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INVITED REVIEW

# Nanotechnology: A Focus on Nanoparticles as a Drug Delivery System

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**Abstract** This review will provide an in-depth discussion on the previous development of nanoparticle-based drug delivery systems (DDS) and discuss original research data that includes the therapeutic enhancement of antiretroviral therapy. The use of nanoparticle DDS will allow practitioners to use drugs to target specific areas of the body. In the treatment of malignancies, the use of nanoparticles as a DDS is making measurable treatment impact. Medical imaging will also utilize DDS to illuminate tumors, the brain, or other cellular functions in the body. The utility of nanoparticle DDS to improve human health is potentially enormous.

**Key words** nanotechnology · review · indinavir · drug delivery system · cancer · HIV treatment

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## Introduction

An important and long-term goal of the pharmaceutical industry is to develop therapeutic agents that can be selectively delivered to specific areas in the body to maximize the therapeutic index. Drugs, given systemically, provide a profound beneficial effect but can also exhibit adverse reactions. Historically, cancer chemotherapy agents have been well-known examples of achieving balance between efficacy and toxicity. Cytotoxic compounds can be highly effective in destroying cancer cells but may also damage normal cells resulting in possible adverse and potentially life-threatening effects.

The "magic bullet" concept, first theorized by Paul Ehrlich in 1891, represents the first early description of the drug-targeting paradigm (Gensini et al. 2006). The aim of drug targeting is to deliver drugs to the right place, at the right concentration, for the right period of time. As drug characteristics differ substantially in chemical composition, molecular size, hydrophilicity, and protein binding, the essential characteristics that identify efficacy are highly complex. All of these are investigated to bring a new compound to market although only a fraction reaches active clinical use (Hoag 2006).

Many promising new compounds are compromised by poor physiochemical properties (Rabinow 2004) that leads to poor solubility and biodistribution and therefore the drug does not interact with the site of action. Poor oral

absorption (i.e., proteins and peptides), low solubility at physiological pH (Persson et al. 2005), insufficient cellular uptake (Lysik and Wu-Pong 2003), and rapid drug elimination (Singh 2006a, b) are impediments for drug development. New drug candidates must provide evidence that they reach the site of action and have an effect. The field of drug delivery designs carriers, excipients, and solubilizers to transport drugs to the site of action.

An answer to poor drug physiochemical properties is to associate the drug with a pharmaceutical carrier [i.e., a drug delivery system (DDS)] (Ferrari 2005; Kim and Lim 2002; Torchilin 2000; Allen and Cullis 2004). A DDS can enhance a drug pharmacokinetics and cellular penetration. Moreover, obstacles arising from low drug solubility, degradation, fast clearance rates, nonspecific toxicity, and inability to cross biological barriers may also be addressed by a DDS. To be useful, a DDS is required to be biocompatible with processes in the body as well as with the drug to be delivered. Overall, the challenge of increasing a drug's therapeutic effect, with a concurrent minimization of side effects, can be optimized through proper design and DDS engineering (Kipp 2004; LaVan et al. 2003; Moghimi et al. 2005).

## Nanoparticles as drug carriers

Nanoparticles were first developed approximately 35 years ago (Ravi Kumar 2000). They were initially developed as carriers for vaccines and cancer chemotherapy agents (Couvreur et al. 1982; Beck et al. 1993; Conway et al. 2001). Nanoparticles are stable, solid colloidal particles consisting of biodegradable polymer or lipid materials and range in size from 10 to 1,000 nm. Drugs can be absorbed onto the particle surface, entrapped inside the polymer/lipid, or dissolved within the particle matrix (Kreuter 1983). An example of a DDS is a liposome. Liposomes are closed bilayered phospholipids first designed 40 years ago (Torchilin 2005a, b). Table 1 lists examples of FDA-approved nanoparticle DDS. These were collectively designed to be taken up and delivered by mononuclear phagocytes (MP).

