Nanoparticles in modern medicine: State of the art and future challenges

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Abstract: Nanoparticles are materials with overall dimensions in the nanoscale, ie, under 100 nm. In recent years, these materials have emerged as important players in modern medicine, with clinical applications ranging from contrast agents in imaging to carriers for drug and gene delivery into tumors. Indeed, there are some instances where nanoparticles enable analyses and therapies that simply cannot be performed otherwise. However, nanoparticles also bring with them unique environmental and societal challenges, particularly in regard to toxicity. This review aims to highlight the major contributions of nanoparticles to modern medicine and also discuss environmental and societal aspects of their use.

Keywords: nanoparticles, contrast agents, drug delivery, tumors, quantum dots, toxicity

Introduction

Nanoparticles are materials with overall dimensions in the nanoscale, ie, under 100 nm. In recent years, these materials have emerged as important players in modern medicine, with applications ranging from contrast agents in medical imaging to carriers for gene delivery into individual cells. Nanoparticles have a number of properties that distinguish them from bulk materials simply by virtue of their size, such as chemical reactivity, energy absorption, and biological mobility.

Nanoparticles are also referred to as "zero-dimensional" nanomaterials. This definition arises from the fact that all of their dimensions are in the nanoscale, as opposed to one-dimensional nanomaterials, which have one dimension larger than the nanoscale (such as nanowires and nanotubes), and two-dimensional nanomaterials, which have two dimensions larger than the nanoscale (such as self-assembled monolayer films).

The benefits of nanoparticles to modern medicine are numerous. Indeed there are some instances where nanoparticles enable analyses and therapies that simply cannot be performed otherwise. However, nanoparticles also bring with them unique environmental and societal challenges, particularly in regard to toxicity. This review aims to highlight the major contributions of nanoparticles to modern medicine and also discuss environmental and societal aspects of their use.

This review is intended to serve as a broad introduction to the role of nanoparticles in medicine rather than as an exhaustive review. Furthermore, this review will focus on technologies that have either already advanced to clinical use or in vivo experimentation. Within the broad categories of medical imaging and drug/gene delivery, this review will discuss examples of medical applications of nanoparticles. Where possible, the reader will be referred to the numerous comprehensive reviews already available within each application area. Lastly, the environmental and societal impact of the use of nanoparticles in modern medicine will also be discussed.

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Nanoparticles in medical imaging

Nanoparticles can provide significant improvements in traditional biological imaging of cells and tissues using fluorescence microscopy as well as in modern magnetic resonance imaging (MRI) of various regions of the body. Chemical composition distinguishes the nanoparticles used in these two techniques. A summary of the applications of nanoparticles in imaging is provided in Table 1

Optical imaging

Conventional imaging of cells and tissue sections is performed by loading organic dyes into the sample. Dyes such as fluorescein isocyanate (FITC) and rhodamine are often tethered to biomolecules that selectively bind to cells or cell components through ligand/receptor interactions. Two problems often encountered in this mode of imaging are inadequate fluorescence intensity and photobleaching. Photobleaching is the gradual decrease in fluorescence intensity often observed over time due to irreversible changes in the molecular structure of the dye molecules that render them nonfluorescent.

Quantum dots (QDs) are nanoparticles composed of inorganic semiconductor molecules. These nanoparticles emit strong fluorescent light under ultraviolet (UV) illumination, and the wavelength (color) of the fluorescent light emitted depends sensitively on particle size. This size dependence is a unique characteristic of these materials. Inorganic semiconductor molecules derive their properties from the presence of a "band gap." The band gap is the difference in energy between the valence band (or energy level), where the electrons primarily reside, and the conduction band, to which they can be "promoted" by the supply of energy of a specific wavelength (excitation), usually in the form of a photon. When an electron moves from the valence band to the conduction band, it leaves behind a "hole" (this is a term given to an energy level lacking an electron, and is not a physical feature). When the excitation ceases, electrons move back to the valence band, releasing their excess energy. In the case of QDs, this energy is released entirely as light. Larger QDs have more electron-hole pairs and are therefore capable of absorbing and releasing more energy. Since energy is inversely related to wavelength ($E = hc/\lambda$), this means that the wavelength of emitted light decreases as QD size increases. QDs can emit light that is far more intense and significantly more stable against photobleaching compared with conventional organic dyes. This is a major advantage in 3-D tissue imaging where photobleaching is a major concern during acquisition of successive sections in the z-direction.

Being inorganic materials, QDs are insoluble in aqueous solutions. An essential part of using QDs in biological and medical applications is therefore coating them with a thin layer of a water-soluble material. Typically, this step is followed by coating with a material that binds preferentially to a particular cell or cell component. The surface of each QD has a large number of sites onto which soluble and/or bioactive molecules can be tethered. Furthermore, more than one type of molecule can be attached to each QD, giving it multiple functionalities. In a review of the application of QDs for live cell and in vivo imaging, Michalet and colleagues (2005) have described different surface modification strategies such as targeting and prolonged retention in the bloodstream.

Kim and colleagues (2004) recently described the use of oligomeric phosphine-coated QDs to map lymph nodes in mice and pigs. These QDs were made of CdTe capped with CdSe, a combination that is capable of light emission under near infrared excitation. The significance of this work is the ability to map lymph nodes up to 1 cm below the skin surface without the need for surgical incisions. The toxicity of the injected QDs was not examined in this study and the authors inferred that the concentrations used were below known toxic levels.

QDs can be targeted to specific organs within the body by coating the QD surface with appropriate molecules. Akerman and colleagues (2002) demonstrated that ZnS-capped CdSe QDs can be directed to the lungs of mice by coating the QD surface with a peptide sequence, CGFECVRQCPERC, which is known to bind to endothelial cells in lung blood vessels. The same methodology was used to direct QDs to blood or lymphatic vessels within tumors in mice. In both instances, the QDs were internalized by the targeted cells by endocytosis but not by cells in surrounding tissue.

