Nanoparticle-Mediated Drug Delivery and Gene Therapy

Sha Jin[†] and Kaiming Ye*[,‡]

DNA Resource Center and Biomedical Engineering Program, College of Engineering, University of Arkansas, Fayetteville, Arkansas 72701

Biomedical application of nanotechnology is a rapidly developing area that raises new prospect in the improvement of diagnosis and treatment of human diseases. The ability to incorporate drugs or genes into a functionalized nanoparticle demonstrates a new era in pharmacotherapy for delivering drugs or genes selectively to tissues or cells. It is envisioned that the transfer of nanoengineering capability into disease therapy will provide constant and concentrated drug delivery to targeted tissues, minimizing systemic side effects and toxicity. We have in this article highlighted the recent state of the art in nanomedicine, focusing particularly on the achievement of nanotechnology in nanoscale drug and gene delivery in vitro and in vivo. In addition, a specific emphasis has been placed on the use of nanotechnology to improve controlled drug release and sustainable drug delivery in solid tumors and on new drug therapies for age-related neurodegenerative disorders.

Nanotechnology focuses on the design, synthesis, characterization, and application of materials and devices at the nano scale. Application of nanotechnology to medicine led to the emergence of a new area called "nanomedicine", which employs molecular knowledge to address medical problems and maintain and improve human health (*1*). The scope of the rapid progress in nanomedicine ranges from in vivo imaging and diagnosis to therapeutics such as drug delivery and gene therapy. One of the most attractive applications of nanotechnology is its ability to significantly improve the sensitivity of biosensors. For example, nanoparticle (NP)-based assay has been demonstrated to be able to detect proteins in the attomolar concentration range, a magnitude of six orders lower than concentrations detected by ELISA (*2*). This improvement will allow early detection of many diseases such as cancer and cardiovascular diseases, saving millions of lives through the prevention and early treatment of these diseases.

Another promising technique developed based on nanotechnology is the nanodrug and/or gene delivery system. This new technology provides greater potential for many applications, including anti-tumor therapy by targeted delivery of therapeutic agents to tumors. Cancer treatment represents an enormous biomedical challenge for drug delivery. The unique properties of cancer require the development of a multifunctional drug delivery system that can be efficiently manufactured to target subtle molecular alterations that distinguish a cancer cell from healthy cells in the body (*3*). A NP-mediated drug delivery system can significantly eliminate drug or drug carrier side effects. A good example is the first biologically interactive agent, albumin-bound paclitaxel, an anti-cancer drug. ABI 007 is encapsulated in a 130 nm NP, designed to avoid solvent-related toxicities and to deliver paclitaxel to tumors via molecular pathways involving an endothelial cell-surface albumin receptor and an albumin-binding protein expressed by tumor cells. The paclitaxel is then secreted into the tumor interstitium (*4*). A phase III study in the U.S. on this technique has already been completed in breast cancer patients and is currently being tested to treat a variety of tumor types (*5*).

It is known that constant drug delivery to a target creates a greater therapeutic effect with lower drug levels than that of traditional dosing methods. Therefore, the design of entirely new classes of micro- and nanofabricated systems is expected for target local transportation of drugs. In this review, we describe a variety of NP modalities including virus, polymer, liposomes, dendrimers, and gold NPs and the development of multifunctional NPs for drug and gene delivery. We emphasize the nanocarrier systems and highlight the significance of these nanocarriers in targeted drug and gene delivery.

Types of Nanoparticles Applied to Drug Delivery

It has been determined that the efficiency of many conventional pharmaceutical therapies can be significantly improved through drug delivery systems. Drug delivery systems are designed to alter the pharmacokinetics and biodistribution of their associated drugs or to function as drug reservoirs or both (*6*). Usually, therapeutic molecules such as proteins and lipids are encapsulated inside or conjugated to particular carriers composed primarily of lipids and polymers. The encapsulation or conjugation allows controlled release rather than a burst of drugs so that a high therapeutic efficiency can be achieved without side effects.

An ideal drug delivery system should be able to localize drug specifically and directly to its target. This is particularly important when drugs made by traditional manufacturing methods are hydrophobic and their solvents are toxic. Nanotechnology promises to improve drug delivery system design and targeting. Nanostructured drugs or delivery carriers allow the continuous and controlled release of therapeutic drugs to maintain drug levels within a desired level. Other advantages of nanostructured drugs include localizing and specifically targeting the drugs to their intended tissues and cells, thereby decreasing drug doses and improving patient compliance.

The size of NPs ranges from 10 to 200 nm, about the size of a protein. Because of their small size, NPs can readily interact

* To whom correspondence should be addressed. Ph: 479-575-5315. Fax: 479-575-7318. Email: kye@uark.edu.
[†] DNA Resource Center.
[‡] Biomedical Engineering Program.

10.1021/bp060348j CCC: $37.00
Published on Web 12/22/2006

Table 1. Applications of Nanoparticles to Drug/Gene Delivery

particle class	materials	size	toxicity	pharmacological application
virus	DNA	30−100 nm	high	gene delivery
liposomes	lipid mixtures	30−200 nm	low	drug/gene delivery
dendrimers	branched polymers	5−50 nm	depends on cell type	drug/gene delivery
polymer carriers	polylactic acid, polysaccharides, poly(cyano)acrylates, poly(lactide-*co*-glycolide)	50−2,000 nm	low	drug/gene delivery
magnetic nanoparticles	iron oxide	5−100 nm	low	diagnosis, drug delivery
silica nanoparticles	SiO_2	10−200 nm	low	gene delivery
gold nanoparticles	chlorauric acid, sodium citrate	30 nm−1.6 μm	low	drug delivery

with biomolecules on the cell surface or inside cells. The small size allows NPs to penetrate tissues such as tumors in depth with a high level of specificity (*7*) improving the targeted delivery of drug/gene. To generate functionalized NP, various layers and coatings need to be made based on the desired NP function (*8*). Table 1 summarizes the types of NPs developed so far for improving drug/gene delivery.

Liposomes are spherical lipid particles in an aqueous compartment formed by an encloseing lipid bilayer (*9*). The fatty layer of the liposome is expected to protect and confine the enclosed drug until the liposome binds to the outer membrane of target cells. The hydrophilic core and hydrophobic phospholipid bilayer coat of liposomes improve the solubility of many amphiphilic drugs. NPs made with liposomes are the simplest form of NPs and have the advantage of a long circulation time, reduced systemic toxicity, increased uptake into tumors, and steady release of their payload. They have been widely used in clinics for decades (*10*).

Dendrimers are another type of NPs. They are made up of different types of polymers such as poly(L-glutamic acid) (PGA), polyamidoamine (PAMAM), poly(ethylene glycol) (PEG), and polyethylenimine (PEI) using either convergent or divergent step growth polymerization (*11*). The dendrimers consist of many branches coming out of an inner core. The dendrimer is hydrophilic and thus can be used as a coating agent for drug delivery. It is easy to prepare and its terminal can be modified for targeted drug delivery and selective imaging of different tissues such as tumors (*12*, *13*). In vivo study reveals that 96% inhibition of tumor growth can be achieved after delivering the anti-angiogenic angiostatin and tissue inhibitor of metalloproteinases gene into animal models using a dendrimer gene delivery system (*14*).

Biodegradable polymeric NPs, typically consisting of polylactic acid, polyglycolic acid, polylactic-glycolic acid (PLGA), and poly(methyl methacrylate) (PMMA) have been studied for both gene and drug delivery for many years. Polymer−drug conjugation promotes tumor targeting through its enhanced permeability and retention effect. Polymer-bound chemotherapy has provided a solid foundation for the second generation of carriers that deliver the recently under-development target-specific anti-cancer agents (*15*). Chitosan is one of the natural nontoxic polysaccharide polymers. It is biodegradable and biocompatible and protects DNA against DNase degradation, leading to DNA condensation (*16*).

Whereas superparamagnetic NPs are developed primarily for noninvasive imaging, especially magnetic resonance imaging (MRI), they have also been exploited for monitoring or guiding NP-mediated drug delivery (*17*−*19*).

Gold NPs have also been utilized as nontoxic drug carriers for selective drug delivery. It has been reported that using PEG-coated colloidal gold NPs (PT-cAu-TNF-α) with an incorporated TNF (tumor necrosis factor)-α payload can delay tumor growth and enhance tumor thermal therapy in mice when they are given intravenous administrations of proper dosage (*20*). Another example is that the colloidal gold NPs bound with thiol-derivatized PEG and recombinant human TNF on their surface can rapidly accumulate in MC-38 colon carcinoma tumors with little or no accumulation in the livers, spleens, or other healthy organs (*21*).

Bio- and Chemical Modification of Nanoparticles for Targeted Drug Delivery and Controlled Release

Generally, NPs used for drug delivery must be made to have the following components: a particle core, a protective layer for biocompatibility, and a linker molecular layer for bioactivity. The linker layer usually has reactive groups at both ends that attach the linker to the core and to the bioactive molecules. Biocompatibility, long circulation times, and selective targeting of NP-mediated drug delivery can be promoted by conjugation. Hydrophilic molecules, such as PEG, can be coated onto the surface of NPs to reduce reticuloendothelial system (RES) uptake and thus increase the circulation time of administrated NP-linked drugs, because nanostructured drugs administrated systematically can be taken up primarily by the RES through the phagocytotic pathway (*22*, *23*). For example, liposomes coated with PEG have been found to have notably long circulation time after intravenous administration in mice (*24*). In the following sections, we will discuss a number of approaches that have been developed to date to functionalize the NPs for targeted drug delivery and controlled release.

