AJNR

Uses of Nanoparticles for Central Nervous System Imaging and Therapy

J.M. Provenzale and G.A. Silva

Uses of Nanoparticles for Central Nervous System Imaging and Therapy

J.M. Provenzale
G.A. Silva

SUMMARY: Applications of nanotechnology to medicine are leading to novel means of imaging living systems and of delivering therapy. Much nanotechnology research is focused on methods for imaging central nervous system functions and disease states. In this review, the principles of nanoparticle design and function are discussed with specific emphasis on applications to neuroradiology. In addition to innovative forms of imaging, this review describes therapeutic uses of nanoparticles, such as drug delivery systems, neuroprotection devices, and methods for tissue regeneration.

In the past decade, rapid advances have been seen in applications of nanotechnology for medical uses, with the emergence of a field termed "nanomedicine." "Nanotechnology" refers to the development of materials and devices on the nanometer scale (ie, 1–100 nm) for manipulation of physical, chemical, or biologic processes.[1] "Nanoparticles" are solid colloidal particles on the nanometer scale, which are frequently composed of insoluble polymers.[2] The size range of nanoparticles can be understood in a biologic context: Viruses range from 20 to 300 nm; the diameter of a cell ranges from 30 μm (3000 nm) to 300 μm (300,000 nm). The small size of nanoparticles in relation to the size of cells allows nanoparticles to be designed for such functions as binding to cell membranes, delivery of agents into cells or across anatomic and physiologic compartments (eg, the blood-brain barrier [BBB]), and monitoring cellular physiologic events. Performance of such functions would be difficult or impossible at a larger range of particle size.

The field of nanomedicine is rapidly developing. Little doubt exists that nanotechnologic research will lead to advances in imaging. If neuroradiologists wish to play a role in promoting and guiding the progress of nanomedicine, they should understand the basics of the techniques and, to whatever extent possible, participate in research. The purpose of this review is to acquaint the neuroradiologist with principles and challenges associated with nanoparticle design and function and to discuss ways in which nanotechnology may be applied to central nervous system (CNS) imaging and treatment of CNS diseases.

General Principles Regarding Nanoparticles

Basic Composition
Nanoparticles can be made from many different types of materials. Besides polymers, nanoparticles can be composed of ceramics, carbon, and various metals. They can be designed in a number of shapes, such as tubes, rods, hollow or solid spheres, and complex strands. Some materials and shapes are optimal for a specific application. Thus, there is no single pre-

ferred type of nanoparticle; rather, the adequacy of the material and shape used is dependent on the use for which the nanoparticle is intended. Nanoparticles for biologic imaging and/or delivery of therapeutic agents often consist of a central structure on which (or within which) an imaging agent or drug is held.[3]

Types of Nanoparticles
The following explanation of nanoparticles solely describes features of 4 of the most commonly used types of nanoparticles. Many more types exist but are not described here for the sake of brevity. The reader is referred to several excellent reviews that provide information about the broader range of nanoparticles in more detail.[1,3]

Iron Oxide Particles. Iron oxide particles were one of the first nanoparticles developed[4] and arguably have had the widest range of applications yet, compared with other nanoparticles.[5] These particles have already received approval by the US Food and Drug Administration (FDA) for some clinical uses, specifically in imaging the bowel and abdominal viscera.[6] Iron oxide nanoparticles contain ultrasmall paramagnetic iron oxide particles, which allow them to produce an increase in signal intensity on T1-weighted images, similar to that seen with commercially available gadolinium-containing MR imaging contrast agents.[7] These agents have the advantage of also producing signal-intensity decrease on T2*-weighted images.[8]

The application of a Dextran coating (Pharmacose, Holbaek, Denmark) on the surface of the nanoparticles provides them with a much longer half-life (on the order of 24–30 hours) than conventional MR imaging contrast agents; as a result, imaging can be performed many hours after infusion.[8] Advantages include allowing a single infusion of contrast material many hours before surgery while still being able to perform intraoperative MR imaging by using the same dose of contrast material. For instance, one type of iron oxide nanoparticle termed ferumoxtran-10 can be detected in surgical and biopsy specimens by using an iron stain. Thus, surgical specimens can be microscopically examined to determine whether the resected specimen was removed from enhancing portions of the tumor. This process allows better identification of sites on imaging studies from which tissue has been resected as well as closer correlation between imaging findings and histologic features than would be available with routine contrast agents.

Nanoshells. "Nanoshells" are composed of a dielectric core (often made of silicon or another metal) covered by a metallic shell, which is often composed of gold. The relative dimen-
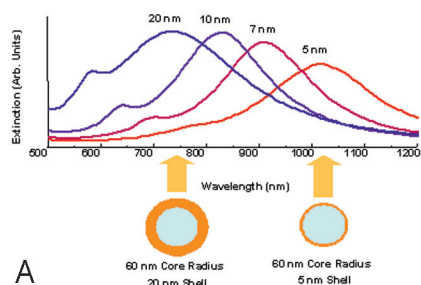
Fig 1. Mie-scattering plot depicting a nanoshell plasmon resonance wavelength shift in relation to variation of the thickness of the gold shell in a nanoparticle having a silica core with a radius of 60 nm. *A,* Graph shows that optical resonances of gold nanoshells (as exhibited by wavelength of light emitted on x-axis) is dependent on the size of the nanoparticle core and shell. Numbers above the wave peaks indicate the thickness of shell. *B,* Image shows vials, each of which contains nanoparticles of a specific composition. Because the light-emitting properties of the nanoparticles depend on nanoparticle composition, each vial emits a unique wavelength of light and thus has a color different from the other vials. Arb indicates arbitrary. Published with permission from: Nanoshell-enabled photonics-based imaging and therapy of cancer. *Technol Cancer Res Treat* 3.33– 40, 2004. Published by Adenine Press.www.adenine-press.com.[9] Photograph courtesy of C. Radloff.

sions of the core and the shell can be modified in such a manner that the particles emit light of a characteristic wavelength, a feature referred to as "spectral tunability" (Fig 1). As a result of these optical properties, nanoshells can serve as optical imaging agents.[9] In addition, gold nanoshells can be designed for absorption of light in the near-infrared portion of the light spectrum. As a consequence, the nanoparticles can be heated on application of light of the appropriate wavelength and the resultant heat can be used for thermal ablation of tumors.[10]

Quantum Dots. Semiconductor quantum dot nanoparticles have unique optical and electronic properties,[11] which make them particularly well-suited for cellular imaging. Quantum dots typically have a heavy metal core composed of materials such as cadmium selenium or cadmium telluride, which is surrounded by an intermediate unreactive metallic shell (typically zinc sulfide). The outer coating of the particle can be chemically functionalized with bioactive molecules that enable targeting of specific molecules (eg, cell membrane receptors).[12]

Advantages of quantum dots include light emission that is size-dependent (ie, the wavelength of emitted light is related to small changes in the semiconductor core of the nanoparticle due to a quantum mechanical phenomenon called quantum confinement), very bright signal intensity, and resistance to photobleaching with time (ie, photostability).[11] This last feature is important because it allows quantum dots to be used for imaging for longer periods of time than is allowed with other optical imaging agents, such as organic fluorophores. The relationship between the size of the nanoparticle and the wavelength of light emission (tunability) is important because it allows different particles to be used simultaneously in the same biologic preparation or tissue; the presence and location of individual quantum dots can be determined by the color they emit. For example, quantum dots of a few different sizes can be used, with a different antibody used for each size of quantum dot, allowing imaging of multiple biologic targets (so-called "multiplexing capability"). For instance, use of 3 different sizes of quantum dots in an experiment, with each size quantum dot having a specific antibody, would allow 3 different

molecular targets (eg, 3 different types of receptor) to be imaged. The 3 different types of quantum dots (bound to their respective 3 targets) will be distinguishable on the basis of the emission of a specific wavelength for each type of quantum dot.