Gao and colleagues (2004) encapsulated semiconductor QDs within an amphiphilic copolymer and modified the polymer surface with targeting ligands (as shown in Figure 1A). The QDs were made of CdSe capped with ZnS and protected from aggregation in solution by a coordinating ligand (tri-*n*-octylphosphine oxide [TOPO]). The copolymer was a triblock, consisting of butylacrylate, ethylacrylate, and methacrylic acid segments; the former two segment types are more hydrophobic than the latter. In solution, the hydrophobic segments of the copolymer are attracted to the TOPO, resulting in the structure shown in Figure 1A, which has the carboxylic acid groups of the hydrophilic segment

Table 1 Summary of current nanoparticle technologies in medicine

Area	Nanoparticle type	Major in vivo applications	Significant characteristics	Selected references
Optical imaging	Quantum dots	Site-specific imaging in-vivo	▪ Imaging of lymph nodes, lung blood vessels, and tumors. ▪ Greater intensity and resistance to photobleaching compared with conventional methods. ▪ Site-specific targeting via surface functionalization. ▪ Sub-cutaneous imaging without surgical incisions.	Akerman et al 2002; Gao et al 2004; Kim et al 2004
MRI	Superparamagnetic iron oxide nanoparticles	Cancer detection	▪ Enhanced contrast for imaging of liver, lymph nodes, and bone marrow. ▪ Paramagnetic properties that can alter magnetic resonance relaxation times of selected regions or fluids *in vivo*.	Harisinghani et al 2003; Huh et al 2005
Drug and gene delivery	Polymer- and liposome-based nanoparticles	Cancer therapy	▪ Targetted delivery by surface functionalization. ▪ Strategies for prolonging residence times in vivo (eg, PEG attachment). ▪ Strategies for solubilizing water-insoluble drugs (eg, paclitaxel). ▪ Multi-layer and multi-functional (eg, chemotherapeutic and anti-angiogenic).	Duncan 2003; Allen and Cullis 2004; Micha et al 2006; Sengupta et al 2005
		Neurodegenerative disease therapy	▪ Transport across blood–brain barrier (eg, by PEG incorporation). ▪ Superior to direct drug administration. ▪ Therapies for diseases unresponsive to small molecule drugs (gene therapy).	Schlachetzki et al 2004; Garcia-Garcia et al 2005; Popovic and Brundin 2006
		HIV/AIDS therapy	▪ Solubilizing water-insoluble drugs by emulsification. ▪ Ability to transfect cells by DNA incorporation in nanoparticle.	De Jaeghere et al 2000; Olbrich et al 2001; Tabatt et al 2004
		Ocular disease therapy	▪ Ability to prolong drug residence times within ocular mucus layer or retina. ▪ Alternative to frequent application of high-drug conc. drops.	Pignatello et al 2002, 2002b; Ludwig 2005
		Respiratory disease therapy	▪ Mitigation of inflammatory responses in respiratory tract.	John et al 2003; Kumar et al 2003

Abbreviations: PEG, poly(ethylene glycol).

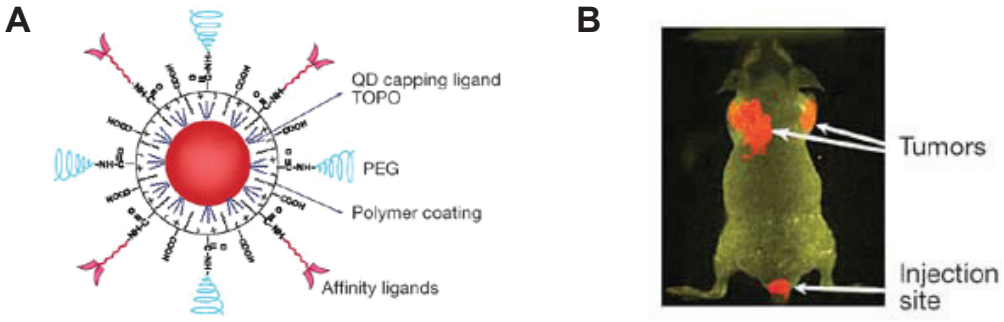

Figure 1 Quantum dots (QDs) used in tumor imaging. (**A**) Surface modification of the CdSe/ZnS QD with a capping ligand TOPO which keeps QDs from aggregating in solution; this assembly is enclosed by an amphiphilic polymer whose hydrophobic segments bind to TOPO and whose hydrophilic carboxylic acid groups can bind to affinity ligands (such as a tumor-specific antibody) or PEG. (**B**) Fluorescence image of a live mouse showing targeted delivery of QDs to a tumor. Adapted from Gao et al (2004) with permission from Macmillian Publishers Ltd: *Nature Biotechnology*. Copyright © 2004.