Functionalization of Nanoparticle for Targeted Drug Delivery. Critical issues for successful NP drug delivery include the NPs' ability to target specific cells or tissues. Targeted drug delivery can achieve a high therapeutic efficiency without side effects. It is known that the surface property of NPs is the key for the determination of the in vivo fate of NP−drug complexes, including their interaction with cells and the intracellular transport machinery, and their drug release properties (*25*). Thus, the modification of surface properties of NPs becomes critical to targeted drug delivery (*26*). Such a modification can be accomplished through NP functionalization, i.e., conjugating or coating ligands that specifically bind to target cells or tissues, onto the surface of NPs. Ligands such as antibodies (*27*), folic acid (*28*−*32*), and peptides (*33*, *34*) can be utilized as appropriate target molecules for targeted drug delivery in cancer therapy, as these ligands can specifically interact with tumor-associated antigens or receptors. For example, aptamer (apt) bioconjugated NPs have shown a very high specificity for drug delivery in prostate cancer chemotherapy (*35*). As the A10 2′-fluoropyrimidine RNA apts recognize the extracellular domain of the prostate-specific membrane antigen (PSMA), a well characterized antigen expressed on the surface of prostate cancer cells, these surface-functionalized NPs can selectively deliver the docetazel (Dtxl), an anti-prostate cancer agent, to prostate cancer cells through the interaction between the NP-conjugated apts and PSMA. In this study, Dtxl was encapsulated in PLGA-PEG copolymer NPs through a nanoprecipitation method. Because of the carboxylic acid group on the terminal end of the PEG,

these NPs have a negative surface charge and thus can be easily conjugated to amine-functionalized A10 PSMA apts by carbodilimide coupling chemistry. In vivo studies demonstrate that these drug-encapsulated NP-apts bioconjugates were able to significantly inhibit prostate tumor growth without systemic toxicity (*35*, *36*).

In order to further enhance the selectivity of therapeutic NPs, a dual-ligand approach has been established. As most commonly used ligands are directed at receptors expressed not only on target cells but also on other cells in the body, dual-ligand conjugation can remarkably augment the selective binding of NPs to target cells. This approach utilizes the fact that some cells, such as tumor cells, typically overexpress multiple types of surface receptors. This approach has been tested in the human KB cell lines, which overexpress both the folate receptor (FR) and the epidermal growth factor receptor (EGFR) (*37*). In this study, liposomal NPs loaded with a chemotherapeutic agent, doxorubicin, and bearing controlled numbers of both folic acid and a monoclonal antibody against EGFR were designed and used to selectively deliver doxorubicin to tumor cells.

Another way to functionalize the NPs for targeted drug delivery is to self-assemble DNA with nanosized colloidal particles such as polymeric micelles that have a hydrophobic core and a hydrophilic shell. The micelles can be formed using amphiphilic block copolymers. This approach allows the solubilization of various poorly soluble pharmaceuticals for targeted drug delivery. It has been found that the drug carried by these lipid-core micelles, formed by conjugates of soluble copolymers with lipids such as poly(ethylene glycol)−phosphatidyl ethanolamine conjugate (PEG-PE), can spontaneously target body areas with compromised vasculature (tumors, infarcts) via the enhanced permeability and retention effect (*38*, *39*). Furthermore, the lipid-core mixed micelles containing certain components such as positively charged lipids are capable of escaping endosomes and delivering incorporated drugs directly into a cellular cytoplasm (*39*). In another study, a class of polymeric micelles was used to incorporate Cisplatin, an anti-tumor agent, to facilitate the application of NPs in oncology, because the in vivo use of Cisplatin showed severe side effects (*40*, *41*). When Cisplatin was encapsulated inside self-assembled PEG-PGA block copolymers and administrated intravenously into tumor-bearing mice, a high chemotherapy activity was detected, and there were no significant side effects (*40*, *42*).

The pathophysiology of tumor tissue, characterized by angiogenesis, hypervasculature, defective vascular architecture, impaired lymphatic drainage, and acid tumor microenvironment, has also been exploited for NP-mediated targeted drug delivery to solid tumors. A paclitaxel-encapsulated NP, formed by pH-sensitive poly(ethylene oxide) (PEO)-modified poly(β-amino ester) (PbAE), has been designed and employed to overcome the toxicity of the paclitaxel aqueous solution when administrated intravenously (*43*). Because of the low pH of the tumor interstitial microenvironment resulting from lactic acid production due to hypoxia or acidic intracellular organelles, the paclitaxel-encapsulated pH-sensitive NPs can selectively release the drugs into the tumor cells (*44*, *45*). It has been demonstrated that the formulated NPs improve therapeutic efficiency when compared to results achieved without NPs, while maintaining low toxicity in solid tumor therapy (*43*).

In addition, various approaches have been taken to differentiate the biochemical properties of the vasculature of tumor from that of normal tissues (*46*, *47*). For example, tumor blood vessels are physically distinct from normal vessels. Hobbs et al. have discovered that tumor vessels have a characteristic pore cutoff size that seems to be maintained by both local microenvironmental factors and the tumor milieu (*48*). In the tumors studied by their groups, the pore cutoff size ranged between 380 and 780 nm. Most long-circulating liposomes and viral vectors proposed for therapeutic use are between 100 and 300 nm, and therefore NPs will be allowed passage into extravascular spaces and accumulate inside tumors. Matsumura et al. as well as Muggia's group have observed a 6-fold higher level of accumulation of biocompatible macromolecules in tumor tissues than in normal tissues or organs due to cancer tissue's enhanced permeability and retention effect (*49*, *50*). By utilizing the leakage of some types of tumor tissues, permeability of the polymeric NP drug to the tumor area through blood flow can be enhanced (*51*). However, the problem with this approach is that not all the tumors have the same porous properties: even in a single vessel there are leakage differences at different regions (*52*).

Biodegradable Nanoparticles for Controlled Release and Sustainable Drug Delivery. Biodegradable and biocompatible PLGA is perhaps the most widely investigated biomaterial for making NPs for controlled-release and sustainable drug delivery (*53*−*57*). Researches have found that PLGA has a solid safety profile and drug sustained release (*58*−*62*). Like other polyesters in nature, the PLGA undergoes hydrolysis upon implantation into the body, forming biocompatible and metabolizable moieties such as lactic acid and glycolic acid that are eventually removed from the body by the citric acid cycle (*63*). PLGA NPs are generally made by emulsion solvent evaporation or by solvent displacement techniques (*64*). Drugs encapsulated inside the NPs can be released at a sustained rate through diffusion and by the degradation of the NPs. Many lines of evidence suggest that the degradation rate of PLGA can be controlled by changing block copolymer composition and molecular weight (*65*). Accordingly, the release rate of encapsulated drugs can be altered from lasting for days to for months. Meinel et al. have shown that new bone encouraging biodegradable PLGA NPs, formed by encapsulating insulin-like growth factor I inside PLGA, can stimulate new bone formation for up to 3 weeks in the drill hole and stimulate the bridging of a segmental defect for up to 8 weeks, indicating the sustainable release of drugs in bone fractures treatment (*66*). In another study, Saito and co-workers synthesized a poly-D,L-lactic acid−*p*-dioxanone−poly-(ethylene glycol) block copolymer (PLA-DX-PEG) that can be used as a biocompatible and biodegradable NP for recombinant human bone morphogenetic proteins (rhBMP) delivery systems to induce bone formation in vivo (*67*). In animal experiments, Saito and co-workers showed that a new bone was efficiently formed and a large bone defect was repaired using PLA-DX-PEG/rhBMP composites by the slow release of rhBMP. Furthermore, Kang and his colleagues encapsulated paclitaxel in PLGA with various molecular weights prepared by a self-microemulsifying method (*68*). Their results indicate that paclitaxel-containing PLGA exhibit sustained release and a higher antitumor activity in contrast to that of a drug without PLGA.

The controlled drug release from biodegradable polymer NPs can be further improved by designing a "nanocell" delivery system (*69*). This nanocell comprises a nuclear NP made from PLGA. The nuclear NPs are coated with a nanoscale PEGylated-phospholipid block-copolymer envelope to form a nanocell structure so that the NPs can be preferentially taken up by tumors. A chemotherapeutic agent such as doxorubicin can be conjugated to the NP and another anti-angiogenesis agent such

as combretastatin-A4 can then be trapped within the lipid envelope, enabling a temporal release of two drugs. The outer envelope first releases an anti-angiogenesis agent, causing a vascular shutdown, and the disruption of this envelope inside a tumor will result in rapid deployment of the second agent, an anti-angiogenesis agent, leading to vascular collapse and intratumoral trapping of the NPs. Studies using these nanocells suggest that the combination of two drugs in one NP is more effective in suppressing tumor growth than a single drug delivery therapy in murine tumor models.

It has been widely speculated that image-guided drug delivery can remarkably enhance the localization and selective delivery of therapeutic agents to target cells and tissues. First, it is essential to develop multifunctional NPs that not only are detectable in vivo through imaging such as MRI but also are capable of drug-targeting delivery. Nasongkla et al. have developed this kind of NP (*17*). They utilized the polymeric micelle structure to construct a multifunctional nanomedicine platform for monitoring the targeted drug delivery. Their constructs are composed of three key components: (1) a chemotherapeutic agent such as doxorubicin that is released from polymeric micelles through a pH-dependent mechanism; (2) a cRGD ligand that can target $\alpha_v\beta_3$ integrins on tumor endothelial cells and subsequently induce receptor-mediated endocytosis for cell uptake; and (3) a cluster of superparamagnetic iron oxide NPs loaded inside the hydrophobic core of each micelle for ultrasensitive MRI detection. The presence of cRGD on the micelle surface results in the cancer-targeted delivery of $\alpha_v\beta_3$-expressing tumor cells. In vitro MRI and cytotoxicity studies demonstrate the ultrasensitive MRI and $\alpha_v\beta_3$-sepcific cytotoxic response of these multifunctional polymeric micelles. Similarly, supermagnetic NPs with a metal core that are bioconjugated with antibodies against ERBB2 have shown promising results for simultaneous imaging and targeting breast cancers therapeutically in vivo (*19*, *70*).

