seen in the twentieth century was not realised until the advent of the computer and the massive increment in computational capacity it facilitated from its predecessor and the new technologies that were built from it; first with the use of thermionic valves, which gave rise to widespread domestic radio as well as the first electronic digital computers, and then with transistor-based modern computing. The transistor, more so than even the thermionic valve which immediately preceded it, allowed for the accelerative progress which is now taken as normality.

This accelerative progress has been embodied in Moore's law which states that computing power will approximately double every 18 months [3]. Needless to say, extrapolation of an exponential function quickly leads to quite dizzying heights relative to a static position. With such increasing computing power, technological intricacy has exploded at an extraordinary rate; an example of this is achieving space flight less just a few short decades after the achieving flight itself. Thus humanity has grown accustomed to such fantastical changes within a lifetime to the extent that it is now expected.

The advent of nanotechnology has renewed this expectation if not increased it even more. It is true to say that this age is truly one of the futurists; never before has a generation looked forward in time with such a degree of expectation. As a generation we have become accustomed to jumping from the shoulders of giants rather than merely standing on them. Though this ethos is extremely useful and the basis of this modern and highly progressive society, the level to which the culture of hyper-expectation should be entertained has always been a hot topic.

Nanomedicine has proven its worth already in its various applications and the projections for its growth and development predict sophistication and effectiveness that will radically change the way medicine is practiced [4–7]. The question that

immediately follows such predictions almost always relates to the time scale involved. With the initial assimilation of nanomedicine into society approaching completion, to some degree, the mystery that had shrouded nanomedicine in the past has lifted to leave a clearheaded realisation of the work that is required before any of the grand promises that nanomedicine has given can be realised.

This being said, the economic implications of first and second generation (Fig. 13.2) nanotechnology-incorporating products can already be seen in the market place. Heavy financial investment has been made in increasingly large instalments on the back of the projected treatments and technologies that nanomedicine will yield. The National Nanotechnology Initiative in the US, having been set up initially in 1998, was designed to encourage collaboration between the fields of science, healthcare, engineering and technology on the nanoscale [9]. As of 2008, the budget requested was $1.5 billion, almost triple that spent in 2001. This figure has been revised to $1.8 billion for the 2013 in a budget proposal put forward by President Obama. This aggressive growth in the funding is indicative of the volume of research that has taken place in that short period of time [10]. Regarding nanomedicine specifically, substantial research initiatives have been set up such as that in the American National Cancer Institute's $144 million cancer nanotechnology initiative, which commenced in 2004, in order to bolster the interest in nano-research. In 2010 this initiative entered into its second phase in which many of the researched nanomedicines would be brought to clinical trial fortified by funding of equal rigor [11].

Indeed, these sums of money were offered in such quantities because of the projected growth and potential that the nanotechnology sector was speculated to have; it was predicted in 2000 that nanotechnology-incorporating devices would reach the one trillion (10^{12}) dollar mark by 2015 at which point it would account for more than two million high skill jobs [8]. In contrast to this, analysts in 2004 projected a

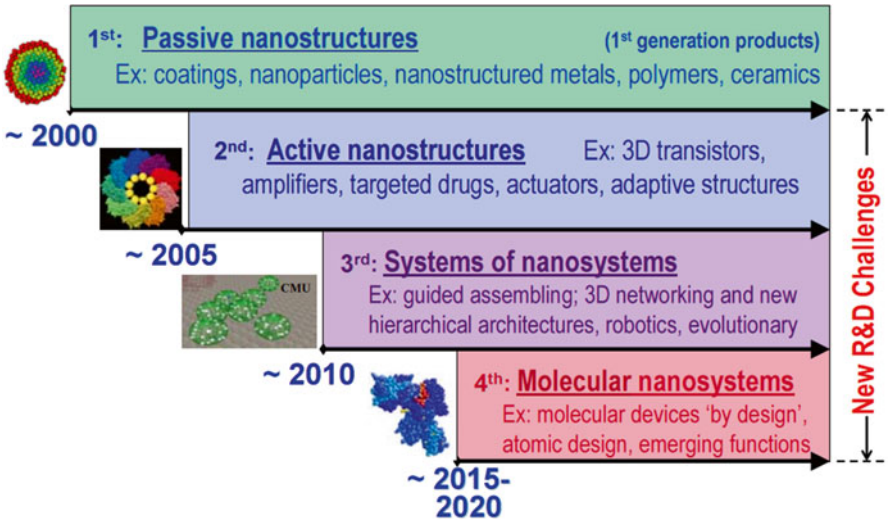

Fig. 13.2 The projected progression of Nanotechnology from 2000 to 2020 (Reproduced from [8])

nanotechnology market worth of 2.6 trillion dollars with ten million associated manufacturing jobs by 2014 [12]. This projection was based on an estimate of the 2004 market value of nanotechnology which was cited as 158 billion dollars, 92 % of which were established materials with nanoscale dimensions. Based on this valuation, a further eight fold increase in market value was projected in the decade following the publication of the report. Within this extrapolated valuation, it was estimated that 89 % of the future revenue of the market would come from new and emerging nanotechnology [13]. As of 2009, nanotechnology was estimated to be worth one quarter of a trillion dollars and, with retrospective analysis, showing a doubling of the market every 3 years as a result of the on-going introduction of new nanotechnology-incorporating products [14]. Such a revision in estimation demonstrates the highly dynamic and fast paced nature of the nanotechnology sector. Though, as demonstrated in the 2004 analysis, a large amount of the initial growth came from an increase in market share through merely nano-sizing existing components rather than through true nanotechnological innovation. Thus, it is often an arduous task to differentiate between true advancement in the field and mere colonisation of an existing market.

Having achieved increasingly complex structures in keeping with the projected timetable, prototypically, the molecular transistor which was predicted circa 2010 [15], predictions for what future nanomedicine will yield can be divided in two. The first is the conceptual research which is discovering the extraordinary applications and capabilities that nanomedicine has to offer. Overlaying this is the stark reality of attempting to translate the complex laboratory ideal of medicine on a nanoscopic scale from concept to clinic.

The nano-age offers a unique opportunity to stand starry eyed idealism, stemming from on-going conceptual research of a young field, next to the cold logistics of implementation through which is discovered the new challenges that nanomedicine will bring which can be represented by the nanotoxicity example (Sect. 13.5). The combination of these two outlooks has created a fascinating cocktail of on-going fundamental research in the face of the emergence of nanotoxicology as the main bottleneck for implementation. The rapidity of the field has also challenged the traditionally conservative and slow-moving gears of bureaucracy in determining the manner in which such an unknown and emerging sector should be governed and regulated.

Thus there is a complex ballet being performed between the hyper-expectation of the applications of nanomedicine and the dawning realisation of what the act of applying it actually entails.

13.2 Popular Media: Feeding Frenzy

There has long been a complex relationship between the popular media and science with both sides looking to the other for inspiration. Such a complexity frequently leads to confusion as to what is science fiction and what is science fact. Through the ages, this has led, to some extent, to a convoluted public perception of the

state-of-the-art. On the other hand, the media often borrows from real scientific phenomena and in doing so, serves to acclimatise the public to such concepts as well as advertising the technology; in this respect, science and the media can be likened to feuding siblings pitted in a perpetual game of chase.

For many years, nanotechnology has provided fertile ground for science fiction to explain their farfetched devices and techniques. The foundations on which nanomedicine are based on can be traced even further back than this. One of the first popular media acknowledgements of a core nanomedicine concept can be seen in the film 'Dr Ehrlich's magic bullet', released in 1940, it documents the arduous journey of Dr Paul Ehrlich, the Nobel laureate often referred to as the father of modern chemotherapy and widely recognised as to be the first to propose the concept of targeted delivery, a central application of nanomedicine [16], to develop the first chemotherapeutic. Further homage, albeit unwittingly, to the concepts of nanomedicine can be seen in the film 'Fantastic Voyage', released in 1966, it came just 7 years after Richard Feynman's landmark talk 'There's plenty of room at the bottom' which can be taken as the conceptual birth of nanotechnology [17]. The Fantastic Voyage, perhaps aided by the pleasant visage of Raquel Welsh, has become one of the most enduring epitomes of the concepts being realised through nanomedicine. The portrayal given by this film can be greatly likened to the concept of the nanofactory which is already under development today (Sect. 13.3.3).

The 'Grey Goo' scenario was first proposed by nanotechnological visionary Eric Drexler in 1986 [18] as a hypothetical scenario whereby he observed the consequence of creating an organism that could proliferate uncontrollably, eventually engulfing all things in 'Grey Goo'. Indeed, the ability of an object to self-replicate was cited as one of the primary factors to make nanotechnologies 'particularly consequential' [19]. This projection built on Feynman's original proposal made almost 30 years previously. The grey goo scenario is a prime example of hyper-expectation of a conceptual field. 1986, close to 30 years ago, was still a long time short of any meaningful realisation of the concepts dealt with by Drexler. Without this 'real world' perspective, the then largely unknown field of nanotechnology could be viewed as the herald for the imminent arrival of Huxley's 1932 novel 'Brave New World' [20] bringing with it some understandable misgivings if not hysteria.

In more recent times, nanotechnology has even acquired its own 'Jaws'. This is Michael Crichton's superbly written 'Prey' [21] in which the more ominous potential of sophisticated nanotechnology is explored. Nanotechnology is also very much present in modern media as well where it is used as a one word explanation of highly evolved and sophisticated technology. It played a pivotal role in the description of many aspects of the 2009 film 'G.I. Joe' and also can be identified throughout modern popular media as the facilitator of the future advancement of medicine. The videogame 'Deus EX', the latest addition to the series having been released in 2011, depicts the central character as being an 'augmented human', heavily modified with bionic appendages all of which contain nanotechnology. There is also reference made to so-called 'nanites' which are nano-robots, not unlike those speculated to perpetrate the 'grey goo' scenario, these robots are introduced into the blood stream of the central character where they serve to repair injuries as they occur [22].

The content of Deus EX, which is cited purely as a prototypical example, can be used as an illustration of the realisation, which has come predominantly through media-facilitated acclimatisation, and corresponding acceptance of the public of nanotechnology as a non-malevolent 'part of the woodwork' of the future.

13.3 The Dawning Implication of Hyper-expectation

What is medicine? A basic definition can be given as the maintenance of the physical body with a primary endpoint being staving off or guarding against death. The next barrier after the conquering of illness is the defeat of death itself or, in a more immediate and plausible way, to keep the reaper at bay for increasingly an increasingly long period of time. Even using the most prudent mind-set, with the medical advances which are occurring in this age, it is not unreasonable to predict a time in the not-entirely-distant future when death, certainly for those of means at least, will be optional.

Given the current trends in population age (Fig. 13.3), it is quite reasonable to state that the coming generation, as a consequence of the advances made within nanomedicine today, will be the first with the true ability to 'outlive' the very bodies which the personality of the person is contained. Indeed, the implications of nanomedicine stretch as far as one would care to look. This is already apparent in the increased prevalence of chronic degenerative conditions including connotative impairments

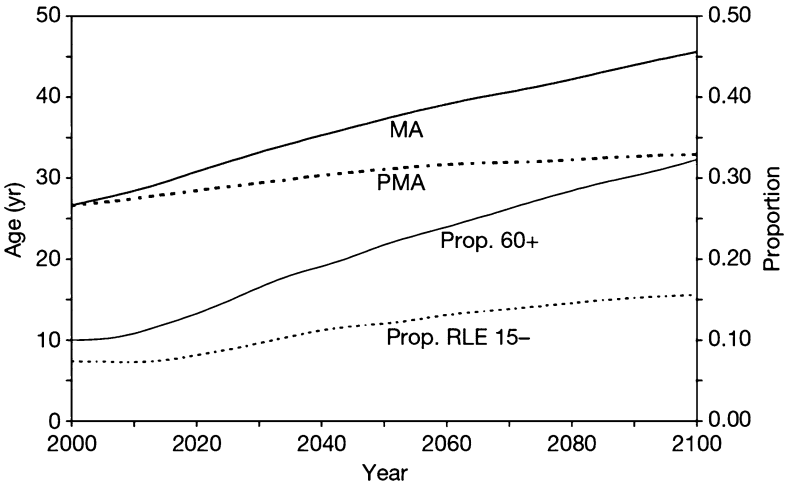

Fig. 13.3 Various projected trends in populating aging with respect to Median Age (MA), population proportion aged 60 or above (Prop. 60+), the proportion of the population with 15 or less years' life expectancy remaining (Prop. RLE 15−) and the prospective median age (PMA) defined as an adjusted median age which takes into account the increased proportion of the population within the Prop. RLE 15− band (Reproduced from [23])

such as dementia as well as physical impairments, such as osteoporosis and arthritis as well as vital organ failure. Increased longevity also increases the risk of cancer which is explained in the theory of aging which states that cancer, in a lifetime, is a statistical inevitability. This is down to the estimated 10^{16} divisions that a cell undergoes within a lifetime which leads to a build-up in DNA replication errors [24].

Indeed, ruling out morbidity from an acute source, death can be predicted to inevitably come from a cancer, organ failure or neurodegeneration [25]. The first two of these, cancer and organ failure, have the potential for onset at a much earlier age than neurodegeneration and thus these have a tendency to monopolise the time and money of research, nanomedicine included. The consequence which has been seen as a result of this, and is being seen at increasing levels, is that an increasing volume of the population is surviving previously terminal encounters with such conditions. Neurodegeneration can be seen as one of the final obstacles in the way of humanity achieving true hyper-extension of the natural life span. As a demonstration of the scale of the issue, it is estimated that six to seven million persons in Western Europe are currently affected by Alzheimer's disease, the main cause of dementia. This number doubles every 5 years within the over 65 age bracket and has an incidence of one in three for those at and above 80 years of age [26].

Within the past decade, nanomedical research has been extremely fruitful. Ground-breaking treatments for all ailments have heralded the move towards a time where humanity will be true masters of their existence in the sense that disease in the convention that it is known today will no longer exist. Indeed, parties in favour of this future eagerly anticipate and speculate about such a time. In this sense, nanomedicine as a concept, albeit indirectly, will force mankind to re-evaluate what it is to be human. It can be argued that nanomedicine as the tool or mechanism will accelerate technological progress to such a level as could affect humanity in a manner so drastic that post-nano mankind would be unrecognisable to the minds of today.

Nanomedicine could eventually chaperone a transition from a definition of humanity that is predominantly based on the physical to more of a disembodied ideal. This will first be confronted when the level of medical care is reached to make it possible for the person, which must be defined as the consciousness within the physical body, to be allowed to exist longer than the flesh in which it is contained. The 'wearing out' of the physical body is clear to see in the elderly. In some ways, the separation of mind and body is quite obvious; for example, the development of a cancer in the abdominal cavity. In this scenario, the person is unquestionably the same person as they were before the tumour. This definition blurs significantly when this 'wearing out' is observed in neurodegenerative diseases. As will be discussed in further depth below (Sect. 1.3.6), the consciousness of a person can be generally isolated to the brain, which is the epicentre of a person's being. Once the brain's neuronal tissue begins to degenerate at the end of their life time, portions of that consciousness are lost with the degradation of the physical tissue to which it was bound.

Because regeneration of neuron cells does not occur in the majority of the adult brain, it can be argued that the consciousness of that person, which is developed over their lifetime, is inextricably bound to the flesh and thus when the flesh degrades at the end of its lifespan, so too does the consciousness. Indeed, this degeneration

occurs throughout the adult life at a rate of 80,000 neurons a day to the extent that, at 80 year of age, the accumulative loss of neurons amounts to 10 % of the brain's original capacity [26].

The advances made by nanomedicine, either through repair or replacement will cause the artificial prolongation of said consciousness longer than the original flesh to which it was once connected. As the flesh is the dominating identifying feature of all living things, the application of nanomedicine to this extent will force what will be for some an extremely uncomfortable discussion as to what it is to be not only a human but a disembodied consciousness.

13.3.1 Nanomedicine: The Cure for Death?

The only things certain in this life are death and taxes. The words of Benjamin Franklin have been reiterated for several centuries and have been proven to be true whenever one cared to test them. Indeed, death was a certainty for all living things long before the initial utterance of Franklin's zealously quoted line. Due to its inevitability, death has always held a revered position within the human psyche alongside which has been placed the desire to overcome it. One of the first high profile casualties of this quest for immortality was Qin Shi Huang, the first emperor of China circa 200 BC who discovered his mortality shortly after ingesting mercury pills ironically intended to endow the immortality he so sorely wanted [27].

In this more modern time, an increased understanding of the physiological mechanisms of aging as well as the advent of transplantation surgery and bionics has made an increase in longevity much more attainable. It is without doubt that nanomedicine will have a substantial role to play in this process however, what can be called into question is to what extent will the 1,000 year old man, as heralded by Professor Aubrey De Grey to be born in the next 10–20 years [28], resemble today's perception of 'man'.

Modern medicine has adopted an increasingly mechanistic stance towards the body in the sense that individual parts can be removed, repaired or replaced. These procedures have been executed with increasing ease with respect to the passage of time, which has been facilitated by increasingly sophisticated surgical tools and autonomous surgical aides. The idea of transplantation is by no means a new one – Saints Cosmas and Damian, patron saints of surgeons, are said to have removed and replaced a cancerous leg of a Roman Deacon in the third to fourth century [29].

Organ replacement and transplantation is a procedure with a similar cornucopia of legends. An example of such transplantation can be seen in the story of the third century BC physician PienCh'iao who performed not only an organ transplant but an organ exchange. The motivation of such a procedure was to balance the constitutions of the two patients since one was strong of will but weak of spirit and the other was strong of spirit and weak of will [30]. Unfortunately for PienCh'iao, he did not publish in a peer-reviewed journal and thus, the validity of such a procedure must be speculated. The first verified human to human heart transplant was successfully

completed some twenty-three centuries later in 1967 by the South African doctor Christiaan Barnard, though the patient died just 18 days later due to complications arising from the obligatory immunosuppression medication administered to guard against organ rejection [31]. Organ rejection was not an issue for the first kidney transplant which was carried out in 1954 between identical 23 year old twins Ronald and Richard Herrick who, as it turned out were ideal candidates for transplantation surgery being immunologically identical and thus absent of the need for immunosuppression [32].

In this modern age, built upon such pioneering actions, this technique of replacing a 'faulty' organ with a healthy one from a donor is now common place with 3,740 organ transplants taking place in the UK in 2011, a record high from previous years [33]; a significant hurdle to this transplantation process is encountered when a suitable match is searched for which is most often contingent on the death of a secondary individual which is then used as the donor. Even at this point, there is still chance of the organ being rejected by the receiver's immune system, as was encountered by Barnard immediately following the first heart transplant, which requires the recipient to take immunosuppressants for the rest of their lives to protect the donor organ through the inhibition of the host immune responses [34].

The consequences of extended immunosuppression can be in intuitively deduced as a decreased resistance to infections and malignancies; a side-effect which has serious connotations on the quality of life of the organ receiver. Even so, the alternative to a transplant for most is organ failure which results in death or reliance on artificial organ assistance and substitutes most commonly recognised in the use of an implanted pacemaker, kidney dialysis, a colostomy bag, respirator use or an artificial larynx. Substitutes have been invented for each of the many components within the human body, all of which seek to restore some quality of life to the patient in the face of the failure of their natural organ. All of these, including those mentioned above, are pale comparisons to the physiological marvels that they attempt to replace.

In this nano-age, medical technology has reached a level which was previously only conceivable on the pages of science fiction publications. This is the emerging world of regenerative medicine. This facet of medicine has long been embodied in the 50 year old application of 'skin farming' to grow epidermal grafts for burn victims [35]. In more recent times, increasingly sophisticated laboratory techniques have facilitated the *ex vivo* synthesis of various more complex organs; already, basic organ and tissue analogues have been reported such as of bladders and urethras [36, 37], as well as more complex organ structures such as hearts and neural tissue [38, 39] and even, most recently, the synthesis of functioning human livers from pluripotent adult stem cells [40].

What must first be noted from these cited breakthroughs is the striking decrease in the separation time between them. This is to say, if it took over 40 years to make the transition from straightforward epidermal tissue cultures to a simple organ such as a bladder, why did the transition from a static and relatively simplistic organ to a complex and active organ such as the heart only take a further 2 years? The answer comes from the accelerative nature at which technology advances – from the shoulders of

giants indeed! New nanomedical techniques such as nano-manipulation, for example, vastly increase the speed at which cellular and even intracellular handling can be done. Techniques such as cell identification and sorting are pivotal steps within the process of organ regeneration; the location and isolation of the viable stem cells of the original organ must be carried out. These must then be introduced onto a prefabricated scaffold and cultured in order to ensure their successful adherence and growth. Efficient cellular and intracellular manipulation has been made possible or enhanced through techniques and tools birthed by nanomedicine. A prototypical example of these techniques can be given by the optoelectronic tweezers which can facilitate the manipulation of cells with a rapidity and resolution never before seen [41, 42]. Armed with such advanced tools, a greatly enhanced understanding of cellular behaviour can be achieved; an understanding essential for the complexity that is implied by the growth of a complex organ structure.

The advantages of being able to 'regrow' an organ from a patient's own organ stem cells lies in the elimination of reliance on immunosuppressants to guard against rejection which in turn will increase the life-years of the patient. Indeed, these advances in organ growth have occurred parallel to breakthroughs in antimicrobial nanoparticle treatments the prototype of which can be seen in the use of nano-silver. The novel use of nanosilver as an antimicrobial is most notable in its application as a wound dressing which have been seen to be effective treatments of otherwise fatal burns and likewise could be used to protect the immunosuppressed from otherwise fatal infections [43].

To complement the progression of organ regeneration and transplantation, there has also been seen an increase in the complexity transplantations successfully executed. Hand transplantation is now relatively commonplace [44]; building on this, the first double leg transplant was also carried out in July 2011 [45] whilst the first full face transplant took place in March 2010 [46] with partial facial transplants having been carried out successfully for half a decade [47]. The increased complexity and precision demonstrated in these landmark procedures are made possible through advances in surgical tools and most apparent in the increased complexity of the robotics used in such procedures.

13.3.2 Cellular Surgery and Beyond

Enhancement of 'conventional' surgeries, taken as manipulation of tissues, can be brushed aside as the mere infancy of nanomedicine. Intracellular surgery is also becoming a reality through the application of nano-procedures. Given that the regeneration of an organ is now becoming a reality through the use of a subject's stem cells, the next hurdle to overcome will be the complete halt of cellular aging which can be defined as the shortening of stem cell telomeres, located at the end of each chromosome, which occurs during every cell division. A possible solution for the slowing of this aging process as proposed by conventional medicine can be taken as the isolation of the resveratrol, the speculated 'anti-aging' ingredient in red

wine [48]. Assuming that this compound does have such an effect, it merely has a retardation effect and does not completely halt aging with respect to the passage of time. Nano-surgery, which is carried out using nano-tipped needles, can probe into the interior of living cells without causing damage to the cell itself [49]. The connotations of such a procedure will again force a radical re-evaluation of convention. While still in its infancy, this technique has been cited as to be used as a great aide in the study of cellular diseases and other cellular components [50]; to bring this idea further, there has already been synthesised artificial human telomere 'nanocircles', designed initially to study the aging process more closely [51].

It is quite plausible that this technique could be carried out ahead of the organ regeneration procedures that were discussed previously. In this manner, theoretical immortality could be achieved in the sense that repairs and replacements for the various corpus components could be manufactured and executed using a subject's stem cells with artificially elongated lifespans. This being said, whilst the idea of cellular surgery is with nano-needles is impressive, even now there is an increasing possibility that this will seem to future generations' eyes as the idea of a witty talking servant-robot seems to this generation (Sect. 13.1). This is because the idea of cellular surgery using a nano-tipped needle is an approach from the top-down which is one of the established and time-proven conventions of technology to date. As was discussed is Sect. 13.1, a shift in the paradigm of medicine is occurring at present embodied by the bottom-up approach. In the case of regenerative medicine, an example of this is the reprogramming of adult cells to their pluripotent and totipotent forms *in vivo* as was reported by Abad and colleagues in September 2013 in *Nature* [52].

Just as it has been seen within the field of particle synthesis, while the top-down approach for nanomedicine is an uncontestable revolution in itself, it is also, at its heart, an extension of existing top-down conventions. The true revolution, in many ways, is still yet to come in the form for the full implementation of bottom-up approaches to the clinical setting.

To use an example within the field of regenerative medicine, the manipulation of cells *in vitro* has already been reported and has been exploited to tailor-make replacement body parts within a clinical setting with the use of the patient's own stem cells thus eliminating the possibility of rejection that plagued conventional organ transplantation. This is embodied in the landmark success of the *in vitro* growth and subsequent transplantation of a tracheobronchial section into a patient in 2011 which was made using a synthetic tissue scaffold and multipotent adult stem cells [53]. While this is extraordinary to see happening in a clinical setting, the report of the *in vivo* manipulation of cells in animal models to reprogram them to an embryonic state, as stated above, has the potential for implications that could dwarf that entailed by the advent of tailored body parts and organs. When such techniques come to clinical fruition, it may be possible to dispense with surgery as it is known today altogether opting instead to reprogram the 'malfunctioning' cells *in vitro* and likewise to have patrolling nano-doctors (Sect. 13.3.3) to detect and prevent disease and other ailments as they occur.

Indeed, if these land mark procedures were not astounding enough, attention must also be drawn to the more bizarre transplantation procedures which have been

completed in the past. Experimentation centred around the transplantation-of-consciousness hypothesis which was carried out in the mid-twentieth century in both America and Russia was seen to be largely successful [54]. In these experiments, the heads of animal subjects were transplanted onto secondary bodies, following the reattachment of the blood supplies, the re-embodied head was seen to regain consciousness and respond to various stimuli thus providing preliminary proof that such a drastic transplantation was possible. The heads of the American and Russian Laboratories, Doctors Robert J. White and Vladimir Demikhov respectively, were greeted with mixed responses to their landmark work. Undoubtedly a good number of these negative responses were due to the drastic contrast between the level of practiced medicine of the day and the complexity which head transplantation implied.

Indeed, as was previously discussed (Sect. 13.2), it is often the case whereby technological research and advancement is obliged to wait for the public perceptions to catch up. An example of this can be taken as either gene or stem cell therapy whose progress experienced notable retardation through financial starvation. This was due both to the ethical grey area which these pioneering therapies had unearthed but also, in the case of gene therapy, due to the death of clinical trial subjects [55, 56]. In the case of the head transplant procedure; the key feature which was missing from the original experiments was a mechanism of reattachment of the spinal cord thus completing the interface between the head with the donor body.

Incorporation of nanomaterials into neuronal therapy has led to the development of techniques for the regeneration of injured tissue. Growth factor-impregnated scaffolds made from aligned polymer nanofibres have been seen to induce rapid regrowth of the spinal cord tissue. The use of this technique could be highly advantageous for such a procedure as head transplantation because within its primary growth-promoting role it also suppresses the formation of scar tissue, an occurrence that severely hampers any further attempt at reattachment [57, 58]. The employment of magnetic nanoparticles has also been proposed as a mechanism for reattachment of spinal cord tissue. The use of the mechanical force provided by the nanoparticles would also void the former dependence on growth factors [57, 59]. It must be noted that such innovative and revolutionary proposals have occurred quite recently, as the date of the cited publications will demonstrate, and are still very much in development; however, they do lay the foundation of a surge of growth in the sector of central nervous system regeneration which will play a key role in humanity's route to immortality.

The concept of head transplantation must not be dismissed as something that could never occur. It must be noted that less than a decade ago the idea of clinical use embryonic stem cells was still somewhat of a taboo. Exhaustive promotion from the late Christopher Reeve seemed, in the eyes of some at least, to be in vain. There was however, a shift in public perception which was coaxed by a greater understanding of what the therapy entailed which was brought about by such campaigning. This education of the public and subsequent change in governmental policy has led to the first embryonic stem cell clinical trial being executed in October of 2010 [60]. The acceptance of this preliminary trial achieved somewhat of a flood gate effect. There are now several trials using embryonic stem cells throughout the world

many of which are achieving notable success; Success which can be personified in the UK based stem cell trial for the treatment of Stroke victims [61]. This trial achieved its initial goal with enough authority to merit its extension which was announced at the start of September 2011 [62]. As of September 2013 the company reiterated its commitment to its targets and projected the start of phase II trials for the aforementioned treatment, codenamed ReN001, as well as the commencement of phase I trials of second treatment, codenamed ReN009, for the vascular condition ischemia by the end of the year [63]. The acceptance of stem cell therapy could be a very good model with which to project the acceptance of the radical procedures that nanomedicine will lead to, in the same way that the success of the ReNeuroncan be an indication as to the potential rewards for the future of healthcare which await such an acceptance.

To return again to the idea of head transplantation, in terms of the complexity that such a procedure would entail one must only look to the progress made in the human genome project. The project, having started in 1990, completed an initial 'draft' in 2000. A further 3 years saw the announcement of the true completion of the human genome [64]. What followed could be likened to the opening of a flood gate for research whereby it was attempted to map the genomes of each demographic. This flood of research has progressed to such a stage that, as of the end of 2011, there was an estimated 30,000 completed human genomes [65].

It must be acknowledged that a procedure such as head transplantation, which is arguably becoming increasingly feasible with the medical advances discussed above, does not seem as outlandish an idea to some as one might think. The practice of cryogenically freezing the body directly following death is becoming somewhat of an accepted phenomenon. This follows the 'cryonic suspension' of the first person almost half a century ago; one James Bedford, a professor of psychology and well-known advocate of the then-theoretical technique. As could be expected, the speculative preservation of his body in January of 1967 was greeted with controversy and several court cases [66]. Since then, there has been an explosion of companies all of which offer this same service, for a fee which is argued to be nominal relative to the benefit it is speculated to provide. A list of which can be seen in Table 13.1.

What is most interesting in the context of this discussion with regard to this speculative freezing process is the option of 'neurosuspension' which is the preservation of the head of the subject only. In contrast to the freezing of the entire body, the

Table 13.1 List of organisations which offer cryonic suspension

Company name	Location	Foundation
Alcor Life Extension Foundation	Arizona, US	1972
American Cryonic Society	California, US	1969
Cryonic Institute	Michigan, US	1976
EUCrio	Braga, Portugal	2010
KrioRus	Alabychevo, Russia	2005
Suspended Animation Inc.	Florida, US	2002
TransTime Inc.	California, US	1972

neurosuspension option offers to suspend the disembodied head or 'consciousness' of the patient in the speculation that, in a time when technology can facilitate 'reanimation' of a patient, it would also be viable to synthesise an entirely new replacement body for the bodiless 'person' [67].

Technological advances have already progressed this reanimation process a great deal; while the complexity of reviving a brain is still beyond the reach of current technology, increasingly complex organs tissues and, more recently, whole organs are being successfully frozen and then replanted [68, 69]. The primary source of damage in this case is the formation of ice crystals within the tissue; again, there have been proposals to bypass the need even to keep the physical remains of the subject by 'downloading' the respective consciousness to a secondary location via the concept of total brain emulation (Sect. 13.3.4). However, this then forces the need to seriously debate and define for the fathomless depths of what it is to be human, an individual and perhaps even, a consciousness itself (Sect. 13.3.6).

13.3.3 Nano-Doctors: The Perpetual House Call

The concept of *in vivo* 'nanofactories' (Fig. 13.4) has also been put forward as a future direction of medical research. These factories, described as pseudo-cells, would exist *in vivo* in order to detect and diagnose microbial infiltration, malignancies or structural damage as it occurs. These structures would then synthesise an appropriate therapeutic in situ utilising ambient physiological molecules to do so [70]. This concept builds on many of the present novel functions of nanomedicine such as targeted delivery, stimulus-dependent execution of function and monocellular specificity. The advantage of these systems would be the prevention of illness or structural damage through the removal or neutralisation of the threat before it can manifest its conventionally recognisable symptoms.

Steps towards the realisation of this programmable nanofactory can be seen in the production of a 'synthetic life' within the laboratory of Craig Venter [71]. In this experiment, bacterial cells were reprogrammed with synthesised DNA to the end that the bacteria would then execute the predetermined function within its environment.

