Chapter 5
Medical Nanomaterials

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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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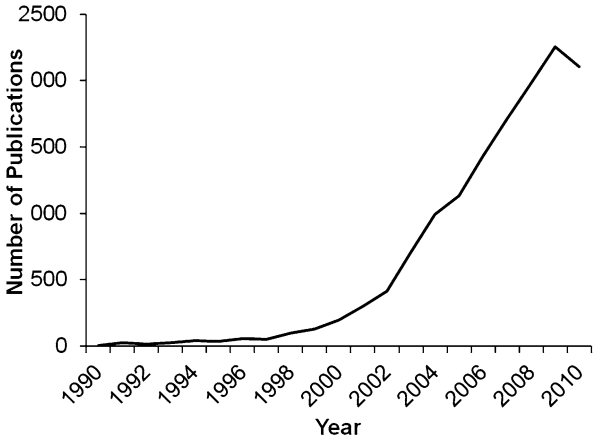

Fig. 5.1 Number of "nanotechnology" publications per year between 1990 and 2010

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.

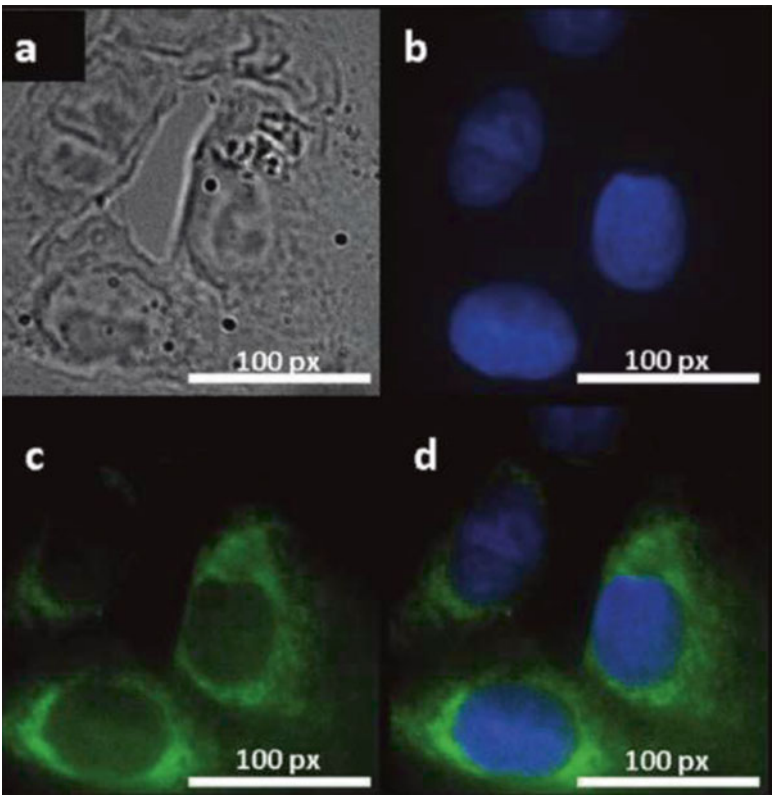

Fig. 5.2 Human breast cancer cells (T47D) exposed to graphene quantum dots. (**a**) Brightfield image of cancer cells. (**b**) Nuclei stained in *blue*, (**c**) *green* fluorescence shows accumulation of quantum dots in the cytoplasm, (**d**) overlay. [17]

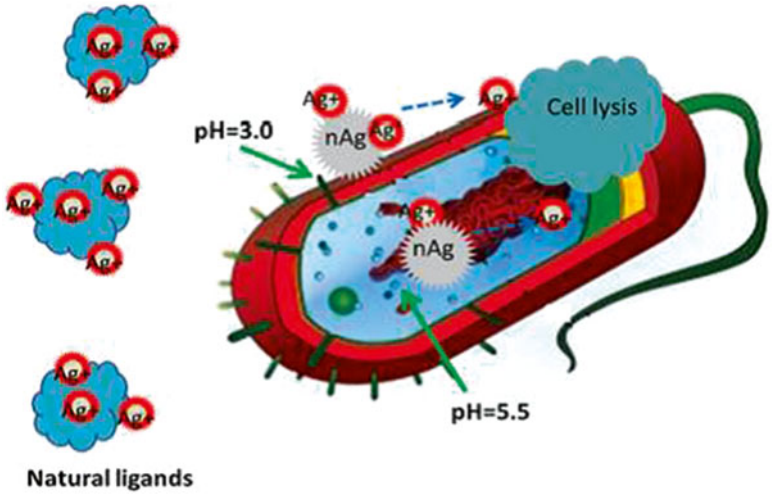

Fig. 5.3 Interaction between silver nanoparticle and the bacterial cell [18]

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.