sticking out. These acid groups can be used as attachment points for molecules such as poly(ethylene glycol) (PEG) or affinity ligands. The composite particles were 20–30 nm in diameter. Tumor targeting was achieved by tethering to the particle surface an antibody against prostate specific membrane antigen (PSMA). As hypothesized, these particles did indeed selectively bind to tumors in mice upon intravenous injection (Figure 1B). No accumulation was observed in the brain, kidney, or lung.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is a technique used to perform 3-D, noninvasive scans of the body. This technique is widely used in modern medicine, particularly in the diagnosis and treatment of most diseases of the brain, spine, and musculoskeletal system. MRI utilizes magnetic resonance spectroscopy to analyze hydrogen atoms that are naturally present in tissue (as water and cell membrane proteins, for example). A sample is placed within a strong static magnetic field and a transverse radiofrequency (RF) signal is use to excite the magnetic dipoles within hydrogen nuclei in the sample. Prior to the RF pulse, the spinning nuclei are aligned with the static field. The RF pulse provides additional energy to these nuclei and causes them to spin at a different frequency and in a different (transverse) direction. Following the RF pulse, the hydrogen nuclei return, or "relax," to a state of equilibrium in alignment with the static magnetic field. The relaxation process is typically characterized by two parameters referred to as T1 and T2. T1 represents the time required for restoration of nuclear spins in alignment with the static field; T2 represents the characteristic time over which the transverse magnetization of the nuclei vanishes. Hydrogen nuclei and different types of tissue can be differentiated on the basis of different T1 and T2 relaxation times. MRI scans involve collection of several images based on spatial location as well as on weighting based on T1 or T2. A sample with low T1 appears bright in a T1-weighted image.

In many clinical applications, however, the natural differences in relaxation times between regions of interest (such as normal versus scar tissue) are small, necessitating the use of contrast agents. Contrast agents are typically paramagnetic molecules that can alter the relaxation times of selected regions or types of tissue or fluid within the body. Compounds of gadolinium have been successfully utilized for several years as contrast agents with the ability to resolve such areas as the kidney and brain (Mornet et al 2004). Gadolinium-based contrast agents act by shortening T1.

Superparamagnetic iron oxide (SPIO) nanoparticles have recently emerged as effective contrast agents for T2-weighting, thereby serving as a complement to gadolinium-based agents. T2 weighting is important for the imaging of the liver, lymph nodes, and bone marrow (Mornet et al 2004). The relaxation times of superparamagnetic nanoparticles (such as iron oxide) are much higher than those of gadolinium-based agents.

Huh and colleagues (2005) recently described how SPIO nanoparticles can be used to detect cancer in vivo using a mouse xenograft model. In this investigation, the nanoparticles were conjugated to herceptin, a cancer-targeting antibody. SPIO nanoparticles were prepared by the thermal decomposition of iron acetylacetonate and made water-soluble by binding with 2, 3-dimercaptosuccinic acid before conjugation with herceptin. When administered intravenously to mice, a rapid change was observed in the T2-weighted MRI signal from the tumor located in the thigh of the animals. The specificity of antibody binding was verified in a control experiment where the same iron oxide nanoparticles were

bound to a nonspecific antibody. These control nanoparticles did not produce any change in MRI signal.

SPIO nanoparticles can also be used to visualize features that would not otherwise be detectable by conventional MRI. Harisinghani and colleagues (2003) utilized SPIO nanoparticles in human patients with prostate cancer to detect small metastases in the lymph node. In this case, the nanoparticles were coated with dextran for retention in the blood stream and gradual uptake into the lymph nodes where they are internalized by macrophages. As shown in Figure 2, SPIO nanoparticles allow the visualization of metastases that can only be vaguely discerned by conventional MRI. The significance of this work is that patients with localized disease have the option of early treatment by surgery without being restricted to radiation therapy, the primary treatment for advanced-stage patients.

Nanoparticles in drug and gene delivery

Among the different application areas of nanoparticles, drug delivery is one of the most advanced. This is large part due to the success of polymer- and liposome-based drug delivery systems (Figure 3), many of which are in clinical use today.

Polymer-based drug delivery systems can be categorized as polymeric drugs, polymer-protein conjugates, polymer-drug conjugates, and polymeric micelles (Duncan 2003). Polymers can also be emulsified into nanometer-size particles within which drugs can be trapped. Polymeric drugs are typically natural polymers that are known to have antiviral or antitumor characteristics. Polymer-protein conjugates most commonly use PEG. PEG is well known for its high water solubility and excellent biocompatibility, and its attachment to drugs results in increased solubility. PEG attachment is also known to reduce the renal clearance of drugs and enhance receptor-mediated uptake by cells. This approach can therefore be utilized to prolong the half life of a drug and reduce dosing frequency. Polymer-drug conjugation is aimed at improving solubility and specificity of low molecular weight drugs. Lastly, polymeric micelles are typically created with amphipilic polymers that form micelles in solution with a drug entrapped inside the micelles.

Liposomes are vesicles formed by the entrapment of fluid by phospholipid molecules which have hydrophobic and hydrophilic components and can form bilayers. A bilayer is formed when two layers of oriented lipid molecules come together such that their hydrophobic sides are in contact with one another. Under certain conditions, lipid molecules form vesicles, in which a volume of fluid is enclosed by lipid bilayers. Vesicles can range in size from tens of nanometers to thousands of nanometers (Torchilin and Weissig 2003). Drug molecules can be incorporated along with the fluid enclosed by vesicles or within lipid bilayers. The structure of these synthetic bilayers, which are biocompatible and biodegradable, is similar to that of biological membranes in the body. Targeting can be achieved by chemical modification of the vesicle surface using ligands or polymers. As such, liposomes are not conventional "particles" in that they do not have a solid core that defines their identity. However, just like nanoscale "particles" of polymers, they are colloidal entities and constitute a significant proportion of nanoscale drug delivery systems. Solid lipid nanoparticles (SLN) are another class of nanoparticles that are made from lipids that are solids at room temperature (Muller et al 2000). The