It must again be acknowledged that achieving such a landmark as the creation a synthetic cell, a feat that for so long was confined within the realm of science fiction, has been on the technological agenda for some time now with tireless research and development being carried out to achieve it. The progress that could be expected within the field of synthetic cell design could be expected to be analogous to the exploitation of viral protein cages as encapsulation and delivery entities, which can be taken as a breakthrough along the same vein and preceding that of the manipulation of a bacterial cell to carry out a specified function, is a technique over a decade old [72]. Following the initial breakthrough, the usage of the technology quietly grew within its primary niche, gene delivery, to such an extent that as of 2011, it was being used in approximately 70 % of the 1,714 gene therapy clinical trials underway worldwide [73]. Indeed, such progression from manipulation of a simple viral structure to

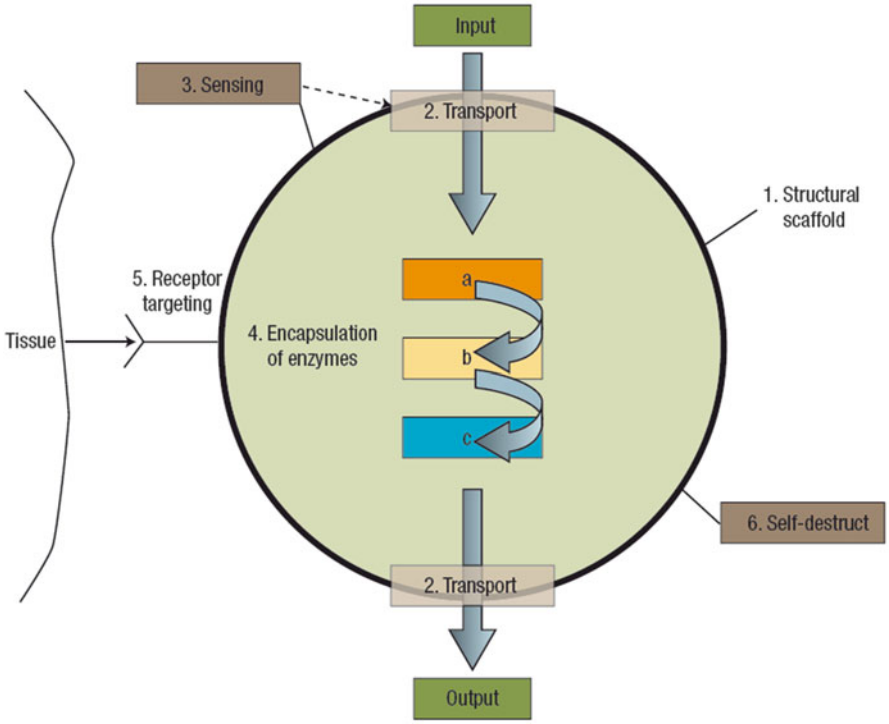

Fig. 13.4 Conceptual structure of a 'nanofactory' consisting of (*1*) a structural membrane or scaffold, (*2*) a mechanism for transportation of biomolecules in and out of the factory interior, (*3*) a sensory application, (*4*) biochemical machinery or enzymes for compound manufacture, (*5*) a targetable ligand attachment for targeting within the body, (*6*) an inherent self-destruct mechanism triggered upon completion of the nanofactory's primary goal (Reproduced from [70])

prokaryotic cell can be used to extrapolate the time to progress from prokaryote to complex eukaryote. The 12 years between these two landmarks could be expected to short relative to predictions, given the experience of the previously discussed human genome project (Sect. 13.3.2) in terms of rapid acceleration in progress following the achievement of the initial goal.

13.3.4 Nanomedicine and Prostheses

The ultimate melding of technology to medicine can be found in the form of increasingly sophisticated interfaces between man and machine. This has long been the goal of prostheses. Two of the main limiting factors within this quest can be given as intricacy and deftness. While each one is nigh on synonymous with the other, the pair can be separated thus: intricacy implying a complex interface, such as a device

'plugging in' to the brain and deftness signifying the extent to which the Darwinian perfection of a biological organ or appendage can be mimicked.

An example of this deftness can be found in one interesting and most recent development which was announced in May of 2013. This was the approval of clinical trials of a biosynthetic hybrid artificial heart, the most advanced of its kind to date [74]. This approval comes approximately 4 years after the granting of the patent rights to this design [75]. The incorporation of a biological element within this synthetic device comes in light of the increased manner in which biological tissues can be manipulated due to technological advances and an increased understanding of the behaviour of such materials as discussed in Sects. 13.3.1 and 13.3.2. In this instance, the use of such 'living' materials reduces complications experienced by previous forays into bio-mimicry such as blood clot formation and device rejection by the host. The manipulation and incorporation of, in this case, animal tissues in to a clinical device can be taken as an indication as to the direction in which this particular field of prosthetics is headed.

In relation to intricacy, there is no organ with a greater inherent intricacy and importance than the brain. It has become possible to interface directly with the brain or nervous system so as to vastly increase the utility of exterior prostheses: eyes and ears for example. The argument in favour of such prostheses has always been foiled due to their lack of sophistication in contrast to the biological component they attempted to mimic, caused predominantly because of the barrier which stood between the user and the appendage. This was due to the stark contrast between the then-current state of the art and computing power as well as electricity required to process the massive amount of data which a biological appendage amasses through its intricate function.

A direct interface with the brain need not be as futuristic a concept as may be initially thought. Indeed, both cochlear and sight-enhancing implants are both well-established concepts. Cochlear implants are perhaps the most basic methods of interface with the brain and certainly one of the least invasive or, at least, one of the most accepted [76]. Their wide spread use can also be contributed to their comparatively basic technological requirements relative to the other senses.

Visual aids implanted directly into the visual cortex can be viewed as something of a threshold because, in contrast to the cochlear implant, which is interfaced through the cochlear nerves within the ear, or indeed the research into interface with the optic nerve [77], such a visual aide requires interfacing directly with the brain. This idea, again, sits upon comparatively ancient roots with the first attempted cortex-camera interface being attempted in the early 1970s [78]. This initial attempt, as one could imagine, was a long way off the capabilities of the biological eye, nonetheless, it was a decisive start. Though there has been notable progress in the time between then and now, the attempt of interfacing such a complex network as the visual cortex with an artificial component highlights the limitations of non-nanotechnology based systems. This limitation is the phenomenal intricacy with which the many tens of thousands of neurons are connected within the cortex [79]. In order to emulate a system with such complexity, a radical rethink must occur at the fundamental level of established computing convention.

This radical rethink is already in motion today with nanotechnology as its primary driver. In order to recreate the exquisite detail and efficiency of the human or indeed any biological entity with cognitive function, an overhaul in the base hardware units of computer processing must occur. An artificial synapse connection has been synthesised with the use of carbon nanotubes [80]. This can be hailed as the beginning the design and synthesis of more complex brain prostheses which in time could be part of routine surgical treatment for degenerative brain conditions. In parallel to this development is the work to replicate hippocampus structure in which would serve to save the memory of a person which would have been lost due to neural degeneration [81]. This breakthrough, perhaps more so than any other has potential connotations in lieu of its proposed application to essentially 'store' a portion of an identity within it. Subsequent to its announcement in 2003 where it was hailed as 'the world's first brain prosthesis', further work carried out proved the concept such that the artificial chip could cause recollection of a predetermined function in the animal models used [82].

Complementarily to these initial animal models and hailing another major jump into the future of medical treatments, actual clinical brain interfacing has also been established for some years. The first direct interfacing device which read an output from the brain was achieved in 2004 when a severely paralyzed patient was interfaced with a small hundred-electrode sensor which interpreted the signal in a manner which could then be used to direct a cursor around a computer screen [83].

Mediation of brain signals has also become an accepted clinical practice for the treatment of 'faulty' brain function arising from debilitating neuronal disorders personified by conditions such as Parkinson's disease or through traumatic brain injury. Though deep brain stimulation for the inhibition of symptoms, as is the case for Parkinson's, has been well established having been introduced more than 20 years ago [84], behavioural modification through the implantation of a brain 'pacemaker' has also been shown quite recently [85]. With the implantation of such a device, many quality-of-life functions can be returned to the patient which were previously been lost through injury. This artificial interface is also being proposed from non-neurodegenerative conditions such as depression and schizophrenia [86, 87].

What is being observed in the progression of the research cited above is a movement towards a sophistication of medical treatment which undoubtedly will not be able to progress without the aid of nanomedicine. To take the goal of total human brain emulation as an ultimate goal, the essentiality of nanomedicine as a chaperone becomes clear. The human brain can be viewed as the ultimate engine from which all human knowledge stems. As a physical entity, its complexity is gargantuan; consisting of a network of 100 billion neurons each of which can have a connectivity of up to 10,000 synapses [80]. The computing power of the human brain has been estimated to be equivalent to 100 teraflops (floating point operations per second) [88]. Though such high performance rates have been achieved as far back as 2003 [89], the energy requirements to achieve this, cited to be enough to power 7,500 domestic homes, are grossly inflated from that of the biological brain, generally given as approximately 20 W depending on cognitive load, which it was attempting to match. If brain prostheses are going to be a viable answer to the problems manifested by

age-related neurodegeneration, incorporation of nanotechnology in unavoidable; with the trend towards neurodegeneration seen in the population now, it can be reasonably speculated that the demographic effected will grow a great deal in the coming years and thus so too will the global dependency on increasingly more complex and innovative neural prostheses.

Increased feasibility of the idea of total brain emulation has led to the publication of speculative predictions of the requirements of such a feat. The most notable of these could be taken as that published by the Future of Humanity Institute based out of Oxford University [90]. In this report, a systematic mapping of all the aspects and connotations of brain emulation is done. What is acknowledged first and foremost is the speculative nature of such report in the sense that such a reality as total human brain emulation is so far off at this point and subject to the advents and changes in technology that a true accurate prediction is not a real possibility.

13.3.5 The Tithonus Error

Just as the direct positive effects of nanomedicine can be traced back to a deep seated desire for extended life echoed on mythology, the indirect implications of nanomedicine can too be traced back to such fabled roots also. Already, the less-than-desirable consequences of having an artificially extended life span can be clearly identified. This is primarily the notable decrease in quality of life which accompanies old age. This concept is known as the Tithonus error [91]. Tithonus was a Trojan prince made famous because of the deal struck between his titan lover, Eos, and Zeus. She implored Zeus to grant eternal life to him though by forgetting to request eternal youth to compliment it, Tithonus was forced to endure eternal life without eternal youth.

As was discussed previously (Sect. 13.3), medicine has brought this generation to a point, as demonstrated by the aging of the population, at which the Tithonus error is more apparent than ever. This can be generically embodied by dementia, which can be likened to the degeneration of the very character of the person through memory loss et cetera. More so than before, an emphasis must be placed on improving quality-of-life in old age at the very least equal to that on curing diseases relating lifestyle such as cancer and organ failure which previously served to shorten life span.

One must only look at the advent and popularisation of the euthanasia movement and its subsequent legalisation in such places as the Netherlands, Belgium, the US state of Oregon, the northern territories of Australia and Switzerland [92] to see the growing need for further research into quality-of-life improvements for sufferers of chronic and debilitating conditions, as discussed below, as well as a greater acknowledgement for the need for further debate for such treatment or action as euthanasia. Indeed, the reality is that with such boarder-dissolving agreements as were made to create the Schengen zone in mainland Europe, it is becoming more and more complicated to enforce an individual nation's laws regarding such an issue as euthanasia. The ability to travel from a country where such a procedure is still illegal, such

as the UK (which incidentally is outside of the Schengen zone though a member-state of the European Union), to a country permitting it, such as Switzerland, was documented by the celebrated British author and diagnosed Alzheimer's disease sufferer Terry Pratchett in the controversial 2011 BBC documentary 'Terry Pratchett: Choosing to Die' [93]. The creation and airing of such a program demonstrates that those with a will to do such a thing could, can and do indeed do it, with or without the approval of their native country as well as the growing awareness of such a practice as euthanasia in the public psyche.

The end of the first decade of the millennium marked the start of the arrival of the Post-World War II 'Baby Boomer' generation to retiring age. With this landmark passed, the world population is set to undergo a dramatic change in demographic. Between 2000 and 2009, the proportion of the world population over 60 increased by 14 %. Indeed, this has followed a tripling of this demographic from 1950 to 2000. Extrapolation of this trend projects a further tripling of this demographic between 2000 and 2050 [94]. This is expected to send dependency ratios, which is measured by the volume of the population aged above 65 and below 15 years versus the proportion of the population within the 'working' band of 15–65, as high as 70 by 2050 [95].

Chronic illnesses, which can be taken as inevitable in an increasingly elongated lifespan, can be divided into three categories. These can be given, as mentioned previously in this section, as cancer, organ failure and dementia [25]. The first two, cancer and organ failure, have received a majority of the attention from nanomedicine research due to their malicious track record of dramatically shortening life spans. While innovation stemming from nanomedicine has caused a greatly increased chance of overcoming these conditions, the repercussions of overcoming these previously terminal illnesses will be profoundly far-reaching. Such a triumph of modern medicine over these illnesses will cause, and to an extent has already caused, an upheaval in medicine towards increased emphasis on age related ailments which have chronic rather than acute onsets.

Such an upheaval in medicine has been a long time coming; palliative care has long been the sole consolation to those afflicted with the Tithonus error; though as stated above, euthanasia, though somewhat of a taboo, has been practiced in throughout history as it is being practiced in this day and age. Palliative care is not something isolated to the comparatively recent advent of nanomedicine; the hospice movement which can be linked to the tripling of the over-1960s demographic between 1950 and 2000 having been started at approximately the same time [96]. In pre-twentieth century medicine, the need for such palliative care was limited due to the shortened life expectancy of the time. The twentieth century saw the advent of better and more sophisticated practices through greater understanding of the diseases and conditions being treated. In hand with this, came medical advancement and new techniques and treatments such as radiotherapy [97], chemotherapy [98] and the first mass-produced antibiotic, Penicillin [99] all of which came to be in the first half of the twentieth centuries.

The acceleration of medical technology within the twentieth century increased numbers requiring such palliative care to the level that provoked the commencement

of the hospice movement which has been carried on since then to the present day. At this point, the advent of nanomedicine has begun a revolution in healthcare not dissimilar to that seen in the twentieth century in the sense that it will remove the death sentence that were for so long associated with certain diseases. This will, again, cause an exponential growth within the medical field in the manner which originally provoked the aforementioned movement; the difference being that in this modern age, mere palliative care will not be an acceptable option. Instead, medicine must embrace the radical change in perspective which will undoubtedly be greater than anything that has been seen before as well as dealing with the ethical dilemmas that will be pushed to the forefront because of it.

13.3.6 A Brave New World of Nanomedicine

Undoubtedly there will be logistical and economic problems that are associated with an increasingly long-lived population stemming from increasing demand and dependency on finite resources as were foreseen in the Malthusian population model, as too will there be ethical issues surrounding the increasingly radical treatments that will be required to ensure the aging populous has a quality-of-life justifying their prolonged longevity and indeed, the radical interventions that may be deemed necessary if this quality-of-life cannot be achieved or sustained namely, the option to choose death rather than a prolonged life of insufficient quality (Sect. 13.3.5) There are, however, far greater connotations that nanomedicine will be involved in as a result of its increased acceptance and application. The most drastic of these stems from the increasingly successful treatment of neural conditions and neurodegenerative diseases via artificial means.

The concept of brain 'pace markers' has already been discussed above (Sect. 13.3.4); while still in their infancy, they are predominantly used as mediators of basic function. This is implicated in their use to treat Parkinson's disease by way of dulling the bodily tremors that are symptomatic of the condition. This form of neurodegeneration does not impinge on the personality of the person and thus can be isolated in the realm of physical debilitation directly caused by a neurological degeneration. Proposals for the treatment of depression and schizophrenia, conversely, can be taken as disorders far more related to genetics and personality of the individual [100, 101]. Given that these proposals are occurring at the relatively young age of the technology can be taken as a sign of the potential scope of such a technique. Personality and behaviour modification or modulation through interface with an electronic controller has the potential for a striking improvement of quality of life for those suffering debilitating conditions which may or may not be related to age. The intimate connection that such an electronic device provides between the brain and the controller, particularly with the intricacy being allowed through nanomedicine, has the potential for invasive mind control, be it intentional or not. Of course it is true that the same can be said on some level about any existing psychoactive drug but the introduction of an electronic device into the brain is a step beyond

the ingestion of a pill since there is the potential for far more direct control of the targeted aspect of the subject.

Progressively more complex proposals of 'mind control' are being proposed as solutions to behavioural disorders. Indeed, promising results have been yielded from the use of deep brain stimulation to combat eating disorders and alcoholism [102, 103]. The ability to artificially control such powerful behavioural traits has implications which have been heralded for quite some time. Indeed, it was a central theme in Anthony Burgess's 1962 dystopian novel 'A Clockwork Orange' in which the protagonist was conditioned to feel violently ill when confronted with violence – or classical music – so as to render him unable to be in the presence of violence via virtual incapacitation; when this secret was found out by one with motivation to harm the protagonist, this conditioning was then used in a malicious way to his apparent-detriment [104]. Willingly surrendering control to a secondary entity, be it behavioural conditioning or an implanted computer chip puts one at the mercy of those who hold the controls. This sentiment is superbly represented in Author Koestler's words [105]:

> Who is to control the controls, manipulate the manipulators?

Indeed, this is not limited to the potential of direct behavioural manipulation; such a question arises with the proposed use of 'nanofactories' whereby a level of intelligence is bestowed upon an entity which is then entrusted with a colossal responsibility: the health of the subject. More so than ever before, there will be potential for mankind to do increasingly deistic acts the repercussions of which may not be seen for some time afterwards. A model for such repercussions can be seen in the emergence of nanotoxicity (Sect. 13.5). A very fine balance must be struck between caution and optimism. Given the cautionary tales of the past as well as the potential for harm which nanomedicine possesses, each step taken in the progression of nanomedicine must not be taken lightly.

Less novel questions must also be confronted with new vigour in the face of the accelerative effect which nanomedicine is having on medical research. This is namely, what does being an individual or indeed a human entail? The idea of a soul may seem to some to be out of place within a scientific document but certainly, the idea of the personality of an individual is a concept which is universal.

In this age of modern medicine, there is an increasingly mechanistic view of the human body in the sense that, if a component is not functioning in a satisfactory manner, it can be repaired, removed or replaced. This attitude of medicine can trace its origins back to and beyond the pioneering work of the renascence anatomists which encouraged its students the view of the body less as the 'image of God' whose defilement could be thought to be sacrilege, and more as a complex mechanism of interdependent components [106]. It is quite interesting to contrast the interpretation of ancient cultures of the body-whole as the 'seat of the soul' to modern attitudes of medicine in the sense that it has not been until quite recently that the mind within the head has been qualified separately from the rest of the body. The roots of this distinction can be recognised in the words of Juvenal, a roman poet who wrote in the early second century:

> Menssana in corporesano

A healthy mind in a sound body. Even with this initial separation, there has always been an inherent link in terms of a person's identity with the physical body. With transplantation surgery becoming increasingly ambitious, as the concept of a full face transplant (Sect. 13.3.1) would indicate, medicine seems on some level to be distancing itself from such an idea. Even so, it cannot be denied that it is common for transplant recipients to experience some manner of change in personality traits in keeping with the foreign organ that has replaced their own [107–109] thus renewing the argument in favour of the ambiguity of the location of a person's 'soul' or consciousness.

The degree to which this testimony will impact the momentum with which transplantation and regenerative medicine is moving is undoubtedly small; however with the increase in population age will come a corresponding increase in the incidence of transplantations. It is a reasonable estimate that by 2050, the science of organ regeneration will have been greatly advanced if not perfected through nanomedical progress. At that point, the question as to how much a personality could truly be affected by an increasing number of replaced or regenerated organs or body-parts perhaps will perhaps be easier to answer, until then only speculation is possible.

More interesting even than this will be the behaviour of the first recipient of an artificial memory implant as has been cited previously (Sect. 13.3.4) whereas the exact location of the seat of the soul if not its entire existence is debatable, the hippocampus as the location of human memory is not. When the first memory implant is implanted, it will mark a profound step in human evolution toward disassociation from the physical body the likes of which has never before been seen. The personality of a person is so much based on the flesh and blood that the implications of the separation of the mind from the body challenge the very notion of human consciousness itself. Indeed, with the report of the successful deletion of targeted memories [110] and the announcement of the creation of false memories through direct manipulation of neurons in animal models, it is not unreasonable to foresee a time where memory, personality or behaviour of a subject could be altered at will until the desired effect was attained. This, coupled with the connotations of artificial implants such as were earlier discussed, is nothing short of a Valhalla for conspiracy theorists.

The next step, if not the definitive answer to this, will come with the first brain emulation whereby a new definition of life must be created to avoid complete indistinction from a non-living computer programme. This will be the ultimate realisation of the philosophical concept first described by Descartes as 'Dualism', or the relationship between mind and matter. Whether a mind can exist in the same manner outside of its physical body has been one of the most perplexing questions in philosophy since its proposition in the seventeenth century. This concept has been challenged by various futurists throughout the years with various degrees of scepticism; perhaps the most relevant to this discussion is the vision of Masamune Shirow portrayed exquisitely in the publication of 'Ghost in the Shell' [111] in which human-computer interfacing has blurred the definition of humanity to the degree where a self-aware computer virus could request diplomatic immunity. Of course, such a scenario is a great way away off but it is the privilege of this generation to be present at the dawning of the age of such significant scientific discoveries.

13.4 The Boy Who Cried … Self-Assembling Semiconductors!

As is the case in so many emerging technologies, it is extremely easy to allow oneself to get swept away by fantastical speculation. This is made all the more relevant given the topic of the preceding section and the seeds of hyper-expectation it sows. It must be acknowledged in the face of such grand predictions that nanomedicine is merely a facilitator or a means to achieve a step forward in science. In the words of Thomas Edison:

> Genius is one per cent inspiration, ninety-nine per cent perspiration

It is the nature of humanity, be it industrial, commercial or the public, to speculate on any up-and-coming market or technology with the potential to yield a profitable end. This mentality has littered history with the remnants of burst bubbles; relics of man's fondness for snake oil, which were superimposed over emerging markets throughout history. Within this generation there have also been several; these are most commonly recognised in the dot com bubble of the late 1990s or the more recent world banking crisis of 2008 respectively, the implications of which are still being felt to this day. The root-cause of all of these market plunges is an inherent hyper-expectation which causes an over confidence in the speculated progression and growth of a trend, be it technology or purely financial. Scandal as a consequence of hyper-expectation has also occurred within nanotechnology and, though it was spared the magnitude of financial grief seen by previous and subsequent bubbles, the academic turmoil it caused has given harsh perspective to any overly zealous speculators in the field.

This scandal occurred at the turn of the millennium. In the late 1990s, Jan Hendrik Schön, a newly graduated and highly promising physicist doctorate, was awarded a position in the world renowned Bell labs. Bell labs' reputation can be indicated by the 11 Nobel laureates associated with the establishment through its history [112]. Schön's research interest centred on condensed matter physics and nanotechnology; true to the reputation of his employer, within a short period of time, Schön reported a revolutionary break through: the successful synthesis of a self-assembling semiconductor using organic die molecules. Retrospectively, the achievements which Schön were claiming had been made a full 20 years ahead of subsequent projections in the case of self-assembling nanostructures (Fig. 13.2) and close to a decade ahead of the final realisation of a molecular transistor [15]. The timing of this discovery did not trouble anyone at the time it was reported due, perhaps, to the reputation and academic weight of the establishment making the claim.

What followed could be described as nothing short of a frenzied fit of publication which lasted nigh on 3 years. In this time, Schön was published several times over in some of the highest ranking and respected journals in the world; Nature, Science, Physical Review, and Applied Physics Letters all willingly and repeatedly published Schön's work to the extent that by 2001, he was being cited as an author in a journal every 8 days [113]. Capitalising on this momentum, he continued on to claim to have

made significant steps towards achieving molecular scaled transistors [114]. Such was the influence of the establishments backing his work, much of the scientific community were accepting, albeit with bemusement, that such prolific publication portfolio was the new benchmark for research and discovery within nanotechnology.

Following Schön's lead, a flurry of activity ensued as different institutions attempted to replicate the results cited in the literature. Following what was undoubtedly a vast investment of time and money from many of researchers in the field, the scandal of the century broke. Just 2 years into the twenty-first century and with nanotechnology still very much in its infancy, it transpired that Schön's work was fraudulent with much of the published graphical data being directly copied and pasted from one article to the next and originating from mathematical functions rather than experimentation. Following a formal inquiry, Bell Labs concluded that the lofty claims which had catapulted Schön to stardom were ungrounded [115]. Before this happened however, citations of Schön's bogus publications were countable in quadruple digits.

In the ensuing controversy in which Schön's explanation for his actions regarding the doctoring of experimental data was to magnify existing trends, when asked to procure this raw data, it transpired that Schön had erased it from his electronic records stating that his computer storage space was insufficient. In response to this, the there was a unanimous retraction of journal articles including seven in Nature [116] and eight in Science [117]. In total, nearly 30 articles from reputable journals had to be retracted whilst several articles had their content thrown into question and the scientific community stood on in disbelief [118].

The success of such a scoundrel to deceive the scientific community will no doubt ring hollow in the ears of researchers and potential investors alike when confronted with any further report of alleged breakthroughs within nanotechnology, in medicine or otherwise. One could quite reasonably argue that such perspective was achieved at a nominal price in comparison to the collapse of such bubble-growth in other fields. It was perhaps the rapid nature with which the situation grew and the scandal was exposed which may have saved many from more painful losses or embarrassment. The worth of such perspective early on in the growth of a field is invaluable for level headed and objective growth and investment based on accurate projections of the outlook of the sector.

With this in mind, in an analysis published in Nature in 2011 [119], it was found that the number of retractions of published articles had increased 10-fold in the preceding decade based on data extracted from Web of Science and PubMed. It is interesting to see how far reaching the Schön scandal has been for the scientific community in the sense that, though post-2005 saw the majority of the retractions coming from lower impact factor journals, the statistics for the years 2000–2005 place Nature and Science on the podium for the most retractions with approximately half the quantity of retractions issued by each coming from the Schön scandal. It is also interesting to note that of the total retractions cited, a full 44 % of these were removed due to misconduct with another 11 % removed because of irreproducible results, leaving 45 % the categories of honest error and 'other'. This would imply that while the Schön scandal was shocking and unprecedented from the point of the sheer volume of fraudulent data published by an individual in an emerging

field, it could be unfortunately argued that regardless of the field, the dynamic and speculative nature of science lends itself to mistakes and null hypotheses. Thus all published results and claims must be greeted with a measure of scientific scepticism in keeping with the magnitude of the claims being made, regardless of the reputation of the institute, individual or journal making and publishing the claim.

13.5 Nanotoxicology and Regulation

A primary attenuator of realisation of the initial hyper-expectation of nanomedicine is the emerging field of nanotoxicology. This field incorporates the less desirable effects of nanomaterials within the physiological environment. Just as nanomedicine has brought a new wave of innovation and growth, the corresponding emergence of an entirely new and previously unknown form of toxicity has also been seen. Nanotoxicology documents material toxicity at a level of complexity far in excess of the simplistic and well established Paracelsus model for modern toxicity whereby the primary variable is dose alone [120].

Indeed, the nanotoxicity paradigm contains several variables within a single material alone. These include size, shape, structure, solubility and surface charge, and chemical composition [121]. The discovery and development of this subdivision of the field came in parallel with on-going research and application of nanotechnology in medical treatments. It is almost two decades since the Food and Drugs Administration (FDA) approved first generation nanomedicines (Fig. 13.2) in the form of liposomal preparations, this approval is widely accepted to have been made before a true understanding of the issues which the application of nanomaterials would present [42].

The discovery of the potential adverse effects of nanomaterials sparked a global scramble to put in place adequate regulation for the sector. This move, whilst very much required for the safe integration of nanotechnologies into medicine can be identified as a significant bottleneck for the transition of nanotechnology from concept to clinic.

With the ever-increasing scope of nanomedicine as well as the two decade time period since the clinical approval of the first nanomedicines, it might be surprising to know that the FDA until did not have guidelines specifically pertaining to nanomaterial-containing products until April 2012. It was at this point that two draft guidelines were issued for nanomaterial-containing food substances [122] and cosmetics [123]. Each of these documents, described as non-binding recommendations, were issued for public comment in the hopes that the previously broad regulatory approach, where new nanoproducts were reviewed alongside non-nanoproducts, would become a more nuanced affair with the aide of increased understanding and information as well as this requested public input [124, 125].

Similar to that in America, in the European Union the initial strategy was to neglect the publication of a clear official definition of ‘Nanotechnology’ instead opting for a ‘broadly inclusive approach’ to regulate nanotechnology under the umbrella of those existing frameworks; This situation arose due to the decision to

initially define nanomaterials under the pre-existing term of a 'substance' [126] up until 2010 when an official definition was released by the European Commission (EC) [127] in which it referred to nanopharmaceutics, for instance, as being covered by the pre-clinical safety precautions of pharmaceuticals. Though the definition of what constitutes a nanomaterial is useful, the move was intended to enable more efficient regulation and application so as to reduce the waiting time for therapies to receive a verdict. In this way, any ambiguity resulting from boundary conditions for nanomaterials such as size limitations were removed [128]. As of September 2013, as was done in the United Sates, a request for public contribution and recommendation for modifications to the REACH annexes on nanomaterials in order to improve regulatory efficiency [129]. Just before this, a request for tenders was issued for a study 'to assess the impact of possible legislation to increase transparency on nanomaterials on the market' [130].

The delay to implement regulatory measures is understandable given the connotations that inadequate legislation could have on research momentum. While the decision to include nanomaterials in an umbrella definition was a good short term solution, such a decision then obliged individual nanomaterials to be reviewed on a case-by-case basis. This lack of definition also permitted certain nanomaterials to be freely sold as medicinal agents under the definition of 'food substances' thus obliging the regulatory powers to redefine such definitions with the advent of nanotoxicology.

Taking nanosilver as a case study of this, the difficulties arising from retrofit, case-by-case legislation can be clearly seen. Colloidal suspensions of silver have been freely available for over 100 years to any member of the public who cared to buy it [131]. It was originally used in a diverse range of applications such as pigments, photographics, wound treatment, conductive or antistatic composites and catalysis. It was after the advent of penicillin and modern antibiotics, that nanosilver's antimicrobial properties were used specifically as a substitute for conventional antibiotics due to its apparent lack of adverse side effects commonly associated with antibiotics. The need for stricter regulation of nanosilver only became clear when it was discovered to potentially be a significant health risk due to its high cytotoxicity [132]. The sale of these nanosilver products, however, continued regardless due to the aforementioned lack of a differentiation between silver's bulk and nanoscopic modulus and corresponding absence of its specific regulation within the public domain. This lack of definition allowed companies to sell such products as health-promoting food supplements [133]. This unchecked domestic sale of nanosilver through this food-supplement loophole was finally stopped at the beginning of 2010 with the implementation of Commission Regulation No. 1170/2009 [134].

Attempts to regulate the nanomedicine sector at such a relatively late time in its growth has resulted in a unique limbo-esque situation which has been artificially created by various regulatory bodies whereby implementation of nanomedicine into daily public life has been, to a large degree, halted or at least slowed significantly from the pace at which it was being distributed. Such attenuation has come as a direct result of the better understanding and realisation of the true power and potential of medicinal nanomaterials which has, for better or worse, dulled the whimsical ideologies which had previously accompanied conceptual nanomedicine.

13.6 Conclusions and Future Outlook

Nanomedicine has yielded technologies and techniques that have and will revolutionise the field of medicine. This has been done utilising the materials, properties and phenomena which are unique to the nano-scale. Nanotechnology has provided the means to continue the progression of computing power and sophistication in keeping with and perhaps eventually even surpassing Moore's law. With the security that such an assurance as this provides, predictions can be made as to the future state-of-the-art. This law can also be used as an example of the hyper-expectation that has become part of this modern society. This generation has grown accustomed to the rapid evolution of technology to the extent that the rapid progression that has been seen and forecasted within the field of nanomedicine can be taken as normality.

Such hyper-expectation transfers to healthcare as well. Increasingly high levels of sophistication in healthcare and treatments have brought about an increased global life expectancy which is projected to increase further with time; in this sense, nanomedicine has a direct hand in the creation of the next crisis in medicine. For many years, chronic illnesses embodied by organ failure and cancer have kept net life expectancy below a certain point. Advances in treatments such as regenerative medicine, more efficient therapy and earlier detection, all of which have intimate connections to nanomedicine, have served to greatly increase the numbers now surviving to the extremities of old age. In this way, nanomedicine is having a direct hand the aging population of the first world which brings with it a radical change in the practice of medicine towards an increased focus on geriatric conditions. This transition also brings with it new challenges to healthcare as well to society and, with the population of over-60s set to triple between 2000 and 2050, will potentially create an 'identity' crisis within the population as an increased reliance on prostheses and pharmaceutics, which will go in hand with the aging population, takes hold.