At present, the potential toxicity associated with the heavy metal content of quantum dots limits their applicability to use solely in such scenarios as tissue culture, ex vivo human tissue specimens, and animals rather than in humans. Researchers developing newer quantum dot formulations in a number of different laboratories are addressing this issue and attempting to develop quantum dots by using materials other than heavy metals to make them suitable for in vivo human use. Nonetheless, even in those restricted venues, quantum dots have already shown great promise in our understanding of both normal physiologic processes and disease states.

Liposomes and Micelles. "Liposomes" are nanoscale vesicles having a phospholipid bilayer membrane and an aqueous core. The aqueous core provides an environment in which therapeutic drugs can be sequestered for transport to target sites, thereby protecting drugs from actions at nonintended targets and from degradation.[13] Like many other types of nanoparticles, ligands that bind biologic targets can be attached to the surface of liposomes for targeted delivery. These particles can be made to provide sustained time-release of their contents.[14] Liposomal formulations of doxorubicin have been approved by the FDA for clinical use in tumors such as ovarian cancer and multiple myeloma; others are presently in the stage of testing of feasibility for human use.[15] Liposomes have recently been developed for treatment of brain tumors but are still in the investigational stage in that setting.[16]

"Micelles" are spheric aggregates of molecules, in which the hydrophilic regions of the molecules face outward and the hydrophobic portions of the molecules face inward. Micelles offer a means of allowing a compound that is normally insoluble in a particular solvent to become soluble by being sequestered in the hydrophobic core of the micelle. The external hydrophilic shell allows the micelle to serve as a nanocarrier, which permits delivery of greater amounts of a drug to a target

tissue compared with the intravenous administration of free drug.[17] Thus, micelles may offer one mechanism for increased distribution of drugs across the BBB, an issue that is discussed further below.

Principles of Nanoparticle Delivery

Issues of Biodistribution

One of the major challenges investigators face is effective delivery of nanoparticles to the organ of interest rather than to unintended targets. One major impediment in the delivery of nanoparticles administered by intravenous infusion is sequestration by the reticuloendothelial system. Nanoparticles will typically be captured in the liver and spleen unless, during the manufacturing process, a deliberate attempt is made to provide a means to escape capture by the reticuloendothelial system. The most commonly used technique is to coat the nanoparticles with a covalent attachment of polyethylene glycol (PEG), which renders the nanoparticle essentially invisible to the reticuloendothelial system; such particles are sometimes referred to as stealth particles. This process (referred to as PEGylation) substantially prolongs circulation time and allows the nanoparticle to be delivered to the organ of interest.

In many instances, nanoparticles can cross an interrupted BBB. For instance, in one study that used a common brain inflammation model (experimental allergic encephalomyelitis), nanoparticle delivery was pronounced in areas where the BBB had been disrupted and in regions of macrophage infiltration.[18] However, most CNS uses of nanoparticles are intended for conditions in which the BBB is intact. In the experimental allergic encephalomyelitis study just cited, the concentration of PEGylated nanoparticles was found to be increased even in brain regions in which the BBB was intact compared with non-PEGylated nanoparticles, especially in white matter.[18] However, in most instances, an intact BBB remains an effective barrier for preventing access of even PEGylated nanoparticles to the CNS.[19]

To address this issue, researchers have used a number of methods to adapt nanoparticles for crossing the BBB, particularly to deliver chemotherapy.[20] One agent of interest is a poly(butylcyanoacrylate) nanoparticle coated with polysorbate 80, which adsorbs apolipoproteins B and E and allows receptor-mediated endocytosis by brain capillary endothelial cells.[19,21] Polysorbate 80 nanoparticles have also been used for CNS drug therapies for nonneoplastic disorders and have similarly been shown to cross the intact BBB in reasonable amounts.[22,23] In a number of studies, doxorubicin bound to nanoparticles has been shown to cross the intact BBB and reach therapeutic levels in the brain as well as to prolong survival times significantly in rats with glioblastomas.[24] A significant challenge facing the use of these nanotechnologies for delivering drugs and other small molecules across the BBB is that in addition to their primary function of having to deliver enough drug to elicit a therapeutic effect, at the same time, the nanoparticle-drug conjugates must curb unintended systemic side effects by limiting undesired molecular interactions with cell types other than those they are designed to target. Thus, simply increasing the concentration of a nanoparticle that crosses the BBB to deliver a greater quantity of drugs may not be possible if the higher concentration results in increased nonspecific molecular interaction events. This is a formidable but unavoidable challenge that faces the development and use of nanotechnologies aimed at CNS drug delivery.

Many other techniques for enhanced delivery across the BBB are being evaluated. However, because the topic is a broad one that cannot be easily summarized here, the reader is referred to a review article specifically addressing the issue of BBB transport.[19]

Nanoparticles Directed against Targets

Nanoparticles can be targeted (ie, modified with specific binding properties directed against molecular targets) or nontargeted. Nontargeted nanoparticles passively accumulate at the site of interest (eg, within a tumor). Thus, nanoparticles administered intravenously are passively delivered under the guidance of normal blood circulation. Two phenomena allow nontargeted nanoparticles to congregate within tumors. First, vessels produced by the angiogenesis that accompanies tumor growth are known to exhibit marked leakiness, allowing extravasation of nanoparticles into the tumor microenvironment (Fig 2). In addition, because tumors typically lack an effective lymphatic drainage system, egress of nanoparticles away from the tumor is impaired.[25] This dual phenomenon has been termed the "enhanced permeability and retention effect."[25]

The term "targeted nanoparticles" refers to those manufactured with a surface ligand or other surface modification designed to allow the nanoparticle to bind to a target such as a cell membrane receptor or other protein. Thus, targeted nanoparticles can selectively bind to sites of interest (eg, tumor cell membranes) (Fig 2). A wide array of modifications of nanoparticles is available; binding of ≥ 1 antibody against the intended target is one of the more common strategies used. Some of these strategies are described in subsequent parts of this review.