References

1. World Health Organization (2009) New HIV infections reduced by 17 % over the past eight years. World Health Organisation. http://www.who.int/mediacentre/news/releases/2009/hiv_aids_20091124/en/index.html
2. National Cancer Institute (2010) Cancer trends progress report – 2009/2010 update. http://progressreport.cancer.gov
3. Moghimi S, Farhangrazi Z (2014) Just so stories: the random acts of anti-cancer nanomedicine performance. Nanomed Nanotechnol Biol Med. doi:10.1016/j.nano.2014.04.011
4. Toy R, Peiris P, Ghaghada K et al (2014) Shaping cancer nanomedicine: the effect of particle shape on the in vivo journey of nanoparticles. Nanomedicine 9(1):121–134
5. Adams G, Weiner L (2005) Monoclonal antibody therapy of cancer. Nat Biotechnol 23:1147–1157
6. Wong M, Ziring D, Korin Y et al (2008) TNFα blockade in human diseases: mechanisms and future directions. Clin Immunol 126:121–136
7. Fischer A, Hacein-Bey-Abina S, Cavazzana-Calvo M (2010) 20 years of gene therapy for SCID. Nat Immunol 11:457–460
8. Touzot F, Hacein-Bey-Abina S, Fischer A et al (2014) Gene therapy for inherited immunodeficiency. Expert Opin Biol Ther 14(6):789–798
9. Davis M, Jonathan Z, Chung Hang C et al (2010) Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nature 464:1067–1070
10. Fan Z, Fu P, Yu H et al (2014) Theranostic nanomedicine for cancer detection and treatment. J Food Drug Anal 22(1):3–17
11. Chaturvedi S, Dave PN (2014) Emerging applications of nanoscience. Mater Sci Forum 781:25–32

12. Goel A, Colcher D, Baranowska-Kortylewicz J et al (2000) Genetically engineered tetravalent single-chain Fv of the pancarcinoma monoclonal antibody CC49: improved biodistribution and potential for therapeutic application. Cancer Res 60:6964–6971
13. Ilium L, Davis S, Wilson C et al (1982) Blood clearance and organ deposition of intravenously administered colloidal particles: the effects of particle size, nature and shape. Int J Pharm 12:135–146
14. Krpetić Ž, Anguissola S, Garry D, Kelly PM, Dawson KA (2014) Nanomaterials: impact on cells and cell organelles. In: Nanomaterial. Springer, Dordrecht, pp 135–156. http://link.springer.com/book/10.1007%2F978-94-017-8739-0
15. Mathaes R, Winter G, Besheer A et al (2014) Influence of particle geometry and PEGylation on phagocytosis of particulate carriers. Int J Pharm 465(1):159–164
16. Lhuillier E, Keuleyan S, Guyot-Sionnest P (2012) Optical properties of HgTe colloidal quantum dots. Nanotechnology 23:175705
17. Peng J, Gao W, Gupta B et al (2012) Graphene quantum dots derived from carbon fibres. Nano Lett 12(2):844–849
18. Xiu Z, Zhang G, Puppala H et al (2012) Negligible particle-specific antibacterial activity of silver nanoparticles. Nano Lett 12:4271–4275
19. Lu Z, Rong K, Li J et al (2013) Size-dependent antibacterial activities of silver nanoparticles against oral anaerobic pathogenic bacteria. J Mater Sci Mater Med 24(6):1465–1471
20. Alkilany A, Thompson L, Boulos S et al (2012) Gold nanorods: their potential for photothermal therapeutics and drug delivery, tempered by the complexity of their biological interactions. Adv Drug Deliv Rev 64:190–199
21. Choi W, Sahn A, Kim Y et al (2012) Photothermal cancer therapy and imaging based on gold nanorods. Ann Biomed Eng 40(2):534–546
22. Moghimi S, Peer D, Langer R (2011) Reshaping the future of nanopharmaceuticals: ad Iudicium. ACS Nano 5(11):8454–8458
23. Harvey A, Brand A, Holgate S et al (2012) The future of technologies for personalised medicine. N Biotechnol 29(6):625–633
24. The Freedonia Group (2009) In vitro diagnostics: US industry study with forecasts for 2013 & 2018. The Freedonia Group, Cleveland
25. Giljohann D, Seferos D, Daniel W et al (2010) Gold nanoparticles for biology and medicine. Angew Chem Int Ed 49(19):3280–3294
26. Davis M, Chen Z, Shin D (2008) Nanoparticle therapeutics: an emerging treatment modality for cancer. Nat Rev Drug Discov 7:771–782
27. Wagner V, Dullaart A, Bock A et al (2006) The emerging nanomedicine landscape. Nat Biotechnol 24:1211–1217
28. Gentleman D, Chan W (2009) A systematic nomenclature for codifying engineered nanostructures. Small 5:426–431
29. Alivisatos A (1996) Semiconductor clusters, nanocrystals, and quantum dots. Science 271:933–937
30. Åkerman M, Chan W, Laakkonen P et al (2002) Nanocrystal targeting in vivo. Proc Natl Acad Sci U S A 99:12617–12621
31. Fischer H, Liu L, Pang K et al (2006) Pharmacokinetics of nanoscale quantum dots: in vivo distribution, sequestration, and clearance in the rat. Adv Funct Mater 16:1299–1305
32. Hauck T, Anderson R, Fischer H et al (2010) In vivo quantum-dot toxicity assessment. Small 6:138–144
33. Tans S, Devoret M, Dai H et al (1997) Individual single-wall carbon nanotubes as quantum wires. Nature 386:474–477
34. Frens G (1973) Controlled nucleation for regulation of particle-size in monodisperse gold suspensions. Nat Phys Sci 241:20–22
35. Cepak V, Martin C (1998) Preparation and stability of template-synthesized metal nanorod sols in organic solvents. J Phys Chem B 102:9985–9990
36. Link S, Mohamed M, El-Sayed M (1999) Simulation of the optical absorption spectra of gold nanorods as a function of their aspect ratio and the effect of the medium dielectric constant. J Phys Chem B 103:3073–3077