With such lofty expectations and projections for nanomedicine, it is easy to find one's head in the clouds whilst speculating the future state-of-the-art. Such a societal ethos brings with it an increased vulnerability to the proverbial snake oil salesman in the sense that, as a consequence of the pace of progress, one must often rely on what is reported in the literature, rather than what is actually directly observed, to interpret the level of technological innovation of the day. This ethos has already proven to be a risky one for nanotechnology. With the conclusion of its first major global nano-related scandal, professionals and laymen alike have been forced to view the grand plans and projections offered by nanomedicine in the harsh light of reality in order to better estimate the legitimate future progression of the field and to differentiate such estimates from the farcical claims made out of context and well before their time.

There must also be a heightened awareness of the far reaching implications of the application of nanomedicine. These implications can already be seen with the advent of nanotoxicology and the scramble to retrofit regulation to an industry experiencing prodigious growth worldwide. A delicate balance must be struck between nanomedicine as a conceptual idea and it as a practiced discipline. The regulatory bodies, which are at the helm of the assimilation of nanomedicine into wide spread use, have

been charged with the intricate task of determining the safety of the nanomaterials used within these new techniques whilst simultaneously not entirely halting or bottlenecking nanomedical progress.

This generation has been granted the great privilege of witnessing the transition of society from the pre to post nano-age and from this vantage point, can be seen the melding of both conceptual hyper-expectation and the dawning realisation that comes with attempts towards implementation.

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Chapter 14
Nanomedicine as a Business Venture

Olivier Fontaine, Bojan Boskovic, and Yi Ge

List of Abbreviations

BIO	Biotechnology Industry Organisation
CAGR	Compound Annual Growth Rate
EC	European Commission
EPO	European Patent Office
ESF	European Science Foundation
ETPN	European Technology Platform on Nanomedicine
EU	European Union
FDA	Food and Drug Administration
FP	Framework Programme
GCP	Good Clinical Practice
GDP	Gross Domestic Product
GMO	Good Manufacturing Organisation
GMP	Good Manufacturing Practice

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IP	Intellectual Property
IPTS	Institute for Prospective Technological Studies
JRC	Joint Research Centre
NDDS	New Drug Delivery Systems
NIH	National Institute for Health
R&D	Research and Development
SME	Small and Medium Enterprise
VC	Venture Capital
WoS	Web of Science

14.1 The Current Nanomedicine Market

In the last few years, many marketing and/or scientific reports on nanomedicine have been published, based on the 3–5 years of data accumulation. Among them, the most highlighted reports in Europe include: (1) Scientific forward look on nanomedicine [1]; (2) Strategic research agenda for nanomedicine [2]; (3) NanoMed round table extended report [3]; and (4) Contribution of Nanomedicine to Horizon 2020 [4].

There is another important report [5] which executive summary was published first in Nature Biotechnology in 2006 [6], in addition to a comprehensive review on nanobiotechnology in the medical sector [7] and a very recent paper [8] on the state of investigational and approved nanomedicine products. Unfortunately, up-to-date data on the market landscape of nanomedicine still could be limitedly found on a free access basis due to the exceptionally rapid development of nanomedicine, intellectual property issue and business information/data protection.

14.1.1 Nanomedicine: A Global Market Analysis

14.1.1.1 A Market Overview

In 2004, there were already 38 nanotechnology-enhanced medical products on the market with estimated total sales of EUR 5.4 billion [5]. According to an European Science Foundation (ESF) Report published in 2005 [1], drug delivery applications account for three-quarters of the total nanomedicine market, with a special emphasis on novel drug delivery systems (NDDS) (23 products on the market). This statement was also reinforced by a French report stating that drug delivery applications are accounted for 58 % of the 36 nanoproducts already on the market (Bionest Partners and LEEM [9]). The reasons of such commercial interest and success will be addressed and discussed later in this chapter.

As further described in Wagner and his co-authors' report, for drug delivery, most products are based on liposomal or virosomal formulations [5]. In vivo imaging is highly represented by iron nanoparticles for liver tumours, whereas colloidal

gold is mostly used for in vitro *diagnostics* and lateral flow tests. Biomaterials on the market are for dental filling and repair or to tackle bone defects, while active implants are to cope with heart failure.

In the field of therapeutics, anticancer drugs seems represent the largest product segment of the nanomedicine market. According to the FP7 EuroNanoMed's Strategic Agenda [10], "of the 65 nanomedicine related trials identified in the. ClinicalTrials. gov registry, 62 were related to cancer treatment". Furthermore, as presented in a market report [11], cancer therapy will continue to be a top priority until 2016.

In terms of commercial nano-products, liposomes have one of the longest development histories, with successful drugs already on the market, such as Ambisome, a liposomal injection against fungi infection produced by Gilead (CA, USA) c.

14.1.1.2 A Breakdown per Types of Companies

207 companies based on nanomedicine activities were reported by the ESF in 2005, with more than 150 startups and small and medium enterprises (SMEs) focusing on nanomedicine R&D projects [5]. Furthermore, for the product pipeline, the nanomedicine related companies seems focus on drug delivery applications. In fact, 56 % of companies are involved in developing new drug delivery systems (NDDS), for 80 % of the nanomedicine market share. However these products represent small market values and focus on diseases of small patient groups, consequently representing niche markets [5]. It is worth noticing that although 16 % of nanomedicine companies are related to in vitro diagnostics, the outcome on the product pipeline is only of 6 %. Overall, 46 % of nanomedicine products are developed or co-developed by US companies, against 37 % for EU companies.

Among successful companies in Europe, there are three companies that are at the centre of attention and were explicitly presented during the EU FP7-funded NanoMed Round Table Extended Report [3]:

- **Nanobiotix:** develops novel therapeutics based on multifunctional nanoparticles and especially to treat cancer
- **Magforce:** proposes a revolutionary way to treat tumours via the heating of nanoparticles by an external magnetic field
- **Sonodrugs:** aims at developing novel drug delivery systems via for example a triggered release of drug by focused ultrasound induced pressure or temperature stimuli. Such NDDS is aimed to treat more efficiently cardiovascular diseases or cancer.

14.1.1.3 Predicting the Nanomedicine Market

In fact, it could be hard to economically evaluate the added value of nanotechnology in a medical product [6, 12–15]. Moreover, there is still not a proper definition of *nanomedicine* and the boundaries to depict nanotechnology-enabled medical products can vary substantially from one report to another. For some sectors however,

Table 14.1 Statements and predictions on the overall nanomedicine market using two types of analysis

	Type of analysis	
Market	Consideration of the total sales	Consideration of nano-specific sales
Nanomedicine market	The global nanomedicine sector will grow from $53 billion in 2009 to more than $100 billion in 2014 [27]	The nanomedicine market will grow to around $12 billion in 2012 [5]
	It actually reached $72.8 billion in 2011 and is expected to grow to $130.9 billion in 2016 [11]	The healthcare nanomarket will grow from $6.8 billion in 2007 to almost $29 billion in 2014 [18]
	The world nanomedicine market will cross $160 billion by 2015 [29]	
Nanobiotechnology market	The National Science Foundation projected the nanobiotechnology-based market to reach $300 billion by 2016 in the US alone and the molecular imaging market to reach $45 billion in 2010 [16, 17]	The Biotechnology Industry Organization (BIO) projected the biotech revenues to grow to $90 billion in 2008 [16, 17]

assumptions can be made and lead to a more specific market analysis. For example, the Institute for Prospective Technological Studies (IPTS) states that: "on average 30 % of the value of a drug is added by a NDDS" [5]. As a general trend, two types of predictions could be highlighted when comparing different scientific papers and marketing reports: (1) some analysts use the total sales to depict the nanomedicine market and its predictions; (2) some others try to estimate more specifically the nano-related commercial outputs of healthcare products.

The following table (Table 14.1) demonstrates the difference made by using two types of prediction analysis:

Despite the divergence in different resources, all these statements highlight how important the nanomedicine market is in terms of revenues, sales and commercial outcomes, and how fast it is growing. Whether analysts refer to the nanomedicine market, the nanobiotechnology market, or one of its leading applications such as anti-cancer products, the compound annual growth rate (CAGR) always exceeds 10 %.

Some other reports, such as IPTS's report [5] and BBC Research LCC's report [11], have emphasized on the predominant role that nanomedicine would own in healthcare applications and its impact on the worldwide economy.

14.1.2 Nanomedicine: An Overview of the Research and Development Landscape

Assessing the publications' activity (i.e. the number of published scientific papers) of a country or organization could be one of the most efficient ways to obtain a good representation of its research trends and perspectives. Important databases are at disposal to search for publications by relevant keywords. One of the most widely

used and comprehensive database is the Web of Science (WoS) database maintained by Thomson Reuters.

By exploring the WoS database, it was found that in 2004 nanomedicine represented 4 % of the nanotechnology research with about 1,400 scientific publications out of 34,300 for nanotechnology. The United States is the leading country in the field of nanomedicine research, accounting for 32 % of the publications. EU follows, with Germany being the most active country with 8 % of the worldwide publications. As for the nanomedicine products' distribution, drug delivery is the top research area, accounting for 76 % of the scientific papers, followed by in vitro diagnostics (11 %) and biomaterials (6 %) [6]. USA is also the only country in 2004 to have its number of patents in the field exceeding the one of publications, showing that nanomedicine is still at an early phase of development with limited commercial outcomes yet [5].

Nanomedicine also receives growing shares of public funding and strategic initiatives supplied and developed by some leading economic bodies/countries, such as USA, EU, Japan and China, to promote the research of nanotechnology. Unfortunately, there are no data available on public funding of nanomedicine research merely. However, the share of nanotechnology publications focusing on healthcare could be used as an indicator in such case. Hence, it is assumed that "in the EU25 about 5 % of the nanotechnology funding is spent on medicine-related research" and about 20 % on life sciences more generally [5].

14.1.3 Nanomedicine: An Overview of the Patent Landscape

14.1.3.1 A New Era of Commercial Development

Studying the patent landscape via official patent database such as the European Patent Office (EPO) could form an efficient way to obtain an overall picture of the development of a novel technology. Since patents are a central milestone to entering the market and a key step towards commercialization, such analysis can provide some significant clues on the current trends and future perspectives of the commercial outcomes and the maturity of the market. The patenting activity thus could be an intermediate step in the supply chain of nanomedicine, following research and publications and preceding commercial output and on-the-market products.

For nanomedicine itself, a symbolic line have already been crossed: since the beginning of the millennium, the trend of patenting activity is now approximately rising to a synchronous pace with the pace of publications [16, 17). The number of filed patents grew from 2160 in 1989 to 7763 in 2002. According to Wagner and his colleagues [6], the related patenting activities have even outpaced research publications since 2001. 2000 patents were filed in the field of nanomedicine sector in 2003, compared to 220 in 1993.

It seems that nanomedicine has entered a new era of commercial development and started to fulfil its initial hype. The increase of commercial outputs of research findings is notably attributed to the increased amount of research investment from government, corporate and private sources, along with numerous initiatives to develop translational nanomedicine.

14.1.3.2 Sectorial and Country Breakdown of Patents in Nanomedicine Worldwide

In the field of nanomedicine, the dominant patent sector is drug delivery with a share of 59 % in 2006, followed by in vitro diagnostics (14 %), Imaging (13 %) and others (e.g. drugs) (14 %) [6].

USA was the leading country for the nanomedicine patents, with a share of 54 % worldwide in 2006. In comparison, Europe held only 25 % share despite the fact that it was the world leader for the publication of nanomedicine papers. Herein, Germany itself had an outstanding activity with a share of 12 %. Meanwhile, Asia held 12 % share [5].

According to another report from Scrip Insights [18], however, Europe gained a substantial increase to 36 % of the overall number of relating patents in 2009, due to the increasing R&D efforts and the greater market participation of European healthcare players.

14.2 The Nanomedicine Business Environment

14.2.1 Introduction: Key Players in the Nanomedicine Business Environment

Nanotechnology is at the interplay between governments, regulatory bodies, start-ups, venture capitalists, start-ups and entrepreneurs [19]. Consequently, nanomedicine could expect a similar environment. However, the environment becomes much more complex when it comes to medical applications and healthcare drivers since (1) public perception and ethical issues have now become crucial to be taken into consideration; (2) pharmaceutical companies as well as clinical trial centres now have entered the dynamic environment as major players in the development of nano-related medical products; and (3) regulation bodies are now at the centre of marketing approval, making impacts on each stakeholder. A re-evaluation of the nanomedicine business environment is thus required.

The key players and their impact on the nanomedicine business environment are given in Table 14.2.

As highlighted in Table 14.2, pharmaceutical companies play a crucial role in the current nanomedicine supply chain. Since they are the only industry structure to provide enough funding for the development costs in clinical trials (accounting for millions of dollars), they are at the core of the business process. Moreover, the uptake by pharmaceutical companies is also very beneficial to start-ups and SMEs:

- The licenses will provide an additional revenue stream, whereas partnerships will offer them the distribution network and structure of the pharmaceutical industry;
- The uptake of on-going products after proof of concept can be seen as a liquidity exit in a much shorter timeline, which could greatly encourage and attract investors to fund start-ups and SMEs based on this business model.

Table 14.2 Key players and their impact on the nanomedicine business environment [19]

Key stakeholder in the nanomedicine supply chain	Impact on the supply chain
Academia (Research)	Innovation driver
	Availability of technology and equipment
Start-ups and SMEs	Risks are assumed in this most upstream step of the business process
	Innovation drivers
	Suppliers of nanomaterials for nano-based devices, drugs and platforms
Pharmaceutical companies	Uptake of on-going products after proof of concept: developing costs in clinical trials are so important that it is hard for SMEs to do without pharmaceutical companies
	Their distribution networks is crucial
Medical and clinical trials centres	Essential to ensure Good Clinical Practice (GCP) and to maximize the chance of marketing approval
Governments	Their policy can impact innovation by attracting companies, minds and projects; and impact a few steps downstream by promoting efficient structures to conduct research ideas into manufactured products
Regulatory bodies	Agencies such as the FDA are responsible for marketing approvals of each novel medical product
	Specific legislation can facilitate technology or IP transfer and promote research
Funding sources/investors	Without capital, no research can be carried out and no innovation can possibly be translated to effective products and medical applications
	Start-ups and SMEs cannot afford by themselves developing costs along with infrastructure investments and IP strategy
Supportive infrastructures (consortia, government initiatives, research and development platforms…)	Essential to promote research, increase public awareness and therefore consumers' dynamics
	Important player in bridging the gap between industry and academia for resources gathering and more efficient translational nanomedicine

The necessity to rely on pharmaceutical companies could therefore explain why NDDS are currently highly dominant in research activities, patents filings and commercial products of nanomedicine.

If pharmaceutical companies are the main resource to monetize intellectual property, governance and venture capitalists could be another two important sources in terms of funding bodies/providers for companies which are willing to undertake risky R&D in nanomedicine. Between these two sources, venture capitalists seems more suitable to invest in nanomedicine-related start-ups since they could not only provide a source of funding, but also offer a "governance structure" [20] and make additional contributions by sharing their expertise in market analysis and management.

14.2.2 Nanomedicine Business Drivers

The study of business drivers delivers another complementary view on the business environment. Drivers could result more or less directly from key players (such as the availability of capital results from funding bodies and venture capitalists) and impact on the dynamics of the business.

Business drivers can be defined loosely as the main factors and resources which provide the essential marketing, sales, and operational functions of a business, are of paramount importance. For nanomedicine business drivers, they should particularly encompass all factors to drive commercialization in nanomedicine.

In a general way, the research and development strategic agenda of nanomedicine could result from two driving forces: (1) technological innovation and science progress (upstream technological push); and (2) healthcare needs (downstream clinical pull). The technological push may lead to products and applications resulting from science/technology breakthrough and will answer "what can be done with such progress?", whereas the clinical pull is able to tailor products to healthcare needs and will answer "how can this urgent need be technologically addressed?".

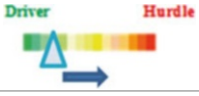
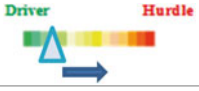
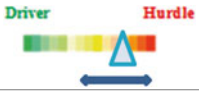
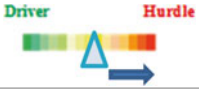
If the research in nanomedicine is highly pushed by technology, governance and stakeholders would then like to see a shift to a more clinically driven development, to ensure optimal healthcare benefits and maximal acceptance by the public, patients and clinicians [7]. However, technological drivers are still crucial, since genomics and proteomics lead a better understand and redefinition of diseases while nanoscale manipulation of proteins and DNA is necessary for enhancing diagnosis and treatments.

There is a need to emphasize that nanomedicine encompasses a highly diversified range of products and technologies, which fall in different markets (size or maturity), industries and regulations. Hence, specific drivers and dynamics would have to be considered for each sector of application, for each type and size of companies, and in each country's regulatory landscape, which is beyond the scope of this study. The following Table (Table 14.3) is a non- exhaustive list of influencing factors for business and commercialization of nanomedicine together with an estimated assessment in terms of their roles as drivers or hurdles based on a self-study in the related fields and some published resources [4, 10, 16, 17, 19, 21–27]. For the intellectual property (IP) landscape, current issues and strategies are focusing on patents as they are most attractive to investors due to the market exclusivity and the high guarantees and protections they offer. However, for IP strategy it can further involve trademarks, trade secrets, and copyrights [19].

14.2.3 Business Models and Strategies

As described earlier, the current nanomedicine research and development is driven by start-ups and SMEs. According to the IPTS Report [5], these innovative companies rely mainly on three types of business models, depending on the category of their novel products (Table 14.4).

Table 14.3 A non-exhaustive list of influencing factors for business and commercialization of nanomedicine together with an estimated assessment in terms of their roles as drivers or hurdles

Influencing factors for business and commercialization of nanomedicine	Roles as drivers or hurdles
IP landscape and strategy	
Healthcare costs	
Aging population	
Availability of funding and venture capital especially	
Structure for translational nanomedicine	
Regulatory landscape	
Public perception and acceptance	
Manufacturing considerations	
Technology status promises	

The crucial role played by pharmaceutical companies in the current development of most nanomedicine-related products is well conveyed in the business models pursued by start-ups and SMEs.

In terms of business strategies, the market players have four main types of strategic initiatives at their disposal to increase revenue and boost market growth in the competitive and fragmented landscape of nanomedicine [18]:

- Investment in R&D activities to upgrade their existing technologies for newer applications or to develop new products (58 % of major players' strategy for the 2007–2009 period);
- Collaboration and agreements with research institutions to explore avenues for new products and applications (25 %);
- Launching of new products (12 %);
- Mergers and acquisitions (5 %);

Table 14.4 Business models of nanomedicine start-ups and SMEs (Modified from [5])

Business model Category of product	Description	Start-ups and SMEs Examples
Development of nano-pharmaceuticals or medical devices	The aim is to develop a proprietary product pipeline by bringing novel medical devices, drugs, or conventional drugs with NDDS to market.	Inex, USA
	The company goes through the development until proof of concept is acquired. Afterwards the partnerships with pharmaceutical companies often take the product through clinical trials and offer the required distribution networks	Starpharma, Australia
		Idea, Germany
Development of nano-platforms	The aim is to provide added value to second-party products. For drug delivery companies, they usually focus on a particular delivery technology.	Pharmasol, Germany
	The technology is then often licensed to pharmaceutical companies, as it may have been directly customised to their needs.	Eiffel Technologies, Australia
Development and manufacturing of high-value nanomaterials	The aim is to manufacture and provide specific nanomaterials (such as carbon nanotubes or quantum dots) that will be used in nano-enhanced drugs or medical devices.	Biogate, Germany (silver nanoparticles)
	Such nanomaterials are often directed to a specific area of application such as implants, sensors and lateral flow tests.	British Biocell, UK (gold nanoparticles for lateral flow tests)
		Raymor Industries, USA (nanosized titanium powders for implants)

As a result of the early stage of nanomedicine's development, R&D activities highly dominant, accounting for 58 % of the business strategies. Whatever the initiative, drug formulation and delivery accounts for the main application, with 77 % of the collaborations and 58 % of the R&D and new products launches [18].

14.2.4 The Nanomedicine Business Environment: An Overview of Its Dynamics

A summary chart is given in Fig. 14.1, illustrating the overview of the nanomedicine business environment and its dynamics based on the earlier discussions.

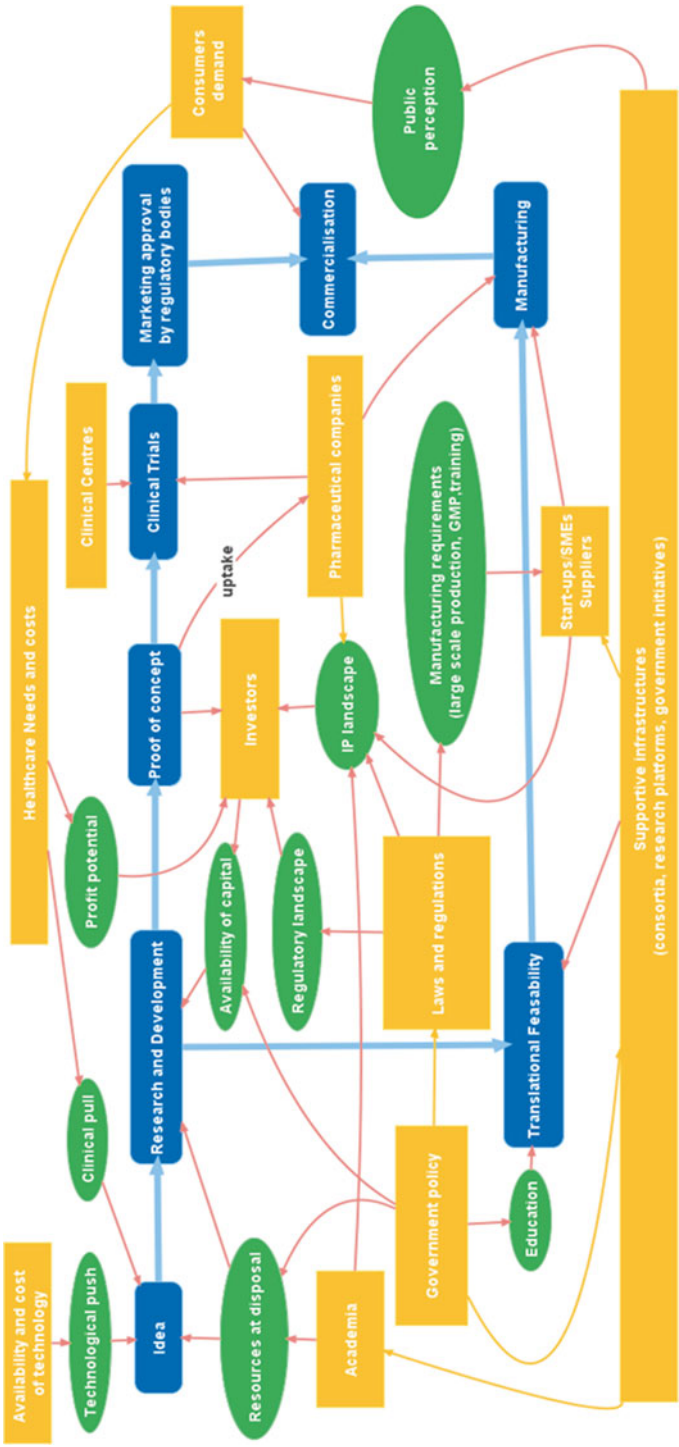


Fig. 14.1 The nanomedicine business environment. *Blue block*: nanomedicine supply chain; *orange block*: business players; *green block*: business drivers; *arrows*: impact

14.2.5 Current Issues and Future Perspectives

14.2.5.1 Current Issues and Strategic Recommendations

At the moment, most of the emerging fields of research and development are often driven by government-funded projects or initiatives, since the venture capital (VC) capitalists usually consider too much about the maturity degree of science/technology and the uncertainty of business environment. According to Jackson and his colleagues' study [19], attractiveness of start-up companies to VCs is embodied by very short time-to-market horizon (less than 2 years), an established customer base, and a successful management structure with experienced executives. Unfortunately, the current development status of nanomedicine does not quite meet those characteristics and conditions. The development of a nanomedicine product could takes 10–15 years and the public perception is highly unstable and impressionable by the toxicological concerns. Furthermore, no golden standard is yet established in the commercial steps towards business success or in the managing structure and expertise to gather. The evolving and uncertain regulatory landscape along with the emerging "patent's thickets" [16] doesn't lighten this picture of the business environment. Finally, VC often enters the funding stream after the proof of concept is established. Thus VC is unable to not represent the main funding body when investments are needed to launch an innovative yet risky start-up.

Although the availability of venture capital seems currently one of the weak parts of the European market, as one of the emerging and most promising fields worldwide, nanomedicine has benefited from a wide diversity of funding resources and ongoing government initiatives to promote its sustainable development. As a result, some highly ambitious programs have already arisen, with the creation of major clusters and centres of excellence for gathering resources and people around nanomedicine, to cope with its multidisciplinary and highly complex dimensions. In this context, governments and supportive infrastructures have initiated a series of initiatives, workshops and round tables to carefully frame the future development and commercialization environment of nanomedicine.

For example, the European Technology Platform on Nanomedicine (ETPN), a supportive infrastructure funded by FP7, established its first strategic research agenda in 2006. In its comprehensive report [2], some general recommendations were provided: focus should be made around increased innovation and reduced toxicity in order to reassure investors and promote a less risky and uncertain business environment; delays in commercialization should be addressed, with adequate and specific manufacturing standards and less complex and stringent regulatory systems; increased collaboration, consultation and cooperation will be crucial as well to avoid fragmentation and lack of coordination in this emerging technology.

In terms of strategic considerations, the following criteria, along with issues to be addressed, were further identified by ETPN (see Table 14.5) [2] as areas of priority to provide nanomedicine with a sustainable developing environment:

There are some other important reports and collaborative initiatives include: (1) Scientific forward look on nanomedicine [1]; (2) NanoMed round table extended

Table 14.5 Strategic considerations and issues to be addressed for a sustainable development of nanomedicine [2]

Strategic consideration	Issues to be addressed
Public acceptance	Transparent dialogue to avoid overflow of negative opinion due to toxicological concern
	Need to speak from the perspective of nanomedicine, not nanotechnology
Risk assessment	Specific research to answer safety-related basic science questions: in vitro models, identification of parameters, and high throughput screening
Regulatory framework	Address adequacy and appropriateness of current system in terms of novel products and not novel enabling technologies
IP rights	New model is to be investigated by ETPN
Required Research Infrastructure	Increase proximity between experts and facilities of different areas by larger clusters and centres of excellence
	Increase translation of research results to clinic of patients
Education and training to facilitate adoption of technology in hospitals	Need to develop and synchronize regional education schemes
	Develop training of industry and clinical personnel at all levels
Manufacturing costs	Commercialized products should have high potency and targeting in order to counter-balance with the complexity of manufacturing

report [3]; and (3) Concept for a European Infrastructure in Nanobiotechnology [25]. More recently, ETPN published a report on Contribution of Nanomedicine to Horizon 2020 [4], which is a White paper to the Horizon 2020 Framework Programme for Research and Innovation. It gave some specific recommendations from the nanomedicine community in order to exploit nanomedicine's full potential and for products to be brought efficiently to the market:

- A specific funding programme should be established for translational nanomedicine topics in order to refocus projects and adjust funding allocation criteria.
- A novel translational infrastructure should be established by federating research centres and clinical centres to de-risk innovation and leverage capabilities.
- Translational know-how should be recognised and more easily accessible to encourage a shift from disruptive and fundamental research to more translation focused projects.

14.3 Conclusions and Future Perspectives

In conclusion, nanomedicine is often seen as the area with the most promising and visible applications of nanotechnology on the short and long term. However, it is still in an early stage of commercialisation and its environment is currently undergoing high scrutiny as products are entering the market. At the moment, drug delivery, imaging and in-vitro diagnostics remain the key applications in terms of recent dynamics, market attractiveness, research interests and level of technology readiness.

Early in 2009, Wiek et al. highlighted that the interplay between very similar context variables (business players and drivers) in nanotechnology could lead to highly different market situations [28]. For the development and commercial outcomes of nanomedicine, there could be three hurdles and threats:

- Health and environmental toxicological effects proven by long-term risk assessments.
- Media influence
- Risk-averse public awareness

The on-going IP landscape may also discourage large and pharmaceutical companies of structural changes to embrace nanomedicine research and development. In addition to different market access to companies, unforeseen future obstacles and highly impacting factors might also come from a major and emerging societal issue: the access of treatments to patients. Costs will substantially vary from one hospital to another due to different budget envelopes per disease, and from one country to another due to different reimbursement policies [3].

Nonetheless, it has become a more and more solid fact that the nanomedicine development is currently witnessing a shift in stakeholders' wills and awareness in the priority issues to address and in the necessary environment to provide. In the near future, deeper understanding of the scientific and technological processes coupled with enhanced analytical tools for early characterization of toxicological profiles and a more established business model for efficient commercialization of nanomedicine products, will be able to provide this emerging "sustainable governance" with all the required knowledge for evidence-based decisions and for promoting the most beneficial applications for our society.

Heavily increasing funding programs and infrastructures, proactive initiatives and collaboration, as well as great efforts toward appropriate regulation and societal formation, have laid the foundation and framed a very bright future for nanomedicine. The next coming years will be crucial in building the path towards the future socio-economic impact of the nanomedicine era.

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Chapter 15
What Can Nanomedicine Learn from the Current Developments of Nanotechnology?

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15.1 Introduction

Nanotechnology is defined as the design, control, manipulation, synthesis, production and application of properties and functionalities of any structure, device, or system, which has one of its dimensions between 1 and 100 nm, by controlling its shape and size [1]. Expanding from its original definition, nanotechnology can be applied to and combined with various fields of science and gives rise to emerging sciences which greatly improves technology and, ultimately, the quality of life. An excellent example to this is the combination between nanotechnology and medicine – known as the emerging 'nanomedicine'. Therefore, nanomedicine can be defined, in general, as the application of nanotechnology to medicine to create advanced diagnostics and therapeutics for disease treatment and prevention from nanoscale, using knowledge, principles, and techniques from nanotechnology [1–4].

The starting point of nanotechnology in the human history began when Richard Feyman gave his infamous lecture in 1959 'There's Plenty of Room at the Bottom' stating the idea of manipulating individual atoms using larger equipment to produce relatively small matters. However, it was only in 1974 that the term 'nanotechnology' was first invented by Norio Tanaguchi and was accepted as an official, new scientific terminology. Nanotechnology and nanosciences has begun to grow at an incredible speed since then, and their applications started to branch out in various fields. However, proper and serious attention in nanomedicine has begun since only

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a few decades ago [5, 6, 7]. Table 15.1 below lists a brief summary of hallmarks relating to the evolution of nanotechnology and nanomedicine.

At nanoscale, substances exhibit unique properties and phenomena which are absent or different from when they are at macroscale [5]; based on the significance of this fact, versatile applications and implications can be made to find solutions to the related unsolved problems at macroscale. The application of nanotechnology in medicine has been brought to attention based on the fact that cellular components, activities and interactions, which are the essence and the most basic level of life, are at nanoscale (Table 15.2); hence working at the same scale might lead to solutions to current medical limitations or, at least, provide better understanding of the situation. Nanotechnology is widely applied to medical imaging, disease detection, medical analysis, drug manipulation, and modelling at nanoscale [5] to develop advanced diagnostic and therapeutic tools for curing and preventing diseases, and ultimately improve the quality of life.

Table 15.1 A brief list of nanotechnology and nanomedicine hallmarks [5]

Year	Event
1959	Richard Feyman's "There's Plenty of Room at the Bottom" lecture – the starting point of nanotechnology
1974	Establishment of the term 'nanotechnology' by Norio Tanaguchi
1979	World's first use of colloidal Au nanoparticles (NPs) in electron microscopy
1987	World's first cancer targeting using NPs coated with monoclonal antibodies
1990	World's first visualisation of atoms by the scanning tunnelling microscope (STM) invented by IBM Zurich Lab
1991	Discovery of carbon nanotube
1994	Establishment of the concept of NP-based drug delivery
1995	Liposome fabrication and usage in drug delivery
1998	Establishment of the term 'nanomedicine'

Table 15.2 Example dimensions of significant biological substances in the body [5]

Average dimensions (nm)	Substances
2,500	Human red blood cell
65–100	Exosome (vesicles from dendritic cells)
1–20	Proteins
2–4	Ribosomes
2.5	DNA (diameter)
1.2	The largest amino acid measured
0.4	A base pair in human genome
0.25	Average individual atom

From a general point of view, the scope of nanomedicine can be categorised into the following three main categories [8]:

- Medical imaging, diagnostics, and therapeutics using engineered nanoparticles and nanomaterials
- Regenerative medicine and other relevant innovative treatments
- Studies for in-depth understanding of activities, functions and mechanisms inside the body at nanoscale, and inside the cell

In this chapter, discussions about what nanomedicine can learn from the current developments in nanotechnology, and relevant topics, such as benefits, concerns, challenges and limitations, are explained. In addition, conclusions and suggestions on possible future opportunities and perspectives are stated.