Drug Delivery Using Nanoparticles

One of the major intended uses of nanoparticles is more efficacious delivery of drugs. As previously mentioned, this use of nanoparticles is especially valuable for drugs that are not easily soluble and for drugs that might be too readily metabolized when administered via other methods. Some advantages of nanoparticle-mediated transport include good carrier stability, high carrier capacity (ie, a large number of drug molecules incorporated into the particle), and the possibility of carrying both hydrophilic and hydrophobic substances, all of which can contribute to efficient drug delivery.[26]

Nanoparticle-mediated selective drug delivery may allow a means to minimize delivery to unintended sites, potentially allowing larger doses of drug to be administered and with a greater percentage of drug reaching the target, thereby possibly lowering toxicity.[27] Many methods of selective delivery exist. In addition to targeting strategies using ligands on nanoparticle surfaces mentioned earlier, strategies have been devised for release of nanoparticle contents by using stimuli external to the body by means of a focused trigger, such as light (so-called "photodynamic therapy") or heat.[28] Antineoplastic therapy by using heat-labile liposomes is one example.[29] After intravenous administration of the liposomes and passive delivery to the tumor, heating of the tumor (by using various
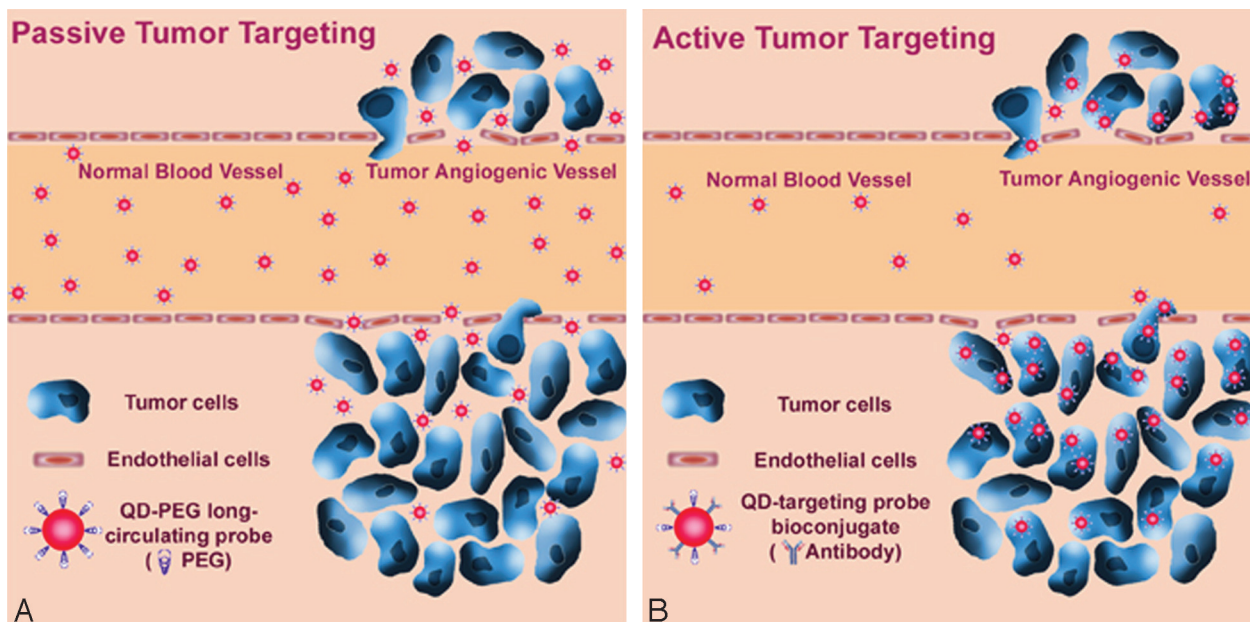
Fig 2. Nanoparticles containing therapeutic drugs against a tumor may reach their target by using either passive targeting or active targeting (ie, by using ligands that are tumor-specific). *A,* Passive tumor deposition by nontargeted nanoparticles (ie, nanoparticles that are not coated with antibody against tumor cells) is accomplished by extravasation from leaky vessels adjacent to the tumor and retention of nanoparticles at the tumor site due to slow clearance. Note that, in this diagram, nanoparticles have accumulated in the extracellular environment, rather than within tumor cells. *B,* Active targeting by the same quantum dots as in *A* but to which an antibody targeted against tumor cells has been added to the surface of the nanoparticle. Note that, in this example, nanoparticles again extravasate through leaky peritumoral vessels, but due to active targeting, they accumulate on tumor cell membranes and are incorporated within tumor cells. QD indicates quantum dots. Reprinted with permission from Macmillan Publishers Ltd; *Nature Biotechnology* 2004;22:969-76, copyright 2004.

means such as focused sonography) can cause targeted release of liposomal contents directly at the tumor, thereby minimizing systemic drug effects.

Adaptation of Nanoparticles for Specific Functions

pH-Sensitive Nanoparticles
For effective nanoparticle-borne antineoplastic therapy, nanoparticles must exit the bloodstream into the tumor interstitium, enter cells, and, in many cases, be taken up within endosomes.[30] However, each compartment has its own pH. For instance, the pH of the endosomal system (ie, range of 5.0–6.0) is substantially lower than physiologic pH (ie, pH of 7.4) and the extracellular pH of solid tumors (ie, pH of 6.8). The differing pH of various compartments can potentially affect transport and stability of nanoparticles. With this in mind, nanoparticles whose structures are altered by the pH of the local environment have been engineered.[31] Specifically, a PEG coating on a nanoparticle is reversibly removed at a pH of 6.8 and reattached at a pH of 7.4. Note that a nanoparticle of this type could potentially be altered to provide information about local pH, which might be important in many scenarios, such as understanding drug resistance to chemotherapeutic agents that are pH-sensitive.

Dual Capability Imaging Using Nanoparticles
Another innovative technique is development of nanoparticles that combine optical nanoprobe systems with the capacity for more conventional imaging techniques, such as MR imaging. As an example, investigators have developed methods to fuse fluorescent dyes and magnetic nanoparticles into a single nanoprobe.[32] In one such probe, a dye-doped silica core is surrounded by water soluble iron oxide particles, which can be detected by using T2*-weighted imaging (Fig 3). When coated with an appropriate antibody (in this case, an antibody against polysialic acids on neuroblastoma cells), the dual-purpose nanoparticles can be used to image the cells by using both fluorescence imaging and MR imaging (Fig 3). Furthermore, synergistic magnetism between the multiple iron oxide molecules markedly increases the T2 relaxivity of the agents and provides greater conspicuity on MR imaging.