Another significant advantage of NP drug delivery is the treatment of age-related neurodegenerative disorders by applying the unique capability of NPs to transport across the blood-brain barrier (BBB) by passive diffusion or carrier-mediated transcytosis pathways (*71*, *72*). Of all the endothelial barriers within the body, the BBB is the tightest, which containing specialized tight junction proteins to minimize paracellular transport (*73*). Drug delivery to the central nervous system (CNS) is a challenge due to the BBB. One potential for overcoming the barrier is to deliver drugs to the brain using receptor-specific monoclonal antibody conjugated NPs. PEG-conjugated antibody-directed liposome NPs are synthesized and used to target rat transferring receptor, which is abundant on the brain microvascular endothelium (*74*). Incorporation of PEG strands on the surface of the liposome enhanced the stability of the liposome in the circulation. The use of the PEG-conjugated antibody NPs in rats resulted in greater brain delivery of NPs with a moderate plasma clearance. The target of exogenous gene expression to the primate brain has been evaluated using encapsulated PEG-conjugated antibody to human insulin receptor by intravenous injection (*75*). It has been concluded that the technology is able to deliver therapeutic genes to the human brain with an intravenous administration without the use of viral vectors. In an approach for Alzheimer's disease treatment, the Cu(I) chelator D-penicillamine was conjugated to NPs via a disulfide bond or a thioether bond to reverse the metal-induced precipitation of the Aβ protein (*76*). The study indicated that the conjugated D-penicillamine NPs have potential to deliver D-penicillamine to the brain and effectively resolubilize copper-Aβ (1-42) aggregates, which slowed down the disease development.

Nanoparticles for Gene Delivery

Viral Vector-Based Gene Delivery. Viral vectors, bionanoparticles with diameters of ~100 nm or less, have long been proven to be the most efficient and stable transgene vectors into the cell and thus are suitable for vaccine and gene therapy (*41*, *77*). Viruses are able to use the host cell machinery for protein synthesis, and some of them are able to stably insert into the host cell genome and provide a long-term transgene expression in transduced cells. Thus, viral gene carriers possess higher gene transfection efficiency than nonviral vectors. Numerous researches have focused on using viral vectors as gene delivery systems. Retrovirus (*41*, *78*), lentivirus (*79*−*82*), adenovirus (*83*−*85*), and adeno-associated virus (*87*−*90*) gene transfers have been comprehensively investigated for the purpose of vaccination and cancer and neurological disorder treatment (*91*−*94*) due to their capability to deliver their genomes into the nucleus where the transgenes can be transcribed. Adenoviruses are the most extensively used vector model for human gene therapy. The performance and pathogenicity of these viral NPs have been evaluated in both animal models and clinical trials. For instance, the phase II clinical testing of a genetically engineered adenovirus ONYX-015, capable of selectively replicating in and lysing p53-deficient cancer cells, demonstrate highly selective tumor tissue destruction and significant tumor regression (>50%) in 58% of the patients with advanced head and neck p53 mutant cancers (*95*).

Despite some successes in clinic trials, viral vector gene transfer still has some safety issues. The immune response caused by the expression of viral proteins has to be overcome. To this end, approaches have been developed to circumvent vector immunity (*96*). The Food and Drug Administration has not approved any viral vector based therapeutics because the viral gene delivery system shows a high transfection yield but has many disadvantages such as immunogenicity, oncogenicity, and potential virus recombination problems inherent in viral vector systems. Recently it has been reported that severe immunodeficiency leukemia patients have died following a retroviral vector treatment. The adenovirus therapy trial raised a red flag for viral vector-mediated gene transfer (*97*).

Safety concerns over the use of viral vectors have stimulated interest in developing substitute gene carriers. Nonviral NPs may offer an alternative and perhaps a better approach, as they are less immunogenic and less cytotoxic than viral vectors. Unlike viral vector-mediated gene transfer, no additional genes will be introduced into the transduced cells in NP-mediated gene transfer.

Functionalization of Nanoparticles for Nonviral Gene Transfer. Nonviral gene delivery has been gaining interest recently. Although the efficacy of DNA transfection is the major concern to date, compared to viral vector-mediated gene transfer, nonviral vectors are relatively easy to prepare, are less immunogenic and oncogenic, and have no potential of virus recombination and limitation on the size of a transferred gene. In addition, they can be vested readily to carry genetic materials to target cells by structurally modifying the vectors. Of many nanoconstructs, NPs are attractive vectors for nonviral gene transfer. They have been successfully tested for both in vitro (*98*) and in vivo gene delivery (*99*). In principle, they can be made to reach a target site by virtue of their size and charge (*100*). Their high surface area to volume ratio make NPs ideal for nonviral gene transfer.

Genetic materials such as DNA plasmids, RNA, and siRNA can be either encapsulated inside (*101*) or conjugated to the NPs (*98*, *102*, *103*). One of the easiest ways to link DNA to a NP is to modify the surface of NPs to a positive charge so that the NP−DNA complexes can be formed simply through electrostatic binding between the positive charges of the NPs and the negative charges of the DNA. This mechanism has been widely used in liposome and other polymer-mediated gene transfer (*99*, *104*−*107*). Nevertheless, these gene transfer vectors suffer from several disadvantages. For example, the delivery using NP vectors is far less efficient than that of viral vectors. The reproducibility of the polymeric vector synthesis is relatively low because of the high polydispersity of the polymers. Besides, these polymers do not tolerate heat, making autoclaving virtually impossible. In contrast, inorganic NPs such as hydroxyapatide and inert silica NPs have low polydispersity and low toxicity as well as high biocompatibility (*103*). Silica NPs are resistant to bile salts and lipase encountered in the gastrointestinal tract, have physical strength during aerolization, and can withstand autoclaving (*103*, *108*). However, their surfaces need to be modified to positive charges so that they can be used as vectors for gene transfer. Kneuer et al. have proposed an approach to create cationic surface modified silica NPs through the synchronous hydrolysis of tetraethoxysilane and *N*-(β-aminoethyl)-γ-aminopropyltyiethoxysilane in a water-in-oil microemulsion method (*102*, *103*). After amino-modification, these silica NPs were sized between 10 and 100 nm and with ξ potential ranging from +7 to +31 mV at pH 7.4 due to the protonation of the amino group on the surface of these NPs. With positive charges on the surface, plasmid DNA was able to link to the NPs through electrostatic binding. The electrostatic-bound DNA can be released from the NP−DNA complexes at alkaline pH (>10) or by the presence of high salt concentrations (>2 mol/L sodium chloride) (*108*). The enzymatic digestion of DNA can be inhibited by these NP−DNA complexes (*109*). It has been demonstrated that DNA protection through binding to amino-modified silica NPs can be due to (1) positive charges that keep Mg^{2+} away from the positively charged NPs on the amino group of the NPs and/or (2) conformational change of DNA structure when they are embedded onto the surface of NPs. Further studies suggest that the positive charge on the amino group of the NPs alone was not the major factor for the protection of DNA from cleavage. Thus, the smallness of the NPs may force the DNA to become bound in such a way that cleavage is either impossible or at least greatly slowed on the NP surface. This hypothesis needs to be further clarified. Using this technique, Bharali et al. successfully delivered a plasmid encoding EGFP (enhanced green fluorescent protein) into mouse ventral midbrain and lateral ventricle, allowing fluorescent visualization of the extensive transfection of neuronal-like cells in substantia nigra and areas surrounding the lateral ventricle (*110*). They also showed that these amino-modified silica NPs can be used to selectively deliver a nucleus-targeting fibroblast growth factor receptor type 1 into the mouse brain, resulting in significant inhibition of incorporation of bromodeoxyuridine into the subventricular zone and the adjacent rostral migratory stream.

In other efforts to utilize silica NPs for gene transfer, hydrated organically modified silica NPs are synthesized by constructing dioctyl sodium sulfosuccinate (Aerosol-OT)/DMSO/water microemulsions as a nonpolar core. Amino groups are added to the surface of the NPs through synchronous hydrolysis of the triethoxyvinylsilane (VETS) precursor and 3-aminopropyltriethoxysilane (APTES). The functionalized silica-NPs are capable of condensing DNA and extensively staining the cytoplasm of tumor cells in vitro (*111*). It has been observed that silica-NPs released the DNA inside the cytoplasm and migrated into the nucleus for gene delivery.

In addition, DNA or RNA can be encapsulated inside biodegradable polymeric NPs for controlled gene release when polymeric NPs are degraded or digested by enzymes. The encapsulation of nucleic acids into the NPs provides the nucleic acids protection from enzymatic digestion during their transit in systemic circulation and allows targeting to tissues or cells in the body through the surface functionalization of the NPs. The encapsulation also avoids uptake of the nucleic acids, such as plasmid DNA, by the mononuclear phagocytic system, which happens all the time when naked plasmid DNA are used in systemic administration (*101*). An experimental approach has been proposed to enclose plasmid DNA using PEGylated gelatin NPs by the acidic or basic hydrolysis of collagen (*112*, *113*). The plasmid DNA can be encapsulated inside the PEGylated gelatin NPs through a mild water−ethanol solvent displacement method under controlled pH and temperature (*114*, *115*). The PEGylated gelatin NPs exhibit the capability of delivering a plasmid DNA into NIH 3T3 murine fibroblast cells (*116*). Furthermore, PEGylated gelatin NPs can target solid tumors through preferentially distributing NPs in the vasculature because of enhanced permeability and retention effect in tumors (*101*, *117*).