15.2 From Nanotechnology to Nanomedicine

Achievement in nanotechnology opens the gate to the new era of medicine, creating opportunities for better understanding, development and invention from new perspectives. It moves medical challenges to the next step. However, everything has two sides: benefits and drawbacks. It is absolutely crucial, and it is always the aim, to minimise the adverse sides of the technology, and maximise its benefits.

Nanomedicine is a massive area, and is relatively new. Despite getting a lot of attention and funding over the past decade, the science and technology still have not been very well established and understood. Certainly, nanomedicine has been claimed to be capable of producing satisfying results and giving hopes for future medicine. However, the "negative" sides and its potential issues as well as the uncertainty have not been thoroughly studied and solved yet. As nanomedicine is closely linked to nanotechnology, it is a good idea to learn from what nanotechnology has already went through, to get a hinted starting point about what are to be marked for consideration when it comes to nanomedicine.

15.2.1 A Quick Look at the Current Development in Nanotechnology with a Critical View

Since its establishment, nanotechnology has been growing rapidly at an amazing rate. Extensive studies and experiments have been going on in various aspects of nanotechnology. Current major attentions are, for example, the development of nanoparticles and nanomaterials, nanofabrication techniques, nanoelectronics, precision engineering, nanofluidics, nanoreactors, advanced microscopy, nanometrology, nanotechnology for energy solution, nanophotonics, and nanotoxicity [9–15]. As studies and experiments go, investigators make discoveries of successful results, as well as problems and limitations. Discussion on how nanotechnologies

have been successfully applied into medicine will be made in the next section, while the problems and issues of the technologies from a nanotechnology perspective will be discussed in this section.

A classic example of problems raised which could be related to nanomedicine is the property inconsistency of products from nanofabrication. In the production of nanoparticles, this problem occurs very frequently, even with the best technique available which produces the finest nanoparticles. With current technologies, nanofabrication still exhibits variation of nanoparticle product sizes in a batch [14]. Having size inconsistency implies that the entire batch of the particle will also have inconsistent property – low production effectiveness and product quality. As a real-case example, Saito et al. carried out the synthesis of single-walled carbon nanotubes (SWCNTs) via gas-phase pyrolytic method using metal nanoparticles as catalysts [16]. In their experiments, particle size inconsistency occurred during fabrication of metal nanoparticles, hence creating SWCNTs size inconsistency. Problems with inconsistency does not confine to only particle sizes, but also other important parameters, such as surface chemistry and concentration, as demonstrated in the work conducted by França et al. [17].

Another example is related to micro- and nanofluidics which are very complicated concepts and are still under investigation [18]. A micro/nanodevice inevitably requires application of micro/nanofluidics. The concepts of micro/nanofluidics are complex and challenging even to engineers as it is, in effect, a scale-down, which is the opposite to their usual work – scale-up. There are several issues that have not been understood thoroughly yet, such as interaction between fluid flow, surface forces and molecular interaction. Uniquely, at these tiny scales, surface forces become dominant. This is one of the reasons which make micro/nanofludics so different from, and much more complicated than, fluidics at the normal scale. The flow regime in these devices has unique characteristics which cannot be considered as a laminar flow, and has to be classified as a separate regime called 'microhydrodynamics'. The flow also has complicated 3-D geometries [19, 20].

A more general problem is that most of the products derived from nanotechnology currently rely on advanced technologies for their preparation and production, such as X-ray lithography for nanopattering; hence, production costs is undoubtedly expensive. Fabricating nanostructures involves several expensive technologies, for instance, as seen in the work of Liu et al. in producing palladium nanosprings [21]. Attempts have been made in trying to find alternative fabrication methods for nanotechnology related products, with the aim of reducing production costs. For example, Choi and Kim succeeded in developing an easy method to fabricate a dense nanoscale array on a large surface [22]. In addition, most of the products involving nanotechnology are still on a laboratory or pilot plant scale (i.e. still being processed in small batches), and their synthetic processes for industrial scale-up have not yet been established due to complexities and other several reasons (such as economics and profitability), which make mass production and commercialisation of these products still not practically and widely feasible.

15.2.2 A Glance of Current Advanced Nanomedicine

Before answering what nanomedicine can learn from nanotechnology so far, it is essential to understand and evaluate how nanomedicine has been making its progress. Several techniques and principles in nanotechnology, such as nanometrology, nanoparticles, nanomaterials, and nanofabrication, have been applied and implied to various medical fields. The technologies have greatly transformed medicine from its conventional practice both in diagnostics and therapeutics. It is worth noting that, several emerging medical concepts have been greatly made closer to clinical use through nanomedicine, such as regenerative medicine, theranostics, and gene therapy, as elaborated below.

15.2.2.1 Medical Diagnostics

Conventional medical diagnostic methods play important roles in medicine. However, they also have some noticeable drawbacks, such as time-consuming process and biological substance degradation. Table 15.3 shows some comparisons between conventional and nanomedical diagnostics in terms of their properties and performances. In addition to helping to modify and improve the conventional methods, nanotechnology has been applied in medical diagnostics to overcome some of their existing problems/drawbacks. Speaking in general, the major areas in nanomedical diagnostics are nanobiosensor, point-of-care (POC) medical diagnostic devices, and medical imaging.

Nanobiosensors Nanobiosensor is generally described as a biosensor at the nanoscale. A typical biosensor consists of three main parts: biological receptor element, physiochemical transducer, and detector. The first two components are critical parameters for a good biosensor. The biological receptor element should selectively and specifically binds to the desired analyte. Transduction process should be efficient so that the generated signal can be translated correctly and accurately. Hence, the design criteria for a good biosensor are selectivity, limit of

Table 15.3 Some comparisons between conventional and nanomedical diagnostics

Conventional diagnostics	Nanomedical diagnostics
Time consuming	Rapid diagnosis
Sample deterioration	Remove problems about sample deterioration
Requires a certain amount of sample to process, hence can be invasive	Requires only a very small amount of sample, hence less invasive
Difficulties from integrating parameters (resulting from various type of tests), hence requires personnel with special skills	Tends to be easy to use, hence does not require any special skill to operate
Can give inaccurate results at time, e.g. when the amount of sample is too small	Produce relatively accurate results instantly
High-cost	Low-cost

detection (LOD), response time, and signal-to-noise (S/N) ratio. Nanomaterials (e.g. nanoparticles) is one of the top candidates for the improvement of both above key components due to their unique physical and chemical properties and the ability to easily control those properties. Nanomaterials can be used to develop improved sensor coating, base, or circuit components. They can also be applied to improve the biological receptor element [23, 24].

Nanoparticles is the popular choice for studies in nanobiosensors due to several reasons, such as its unique optical property, high surface-to-volume ratio, tunable properties, high stability and biocompatibility, and non-complicated synthesis. When bound with an analyte, the overall physical and chemical property of the nanoparticle changes, thus producing detectable signal sent to the transducer. These changes, such as changes in surface plasmon resonance (SPR), electrical conductivity, or redox activity [25], could dramatically increases the sensor sensitivity. A recent example could be found from the work of Cao et al. where they reported that the plasmon shift produced from the sandwich system, having gold nanoparticles instead of secondary free antibody, was 28 times increased; hence, the signal was greatly amplified in such a manner so that it can detect the analyte at a picomolar level of concentration [26].

Several sensors can be integrated into an array called 'integrated biosensor' which is able to take different, parallel measurements simultaneously from one sample [27]. These nanobiosensors can also be combined with other nanotechnologies, such as atomic force microscopy (AFM) [28], fluorescence resonance energy transfer (FRET) [29, 30], and DNA technology [31], to improve the quality of contrast and/or add additional properties to the sensor. Recently, a novel paper-based nanobiosensor has been developed for medical diagnostic purpose by Parolo and colleagues [32].

Plastic antibody is a novel and powerful concept which greatly helps reducing several problems with biosensors. Plastic antibody is the synthetic and imprinted polymer with an affinity to bind with a specific analyte. It is produced by polymerisation of cross-linkers and functional monomer with a target molecule acting as a template. Popular templates in biological applications including proteins and small peptides. Plastic antibody has several advantages over natural antibodies. From a production point of view, it is cheaper and more stable. The product properties, such as particle size and molecular weight, can also be controlled easily during the production. For instance, [33] successfully created a nanoscale plastic antibody for the detection of a bee toxin called melittin.

Point-of-Care (POC) Medical Diagnostic Devices Compared with normal diagnostic tools, POC devices are for patients to be able to take measurement and see the results by themselves without having to visit the hospital and have their samples taken to the laboratory. Nanotechnology has made an impact on this area of medical diagnostics by offering micro/nanofluidics and nanoelectronics. POC devices tend to be of a portable size. One of the major POC principles is to take the sample from the patient as little as possible per measurement. A micro/nanofluidic system would be able to make this feasible and practical. The knowledge from nanoelectronics

could greatly help with circuit and circuit component fabrication for an electronic nanoscale device. There are many researches and studies on developing 'lab-on-a-chip' devices of which mixing, separation, identification and analysis of sample fluid can be done on a small, single device [34]. Attention has been paid to the 'lab-on-a-chip' concept due to its high possibility of providing early diagnosis and therapy monitoring [35]. POC development is unfortunately not in the main stream yet, but it is a good candidate in a long term strategy [2]. Good and encouraging examples in this area include the highly-integrated lab-on-a-chip which simultaneously analyses several parameters developed by Schumacher and colleagues [36] and an attempt to create an inexpensive POC microfluidic device for viscous sample [37].

Medical Imaging Nanoparticles, often with modified surfaces, have unique and useful properties which can be used to improve in vivo medical diagnostics by generating contrast through a selection of paths, such as radiation and magnetic field. They have been investigated and applied in various medical imaging technologies, such as magnetic resonance imaging (MRI), X-ray imaging and computer tomography (CT) to improve the efficiency of imaging tools and their contrast agents. The main purposes of the application are to make early diagnosis, track therapeutic efficiency, and obtain knowledge regarding disease development and pathology. Usually, the materials used for making contrast agents are those which are fluorescent, magnetic or paramagnetic. They can be used to localise and verify the current stage of tumour, identify the location of inflammation, verify stages of particular diseases, visualise structure of a blood vessel, and assess drug distribution and accumulation inside the body [38]. For a more advanced medical imaging, it is aimed for a method capable of detecting a single specified molecule or cell in the complex environment of human body. Since high dose of contrast agent is required for CT scan, inert materials, such as iodine-based, gold, lanthanide and tantalum nanoparticles have been chosen to make a suitable CT scan contrast agent [39]. The main focus of using nanomedical imaging technology currently is in cancer detection. Recently Chien et al. reported that administration of gold nanoparticle together with heparin produced contrast in X-ray imaging which was sufficient to see tumour microvessel (3–5 μm diameter) or extravascular diffusion [40]. For MRI contrast agents, the use of iron oxide nanoparticles is a classic example. Bae et al. developed carbon-coated iron oxide nanoparticles, improving the availability of the contrast agent [41].

15.2.2.2 Nanomedical Therapeutics

In general, the on-going research and development in nanopharmaceutics can be grouped into the following categories:

- Single, specific aspect: targeted delivery, stimuli responsive systems, controlled release, imaging, disease detection, and gene therapy
- Multifunctional nanoparticles (MFNPs): non-hybrid MFNPs and hybrid MFNPs
- Synthesis and fabrication method
- Therapeutic medical devices (e.g. cardiovascular stent)

There are further three main factors which make nanoparticles and other proper nanomaterials appealing to their application in therapeutics: very small size, unique behaviour and designable properties. Having a very small size means that they can reach sites which are previously unreachable, thereby increasing treatment effectiveness. Through several available modification techniques for nanoparticles, the particle biocompatibility, bioavailability, half-life can be increased, while the toxicity can be minimised. Apart from receiving the most attentions in cancer therapy, nanomedical therapeutic products have also been intensively investigated for providing solutions for some other major diseases, such as neurological diseases (e.g. Alzheimer's and Parkinson's diseases) cardiovascular diseases, respiratory diseases, infectious diseases (e.g. HIV and meningitis), and chronic diseases (e.g. diabetes). The field of nanomedical therapeutics generally involves nanoparticle/nanomaterial drug-delivery systems, nano-therapeutic medical devices, and special nanomedical treatments.

Nanoparticle/Nanomaterial Drug-Delivery Systems Conventionally, drugs are administered into the body and the drug molecules float around inside the body. However, not all drug molecules could efficiently arrive at the desired location: some degrade, some trapped and some cleared by the body defence mechanism. In order to cope with these problems, nanoparticles and some other nanomaterials have been widely investigated and studied. They have been successfully incorporated into the drug molecules to add or enhance properties such as biocompatibility, bioavailability, half-life, target specificity, payload, and controlled release mechanism, while minimising its toxicity effect. Coating the system with certain polymers, such as polyethylene glycol (PEG), has been shown to increase biocompatibility, bioavailability and half-life [42]. Very recently Liu et al. fabricated a complex of gold nanoshells on silica nanorattles showing increased permeability, enhanced permeability and retention (EPR) effect in tumour tissues and light conversion in vivo, while having less toxicity [43]. The concepts of antigen-antibody binding can further be applied to create target specificity by incorporating suitable ligands, such as antibody, DNA strands, and peptides, on the surface of the drug-nanoparticle complex [44]. In 2007, Hatakeyama et al. reported an anti-MT1-MMP immunoliposome complex carrying doxorubicin [45]. The complex greatly reduced tumour growth in vivo in mice. They suggested that the cellular uptake of the complex was increased due to a resulting immunoconjugation. In addition, several nanofabrication and nanoencapsulation techniques could be applied to create layers or appropriate structures so that the complex can be loaded with desired drugs of different properties, such as hydrophobicity and hydrophilicity, on a single complex, as demonstrated by Hammond [46]. In summary, the nanoparticles and other nanomaterials which have been commonly used for drug-delivery systems include [47–55]:

- Carbon nanomaterials (e.g. carbon nanotubes, fullerenes)
- Magnetic nanoparticles (e.g. iron oxide nanoparticles)
- Metal/inorganic nanoparticles (e.g. gold, silver, silica nanoparticles)
- Quantum dots
- Polymeric nanoparticles (e.g. PLGA nanoparticles)
- Solid-lipid nanoparticles

- Micelles
- Liposomes
- Dendrimers
- Multifunctional nanoparticles (MFNPs)

There are about 22 nanoparticle-content drugs which have been approved by the Food and Drug Administration (FDA) in the United States of America. Furthermore, there are about 25 nanoparticle-content drugs being investigated in clinical trials in Europe [2].

Nano-therapeutic Medical Devices Nanotechnology has been applied not only in medical diagnostic devices, but also in therapeutic devices by improving the device's therapeutic efficiency, biocompatibility, strength or flexibilty, while minimising its adverse effects. An excellent example is the cardiovascular stent. The knowledge of nanomaterial fabrication has been used in mechanical improvement of the material used to build the stent itself [56]. Certain fabrication methods are used to improve the structure of the stent body. For example, micro-wells can be created on the surface of the metal that is used to make a stent, in order to increase drug-loading capacity of the drug eluting stent (DES). Other fabrication methods can also be used to create thin layers of polymer coating on the metal surface in order to increase the stent's biocompatibility and reduce the side effects from restenosis and thrombosis, which are side effects as a result of the interaction between the body immunological response and the stent (considered as a foreign object inside the body) [56]. There further has been a report on a successful sustained and controlled release of a DES using the layer-by-layer thin-film coating consisting of different materials for different functions [46]. Layers of drugs with different opposite charges were coated. The properties of the materials in each layer govern the kinetics of layer degradation and drug release based on the principle of mass transfer. Moreover, the technology of making a particular nanocomposite, together with surface modification, could add desired properties onto a cardiovascular stent. For example, after coating the stent with a layer of polyhedral oligomeric silsesquioxane poly (carbonate-urea) urethane (POSS-PCU), which is a nanocomposite, endothelial progenitor cell (EPC) specific antibody was successfully grafted on the composite [57]. Consequently, this device could facilitate endothelialisation of the stent to blood vessel wall, reducing problems with restenosis and thrombosis.

Innovative Medical Treatment for Specific Diseases The unique behaviours of substance at nanoscale can be selectively used to enhance treatment efficiency. For example, there are several nanoparticles which respond to certain external stimuli. They could be applied in hyperthermia for cancer treatment. Hyperthermia literally means the condition where temperature is higher than normal. However, it can also refer to a method in cancer treatment using heat. Hyperthermia cancer treatment is a non-invasive medical treatment in which body tissue is exposed to higher temperatures to damage and kill cancer cells or to make cancer cells more sensitive to the effects of radiation and certain anti-cancer drugs. Since a too high temperature might also kill neighbouring normal cells; hence the heating process must be carefully

controlled. There were problems regarding keeping the heat at the suitable level and location (problems with consistency and accuracy). For the conventional hyperthermia, heating is supplied from an external source and has to struggle through several barriers, which can be considered as resistance to heat transfer. Heat is lost during the way and the amount of heat that reaches the target is obviously less than the heat originally emitted from the heat source. However, by utilising nanoparticles in hyperthermia, it has now become feasible to achieve better consistency and accuracy [58–60]. Bhayani et al. successfully developed a nanoscale complex of dextran-iron oxide nanoparticles which responds to a certain radio frequency [61]. The complex, activated by the certain radio frequency, provides the same changes to the tumour cells as seen from externally heating (43 °C for 60 min) in terms of cell morphology, proliferation patter, and measurement of protein associated with heat shock. Similarly, magnetic nanoparticles can also be used in hyperthermia where the magnetic nanoparticles generate heat after an alternating magnetic field is applied. For example, Sadhukha et al. successfully achieved a significant in vivo inhibition of lung tumour growth by using super-paramagnetic iron oxide nanoparticles (SPIONs) [62].

15.2.2.3 Multifunctional Nanoparticles: Diagnostics and Therapeutics in a Single System

Equipped and stimulated by the rapid development of advanced nanotechnology, it is able to fabricate a nanoscale complex which has multifunctionalities, such as both therapeutic and diagnostic features. A selection of techniques such as nanofabrication, nanoencapsulation, surface grafting and layer-by-layer coating can transform simple nanoparticles to all-purpose multifunctional nanoparticles or nanoplatforms. Multifunctional nanoparticles have been established aiming to improve the particle's stability, biocompatibility, half-life, and add miscellaneous properties (e.g. stimuli responsive, target-specific, disease detecting, or imaging). Particle functionalisation is normally achieved by surface modification [63, 64].

For cancer treatment, attempts have been made to try to create a stable, safe and biocompatible nanoscale system which is capable of (1) accurately targeting tumour cells or tissues; (2) releasing therapeutic agent or performing appropriate treatment in a controlled manner to destroy the tumour cells directly or inhibit their growth; and (3) safely self-degrading or getting itself out of the body through body clearance mechanism. Usually, the system (e.g. non-hybrid multifunctional nanoparticles) consists of a nanoparticle core, shell(s), and surface ligands. non-hybrid multifunctional nanoparticles. Recently, a new class of multifunctional nanoparticles has been created by combining more than one nanomaterials as the system's backbone [63]. They are classified as hybrid multifunctional nanoparticles which possess properties of different backbone materials. Furthermore, they seem to offer possible solutions to current limitation from the non-hybrid systems in terms of the suspension and size stability (once administered into the body), encapsulation effectiveness, controlled release mechanisms, and biocompatibility issues (multi drug resistance and blood compatibility).

Cheng et al. recently a multifunctional system of upconversion nanoparticles (e.g. nanoparticles of lanthanide elements) offering in vivo dual medical imaging: fluorescence and MRI [65]. The system also has the magnetic targeting ability which can increase its accumulation on tumour sites by approximately eight times when compared to the system without the presence of magnetic element. In addition, the system is capable for hyperthermia via near-infrared (NIR) light stimulating which is its therapeutic feature, specific to cancer. Another recent example of multifunctional nanoparticles for the treatment of other diseases was reported by Lee et al. who successfully fabricated targeted gold half-shell nanoparticles for chemo-photothermal therapy of rheumatoid arthritis [66]. Arginine-glycine-aspartic acid (RGD) was conjugated to the nanoparticles for its rheumatoid arthritis-specific targeting ability. The system was loaded with methotrexate, the most effective drug of choice for treating rheumatoid arthritis. The gold nanoparticles further provide hyperthermia ability. When stimulated with NIR radiation, those gold nanoparticles generate heat effectively. The generated heat acts as a trigger for both drug release and direct hyperthermia to cure the diseased sites inside the body. This system further greatly increased the therapeutic efficiency for arthritis using a dramatically reduced dose of methotrexate and its side effects.

15.2.2.4 Nanotechnology in Regenerative Medicine

Regenerative medicine has been increasingly exploited and developed where nanotechnology is utilised in cell therapy, in vivo real-time labelling and imaging, 2D-nanotopography, 3D-nanoscaffold, and growth factor delivery [67, 68]. Nanomaterials have been investigated for their effectiveness, in mechanical, chemical and biological aspects, for making regenerative scaffolds. Furthermore, nanotechnology is able to create opportunities for scientists to develop biomaterials which can mimic various types of extracellular matrices in tissues, generating suitable conditions for triggering cell repair or growth [2, 69].

However, further studies are still required to develop nanomaterials used for regenerative medicine and investigate if they possess the following conditions [2]:

- Non-toxic
- Biocompatible
- Simultaneously facilitate regeneration
- Maintain physical properties (even after being conjugated at the surface)
- Interact with desired target (protein or cell) but not disturbing its normal biological activities

The ultimate goals of regenerative medicine are to induce cell or tissue repairmen without causing other complication from immunological respond or dependence of donors [67].

15.2.2.5 Nanomedicine in Gene Therapy

Gene therapy is defined as a method which utilises appropriate genetic materials, such as fragments of DNA or RNA, to selectively repair faulty genes causing diseases [70]. It is seen as a promising solution as a cure to diseases which are currently incurable or difficult to be cured, such as genetically inherited disorders, certain types of cancer, and viral infections [71]. There are several approaches to cure diseases using different techniques in gene therapy. The most common approach is to replace a non-functional gene on a specific location with a normal gene. Therapy target can be set on the faulty gene too. Homologous recombination can be used to swap a problematic faulty gene with a normal healthy gene. Alternatively, selective reverse mutation on the faulty gene can result in gene repair and the gene is turned into a normal gene [70].

To carry out gene therapy, a messenger called 'gene vector' is required to deliver desired genetic material into the nucleus. Gene therapy starts with the transfection of the target cells by the gene vectors. Genetic materials inside the vectors are then released into the cell. After the genetic materials pass through nuclear membrane into the nucleus, the desired proteins can be synthesised, which will ultimately bring the cell back to its normal condition. There are usually two main types of gene vectors: viral vectors and non-viral vectors. At present, viral gene vectors have a much higher rate of successful delivery compared with non-viral vectors. However, there are issues and concerns using viruses inside the body, despite the fact that they are genetically modified to contain only the desired human genetic materials for the therapy. As a foreign body, it would activate the body's immunological response and even inflammation. Concerns have been raised regarding the fact that there has been no solid evidence to approve: after being administered into the body, the modified viruses would be able to resume their original pathogenic activities and not to cause complication to the patient [70]. As a result, efforts have been continuously made to apply safe and effective non-viral gene vectors. However non-viral vectors have several limitations which are needed to be overcome: stability in biological condition, extracellular obstructions, intracellular obstructions and targeted delivery [72, 73].

Recently nanotechnology has been applied to gene therapy in order to overcome these limitations by creating a stable, safe, and biocompatible non-viral gene vector for effective targeted intracellular gene delivery. Both inorganic and organic (biodegradable) nanoparticles/nanomaterials have been used to create non-viral gene vectors for gene therapy.

Labhasetwar and Panyam described a successful escape of gene-encapsulated PLGA nanoparticles from endosome into the cytoplasm [74]. Yamashita et al. made a photothermally controlled gene delivery system by conjugating double-strand DNA onto gold nanorods [75]. The system has a unique thermal conductivity in response to near-infrared radiation, causing the release of single-strand DNA in a controlled manner. A system of chloroquine-encapsulated polycationic mesoporous silica nanoparticles containing siRNA was shown to have successfully delivered both siRNA and chloroquine [76]. In addition, Chen et al. reported an up-to 50 % gene silencing ability after 48-h post-administration of chitosan-siRNA nanoparticles in

PLGA nanofibers [77]. An approximately 1 week of sustained therapeutic genetic expression was observed by Kwon et al. employing a complex of DNA and cationic lipid-based nanoemulsion [78].

15.3 What Lessons Nanomedicine Can Learn from Nanotechnology?

Nanomedicine incubates and develops from nanotechnology. Broadly speaking, nanomedicine could be regarded as one of the divisions of nanotechnology. Thus, all matters inherited and/or transferred from nanotechnology would certainly affect the progress of nanomedicine.

Lessons, depending on their nature, influence and impact, could be either positive or negative. In the previous sections of this chapter, detailed discussions and sufficient examples are given to demonstrate the positive lessons and results from nanotechnology. It has shown that nanotechnology has broken down several crucial barriers previously existed in medicine, bringing the chance and reality of curing hopeless diseases. It also has improved effectiveness of current medical technologies, such as medical imaging, by enhancing the efficiency of the contrast agents or creating a novel approach of multi-modal medical imaging. Furthermore, the appropriate use of unique physicochemical properties of nanoparticles/nanomaterials enables modulation and control of biological activity at nanoscale, which has been proved to be a promising approach in drug delivery, regenerative medicine, gene therapy and other innovative medical treatments. The following table (Table 15.4)

Table 15.4 Summary of current positive and negative aspects in nanomedicine

Positive aspects	Negative aspects
Smaller devices, hence they are less invasive	Smaller devices require sophisticated technology which may not be economically feasible to everybody
Nanomedical diagnostic devices and methods only require a small amount of sample	Several pre-analysis preparations of the sample are required
Comes in small size and operates at the same level as interaction inside the body	Early developed NPs might accidentally affect various biological barriers, hence results in unexpected toxicities
	Difficult to monitor exposure from outside
Drug delivery technology using nanoparticles protects the drug from being degraded inside the body	The used nanoparticle might be difficult to degrade or come out of the body via normal excretion pathways or mechanisms
NPs-based drugs come in small quantities but with increased efficiency	Damages could be done to healthy tissues or cells if NPs accidentally accumulate at the unexpected areas
Cheaper	More expensive
More accessible to general public	Limited access to affordable population only

summarises the current positive and negative aspects in nanomedicine, leading to a further discussion next on the constructive lessons that nanomedicine can learn from nanotechnology.

15.3.1 Inconsistency Issues in Nanofabrication

As mentioned earlier, there are issues and concerns with respect to the inconsistency of particle sizes and properties, even within the same batch production. The inconsistency could greatly reduce the product quality, and further especially affect the safety of product to be effectively used inside human bodies since it is difficult to confidently control or monitor the effectiveness of the treatment which is not uniform. Serious undesired complications might even occur from this uncontrolled variation.

15.3.2 Industrial Scale-up and Commercialisation Limitations

The techniques and methods evolving nanotechnology could be very specific and expensive. Some of them are also limited to only batch production. Furthermore, some synthetic routes of nanoparticles and other nanomaterials could be too sophisticated so that the product yield might be relatively low and that overall product cost is high. These issues have been preventing several nanotechnology related products from being industrially scaled-up and commercialised as they are not economically feasible with reasonable profitability. It is suggested that researches should be carried out in order to find better and optimised (scaled-up and economic) synthetic routes. Generally speaking, an ideal synthetic route of nanoparticles and other nanomaterials for industrialisation should have the following features:

- Safe
- Easily accessible raw materials
- Including sophisticate steps or equipment as least as possible
- Reasonable capital and operating costs
- Reasonable yield
- Reasonable production timescale
- Useful by-products (if any)

15.3.3 Complex Nature of Biological Phenomena: Additional Complication to the Complex Concept of Nanoscience, Nanotoxicity and Related Matters

Biological phenomena are much more complicated than physical phenomena. Several activities occur simultaneously in a set manner in the biological environment. This is an additional complication to the study of nanoscale phenomena.

Nanotechnology is already a very broad, emerging and extensive field. As discussed earlier, it is somehow difficult to set topics or areas of nanomedicine into discrete categories since everything seems link together.

A critical limitation to the development of nanomedicine at present is the lack of thorough and well-established knowledge regarding interaction between nanomaterials and biological environment inside and outside the body. It has raised public concerns about safety issues regarding the use of nanotechnology in health care, and this is why the study of nanotoxicology will play an more and more important role [2, 79, 69]. The classic examples regarding the nanotoxicity issues include the cases of using silver nanoparticles and carbon nanotubes. Silver nanoparticles have many interesting properties involving the anti-bacterial property which has received extensive interests and investigations. However, it was discovered later that silver nanoparticles has undesired adverse effect in vivo [80]. For carbon nanotubes, it was once at the centre of attraction as a promising drug delivery method and platform. However, it was also discovered later that it could become harmful [81]. Concerns have also been raised with respect to the nanomaterials' fate inside the body and body clearance. In theory, nanomaterials for clinical application has to undergo absorption, distribution, metabolism and excretion (ADME) studies first. However, since it is heterogeneous in content with size distribution, it is difficult to describe ADME properties of nanomaterials [79].

15.3.4 Establishment of Standards and Protocols for Nanomaterial Characterisation

Despite the amazing developments in nanotechnology, there are still limitations of achieving accurate and reliable characterisation of the physical and chemical properties of nano-products. Furthermore, there is no solid standards and protocols for a full/comprehensive and reliable nanomaterial (medical nanomaterial in particular) characterisation mainly due to the diversity, complexity and uncertainty of nanomaterials. There have been suggestions to first develop ex vivo studies of activities of nanoparticles and the body thoroughly from the possible ways of administration, their routes and journey inside the body, and their fate, before performing in vivo studies [11]. However, more issues will rise due to the complexity in fabricating ex vivo system for the study. It is also essential to trace if the nanoparticles are degraded, excreted or accumulated inside the body, as it will affect the toxicity of such particles [79].

15.3.5 Nanomedicine Regulating Bodies: A Demand for Proper Regulations

Nanomedicine has recently come into our visions attributed to the rapid and exciting development of nanotechnology. Researchers are even more optimistic for the future of nanomedicine but it also consequently brings us a new task of determining

how to best regulate it so that it is both safe and effective. Similarly occurred during the development of biotechnology, the national governments/bodies worldwide are now struggling with balancing the competing benefits and risks of nanotechnology in the medical and other sectors. It is becoming increasingly clear that reasonable, effective and predictable regulatory structures will be critical to the successful implementation of nanotechnology [82]. When it comes to developing a regulation plan for nanomedicine, the focus needs to be on who will be given the responsibility to oversee regulation and whether to operate under the current regulations or write new regulations [83].

Hence, there have been some discussions on the challenge and suitable role of regulatory bodies such as FDA [84, 85]. It was suggested that whether the FDA should at least look at nanoproducts on a case-by-case basis and should not attempt regulation of nanomedicine by applying existing statutes alone, especially where scientific evidence suggests otherwise. Incorporating nanomedicine into the current regulatory scheme is a poor idea. Hence, regulation of nanomedicine must balance innovation and R&D with the principle of ensuring maximum public health protection and safety. The FDA should also consider implementing several reforms to ensure that it is adequately prepared to regulate nanomedicine.

Chowdhury further recently discussed the regulation of nanomedicine in Europe [86]. Due to the fact that the nanomedicine market in EU is poised at a critical stage wherein clear regulatory guidance is lacking in providing for clarity and legal certainty to manufacturers of nanomedicine, it is imperative to establish suitable regulatory structures for nanomedicine. It was suggested that both the pediatric and the advanced therapies medicinal products regimes offer important regulatory guidance that could be adopted for the regulation of nanomedicines in the EU first.

15.3.6 Ethical Issues

For nanomedicine, an evolved area from nanotechnology receiving increasing attentions from our society, it is crucial to proactively address the ethical, social and regulatory aspects of nanomedicine to minimize its adverse impacts on the environment and public health and to avoid a public backlash [87]. The most significant concerns involve risk assessment, risk management of engineered nanomaterials, and risk communication in clinical trials. Other concerns have been raised regarding privacy violation from generating genetic data of the patient and social justice. Accessibility to health care is also an important issue. Due to generally high-cost of the nanotechnology related products, economic and equity issues have also been pinpointed that only a few people who are financially capable can access this high-end health care but the diseases do not selectively occur in rich population [2, 87]. Educating members of society about the benefits and risks of nanomedicine is thus important to gain and maintain public support.