Uses of Nanoparticles in CNS Processes

Tracking Functional Responses in Neurons
One advantage of nanoparticles is the enhanced ability to study and track molecular events within neurons and glia over the course of many seconds or even minutes. Types of cells can be identified, and their number, development, and physiologic responses can be monitored using fluorescence microscopy with quantum dots that are coated with an antibody directed against specific cellular features, (Fig 4).[12]

For instance, nanoparticles bound to effector compounds (ie, molecules that initiate a response in cells) can be used to interrogate functional capabilities of cells in the CNS. Such nanoparticles can serve the dual function of both an imaging probe and a functional probe that induces a cellular response, such as a change in cell metabolism. In one study, investigators bound quantum dots to nerve growth factor, a peptide hormone that targets motor, sensory, and autonomic neurons and is important for neuronal development and survival.[33] Exposure of cells from a highly tumorigenic teratoma-derived cell line to such quantum dots over the period of a few days induced changes in cell-signaling mechanisms and cell behavior as well as development of neurites extending from cell bodies.
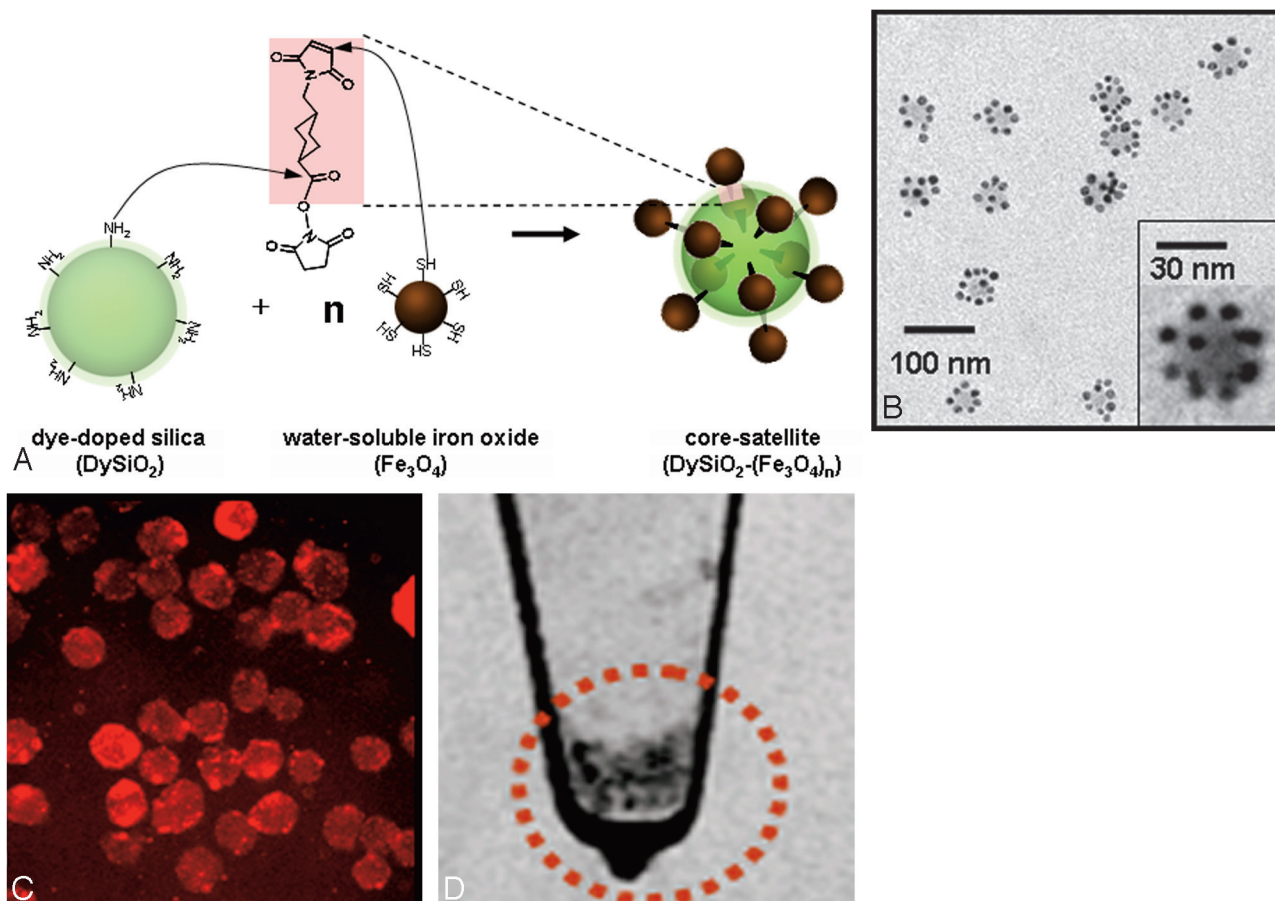
Fig 3. Use of dual-purpose nanoparticles to image neuroblastoma cells by both fluorescence imaging and MR imaging. The nanoparticles are coated with an antibody that binds to polysialic acids on neuroblastoma cell surfaces. *A*, Diagram showing components of nanoparticle, with water soluble iron oxide particles conjugated to the surface of a rhodamine dye-doped silica core by using cross-linkers (shown in pink). *B*, Transmission electron microscopy image of nanoparticles shows that the constellation of iron oxide particles (black) bound to the surface dye-doped silica core (gray) measures approximately 30 nm in diameter (inset). *C*, Fluorescence imaging of dual-purpose nanoparticles bound to neuroblastoma cells that overexpress polysialic acids shows the nanoparticle-bound cells in red. *D*, T2*-weighted MR image shows the same cells as shown in *A* as dark regions due to susceptibility effect of Fe_3O_4 molecules on nanoparticle surface. Reprinted with permission from Lee JH, Jun YW, Yeon SI, et al. Dual-mode nanoparticle probes for high-performance magnetic resonance and fluorescence imaging of neuroblastoma. *Angew Chem Int Ed Engl* 2006;45:8160–62. Copyright Wiley-VCH, Verlag GmbH & Co. KGaA.
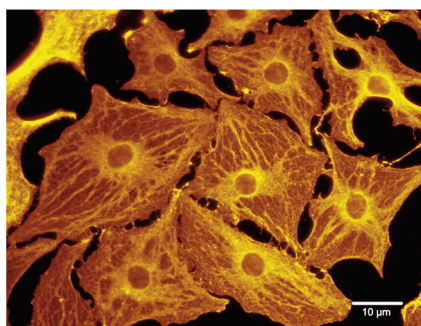

Fig 4. Use of nanoparticles to image astrocytes. Staining of cortical astrocytes by antibody-conjugated nanoparticles that cross-react with glial fibrillary acidic protein is depicted. Reprinted with permission from *J Neurosci* 2006;26:1893–95.