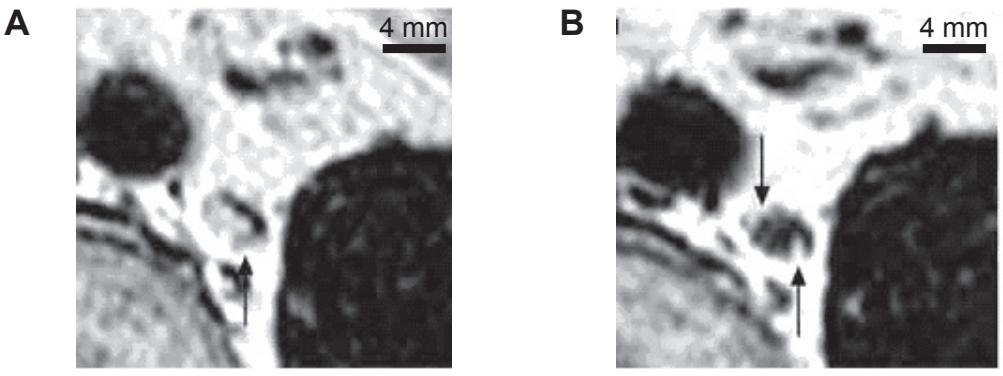

Figure 2 Visualization of lymph node metastases in prostate cancer using iron oxide nanoparticles as MRI contrast agents. (**A**) A conventional MRI image can only vaguely indicate the presence of metastases. (**B**) Two metastases, indicated by arrows, can be clearly seen when the iron oxide nanoparticles are used. Scale bars = 4 mm (added based on the authors' description of 2 mm metastases). Adapted from Harisinghani et al (2003) with permission.

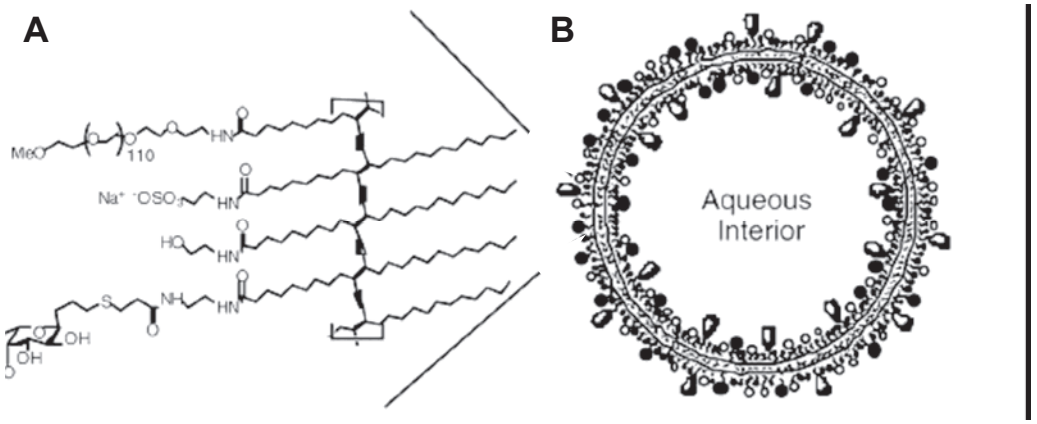

Figure 3. Schematic representations of (**A**) a polymeric matrix and (**B**) a liposome, both of which can enclose a drug. Reprinted with permission from Brigger I, Dubernet C, Couvreur P. 2002. Nanoparticles in cancer therapy and diagnosis. *Adv Drug Deliv Rev*, 54:631–51 (Elsevier) and John AE, Lukacs NW, Berlin AA, et al 2003. Discovery of a potent nanoparticle P-selectin antagonist with anti-inflammatory effects in allergic airway disease. *FASEB J*, 17:2296-8.

nanoparticles are synthesized by emulsifying a molten lipid mixed with drug and surfactant which is then cooled.

The utilization of polymer- and liposome-based nanoparticles in drug delivery is illustrated below with a few examples organized by disease type. The list of diseases or the number of examples within each disease is by no means exhaustive; the intent here is to illustrate the breath of nanoparticle drug delivery systems rather than cover all areas in depth. A summary of key examples is included in Table 1.

Cancer

Nanoparticles have made a tremendous impact in the treatment of various types of cancer, as evidenced by the numerous nanoparticle-based drugs and delivery systems that are in clinical use. Examples of numerous liposome- and polymer-based drugs or therapeutic agents have been presented in recent reviews (Duncan 2003; Allen and Cullis 2004).

Paclitaxel is a well-known anti-cancer agent used to treat several types of cancer (such as ovarian, skin, esophageal, and lung) (Kikuchi et al 2005; Abratt et al 2006; Chao et al 2006; De Giorgi et al 2006; Roof et al 2006; Worden et al 2006). This drug interferes with the functions of cancer cells by microtubule stabilization, resulting eventually in apoptosis (Koziara et al 2006). The most common mode of administration of this water-insoluble drug is as a solution in ethanol (Taxol®), administered together with a solvent, polyoxyethylated castor oil (Cremophor® EL). A major shortcoming of this approach has been the side effects associated with Cremophor®, including hypersensitivity reactions, necessitating the administration of steroids and antihistamines as premedications (Zhang et al 2005a; Micha et al 2006). In early 2005, a different form of paclitaxel known as Abraxane® was approved for clinical use. In this form, paclitaxel is loaded within nanoparticles of a natural polymer, albumin, using a high-pressure emulsification process. This soluble form of paclitaxel has been shown not only to eliminate the side effects associated with the use of Cremophor® (Micha et al 2006) but also provides some additional benefits. The albumin carrier improves transport of the drug from the bloodstream to the tumor site and allows higher drug dosing compared with Taxol® (Ibrahim et al 2002).