The majority of pharmaceutical research into the utility of nanoparticle DDS has been in the area of oncology. Nanocarriers can concentrate preferentially in tumor masses, inflammatory sites, and infectious sites by virtue of the enhanced permeability and retention (EPR) effect on the vasculature (Shenoy et al. 2005). Characteristics of the EPR effect include extensive angiogenesis, defective vascular architecture, impaired lymphatic drainage/recovery system, and increased production of a number of permeability mediators. These EPR characteristics are essential components of solid tumors and are not associated with normal tissues or organs. The EPR effect provides an opportunity for more selective targeting of nanoparticles (lipid or polymer conjugated anticancer drugs) to the tumor (Maeda et al. 2000; Vicent and Duncan 2006; Sengupta et al. 2005). Additionally, it is possible to fabricate nanoparticles with several different drugs and allow for the nanocarriers to selectively deliver the drugs to the malignant tissue, be ingested by the malignant cells, and then release the now intracellular anticancer drugs killing malignant cells. Nanocarriers can also use more than one drug within the particles. An anticancer drug incorporated within a biodegradable colloidal shell and another antiangiogenesis drug that is placed within a lipid layer that surrounds the colloidal shell. When the nanocarrier is administered intravenously, the malignant cells internalize the nanoparticle. Once intracellular, the antiangiogenesis drug within the lipid layer of the

**Table 1** FDA approved nanoparticle DDS (adapted in part from Allen and Cullis 2004)

| Drug or therapeutic agent (trade name) | Indication | Reference |
|---|---|---|
| Liposomal amphotericin B (Ambisome, Ablecet, Amphotec) | Fungal infections, Leishmaniasis | Alder-Moore (1994) |
| PEG-adenosine deaminase (Pegademase) | Severe combined immunodeficiency disease | Bory et al. (1991) |
| PEG-stabilized liposomal doxorubicin (Doxil, Evacet) | Kaposi's sarcoma, refractory ovarian cancer | Muggia and Hamilton (2001), Northfelt et al. (1996) |
| Liposomal cytosine arabinoside (DepoCyt) | Lymphomatous meningitis, neoplastic meningitis | Glantz et al. (1999a), Glantz et al. (1999b) |
| Interleukin 2-diptheria toxin fusion protein (Denileikin Diffitox) | Cutaneous T cell lymphoma | Olsen et al. (2001) |
| Liposomal verteporfin (Visudyne) | Wet macular degeneration | Bressler (2001) |
| PEG-interferon α-2b (Pegasys) | Hepatitis C | Glue et al. (2000) |
| PEG-granulocyte colony stimulating factor (Neulasta) | Chemotherapy associated neutropenia | Siena et al. (2003) |
| Protein bound paclitaxel (Abraxane) | Metastatic breast cancer | Nyman et al. (2005) |
| PEG L-asparaginase (Oncaspar) | Acute lymphocytic leukemia | Rosen et al. (2003) |
| PEG aptanib (Macugen) | Wet macular degeneration | Lee et al. (2005a, b) |
| Pemetrexed (Alimta) | Malignant pleural mesothelioma | Ceresoli et al. (2006) |

nanocarrier is released, inhibiting the mediators for blood vessel production. Then, the core anticancer drug within the colloidal shell is released, effectively killing the malignant cell. All of this can be fabricated in a "nanocell" that is less than 250 nm (Sengupta et al. 2005). This is truly an optimized delivery of anticancer drugs to the tumor site.

Doxorubicin and paclitaxel are FDA-approved anticancer drugs that are limited by physiochemical properties that make them difficult to administer or have intolerable side effects. Paclitaxel is a difficult drug to keep in an aqueous solution. Cremophor and alcohol are added to the product as solubilizers to prevent paclitaxel from precipitating out of aqueous solution. However, toxicities (i.e., hypersensitivity reaction and neurotoxicity) associated with Cremophor are problematic for patients to tolerate (Nyman et al. 2005). Additionally, polyethylene glycol (PEG)-coupled paclitaxel has been responsible for some infusion reactions. Therefore, albumin-bound paclitaxel has been developed to enhance product solubility and improve patient tolerability. This formulation has an improved side effect profile compared to the conventional paclitaxel (Cabanes et al. 1998). Doxorubicin has a potential life-threatening side effect, cardiotoxicity, related to the cumulative dose. When administered to patients, pegylated liposomal doxorubicin dose and frequency of administration are reduced because of the improved penetration to the malignant tissue. The cumulative dose is reduced. Therefore, the potential life-threatening cardiotoxicity associated with this drug is reduced. Using pegylated doxorubicin in patients with breast metastases has shown improved tissue concentrations and survival at a median initial dose of 42.5 mg/m$^2$ every 4 weeks (Symon et al. 1999; Perez et al. 2002). The development of the liposome-based, slow-release formulation of cytarabine (ara-C) has been a step forward in the treatment of lymphomatous meningitis (Murry and Blaney 2000). The product is encapsulated in multivesicular, lipid-based particles with diameters of 3–30 μm and consists of numerous nonconcentric vesicles (Ye et al. 2000). When the liposomal ara-C was administered intrathecally to patients, the plasma concentration of ara-C was nondetectable (Kim et al. 1993). Treatment modalities for another tumor, malignant pleural mesotheolioma, have not produced any impact on median survival, and many toxicities associated with standard chemotherapy have been found to be harsh and result in increased mortality and morbidity. Liposomal pemetrexed was recently approved for mesotheolioma (Ceresoli et al. 2006). Other liposomal anticancer drugs are currently in various stages of development (Hofheinz et al. 2005).