Nanotechnology offers a number of very interesting methods to monitor the fundamental features of cellular organization in real-time. An important issue in the understanding of synaptic development and neurotransmitter function is the mobility of neurotransmitter receptors along cell surfaces. Investigators have used quantum dots to track the lateral motion of glycine receptors (the major inhibitory neurotransmitter in the adult spinal cord) within the membranes of living cells for many minutes (Fig 5).[34] Here, the photostability (ie, sustained ability to provide signal intensity with time) and improved signal intensity–to-noise ratio (ie, fluorescence signal intensity from the quantum dot relative to nonspecific background fluorescence) of quantum dots provides a real advantage relative to standard fluorophores, which are more photolabile (ie, their fluorescent properties degrade with time).[34] In addition, the in-plane resolution offered by quantum dots is markedly superior to that offered by standard fluorophores.

Iron Oxide Nanoparticles for MR Imaging of Brain Tumors

Because ultrasmall paramagnetic iron oxide−labeled nanoparticles were among the first nanoparticles to be used in humans, investigators have gained a fair amount of experience with the use of these particles. MR imaging agents based on such technology have been used for preoperative and intraoperative imaging of human brain tumors.[8] MR imaging contrast agents that use these nanoparticles have the unique capability of contrast enhancement of brain tumors for days after administration. Most interesting, the nanoparticles appear to accumulate in macrophages and reactive astrocytes immediately adjacent to tumor cells rather than within tumor cells.

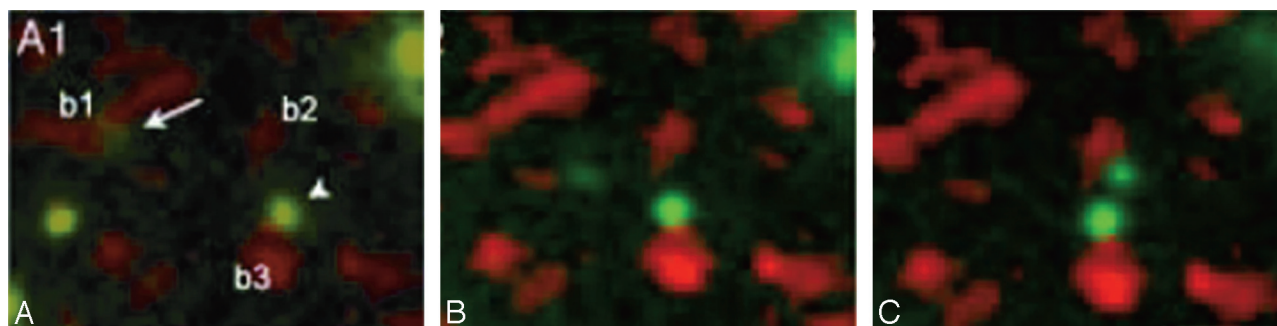Superparamagnetic nanoparticles have been developed for

Fig 5. Use of quantum dots to track the lateral mobility of neurotransmitter receptors over the surface of a neurite, which is important for understanding development and plasticity of synapses. Images are derived from a series of 850 images obtained during 60 seconds. Quantum dots coated with antibody to glycine receptor are shown in green, and synaptic boutons marked by FM4−64 dye are depicted in red. *A*, Early in the sequence, a glycine receptor (*arrow*) is seen adjacent to a synaptic bouton (b1). Two other synaptic boutons are labeled b2 and b3. Another glycine receptor (*arrowhead*) is seen adjacent to synaptic bouton b3. *B*, Approximately 8 seconds later, the glycine receptor previously located at synaptic bouton b1 has migrated to a position in the center of the field. The glycine receptor at synaptic bouton b3 has remained stationary. *C*, At the end of 1 minute, the glycine receptor originally located at synaptic bouton b1 in *A* has migrated to synaptic bouton b2. From Dahan M, Levi S, Luccardini C, et al. Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking. *Science* 2003; 302:442–45. Reprinted with permission from AAAS.

targeted therapy of brain tumors. Conjugation of the surface of the nanoparticle with chlorotoxin allows targeting of tumors of neuroectodermal origin (including gliomas) that contain membrane-bound matrix metalloproteinase-2.[7] Experiments have shown selective targeting of tumors by the chlorotoxin-conjugated nanoparticles compared with nanoparticles that are not chlorotoxin-bound. Although a very small amount of nontargeted nanoparticles were found in tumor, the amount of targeted nanoparticles in tumor was markedly greater (Fig 6). In addition, nanoparticles were not reported in organs other than the brain.

Nanoparticles as Neuroprotection Devices

In the past few decades, increased emphasis has been placed on the development of agents that could limit the effect of injurious CNS events such as cerebral infarction and brain trauma. Following such injuries, a number of chemical species are released that contribute to ongoing tissue damage as part of secondary injury mechanisms; examples include oxygen free radicals and superoxide and peroxide molecules (Fig 7). The accumulation of these products leads to a number of processes such as impaired mitochondrial energy production, inactivation of transporter proteins, increases in intracellular calcium concentrations promoted by elevated glutamate levels, and promotion of apoptosis. Various approaches, mostly pharmacologic in nature, have been attempted to diminish the local concentrations of such substances. Nanoparticles represent another possible method for limiting brain injury.[35] For example, fullerenes (nanoparticles comprising arrays of regularly spaced carbon atoms) have been investigated for this purpose and have shown promise as antioxidants with the capacity to scavenge free radicals.[36] In theory, nanoparticles can be developed that are capable of releasing therapeutic agents directly at the site of CNS injury and are a potential novel approach for achieving neuroprotection.

Monitoring of Stem Cell Migration within the CNS

Application of stem cells for treatment of CNS diseases is a topic that is gaining intense interest among neuroscientists and clinicians alike because it may provide a means to regenerate damaged brain tissue (eg, after trauma, degeneration, or cerebral infarction) or replace missing enzymes in enzymatic disorders.[37,38] As an example, stem cells are already in clinical use as a form of enzyme replacement for treatment of pediatric leukodystrophies such as Krabbe disease.[39] At present, stem cells are typically provided intravenously, but they could potentially be stereotactically placed within CNS tissue.

Iron oxide nanoparticles are a means by which migration of nanoparticle-tagged cells can be monitored by MR imaging. In one study, investigators produced cerebral infarction in mice and then implanted stem cells containing iron oxide nanoparticles.[40] MR imaging was successfully used to detect the location of transplanted cells. In another study of mice in which brain or spinal cord injury had been produced, researchers followed migration of iron oxide−labeled stem cells implanted in the CNS to sites of injury.[41] In combination, these studies show a potential role for MR imaging surveillance of labeled stem cell migration for CNS therapies in humans.