37. Link S, El-Sayed M (2000) Shape and size dependence of radiative, non-radiative and photothermal properties of gold nanocrystals. Int Rev Phys Chem 19:409–453
38. Jana N, Gearheart L, Murphy C (2001) Wet chemical synthesis of high aspect ratio cylindrical gold nanorods. The Journal of Physical Chemistry B 105:4065–4067
39. Subramanian V, Wolf E, Kamat P (2004) Catalysis with TiO_2/gold nanocomposites. Effect of metal particle size on the fermi level equilibration. J Am Chem Soc 126:4943–4950
40. Yuan F, Dellian M, Fukumura D et al (1995) Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. Cancer Res 55:3752–3756
41. Perrault S, Walkey C, Jennings T et al (2009) Mediating tumor targeting efficiency of nanoparticles through design. Nano Lett 9:1909–1915
42. Cabral H, Matsumoto Y, Mizuno K et al (2011) Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size. Nat Nanotechnol 6:815–823
43. Fang C, Shi B, Pei Y et al (2006) In vivo tumor targeting of tumor necrosis factor-α-loaded stealth nanoparticles: effect of MePEG molecular weight and particle size. Eur J Pharm Sci 27:27–36
44. Chithrani B, Ghazani A, Chan W (2006) Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. Nano Lett 6:662–668
45. Jiang W, Kim B, Rutka J et al (2008) Nanoparticle-mediated cellular response is size-dependent. Nat Nanotechnol 3:145–150
46. Boyer J, Vetrone F, Cuccia L et al (2006) Synthesis of colloidal upconverting NaYF4 nanocrystals doped with Er^{3+}, Yb^{3+} and Tm^{3+}, Yb^{3+} via thermal decomposition of lanthanide trifluoroacetate precursors. J Am Chem Soc 128:7444–7445
47. Boyer J, Cuccia L, Capobianco J (2007) Synthesis of colloidal upconverting NaYF4: Er^{3+}/Yb^{3+} and Tm^{3+}/Yb^{3+} monodisperse nanocrystals. Nano Lett 7:847–852
48. Chan W (1998) Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science 281:2016–2018
49. Horne R, Bangham A, Whittaker V (1963) Negatively stained lipoprotein membranes. Nature 200:1340
50. Baish J, Gazit Y, Berk D et al (1996) Role of tumor vascular architecture in nutrient and drug delivery: an invasion percolation-based network model. Microvasc Res 51:327–346
51. Hashizume H, Baluk P, Morikawa S et al (2000) Openings between defective endothelial cells explain tumor vessel leakiness. Am J Pathol 156:1363–1380
52. Morikawa S, Baluk P, Kaidoh T et al (2002) Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. Am J Pathol 160:985–1000
53. Matsumura Y, Maeda H (1986) New concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res 46:6387–6392
54. Klibanov A, Maruyama K, Torchilin V et al (1990) Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes. FEBS Lett 268:235–237
55. Cohen S, Yoshioka T, Lucarelli M et al (1991) Controlled delivery systems for proteins based on poly(lactic glycolic acid) microspheres. Pharm Res 8:713–720
56. Jain R (2000) The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials 21:2475–2490
57. Faraday M (1857) Experimental relations of gold (and other metals) to light. Philos Trans R Soc Lond 147:145–181
58. Turkevich J, Stevenson P, Hillier J (1951) A study of the nucleation and growth processes in the synthesis of colloidal gold. Discuss Faraday Soc 11:55–75
59. Jana N, Gearheart L, Murphy C (2001) Evidence for seed-mediated nucleation in the chemical reduction of gold salts to gold nanoparticles. Chem Mater 13:2313–2322
60. Perrault S, Chan W (2009) Synthesis and surface modification of highly monodispersed, spherical gold nanoparticles of 50–200 nm. J Am Chem Soc 131:17042–17043
61. Mie G (1908) Articles on the optical characteristics of turbid tubes, especially colloidal metal solutions. Ann Phys 25:377–445