To improve the efficiency of targeted gene delivery, further efforts have been made by ligands conjugate antibodies to biodegradable NPs in which DNA are encapsulated. The linkage of ligands to the hydrophilic coat around the NPs for specific binding to cell surface receptors mimics the viral structure so that a high level of transfection efficiency can be achieved, as the surface modified NPs can enter into target cells by a receptor-mediated endocytosis pathway that is similar to that used by most of the viral vectors. There are a number of ligands that have been tested in vitro and in vivo. For example, a DNA/polycation complex has been constructed by simply mixing plasmid DNA with PEI at varying ratios (*118*). To target breast cancer cells, trastuzumab (herceptin), a human epidermal growth factor receptor-2 (HER-2) monoclonal antibody, was conjugated to DNA/polycation complexes. The experimental results show that these surface functionalized complexes had up to 20-fold higher transfection activity compared with noncationic polymer based gene transfer in breast cancer cells.

In another study, an integrin $\alpha_v\beta_3$ ligand was conjugated onto the surfaces of cationic polymerized lipid-based NPs for targeted gene delivery in solid tumors (*119*). Integrin plays a key role in endothelial cell survival during angiogenesis in vivo and is preferentially expressed in the angiogenic endothelium. Thus, the linkage of the integrin $\alpha_v\beta_3$ ligand to the cationic polymerized lipid-based NPs considerably enhances selective gene delivery to the angiogenic blood vessels in tumor-bearing mice.

In addition, Li and his co-workers investigated the feasibility of using a controlled release system formed by polyaminoethyl propylene phosphate (PPE-EA) NPs to achieve gene transfer in the brain (*120*). A unique feature of this gene delivery system is the biodegradability of PPE-EA, which provides a sustained release of DNA at different rates depending on the charge ratio of the polymer to the DNA. PPE-EA displays much lower toxicity in cultured neural cells when compared to PEI and did not cause detectable pathological changes in the CNS. The results establish the potential of PPE-EA as a new and biocompatible gene carrier to realize sustained gene expression in the CNS.

Except for charge and surface characteristics (*121*, *122*), the particle size of NPs can significantly impact gene transfer efficiency in vivo. It has been reported that the optimization of particle size can dramatically improve the clearance behavior and the tissue distribution of intravenously injected NPs and hence ameliorate the efficiency of drug or gene delivery (*121*, *123*, *124*). Transfection efficiency of the dendrimers can be dramatically elevated more than 50-fold by the heat treatment of NPs in a variety of solvolytic solvents, e.g., water or butanol (*125*). The smaller-sized PLGA NPs with a mean diameter of 70 ± 2 nm containing a plasmid DNA show 27-fold higher transfection than larger-sized NPs with a mean diameter of 202 ± 9 nm in the COS-7 cell line and a 4-fold higher transfection in the HEK-293 cell line (*124*).

Self-Assembly of Nanoparticles for Gene Transfer. Although the positive charge of NPs helps improve the gene transfer efficiency in vitro, it will be a concern in vivo. It is understood that a high cationic charge density can lead to the aggregation of NPs in the microvasculature of "first pass" organs such as the spleen, liver, and particularly the lung (*126*, *127*). To diminish these disadvantages, a nucleic acid-lipid self-assembly approach has been developed and used to provide small colloidally stable NPs for targeted gene delivery in solid tumors (*128*). To self-assemble DNA into lipid NPs, cationic and neutral lipids such as dioctadecyldimethylammonium bromide (DDAB) or 1,2-dioleoyl-3-trimethylammonio)propane (DOTAP), a phosphattidyl-choline (e.g., 1-palmitoyl-2-oleoyl phosphatidylcholine-POPC), can be combined with cholesterol in 100% ethanol and diluted with an equal volume of 5 mM HEPES at pH 7.4. Subsequently, the equal volume of DNA plasmids may be mixed with a lipids solution at 60 °C, followed by a gradual cooldown to room temperature. The ethanol can be removed by rotary evaporation or dialysis against unbuffered 144 mM NaCl. An antibody such as the F5-cys-mal-PEG(2000)-DSPE can be conjugated to the NPs for targeted gene delivery. The self-assembled NPs have shown remarkable improvement in their pharmacokinetic characteristics in comparison to early lipoplexes. The antibody-conjugated self-assembled NPs can selectively deliver plasmid DNA into HER2 overexpressing SK-BR-3 breast cancer cells (*128*). One of the significances of this approach is the scaleable preparation of highly stable immunotargeted nucleic acid delivery vehicles capable of achieving a high degree of specific transfection activity.

siRNA Delivery Using Nanoparticles. Gene silencing mediated by double-stranded small interfering RNA (siRNA) has been widely investigated as a potential therapeutic approach for diseases of genetic defects. However, siRNA therapy is hindered as a result of the poor stability of siRNAs in physiological fluids and their limitations in intracellular uptake (*129*). NPs have been exploited to maintain a sufficient local concentration of siRNA. For example, RhoA is one of the proteins overexpressed in cancer cells. Studies on intravenously administering chitosan-coated polyisohexylcyanoacrylate (PIHCA) NPs encapsulated anti-RhoA siRNA in nude mice with aggressive breast cancer demonstrated that tumor growth can be inhibited by 90% without toxic effect (*130*). In another study, the intraperitoneal administration of PEI-complexed siRNA targeting the HER-2 receptor into experimental mice resulted in the delivery of the intact siRNA into the tumors and a significant reduction in tumor growth (*131*). Similarly, PEI was employed to construct NPs bearing siRNA targeting vascular endothelial growth factor receptor-2 in order to target tumor neovasculature expressing integrins (*132*). The NPs with the combination of neovasculature ligands and siRNA led to selective siRNA delivery to tumor tissue and inhibition of tumor growth via selective gene pathway in tumor-bearing mice.

NP-mediated antisense RNA delivery has also been examined. A 12-mer antisense oligonucleotide directly against *ras* oncogenes was loaded onto polymeric NPs (*106*). It has been observed that low quantities (less than 100 μg) of NP-absorbed oligonucleotides inhibit neoplastics growth remarkably both before and after the onset of tumor in nude mice.

Health Risks and Safety Evaluation of Nanoparticles

The fast growth of NP-mediated drug or gene delivery calls for concerns about the potential health and environmental risks related to the use of the NPs and widespread nanomaterial production created by these technologies. Several issues related to the health risks during NPs usage including the deposition and clearing of solid NPs, biocompatibility, systemic translocation and body distribution of NPs, intestinal tract involvement, and direct effects on the central nervous system have been studied so far (*133*). It is clear that the change in the physicochemical and structural properties of synthesized NPs with a decrease in size can be responsible for a number of material interactions that can lead to toxic effects (*134*).

It has also been confirmed that NPs can have pronounced environmental effects even at very low aqueous concentrations (*135*). For instance, the utility of nanocrystal quantum dots (QDs) in humans may be limited because of the heavy metals used in them, which are reportedly toxic to cells at concentrations as low as 10 μg/mL (*136*). QD size, charge, concentration, capping material, functional groups, and mechanical stability have been associated as determining factors in QD toxicity (*137*).

However, there is a large gap between research on NPs in inhalation toxicology and in nanoscaled drug/gene delivery (*138*), although the toxicological study of NPs is a critical branch of nanomedicine research.

Perspective

Nanotechnology has been proved to be extremely important for future medicine. The ideal NPs for a drug or gene carrier system can achieve long circulation time, low immunogenicity, good biocompatibility, selective targeting, and the efficient penetration of barriers such as the vascular endothelium and the BBB, self-regulating drug release without clinical side effects (*139*). The penetration of NPs into endothelial barriers to reach tumor sites opens many avenues for using NPs as delivery vectors in neuron-related disease therapies. However, more studies are required to determine the efficacy and safety for their use in humans. Interestingly, it has been discovered recently that gold NPs can bind specifically to heparin-binding growth factors such as VEGF165 and bFGF, the two critical cytokines for the induction of angiogenesis, inhibit these cytokine activity in vitro, and inhibit VEGF-induced angiogenesis in vivo. However, the toxicity of gold NPs was not observed in the study (*140*). This finding indicates that gold NPs may be excellent nanomaterials for further modification and development of targeted drug or gene delivery. Future emphasis is to generate a common platform that can be modified easily to conjugate different molecules for drug or gene delivery.

NP-mediated gene delivery is deficient in the ability of translocation to the nucleus. Thus, its efficiency as a gene transfer system is significantly lower than that of a viral vector-mediated gene delivery system. The better design of NP structure to increase gene transfection efficiency is desired to overcome extracellular and intracellular transfection barriers: the blood-

stream, the cellular membrane, endosomes, and the nuclear membrane. Furthermore, the modifications of NPs for enhancing localization and retention in target tissue, local delivery of agents to a large volume of tissues, and controlled release of drug must be further improved along with searching for better tissue-specific targeting molecules. In addition, NPs with higher loading capacity of therapeutic agents are desired. Development of multifunctional NPs will further promote the medical application of nanotechnology in the next decade. The toxicological issue of NPs and their degradation products remain major concerns and must be addressed before being applied to humans.