Nanoparticle loading of paclitaxel, however, did not address multidrug resistance, a common problem in tumor therapy that arises when cancer cells adapt to stimuli by expressing efflux transporters or other proteins on the surface (Gottesman et al 1996 ; Tomonaga et al 1996). Koziara and colleagues (2006) have attempted to overcome this problem by loading paclitaxel into emulsifying wax nanoparticles. The wax is a commercially available product (Tween 80®) alternatively known as polyoxyethylene 20-sorbitan monooleate. The nanoparticles were prepared by heating a mixture of the wax, drug, and a surfactant and then emulsifying. The efficacy of these drug-loaded nanoparticles was assessed in a murine xenograft model (HCT-15) in which tumor cells express p-glycoprotein, an efflux transporter. With the help of a control experiment using Taxol®, the resulting cessation of tumor growth was judged to be due to a combination of overcoming resistance (by nonspecific cytoskeletal disturbance) and the antiangiogenic effect of paclitaxel. These examples of different versions of paclitaxel serve to illustrate how differ-

ent nanoparticle-based drug delivery strategies can be utilized to modulate and improve the performance of a drug.

An important consideration in tumor therapy is the interplay between chemotherapeutic and antiangiogenic agents. As Sengupta and colleagues (2005) have pointed out, disruption of tumor blood vessels can impact delivery of the chemotherapeutic agent and also cause increased expression of factors associated with drug resistance. These investigators synthesized a nanoparticle drug delivery system with two layers: a core of poly-(lactic-co-glycolic) acid (PLGA) conjugated with doxorubicin enclosed within a liposome composed of phospholipids conjugated with PEG and combretastatin. Here, doxorubicin is the chemotherapeutic agent and combretastatin is the antiangiogenic agent. These multilayered particles ranged in size from 80–120 nm. The underlying strategy was to deliver the particles to the tumor site and then release the drug slowly by degradation of the PLGA core. When administered intravenously to mice with tumors induced by carcinoma or melanoma cells, the particles were readily taken up by the tumor, consistent with the increased residence time resulting from PEG conjugation (Harris and Chess 2003) and the known 'leakiness' of tumor vessels (also termed the enhanced permeability and retention, or EPR, effect; tumor vessels have 400–600 nm pores) (Yuan et al 1995). The nanoparticles induced significant inhibition of tumor growth and prolonged the lifespan of the animals.

Neurodegenerative diseases

Drug delivery to the central nervous system remains a challenge in developing effective treatments for neurodegenerative diseases (Garcia-Garcia et al 2005; Popovic and Brundin 2006). An important part of this challenge is overcoming the natural tendency of the blood–brain barrier (BBB) to block to drug transport. This barrier is designed to protect the brain from foreign substances and blood-borne infections but it cannot recognize many therapeutic compounds. As a result, high doses must be administered, with increased risks of adverse side effects. Among the different approaches explored in recent years to overcome this limitation are nanoparticle-based systems ranging from polymer particles to liposomes. A thorough review of work in this area has been published by Garcia-Garcia and colleagues (2005).

Nanoparticles made from poly(hexadecyl cyanoacrylate) and related compounds have been shown to facilitate drug transport across the BBB. Kreuter and colleagues (2003) adsorbed dalargin (an analgesic) onto poly(butyl cyanoacrylate) (PBCA) nanoparticles and demonstrated penetration across the BBB in rats. More recently, Siegemund and colleagues (2006) showed how PBCA nanoparticles loaded with thioflavins can target fibrillar amyloid β in a murine model of Alzheimer's disease. Calvo and colleagues (2002, 2001) synthesized a nanoparticle system composed of a copolymer of PEG and poly(hexadecyl cyanoacrylate) (PHDCA). Since PEG is hydrophilic and PHDCA is hydrophobic, an aqueous environment causes the copolymer molecules to arrange themselves as particles with an insoluble PHDCA core and a surface layer of PEG. The incorporation of PEG is common in many drug delivery systems because it is not recognized as a foreign material by macrophages in blood and can therefore increase the half life of drug carriers in blood (Harris and Chess 2003). Indeed the incorporation of PEG enhances the ability of PHDCA to cross the BBB. Polymeric micelles can be formed by copolymers of PEG and materials similar to PEG, such as poly(propylene oxide). The commercially available Pluronic® P-85 polymer is an example, and P-85 micelles have been utilized to transport analgesics across the BBB in mice (Witt et al 2002).

Liposome-based drug delivery systems have also been extensively investigated for drug delivery to the central nervous system (Garcia-Garcia et al 2005). Surface coverage with PEG is also effective in these systems. Schmidt and colleagues (2003) prepared liposomes with diameters ranging from 90–100 nm to encapsulate prednisolone, a drug used in the treatment of multiple sclerosis (MS). Following intravenous injection in mice with experimental autoimmune encephalomyelitis (an animal model for MS), the liposomes were observed to accumulate to high levels in the central nervous system within 2 h. Figure 4 shows gold-(black) labeled liposomes among astrocytes and microglia in a spinal cord section, indicating BBB penetration. Treatment with the drug-loaded liposomes resulted in restoration of BBB integrity and reduction in inflammation as well as macrophage infiltration. This treatment was judged to be superior to the administration of free glucocorticosteriods, which is a conventional therapy for MS.

A further application of PEG-conjugated liposomes is in gene delivery across the BBB. This approach is being followed to develop therapies for chronic neurological diseases that do not respond to small molecule drugs (such as Huntington's disease, Rett syndrome, and Fragile-X syndrome, to name only a few) (Schlachetzki et al 2004). Shi and colleagues (2001) delivered plasmid DNA encoding β-galactosidase across the BBB in rats. Some of the PEG molecules on the liposome surfaces were attached to a target-