62. Oldenburg S, Jackson J, Westcott S et al (1999) Infrared extinction properties of gold nanoshells. Appl Phys Lett 75:2897–2899
63. Storhoff J, Elghanian R, Mucic R et al (1998) One-pot colorimetric differentiation of polynucleotides with single base imperfections using gold nanoparticle probes. J Am Chem Soc 120:1959–1964
64. Anger P, Bharadwaj P, Novotny L (2006) Enhancement and quenching of single-molecule fluorescence. Phys Rev Lett 96:113002
65. Kuehn S, Hakanson U, Rogobete L, Sandoghdar V (2006) Enhancement of single-molecule fluorescence using a gold nanoparticle as an optical nanoantenna. Phys Rev Lett 97(1):017402
66. Mu C, LaVan D, Langer R et al (2010) Self-assembled gold nanoparticle molecular probes for detecting proteolytic activity in vivo. ACS Nano 4:1511–1520
67. Dickerson E, Dreaden E, Huang X et al (2008) Gold nanorod assisted near-infrared plasmonic photothermal therapy (PPTT) of squamous cell carcinoma in mice. Cancer Lett 269:57–66
68. Hauck T, Ghazani A, Chan W (2008) Assessing the effect of surface chemistry on gold nanorod uptake, toxicity, and gene expression in mammalian cells. Small 4:153–159
69. Paciotti G, Myer L, Weinreich D et al (2004) Colloidal gold: a novel nanoparticle vector for tumor directed drug delivery. Drug Deliv 11:169–183
70. Visaria R, Griffin R, Williams B et al (2006) Enhancement of tumor thermal therapy using gold nanoparticle-assisted tumor necrosis factor-alpha delivery. Mol Cancer Ther 5:1014–1020
71. Goel R, Shah N, Visaria R et al (2009) Biodistribution of TNF-alpha-coated gold nanoparticles in an in vivo model system. Nanomedicine 4:401–410
72. Farokhzad O, Jon S, Khademhosseini A et al (2004) Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. Cancer Res 64:7668–7672
73. Gao X, Cui Y, Levenson R et al (2004) In vivo cancer targeting and imaging with semiconductor quantum dots. Nat Biotechnol 22:969–976
74. Park J, von Maltzahn G, Xu M et al (2010) Cooperative nanomaterial system to sensitize, target, and treat tumors. Proc Natl Acad Sci U S A 107:981–986
75. von Maltzahn G, Park J, Lin K et al (2011) Nanoparticles that communicate in vivo to amplify tumour targeting. Nat Mater 10:545–552
76. Perrault S, Chan W (2010) In vivo assembly of nanoparticle components to improve targeted cancer imaging. Proc Natl Acad Sci 107:11194