References and Notes

(1) Freitas, R. A., Jr. The future of nanofabrication and molecular scale devices in nanomedicine. *Stud. Health Technol. Inf.* **2002**, *80*, 45−59.

(2) Fortina, P.; Kricka, L. J.; Surrey, S.; Grodzinski, P. Nanobiotechnology: the promise and reality of new approaches to molecular recognition. *Trends Biotechnol.* **2005**, *23* (4), 168−173.

(3) Citrin, D.; Scott, T.; Sproull, M.; Menard, C.; Tofilon, P. J.; Camphausen, K. In vivo tumor imaging using a near-infrared-labeled endostatin molecule. *Int. J. Radiat. Oncol. Biol. Phys.* **2004**, *58* (2), 536−541.

(4) Nyman, D. W.; Campbell, K. J.; Hersh, E.; Long, K.; Richardson, K.; Trieu, V.; Desai, N.; Hawkins, M. J.; Von Hoff, D. D. Phase I and pharmacokinetics trial of ABI-007, a novel nanoparticle formulation of paclitaxel in patients with advanced nonhematologic malignancies. *J. Clin. Oncol.* **2005**, *23* (31), 7785−7793.

(5) Gradishar, W. J.; Tjulandin, S.; Davidson, N.; Shaw, H.; Desai, N.; Bhar, P.; Hawkins, M.; O'Shaughnessy, J. Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. *J. Clin. Oncol.* **2005**, *23* (31), 7794−7803.

(6) Allen, T. M.; Cullis, P. R. Drug delivery systems: entering the mainstream. *Science* **2004**, *303* (5665), 1818−1822.

(7) Cuenca, A. G.; Jiang, H.; Hochwald, S. N.; Delano, M.; Cance, W. G.; Grobmyer, S. R. Emerging implications of nanotechnology on cancer diagnostics and therapeutics. *Cancer* **2006**, *107* (3), 459−466.

(8) Groneberg, D. A.; Giersig, M.; Welte, T.; Pison, U. Nanoparticle-based diagnosis and therapy. *Curr. Drug Targets* **2006**, *7* (6), 643−648.

(9) Rawat, M.; Singh, D.; Saraf, S.; Saraf, S. Nanocarriers: promising vehicle for bioactive drugs. *Biol. Pharm. Bull.* **2006**, *29* (9), 1790−1798.

(10) Park, J. W. Liposome-based drug delivery in breast cancer treatment. *Breast Cancer Res.* **2002**, *4* (3), 95−99.

(11) Aulenta, F.; Drew, M. G.; Foster, A.; Hayes, W.; Rannard, S.; Thornthwaite, D. W.; Worrall, D. R.; Youngs, T. G. Synthesis and characterization of fluorescent poly(aromatic amide) dendrimers. *J. Org. Chem.* **2005**, *70* (1), 63−78.

(12) Quintana, A.; Raczka, E.; Piehler, L.; Lee, I.; Myc, A.; Majoros, I.; Patri, A. K.; Thomas, T.; Mule, J.; Baker, J. R., Jr. Design and function of a dendrimer-based therapeutic nanodevice targeted to tumor cells through the folate receptor. *Pharm. Res.* **2002**, *19* (9), 1310−1316.

(13) Padilla De Jesus, O. L.; Ihre, H. R.; Gagne, L.; Frechet, J. M.; Szoka, F. C. Jr. Polyester dendritic systems for drug delivery applications: in vitro and in vivo evaluation. *Bioconjugate Chem.* **2002**, *13* (3), 453−461.

(14) Vincent, L.; Varet, J.; Pille, J. Y.; Bompais, H.; Opolon, P.; Maksimenko, A.; Malvy, C.; Mirshahi, M.; Lu, H.; Vannier, J. P.; Soria, C.; Li, H. Efficacy of dendrimer-mediated angiostatin and TIMP-2 gene delivery on inhibition of tumor growth and angiogenesis: in vitro and in vivo studies. *Int. J. Cancer* **2003**, *105* (3), 419−429.

(15) Vicent, M. J.; Duncan, R. Polymer conjugates: nanosized medicines for treating cancer. *Trends Biotechnol.* **2006**, *24* (1), 39−47.

(16) Mansouri, S P. L.; Corsi, K.; Benderdour, M.; Beaumont, E.; Fernandes, J. C. Chitosan-DNA nanoparticles as non-viral vectors in gene therapy: strategies to improve transfection efficacy. *Eur. J. Pharm. Biopharm.* **2004**, *57* (1), 1−8.

(17) Nasongkla, N.; Bey, E.; Ren, J.; Ai, H.; Khemtong, C.; Guthi, J. S.; Chin, S. F.; Sherry, A. D.; Boothman, D. A.; Gao, J. Multifunctional polymeric micelles as cancer-targeted, MRI-ultrasensitive drug delivery systems. *Nano Lett.* **2006**, *6* (11), 2427−2430.

(18) Hirsch, L. R.; Stafford, R. J.; Bankson, J. A.; Sershen, S. R.; Rivera, B.; Price, R. E.; Hazle, J. D.; Halas, N. J.; West, J. L. Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100* (23), 13549−13554.

(19) Artemov, D.; Mori, N.; Okollie, B.; Bhujwalla, Z. M. MR molecular imaging of the Her-2/neu receptor in breast cancer cells using targeted iron oxide nanoparticles. *Magn. Reson. Med.* **2003**, *49* (3), 403−408.

(20) Visaria, R. K.; Griffin, R. J.; Williams, B. W.; Ebbini, E. S.; Paciotti, G. F.; Song, C. W.; Bischof, J. C. Enhancement of tumor thermal therapy using gold nanoparticle-assisted tumor necrosis factor-alpha delivery. *Mol. Cancer Ther.* **2006**, *5* (4), 1014−1020.

(21) Paciotti, G. F.; Myer, L.; Weinreich, D.; Goia, D.; Pavel, N.; McLaughlin, R. E.; Tamarkin, L. Colloidal gold: a novel nanoparticle vector for tumor directed drug delivery. *Drug Delivery* **2004**, *11* (3), 169−183.

(22) Brigger, I.; Dubernet, C.; Couvreur, P. Nanoparticles in cancer therapy and diagnosis. *Adv. Drug Delivery Rev.* **2002**, *54* (5), 631−651.

(23) Otsuka, H.; Nagasaki, Y.; Kataoka, K. PEGylated nanoparticles for biological and pharmaceutical applications. *Adv. Drug Delivery Rev.* **2003**, *55* (3), 403−419.

(24) Blume, G.; Cevc, G. Liposomes for the sustained drug release in vivo. *Biochim. Biophys. Acta* **1990**, *1029* (1), 91−97.

(25) Labhasetwar, V. Nanotechnology for drug and gene therapy: the importance of understanding molecular mechanisms of delivery. *Curr. Opin. Biotechnol.* **2005**, *16* (6), 674−680.

(26) Sahoo, S. K.; Ma, W.; Labhasetwar, V. Efficacy of transferrin-conjugated paclitaxel-loaded nanoparticles in a murine model of prostate cancer. *Int. J. Cancer* **2004**, *112* (2), 335−340.

(27) Kirpotin, D. B.; Drummond, D. C.; Shao, Y.; Shalaby, M. R.; Hong, K.; Nielsen, U. B.; Marks, J. D.; Benz, C. C.; Park, J. W. Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models. *Cancer Res.* **2006**, *66* (13), 6732−6740.

(28) Zheng, G.; Chen, J.; Li, H.; Glickson, J. D. Rerouting lipoprotein nanoparticles to selected alternate receptors for the targeted delivery of cancer diagnostic and therapeutic agents. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102* (49), 17757−17762.

(29) Hattori, Y.; Maitani, Y. Folate-linked lipid-based nanoparticle for targeted gene delivery. *Curr. Drug Delivery* **2005**, *2* (3), 243−252.

(30) Hattori, Y.; Maitani, Y. Enhanced in vitro DNA transfection efficiency by novel folate-linked nanoparticles in human prostate cancer and oral cancer. *J. Controlled Release* **2004**, *97* (1), 173−183.

(31) Kukowska-Latallo, J. F.; Candido, K. A.; Cao, Z.; Nigavekar, S. S.; Majoros, I. J.; Thomas, T. P.; Balogh, L. P.; Khan, M. K.; Baker, J. R., Jr. Nanoparticle targeting of anticancer drug improves therapeutic response in animal model of human epithelial cancer. *Cancer Res.* **2005**, *65* (12), 5317−5324.

(32) Dixit, V.; Van den Bossche, J.; Sherman, D. M.; Thompson, D. H.; Andres, R. P. Synthesis and grafting of thioctic acid-PEG-folate conjugates onto Au nanoparticles for selective targeting of folate receptor-positive tumor cells. *Bioconjugate Chem.* **2006**, *17* (3), 603−609.

(33) Gu, X. G.; Schmitt, M.; Hiasa, A.; Nagata, Y.; Ikeda, H.; Sasaki, Y.; Akiyoshi, K.; Sunamoto, J.; Nakamura, H.; Kuribayashi, K.; Shiku, H. A novel hydrophobized polysaccharide/oncoprotein complex vaccine induces in vitro and in vivo cellular and humoral immune responses against HER2-expressing murine sarcomas. *Cancer Res.* **1998**, *58* (15), 3385−3390.

(34) Shiku, H.; Wang, L.; Ikuta, Y.; Okugawa, T.; Schmitt, M.; Gu, X.; Akiyoshi, K.; Sunamoto, J.; Nakamura, H. Development of a cancer vaccine: peptides, proteins, and DNA. *Cancer Chemother. Pharmacol.* **2000**, *46* Suppl, S77−82.