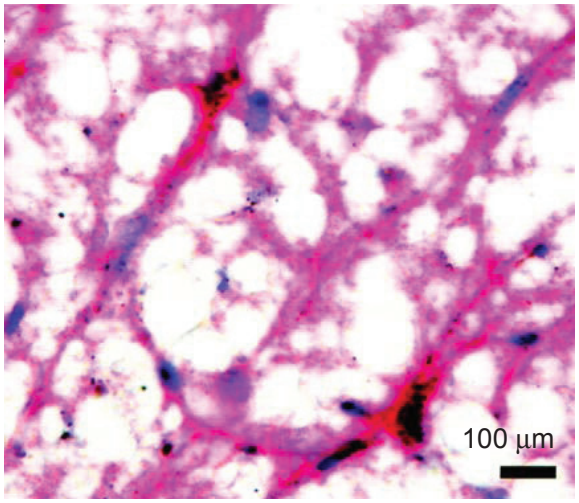

Figure 4 Liposome-based drug delivery to the nervous system. Gold-labeled liposomes (colored black in image) among astrocytes and microglia in rat spinal cord sections indicating penetration of the blood–brain barrier (astrocytes and microglia stained red); scale bar = 100 μm. Adapted from Schmidt et al (2003) by permission of Oxford University Press.

ing monoclonal antibody anti-TFR, which targets the brain, liver, and spleen. Antibody attachment allowed targeted delivery of the liposomes to specific regions, and plasmid-induced gene expression in the brain was observed for at least 6 days following liposome administration. The significance of this approach is the ability to transport genes that would normally be degraded by endonucleases in vivo by loading them within liposomes with targeting capability.

HIV/AIDS

De Jaeghere and colleagues (2000) investigated the delivery of an HIV-1 protease inhibitor, CGP 70726, using pH-sensitive nanoparticles made from a copolymer of methacrylic acid) and ethyl acrylate. This copolymer is commercially available under the name Eudragit® L100–55. The copolymer was chosen because of its pH-dependent solubility. CGP 70726 and other similar anti-viral agents are known to disrupt the replication cycle of HIV-1 (Robins and Plattner 1993). A major challenge in delivering agents such as CGP 70726 is poor water solubility. De Jaeghere and colleagues synthesized nanoparticles by emulsifying a solution of the copolymer with a mixture of CGP 70726 and benzyl alcohol. The nanoparticles were administered orally to dogs and successful drug release was observed by analysis of blood samples.

The HIV-1 Tat protein has recently emerged as a potential candidate for a prophylactic or therapeutic vaccine against HIV-1/AIDS (Cafaro et al 1999; Caputo et al 2004). Rudolph and colleagues (2004) recently described an SLN-based system consisting of DNA compacted with a Tat protein. This work built on earlier studies by the same group in which SLNs loaded with DNA were shown to transfect mammalian cells in vitro (Olbrich et al 2001; Tabatt et al 2004). SLNs were prepared from a cationic lipid in addition to a surfactant. DNA and Tat peptide were subsequently adsorbed onto the nanoparticle surface by electrostatic forces. When administered to the lungs of mice by either intratracheal instillation or aerosol application, increased gene expression was observed indicating successful transfection of the SLNs, but some DNA degradation was observed.

While there are comparatively fewer reports of in vivo nanoparticle-based drug delivery in the area of HIV/AIDS than in such areas as cancer and neurodegenerative diseases, activity in the area can certainly be gauged by numerous recent in vitro studies (Berton et al 1999, 2001; Nam et al 2002; Cui and Mumper 2003; Becker et al 2004; Sawant et al 2006).

Ocular diseases

The primary motivation for using nanoparticle-based drug delivery systems in ophthalmic applications is the ability to prolong drug residence times by trapping the drug in the ocular mucus layer (Ludwig 2005). This layer, which is considered to be a diffusion barrier to macromolecules, is secreted by goblet cells in the conjunctiva and protects the epithelial layer of the cornea. Most ocular diseases are treated with drug solutions administered as eye drops. These solutions are usually highly concentrated and require frequent application because of rapid precorneal loss caused by the movement of mucus during blinking. Nanoparticles have provided an effective way to overcome this difficulty, as illustrated by a comprehensive review by Ludwig (2005); this review includes a summary of *in vivo* drug delivery studies in this area.

Pignatello and colleagues (2002a; 2002b) have used commercially available Eudragit® polymers to deliver nonsteroidal and anti-inflammatory drugs (flurbiprofen and ibuprofen) to rabbit eyes. These drugs are typically used to mitigate the inflammatory response that typically occurs following ophthalmic surgery. The Eudragit® RS and RL polymers used in these investigations were copolymers of poly(ethyl acrylate), poly(methyl methacrylate), and poly(chlorotrimethyl-aminoethyl-methacrylate). These polymers are insoluble and capable of swelling under physiological conditions, making them suitable platforms for controlled release. Mixtures of drug

and polymer were dissolved in ethanol and emulsified (with the help of water and a surfactant) to form drug-embedded nanoparticles ~100 nm in size. Saline suspensions of these nanoparticles were instilled in the conjunctive sac of the rabbit eyes. Nanoparticles loaded with both drugs (flurbiprofen and ibuprofen) effectively inhibited inflammatory responses after surgical trauma and were comparable with conventional eye-drop controls. The significance of this result is that the nanoparticle system was assembled with a lower drug concentration compared to the eye-drop control. Secondly the nanoparticle system was able to generate higher drug levels in the vitreous humor, which arises from the longer residence time of the drug in the polymer matrix.

Certain disease conditions, such as cytomegalovirus (CMV) retinitis require administration of drugs to the retinal region of the eye. Infection with CMV can lead to permanent damage of the retina, choroid (the region behind the retina), iris, and adjacent tissue. Merodio and colleagues (2002) have described the use of bovine serum albumin (BSA) nanoparticles to deliver ganciclovir, a drug used to treat CMV infection. The drug was incubated with BSA in an aqueous solution and droplets were subsequently generated by the additional of ethanol in an emulsification process; the resulting nanoparticles were approximately 280 nm in diameter. These nanoparticles were resuspended in saline and administered by intravitreal injection. The authors observed that the nanoparticles remained in a thin layer on the retina for up to two weeks post-injection and histological analysis indicated the absence of any inflammatory responses or changes in tissue morphology compared with normal eye controls.