Polyethylene glycol-filgrastim, used to treat neutropenia (low WBC) associated with cancer chemotherapy, is another example of nanoparticulate DDS. As a result of the addition of PEG, circulating proteins do not complex with PEG-filgrastim and eliminate it from the circulation. This keeps PEG-filgrastim in the circulation longer than filgrastim without pegylation. Therefore, this form of filgrastim can be given less often compared to conventional filgrastim. A clinical study was performed to evaluate the efficacy of pegfilgrastim compared to conventional filgrastim in the development of febrile neutropenia in patients with breast cancer (Vogel et al. 2005). In the pegfilgrastim group, significantly fewer patients (1%) developed neutropenia compared to conventional filgrastim (17%).

The ERP effect can be exploited for infectious diseases or other inflammatory diseases. Pegylated interferon alpha 2a is used in the treatment of patients with hepatitis C. The recommended dose, a once-a-weekly injection, is primarily attributable to the PEG masking the protein from MPS clearance, thereby allowing serum concentrations to be relatively constant over the dosing period (Caliceti 2004; Kamal et al. 2006; Jacobson et al. 2005). An example of an experimental DDS is the drug rolipram, an anti-inflammatory drug, which was incorporated into nanoparticles and tested in a rat model of inflammatory bowel disease (Lamprecht et al. 2001). Animals that received the nanoparticle formulation demonstrated a better response based on myleoperoxide activity and lack of weight loss compared to rolipram solution.

Incorporating aminoglycoside antibiotics into nanoparticles or microparticles have reduced drug-induced nephrotoxicity (Schiffelers et al. 2001; Pinto-Alphandary 2000). Amikacin-liposome product has undergone clinical trials in an attempt to maximize the therapeutic window and minimize the toxicity of this antibiotic. Overall therapeutic index is improved compared to conventional amikacin. Amikacin encapsulated in unilamellar liposomes (MiKasome®) has prolonged circulation time and sustained efficacy in animals infected with *Mycobacterium* spp., *Klebsiella* spp., and *Pseudomonas* spp. (Fielding et al. 1998). It is hypothesized that the acidic intracellular pH promotes release of the amikacin after phagocytosis by the macrophage effectively delivering drug to the intracellular pathogen (Schiffelers et al. 2001). Indeed, treatment of intracellular organisms such as mycobacterial organisms using liposomal technology and the macrophage as the carrier is improved compared to free drug (Cynamon et al. 1989; Sharma et al. 2004).

Important examples of effective DDS used outside of the treatment of oncology and infectious diseases include advances in the treatment of eye disease. Age-related macular degeneration causes progressive loss of central vision leading to blindness that is a result of abnormal growth of blood vessels under the retina and macula. The walls of these blood vessels easily rupture and bleed into the surrounding retinal tissues causing the macula to bulge and distort/destroy central vision. Verteporfin therapy is part of a two-step process that incorporates intravenous liposomal verte-

porfin along with light from a diode laser that is FDA-approved for wet macular degeneration (Gryziewicz 2005). Verteporfin, once activated by light, is highly reactive and produces singlet oxygen and reactive oxygen radicals that disrupts the abnormal neovascular endothelium and causes occlusion of the leaky vessels. The damaged endothelium releases vasoactive factors and procoagulants, resulting in platelet aggregation, fibrin clot formation, and vasoconstriction. Another drug, pegaptanib sodium, has recently gained FDA approval for the same indication. This drug is a pegylated synthetic oligonucleotide that acts as an antagonist of vascular endothelial growth factor isoform 165 (Gryziewicz 2005). This isoform is associated with ocular disease. Pegaptanib is injected into the vitreous fluid of the eye every 6 weeks to deliver the drug to the site of action.