Nanoparticles as a Means for CNS Tissue Regeneration

Nanoparticles offer a potential means to enhance the body's own reparative mechanisms by providing building-block molecular materials or products needed for CNS repair (Fig 7). Alternatively, self-assembling nanodevices can be administered that can serve as platforms for regenerative processes.[42,43] One example is a constellation of nanofiber scaffolds that can self-assemble within the CNS to promote growth of neurites. For instance, peptide amphiphile molecules have been designed that self-assemble in a physiologic environment in response to appropriate cation concentrations in a configuration that allows physiologically active peptide sequences to act as ligands for cell surface receptors.[43] The peptides can then engage in cell signaling, which promotes cell growth and differentiation. In one experiment, scaffolds of the type outlined above trap water molecules following self-assembly into nanofibers and form a gel-like material in which neural progenitor cells are encapsulated.[43] Rapid (ie, within a day) and robust differentiation of the neural progenitor cells into mature neuronal cells was induced by a combination of the 3D nanostructure of the gel and the bioactive signaling peptide on the surface of the nanofibers, thereby potentially providing a method to regenerate CNS tissues. Furthermore, very little astrocyte development was noted, which suggests that reactive gliosis and glial scarring have been minimized.
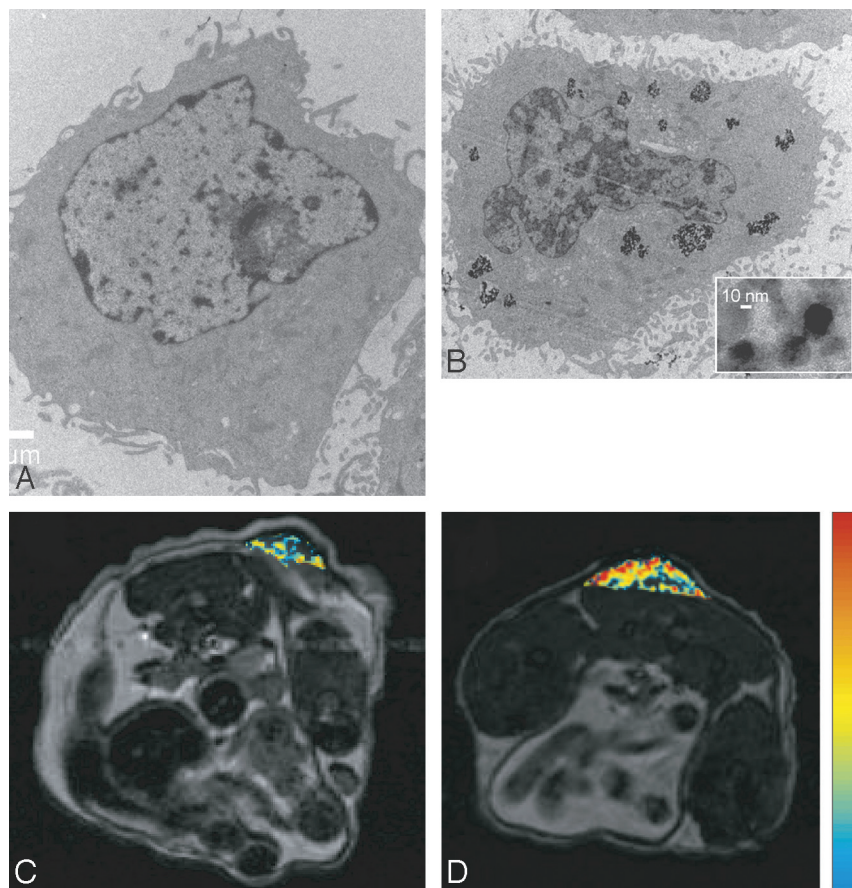
Fig 6. Comparison of tumor-targeted (chlorotoxin-conjugated) and nontargeted superparamagnetic nanoparticles for detection of a 9L glioma xenograft grown in the flank of a mouse. *A*, Transmission electron microscope image of a 9L tumor cell incubated with superparamagnetic nanoparticles that are not targeted specifically against tumor cells shows relatively few nanoparticles (black dots) within the cell. *B*, Transmission electron microscope image of a 9L tumor cell incubated with tumor-targeted superparamagnetic nanoparticles shows prominent intracellular uptake of nanoparticles (black dots). *C*, Axial MR image of a mouse shows relatively sparse uptake of nontargeted nanoparticles (colored regions) within the tumor. Relatively low uptake is depicted in blue and slightly higher uptake, in yellow. *D*, Axial MR image of a different tumor-bearing mouse than that depicted in *C* shows marked uptake of tumor-targeted nanoparticles (colored regions) within the tumor. Note the relatively large percentage of red and orange pixels indicating high uptake (see color scale). Reprinted with permission from Sun C, Veiseh O, Gunn J, et al. In vivo MRI detection of gliomas by chlorotoxin-conjugated superparamagnetic nanoprobes. *Small* 2008; 4:372-79. Copyright Wiley-VCH Verlag GmbH & Co. KGaA.

Nanoparticles as Sensors within the CNS

One intriguing possible use of nanoparticles involves indwelling sensors within the CNS, which could provide intermittent or continuous measurement of physiologic processes within the local environment. As an example, a nanosensor that is based on the principle of a magnetic nanoswitch has been proposed.[44] A nanoswitch is a functionalized iron oxide particle that can be used to monitor in vivo dynamic events such as changes in concentrations of molecules in the local nanoenvironment.[45] These nanoparticles undergo reversible assembly and disassembly in the presence of specific molecules (eg, glucose, enzymes, and other proteins). The assembly state and the disassembly state are each associated with a different transverse magnetic relaxivity. The different relaxivities can be detected by using MR imaging based on T2 relaxation times, thus providing a noninvasive means for detecting physiologic changes that are reflected by varying concentrations of specific molecules.

Nanoparticles for Imaging of Angiogenesis

One of the major applications of molecular imaging for assessment of tumors is development of nanoparticles for imaging of angiogenesis. One interesting advance is the development

of a lipid-encapsulated perfluorocarbon nanoparticle that can be modified for imaging by using sonography, nuclear medicine techniques, or MR imaging.[46] The nanoparticle is optimized for MR imaging through conjugation with very large numbers of gadolinium particles, thereby compensating for the poor signal-intensity contrast, which is one of the inherent limitations of MR molecular imaging agents. By conjugating ≥1 ligand to the surface of the nanoparticle, targeted imaging can be accomplished. Specifically, for imaging of angiogenesis, the nanoparticle is targeted against a protein that is expressed on the luminal surface of angioblasts within neovasculature (ie, $\alpha_{\nu}\beta_{3}$-integrin). With this technique, imaging of small animals has been successfully conducted on a 1.5T scanner, raising the promise that if such imaging could be performed in humans, MR imaging scanners that are presently clinically relevant in humans could be used (as opposed to the very high-field-strength scanners often used for small-animal imaging).