(35) Farokhzad, O. C.; Cheng, J.; Teply, B. A.; Sherifi, I.; Jon, S.; Kantoff, P. W.; Richie, J. P.; Langer, R. Targeted nanoparticle-

aptamer bioconjugates for cancer chemotherapy in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103* (16), 6315−5320.
(36) Cheng, J.; Teply, B. A.; Sherifi, I.; Sung, J.; Luther, G.; Gu, F. X.; Levy-Nissenbaum, E.; Radovic-Moreno, A. F.; Langer, R.; Farokhzad, O. C. Formulation of functionalized PLGA-PEG nanoparticles for in vivo targeted drug delivery. *Biomaterials* **2007**, *28* (5), 869−876.
(37) Saul, J. M.; Annapragada, A. V.; Bellamkonda, R. V. A dual-ligand approach for enhancing targeting selectivity of therapeutic nanocarriers. *J. Controlled Release* **2006**, *114* (3), 277−287.
(38) Shiah, J. J.; Sun, Y.; Peterson, C. M.; Kopecek, J. Biodistribution of free and N-(2-hydroxypropyl)methacrylamide copolymer-bound mesochlorin e(6) and adriamycin in nude mice bearing human ovarian carcinoma OVCAR-3 xenografts. *J. Controlled Release* **1999**, *61* (1−2), 145−157.
(39) Torchilin, V. P. Lipid-core micelles for targeted drug delivery. *Curr. Drug Delivery* **2005**, *2* (4), 319−327.
(40) Nishiyama, N.; Okazaki, S.; Cabral, H.; Miyamoto, M.; Kato, Y.; Sugiyama, Y.; Nishio, K.; Matsumura, Y.; Kataoka, K. Novel cisplatin-incorporated polymeric micelles can eradicate solid tumors in mice. *Cancer Res.* **2003**, *63* (24), 8977−8983.
(41) Verma, I. M.; Weitzman, M. D. Gene therapy: twenty-first century medicine. *Annu. Rev. Biochem.* **2005**, *74*, 711−738.
(42) Nishiyama, N.; Kato, Y.; Sugiyama, Y.; Kataoka, K. Cisplatin-loaded polymer-metal complex micelle with time-modulated decaying property as a novel drug delivery system. *Pharm. Res.* **2001**, *18* (7), 1035−1041.
(43) Devalapally, H.; Shenoy, D.; Little, S.; Langer, R.; Amiji, M. Poly(ethylene oxide)-modified poly(beta-amino ester) nanoparticles as a pH-sensitive system for tumor-targeted delivery of hydrophobic drugs: part 3. Therapeutic efficacy and safety studies in ovarian cancer xenograft model. *Cancer Chemother. Pharmacol.* **2006**, Epub Jul 22, DOI 10.1007/s00280-006-0287-5.
(44) Helmlinger, G.; Yuan, F.; Dellian, M.; Jain, R. K. Interstitial pH and pO2 gradients in solid tumors in vivo: high-resolution measurements reveal a lack of correlation. *Nat. Med.* **1997**, *3* (2), 177−182.
(45) Wike-Hooley, J. L.; Haveman, J.; Reinhold, H. S. The relevance of tumour pH to the treatment of malignant disease. *Radiother. Oncol.* **1984**, *2* (4), 343−366.
(46) Dobbs, S. P.; Hewett, P. W.; Johnson, I. R.; Carmichael, J.; Murray, J. C. Angiogenesis is associated with vascular endothelial growth factor expression in cervical intraepithelial neoplasia. *Br. J. Cancer* **1997**, *76* (11), 1410−1415.
(47) Rettig, W. J.; Garin-Chesa, P.; Healey, J. H.; Su, S. L.; Jaffe, E. A.; Old, L. J. Identification of endosialin, a cell surface glycoprotein of vascular endothelial cells in human cancer. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89* (22), 10832−10836.
(48) Hobbs, S. K.; Monsky, W. L.; Yuan, F.; Roberts, W. G.; Griffith, L.; Torchilin, V. P.; Jain, R. K. Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95* (8), 4607−4612.
(49) Matsumura, Y.; Maeda, H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Res.* **1986**, *46* (12 Pt 1), 6387−6392.
(50) Muggia, F. M. Doxorubicin-polymer conjugates: further demonstration of the concept of enhanced permeability and retention. *Clin. Cancer Res.* **1999**, *5* (1), 7−8.
(51) Heuser, L. S.; Miller, F. N. Differential macromolecular leakage from the vasculature of tumors. *Cancer* **1986**, *57* (3), 461−464.
(52) Jain, R. K. Barriers to drug delivery in solid tumors. *Sci. Am.* **1994**, *271* (1), 58−65.
(53) Blanco, M. D.; Sastre, R. L.; Teijon, C.; Olmo, R.; Teijon, J. M. 5-Fluorouracil-loaded microspheres prepared by spray-drying poly-(D,L-lactide) and poly(lactide-co-glycolide) polymers: characterization and drug release. *J. Microencapsulation* **2005**, *22* (6), 671−682.
(54) Kilic, A. C.; Capan, Y.; Vural, I.; Gursoy, R. N.; Dalkara, T.; Cuine, A.; Hincal, A. A. Preparation and characterization of PLGA nanospheres for the targeted delivery of NR2B-specific antisense oligonucleotides to the NMDA receptors in the brain. *J. Microencapsulation* **2005**, *22* (6), 633−641.
(55) Olivier, J. C. Drug transport to brain with targeted nanoparticles. *NeuroRx* **2005**, *2* (1), 108−119.
(56) Eley, J. G.; Pujari, V. D.; McLane, J. Poly (lactide-co-glycolide) nanoparticles containing coumarin-6 for suppository delivery: in vitro release profile and in vivo tissue distribution. *Drug Delivery* **2004**, *11* (4), 255−261.
(57) Samlowski, W. E.; McGregor, J. R.; Jurek, M.; Baudys, M.; Zentner, G. M.; Fowers, K. D. ReGel polymer-based delivery of interleukin-2 as a cancer treatment. *J. Immunother.* **2006**, *29* (5), 524−535.
(58) Lupi, A.; Perugini, P.; Genta, I.; Modena, T.; Conti, B.; Casado, B.; Cetta, G.; Pavanetto, F.; Iadarola, P. Biodegradable microspheres for prolidase delivery to human cultured fibroblasts. *J. Pharm. Pharmacol.* **2004**, *56* (5), 597−603.
(59) Gavini, E.; Chetoni, P.; Cossu, M.; Alvarez, M. G.; Saettone, M. F.; Giunchedi, P. PLGA microspheres for the ocular delivery of a peptide drug, vancomycin using emulsification/spray-drying as the preparation method: in vitro/in vivo studies. *Eur. J. Pharm. Biopharm.* **2004**, *57* (2), 207−212.
(60) Aukunuru, J. V.; Ayalasomayajula, S. P.; Kompella, U. B. Nanoparticle formulation enhances the delivery and activity of a vascular endothelial growth factor antisense oligonucleotide in human retinal pigment epithelial cells. *J. Pharm. Pharmacol.* **2003**, *55* (9), 1199−1206.
(61) Walter, E.; Dreher, D.; Kok, M.; Thiele, L.; Kiama, S. G.; Gehr, P.; Merkle, H. P. Hydrophilic poly(DL-lactide-co-glycolide) microspheres for the delivery of DNA to human-derived macrophages and dendritic cells. *J. Controlled Release* **2001**, *76* (1−2), 149−168.
(62) Panyam, J.; Zhou, W. Z.; Prabha, S.; Sahoo, S. K.; Labhasetwar, V. Rapid endo-lysosomal escape of poly(DL-lactide-co-glycolide) nanoparticles: implications for drug and gene delivery. *FASEB J.* **2002**, *16* (10), 1217−1226.
(63) Panyam, J.; Labhasetwar, V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. *Adv. Drug Delivery Rev.* **2003**, *55* (3), 329−347.
(64) Jain, R. A. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. *Biomaterials* **2000**, *21* (23), 2475−2490.
(65) Lin, S. Y.; Chen, K. S.; Teng, H. H.; Li, M. J. In vitro degradation and dissolution behaviours of microspheres prepared by three low molecular weight polyesters. *J. Microencapsulation* **2000**, *17* (5), 577−586.
(66) Meinel, L.; Zoidis, E.; Zapf, J.; Hassa, P.; Hottiger, M. O.; Auer, J. A.; Schneider, R.; Gander, B.; Luginbuehl, V.; Bettschart-Wolfisberger, R.; Illi, O. E.; Merkle, H. P.; von Rechenberg, B. Localized insulin-like growth factor I delivery to enhance new bone formation. *Bone* **2003**, *33* (4), 660−672.
(67) Saito, N.; Murakami, N.; Takahashi, J.; Horiuchi, H.; Ota, H.; Kato, H.; Okada, T.; Nozaki, K.; Takaoka, K. Synthetic biodegradable polymers as drug delivery systems for bone morphogenetic proteins. *Adv. Drug Delivery Rev.* **2005**, *57* (7), 1037−1048.
(68) Kang, B. K.; Chon, S. K.; Kim, S. H.; Jeong, S. Y.; Kim, M. S.; Cho, S. H.; Lee, H. B.; Khang, G. Controlled release of paclitaxel from microemulsion containing PLGA and evaluation of anti-tumor activity in vitro and in vivo. *Int. J. Pharm.* **2004**, *286* (1−2), 147−156.
(69) Sengupta, S.; Eavarone, D.; Capila, I.; Zhao, G.; Watson, N.; Kiziltepe, T.; Sasisekharan, R. Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system. *Nature* **2005**, *436* (7050), 568−572.
(70) Yezhelyev, M. V.; Gao, X.; Xing, Y.; Al-Hajj, A.; Nie, S.; O'Regan, R. M. Emerging use of nanoparticles in diagnosis and treatment of breast cancer. *Lancet Oncol.* **2006**, *7* (8), 657−667.
(71) Alyaudtin, R. N.; Reichel, A.; Lobenberg, R.; Ramge, P.; Kreuter, J.; Begley, D. J. Interaction of poly(butylcyanoacrylate) nanoparticles with the blood-brain barrier in vivo and in vitro. *J. Drug Targeting* **2001**, *9* (3), 209−221.
(72) Schroeder, U.; Sommerfeld, P.; Ulrich, S.; Sabel, B. A. Nanoparticle technology for delivery of drugs across the blood-brain barrier. *J. Pharm. Sci.* **1998**, *87* (11), 1305−1307.
(73) Borm, P. J.; Robbins, D.; Haubold, S.; Kuhlbusch, T.; Fissan, H.; Donaldson, K.; Schins, R.; Stone, V.; Kreyling, W.; Lademann, J.; Krutmann, J.; Warheit, D.; Oberdorster, E. The potential risks of nanomaterials: a review carried out for ECETOC. *Part. Fibre Toxicol.* **2006**, *3*, 11.