Small molecular weight drugs fabricated into a DDS using nanoparticles have been shown to be useful for the treatment of malignancies and infectious diseases (Walsh et al. 2000). Other investigators have fabricated anti-HIV drug nanoparticles (Dembri et al. 2001; Lobenberg and Kreuter 1996; Lobenberg et al. 1998). Our laboratory has investigated the plasma pharmacokinetics of indinavir nanoparticles versus free indinavir after a one-dose intraperitoneal (i.p.) administration.

Indinavir (IDV) nanoparticles developed by Baxter Healthcare (Deerfield, IL, USA) and our laboratories have begun to change the concept for antiretroviral delivery. Our previous works demonstrated that nanoparticle IDV packaged in bone marrow-derived macrophages (BMM) could markedly improve the bioavailability and pharmacokinetics of this drug and its present formulation (Dou et al. 2006). Such delivery system brings the drug to sites of active viral replication with limited toxicity and restoration of immune suppression in humanized mice with active viral infection. Proof of concept of utilizing IDV nanoparticles as a treatment modality in a nonobese severe combined immunodeficient (NOD-SCID) mouse model of HIV-1 infection (Dou et al. 2006) using harvested donor mouse bone marrow macrophages (Nano-IDV-BMM) as the carrier vehicle. The Nano-IDV-BMM was intravenously infused into the recipient NOD-SCID mice. High-performance liquid chromatography (HPLC) was used to assess the indinavir levels from serum and urine, as well as tissues from lung, liver, lymph node, and kidney over the 14-day time frame. Indinavir levels were still present 10 days after the initial, one-time infusion. Additionally, in the Nano-IDV-BMM mice with HIV-1 infection, $CD4^+$ lymphocyte counts remained stable and HIV viral loads were reduced at day 10 after the one infusion of Nano-IDV-BMM IV. Moreover, we now demonstrate that direct routes of administration can also improve drug delivery.

We compared a 10 mg/kg (i.p.) indinavir dose given both as free drug and nanoparticles to BALB/c mice. Blood for IDV plasma concentrations were withdrawn at selected times over a 14-day time frame after IDV administration and analyzed by HPLC (Jayewardene et al. 1998).

The results show a significant prolongation in the circulation time for the nanoparticle indinavir [elimination half-life of 11.5 min (free drug) and 71 h for 10 mg/kg (nanoparticle); Figs. 1 and 2, respectively]. Both free and nanoparticle IDV produced similar peak concentrations with the same dose. Absorption of both free IDV and nanoparticle IDV from i.p. administration is virtually complete within 1 h. Currently, investigations are in progress to determine the most appropriate dose and route of administration for HIV-positive patients. If these results can be confirmed in human studies, the nanoparticle IDV could be fabricated with other antiretroviral agents. This could launch a successful delivery of antiretroviral therapy to HIV-positive patients in the developing world. We speculate that the i.p. administration of IDV nanoparticles may provide a vehicle for MP upta ke and distribution. The MP engulfs the nanoparticles by endocytosis and carries the nanoparticles to macrophage-rich organs, including liver and spleen. Over time, these nanoparticles released IDV into the intracellular environment, and eventually the drugs were released from the phagocyte into the plasma circulation. These results demonstrate the potential utility of nanotechnology in the treatment of HIV-1 infection. Other investigators have previously utilized nanoparticles for enhanced drug delivery against HIV infection including works performed in our laboratories and others (Dou et al. 2006, unpublished data, 2006; Gorantla et al., unpublished data, 2006; Dembri et al. 2001; Bender et al. 1994; Leroux et al. 1995). Our results are similar to prior investigations demonstrating prolonged elimination half-life and reduced clearance of nanoparticle-incorporated antiviral drugs compared to standard free-drug products.