In addition to serving as an imaging agent, angiogenesis-targeted nanoparticles can serve as a therapy-delivery vehicle. In one study, investigators developed a polyacrylamide nanoparticle containing iron oxide particles (thereby allowing use as an MR imaging contrast agent) and also having a tumor vasculature−targeting peptide on its surface.[47] The nanopar-
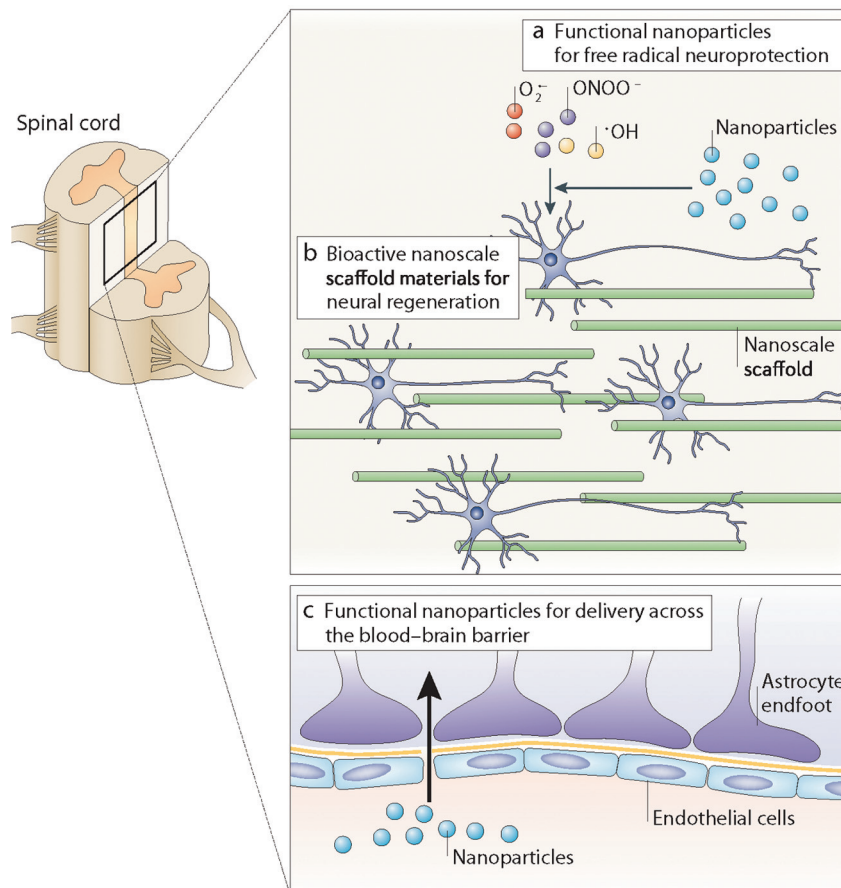
Fig 7. Diagram showing the scope of potential therapeutic applications for use of nanoparticles in the central nervous system. *A*, Nanoparticles for limiting the degree of tissue injury following events such as cerebral infarction or trauma. Nanoparticles could potentially be used as neuroprotective agents by limiting the effect of substances produced by injury, such as free radicals. In principle, nanoparticles could be loaded with materials that negate the injurious effects of free radicals or could actually serve as scavengers of free radicals. *B*, The use of nanoparticles to produce self-assembled scaffold materials that can provide the structural environment for neural regeneration, such as a medium for regrowth of neurons. *C*, Enhanced delivery of therapeutic agents by nanoparticles specifically designed to cross the BBB. Reprinted with permission from Macmillan Publishers Ltd. *Nat Rev Neurosci* 2006;7:65–74. Copyright 2006.

ticle also contained a photosensitizing molecule that, when activated by light, produces microvascular injury and tumor cell death. The study showed good deposition of the nanoparticles within 9L glioma tumors in rats and substantial improvement in the survival rate of treated animals after administration of phototherapy.

Summary

This review has provided an introduction to a number of the most important applications of nanotechnology to imaging of the CNS. The topics here are necessarily presented in limited detail; the reader is encouraged to learn more about this important and exciting field of investigation by reading articles that have been cited. The fact that many of the topics covered here are still in discovery mode should not discourage neuroradiologists from taking a serious interest in topics in nanotechnology related to imaging. Although the nanotechnology applications are not ready for clinical translation at this time, little doubt can be entertained that, in one form or another, some of the general principles described here will prove clinically relevant. After all, many techniques that are presently the mainstay of neuroradiologic practice were once also regarded (as nanotechnology applications might now be considered) as solely exciting theoretic principles without practical application.

References

1. Leary SP, Liu CY, Apuzzo MLJ. **Toward the emergence of nanoneurosurgery. Part III. Nanomedicine: targeted nanotherapy, nanosurgery, and progress toward the realization of nanoneurosurgery**. *Neurosurgery* 2006;58:1009–26
2. Gilmore JL, Yi X, Quan L, et al. **Novel nanomaterials for clinical neuroscience**. *J Neuroimmune Pharmacol* 2008;3:83–94. Epub 2008 Jan 22
3. Ferrari M. **Cancer nanotechnology: opportunities and challenges**. *Nat Rev Cancer* 2005;5:161–71
4. Enochs WS, Harsh G, Hochberg F, et al. **Improved delineation of human brain tumors on MR images using a long-circulating, superparamagnetic iron oxide agent**. *J Magn Reson Imaging* 1999;9:228–32
5. Varallyay CG, Muldoon LL, Gahramanov S, et al. **Dynamic MRI using iron oxide nanoparticles to assess early vascular effects of antiangiogenic versus corticosteroid treatment in a glioma model**. *J Cereb Blood Flow Metab* 2009;29: 853–60. Epub 2009 Jan 14
6. Wang YX, Hussain SM, Krestin GP. **Superparamagnetic iron oxide contrast agents: physicochemical characteristics and applications in MR imaging**. *Eur Radiol* 2001;11:2319–31
7. Sun C, Veiseh O, Gunn J, et al. **In vivo MRI detection of gliomas by chlorotoxin-conjugated superparamagnetic nanoprobes**. *Small* 2008;4:372–79
8. Neuwelt EA, Várallyay P, Bagó AG, et al. **Imaging of iron oxide nanoparticles by MR and light microscopy in patients with malignant brain tumours**. *Neuropathol Appl Neurobiol* 2004;30:456–71
9. Loo C, Lin A, Hirsch L, et al. **Nanoshell-enabled photonics-based imaging and therapy of cancer**. *Technol Cancer Res Treat* 2004;3:33–40
10. Dickerson EB, Dreaden EC, Huang X, et al. **Gold nanorod assisted near-infra-**

red plasmonic photothermal therapy (PPTT) of squamous cell carcinoma in mice. *Cancer Lett* 2008;269:57–66. Epub 2008 Jun 9

11. Xing Y, Chaudry Q, Shen C, et al. **Bioconjugated quantum dots for multiplexed and quantitative immunohistochemistry.** *Nat Protoc* 2007;2:1152–65

12. Pathak S, Cao E, Davidson MC, et al. **Quantum dot applications to neuroscience: new tools for probing neurons and glia.** *J Neurosci* 2006;26:1893–95

13. Ruiz MA, Clares B, Morales ME, et al. **Vesicular lipidic systems, liposomes, PLO, and liposomes-PLO: characterization by electronic transmission microscopy.** *Drug Dev Ind Pharm* 2008;34:1269–76

14. Ahmed F, Discher DE. **Self-porating polymersomes of PEG-PLA and PEG-PCL: hydrolysis-triggered controlled release vesicles.** *J Control Release* 2004;96:37–53