(74) Huwyler, J.; Wu, D.; Pardridge, W. M. Brain drug delivery of small molecules using immunoliposomes. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93* (24), 14164−14169.
(75) Zhang, Y.; Schlachetzki, F.; Pardridge, W. M. Global non-viral gene transfer to the primate brain following intravenous administration. *Mol. Ther.* **2003**, *7* (1), 11−18.
(76) Cui, Z.; Lockman, P. R.; Atwood, C. S.; Hsu, C. H.; Gupte, A.; Allen, D. D.; Mumper, R. J. Novel D-penicillamine carrying nanoparticles for metal chelation therapy in Alzheimer's and other CNS diseases. *Eur. J. Pharm. Biopharm.* **2005**, *59* (2), 263−272.
(77) Lundstrom, K. Latest development in viral vectors for gene therapy. *Trends Biotechnol.* **2003**, *21* (3), 117−122.
(78) Ye, K.; Jin, S. Potent and specific inhibition of retrovirus production by coexpression of multiple siRNAs directed against different regions of viral genomes. *Biotechnol. Prog.* **2006**, *22* (1), 45−52.
(79) Oka, M.; Chang, L. J.; Costantini, F.; Terada, N. Lentiviral vector-mediated gene transfer in embryonic stem cells. *Methods Mol. Biol.* **2006**, *329*, 273−281.
(80) Fedorova, E.; Battini, L.; Prakash-Cheng, A.; Marras, D.; Gusella, G. L. Lentiviral gene delivery to CNS by spinal intrathecal administration to neonatal mice. *J. Gene Med.* **2006**, *8* (4), 414−424.
(81) Dodart, J. C.; Marr, R. A.; Koistinaho, M.; Gregersen, B. M.; Malkani, S.; Verma, I. M.; Paul, S. M. Gene delivery of human apolipoprotein E alters brain Abeta burden in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102* (4), 1211−1216.
(82) Naldini, L.; Blomer, U.; Gage, F. H.; Trono, D.; Verma, I. M. Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93* (21), 11382−11388.
(83) Yu, J. H.; Schaffer, D. V. Advanced, targeting strategies for murine retroviral and adeno-associated viral vectors. *Adv. Biochem. Eng. Biotechnol.* **2005**, *99*, 147−167.
(84) Roberts, D. M.; Nanda, A.; Havenga, M. J.; Abbink, P.; Lynch, D. M.; Ewald, B. A.; Liu, J.; Thorner, A. R.; Swanson, P. E.; Gorgone, D. A.; Lifton, M. A.; Lemckert, A. A.; Holterman, L.; Chen, B.; Dilraj, A.; Carville, A.; Mansfield, K. G.; Goudsmit, J.; Barouch, D. H. Hexon-chimaeric adenovirus serotype 5 vectors circumvent pre-existing anti-vector immunity. *Nature* **2006**, *441* (7090), 239−243.
(85) Glasgow, J. N.; Bauerschmitz, G. J.; Curiel, D. T.; Hemminki, A. Transductional and transcriptional targeting of adenovirus for clinical applications. *Curr. Gene Ther.* **2004**, *4* (1), 1−14.
(86) Noureddini, S. C.; Curiel, D. T. Genetic targeting strategies for adenovirus. *Mol. Pharm.* **2005**, *2* (5), 341−347.
(87) Wu, Z.; Asokan, A.; Samulski, R. J. Adeno-associated virus serotypes: vector toolkit for human gene therapy. *Mol. Ther.* **2006**, *14* (3), 316−327.
(88) Burger, C.; Nash, K.; Mandel, R. J. Recombinant adeno-associated viral vectors in the nervous system. *Hum. Gene Ther.* **2005**, *16* (7), 781−791.
(89) Kapturczak, M. H.; Chen, S.; Agarwal, A. Adeno-associated virus vector-mediated gene delivery to the vasculature and kidney. *Acta Biochim. Pol.* **2005**, *52* (2), 293−299.
(90) Miller, D. G.; Wang, P. R.; Petek, L. M.; Hirata, R. K.; Sands, M. S.; Russell, D. W. Gene targeting in vivo by adeno-associated virus vectors. *Nat. Biotechnol.* **2006**, *24* (8), 1022−1026.
(91) Wu, Q.; Xia, D.; Carlsen, S.; Xiang, J. Adenovirus-mediated transgene-engineered dendritic cell vaccine of cancer. *Curr. Gene Ther.* **2005**, *5* (2), 237−247.
(92) Young, L. S.; Searle, P. F.; Onion, D.; Mautner, V. Viral gene therapy strategies: from basic science to clinical application. *J. Pathol.* **2006**, *208* (2), 299−318.
(93) Wong, L. F.; Goodhead, L.; Prat, C.; Mitrophanous, K. A.; Kingsman, S. M.; Mazarakis, N. D. Lentivirus-mediated gene transfer to the central nervous system: therapeutic and research applications. *Hum. Gene Ther.* **2006**, *17* (1), 1−9.
(94) Feng, X.; Eide, F. F.; Jiang, H.; Reder, A. T. Adeno-associated, viral vector-mediated ApoE expression in Alzheimer's disease mice: low CNS immune response, long-term expression, and astrocyte specificity. *Front. Biosci.* **2004**, *9*, 1540−1546.
(95) Nemunaitis, J.; Khuri, F.; Ganly, I.; Arseneau, J.; Posner, M.; Vokes, E.; Kuhn, J.; McCarty, T.; Landers, S.; Blackburn, A.; Romel, L.; Randlev, B.; Kaye, S.; Kirn, D. Phase II trial of intratumoral administration of ONYX-015, a replication-selective adenovirus, in patients with refractory head and neck cancer. *J. Clin. Oncol.* **2001**, *19* (2), 289−298.
(96) Bangari, D. S.; Mittal, S. K. Current strategies and future directions for eluding adenoviral vector immunity. *Curr. Gene Ther.* **2006**, *6* (2), 215−226.
(97) Mastrobattista, E.; van der Aa, M. A.; Hennink, W. E.; Crommelin, D. J. Artificial viruses: a nanotechnological approach to gene delivery. *Nat. Rev. Drug Discovery* **2006**, *5* (2), 115−121.
(98) Tan, W.; Wang, K.; He, X.; Zhao, X. J.; Drake, T.; Wang, L.; Bagwe, R. P. Bionanotechnology based on silica nanoparticles. *Med. Res. Rev.* **2004**, *24* (5), 621−638.
(99) Singh, M.; Briones, M.; Ott, G.; O'Hagan, D. Cationic microparticles: A potent delivery system for DNA vaccines. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97* (2), 811−816.
(100) Nomura, T.; Koreeda, N.; Yamashita, F.; Takakura, Y.; Hashida, M. Effect of particle size and charge on the disposition of lipid carriers after intratumoral injection into tissue-isolated tumors. *Pharm. Res.* **1998**, *15* (1), 128−132.
(101) Kaul, G.; Amiji, M. Tumor-targeted gene delivery using poly(ethylene glycol)-modified gelatin nanoparticles: in vitro and in vivo studies. *Pharm. Res.* **2005**, *22* (6), 951−961.
(102) Kneuer, C.; Sameti, M.; Bakowsky, U.; Schiestel, T.; Schirra, H.; Schmidt, H.; Lehr, C. M. A nonviral DNA delivery system based on surface modified silica-nanoparticles can efficiently transfect cells in vitro. *Bioconjugate Chem.* **2000**, *11* (6), 926−932.
(103) Kneuer, C.; Sameti, M.; Haltner, E. G.; Schiestel, T.; Schirra, H.; Schmidt, H.; Lehr, C. M. Silica nanoparticles modified with aminosilanes as carriers for plasmid DNA. *Int. J. Pharm.* **2000**, *196* (2), 257−261.
(104) Reszka, R.; Zhu, J. H.; Weber, F. Liposome mediated transfer of marker and cytokine genes into rat and human Glioblastoma, cells in vitro and in vivo. *J. Lipsome Res.* **1995**, *5*, 149−154.
(105) Junghans, M.; Kreuter, J.; Zimmer, A. Antisense delivery using protamine-oligonucleotide particles. *Nucleic Acids Res.* **2000**, *28* (10), E45.
(106) Schwab, G.; Chavany, C.; Duroux, I.; Goubin, G.; Lebeau, J.; Helene, C.; Saison-Behmoaras, T. Antisense oligonucleotides adsorbed to polyalkylcyanoacrylate nanoparticles specifically inhibit mutated Ha-ras-mediated cell proliferation and tumorigenicity in nude mice. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91* (22), 10460−10464.
(107) Erbacher, P.; Zou, S.; Bettinger, T.; Steffan, A. M.; Remy, J. S. Chitosan-based vector/DNA complexes for gene delivery: biophysical characteristics and transfection ability. *Pharm. Res.* **1998**, *15* (9), 1332−1339.
(108) He, X.; Wang, K.; Tan, W.; Liu, B.; Liu, X.; Huang, S.; Li, D.; He, C.; Li, J. A novel gene carrier based on amino-modified silica nanoparticles. *Chin. Sci. Bull.* **2003**, *48* (3), 223−228.
(109) He, X.; Wang, K.; Tan, W.; Liu, B.; Liu, X.; Huang, S.; Li, D.; He, C.; Li, J. Bioconjuated nanoparticles for DNA protection from cleavage. *J. Am. Chem. Soc.* **2003**, *125*, 7168−7169.
(110) Bharali, D. J.; Klejbor, I.; Stachowiak, E. K.; Dutta, P.; Roy, I.; Kaur, N.; Bergey, E. J.; Prasad, P. N.; Stachowiak, M. K. Organically modified silica nanoparticles: a nonviral vector for in vivo gene delivery and expression in the brain. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102* (32), 11539−11544.
(111) Roy, I.; Ohulchanskyy, T. Y.; Bharali, D. J.; Pudavar, H. E.; Mistretta, R. A.; Kaur, N.; Prasad, P. N. Optical tracking of organically modified silica nanoparticles as DNA carriers: a nonviral, nanomedicine approach for gene delivery. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102* (2), 279−284.
(112) Kaul, G.; Amiji, M. Protein nanospheres for gene delivery. In *Polymeric Gene Delivery: Principles and Applications*; Amiji, M. M., Ed.; CRC Press: Boca Raton, FL, 2004; pp 429−447.
(113) Veis, A. *The Macromolecular Chemstry of Gelatin*; Academic Press: New York, 1964.
(114) Kaul, G.; Amiji, M. Long-circulating poly(ethylene glycol)-modified gelatin nanoparticles for intracellular delivery. *Pharm. Res.* **2002**, *19* (7), 1061−1067.
(115) Kaul, G.; Lee-Parsons, C.; Amiji, M. poly(ethylene glycol)-modified gelatin nanoparticles for intracellular delivery. *Pharm. Eng.* **2003**, *23*, 108−114.