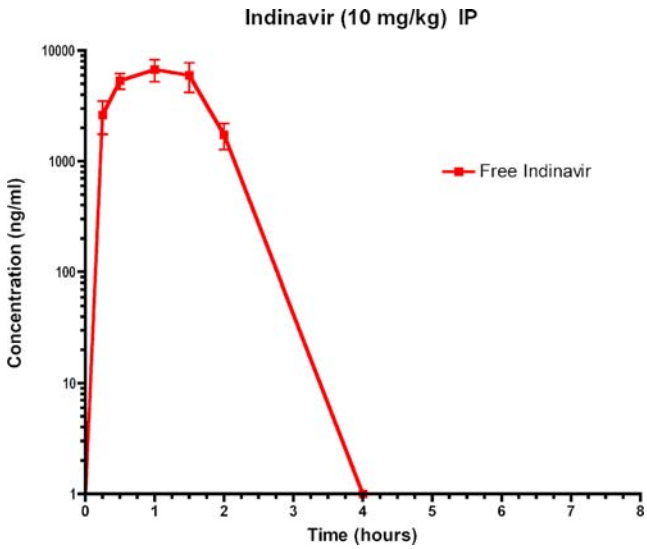


**Fig. 1.** Free indinavir (10 mg/kg) i.p. plasma concentration–time profile.

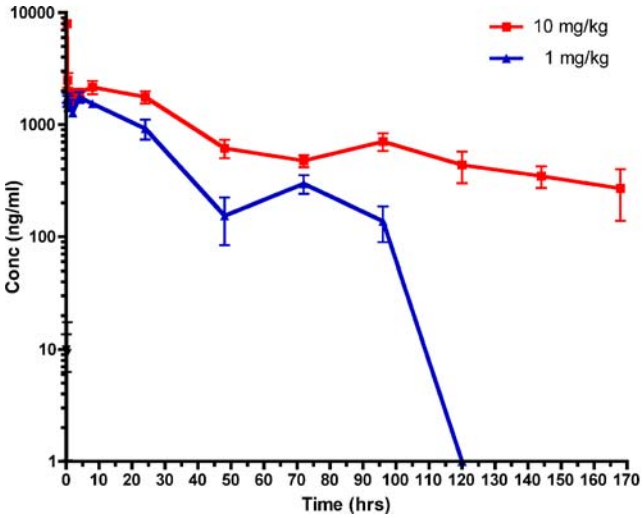


**Fig. 2.** Nanoparticle indinavir (1 and 10 mg/kg) i.p. plasma concentration–time profile.

## Evading phagocytosis

Colloids larger than a few micrometers accumulate in the lung capillaries and remain there (Fiegel et al. 2004). Sufficiently smaller structures can passively migrate through intercellular junctions of healthy endothelium or may be removed by the bone marrow (Moghimi et al. 2005). Circumventing the MPS (monocytes, macrophages, microglia, Kuppfer liver cells, and spleen red pulp macrophages) is an additional step toward successful targeted delivery. The MPS migrate throughout the body guided by chemoattractant gradients to encounter and ingest foreign particles or organisms (Luster and Rothenburg 1997). After encountering foreign particles, the MPS recognize specific surface ligands and become activated. Activation occurs through intracellular cytokine priming mechanisms. Activated cells then ingest the particles and partition them within intracellular phagosomes that contain granule-associated lysosomal enzymes and reactive oxide intermediates that all function to degrade the ingested material (Aderem and Underhill 1999; Hirsch 1962; Cline et al. 1978; Henson et al. 1988). The activated macrophages secrete cytokines that recruit other macrophages and neutrophils into the area.

Hepatic and splenic cells can rapidly phagocytize polymeric nanoparticles (Reddy et al. 2004), and fabrication of nanoparticles that can avoid or minimize phagocytosis is important for sustained circulation and effect. Drug carriers, recognized as foreign objects, are removed from the circulation by surface interactions between cells and the carrier. Blood protein absorption onto colloids is dependent on surface physicochemical properties (size, shape, charge density, hydrophobicity) (Luck et al. 1998). The methods used (spray-dried or w/o/w emulsion solvent evaporation) to create the particles may influence protein absorption (Luck et al. 1998). Opsonins are proteins that promote the activation of the complement system and assist in phagocytic uptake by macrophages. Prevention of opsonin adsorption, via carrier surface modification, has proven to be a successful strategy for sustained circulation. Among the surface modifiers examined, poly(ethylene glycol) (PEG) is certainly the most well known (Moghimi and Hunter 2001; Gbadamosi et al. 2002). PEG chains form a protein-repellent layer around the carrier (Moghimi et al. 1993, 2001). It is the limited concentration of blood opsonin that partly contributes to the prolonged circulation time for PEG particles (Moghimi and Szebeni 2003). In contrast to PEG, dysopsonins are proteins that suppress recognition by macrophages (Moghimi and Szebeni 2003). Favorable interactions between surface-bound polymers and blood dysopsonins can also contribute to the prolonged circulation time. Optimization of the use of dysopsonins to diminish blood opsonin absorption would be advantageous at increasing the residence time of nanoparticles. This is a promising approach toward higher plasma retention (Moghimi and Szebeni 2003).