15.4 Conclusions and Future Outlook

Nanomedicine bridges the gap between nanotechnology and medicine. It is a truly interdisciplinary science which requires cooperation and contribution from engineers, scientists and medical staffs to appropriately, effectively, safely and successfully apply nanotechnology in medicine to move to the next generation of health care. Several discoveries and achievements in nanomedicine have been made, making significant medical advancement and bringing medicine closer to the new era. However, in order to drive nanomedicine further in the right direction with confidence, lessons from nanotechnology must be considered and should be learnt and used wisely to overcome problems and limitations. Complete knowledge and understanding of nanoscale phenomena, such as interaction between nanoparticles and biological environment, nanotoxicity, and nanomaterial physical and chemical properties characterisation are all needed to better refine and catalyse the successful implementation of nanomedicine. It is also very crucial to establish suitable regulating bodies for controlling and monitoring the use of nanotechnology and its products, as well as for providing clarity and legal certainty to manufacturers. The new era of nanomedicine is coming and the potential of nanomedicine seems infinite along with more public awareness and support.

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Chapter 16
Intersection of Nanotechnology and Healthcare

Swasti Gurung, Dan Fei, and Yi Ge

16.1 Introduction – Brief Background of Nanotechnology

Nanotechnology is the engineering of functional systems and manufacturing of materials at atomic and molecular scale. Modern word *nanotechnology* is derived from Greek term 'nano' meaning dwarf. Dr. Richard Feynman, the Nobel Prize winner in Physics in 1959, is widely recognised as the "father" of the subject, but the term *nanotechnology* was formally introduced by Professor N. Taniguchi in the year 1974 who defined nanotechnology as "the processing of separation, consolidation and deformation of materials by one atom or one molecule" [1]. Nano in the International Systems of Units (SI) is known as one billionth of a meter (10^{-9} m). The size of a structure to be classified as 'nano' usually needs to be roughly between 1 and 100 nm (nano-meter) in size at any one dimension. Regardless of the size restriction, nanotechnology implies to any structures that are developed by top-down or bottom-up engineering of individual components even if it is several hundred nanometers in size [2]. The top-down fabrication is normally applied for achieving nanometric precision and accuracy in an artefact by material removal or

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deposition (e.g. nanolithography and energy beams). In comparison, the bottom-up fabrication is a technique used for assembling components of nanometric or sub-nanometric size in creation of an artefact with functions such as molecular assembly or manipulation. According to the National Nanotechnology Initiatives (NNI), defining features of nanotechnology are as follows [3]:

- Any research and technology involving development at 1–100 nm range.
- Creation and usage of structures that have novel properties because of their size.
- Ability to control and manipulate at atomic scale.

Nanometre sized objects possesses remarkable and noble properties such as self-assembly and self-ordering under control of forces which macro objects are not capable of doing [4]. The unique features of nano objects make nanotechnology feasible for altering and improving properties and performances of conventional objects and products.

16.2 Healthcare

Healthcare is the diagnosis, treatment, and prevention of disease, illness, injury, and other physical and mental impairments in humans. It is one of those sectors in any place of any country in the world that is always looking for ways to improve and out perform their existing records. Healthcare is also among the highly invested sectors publically or privately in most of the countries since the health of a citizen is directly related to the country's level of development and progress. According to the official information supplied by the National Health Service (NHS) which is one of the largest publically funded services in the world, around £108.9 billion was budgeted for the financial year of 2012/13 in the UK [5]. This shows the calibre of healthcare investment required in order for it to function smoothly. It also shows the potential and opportunity it holds to cut down the running cost yet maintaining the quality of service or even improving it.

Nanotechnology comes into association with healthcare in various known or unknown forms. For example, one of the most well-known or prominent services where nanotechnology could contribute and aid is the imaging service/technology, such as X-rays, MRI (Magnetic Resonance Imaging) scan, and CT (Computerised Tomography) scan. According to the statistical data released from the Department of Health in the UK, the total number of imaging examinations or tests was 40.1 million during the period of 1st April 2011 to 31st March 2012 (DH, [6]). That is a total of 3.3 % (1.3 million tests) increase in the amount of examinations performed in comparison to previous year 2010–2011. Among those, X-rays (radiographs) totalled to 22.5 million, ultrasound of 9.0 million, CT scan of 4.4 million, MRI scan of 2.3 million, fluoroscopy of 1.3 million and 0.6 million were radio-isotopes. This is just an example of a fraction of the entire healthcare service provided but the amount is ample enough to demonstrate the scale of operation.

16.3 Advances of Nanotechnology in Healthcare

From engineering to designing, environmental science to textile manufacturing, many fields are able to change and benefit from a novel technology, such as nanotechnology, that is garnering pace and success at an incredible speed. Healthcare is no stranger to such revolutionary technology either and the conjugation of nanotechnology in relation to medicine is favourably named 'nanomedicine'. Nanomedicine has brought many commendable advancements in healthcare and what seemed like scientific fiction a few years back is now almost within grasp of reality. Among various fields of medicine, the pharmaceutical industry is the most adaptive, evolutionary and fast paced one getting into holds with precognitive and innovative idea such as nanotechnology, and has made a significant breakthrough as well. Nanomedicine at present is a profound part of pharmaceutical industry and is making a major breakthrough in medicine.

16.3.1 Applications of Nanotechnology in Imaging

Molecular imaging is an emerging technology which intends to improve the accuracy of disease diagnosis, and the association of nanotechnology has aided molecular imaging to further have the capacity of disease pathology characterisation. This has been achieved by using a number of nanoparticles (NPs), such as liposomes, dendrimers, gold, iron oxide and perfluorocarbon NPs, which are very sensitively and selectively responsive to specific disease [7, 8]. In addition, in combination with other advantages such as small size, high surface area to volume ratio, long circulating hour, high affinity for target tissue, easy production, less toxicity and immunogenicity, it makes NPs highly attractive to be used as the contrast agents [9, 10]. For example, tailored NPs (e.g. dendrimers) could be used as advanced contrasting agents for MRI scan by shortening the spin–lattice relaxation time T1 and spin-spin relaxation time T2, resulting in sharper and brighter images. Herein, T1 and T2 are two different types of MRI scans that help differentiates types of abnormalities in accordance to density. T1 is effective in imaging solid organ pathology (e.g. liver and spleen), whereas T2 is effective in imaging soft tissues. Furthermore, the NPs (e.g. iron oxide NPs) that have superparamagnetic properties are able to change the spin-spin relaxation time of neighbouring water molecules. Thus, they could be applied to monitor the expression of genes, detect tumours, artherosclerotic plaques, and tissue inflammation, etc. The NPs can also be targeted actively or passively in favour according to the subject of interest to differentiate normal and diseased tissues.

Furthermore, NPs could have multiple binding sites which increases affinity for target tissues immensely [10]. Intracellular imaging is possible with NPs like quantum dots which have high-fluorescence intensity making it easier for tracking of cells throughout the body. They are more desirable then conventional fluorescent since they are more stable, allowing images to be sharper and crisp over long period

of time. In addition, they have the ability to detect multiple signals at the same time [11]. They emit bright lights which mean small amount of quantum dots can be sufficient to produce desired signal which makes them promising candidate for detection and diagnosis of various diseases. Due to their small size it is easier for them to enter and interact with biomolecules within the cells.

Atomic force microscopy (AFM) is another powerful tool for imaging which incorporates nanotechnology providing results with high-resolution and three-dimensional images [12]. An AFM functions with a microscale cantilever which has a sharp tip, used as a probe to scan specimen surfaces under focus. Deflection of the probe is detected and recorded as a result which happens due to the presence of attraction and repulsion force between the close contact of probe and surface of the specimen which is normally within 1–10 nm in distance. Deflection of the probe is quantified by the beam bounce method where lasers beam on top of the cantilever into range of photodiodes providing a three-dimensional profile of the specimen on scan. The development progression in AFM has allowed tumour detection, detection of erythrocytes influenced by diabetes and studying the structure of C-reactive protein that are a risk for coronary artery disease and peripheral arterial diseases [12]. Also the application of nanotechnology in molecular imaging is giving ways for further development of specialised medicine like personalised medicine and is sure to bring about a huge revolution and transform the way of disease diagnosis, treatment and prevention.

16.3.2 Applications of Nanotechnology in Drug Delivery

When talking about nanotechnology in healthcare, drug delivery system is one of the most conspicuous topics that attract a huge amount of interests from both civil society and industry. Pharmaceutical company's quest to develop targeted drug delivery systems with existing drugs whilst incorporating nanotechnology for effective medical treatment is evolving on daily basis. Effective drug-targeting system based on therapeutic efficacy, appropriate concentration and longer circulation time, could be achieved by utilising nanotechnology [2, 7, 13, 14]. For example, nanoparticles (NPs) could be employed to deliver drugs to specific types of cells (e.g. cancer cells) whilst overcoming barriers such as heat, light and various physiochemical environments. These NPs are engineered in specific way such that it is able to adhere to targeted diseased cells and delivers direct treatment to those cells alone. They could further help to reduce damage to healthy cells significantly, decrease side effects and even allow earlier detection of diseases.

In terms of the improved drug delivery system via a better and innovative formulation, some drug nanocrystals have already been commercially developed [15]. For instance, Elan Nanosystems developed a process called nanonisation to solve the problem of poor water solubility of a drug. This was achieved by reducing the drug crystals until they became particles of 400 nm in diameter or less. A thin layer of polymeric surface modifier was used for absorptions onto the crystal surfaces to

prevent aggregation and for stabilisation of the particles produced. The result was a suspension that looked and functioned like a solution which can be used in various forms of dosage such as pills, sprays or creams.

Researchers in Kyoto University developed a smart drug that got activated in specific circumstances [16]. In this case, the novel drug molecule released antibiotic only in presence of an infection. A molecule of gentamicin was bound to a hydrogel with a peptide linker which is cleavable to a proteinase enzyme produced by *Pseudomonas aeruginosa. Pseudomonas aeruginosa* is a common bacterium that can cause disease in animals and humans. This smart drug was tested on rats, which showed that in presence of a bacterium, the enzymes produced by the microbes cleaved the linker releasing gentamicin which then killed the bacteria. But in the case where there was no presence of bacteria or the enzyme produced, the drug remained unaffected.

Freitas Jr Robert A. designed a spherical nanorobot the size of a bacterium and made up of 18 billion atoms which were arranged precisely in a crystalline structure to form a miniature pressure tank (Freitas Jr, [17]). The design was of an artificial red blood cell called respirocyte. The miniature tank would hold as much as nine billion oxygen and carbon dioxide molecules. When the artificial blood was to be injected into an individual's bloodstream, the sensors on the surface of the robots would detect the level of oxygen and carbon dioxide in the blood and signal the time to load oxygen and unload carbon dioxide and vice-versa. These nanobots could store and transport gas 200 times more than red blood cells and also consists of glucose engine which releases glucose when there was a deficiency in the body. Even though this is a conceptual idea, there have been uses of artificially engineered microbes already to produce human hormones. Such example is incorporation of human DNA in the genome of bacteria which then starts producing human hormones used for curing endocrine diseases.

16.3.3 Applications of Nanotechnology in Gene Delivery

Nanotechnology has been applied in gene delivery with help of NPs such as liposomes and dendrimers [14, 18, 19]. In able to be successful in this venture, understanding gene therapy is important. Gene therapy is a technique which involves altering, removing or inserting gene at particular loci in order to treat various genetic disorders. In order to do so, a factor able to transfer the gene to a desired location is required which is known as a vector. A vector can be viral or non-viral of origin and mostly includes retroviruses, adenoviruses, lentiviruses and adeno-associated viruses which are very useful in utilising the natural mechanism of an infection [7].

Gene delivery replaces defective gene with a normal one or delivers genes into the disease cells to cure and treat diseases. It was applied as a method to treat hereditary diseases earlier but now have been proven very helpful in treating diseases like cancer. Even then, there are certain limitation points that the technique faces which have been able to overcome by help of nanotechnology, with introduction of non-viral

vectors like liposomes and dendrimers which are less immunogenic than the conventional viral vectors. The properties that NPs behold which make them better vectors than currently used vectors are as follows [20, 19]:

- They are cationic in nature and encapsulates negatively charged DNA by electrostatic interactions;
- Safe and simple in use;
- Easily reproducible;
- Even with decreased efficiency in transfection compared to viral vectors, adjustments are easy to make which overcomes the shortcoming.

Dendrimers are known for being efficient in gene delivery and have the ability to protect DNA from the action of DNAse enzyme. The transfection efficiency can be increased by performing heat treatment with solvents like water and butanol which enhances flexibility, allowing dendrimers to become compact when compounded with DNA. The dendrimer that is most commonly used is Polyanidoamine (PAMAM) because it has the highest transfection efficiency [14, 19].

Another such use of NPs, as gene delivery carriers, are liposomes which have certain advantages [19]. Their size can be easily controlled and modified to add a targeting agent but they do have a downfall of having low efficiency in encapsulating DNA. However, the issue of low efficiency can be solved or avoided by using cationic liposomes because they consist of lipid bilayers which are positively charged and combine spontaneously with the negatively charged DNA. The liposomes are mixed with cholesterol and further modified with functional ligands to increase transfection efficiency.

16.4 Impacts of Nanotechnology on Healthcare

The Impact of nanotechnology is extending, from medical, environmental, biology, computing, material science to even communications and military applications. Even though nanotechnology is showing promising development and positive results are being demonstrated steadily, the fact that it is still an emerging field which cannot be blindsided easily. The sole fact that it is an emerging field has roused numerous heated debates about the extent till where the technology benefits and the risks for human health it may bear. Based on the impact of nanotechnology on human health strictly, the subject could be divided into two categories: (1) Potential of nanotechnology holds for innovative medical applications in curing diseases; (2) Potential health hazard it may pose with exposure to nanomaterials. The sustainability of nanotechnology would thus depend on social acceptance, minimised risks and maximised benefits.

The biggest concern related with nanotechnology in regards to its applications in healthcare would be the unknown outcome when exposing to nanomaterials. Due to the scarcity of systematic studies and established regulations, nanotechnology is not easily accepted by many fields even with its promising and positive results.

Healthcare is in no exception since it holds responsibility of millions and billions of people and their health with any small decision they make that has anything to do with treatment, diagnosis and cure. Showing promising results is still not convincing enough for medical society to accept nanotechnology without hesitance because of the technology lacking long documented track record like those of conventional and traditional technologies. Nanotoxicity is being pursued with rigorous pace and through experimentation but it still is not being able to reach the standard required by many healthcare organisations. Especially with the case like asbestosis where the symptoms usually appeared 30-40 years after the exposure period when the damage was catastrophic and unmanageable, the requirement for a more comprehensive investigation of nanotoxicity in relation to nanomaterial exposure is urged and becomes imperative in healthcare.

16.4.1 Nanotoxicity

Materials possess very different properties in comparison to their initial bulk form, such as surface area, surface properties and chemical properties. These different properties have intrigued many scientific innovation and experiments which have made many ground-breaking progress over the years. Nanomaterials have some unique properties in comparison to their larger counterparts due to the quantum size effects and large surface area to volume ratio. Hence, manipulation of substance at nanoscale will have variety of effects in manufacturing, engineering, environmental technology, information technology, health, pharmaceuticals and many other industries. In other words, the resulting nano-sized materials may offer a safe solution or pose a threat to the environment and to human beings [21]. Since there still are no sufficient data available for identifying, monitoring, and controlling the toxicity of nanomaterials, this concern gets brought upon time and again by environmental activist and regulatory bodies, etc. [22].

With respect to our body system in particular, nanomaterials which are very fine could easily be inhaled. They can re-disperse within body to different organs after initial stage of introduction inside the body. Some of the routes which nanomaterials adapted to enter the body are as follows [23, 24]:

- Respiratory system
- Ingestion
- Dermal exposure
- Medical implants (e.g. orthopaedic)

Since there is no cut-off point below about which particles are suddenly classed harmful, in relation to NPs, two factors in mixture may determine the potential harm caused (esp. in lung injury).

1. Large surface area and reactivity of the surface
2. Smaller particles which are more likely to be harmful

Once NPs are inside the human body, they could mix with blood during gas exchange and get transported to different organs of the body during circulation. They could get deposited even in nervous system due to their ability to overcome the blood brain barrier. Collectively, some in vitro studies have already identified that the oxidative stress related changes of gene expression and cell signalling pathways may underlay the toxic effects of NPs [24, 25]. Similar effect in role of transition metals and certain organic compounds on combustion generated NPs were also found [25, 26]. Recently, according to Health and Safety Executive [27] nanomaterials are classified hazardous under following criteria:

- Thinner than 3 μm
- Longer than 10–20 μm
- Biopersistent
- Do not dissolve/break into shorter fibers

16.4.2 Nanopharmaceuticals and Food and Drug Administration (FDA)

Lots of pharmaceutical companies are in trouble with patent expirations on numerous 'blockbuster' drugs, resulting in a loss of multi-billion dollars [28]. There has been an argument over big pharma companies being more focused on shareholder profits than innovative therapies. In today's global economy, big pharmaceutical companies face huge pressure to deliver high-quality products while maintaining profitability. Because of this rising issue, nanotechnology has been applied by numerous pharmaceutical companies to revisit their shelved drugs that were difficult to formulate due to their solubility profiles. The existing nanopharmaceuticals in market that have been approved by the FDA are in absence of any special testing in accordance to the pre-existing laws [28, 29]. However, the approval of new nanodrugs and 'nano-reformulations' has challenged FDA's regulatory framework, which as forced FDA to evaluate submitted products for market approval on the category based-system. A drug, biologic or device has been assigned to Centre for Drug Evaluation and Research (CDER), the Centre for Biologics Evaluation and Research (CBER) or the Centre for Devices and Radiological Health (CDRH) for evaluation respectively. Certain therapies which comprises of two or more components (drug, biologic or a device) that are physically, chemically or otherwise combined or mixed to produce a single entity is 'combination entity'. However, this arrangement has resulted in inconsistency when approved by the FDA in basis of category-based approval which had been deemed "arbitrary and capricious". With issues such as this, nanopharmaceutical is more likely to complicate the combinational products with potential to further blur the lines in distinguishing these categories [29]. In addition, nanopharmaceuticals may also present safety issues for FDA knowing the unpredictable nature of interactions between nanoparticles and biological systems since the surface charge and shape associated with a NP is known to influence its

toxicity. Another particular safety issue to be raised by nanopharmaceuticals is the potential for bioaccumulation of NPs with prolonged use [28]. For example, buckminsterfullerene has shown to impair DNA repair mechanism with additional report of certain NPs shown to cause brain damage in fish and lung toxicity in mice.

16.5 Regulatory Challenges to Nanotechnology

Application of existing regulatory frameworks and space for tailoring rules implementing new technologies and products development is questionable when it comes to nanotechnology. This puts pressure on regulators capacity to keep in pace with developments such as nanomedicine and other new applications. Difficulties arise in balancing technological benefits to risks for expertise in regulatory bodies. New innovations such as nanotechnology require practical regulators, who are able to facilitate responsible development in order to gain trust of stakeholders for such areas to prosper.

As a fact, the knowledge gaps in product formulation and concentration of NPs have raised questions about the applicability of European regulations on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) [30]. Another debatable issue involving nanotechnology is to do with uncertainty and ambiguous risk that rise dilemma in regulators on either to wait until there is sufficient knowledge available or to act promptly. Another knowledge gap in nanotechnology is to do with the toxicological aspect of nanotechnology and the potential risk it pose on health and environment, which challenges safety regulations to its limit. As recorded, toxicological studies conducted with NPs have indicated that free NPs could penetrate through the blood–brain barriers or remaining lodged in capillaries [25]. There has been prediction for possible impact of these particles on immune system and consumption by macrophages. Uncertain biocompatibility imposed by NPs in relation to it being used in medical products and materials is another challenge on its own. It has been evaluated that the toxicological risks of NPs depend on material properties, exposure route, dose and frequency of doses which accounts for risks with usage of NPs among other things such as distribution of particles in the body [24, 25]. Even worse, further toxicological risks of NPs are yet to be known. Previous drug disaster in late 1950s with thalidomide, of which effects are still being projected till today, regulation bodies have thereafter ensured to higher the level of public health protection in terms of safety, efficacy and quality.

16.6 Rules Governing Nanomedicine

There is still no specific rule regarding nanomedicine by the European Union to date. Having said that, nanopharmaceuticals have been classed as advanced therapy medicinal products and can only be approved by centralised procedure. In cases

where nanomedicinal products are in combination with medical devices and/or regular medical products, certain aspects of regulatory regimes for both medical devices and pharmaceuticals apply, regardless of the manner in which the other features have been combined in the product [30]. The application of regulatory regime depends on the category in which the product falls in regards to the definition of medicinal products, advanced therapies medicinal products or medical devices. The primary mode of action depends on the criteria in which the products falls majorly in application to the regulatory regime. The market authorisation depends on positive outcome of risk-benefit balance. Applicant must be able to demonstrate sufficient product safety, quality and efficacy in comparison to large set of objective scientific data. It is mandatory for scientific evaluation of applications to be based on highest level of expertise and standards.

16.7 Marketing Prospects of Nanotechnology

Nanotechnology is bound to have a substantial impact on the world's economy and market volumes, which are a good indicator for such economic significance. Despite all the controversies and hesitance in its acceptance, if successful the technology will contribute substantially. There were plenty of market forecast originated for nanotechnology during the early 2000s with timeline going up to the year of 2015. Among all, the best compiled forecast has to be the one published by the National Science foundation (NSF) of US in 2001. The NSF forecasted that the estimated world market of nanotechnology would worth 1 trillion US dollars by 2015 [31]. In addition, by combination of other technologies, nano-enabled products and markets are expected to be of the largest share in the world. Nanotechnology has already been attracting significant amount of investments from government and various business communities around many parts of the world. In 2007, it was estimated that the total global investment nanotechnology held was around five billion Euros of which two billion Euros were from private sectors [32].

In addition to the booming nanotechnology assisted market, there was also a remarkable increase in published patents of nanotechnology, which ranged from 531 total patents in 1995-1976 total published patents by 2001 (Royal Society [33]).

16.8 Public's Concern & Prospects on Nanotechnology

It is evident that nanotechnology have bought about remarkable differences in ways of diagnosis, patient care and other medical and non-medical implications, yet it has not been able to establish itself in full positive light within the general public's eye due to the lack of communication in interpretation of it. There are very few studies that have been carried out about the media coverage of nanotechnology [34]. Mass media plays a significant role in shaping public attitude towards nanotechnology or

any other field of discovery and development since they are the major source of information to the general public. According to a survey conducted in the US and the UK, some common conclusions were drawn [34]:

- Media interest in nanotechnology has grown immensely since 1999 and in 2003 it began spreading from opinion-leading elite press to the general press hence addressing wider population;
- Media coverage of nanotechnology throughout the period of analysis (1984–2004) has been overwhelmingly positive although there are articles about risks nanotechnology bear;
- Majority of media had presented nanotechnology in terms of progress and economic prospects.

Even when media has been positive towards nanotechnology, the public can be more sensitive to possible impacts of new technologies. A good example of it is the toxicity of NPs. The word 'nano' has been embedded in the national consciousness and is an area of public debate and often concern. From scientific fictional tales of self-replicating 'nanobots' engulfing the word to legit concerns on effect of NPs used in everyday products such as sun creams, it is inevitable for nanotechnology to be out of public view. Factors such as emotive, ethical and political implications also come in to play a major role. One of the best known example of such an issue is the stem cell research. It has managed to gain the highest scientific profiles in both medical community and the general public in the past decade.

Due to the novelty of the technology, a full acceptance is yet to be achieved and acknowledged. Main concern lies in public's view on the development and effective way to convey the novel method. It always is our human nature to have curiosity around new subject and have the expectation to know more about it. There are a few of ways where the message can be conveyed efficiently as follows [34]:

- Learning from previous cases, avoid the same mistakes;
- Aim to elucidate the public's knowledge and attitude towards the technology;
- Public workshops;
- Focus groups;
- Sources that give further information about genuinely considered beliefs of public towards nanotechnology instead of currently uninformed opinions;
- Assessments of nanotechnology as positive and major benefits, especially from health application of nanotechnology;

On the other hand, from the academic point of view, there are also a few of things which could help to dilute the issue and enhance a better future for nanotechnology, including nanomedicine in healthcare [34]:

- Possible ways to deal with inherent uncertainty concerning the potential impacts and future developments;
- Urge the governmental bodies as well as industry to take decisions for the benefit of general public;
- The potential risks and risk management of nanotechnology.

16.9 Conclusions and Future Perspectives

Nanotechnology has come up with many solutions to previously unsolved pharmaceutical, medical and technical problems. It has revolutionised healthcare with its contribution to the betterment of biomarkers, imaging, drug discovery, development and delivery, etc. The applications of nanotechnology in healthcare thus have been growing exponentially, along with increasing interests in investment from both government and industry [35].

However, in healthcare, the unique and novel properties of nanomaterials could become a particular issue in regulatory department due to insufficient information of their toxicity profile and being very different to regular pharmaceuticals. This has caused dilemma among expertise in regulatory body to categorise the nano-related products before getting evaluated for the market approval. The line among many categories are merged and blurred when coming to evaluating nanopharmaceutical. The knowledge gaps on the subject and lack of expert in the field employed among regulation institutes has also created numerous obstacles.

It has also been shown that the public awareness and understanding of nanotechnology in healthcare is still immature and sometimes may be biased and prejudiced due to the concerns of nanotoxicity. Meanwhile, the use of nanotechnology may be promoted too extensively in a sense that it becomes a hype far detached from the reality [36].

Despite such dilemmas, there is no doubt that the future of health will be closely interlinked with developments in nanotechnology which is being used in an evolutionary manner to improve and/or replace many existing therapeutics and healthcare products. It shouldn't be a surprise that in the near future we will have smart 'nanobots' which could be safely be taken by the human body and then automatically repair or destroy specific diseased cells/tumours.

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Chapter 17
The Impact of Nanopharmaceuticals on Healthcare and Regulation

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17.1 Introduction

The world is coming to a new era of nanoscience, with various nanotech-based commercial products mushrooming in every sector of the market [1]. For example, the rapid development of nanotechnology offers a broad selection of nanomaterials with precisely controlled manufacture techniques that provide unprecedented advantages in various medical applications [2]. The integration of nanotechnology into medical field, termed as nanomedicine, has been on the European and the U.S. markets for almost two decades (Dorbeck-Jung et al. 2011). Considered as a revolutionary change to the future of medicine, nanomedicine has been developed into a billion-dollar industry enjoying an everlasting rapid boost [3]. Its worth is estimated to reach around $131 billion by 2016 [4].

The appearance of nanotechnology in pharmaceutical world has provided great potentials for the improvement of current medical service. Nanopharmaceuticals already has shown some superior performance over conventional ones with better biocompatibility, bioavailability, and system stability. Furthermore, pharmaceuticals developed at the nano-scale could induce different pharmacokinetics, and have better

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solubility, efficacy circulation half-life and lower toxicity with decreased dosage. These advantages would thus significantly benefit patients, who suffer from uncomfortable drug administration and drug resistance. Furthermore, with a smart structure design and functionalisation at nano-scale, clinical therapeutics such as drug delivery could be remarkably enhanced by specific targeting and better penetration, precisely detecting diseases at very early stages before situation deteriorates [3].

On the other hand, the rapid development of science and technology brings about higher requirements for survival and quality of life. Traditional regime is not capable of fulfilling the emerging need for personalized medicine [5]. As the most regulated sector in industry, pharmaceutical manufacture faces risky hedges in marketing and compensation [6]. Although current pharmaceutical regime in the EU is a well-established, flexible and comprehensive system, its appropriateness toward the regulation of nanomedicine has been questioned. In the late 1950s, drug disasters such as thalidomide crisis triggered the launch of new regulations for medicine in Europe to ensure the safety as well as efficiency and quality (Dorbeck-Jung et al. 2011). It is worth noting that nano-based drugs are regulated as conventional products in the process of commercialization at the moment (Dorbeck-Jung et al. 2011). In fact, current guidelines contain no specific standard for nanoparticles, despite more issues (e.g. toxicity) have been generated for their medicinal applications. Thus, specific regulations for the assessment and approval of nanomedicine (including nanopharmaceuticals) products are urged [7].

Overall speaking, nanopharmaceuticals, as a part of nanomedicine engaged in therapeutics, is regarded as one of the most promising subfields of nanomedicine (Dorbeck-Jung et al. 2011). It has generated numerous and increasing interests in the past decade, filling in the communication gaps of nanotechnology, material science biomedical science and pharmaceutical science. Since nanopharmaceuticals could greatly alter the some nature of existing pharmaceuticals (e.g. way of administrating therapeutic agents) [8], inevitably, new considerations for healthcare and corresponding regulations have been raised simultaneously.

17.2 Nanopharmaceuticals in Healthcare

17.2.1 Techniques and Advances

The molecular-levelled interactions of nanomaterials with targets could moderate cellular microenvironment at specific spot, providing a new approach to therapeutics [2]. The diversity of nano-scale materials (Fig. 17.1) provides more options for various applications of nanomedicine, such as disease diagnose and drug delivery. Some commonly used nanopharmaceutical materials are particularly listed in Table 17.1, together with their advantages and pharmaceutical applications.

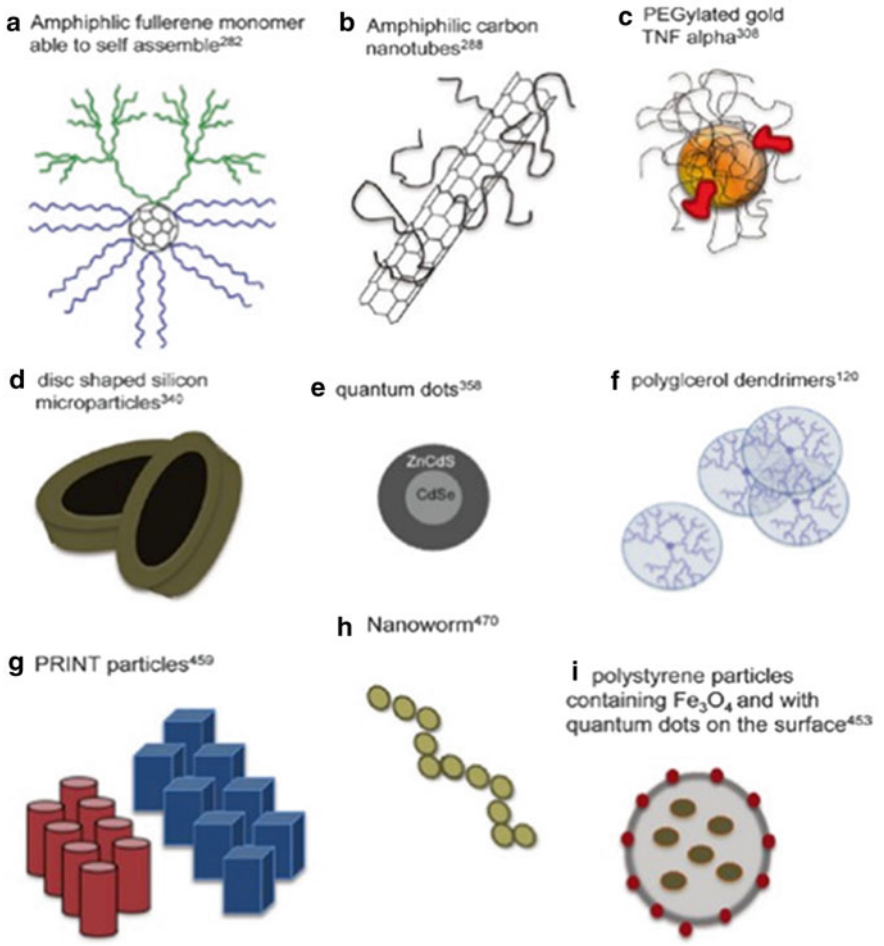

Fig. 17.1 Emerging nanomaterials (Adapted from [9])

As further summarized by Mansour et al. [11] and Hossain Saad et al. [12] recently, nanopharmaceuticals could bring the following advantages:

- Nano size, which increase the surface area, thus enhancing the dissolution rate.
- Surface charge
- Improve drug targeting ability
- Increase the drug stability and improve formulation
- Reduce the dose needed and toxicity related to the drug molecules
- Enhance solubility and bioavailability
- Increase patient compliance since drugs can be deliver by different routes (oral, topical, intravenous, intranasal, etc.)