Respiratory diseases

The application of nanoparticle-based drug delivery approaches in respiratory diseases has been somewhat limited. The literature nevertheless contains several examples of therapies that have been effectively demonstrated for the treatment of allergic, genetic, and infections diseases of the respiratory system (Pison et al 2006).

John and colleagues (2003) demonstrated the use of a liposome-based nanoparticle system to inhibit inflammation in a murine model of allergic asthma. The strategy employed was to inhibit P-selectin receptors on activated endothelial cells in circulation, which mitigates interactions between endothelial cells and leukocytes. This, in turn, attenuates the development of peribronchial inflammation. The nanoparticles (average diameter of 73 nm) were designed to mimic the physiological P-selectin super ligand (PSGL-1) by incorporating fucose and sulfate ester groups on the liposome surface. Lung inflammation and airway hyperreactivity were induced in mice by LPS and cockroach antigen. In both instances, the liposomal nanoparticles were observed to bind preferentially to selectins on activated endothelial cells (Figure 5). Histological analysis indicated significant reduction in peribronchial inflammation and airway hyperreactivity in mice treated with the nanoparticles compared with controls.

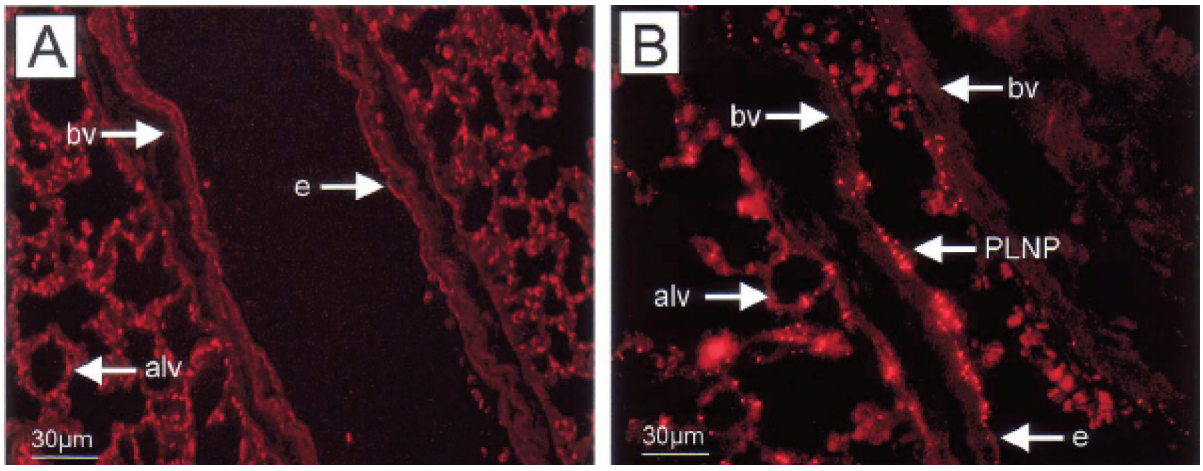

Figure 5 Selective binding of liposomes presenting fucose and sulfate ester groups to activated endothelial cells in mouse lungs following allergen challenge. (**A**) Negative control (liposomes without fucose and sulfate ester groups). (**B**) Liposomes with fucose and sulfate ester groups. Scale bars in both images = 30 µm. Adapted with permission from John et al (2003).
Abbreviations: alv, alveolar wall; bv, blood vessel; e, endothelium; PLNP, liposomes.

Kumar and colleagues (2003) have described how a polymer-drug conjugate, chitosan/interferon-γ pDNA nanoparticles, can reduce allergen-induced airway inflammation. It is known that allergic diseases (such as asthma) cause a drop in production of interferon-γ (IFN-γ) in patients, leaving the patients susceptible to airway inflammation and hyperresponsiveness. The approach of Kumar and colleagues (2003) aims to overcome IFN-γ deficiency by supplying it intranasally as a polymer-drug conjugate. In allergen-challenged mice, nanoparticle therapy resulted in increased IFN-γ expression by epithelial cells, thereby facilitating reduction in inflammation and restoration of lung morphology within 3–6 hours.

Environmental and societal considerations

The impact of nanomaterials on the environment and on public health has received considerable attention in recent years. As technologies in nanomedicine and the broader field of nanotechnology mature, however, much more needs to be done, particularly because different nanomaterials have different kinds of risks associated with them. This section will focus on the nanoparticle types described in the applications above. As in the previous sections, in vivo studies will be given particular attention; however significant in vitro work will also be described.

Toxicity of quantum dots

As described in the section on medical imaging, QDs are inorganic nanoparticles that typically have an organic coating them makes them biocompatible or bioactive. The main toxicological risk associated with the use of QDs in vivo is the exposure of the inorganic core by deterioration of the organic layer. QDs can be made from a large variety of inorganic-metal complexes, such as CdSe, ZnS, CdTe, InP, InAs, GaAs, to name only a few. Each such compound has unique chemical properties that can profoundly influence its toxicology. Although the literature on the toxicity of such compounds in vivo is not extensive, there are reports that highlight major concerns and illustrate the need for more work. A detailed review of the toxicology of quantum dots was recently published by Hardman (2006). A summary of recent toxicogical investigations on QDs is given in Table 2.