## Active drug or device targeting

Active targeting is accomplished by attachment of specific molecules on the carrier's surface, thereby enhancing the binding and interactions with antigens or receptors expressed on target cell populations. One approach to deliver compounds to specific target sites is to attach ligands to the carrier. Some ligands include monoclonal antibodies or peptides (Nobs et al. 2004; Cheng et al. 2005; Douglas et al. 1987). The choice of the appropriate ligand is based on its specificity, stability, availability, and selectivity on the target cells. Liposomes have been extensively studied for this purpose and can deliver a high drug payload, protect the drug from degradation, and improve pharmacokinetics (Kontermann 2006).

With the exception of topical administration and local (joint) injection of drugs, most other routes are, by nature, systemic routes of administration. Currently, a typical dose of a drug is usually tens to hundreds of micrograms. For example, the adult EpiPen used for anaphylactic shock delivers 300 μg of epinephrine. Fentanyl, a widely used narcotic, is usually given as 50 μg for a typical 70-kg adult. Common antibiotics are considerably higher for adults. A device that contains a reservoir that is a cube of 1 mm contains a volume of 1 μl, a cube 100 μm on a side holds a volume of 1 nl (LaVan et al. 2003). Small device fabrication would allow for the implantation of a reservoir of localized drug that could be released over time. A good example of this is the clinical use of a device that releases leuprolide acetate over a 12-month time frame that is 45 ×

4 mm and holds 74 mg of drug. Using nanotechnology, Galeska et al. (2005) have fabricated a drug delivery platform using poly(vinyl alcohol) microgels that entrap dexamethasone-loaded poly-(DL-lactide-*co*-glycolide) (PLGA) microspheres for controlled delivery over a 1-month period. The rate-limiting step for drug delivery devices that release drugs over an extended period is the associated microelectromechanical system (MEMS) themselves.

Activating drugs by radio frequency, magnetic field, or ultrasound are interesting options to control the delivery of a drug. Ferromagnetic rods could be placed within a target tumor and thermal treatment be initiated by using radio frequency or ultrasound (Tucker et al. 2000; Kato et al. 1984). Magnetic liposomes containing doxorubicin were administered to osteosarcoma-bearing hamsters. When the tumor-limb was placed between two poles of a 0.4-T magnet for 60 min, it resulted in a fourfold increase in tumor drug levels (Nobuto et al. 2004). Ferromagnetic microparticles are also used as a magnetic resonance imaging contrast agents (Petersein et al. 1996; Anzai 2004). Liposomal contrast agents have been utilized for experimental diagnostic imaging of liver, spleen, brain, cardiovascular system, tumors, inflammation, and infection (Torchilin 1997). Using polymers that containing metals ($^{111}$In or Gd) enhances the signal intensity. For magnetic resonance imaging (MRI), iodinated organic compounds can be incorporated into the liposomal membrane (Torchilin 1997). A preliminary investigation with infectious organisms has shown systemic locations where nanoparticles migrate (Laverman et al. 2001). Intramuscular *Staphylococcus aureus* abscess was induced in rats and colloidal gold nanoparticles have been shown to concentrate in the blood vessels surrounding the abscess, as well as in the liver and spleen (Laverman et al. 2001). Another nanoparticle used in cell trafficking studies and tumor targeting are PEG-superparamagnetic iron oxide and PEG-quantum dots (Tkachenko et al. 2004; Kircher et al. 2003; Gao et al. 2004; Kim et al. 2004).