Table 17.1 Common nanomaterials used in pharmaceutical applications (Modified from [10])

Nanomaterial	Advantage	Application
Carbon nanotubes	Unique thermal, spectroscopic, electrochemical properties	Drug carrier, imaging agents, hybrid theranostics
Liposomes	Low toxicity composition, can incorporate both hydrophilic and lipophilic drugs	Drug delivery, triggered drug release, develop novel probe
Polymer conjugates/micelles	Biodegradable & biocompatible	Drug delivery for a wide range of disease treatment, especially require chronic administration ones
Dendrimers	Capable of penetrating biological barriers, non-immunogenic, surface chemistry is tailored	Drug delivery, MRI imaging, gene therapy, receptor-mediated / passive tumour targeting
Silver nanoparticles	Strong antibacterial action	Aid wound healing, treating atopic dermatitis

The nanotech-incorporated cancer therapeutics is among the top therapeutic/pharmaceutical applications of nanotechnology [13, 14]. As one of the leading causes of death worldwide without efficient treatment, cancer poses a great threat toward our modern society. The conventional chemotherapy and/or drug treatment could result in severe side effects by non-selectively attacking both healthy and cancer cells. As a solution, nanotechnology could enable a cell-targeting therapy, preventing healthy cells from attack. The unique properties possessed by nanoparticles could also enhance the efficiency of delivery, increase the payload of the drug and further monitor the therapeutic performance [15]. In fact, the second generation of anti-cancer nanomedicine products have started to demonstrate their merits to more stakeholders [8].

Some pH-sensitive nanocarrier systems, for instance, have been successfully developed for targeted therapy in cancer treatment (Manchun et al. 2011). Being different from pH 7.4 for normal tissues, the extracellular pH value of cancer tissues is 6.8 due to the increased extracellular lactate and protons in the microenvironment led by the up-regulated glycolysis. In such systems, the loaded drugs could retain within the nanocarriers at pH 7.4 for normal tissues and be specifically released at an acidic condition near the cancer tissues as a result of structure and/or formation change of the nanocarriers. For example, Filippov and his colleagues [16] recently reported a HPMA (N-(2-hydroxypropyl)-methacrylamide)-based nanoparticle-drug conjugate for targeted drug delivery. The drug doxorubicin (Dox) was conjugated to the nanoparticle via a pH-responsive hydrazine bond. Moreover, the size and shape along with internal structure of the conjugate could be monitored and controlled precisely.

Apart from anticancer therapy, nanopharmaceuticals also has successfully provided promising treatments for other clinical conundrums such as Alzheimer's disease [17], diabetes [18], and ocular delivery (Vadlapudi et al. 2013).

In terms of the fabrication of nanoparticles in pharmaceutical manufacturing, various techniques have been engaged, each with its own advantages and drawbacks [19]:

- Milling: Traditional milling techniques can be modified to operate under certain speed to reduce the particle size to nano size. This technique faces the challenges of balancing the type and amount of the additives (influence toxicity) with the stability of the system, purification of the product, and the variety of time consumed for an aimed range of sizes.
- Polymerisation: This is a widely adapted bottom-up technique for fabricating nanoparticles by using monomers as starting materials for polymerisation. The main disadvantage of this technique is the (potential) toxicity of the monomers and resulting products.
- Emulsification (/Precipitation/Coacervation): In this technique, the drug is dissolved and mixed in an organic, miscible anti-solvent to form a stable emulsion. The product is received after removing the solvent. The drawbacks of this technique are variable mixing processes resulting in various range of size distribution of the nanoparticles, and the spontaneous growth of crystals in presence of a nucleation that increases the difficulty to control the size distribution. Also, the removal of solvent must be sufficient; otherwise the residue of it in the nanoparticles may cause the degradation of nanoparticles.
- Microfluidisation: Also termed as piston-gap homogenizer, this technique prepares nanoparticles/nanosuspensions via homogenization under a high pressure. Problems for this technique include requiring larger amounts of energy for further reduction in size, blocking of the piston, the difficulty for scale-up, and contamination by heavy metal in some cases.
- Supercritical Fluid Technique: This is an environment-friendly technique manufacturing nanoparticles via a procedure free from solvent. It has become an alternative for producing nanoparticles in recent years on the basis of that of micro-scale particles. The restriction of its utility is the limited choices for the polymer since only few polymers have shown to be soluble in supercritical fluid.
- Spray drying: This method fabricates nanoparticles by spray drying of the suspension of nanoparticles, which is prepared through wet comminution using stabilizers.

17.2.2 Positive Impacts

Nanotechnology has already demonstrated its power and capability in revolutionising many healthcare practice and treatments, such as cancer and cardiovascular diseases treatment, gene therapy, and orthopaedic implants. For pharmaceuticals, the unique properties and advantages of nanomaterials have remarkably affected and enhanced the whole production line of this business, from drug discovery and development to drug delivery. As a result, great positive impacts have been made on healthcare, in terms of higher efficiency of treatment, decreased healthcare cost, and less patient suffering.

17.2.3 Safety and Other Concerns

Apart from the great potentials, nanopharmaceuticals has to be carefully examined against uncertainties, such as knowledge gaps on nanomaterials' physicochemical properties and toxicity aspects. The detection and characterisation methods as well as toxicological data of nanopharmaceutical products are still lacking. As a result, the related risk assessment is incomplete. In recent years, increasing concerns have been raised about the long-term potential hazard of employing nanomaterials, resulting in possible toxicity (e.g. cytotoxicity and genotoxicity). As it is well known that the penetration of conventionally impermeable biological barriers can sometimes trigger neurotoxicity, carbon nanotubes, for instance, have been reported to have similar pathogenic phenomenon as asbestos in mice [20]. Intratracheal administration of single-walled carbon nanotubes has also been proven to exacerbate allergen-related airway inflammation (Syed et al. 2012). The specificity of nanomaterials undoubtedly leads to a necessity of the multidisciplinary investigation of nanopharmaceuticals that involves various stages [21]. Unfortunately, the progress is hampered by the unclear and yet-to-reveal hidden mechanism of interactions between nanomaterials and biosystems (e.g. cells, tissues, and organs).

Because of the vital role of pharmaceutical products plays in the battle of saving life, safety needs to be considered as a priority in assessing a new nanopharmaceutical product. Early assessments of safety therefore shall be the open-shut gate deciding whether the product is worthwhile of a further clinical development (Fig. 17.2) [9].

17.3 Nanopharmaceuticals and Regulation

Nanopharmaceuticals are creating new challenges for the regulatory bodies – such as the Food and Drug Administration (FDA) and the European Medicines Agency (EMA). The first challenge is about a universal and international definition of what is nanotechnology [22]. Without a proper and universally agreed definition, it is difficult to put proper regulations in place that take into account all aspects of the use of nanomaterials in pharmaceuticals. For example, nanomaterials could be defined as materials with a size smaller than 100 nm, while some others accept larger materials with a size up to 500 nm as nanomaterials [23].

Pharmaceutical industry is one of the most regulated and controlled industrial sectors. There are already many commercially available nanopharmaceutical products (e.g. Myocet and Rapamune). Most of them have been approved by the regulatory body based on the pre-existing laws/regulations without a further special assessment. Due to the amazing development of nanopharmaceuticals in recent years and its safety concerns discussed earlier in the chapter, the new/refined regulation is urged with a special assessment both in vivo and in vitro.

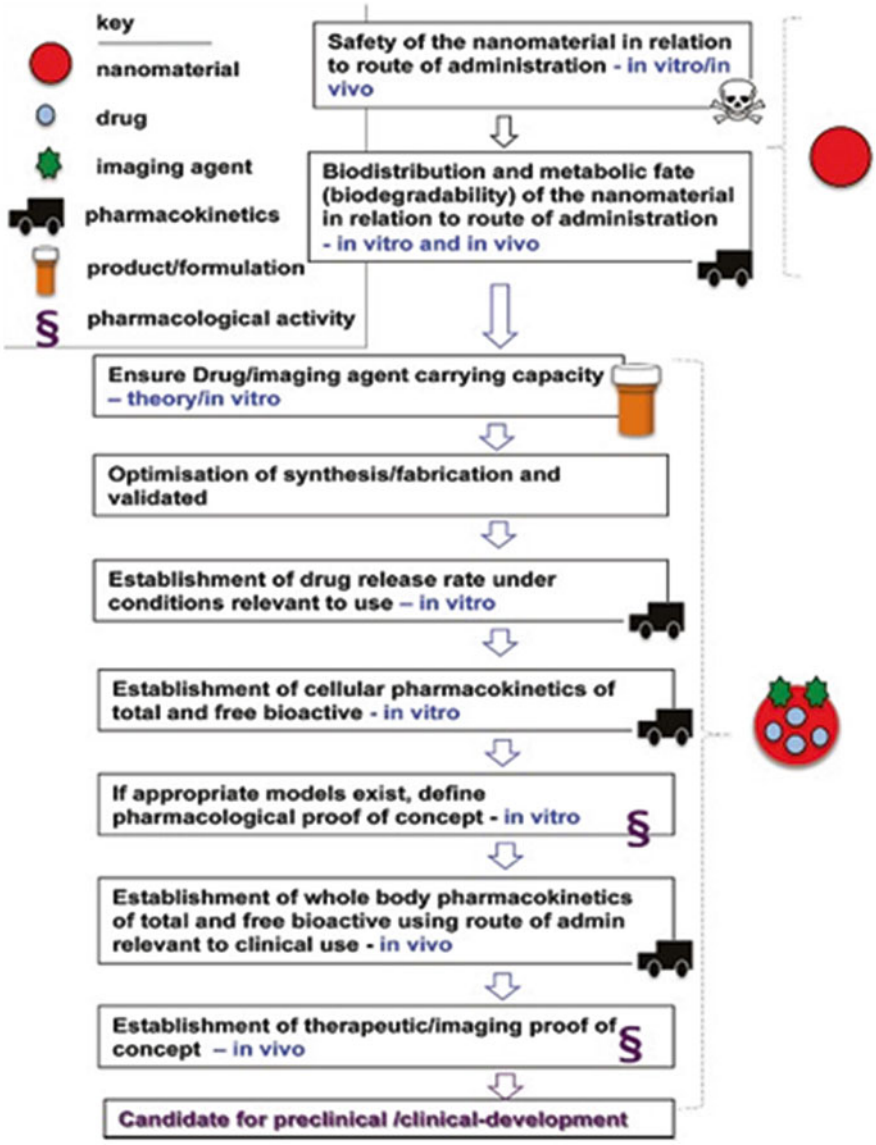

Fig. 17.2 Summary of stop-go checkpoints for the preclinical development of nanomedicine (Adapted from [9])

Excellent progress has been made. Under FDA's updated guidance, the following points should be considered by industry, when a product involves the application of nanotechnology or contains nanomaterials [24]:

- Whether a material or end product is engineered to have at least one external dimension, or an internal or surface structure, in the nanoscale range (approximately 1–100 nm). They are relevant to evaluations of safety, effectiveness, performance, quality, public health impact, or regulatory status of products;
- Whether a material or end product is engineered to exhibit properties or phenomena, including physical or chemical properties or biological effects, that are attributable to its dimension(s), even if these dimensions fall outside the nanoscale range, up to one micrometer (1,000 nm). They emphasized that "such evaluations should include a consideration of the specific tests (whether traditional, modified, or new) that may be needed to determine the physicochemical properties and biological effects of a product that involves the application of nanotechnology".

By contrast, REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals), which is a regulation of the European Union adopted to improve the protection of human health and the environment from the risks that can be posed by chemicals, entered into force on 1 June 2007. Based on the communication of second regulatory review on nanomaterials in 2012, a study has recently be undertaken to support the impact assessment of relevant regulatory options for nanomaterials in the framework of REACH [25]. It was suggested that "those nanomaterials currently falling outside existing notification, registration or authorisation schemes" would be considered and included.

17.4 Summary and Future Outlook

Nanotechnology is revolutionising the market of pharmaceuticals [26]. In general, the expanding application of nanopharmaceuticals in medical service is changing our healthcare system as well as the regulatory system.

However, as discussed earlier, there are still many challenges for nanopharmaceuticals with respect to its application and fate in healthcare and regulation, respectively:

- Apart from the challenges of fabrication of nanomaterials used for nanopharmaceuticals, there are also challenges on the more comprehensive characterisation of nanomaterials such as the determination of structure, aggregation, and purity etc. In addition, the safety assessment of nanoproducts is a key for their applications in pharmaceuticals [19]. By considering the large investment and long period of product development in pharmaceuticals, the prediction and/or initial pre-assessment about the biological performance and toxicity outcome of nano pharmaceutical products would become significantly valuable and rewarding.

- Despite the great efforts made on regulation from FDA and the European Commission etc., so far there has been no thorough regulatory system specific for nanopharmaceuticals. The challenges met to obtain sufficient information about the physicochemical properties of nanomaterials, and to understand the mechanism of their molecular and cellular interactions with the internal environment, would underlay and catalyse the formation of a unified regulatory system. At the moment, the most direct challenge for nanopharmaceuticals is to meet all safety requirements in the guidelines set by the regulatory bodies, in order to gain the entrance for clinical use [27].

Inspired by the great impacts of nanopharmaceuticals on both healthcare and regulations, it is reasonable to believe that nanopharmaceuticals holds great potential to form the mainstream of pharmaceutical industry in the future.

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Chapter 18
Nanomedicine in Cancer Diagnosis and Therapy: Converging Medical Technologies Impacting Healthcare

Maya Thanou and Andrew D. Miller

18.1 Introduction

Nowadays cancer diagnosis and therapy is the primary preoccupation of nanomedicine. This focus has given rise to the new field of cancer nanotechnology that involves multidisciplinary, problem driven research cutting across the traditional boundaries of biology, chemistry, engineering and medicine with the aim of creating major advances in cancer detection, diagnosis and treatment [1–4]. The field has received strong support especially in the US where several nanotechnology for cancer centres have been launched and operated since 2004. There is no better definition and overview of this field, than that given in http://nano.cancer.gov/, which outlines the National Cancer Institute's (NCI's) alliance for nanotechnology for cancer. This alliance aims to create a multidisciplinary nanotechnology approach for the creation of solutions for cancer detection, imaging and diagnosis [5]. In Europe a number of academic groups are interested in cancer nanotechnology as well. However only with the advent of Europe FPVII programs have specific calls been announced to support multidisciplinary research in cancer nanotechnology. In the UK, the major cancer research organisation (Cancer Research UK) appears hesitant to support this emerging field, possibly due to the perceived safety risk from nanomaterials currently untested in man. This hesitation is unfortunate. In a recent report "Roadmaps

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in Nanomedicine towards 2020" [6], specialists are now predicting that imaging and therapy in oncology by means of cancer nanotechnology will be a primary opportunity for various "designer" type nanomaterials, nanodevices and nanoparticles currently in discovery and development. Indeed, the global market size for cancer nanotechnology products is predicted to be €30bn by 2015. The particular opportunity presented by cancer nanotechnology is the eventual likelihood of personalised cancer diagnosis and treatment regimes [3].

Personalized therapy of cancer begins with molecular profiling. Golub et al. were first to report how molecular profiling studies, that show variations in gene expression patterns with time and disease status, could be used to inform on the stage, grade, clinical course and response to treatment of tumours [7]. From then on, increasing numbers of such studies have been performed showing that any given metastatic lesion results from a corresponding combination of tumoral, stromal, and inflammatory factors [8, 9]. Following this, causality in cancer has become associated with cancer disease-specific biomarkers validated by histochemical studies of diseased tissue [10]. The identification of such biomarkers by molecular profiling provides the foundation for personalized cancer diagnosis and therapy [11, 12]. In a prime early example of this principle, Erbb2 (HER2) is a tyrosine kinase receptor and cancer disease-specific biomarker found in 25–30 % of breast cancers. Overexpressed HER2 can be targeted for breast cancer therapy using Herceptin that is a potent, anti-HER2 therapeutic monoclonal antibody (biopharmaceutical agent). However, Herceptin has significant drug-use side effects that can be very severe. Accordingly, the Federal Drug Administration (FDA) now requires the proven identification of over-expressed HER2 in breast cancer patients before Herceptin can be prescribed. Typical in vitro diagnostic tests for HER2 that may be used to diagnose the presence of HER2 in breast tumour development include an immunohistochemistry assay and a nucleic acid fluorescence in situ hybridisation (FISH) test. Once these tests can be shown positive, then breast cancer patients may then be prescribed Herceptin with real confidence in probable therapeutic outcomes. In summary, cancer disease-specific biomarker, HER2, is detected as a diagnosis for breast cancer and disease mechanism. Afterwards a biomarker selective biopharmaceutical agent can be administered.

Relevant cancer disease-process biomarkers are many and various. They range from mutant genes, non-coding RNAs (ncRNAs), proteins, lipids, to carbohydrates and may even be small metabolite molecules. The key is that a link(s) should be established clearly from a given biomarker to tumour growth and development. Following on from this, there is a definite requirement for hyper-flexible, platform technologies that can mobilize diagnostic agents for a given biomarker and then deliver biomarker selective therapeutic agents to disease-target cells, also with selectivity. From the various options open to cancer nanotechnology, multifunctional nanoparticles are potentially ideal to meet these twin requirements. Indeed nanoparticles could be envisaged for (a) the detection of biomarkers, (b) the imaging of tumours and their metastases, (c) the functional delivery of therapeutic agents to target cells, and (d) the real time monitoring of treatment in progression. Therefore, if this is the potential, how close are we really?

Where nanoparticles are to be created for the functional delivery of imaging and/or therapeutic agents specific to cancer biomarkers, many factors have to be taken into consideration. This fact can be illustrated with reference to the fields of gene therapy and RNA interference (RNAi) therapeutics where nanoparticles have been devised for functional delivery of therapeutic nucleic acids with some success [13–15]. Where nanoparticles have been successfully designed and used to mediate the functional delivery of therapeutic nucleic acids, an **ABCD** nanoparticle paradigm can be invoked (Fig. 18.1). According to this general paradigm, functional nanoparticles comprise active pharmaceutical ingredients (APIs) (**A**-components) surrounded initially by compaction/association agents (**B**-components – typically lipids, amphiphiles, proteins or even synthetic polymers etc.) designed to help sequester, carry and promote functional delivery of the **A**-components. Such core **AB** nanoparticles may have some utility in vivo but more typically require coating with a stealth/biocompatibility polymer layer (**C**-layer; primary **C**-component – most often polyethylene glycol [PEG]) designed to render resulting **ABC** nanoparticles with colloidal stability in biological fluids and with immunoprotection from the reticuloendothelial system (RES) plus other immune system responses. Finally, an optional biological targeting layer (**D**-layer; primary **D**-components – *bona fide* biological receptor-specific targeting ligands) might be added to confer the resulting **ABCD** nanoparticle with target cell specificity. A key design principle here is that tailor-made nanoparticles can self-assemble reliably from tool-kits of

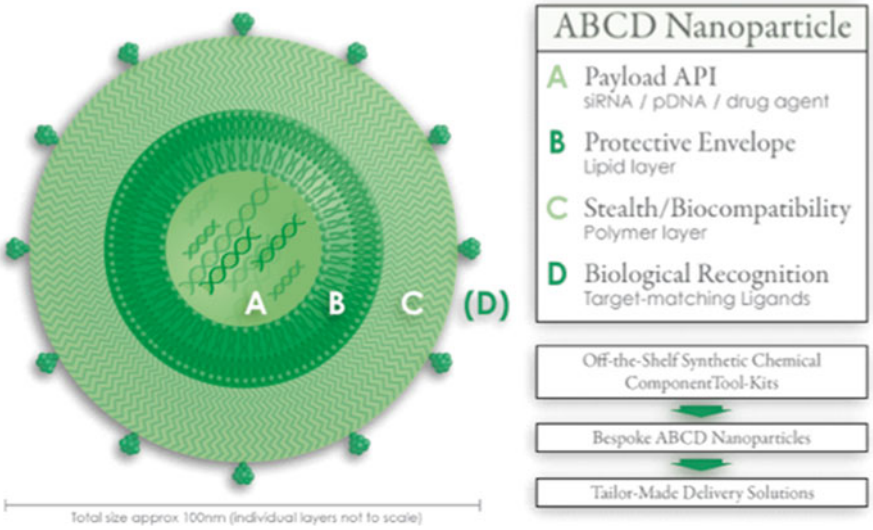

Fig. 18.1 Active pharmaceutical ingredients (APIs) (therapeutic bio-actives or intractactable drugs) are condensed within functional concentric layers of chemical components making up nanoparticle structures designed to enable efficient delivery (trafficking) of active therapeutic agents to disease-target cells. **ABCD** nanoparticle is drawn here assuming that **A**-components are nucleic acids and that **B**-components employed are lipids

purpose designed chemical components [16–26]. Accordingly, the concept of a personalized nanoparticle formulation, assembled in the pharmacy for an individual patient does not seem so far removed from reality.

The **ABCD** nanoparticle paradigm represents a set of well-found principles of design that are being implemented in the real world with the formation of actual nanoparticles leading to actual demonstrated functional properties at least in pre-clinical studies. As such, the design principles laid out in the **ABCD** nanoparticle paradigm are widely corroborated in the literature [1, 27–35]. Clearly functional nanoparticles need to be constructed from a range of chemical components designed to promote functional delivery of different diagnostic and/or therapeutic agents in vivo. In practise this means that nanoparticles need to be equipped to overcome relevant "bio-barriers" in accordance with pharmacological requirements of API use such as site, time and duration of action. Importantly too, with clinical goals in mind, nanoparticles have to be considered different to small and large molecular drugs. For instance, regulations from the FDA state that Absorption, Distribution, Metabolism and Excretion (ADME) studies need to be redesigned in the case of nanoparticles to take into consideration their aggregation and surface chemical characteristics [36].

In terms of cancer diagnosis and therapy, there is one factor that is very much in favour of multifunctional nanoparticle use. Nanoparticles administered in the blood stream (i.v.-administration) frequently accumulate in tumours anyway due to the enhanced permeability and retention (EPR) effect, a behaviour that was identified by Maeda as a means to target anticancer therapeutic agents to tumours [37, 38]. Nanoparticle accumulation in tumours takes place due to the presence of highly permeable blood vessels in tumours with large fenestrations (>100 nm in size), a result of rapid, defective angiogenesis. In addition tumours are characterised by dysfunctional lymphatic drainage that helps the retention of nanoparticles in tumour for long enough to enable local nanoparticle disintegration in the vicinity of tumour cells. The phenomenon has been used widely to explain the efficiency of nanoparticle and macromolecular drug accumulation in tumours [39]. Unfortunately, knowledge of nanoparticle biokinetics, metabolism and clearance is otherwise poor since too few nanoparticle products have been clinically tested. This is a major limitation in the growth of the field of cancer nanotechnology. Nevertheless, cancer nanotechnology is a fast growing field and new data is arriving all the time. In the following sections, the status of nanoparticle use in cancer diagnosis and therapy will be surveyed.

18.2 Nanoparticles for Cancer Imaging and Therapy in Clinical Trials and at Advanced Preclinical Phases of Evaluation

The first nanoparticles used and approved for clinical therapy use were lipid-based nanoparticles (LNPs). Selected structural lipids self-assemble into liposomes that are typically approx. 100 nm in diameter and consist of a lipid bilayer surrounding

an aqueous cavity [40–43]. This cavity can be used to entrap water-soluble drugs in an enclosed volume resulting in a drug-**AB** nanoparticle system [44, 45]. The first reported LNPs of this type were designed to improve the pharmacokinetics and biodistribution of the anthracycline drug doxorubicin. Doxorubicin is a potent anti-cancer agent but is cardiotoxic. In order to minimize cardiotoxicity, doxorubicin was encapsulated in anionic liposomes giving anionic doxorubicin drug-**AB** nanoparticles that enabled improved drug accumulation in tumours and increased antitumour activity while diminishing side effects from cardiotoxicity [46, 47]. This nanoparticle formulation has since been used efficiently in clinic for the treatment of ovarian and breast cancer [48, 49]. Thereafter, Doxil® was devised corresponding to a drug-**ABC** nanoparticle system, comprising PEGylated liposomes with encapsulated doxorubicin. These Doxil® drug-**ABC** nanoparticles (also known as PEGylated drug-nanoparticles) were designed to improve drug pharmacokinetics and reduce toxicity further by maximizing RES avoidance [50–52], making use of the PEG layer to reduce uptake by RES macrophages of the mononuclear phagocyte system (MPS) [53, 54].

The second nanoparticle system used and approved for clinical use were nanoparticles prepared using albumin as a compaction/association agent for sparingly water soluble Taxol®, one of the most potent anticancer drugs known. The resulting protein-based drug-**AB** nanoparticles (130 nm diameter) were christened nab-paclitaxel or Abraxane®. This Abraxane® system was designed to avoid the use of Cremophor EL® solvent (polyethoxylated castor oil) most frequently used to solubilise Taxol® [55–57]. Abraxane® is the first albumin nanoparticle system approved for human use by the FDA. This use of albumin is inspired. Albumin is a natural carrier of endogenous hydrophobic molecules that associate through non-covalent interactions. In addition, albumin assists endothelial trancytosis of protein bound and unbound plasma constituents principally through binding to a 60 kDa glycoprotein cell surface receptor, gp60. The receptor then binds to caveolin-1 with subsequent formation of transcytotic vesicles (caveolae) [58]. In addition, albumin binds to osteonectin, a secreted protein acid rich in cysteine (SPARC), that is present on breast lung and prostate cancer cells, so allowing albumin nanoparticles to accumulate readily in tumours [57, 59]. Currently there are more than 50 clinical trials ongoing using nanoparticles for cancer therapy. Indeed, the majority of these nanoparticles are nab-type (nanoparticle albumin bound) tested for the treatment of various cancer types (http://clinicaltrials.gov).

Otherwise, in terms of leading edge cancer clinical trials, LNPs have also been used in clinical trials for the delivery of biotherapeutic agents in cancer therapy corresponding to leading RNAi effectors known as small interfering RNAs (siRNAs). For instance LNPs corresponding to siRNA-**ABC** nanoparticles, Atu027, ALN-VSP02 and TKM-PLK1 are or have been in various stages of Phase I clinical trials. Moreover, one polymer-based nanoparticle (PNP) system, corresponding to a siRNA-**ABCD** nanoparticle system and christened CALAA-01, has appeared in Phase I clinical trials, with a Phase IIa clinical trial reportedly underway [60]. CALAA-01 employs a cyclodextrin polymer scaffold to entrap RNAi effectors and transferrin as a receptor-specific targeting ligand. Otherwise, advanced LNP (and

even PNP) prototypes, that are either nucleic acid-**AB**, **ABC** or **ABCD** nanoparticle systems, continue to be tested for functional delivery of therapeutic nucleic acids to target cells in animal models of human disease (to liver for treatment of hepatitis B and C virus infection, to ovarian cancer lesions for cancer therapy) and to target cells in murine lungs [61–67]. Rules for enhancing efficient delivery through receptor-mediated uptake of **ABCD** nanoparticles into target cells are also being studied and appreciated [68–71].

From the point of view of using nanoparticle technologies for the imaging of cancer, the ability to combine imaging agents with nanoparticles is central. In terms of the **ABCD** nanoparticle paradigm, the **A**-component now becomes an imaging agent(s) instead of a therapeutic agent. Fortunately, progress with imaging nanoparticles has also been brisk and a number of clinical trials have been expedited. For instance, a heterogeneous LNP system has been described in clinic that consists of a superparamagnetic iron oxide (SPIO) core particle lipid-coated to confer biological function [72]. This LNP system been used as a diagnostic tool for the pre-operative stage(s) of pancreatic cancer [73]. LNPs have also been described for radionuclide delivery to tumour lesions. Typically, these consist of a central liposome, that entraps a radionuclide of interest by analogy to drug-**AB/C** nanoparticles, and whose surface may be modified by targeting antibodies or peptides (**D**-components) in order to derive receptor-targeted nanoparticles [74]. Nanoparticles of this type have been used to entrap the chelate ^{111}In-diethylenetriamine-pentaacetic acid (^{111}In-DTPA). These were administered to 17 patients with locally advanced cancers. Post administration, patients were examined by means of a whole body gamma camera in order to verify pharmacokinetics and biodistribution behaviour. The $t_{1/2}$ of these ^{111}In-labelled nanoparticles was 76.1 h, and levels of tumour LNP uptake were estimated to be approximately 0.5–3.5 % of the injected dose at 72 h. The greatest levels of uptake were seen in the patients with head and neck cancers. However, significant uptake was also seen in the tissues of the RES (namely, liver, spleen, and bone marrow). Nevertheless data support the use of these ^{111}In-labelled nanoparticles for the imaging of solid tumors, particularly those of the head and neck, [75]. Moreover, once delivered to such tumour lesions, the radionuclide may then be used as a therapeutic agent to destroy tumour mass by radiation according to the principles of nuclear medicine.

Potentially important preclinical studies have been carried out recently with imaging LNPs set up for positive contrast magnetic resonance imaging (MRI) [76, 77]. The first described LNPs of this class were formulated by trapping water-soluble, paramagnetic, positive contrast imaging agents [such as $MnCl_2$, gadolinium (III) diethylenetriamine pentaacetic acid (Gd.DTPA), and the manganese (II) equivalent (Mn.DTPA)] in the enclosed volume of a liposome resulting in prototype lipid-based, positive contrast imaging-**AB/C** nanoparticles [78, 79]. Disadvantages were quickly reported such as poor encapsulation efficiency, poor stability, and clear toxicities due to importune contrast agent leakage and poor relaxivity [80]. These problems were obviated when hydrophobic lipidic chains were “grafted” on to contrast agents, thereby enabling these agents to be hosted by a lipid-bilayer [81]. Such lipidic contrast agents formulated in association with the bilayer of a liposome

exhibit improved ionic relaxivity and could therefore be used for dynamic MRI experiments in mice in vivo [82].

A potentially significant variation on this theme involves gadolinium (III) ions complexed with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) to which hydrophobic lipidic chains are attached. In particular, gadolinium (III) 2-(4,7-*bis*-carboxymethyl-10-[(*N*,*N*-distearylamidomethyl)-*N*'-amidomethyl]-1,4,7,10-tetraazacyclododec-1-yl)-acetic acid (Gd.DOTA.DSA) was prepared and formulated into passively targeted Gd-**ABC** (no biological targeting layer) and folate-receptor targeted Gd-**ABCD** nanoparticles in conjunction with a number of other naturally available and synthetic lipid components such as (ω-methoxy-polyethylene glycol 2000)-*N*-carboxy-distearoyl-L-α-phosphatidylethanolamine (PEG^{2000}-DSPE) or its folate variant (folate-PEG^{2000}-DSPE), and fluorescent lipid dioleoyl-L-α-phosphatidylethanolamine-*N*-(lissamine rhodamine B sulphonyl) (DOPE-Rhoda) (Fig. 18.2). These bimodal imaging nanoparticle systems demonstrated excellent tumour tissue penetration and tumour MRI contrast imaging in both instances [83–85]. Interestingly, the folate-receptor targeted Gd-**ABCD** exhibited a fourfold decrease in tumor T_1 value in just 2 h post-injection, a level of tissue relaxation change that was observed only 24 h post administration of passively targeted Gd-**ABC** nanoparticles [83, 84]. Preparations for clinical trial are now underway beginning with cGMP manufacturing and preclinical toxicology testing. These Gd-**ABC/D** nanoparticles are potentially excellent nanotechnology tools for the early detection and diagnosis of primary and metastatic cancer lesions. How effective remains to be seen when clinical trials can be performed. On the other hand, these LNPs may well enter into direct comparison with alternative LNPs that have been described by Müller et al. and are known as solid lipid nanoparticles (SLNs). These SLNs could certainly offer an alternative LNP platform for imaging [86–88]. For instance, under appropriate optimised conditions SLNs can carry MRI contrast agents [89], and SLNs containing $[Gd\text{-}DTPA(H_2O)]^{2-}$ and $[Gd\text{-}DOTA(H_2O)]^{-}$have even been prepared for preclinical studies.

In complete contrast, a variety of PNP systems are also beginning to be realized for the delivery of therapeutic agents and/or imaging agents. For instance, dendrimers are a unique class of repeatedly branched polymeric macromolecules with a nearly perfect 3D geometric pattern. They can be prepared with either divergent methods (outward from the core) or convergent methods (inward towards the core). Tomalia was the first to synthesise the 3D polyamidoamine (PAMAM) dendrimers using divergent methods [90]. The methods of Frechet [91] are characterised by generation (G) building using monomers added to a central core. Controlled synthesis results in molecular diameters between 1.9 nm for G1 to 4.4 nm for G4 dendrimers. These G1-G4 dendrimers represent the smallest known nanocarriers yet developed for pharmaceutical and imaging applications associated with cancer [92], including photodynamic therapy (activation therapies) [93], boron neutron capture therapy [94] and hyperthermia therapies in combination with gold nanoparticles [3]. These Gd-**AB** nanoparticles, known as gadolinium (III) dendrimer conjugates, have proven of provisional value in MRI experiments [95]. Unfortunately as delivery systems for therapeutic agents, dendrimers have a tendency post administration to release conjugated drugs before reaching disease target sites.