Derfus and colleagues (2004) examined the toxicity of a range of cadmium-based QDs using an in vitro model consisting of primary rat hepatocytes. The choice of this cell type was motivated by the fact that the liver is the primary target of Cd exposure. QDs with CdSe cores capped with mercaptoacetic acid (MAA) and TOPO were determined to be acutely cytotoxic at a QD concentration of 62.5 μg/mL. This behavior was correlated with the liberation of Cd^{2+} ions following oxidation of the CdSe lattice by air and ultraviolet (UV) light. Surface oxidation and cytotoxicity were nearly eliminated by coating the CdSe particles with ZnS and further improvement was observed by polymer or protein coating on top of the ZnS. While this was an encouraging result, some Cd release was also observed from commercial QDs made with CdSe/ZnS with an overcoat of polyacrylate and streptavidin.

Ballou and colleagues (2004) examined the in vivo toxicity of CdSe/ZnS QDs coated with either amphiphilic poly(acrylic acid) or PEG in mice. QDs were administered to the animals at a concentration of 20 pmol/g animal weight. No necrosis was

Table 2 Toxicological effects of nanoparticles associated with medical applications

Nanoparticle type	Toxicological effects	References
Quantum dots	Potential for exposure to inorganic core (eg, cadmium) and resulting cytotoxic effects (eg, liver damage).	Derfus et al 2004
	Toxicity risk greatly reduced by coating with ZnS and soluble polymers (such as PEG).	Ballou et al 2004
	Risks associated with production, handling, and storage of QDs need to be evaluated.	Oberdorster et al 2005; Hardman 2006
Metallic	Iron oxide and gold nanoparticles are not toxic.	Weissleder et al 1989; Connor et al 2005; Muldoon et al 2005; Hainfeld et al 2006;.
	Surface functionalization may influence toxicity.	Goodman et al 2004
Polymeric/liposomal	Not toxic since these nanoparticles have natural or highly biocompatible components (eg, chitosan, PEG).	Alonso 2004; de Campos et al 2004

Abbreviations: PEG, poly(ethylene glycol); QD, quantum dots.

observed in liver, spleen, and bone marrow, where the QDs were observed to deposit, and the animals remained viable for 133 days when tissue analysis was performed.

The above two examples illustrate the complexities of measuring the toxicity of QDs. The in vitro and in vivo studies cannot be directly compared because of inherent differences in experimental design (QD concentration measurement being just one of many aspects of this) and differences in the organic coating. Nevertheless, they point to the need for long-term animal studies before QDs can be approved for commercial use; they also point to the potential need for controlled conditions in storing and handling QDs. Questions that arise with regard to the safety of QD manufacturing processes, such as the risk of QD exposure by inhalation or dermal contact, also need to be answered definitively (Oberdorster et al 2005; Hardman 2006).

Toxicity of metallic nanoparticles

Muldoon and colleagues (2005) investigated the toxicity of superparamagnetic iron oxide nanoparticles used as MRI contrast agents in rats. The nanoparticles were administered to the brain by either intracerebral inoculation or intraarterially. Although the MRI signal intensity dropped over time (weeks to months), no pathological changes were observed in brain tissue in normal rats. These findings are consistent with a toxicity study of iron oxide nanoparticles in mice and dogs performed nearly two decades ago by Weissleder and colleagues (1989). In this work, the nanoparticles were administered intravenously and no acute or subacute toxicity responses were found in the histology of targeted tissues or in blood tests. The safety of various iron oxide-based nanoparticles used as contrast agents in clinical use is well-established (Lubbe et al 1999, 2001; Neuberger et al 2005).

Hainfeld and colleagues (2006) examined the toxicity of gold nanoparticles within the context of their use as X-ray contrast agents. When injected intravenously into mice, accumulation was observed in kidneys and within tumors (retention was low in the liver and spleen). Organ histology and blood analysis did not show any indication of toxicity up to 30 days following injection. These observations are consistent with the findings from an in vitro study of gold nanoparticle toxicity performed by Connor and colleagues (2005) with a human leukemia cell line.

The nanoparticles were taken up by the cells but did not cause cytotoxicity. The chemical surface modification of gold nanoparticles can, however, impact toxicity. Goodman and colleagues (2004) recently demonstrated that attachment of a cationic polymer monolayer (alkyl thiol with a quaternary ammonium group) onto gold nanoparticles can render them cytotoxic. Attachment of an anionic monolayer (alkyl chain with carboxylate end group), however did not result in cytotoxic behavior.

Toxicity of polymeric and liposomal nanoparticles

This category of nanoparticles is probably the least problematic with respect to toxicity because the particles are very often typically either made from or covered with natural or highly biocompatible polymers (such as PEG). In drug delivery applications, these particles often carry drugs that are cytotoxic by design (to kill cancer cells) but they are prevented from attacking other regions of the body by the selective targeting described earlier in this review.

The incorporation of natural polymers such as chitosan or natural lipids in the assembly of polymer- or liposome-based nanoparticles is beneficial because these polymers are not recognized as being foreign by the body and are readily metabolized (Alonso 2004; de Campos et al 2004). Nanoparticles made from synthetic polymers can vary widely in the rate of clearance from the blood stream and accumulation in mononuclear phagocytic system (MPS) organs (MPS) organs (such as the liver and spleen) depending on polymer type and composition (Moghimi et al 2001; Owens and Peppas 2006). The incorporation of PEG in the nanoparticle structure can delay the removal of nanoparticles from the blood stream, as discussed earlier. PEG-coated particles are therefore considered to be less toxic than uncoated particles because they are less likely to saturate the MPS (Peracchia et al 1999; Plard and Bazile 1999).

Summary

Nanoparticles have made major contributions to clinical medicine in the areas of medical imaging and drug/gene delivery. While several innovations such as iron oxide contrast agents and many drug delivery systems are by now well-established, newer technologies continue to emerge following the same basic concepts of design. As these innovations advance to clinical application, attention must be paid to environmental and societal implications, particularly in areas such as quantum dots.

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