## Intracellular trafficking

Once the drug delivery vehicle has reached the site, subsequent drug release may occur within the extracellular space, or following internalization of the carrier into the target cell. Drugs with intracellular action, incapable of crossing cell membranes, need to be assisted in reaching their target. Cellular uptake mechanisms vary according to cell type, physicochemical properties of the internalized compound, and mode of activation (Gruenberg 2001). Intracellular targeting is feasible through the use of ligands that trigger receptor-mediated endocytosis. Panyam and Labhasetwar (2003) investigated PLGA nanoparticles and localized delivery of plasmid DNA, proteins and peptides and small molecular weight drugs. Studies in their laboratory revealed that PLGA nanoparticles uptake is both concentration- and time-dependent. The uptake of nanoparticles occurs by nonspecific fluid phase endocytosis and can be saturated as uptake decreases with higher doses. PLGA nanoparticles are transported into primary endosomes and sorted to either recycling endosomes or secondary endosomes. In the acidic environment of the secondary endosome, the nanoparticle surface changes from anionic to cationic leading to the escape of the nanoparticles into the cytoplasm. While intracellular nanoparticle levels fall, extracellular nanoparticle levels may not fall rapidly. A previous study has demonstrated that when nanoparticles are delivered locally, small molecular weight drug levels in the associated tissues are sustained for up to 7–14 days (Fishbein et al. 2001; Panyam et al. 2002). Pentamidine-laden nanoparticles were investigated in *Leishmania* (parasite)-infected mice (Paul et al. 1998). Ultrastructural studies showed drug-loaded nanoparticles trafficking inside *Leishmania*-infected Kuppfer cells. Within these Kuppfer cells, the nanoparticles were located within vacuoles and primary lysosomes to form secondary lysosomes. Secondary lysosomes containing nanoparticle with parasitophorous vacuoles were observed. Of clinical significance, liposomal amphotericin B has been shown to cure virtually all patients, with little side effects and significantly reduced length of treatment (Sundar et al. 2003, 2004). This therapy has replaced antimony and amphotericin B deoxycholate as the drug of choice for this parasite.

Studies using indinavir nanoparticles in our laboratory confirm the above findings of intracellular drug release (Dou et al. 2006, unpublished data). When in an alkaline environment, indinavir can be fabricated into nanoparticles and remain stable. *In vitro* experiments revealed internalization of the nanoparticles into macrophage lysosomes. Within the lysosomes, the acidic environment allows indinavir to be released from the spherical nanoparticles. Our studies have demonstrated that the extracellular release of indinavir is dependent on steady-state drug equilibrium within and outside the macrophages. Multiple washings of cells with media allowed for a continued release of the drug in cell culture. Results of these experiments reveal that indinavir-laden nanoparticle macrophages have the ability to carry the nanoparticles and release the indinavir payload throughout a 7-day period.

## Inhaled DDS

There are difficulties associated with the treatment of tuberculosis (TB). The organism grows slowly and therefore, anti-TB drugs must be given for an extended period of time to be effective. Because of this extended treatment, adherence is problematic for patients (Volmink and Garner 2000).

More than 80% of TB cases involve the pulmonary tissue alone. High doses of anti-TB drugs are required because only a small fraction of the total dose reaches the lungs after oral administration (Gelperina et al. 2005). Adherence to treatment and patient outcome could be optimized with the introduction of long-acting drug formulations that would release drugs at a slow and consistent rate. Investigators have utilized drug-loaded nanoparticles and investigated an inhaled delivery system in a guinea pig model. The particles ranged from 186 to 290 nm in diameter, and the anti-TB drugs that were loaded included rifampin, isoniazid, and pyrazinamide (Pandey et al. 2003). A single nebulization of this formulation was able to maintain a therapeutic plasma concentration for 6–8 days and a lung concentration for 9–11 days. In the animal model inoculated with *M. tuberculosis* $H_{37}Rv$, five nebulized nanoparticle doses administered 10 days apart resulted in undetectable colony-forming units (CFU) in the lungs (Pandey and Khuller 2004). Advantages of this form of therapy, if the nanoparticles can be delivered as a nebulization to humans, are readily apparent. Other avenues of research to optimize the delivery of nanoparticles to the lung tissue are being actively investigated (Pandey and Khuller 2005; Johnson et al. 2005).