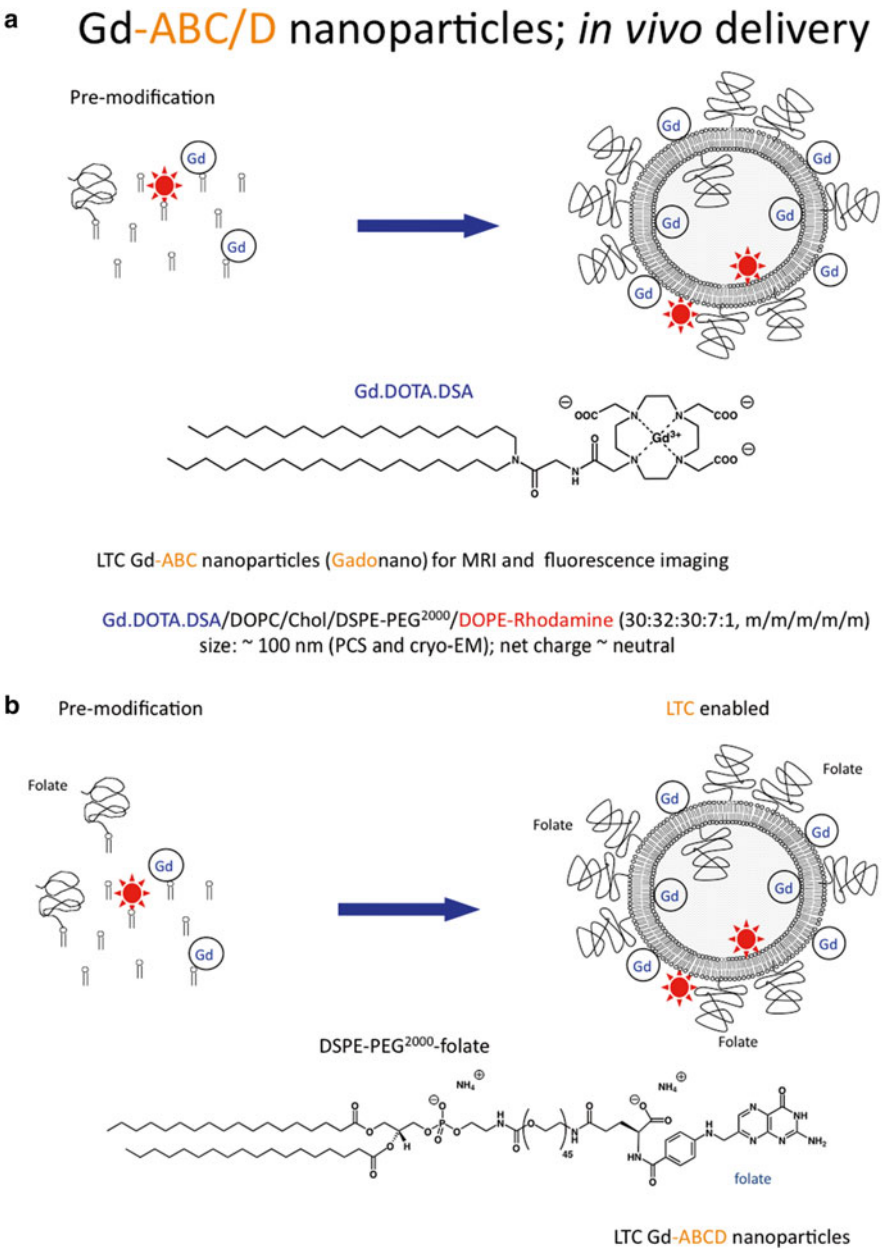

Fig. 18.2 Passively targeted Gd-**ABC** (*top*) and folate-receptor targeted Gd-**ABCD** (*bottom*) nanoparticles for IGROV-1 tumour imaging [83]. These LNPs are long-term circulation (*LTC*) enabled by virtue of the use of bilayer stabilizing lipids and 7 mol% PEG-lipid in the outer leaflet membranes of lipid-based nanoparticle structures

Finally, we turn to inorganic "hard" nanoparticles. Of these the most advanced already in clinical practice are the dextran coated iron oxide nanoparticles that correspond in form to imaging-**AB/C** nanoparticle systems. Ferumoxtran-10 ® is a commercially available ultra-small-superparamagnetic iron oxide particle (USPIO) product [96, 97]. After systemic injection, these nanoparticles collect in lymph nodes, liver, spleen, or brain tissue where are visualized by MRI. In a lymph node with proper architecture and function (healthy), macrophages take up a substantial amount of ferumoxtran-10. This uptake results in a marked reduction in signal intensity and turns the lymph nodes dark when seen by MRI. Infiltration of lymph nodes with malignant cells replaces the macrophages and changes the architecture of the lymph nodes. In malignant lymph nodes there is no ferumoxtran-10 macrophage uptake and they can retain the high signal intensity or display heterogenous signal intensity if micrometastases are involved. This way the grade of tumours and prognosis can be assessed by the presence of micro-metastases [98]. Additionally, iron oxide nanoparticles can be guided in principle to target sites (i.e. tumour) using external magnetic field and they can be also heated to provide hyperthermia for cancer therapy [99].

On another tack, Yu et al. have reported how dextran-coated iron oxide nanoparticles bearing a Cy5.5 near infrared (NIR) probe could also carry doxorubicin thereby allowing both the imaging and drug treatment of cancer lesions. The administration of these bimodal imaging drug-**AB/C** nanoparticles allowed for simultaneous real-time imaging of nanoparticle biodistribution and the measurement of drug pharmacokinetic behaviour alongside the observation of a substantial phenotypic (pharmacodynamic) reduction in tumour size [99]. Similarly bimodal imaging RNAi-**AB/C** nanoparticle systems were realized by coupling RNAi effectors to the dextran coat alongside Cy5.5 near infrared dye. These bimodal imaging nanoparticles were also seen to enable functional delivery of the RNAi effectors to target cells with real-time/diagnostic imaging [100, 101]. Where nanoparticles have a dual function for imaging and therapy, they are increasingly known as theranostic (i.e. *thera*py + diag*nostic*) nanoparticles. Moreover, what was achieved with inorganic "hard" iron oxide nanoparticles was subsequently reported using LNPs. For instance, a multimodal imaging theranostic siRNA-**ABC** nanoparticle system was recently described that had been assembled by the stepwise formulation of PEGylated cationic liposomes (prepared using Gd.DOTA.DSA and DOPE-Rhoda amongst other lipids), followed by the encapsulation of Alexa fluor 488-labelled anti-survivin siRNA. These multimodal imaging theranostic nanoparticles were found able to mediate functional delivery of siRNA to tumours giving rise to a significant phenotypic (pharmacodynamic) reductions in tumour sizes relative to controls, while at the same time nanoparticle biodistribution (DOPE-Rhoda fluorescence plus MRI), and siRNA pharmacokinetic behaviour (Alexa fluor 488 fluorescence) could be observed by means of simultaneous real-time imaging [65]. This concept of multimodal imaging theranostic nanoparticles for cancer imaging and therapy is certain to grow in importance in preclinical cancer nanotechnology studies and maybe in the clinic too.

18.3 Nanoparticle Applications in Triggered and Image-Guided Therapies

Multimodal imaging theranostic nanoparticles may offer substantial benefits for cancer diagnosis and therapy going forward, but only in combination with further advances in nanoparticle platform delivery technologies. What might these advances be and how might they be implemented? As far as imaging nanoparticles are concerned for detection of cancer, provided that all that is required for diagnosis is nanoparticle accumulation within cancer lesions, then current imaging nanoparticle technologies may well be sufficient. However, for personalized medicine to really take off, the detection of cancer disease specific biomarkers in vivo is really required. In order to achieve this, considerable attention may well have to be paid to the appropriate design and selection of ligands (**D**-components) for the biological targeting layer (or **D**-layer).

As far as nanoparticles for cancer therapy are concerned, the opportunities for delivery are relatively limited at this point in time, primarily due to the facile partition of current nanoparticles post-administration to liver and to solid tumours in vivo and in clinic. In order to enable partition to other organs of interest and even to diseased target cell populations within, there is now an imperative to introduce new design features involving new tool-kits of chemical components. Moreover the **ABCD** nanoparticle paradigm itself has one primary design weakness in that the stealth/biocompatibility polymer layer (or **C**-layer) (typically PEG, main **C**-component) does not prevent nanoparticle entry into cells but may substantially inhibit functional intracellular delivery of the therapeutic agent, unless sufficiently removed by the time of target cell-entry or else during the process of cell-entry. Learning the rules for the control of nanoparticle biodistribution and of therapeutic agent cargo pharmacokinetics may take several years yet even though rule sets are emerging. Therefore, overcoming the **C**-layer paradox should be the primary focus for therapeutic nanoparticle development over the next few years. Accordingly, there has been a growing interest in the concept of nanoparticles that possess the property of triggerability. Such nanoparticles are designed for high levels of stability in biological fluids from points of administration to target cells whereupon they become triggered for the controlled release of entrapped therapeutic agent payload(s) by changes in local endogenous conditions (such as pH, $t_{1/2}$, enzyme, redox state, and temperature status) [61–66, 102], or through application of an external/exogenous stimulus (Rosca et al. 2014, manuscript in submission). While much of previous work on this topic has revolved around change(s) in local endogeneous conditions [61–66, 102], the development of appropriate exogeneous stimuli looks to be a real growth area for the future. In principle, all **ABC** and **ABCD** nanoparticle systems could be triggered to exhibit physical property change(s) appropriate for controlled release through interaction with light, ultrasound, radiofrequency and thermal radiation from defined sources. So how might this be harnessed using “soft” organic and “hard” inorganic nanoparticles?

Today the journey towards "soft" organic LNPs for cancer therapy that can be described as truely triggered multimodal imaging theranostic drug-nanoparticles appears well underway. A few years ago thermally triggered drug-**ABC** nanoparticles (now known as Thermodox®, Celsion) were described based upon Doxil®. Thermodox® nanoparticles are formulated with lipids that included lysophospholipids in order to encapsulate doxorubicin within thermosensitive, nanoparticle lipid bilayer membranes [103, 104]. At induced temperatures above 37 °C, these membranes appear to become unexpectedly porous allowing for substantial local controlled release of drug. Needham et al. were first to demonstrate the use of such thermally triggered drug-**ABC** nanoparticles for the controlled local release of drug into target tissues in vivo [105], thus allowing for the potential treatment of tumours more efficiently than was achieved following administration of the thermally insensitive, Doxil® parent system [106]. Thermodox® is currently the subject of phase III HEAT studies and phase II ABLATE studies. In the latter studies, Thermodox® is administered intravenously in combination with Radio Frequency Ablation (RFA) of tumour tissue. In this case, the RFA acts as an exogenous source of local tissue hyperthermia (39.5–42 °C) that simultaneously acts as a thermal trigger for controlled release of encapsulated doxorubicin from the central aqueous cavity of Thermodox® nanoparticles. The company's pipeline going forward is focused on the use of Thermodox® nanoparticles under thermal triggered release conditions for the treatment of breast, colorectal and primary liver cancer lesions [107, 108]. This is the first time that thermally triggered drug-**ABC** nanoparticles have been devised and used in clinical trials.

A further evolution of this concept has now been more recently reported with the simultaneous entrapment of both doxorubicin and a MRI positive contrast agent, $Gd(HPDO_3A)(H_2O)$, into thermally triggered drug-**ABC** nanoparticles [109]. High Frequency Ultrasound (HIFU) was used as an alternative thermal trigger for the controlled release of encapsulated drug at 42 °C. The simultaneous release of MRI contrast agent enabled the observation of release in real time and led to an estimation of doxorubicin nanoparticle release kinetics. Researchers involved in Thermodox® have similarly reported on the development of thermally triggered drug-**ABC** nanoparticles with co-encapsulated doxorubicin and the MRI contrast agent Prohance® [110]. Using HIFU as a thermal trigger once more, they were able to promote controlled release of drug in rabbits with Vx2 tumours, and monitor drug release in real time by MRI [111]. The same researchers also developed an algorithm to simulate the thermal trigger effects of HIFU [112]. Simulation data were in agreement with mean tissue temperature increases from 37 °C to between 40.4 °C and 41.3 °C, resulting in quite heterogeneous drug release kinetic behaviour [112]. By contrast, we have striven to draw inspiration from the Gd-**ABC** and Gd-**ABCD** imaging nanoparticle systems described above [83–85, 113, 114], and Thermodox® data, in order to derive thermally triggered theranostic drug-**ABC** nanoparticles. These might also be described as thermal trig-anostic drug-**ABC** nanoparticles (shortened to the acronym thermal TNPs) (Fig. 18.3). By description, these nanoparticles are enabled for thermally triggered release of encapsulated drug in tumours by means of ultrasound together with real time, diagnostic imaging of nanoparticle

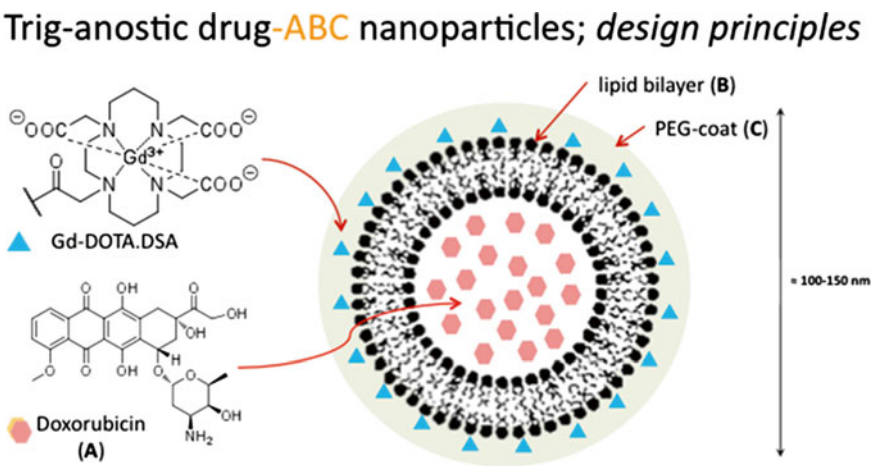

Fig. 18.3 Schematic of thermal trig-anostic drug-**ABC** nanoparticles (thermal TNPs) enabled for thermally triggered release of encapsulated drug in tumours by means of ultrasound together with real time, diagnostic imaging of nanoparticle biodistribution by MRI with drug pharmacokinetics

biodistribution with drug pharmacokinetics. Critical to this proposition is the use of Gd.DOTA.DSA once again. Going forward, lipidic MRI agent use should be supplemented with other imaging agents leading to new families of triggered multimodal imaging theranostic drug-**ABC** nanoparticles. These could also be described as trig-anostic^{n} drug-**ABC** nanoparticles where n is number of imaging modes employed, a description that could also be shortened to the acronym ^{n}TNPs.

In the case of "hard" inorganic nanoparticle systems, gold nanoparticles provide for a useful illustration. These belong to a class of nanoparticles known as nanoshells with tunable optical resonances. These nanoshells consist of a core, in this case silica that is surrounded by a thin metal shell, in this case gold [115]. These particles exhibit highly tunable surface plasmon resonances that absorb NIR radiation from a bespoke laser source and then transmit locally causing local tissue damage while leaving surrounding tissue intact [116]. Nanoshells are currently under evaluation in a number of clinical settings after a 5 years period of intensive preclinical development [117]. Obviously, in this instance, nanoshells are triggered to act in effect as their own "therapeutic agent", but nanoshells can also be administered in combination with anti-cancer therapeutic antibodies opening up options of combining

anti-cancer antibody therapy with hyperthermia therapy [118]. In hyperthermia treatment, nanoshells may be replaced shortly by nanorods in the next steps of development in these "hard" nanoparticle systems [119].

A peak of design must then be represented by the development of targeted trig-anostic^{n} therapeutically multifunctional drug-**ABCD** nanoparticles. These might also be described as targeted trig-anostic^{n} drug^{m}-**ABCD** nanoparticles, where *n* is number of imaging modes employed in nanoparticle and *m* is the number of active therapeutic agents (APIs) encapsulated/entrapped, a description that reduces to the corresponding acronym of targeted $^{n}T_{m}$NPs. Amazingly, while LNP and PNP systems of this type have yet to be devised, nanoshell structures have now been reported that have been pre-doped with MRI probes (by introduction of a 10 nm iron oxide layer over the silica core) and/or NIR probes (indocyanine green dye), then set up (with streptavidin) for surface conjugation of anti-HER2 antibodies (biotin labelled) with an additional surface PEG biocompatibility layer (introduced by disulphide post coupling bond formation). Such ensembles can be described readily as targeted trig-anostic^{2} drug^{2}-**ABCD** nanoparticle systems (or targeted $^{2}T_{2}$NPs) enabled for real time/diagnostic bimodal MRI and NIR contrast imaging accessed in combination with the capability for dual targeted and triggered chemotherapy (by anti-HER-2 antibodies) and photo-thermal ablation therapy (post illumination with a 808 nm wavelength NIR laser) either in vitro or in vivo [120, 121].

18.4 Conclusions and Future Perspective

Nanotechnology is revolutionising research and development in healthcare. Currently, the most advanced clinical-grade nanotechnologies in cancer are lipid-based and some "hard inorganic" nanoparticles. Recent studies show more evidence that biocompatibility and safety of nanoparticles depends on the material, and surface chemistry properties. Even quantum dots that have been previously characterised as toxic can be adapted for apparently safe use in non-human primates [122]. Unfortunately, there is still some scepticism from the big pharma industry and from clinicians themselves regarding the efficacy and safety of nanoparticle technologies. Such scepticism will only be solved with the advent of reliable cGMP-grade manufacturing processes and reliable preclinical ADME/toxicology data, followed by a range of successful first in man-studies. While these data are being acquired, nanoparticle technologies continue to be innovated in the laboratory. In this case, there appears to be an increasing push towards targeted trig-anostic^{n} drug^{m}-**ABCD** nanoparticles ($^{n}T_{m}$NPs) enabled for both targeted and triggered release of *m* active therapeutic agents (APIs) (including small molecule drug entities), all monitored simultaneously by real time/diagnostic imaging using *n* different imaging agent probes integrated into individual nanoparticles. Of the latter, both NIR and ^{19}F-NMR spectroscopy probes [123], could have real clinical potential alongside MRI. Such functional multiplicity offers the very real opportunity for highly personalized, hyper-functionalized drug-nanoparticles tailor-made (designed and assembled) from

tool-kits of chemical components that have themselves been highly refined for specific, personalized delivery applications. As this vision takes shape, so we will be looking on a very different world of innovative, interactive healthcare products with vastly more potential to treat and even to cure cancer than has ever been seen before.

And what of routine personalized cancer diagnosis and therapy? Do current advances in nanoparticle development allow us to close the virtuous circle of molecular profiling to personalized cancer nanomedicine? At this stage the answer must be, "not yet" or "status unproven". Clearly cancer imaging and therapy using nanoparticle technologies looks and is entirely becoming clinically realistic. But we are not yet at the point where patient specific, cancer disease-specific biomarkers can be detected in vivo using nanotechnology followed in the clinic by nanoparticle-mediated functional delivery of biomarker specific therapeutic agents. However, at least where ncRNAs are concerned, the prospect of such a cycle does appear imminent. As ncRNA profiling of cancers take place, so one can envisage a time when the follow on design of nanoparticles for the functional delivery of RNAi effectors targeted against specific cancer biomarker ncRNAs could become routine. Once this can be achieved, then the virtuous circle of personalized cancer nanomedicine will be properly closed.

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Chapter 19
Challenges to Nanomedicine

Richard Moore

19.1 Introduction

The recent past has seen a period of considerable financial upheaval and constraint that has affected healthcare and healthcare provision like many other sectors. There is an increasing pressure on those bringing forward new medical technologies to ensure that they are capable of outperforming existing, established technologies, that they have a high benefit-to-risk ratio and that they are affordable or can otherwise lead to cost-savings in healthcare systems where resource availability is a constant concern.

While economic factors are particularly sensitive in the current financial climate, there are a number of other important hurdles to be negotiated in bringing any new medical technology to the clinic. These include

- taking account of demographic trends and associated changes in healthcare priorities
- addressing and minimising risks
- understanding which regulatory system(s) apply and ensuring product compliance
- understanding and negotiating reimbursement systems
- preparing for healthcare technology assessment
- considering the impact that emerging technologies may have on established medical practice
- ensuring that there is professional uptake of new technologies and addressing training issues that may arise
- addressing public understanding and perception issues
- in some cases, addressing new ethical challenges that the technology may bring

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While some of these topics, such as risk and ethical issues, are explored in greater depth in other chapters, they are reviewed and discussed in the following sections in order to provide a broad overview of some of the important challenges and milestones towards successful commercialization and utilization of medical products based on nanotechnologies.

19.2 The Rising Costs of Healthcare

It is estimated that, in all countries, both health and long-term care will drive up public spending. In the recent OECD Economic Report No. 6, De la Maisonneuve and Oliveira Martins (2013) project that, for OECD countries, average public healthcare expenditure will increase from 5.5 % of GDP in 2010 to 8 % of GDP in 2060; whereas public long-term care expenditure will increase from 0.8 % to 1.6 % of GDP in 2060 [1]. The report projects that healthcare spending will be pushed up mostly by the combined effect of technology, relative prices, and factors such as institutions and policies, while pressures on long-term care costs will originate mostly from weaker productivity gains than in the economy as a whole.

Given the competing pressures from other social spending programmes, the report concludes that projected trends in public health and long-term care spending are likely to be a major source of concern for most governments.

A key challenge for nanomedicine will be to demonstrate that it can contribute towards containing these rising costs. Given that the implementation of technology is frequently cited as contributing to rising healthcare costs this may at first seem paradoxical. However, a major component of the cost of healthcare is hospitalization and reducing the length of stay (LoS) in hospital is a major objective for new treatments and for healthcare planners. Nanomedicine may be able to contribute to reducing the duration of in-patient stays, or to eliminating them altogether, in various ways including:

- facilitating earlier, faster or more accurate diagnosis thereby potentially reducing the length of treatment required;
- contributing to the efficacy of treatment and improving the prognosis for the patient;
- facilitating treatment at home, at the GP's surgery or as an outpatient;
- improving the performance of individual drugs and medical devices;
- contributing to personalized medicine, e.g. by selecting and tailoring treatment to suit the individual patient and their condition.

Robinson and Smith [2] suggest, for example, that there are numerous examples of new products and processes in healthcare that reduce rather than increase the rate of spending growth and that, without these, total costs would be increasing even more rapidly than they are. These include:

- innovative new drugs, tests, devices, and other products (as distinct from services) that are cheaper to manufacture or use than those they replace;

– changes in processes that allow less highly trained but sufficiently competent workers to substitute for more highly trained and expensive staff thereby releasing them for more complex and demanding procedures. Examples could include substituting physician generalists for specialists, nurse practitioners and pharmacists for physicians, non-licensed staff for nurses, and family members and patients themselves for paid staff of any kind.
– sites of care that are less elaborate but which are adequate for the tasks under consideration, including the home itself as an effective site for care in the area of chronic illness.

They further suggest that synergies between changes in one dimension of care and changes in the others may be the most disruptive in terms of channelling patients in new directions and forcing major but desirable changes on both manufacturers and medical practitioners – more so than individual changes in products, personnel, or facilities.

Citing experience in other sectors they suggest that cost-reducing innovations are attributable to both new technology, and to new types of business model that are simpler and cheaper than those they replace, resulting in an expansion of the market due to the increased affordability of these services.

Krishna Kumar (2011), with reference to new medical technologies, makes the point that much of the effort of companies relates to providing additional features to score over their competitors' products but that matter very little in day-to-day decisions, while there is very little focus on making technology widely accessible and inexpensive [3].

Health technology assessment and reimbursement schemes, which are discussed later in this chapter, also increasingly focus on cost-containment and value-for-money. Therefore it is important that nanomedicine is able to demonstrate a contribution towards cost-containment within healthcare systems through diagnosing disease at an earlier and more treatable stage, providing more effective treatments, reducing the costs of or extending the life of products, facilitating efficiencies in the delivery of healthcare and the use of professional resources, shortening hospital stays or improving recovery times, or enabling treatment or care to be carried out in less expensive settings. In some cases, cost savings may be realized in the longer term or in parts of the healthcare system other than that where the technology is deployed, necessitating the development of a strong evidence-based case that explains the overall benefits and savings to the system.

A further factor that further exacerbates cost considerations is the demographic shift to an ageing population coupled with a reduction in the proportion of those actively contributing financially to healthcare systems.

19.3 The Demographic Shift Towards an Ageing Population

The European Commission's 2009 Ageing Report [4] estimates that, between now and 2060 within the European Union, the population will shift from a ratio of four people aged between 15 and 64 for each person aged over 65, to a ratio of only two to one.

The largest change is expected to occur between 2015 and 2035 when the current baby-boomer generation will be entering retirement. Between 2010 and 2030, the number of Europeans aged over 65 is expected to rise by nearly 40 % and, by the mid-2030s, the number of people aged 85 and over is projected to double in most European countries. Furthermore, it is estimated that around 50 % of babies born today are likely to live to 100 due to improvements in healthcare and living standards.

These demographic changes are likely to have a dramatic effect on society and to lead to new clinical challenges in relation to a wide range of health conditions associated with the elderly such as cardiovascular diseases, cancers, arthritis, osteoporosis and other orthopaedic conditions, dementias and other neurodegenerative diseases, hearing and balance disorders, and some forms of blindness.

According to the 2012 World Alzheimer Report [5] the costs associated with dementia alone were estimated to be around 1 % of the world's gross domestic product at around $604bn (€421bn) and it is likely that these costs will increase in proportion to the number of people with dementia. The report goes on to suggest that dementia, which comprises a range of neurodegenerative disorders of which Alzheimer's Disease accounts around two-thirds, poses the most significant health and social crisis of the century as its global financial burden continues to escalate, with the number of people with dementia expected to double by 2030, and more than triple by 2050. Around 682 million people will live with dementia in the next 40 years, significantly more than the population of the whole of North America (542 million) and nearly as much as the whole of Europe (738 million).

According to OECD Economic Policy Paper No. 6 [1], in 2010, 60 % of global healthcare expenditures were directed towards people below 65 years old. In 2060, roughly the same percentage of expenditures will be directed to people aged above 65, reflecting an increase from 15 % to 30 % of their share in the total population.

As the proportion of the population at retirement age and beyond increases, the proportion in work is simultaneously decreasing, reducing the tax and national insurance base that supports healthcare services and further compounding the problem of supporting the increasing costs of treating and caring for the elderly. It is also important to note that, as people live longer, they have an increasing and justifiable expectation also to be able to maintain their dignity, independence and quality of life.

Will nanomedicine be able, therefore, to play a role where clinical and care needs are increasing due to this demographic shift and whilst health and care systems are under enormous pressure and costs are increasingly constrained? The following paragraphs provide some examples of research that has been funded by the European Commission and which is aimed at using nanotechnology, sometimes coupled with other enabling technologies, to address the health needs of an ageing population.

The European FP7 project NAD (*N*anoparticles for Therapy and Diagnosis of *A*lzheimer's *D*isease), which commenced in 2008 and conclude in August 2013 is currently evaluating dendrimer nanocomposites for imaging and therapy, nanoliposomes for therapeutic agent delivery and other functionalized nanoparticles for applications in Alzheimer's disease [6].

The FP7 project Development of Novel *Nano*technology Based *Dia*gnostic Systems for *R*heumatoid *A*rthritis and Osteoarthritis (NanoDiaRA), which commenced in 2010 and was due to conclude in January 2014, is developing nanoparticle-based imaging and blood and urine-based diagnostic tools, and biomarkers, for the early detection of osteo- and rheumatoid arthritis. The research may also offer insights into the development of controlled nanoscale drug release and will consider the social, ethical and legal aspects of applying nanotechnology for medical purposes [7].

The FP6 integrated project Lidwine, which concluded its work in August 2010, developed novel approaches, including nanotechnology-treated textiles, for treating decubitus (pressure) ulcers, a painful and serious and, in terms of treatment, very common and expensive condition affecting many elderly bed- or chair-bound patients [8].

Moore (2011) reports other examples of nanotechnology research geared towards conditions affecting the elderly including the development of multifunctional nanoparticles capable of delivering controlled-release therapeutic agents to the inner ear for the treatment of age-related hearing loss and balance problems, and the use of nanotechnology in novel devices such as retinal implants for potential use in serious eye conditions such as macular degeneration [9].

Nanotechnology may also play a role in promoting the efficiency of care of the elderly through networked monitoring and telecare solutions which can be often be interfaced with novel biosensors incorporating micro- and nanotechnology. A variety of sensors can be embedded in the home, e.g. to monitor energy usage, movement or falls, or can be worn by the elderly person to monitor their physiological condition and provide a continuous feedback regarding their well-being or state of health to a remote monitoring station. Such networked systems can be used to alert health services or carers to react where there is an urgent or identified need, thereby allowing limited resources to be targeted more effectively as well as contributing to the independence of the patient.

Rather than being seen merely as an added cost, nanotechnologies should perhaps instead be viewed as a means of enabling novel healthcare and social care solutions and reducing the burden of long-term and expensive treatment of chronic conditions associated with ageing, as well as contributing to the dignity and independence of elderly persons.

19.4 Disruptive Innovation?

Clayton Christiansen (1997) defined several distinct types of innovation as follows:

Sustaining innovation: an innovation that does not affect existing markets.

Evolutionary innovation: an innovation that improves a product in an existing market in ways that customers are expecting.

Revolutionary (radical) innovation: an innovation that is unexpected, but which does not affect existing markets.

Disruptive innovation: an innovation that creates a new market by applying a different set of values, and which ultimately (and unexpectedly) overtakes an existing market [10].

Nanotechnology has the potential to impact medical products and processes at each of these levels. In many cases, the effects will be incremental such as improving the coating on an orthopaedic implant to improve its performance or lifetime or reformulating the delivery system of a drug to provide gradual release of that drug over an extended period.

The use of nanotechnology in new generations of devices such as retinal implants [11] could be considered an example of a revolutionary innovation in that it might have the potential to address currently unmet clinical needs such providing at least a limited level of vision for patients with macular degeneration or retinitis pigmentosa.

However, nanomedicine also has the potential for disruptive innovation. One example is its potential major contribution to the emerging field of regenerative medicine, for example the implantation of a nanostructured material into the body that can stimulate the body into self-repair producing new tissue such as in the regeneration of a damaged peripheral nerve [12] or the production of autologous bone that can be used elsewhere in the body for reconstructive surgery [13]. This type of emerging application may help shape a new future paradigm of medical treatment that could replace conventional treatments and for which major changes in procedures and training could be envisioned. Likewise the coupling of diagnostic and "-omics" tests (genomic, proteomic, metabolomic) with therapies (a concept sometimes referred to as *theranostics* [14–17] could herald a new, highly personalized form of medicine where, for example, the selection of drugs is matched to the individual patient and their condition, potentially reducing the considerable costs of prescribing drugs to patients for whom they have limited efficacy.

Whether the innovation brought about by nanomedicine is incremental, revolutionary or disruptive, there remains the potential for better treatments and lower costs but it is nevertheless important to consider the potential impacts on medical practice and procedures. Furthermore, while a progression towards a more personalized form of medicine may be strongly welcomed by both patients and medical professionals, it may not necessarily match the current business models of the major pharmaceutical and medical technology companies.

19.5 Risks and Regulatory Compliance

Protecting patients from risk is a primary objective of all medical product regulations but how this is actually achieved can vary widely in practice. In Europe, the regulation of the placing on the market of medical technologies is addressed

primarily at the European level. In the US, the Food and Drug Administration (FDA) is primarily responsible. In nearly all countries around the world there are responsible national agencies or government departments.

Long-established product legislation was often drafted in a quite prescriptive style with a form of wording such as "you must not do this", "you shall do that and in this specific way". Many so-called "old approach" European Directives were drafted in this technical style and, as such, were not always adapted very well to areas of rapid innovation as the detailed requirements contained within the legal texts themselves could not always be changed quickly as new technological developments emerged. As this was recognized, newer types of product legislation, such as European "new approach" Directives, were developed which tended to be based around broad safety- and performance-based "essential requirements" rather than detailed prescriptive text, with the technical aspects being addressed in accompanying "harmonized" European standards drafted to support the broad essential requirements of the Directives. Such standards are, in theory, easier to revise if required although this can still be a lengthy process.