15. Poletti P, Bettini AC, Caremoli ER, et al. **Liposomal-encapsulated doxorubicin (Myocet; D-99) and vinorelbine in previously treated metastatic breast cancer patients: a feasibility study.** *Tumori* 2008;94:686–90

16. Di Paolo D, Pastorino F, Brignole C, et al. **Drug delivery systems: application of liposomal anti-tumor agents to neuroectodermal cancer treatment.** *Tumori* 2008;94:246–53

17. Liu L, Venkatraman SS, Yang YY, et al. **Polymeric micelles anchored with TAT for delivery of antibiotics across the blood-brain barrier.** *Biopolymers* 2008;90:617–23

18. Calvo P, Gouritin B, Villarroya H, et al. **Quantification and localization of PEGylated polycyanoacrylate nanoparticles in brain and spinal cord during experimental allergic encephalomyelitis in the rat.** *Eur J Neurosci* 2002;15:1317–26

19. Silva GA. **Nanotechnology approaches for drug and small molecule delivery across the blood brain barrier.** *Surg Neurol* 2007;67:113–16

20. Reimold I, Domke D, Bender J, et al **Delivery of nanoparticles to the brain detected by fluorescence microscopy.** *Eur J Pharm Biopharm* 2008;70:627–32

21. Gulyaev AE, Gelperina SE, Skidan IN, et al. **Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles.** *Pharm Res* 1999;16:1564–69

22. Olbrich C, Gessner A, Kayser O, et al. **Lipid-drug-conjugate (LDC) nanoparticles as novel carrier system for the hydrophilic antitrypanosomal drug diminazenediaceturate.** *J Drug Target* 2002;10:387–96

23. Alyaudtin RN, Reichel A, Lobenberg R, et al. **Interaction of poly(butylcyanoacrylate) nanoparticles with the blood-brain barrier in vivo and in vitro.** *J Drug Target* 2001;9:209–21

24. Steiniger SC, Kreuter J, Khalansky AS, et al. **Chemotherapy of glioblastoma in rats using doxorubicin-loaded nanoparticles.** *Int J Cancer* 2004;109:759–67

25. Gao X, Cui Y, Levenson RM, et al. **In vivo cancer targeting and imaging with semiconductor quantum dots.** *Nat Biotechnol* 2004;22:969–76

26. Gelperina S, Kisich K, Iseman MD, et al. **The potential advantages of nanoparticle drug delivery systems in chemotherapy of tuberculosis.** *Am J Respir Crit Care Med* 2005;172:1487–90

27. Vyas SP, Gupta S. **Optimizing efficacy of amphotericin B through nanomodification.** *Int J Nanomedicine* 2006;1:417–32

28. Wieder ME, Hone DC, Cook MJ, et al. **Intracellular photodynamic therapy with photosensitizer-nanoparticle conjugates: cancer therapy using a "Trojan horse."** *Photochem Photobiol Sci* 2006;5:727–34. Epub 2006 Jun 21

29. Kong G, Braun RD, Dewhirst MW. **Hyperthermia enables tumor-specific nanoparticle delivery: effect of particle size**. *Cancer Res* 2000;60:4440–45

30. Walker GF, Fella C, Pelisek J, et al. **Toward synthetic viruses: endosomal pH-triggered deshielding of targeted polyplexes greatly enhances gene transfer in vitro and in vivo.** *Mol Ther* 2005;11:418–25

31. Gu J, Cheng WP, Liu J, et al. **pH-triggered reversible "stealth" polycationic micelles.** *Biomacromolecules* 2008;9:255–62

32. Lee JH, Jun YW, Yeon SI, et al. **Dual-mode nanoparticle probes for high-performance magnetic resonance and fluorescence imaging of neuroblastoma.** *Angew Chem Int Ed Engl* 2006;45:8160–62

33. Vu TQ, Maddipati R, Blute TA, et al. **Peptide-conjugated quantum dots activate neuronal receptors and initiate downstream signaling of neurite growth**. *Nano Lett* 2006;5:603–07

34. Dahan M, Levi S, Luccardini C, et al. **Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking.** *Science* 2003;302:442–45

35. Yin JJ, Lao F, Fu PP, et al. **The scavenging of reactive oxygen species and the potential for cell protection by functionalized fullerene materials.** *Biomaterials* 2009;30:611–21

36. Dugan LL, Lovett EG, Quick KL, et al. **Fullerene-based antioxidants and neurodegenerative disorders.** *Parkinsonism Relat Disord* 2001;7:243–46

37. Jeffery ND, McBain SC, Dobson J, et al. **Uptake of systemically administered magnetic nanoparticles (MNPs) in areas of experimental spinal cord injury (SCI).** *J Tissue Eng Regen Med* 2009;3:153–57

38. Delcroix GJ, Jacquart M, Lemaire L, et al. **Mesenchymal and neural stem cells labeled with HEDP-coated SPIO nanoparticles: in vitro characterization and migration potential in rat brain.** *Brain Res* 2009;1255:18–31. Epub 2008 Dec 11

39. Escolar ML, Poe MD, Provenzale JM, et al. **Transplantation of umbilical-cord blood in babies with infantile Krabbe's disease.** *N Engl J Med* 2005;352:2069–81

40. Rice HE, Hsu EW, Sheng H, et al. **Superparamagnetic iron oxide labeling and transplantation of adipose-derived stem cells in middle cerebral artery occlusion-injured mice.** *AJR Am J Roentgenol* 2007;188:1101–08

41. Jendelová P, Herynek V, Urdzíková L, et al. **Magnetic resonance tracking of transplanted bone marrow and embryonic stem cells labeled by iron oxide nanoparticles in rat brain and spinal cord.** *J Neurosci Res* 2004;76:232–43

42. Silva GA. **Neuroscience nanotechnology: progress, opportunities and challenges.** *Nat Rev Neurosci* 2006;7:65–74

43. Silva GA, Czeisler C, Niece KL, et al. **Selective differentiation of neural progenitor cells by high-epitope density nanofibers.** *Science* 2004;303:1352–55

44. Sun EY, Weissleder R, Josephson L. **Continuous analyte sensing with magnetic nanoswitches.** *Small* 2006;2:1144–47

45. Sosnovik DE, Weissleder R. **Emerging concepts in molecular MRI.** *Curr Opin Biotechnol* 2007;18:4–10

46. Winter PM, Caruthers SD, Kassner A, et al. **Molecular imaging of angiogenesis in nascent Vx-2 rabbit tumors using a novel $\alpha v \beta 3$-targeted nanoparticle and 1.5 Tesla magnetic resonance imaging**. *Cancer Res* 2003;63:5838–43

47. Reddy GR, Bhojani MS, McConville P, et al. **Vascular targeted nanoparticles for imaging and treatment of brain tumors.** *Clin Cancer Res* 2006;12:6677–86