## *In vivo* imaging and diagnostics

The utility of quantum dots for *in vivo* imaging, cellular functional assays, and diagnostics has the potential to revolutionize medical research (Michalet et al. 2005; Wu et al. 2003; Lidke et al. 2004). Quantum dots are nanocrystals or nanotubes and are only a few nanometers in diameters and made of semiconductor materials. Quantum dots work by fluorescence resonance (blinking) visible at the single-molecule level (Nirmal et al. 1995). These dots can be observed and tracked over an extended period of time with confocal microscopy (Lacoste et al. 2000), total internal reflection microscopy (Michalet et al. 2005), or wide-field epifluorescence microscopy (Dahan et al. 2003; Hohng and Ha 2004). A utility of quantum dots is their ability to tag these to a target molecule (i.e., ligand, DNA, antibody, etc.) (Dahan et al. 2003; Pinaud et al. 2004; Wu et al. 2003; Jaiswal et al. 2003; Mansson et al. 2004) used in cellular mechanisms, for *in vivo* imaging of a tumor, or to have a readout of a functional assay for diagnostic purposes. Many other uses are currently being investigated for cellular assays, imaging, and diagnostics.

## The future of nanotechnology drug delivery

The therapeutic advantages of nanotechnology-derived drug delivery are becoming apparent and will soon be associated with every route of drug administration. The advantages over current treatment modalities include lower drug toxicities, improved bioavailability, reduced economic costs of treatment, and increased patient adherence to treatment. The medical management of malignancies has already been greatly impacted by nanotechnology, but soon other medical specialties will utilize these novel forms of drug delivery to achieve optimal treatment success. Additionally, innovative research and development of more therapeutically effective nanocarriers will continue including improved forms of polymer–drug conjugates (Vicent and Duncan 2006), liposomes (Allen 1998), dendrimers (Lee et al. 2005a, b), micelles (Torchilin 2005a, b), polymeric vesicles (Hitzman et al. 2006), and nanocapsules (Kreuter 1978). Finally, implantable drug delivery systems will open up many more opportunities for nanotechnology utilization. Optimized drug release from implantable delivery systems is preferable to intravenous administration. The extended duration of action, reduced frequency of redosing, and improved patient acceptance are all positive attributes of implantable drug delivery systems. Although the future of nanotechnology is promising, it is important to consider the toxicological aspects of nanoparticles (Nel et al. 2006). Toxicity screening of nanotechnology products should include physiochemical characterization of the nanomaterial, *in vitro* assays, and *in vivo* studies to assure the public that these materials will not influence cellular reactions within the human body.

Nanotechnology will become more commonplace in the delivery of drug therapy as pharmaceutical innovation continues. Indeed, searching PubMed for 2005 revealed more than 1,650 articles related to nanotechnology and drug delivery for that year. The increasing interest in this field of drug research demonstrates the implicit promise and potential of nanotechnology-derived drug delivery paradigms. Indeed, the preliminary data described in this review reveal that after one intraperitoneal injection, a sustained therapeutic plasma level of indinavir can be maintained for more than 1 week through the use of the nanoparticle formulation compared to the free drug. This finding, along with the apparent dose-dependent variations in duration of therapeutic plasma drug levels, provides strong support for the advancement of nanotechnology-derived drug delivery for antiretroviral drugs.

The use of nanotechnology for the purposes of diagnostics and imaging will also become increasingly important. The use of MEMS to mimic secretion of hormones, insulin, and other closed systems is a central goal of nanotechnology-derived devices. Additionally, the release of narcotic drugs for severe pain relief would be advantageous if an implantable device can fabricated. Research into the fabrication of these much-needed devices may soon lead to possible alternatives to current treatment modalities. The future of quantum dots for imaging, diagnostics, and

cellular applications will continue to develop. The quantum dots possibly could be injected intravenously and target a diseased tissue (cancer). These nanosized particles could be used as contrast agents for MRI, positron emission tomography (PET), and computed tomography to view the malignant tissue. An optical biopsy could be performed that confirms the pathology. A nanoparticle DDS could then be utilized to treat the malignant cells with minimal side effects to the patient.

Certainly, there will be other uses associated with this evolving technology. However, in order for nanotechnology to continue the evolution of medical management, it will require the multidisciplinary approach from both basic and clinical research backgrounds to achieve sustained innovation. Many clinical investigators will collaborate with material scientists, engineers, and polymer chemists to bring this technology to the forefront in the quest to optimize essential medical therapies for patients. It is quite likely that nanotechnology will become the next frontier of medical research.

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