(116) Kaul, G.; Amiji, M. Cellular interactions and in vitro DNA transfection studies with poly(ethylene glycol)-modified gelatin nanoparticles. *J. Pharm. Sci.* **2005**, *94* (1), 184−198.
(117) Kaul, G.; Amiji, M. Biodistribution and targeting potential of poly(ethylene glycol)-modified gelatin nanoparticles in subcutaneous murine tumor model. *J. Drug Targeting* **2004**, *12* (9−10), 585−591.
(118) Chiu, S. J.; Ueno, N. T.; Lee, R. J. Tumor-targeted gene delivery via anti-HER2 antibody (trastuzumab, Herceptin) conjugated polyethylenimine. *J. Controlled Release* **2004**, *97* (2), 357−369.
(119) Hood, J. D.; Bednarski, M.; Frausto, R.; Guccione, S.; Reisfeld, R. A.; Xiang, R.; Cheresh, D. A. Tumor regression by targeted gene delivery to the neovasculature. *Science* **2002**, *296* (5577), 2404−2407.
(120) Li, Y.; Wang, J.; Lee, C. G.; Wang, C. Y.; Gao, S. J.; Tang, G. P.; Ma, Y. X.; Yu, H.; Mao, H. Q.; Leong, K. W.; Wang, S. CNS gene transfer mediated by a novel controlled release system based on DNA complexes of degradable polycation PPE-EA: a comparison with polyethylenimine/DNA complexes. *Gene Ther.* **2004**, *11* (1), 109−114.
(121) Ahl, P. L.; Bhatia, S. K.; Meers, P.; Roberts, P.; Stevens, R.; Dause, R.; Perkins, W. R.; Janoff, A. S. Enhancement of the in vivo circulation lifetime of L-alpha-distearoylphosphatidylcholine liposomes: importance of liposomal aggregation versus complement opsonization. *Biochim. Biophys. Acta* **1997**, *1329* (2), 370−382.
(122) Porter, C. J.; Moghimi, S. M.; Illum, L.; Davis, S. S. The polyoxyethylene/polyoxypropylene block co-polymer poloxamer-407 selectively redirects intravenously injected microspheres to sinusoidal endothelial cells of rabbit bone marrow. *FEBS Lett.* **1992**, *305* (1), 62−66.
(123) Kong, G.; Braun, R. D.; Dewhirst, M. W. Hyperthermia enables tumor-specific nanoparticle delivery: effect of particle size. *Cancer Res.* **2000**, *60* (16), 4440−4445.
(124) Prabha, S.; Zhou, W. Z.; Panyam, J.; Labhasetwar, V. Size-dependency of nanoparticle-mediated gene transfection: studies with fractionated nanoparticles. *Int. J. Pharm.* **2002**, *244* (1−2), 105−115.
(125) Tang, M. X.; Redemann, C. T.; Szoka, F. C., Jr. In vitro gene delivery by degraded polyamidoamine dendrimers. *Bioconjugate Chem.* **1996**, *7* (6), 703−714.
(126) Barron, L. G.; Gagne, L.; Szoka, F. C., Jr. Lipoplex-mediated gene delivery to the lung occurs within 60 minutes of intravenous administration. *Hum. Gene Ther.* **1999**, *10* (10), 1683−1694.
(127) Zhang, J.-S.; Liu, F.; Huang, L. Implications of pharmacokinetic behavior of lipoplex for its imfalammatory toxicity. *Adv. Drug Delivery Rev.* **2005**, *57*, 689−698.
(128) Hayes, M. E.; Drummond, D. C.; Kirpotin, D. B.; Zheng, W. W.; Noble, C. O.; Park, J. W.; Marks, J. D.; Benz, C. C.; Hong, K. Genospheres: self-assembling nucleic acid-lipid nanoparticles suitable for targeted gene delivery. *Gene Ther.* **2006**, *13* (7), 646−651.
(129) Toub, N.; Malvy, C.; Fattal, E.; Couvreur, P. Innovative nanotechnologies for the delivery of oligonucleotides and siRNA. *Biomed. Pharmacother.* **2006**, *60* (9), 607−620.
(130) Pille, J. Y.; Li, H.; Blot, E.; Bertrand, J. R.; Pritchard, L. L.; Opolon, P.; Maksimenko, A.; Lu, H.; Vannier, J. P.; Soria, J.; Malvy, C.; Soria, C. Intravenous delivery of anti-RhoA small interfering RNA loaded in nanoparticles of chitosan in mice: safety and efficacy in xenografted aggressive breast cancer. *Hum. Gene Ther.* **2006**, *17* (10), 1019−1026.
(131) Urban-Klein, B.; Werth, S.; Abuharbeid, S.; Czubayko, F.; Aigner, A. RNAi-mediated gene-targeting through systemic application of polyethylenimine (PEI)-complexed siRNA in vivo. *Gene Ther.* **2005**, *12* (5), 461−466.
(132) Schiffelers, R. M.; Ansari, A.; Xu, J.; Zhou, Q.; Tang, Q.; Storm, G.; Molema, G.; Lu, P. Y.; Scaria, P. V.; Woodle, M. C. Cancer siRNA therapy by tumor selective delivery with ligand-targeted sterically stabilized nanoparticle. *Nucleic Acids Res.* **2004**, *32* (19), e149.
(133) Hoet, P. H.; Bruske-Hohlfeld, I.; Salata, O. V. Nanoparticles - known and unknown health risks. *J. Nanobiotechnol.* **2004**, *2* (1), 12.
(134) Nel, A.; Xia, T.; Madler, L.; Li, N. Toxic potential of materials at the nanolevel. *Science* **2006**, *311* (5761), 622−627.
(135) Colvin, V. L. The potential environmental impact of engineered nanomaterials. *Nat. Biotechnol.* **2003**, *21* (10), 1166−1170.
(136) Lovric, J.; Bazzi, H. S.; Cuie, Y.; Fortin, G. R.; Winnik, F. M.; Maysinger, D. Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots. *J. Mol. Med.* **2005**, *83* (5), 377−385.
(137) Hardman, R. A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. *Environ. Health Perspect.* **2006**, *114* (2), 165−172.
(138) Borm, P. J.; Kreyling, W. Toxicological hazards of inhaled nanoparticles−potential implications for drug delivery. *J. Nanosci. Nanotechnol.* **2004**, *4* (5), 521−531.
(139) Portney, N. G.; Ozkan, M. Nano-oncology: drug delivery, imaging, and sensing. *Anal. Bioanal. Chem.* **2006**, *384* (3), 620−630.
(140) Mukherjee, P.; Bhattacharya, R.; Wang, P.; Wang, L.; Basu, S.; Nagy, J. A.; Atala, A.; Mukhopadhyay, D.; Soker, S. Antiangiogenic properties of gold nanoparticles. *Clin. Cancer Res.* **2005**, *11* (9), 3530−3534.

Received November 15, 2006. Accepted December 11, 2006.

BP060348J