The approach taken by the various international agencies responsible for drug and device regulation varies. In the United States, the Food and Drug Administration (FDA) is responsible for determining the *primary mode of action* of the product and this decision will determine the regulatory framework for the product, i.e. a drug, medical device or biological product. The product regulatory application is thereafter managed by the appropriate FDA Center (Center for Drug Evaluation and Research – CDER; Center for Devices and Radiological Health – CDRH; Center for Biologics Evaluation and Research – CBER) with consultations from the other Centers.

In Europe, the *primary mode of action* of the product also determines the regulatory path(s) that will apply. Because European Directives are transposed into national legislation, national agencies and government departments have a responsibility for compliance within their jurisdiction.

The differences between what constitutes a medicinal product and what constitutes a medical device are similar in the US and Europe. In the US, products that have a primarily chemical/metabolic mode of action within the body are defined as drugs and, in Europe, products that have pharmacological, immunological or metabolic primary mode of action are defined as drugs and fall under the Medicinal Products Directive (2001/83/EC) or its related sister Directives or Regulations such as the Advanced Therapy Medicinal Products Regulation (Regulation EC No. 1394/2007). Similarly, in both regions, products that achieve their primary mode of action through physical or mechanical means are defined as medical devices and fall under their own regulatory pathways (the Medical Device Directives in the case of Europe). The European definition of a medical device (Article 1.2(a) of Directive 93/42/EEC) is as follows:

> *… any instrument, apparatus, appliance, software, material or other article, whether used alone or in combination, together with any accessories… intended by its manufacturer to be used specifically for……*
>
> – *diagnosis, prevention, monitoring, treatment or alleviation of disease,*
> – *diagnosis, monitoring, treatment, alleviation of or compensation for an injury or handicap,*

- *investigation, replacement or modification of the anatomy or of a physiological process,*
- *control of conception,*

and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means. [18]

The FDA predicts that many nanotechnology-based products will span the regulatory boundaries between pharmaceuticals, medical devices and biologicals. It has stated [19] that technical assessments will be product-specific, taking into account the effects of nanomaterials in the particular biological and mechanical context of each product and its intended use, and that the particular policies for each product area, both substantive and procedural, will vary according to the statutory authorities. It also advises manufacturers to consult with the FDA early in their development process to facilitate a mutual understanding of the scientific and regulatory issues for their nanotechnology products.

With these points in mind, the FDA has issued guidelines describing its current thinking concerning regulated products containing nanomaterials or otherwise involving the application of nanotechnology. This guidance states that, based on its current scientific and technical understanding of nanomaterials and their characteristics, the FDA believes that evaluations of safety, effectiveness or public health impact of such products should consider the unique properties and behaviors that nanomaterials may exhibit [20].

In Europe, similar provisions exist for addressing combination products that may fall under more than one regulatory pathway. Since the primary mode of action may sometimes be difficult to determine for materials that exert an effect by virtue of novel properties arising at the nanoscale, determining the appropriate regulatory pathway(s) at an early stage of product development is of key importance.

In Europe, neither the Medicinal Products Directive nor the three Medical Device Directives (addressing medical devices, active implantable medical devices and in-vitro diagnostic medical devices, respectively) were originally drafted with nanotechnology in mind. The Medicinal Products Directive currently has no specific provisions relating to nanotechnology although a number of drugs containing nanomaterials have already been approved onto the European market. However, specific guidance on quality, toxicology, clinical development and monitoring aspects that have a bearing on nanotechnology are planned. Those developing drugs based on nanotechnology are strongly encouraged to interact with the relevant European Agency, the European Medicines Agency based in London which has an Innovation Task Force that addresses nanomedicine, from the earliest stages of development.

The European Medical Device Directives are based on broad “essential requirements” and the European Commission’s Medical Devices Experts’ Group has concluded that the provisions of the Directives broadly address nanotechnology-based medical devices. Essential requirements (ERs) of the Medical Device Directive [18]

that are of general relevance to any technology and which can therefore apply equally to products based on nanotechnologies include the following:

> *ER 1*: *The devices must be designed and manufactured in such a way that, when used under the conditions and for the purposes intended, they will not compromise the clinical condition or the safety of patients, or the safety and health of users or, where applicable, other persons, provided that any risks which may be associated with their use constitute acceptable risks when weighed against the benefits to the patient and are compatible with a high level of protection of health and safety.*
>
> *ER 2: The solutions adopted by the manufacturer for the design and construction of the devices must conform to safety principles, taking account of the generally acknowledged state of the art. In selecting the most appropriate solutions, the manufacturer must apply the following principles in the following order:*
>
> – *eliminate or reduce risks as far as possible (inherently safe design and construction),*
> – *where appropriate take adequate protection measures including alarms if necessary, in relation to risks that cannot be eliminated,*
> – *inform users of the residual risks due to any shortcomings of the protection measures adopted.*
>
> *ER 3: The devices must achieve the performances intended by the manufacturer and be designed, manufactured and packaged in such a way that they are suitable for one or more of the functions…. as specified by the manufacturer. Any undesirable side-effect must constitute an acceptable risk when weighed against the performances intended.*

Other essential requirements address aspects such as chemical, physical and biological properties; infection and microbial contamination; construction and environmental properties; devices with a measuring function; protection against radiation; devices with an energy source; and accompanying information.

Two key themes in essential requirements 1–3 are those of *acceptable risk* and the *reduction of risk*. As there are broad knowledge gaps concerning the risks of many manufactured nanomaterials and, in many cases, a poor understanding of their novel properties and mechanisms of interaction with the body, this subject becomes an extremely important one in terms of compiling relevant information for regulatory approval, and the active collection or generation of appropriate data concerning risk and safety an essential activity in developing nanomedical products.

The European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) concluded in 2009 that a key limitation in the risk assessment of nanomaterials was the general lack of high quality exposure data both for humans and the environment. They noted that risk assessment procedures for the evaluation of potential risks of nanomaterials were still under development and could be expected to remain so until there is sufficient scientific information available to characterise the possible harmful effects on humans and the environment. They concluded that methodologies for both exposure estimations and hazard identification need to be further developed, validated and standardised [21].

A range of nanomaterial characteristics can give rise to novel hazards and their associated risks and these include particle size, shape, surface area, surface charge, surface chemistry, catalytic properties, solubility, crystalline phase, composition, zeta potential and other parameters. A useful overview of the issues surrounding the

risk assessment of manufactured nanomaterials is given in the Organisation for Economic Co-operation and Development's 2012 Report *Series on the Safety of Manufactured Nanomaterials No. 33* [22]. Furthermore, international standards are currently in preparation that are intended to address some of these needs, such as those in ISO/TC 229 *Nanotechnologies* [23].

For medical devices, the harmonised standard EN ISO 14971 [24] describes a systematic risk management process that can be used as the basis for identifying hazards; analysing, estimating and reducing risks; deciding on the acceptability of risks; providing for post-manufacturing risk review; risk communication and risk documentation. While not specifically addressing nanotechnology or nanomedicine, with the addition of data on hazards and risks arising from the nanoscale characteristics of materials, it may nevertheless provide a useful basis for addressing risks for many medical devices incorporating nanotechnology .

One particularly important conclusion of this brief regulatory review is that there are still data gaps concerning the safety of many manufactured nanomaterials and, in the case of highly-regulated product sectors such as nanomedicine, that there is an urgent need to characterise nanomaterials and identify novel hazards and risks that arise from their nanoscale properties. In many cases this may also have implications for the development of new measurement and test methods, particularly those that can contribute towards characterising the interactions between nanoscale materials and biological systems *in-vivo* for nanomaterials that may come into contact with cells and tissues. This will form an important part of compiling risk data that will be required for subsequent regulatory approval.

19.6 Health Technology Assessment

Health technology assessment (HTA) has been defined as "a multi-disciplinary field of policy analysis that examines the medical, economic, social and ethical implications of the incremental value, diffusion and use of a medical technology in healthcare" [25]. Health technology assessment works together with, and relies on, many scientific disciplines such as epidemiology, biomedical sciences, behavioural sciences, clinical effectiveness studies, health economics, implementation science, health impact analysis and evaluation. As in the case of reimbursement systems, HTA systems vary from country to country.

Health technology assessment is intended to provide a bridge between research and decision-making. It is a growing field and is intended to provide the data to support management, clinical, and policy decisions. It is also underpinned by the development of various disciplines in the social and applied sciences, especially clinical epidemiology and healthcare economics. Health policy decisions are increasingly seen as important as the risk of incurring substantial costs from making wrong decisions grows with the rising costs of providing treatment. Evidence-based data and cost-effectiveness information from HTA is therefore increasingly-used in many countries to underpin such decision-making.

In 2004, the European Commission and Council of Ministers identified Health Technology Assessment as a political priority and decided that there was an urgent need to establish a sustainable European HTA network. A European network, EUnetHTA, was established to "…help develop reliable, timely, transparent and transferable information to contribute to HTAs in European countries". EUnetHTA comprises government-appointed organisations from the EU Member States, EEA and Accession countries, together with various regional agencies and non-for-profit organisations that produce or contribute to HTA in Europe [26].

At the global level, the International Network of Agencies for Health Technology Assessment (INAHTA) was established in 1993 and has now grown to include 57 member agencies from 32 countries including North and Latin America, Europe, Africa, Asia, Australia, and New Zealand. All its members are non-profit making organizations producing HTA and are linked to regional or national government. At a national level, most countries have a range of organisations dedicated to developing and implementing HTA methodologies. Notable examples of such bodies include the National Institute for Clinical Excellence (NICE) in the UK, the Institute for Quality and Efficiency in Healthcare (IQWIG) in Germany and the Agency for Healthcare Research and Quality (AHRQ) in the US.

Griffin [27] suggests that access to many European markets, following regulatory approval of a healthcare product, is controlled or influenced by HTA agencies whose decisions depend heavily on value arguments informed by evidence on relative benefits compared with existing standards of care, and by economic modelling. While the regulatory decision to approve a product onto the market or not is based on a scientific judgement of its risks and benefits, the HTA decision, which often also influences whether a technology will be reimbursed or not, is a value judgement, although one based on scientific evidence and economic data. This has broad implications for medical technology companies, whether in the pharmaceutical, device or diagnostic sectors. The intention of healthcare services (e.g. the UK National Health Service) that use HTA is for it to help contribute towards the most effective use of limited resources.

Following a survey of stakeholders, Stephens et al. [28] found that the most common type of cost analysis in HTA is cost-effectiveness, with the primary methodology being decision models. Common end points included cost/life-years saved, cost/event avoided and cost/quality-adjusted life years (QALY). European HTA agencies generally have defined national guidelines they follow, while US agencies are less consistent in this respect.

The same report goes on to conclude that the use of different research methods and their conformity to published HTA principles varies significantly from country to country. Despite the study's relatively small sample size, the results suggest that HTA, using evidence-based medicine, will continue to rapidly evolve and will need standardized research methods and principles to guide assessment and decision-making around novel drug therapies, medical devices, and emerging technologies. It suggests also that a process for information sharing among HTA bodies may be needed to achieve this standardisation in research methods.

The quality-adjusted life year (QALY), as used by NICE in the UK [29], is a measure of disease burden, including both the quality and the quantity of life lived. It is used in assessing the value-for-money of a medical intervention under consideration. The QALY is based on the number of years of life that would be added by the intervention. Each year in perfect health is assigned the value of 1.0 down to a value of 0.0 for death. If the extra years would not be lived in full health, then the extra life-years are given a value between 0 and 1 to account for this.

The measure is then used in a cost-utility analysis to calculate the ratio of cost to QALYs saved for a particular health care intervention. This is then used to allocate healthcare resources, with an intervention with a lower cost to QALY saved ratio being preferred over an intervention with a higher ratio.

The measure is not universally accepted – some opponents suggesting that it means that some people will not receive treatment where it is calculated that the cost is not warranted by the benefit to their quality of life. However, its supporters argue that since healthcare resources are inevitably limited, the measure enables them to be allocated in the way that is most beneficial to society rather than to an individual patient.

This review makes no value judgement about the use of QALYs or other HTA methodologies. Rather, attention is drawn to the increasing application of health technology assessment around the world as a process used to justify expenditure on novel medical technologies, and one that will certainly be applied to the emerging field of nanomedicine. There is, therefore, a clear need for companies to generate data during product development that can contribute towards this process.

19.7 Reimbursement and Novel Medical Technologies

In the development of any new medical technology, attention needs to be paid at an early stage to how that product will be taken up and paid for by healthcare systems and providers. In Europe, the reimbursement and pricing of medical products is determined on a country-by-country, rather than European-wide, basis, leading to significant variations in systems, costs, and availabilities.

Many developments in nanomedicine may facilitate progress towards personalizing treatment towards individual patients. In a review of the reimbursement of personalized medicine products in Europe, on behalf of the Personalized Medicine Coalition, Garfield (2011) found significant differences in the ability of different country's reimbursement infrastructures to effectively assess and provide access to novel personalized medicine technologies [30]. The report suggested that, as a result, healthcare systems in many countries have been failing to appropriately evaluate and pay for personalized medicine technologies, with patients often being denied access to the most advanced drug and diagnostic treatments, while those healthcare systems continue to bear the costs of outdated trial-and-error approaches to medicine.

Inbar (2012) suggests that the clinical data required for regulatory approval does not necessarily encompass the clinical data required for successful reimbursement of a medical product and there are large differences also in terms of cost and effort between fitting into an existing reimbursement mechanism and developing a new code. He states, however, that, in many cases, the data required for the reimbursement process can be developed in parallel to the required regulatory data during the same clinical trials and that companies that consider regulatory and reimbursement as serial processes may reach the market with insufficient funds and time to finance another clinical trial just to develop reimbursement-related data. He concludes that reimbursement needs to be viewed as one of the issues that needs to be dealt with in parallel and early in the device development process, adding that some mistakes may be very difficult and expensive to correct later on [31].

19.8 Professional Uptake of Nanomedicine

At the 2008 conference *The Future Delivery of Medicine: 2020*, hosted at University College London (UCL), one key finding was that the potential benefits of a range of new medical technologies were being delayed by slow uptake in many European national healthcare systems. It was noted that healthcare budgets were under pressure across Europe while, at the same time, new developments in science and technology have emerged that could transform medicine. It was further suggested that delivering this potential in an affordable way will require healthcare to be more patient-centered and for medical professionals to think beyond their specialities and take a far more holistic view [32].

In addition, at a meeting before the start of the main conference, a group comprising speakers and other experts discussed potential guidelines for future policy formulation, including

- a need for changes across the value chain, from basic research through to delivery of medical care at the bedside and in the home;
- fundamental rethinking and reshaping of all the processes that currently underpin healthcare systems;
- challenging healthcare professionals to look outside their specialities;
- requiring regulators to rethink their views of risk and reimbursement authorities to take a different view of value and affordability.

The participants at the meeting also suggested that that there was a need for a new view of value and noted that, while advanced treatments may be expensive, they can lead to cost savings elsewhere and that health technology assessments need to take a broader view in the face of this new paradigm.

While nanotechnology, as an enabling technology, and a continually-evolving understanding of how nanomaterials and biology interact at the nanoscale is beginning to revolutionise medicine and medical products in areas such as screening,

prognosis, diagnosis, treatment planning, therapy, follow-up, and translational research, there is at present limited training available on nanomedicine, both within the curricula at medical schools and at a professional level thereafter.

A 2010 proposal to the European Commission's Directorate-General (DG) Research and Innovation Health Directorate by the European Alliance for Medical and Biological Engineering and Science (EAMBES) [33] suggested that the medical world could potentially become confused by the breadth and depth of the possible emerging medical technology interventions available. As a result, non-suitable solutions could be adopted that do not have the expected impact and thus do not constitute the correct way to approach the issue of preparing a framework for innovative therapeutic approaches. It suggested that this situation had already caused a number of problems in relation to the actual uptake of medical technology research and products resulting in a lower than expected synergy between the biological and medical engineering (BME) industry and the health sector.

The proposal went on further to suggest that a major impeding factor in the adoption of novel medical technology products is that they imply changes not only on the way the doctor thinks but also changes in the medical organizational and regulatory frameworks.

Many novel medical technologies have the potential to change the way medical practice is organised. Currently, typical diagnostic tests conducted by a General Practitioner might comprise taking a blood or bodily fluid sample from the patient, labelling and packaging it, sending it away to a central laboratory facility, waiting for several days for the results to come back and then recalling the patient to the surgery for a further consultation, discussion of the results of the tests and treatment. This multi-step procedure could potentially be replaced in the future by the use of a "smart" diagnostic device, designed for application in a variety of disease or metabolic tests, based on nanobiosensor and microfluidic technologies, and capable of being used in a GP's consulting room and of giving accurate results in a couple of minutes. Such novel diagnostic devices are currently in development and would, in all probability, be welcomed by GPs but there are a number of potential implications such as:

- diagnosis is changed from a remote dedicated laboratory facility/expert to a local "smart" device/medical generalist;
- while there may be costs in implementing such a technology, costs elsewhere, such as handling/packing/transport and laboratory costs would be minimized;
- long-established and familiar procedures would be changed;
- as diagnostic results could be immediately available, there would be implications for both GP, perhaps in terms of training on the interpretation of data and subsequent actions, and for patient;
- issues of trust in the quality and reliability of diagnostic data.

Therefore, in addition to the technological development of the device itself, attention needs to be paid to a broad spectrum of issues such as: the way and situation in which it will be used, e.g. by a patient at home, by a field worker or paramedic, by a qualified nurse, at a generalist's surgery or by a specialist at a hospital; whether

existing practice or organisational aspects are altered; what implications this has for training, interpretation of results and consequent actions; impacts on costs and cost points; storage of confidential data; and many other aspects.

It is important, therefore, for researchers and companies to work with medical professionals at an early stage of product development. Nanomedicine, in particular, has implications for implementation by medical professionals as it utilizes properties of materials that manifest at the nanoscale and which may not be readily apparent or understood, or addressed in their training. Furthermore this understanding of the principles of nanomedicine by medical professionals is important as they form a key and trusted route of communication to patients.

19.9 Public Perception

Usually, the general public, as patients, will first come into contact with nanomedical products via medical professionals, with whom there is generally a high degree of trust and which, again, reinforces the importance of building relationships and trust with the medical profession during development of the product, as previously discussed.

The public's own perception of emerging technologies may be, however, influenced by previous scientific debates or controversies, such as "Mad Cow" Disease (bovine spongiform encephalopathy) (nvCJD), GMO foods, contaminated blood, etc., and how these have been represented, or misrepresented, in the popular media. The public cannot be expected to fully perceive and understand scientific risks arising from new technologies. The same public, however, are perfectly happy to take a risk/benefit decision where they broadly understand the factors involved and perceive the expected benefit as outweighing the risk, e.g. crossing the road, driving a car or travelling by air, or to choose one risk over another ("the lesser of two evils"). Many medical treatments are known by the public, as patients, to involve some measure of risk, e.g. X-rays or aggressive chemotherapy, but they are prepared to undergo such procedures as they perceive the benefits to be gained as outweighing those risks and trust those professionals that carry out such procedures.

The perception of a risk amongst the general public can vary greatly depending upon factors such as:

- the cultural, socio-economic and educational background of the person(s) involved
- whether exposure to the hazard is
  - involuntary;
  - avoidable;
  - from a man-made or natural source;
  - due to negligence;
  - arising from a poorly understood cause;
  - affecting a vulnerable group within society;
- whether there is an obvious benefit to be gained from exposure to the risk.

Furthermore there may be a tendency to distrust "big industry" in some sectors where profits may be seen to outweigh safety concerns. All of these factors taken together may colour attitudes towards the acceptance of new technologies, especially if there has been poor communication about them.

Kahan and co-workers (2007) carried out a study amongst a recruited sample of United States subjects to assess their opinions about nanotechnology [34]. The responses of 1,500 subjects not exposed to additional information suggested that Americans were largely uninformed about nanotechnology: 81 % of subjects reported having heard either "nothing at all" (53 %) or "just a little" (28 %) about nanotechnology prior to being surveyed, and only 5 % reported having heard "a lot." Nevertheless, most of the same group of subjects, 89 %, were reported as having an opinion on whether the benefits of nanotechnology outweigh its risks or vice versa with slight majority (53 %) appearing to view benefits as outweighing risks. When subgroups were examined, however, more divisions were revealed. Men (59 % to 36 %) were significantly more likely than women (47 % to 40 %) to think that risks outweigh benefits. Moreover, whereas a majority of whites (54 %) believed that benefits outweighed risks, 49 % of African-Americans of viewed risks as outweighing benefits. White males were the most pro-benefit orientated (61 % to 30 %).

The study also backed up conclusions from previous studies that *affect* (a person's positive or negative emotional orientation) is one of the most powerful influences on individuals' perceptions of risk – subjects in the survey were asked to indicate whether nanotechnology made them feel "very bad," "bad," "neither good nor bad," "good," or "very good." Furthermore the study suggested how people react to information depends largely on their *values*. One of the major findings was that dissemination of scientifically-sound information is not by itself sufficient to overcome the divisive tendencies of cultural cognition. The authors concluded that those in a position to educate the public, including government, scientists and industry, need also to intelligently frame that information in ways that make it possible for persons of diverse cultural orientation to reconcile it with their values.

A later study by Bottini and colleagues (2011) amongst 790 citizens chosen randomly from four different urban areas of Rome reported that those surveyed exhibited optimism towards nanomedicine despite low awareness of currently available nanodrugs and nanocosmetics, and limited understanding of biocompatibility and toxicity aspects. The study concluded that, if such public optimism justifies the increase in scientific effort and funding for nanomedicine, it also obliges toxicologists, politicians, journalists, entrepreneurs, and policymakers to be more responsible in their dialogue with the public [35].

While there would seem, therefore, to be no major widespread prior distrust of the application of nanotechnology to medicine despite concerns in other areas of technology there is, nevertheless, a need for clear information to be made available to the public and other stakeholders about the benefits and risks of nanomedicine in a language that can be clearly understood and through channels that are trusted.

19.10 Ethical Considerations and Safeguards

While an in-depth review of many of the potential ethical issues associated with nanomedicine is provided by Donald Bruce within this book, it is nevertheless useful to consider some key points here as part of an overview of the challenges facing its widespread implementation.

19.10.1 What Do We Understand by Healthcare?

The increasing ability that we have to manipulate matter precisely at the nanoscale, combined with our improved understanding of biology, may influence our perception of what medicine and what a well person is, e.g.

- Just the treatment of disease?
- The correction of any deviation from what is considered "normal" function?
- What do we mean by "well" if we will be able to monitor at so many levels?
- What is the borderline between impaired function correction and performance enhancement?
- What are the expected limits of a "cure"?

While most people would probably accept the use of medicine for treatment of a disease or the correction of a physiological condition or impairment, they may not readily accept its application for enhanced performance, e.g. strength, senses, endurance for sports, military or other non-medical purposes.

19.10.2 The Changing Face of Medicine

Over the past several centuries medicine has changed beyond all recognition from the seventeenth century where treatments were largely palliative with the doctor focusing mainly on nonphysical supportive measures, through the development of hospital medicine in the nineteenth century and "laboratory medicine" in the twentieth century to the current twenty-first century scenario where we are now beginning to understand the human body as an intricately structured machine with billions of complex interacting parts, with each part (and each subsystem of parts) potentially subject to individual investigation, repair, and possibly replacement by artificial technological means. Along with this transformation of medicine over the centuries, the role of the medical professional has also changed enormously and we might reasonably expect medicine to become even more technological. But do good scientists or engineers make good doctors?

19.10.3 A Data Overload?

The development of novel diagnostic and imaging technologies, coupled with advances in genomics, proteomics and metabolomics (now commonly referred to collectively as the "-omics") means that there is a huge amount of data becoming available to medical professionals. This begs important questions such as

- Who can interpret all of this data?
- How much of the information is clinically significant?
- Who does the data belong to? The healthcare provider? The patient?
- How will the data be stored and transmitted safely?
- Where will the data be stored?
- What about patient confidentiality issues?
- What about the patients right to *know* and, equally, *not to know* certain information?

One particularly important element is maintaining the confidentiality of medical data… much of it could be of value to third parties other than the patient and doctor, e.g. employers, the government and commercial organisations such as insurance companies.

A study by Erlich and colleagues (2012) at the USA's Whitehead Institute demonstrated that the supposedly confidential names of research study participants could be traced from de-identified genetic data [36]. The researchers identified nearly 50 men and women who had submitted samples and had their genomes sequenced for a study performed by the Center for the Study of Human Polymorphisms (CEPH).

By matching short tandem repeats that they found on the Y chromosomes of men in the CEPH study to Y-STRs in publicly-available genetic genealogy databases, the researchers were able to recover the family names of men in the CEPH dataset who had submitted their Y-STRS to these repositories. With this information, they searched other free online information sources including record search engines, obituaries, genealogy websites, and public demographic data from the National Institute of General Medical Sciences' Human Genetic Cell Repository, housed at the Coriell Institute, and were able to track down the participants.

This study suggests that it may be difficult in practice to guarantee the security of medical and genomic data and that there is a need to balance research participants' privacy rights with the societal benefits to be realized from the sharing of biomedical research data.

19.10.4 Non-discrimination and Equity

Non-discrimination is a widely-accepted principle that people deserve equal treatment unless there are reasons that justify difference in treatment. In this context it primarily relates to the distribution of healthcare resources. Equity is the ethical principle that everybody should have fair access to the benefits under consideration.

Earlier commentary indicates, however, that access to treatment may vary from country to country because of regulatory, health technology assessment and reimbursement issues and, within some countries, access may even vary between different regions due to differing practices, priorities or availability of resources.

19.10.5 The Precautionary Principle

This principle entails the moral duty of continuous risk assessment with regard to the not fully foreseeable impact of new technologies. While the Precautionary Principle is already enshrined within European legislation, there are concerns from some quarters that could it be used as the justification to block potentially life-saving technologies on the grounds that the science is not yet fully understood.

19.11 Training

In formal medical education, very few medical schools currently offer modules on nanomedicine as part of their curricula. At the same time, massive levels of investment into research on the application of nanotechnologies to medicine, at both academic and commercial levels, means that there are increasing numbers of products incorporating nanotechnology appearing on the market with many more in the product pipeline or at the stage of clinical trials or awaiting regulatory approval.

While an increasing number of universities are now offering nanotechnology-based undergraduate or postgraduate level courses, there are still only a limited number specifically addressing nanomedicine or specific medical disciplines with a significant medical nanotechnology component.

At a professional level, organisations such as such as the Institute of Nanotechnology (IoN) and universities such as Cranfield and Oxford have developed short courses aimed at addressing training needs in nanomedicine and the application of nanotechnology to topics such as medical diagnostics, imaging, drugs and biosensors, as well as nano- risk and safety issues. These have attracted interest from a range of participants including those working in academia and research, industry, medical professionals and medical students, healthcare providers and regulatory authorities.

The successful adoption and implementation of nanomedical solutions in the clinic will depend on the presence of informed decision-makers who understand the underlying science, opportunities and benefits that the technologies can bring, short and long terms costs and savings, and how nanomedicine can be integrated safely and effectively into everyday healthcare. This includes those working in research funding, commercial strategy, regulatory affairs, health technology assessment, reimbursement and healthcare provision professionals, insurers, and amongst the medical professions. There is, therefore, an ongoing need for training in nanomedicine at both academic and in-service, professional levels.

19.12 Conclusions and Perspectives for the Future

This chapter intends to highlight some of the non-technical challenges that researchers and developers are likely to face in bringing medical products based on nanotechnology to the market and clinic. Many of these challenges are not exclusive to nanomedicine but apply generally to emerging medical technologies. However, some of these challenges may be compounded by the fact that nanoscale materials frequently exhibit novel properties that can provide both benefits and opportunities but that, at the same time, may present novel hazards and risks that are poorly understood. Characterisation of novel nanomaterials and the establishment of a widely-available repository of safety data will therefore be vital to the success of nanomedicine.

From the author's personal experience, the attitude towards nanomedicine from a wide variety of stakeholders who have attended professional training courses, workshops and conferences on the topic, including medical professionals, regulators, industry professionals and others, has been positive. There, however, remains a widespread lack of awareness on the subject in the wider medical community and much needs to be done to engage with these professionals to impart knowledge, build trust and promote the uptake of novel nano-based products.

It is also clear that better communication is needed with health technology and reimbursement professionals. In healthcare systems where cost containment is increasingly critical to healthcare delivery, it must clearly be demonstrated that nanomedicine can deliver better treatments while reducing costs in the short, middle or long term, for example by earlier or more accurate diagnosis, more effective treatments, or by reducing lengths of stay in hospitals. In addition, there is clear scope for a contribution towards more personalised form of medicine rather than a one-size-fits-all approach, although this may well necessitate the development of new business and professional practice models.

Because of the comparative timescales required for regulatory approval, it is likely that the fastest progress to market for nanomedicine will be seen in the areas of diagnostics, biosensors and other medical devices. However, developments in the pharmaceutical and regenerative medicine sectors, although possibly longer term, are likely to be significant and potentially disruptive in terms of contributing to new paradigms of treatment.

In the longer term, there is also potential for massive synergy between nanomedicine and other emerging field such biomimetics, particularly in terms of integrating nano- and biological structures for biosensing, drug delivery and regenerative medicine, and designing new generations of novel nano-based devices.

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Dr. Yi Ge obtained his bachelor's degree (first Class Hons) in Biopharmaceutics and went on to an MPhil degree in Pharmaceutical Chemistry at Aston University. Afterwards, he moved to the University of Sheffield for a PhD in Chemistry. He was later employed as a research scientist in a UK pharmaceutical company and was then a postdoctoral research associate at Imperial College London, before joining Cranfield University as a member of academic staff in 2006. His research interests and activities have a focus on the interdisciplinary areas, at the interfaces of nanotechnology, medicine, materials and pharmaceutics. In 2008, as the founder, he was appointed as the Course Director of Nanomedicine MSc Course Programme, which is a unique postgraduate course and the first of its kind in all of Europe. He is a Member of the Royal Society of Chemistry and a Member of the British Society for Nanomedicine. His activity in the field of nanotechnology and nanomedicine was recognized by the Institute of Nanotechnology and he was admitted as a Fellow of the Institute in 2009. He is a Visiting Professor at the University of Jinan (China) and serves as the Associate Editor of *Smart Materials*, as well as the Editorial Board Members of the *Journal of Nanotechnology: Nanomedicine and Nanobiotechnology*, *Austin Journal of Nanomedicine and Nanotechnology* and *Austin Journal of Biosensors and Bioelectronics*. He has also been the Editor of two books on Smart Nanomaterials for Sensor Applications (Bentham Science) and Molecularly Imprinted Sensors (Elsevier).

Dr. Songjun Li was appointed as an Associate Professor Central China Normal University after receiving his PhD degree from the Chinese Academic of Sciences in 2005. In 2008, he took up a position at the University of Wisconsin-Milwaukee (USA) as a Postdoctoral Associate until 2009 when he received a prestigious Marie Curie International Incoming Fellowship to undertake a 2-year research project at Cranfield University (UK). He is now a Distinguished Professor of Functional Polymers at Jiangsu University in China. Dr. Li's research interests and activities, in relation to nanomedicine, includes nanomaterials, nanobiosensors and smart nanoreactors. He has published over 60 papers in peer-reviewed journals and has edited

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Richard Moore has been active in the fields of medical technology and nanotechnology for over 20 years. His experience includes: (1) 6 years working as Scientific Director at the Institute of Nanotechnology, UK, with a particular focus on the application of nanotechnologies to medicine and the life sciences, risk management, the regulation and governance of nano- and other novel technologies and the development of professional training courses in nanomedicine; (2) 10 years working as Director, Science and Innovation at Eucomed (European Medical Technology Association), Brussels, with a key focus on providing technical and scientific support services to Eucomed's national and corporate members; (3) 6 years working as Project Manager, Healthcare at the European Committee for Standardisation (CEN), Brussels, with a main focus on overseeing the development of the platform of European harmonized standards supporting the Medical Device Directives. Since early 2012, Richard Moore has run his own consultancy specialising in analysis, foresighting, and advisory services in the field of emerging technologies including nanotechnology. He is also a Project Technical Advisor to a number of large EC-funded research projects in the areas of nanosafety and advanced nanomaterials, and is an external lecturer on nanotechnology and nanomedicine topics at several UK universities.

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