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Novel Nanomaterials for Clinical Neuroscience

Jamie L. Gilmore,
Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, 986025 Nebraska Medical Center, Omaha, NE 68198, USA

Xiang Yi,
Center for Drug Delivery and Nanomedicine, Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, 985830 Nebraska Medical Center, Omaha, NE 68198, USA

Lingdong Quan, and
Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, 986025 Nebraska Medical Center, Omaha, NE 68198, USA

Alexander V. Kabanov
Center for Drug Delivery and Nanomedicine, Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, 985830 Nebraska Medical Center, Omaha, NE 68198, USA

Abstract

Neurodegenerative disorders including Alzheimer's and Parkinson's diseases, amyotrophic lateral sclerosis, and stroke are rapidly increasing as population ages. The field of nanomedicine is rapidly expanding and promises revolutionary advances to the diagnosis and treatment of devastating human diseases. This paper provides an overview of novel nanomaterials that have potential to improve diagnosis and therapy of neurodegenerative disorders. Examples include liposomes, nanoparticles, polymeric micelles, block ionomer complexes, nanogels, and dendrimers that have been tested clinically or in experimental models for delivery of drugs, genes, and imaging agents. More recently discovered nanotubes and nanofibers are evaluated as promising scaffolds for neuroregeneration. Novel experimental neuroprotective strategies also include nanomaterials, such as fullerenes, which have antioxidant properties to eliminate reactive oxygen species in the brain to mitigate oxidative stress. Novel technologies to enable these materials to cross the blood brain barrier will allow efficient systemic delivery of therapeutic and diagnostic agents to the brain. Furthermore, by combining such nanomaterials with cell-based delivery strategies, the outcomes of neurodegenerative disorders can be greatly improved.

Keywords

nanomaterials; neuroregeneration; blood–brain barrier; neuroscaffolding; nanotubes/fullerenes; cell-based delivery

Introduction

Disorders of the central nervous system (CNS) are numerous, diverse, frequently severe, and affect a large portion of the world's population. Epilepsy, migraine, chronic pain and psychiatric

e-mail: akabanov@unmc.edu.
Jamie L. Gilmore and Xiang Yi made equal contributions.

disorders such as anxiety, depression, and schizophrenia are debilitating conditions that significantly affect the morbidity and mortality of modern society. In neurodegenerative diseases, such as Alzheimer's diseases (AD), Parkinson's diseases (PD) and multiple sclerosis, patients experience symptoms related to movement, memory, and dementia due to the gradual loss of neurons. Brain tumors constitute a profound and unsolved clinical problem and are a common cause of cancer-related death especially for children. The causes of CNS disorders are complex and associated with many factors such as advancing age, environmental cues, and disordered immunity and less with the host genetics (Mayeux 2003).

Diagnosis and treatment of CNS disorders represents a considerable challenge. There are many devastating and pervasive disorders, for which there are few safe and effective therapeutic options. This is mainly due to the unique and complicated environment imposed by the CNS. The restricted anatomical access of the CNS makes any diagnosis or surgery-based therapy more difficult than any other disease site. The blood–brain barrier (BBB) represents a formidable obstacle for most macromolecules and small molecules to enter the brain (de Boer and Gaillard 2007). Thus, treatment of CNS disorders by systemic administration or local delivery of drugs is currently inefficient in many cases. Furthermore, clinical neuroscience faces great challenges due to the extremely heterogeneous cellular and molecular environment and the complexities of the brain's anatomical and functional “wiring” and associated information processing (Silva 2005). However, the emergence of nanotechnology provides hope that it will revolutionize diagnosis and treatment of CNS disorders.

The origin of nanotechnology can be traced back to 1959 when physicist Richard Feynman (1960) recognized the potential of manipulating individual atoms and molecules at the nanometer scale and suggested that materials at this scale possess unique physical properties. The nanotechnology field encompasses concepts and approaches deeply rooted in physics, polymer and colloidal chemistry, pharmaceutics, biomaterials, as well as cell and molecular biology and biophysics. The main theme of nanotechnology is the development and use of nanometer-scale materials that display unique functional properties not shown by bulk materials. Examples of nanomaterials include nanotubes and nanofibers, liposomes, nanoparticles, polymeric micelles, block ionomer complexes, nanogels, and dendrimers. Such nanomaterials can interact with biological systems at a molecular and supra-molecular level, can be tailored to respond to specific cell environments and even to induce desired physiological responses in cells, while minimizing unwanted side effects. The unique structural organization and function of these nanomaterials, as well as their potential applications for treatment of CNS disorders, are discussed in this review.

Nanomaterial scaffolds for neuroregeneration

Neurodegenerative diseases are usually linked to a loss of brain and spinal cord cells. For example, the neuronal damage in AD and PD is associated with abnormal protein processing and accumulation and results in gradual cognitive and motor deterioration (Shaw et al. 2007). Neuronal deterioration is also found in the conditions of stroke, heat stress, head and spinal cord trauma, and bleeding that occurs in the brain. One promising strategy for treatment of neurodegenerative diseases is to support and promote neurite and axonal growth by implanting nanometer-scale scaffolds using tissue-engineering approaches.

Some promising nanomaterials to use for this application are nanotubes and nanofibers. These materials mimic tubular structures that appear in nature, such as rod shaped bacteria or viruses, microtubules, ion channels, as well as axons and dendrites. They are low-dimensional nano-structures, having a very large axial ratio. Current methods for synthesis of these nanomaterials allow for manipulation of their length-to-volume ratio. For example, the diameter of semi-conducting nanotubes and nanofibers can be reduced to enhance conductivity. Variation in the

surface curvature of these materials can cause different levels of strain on the constituent molecules. In this way, properties of a molecule in a nanotube or nanofiber structure can be different from those in the bulk or in other nanomaterials, such as spherical nanoparticles. Also, these materials have a large surface–volume ratio, which results in a high exposure of the material components to the surrounding environment. This makes nanotubes and nanofibers promising structures for biosensing and molecular recognition. Also, it provides a way to control drug release through the nanotube wall, while the large hollow area inside nanotubes provides an excellent storage for drugs and other agents. Furthermore, nanotubes can be synthesized to be open-ended, which can be exploited for certain biological applications (Hartgerink et al. 1996; Martin and Kohli 2003; Greiner et al. 2006; Tsai et al. 2006).

Discovery of carbon nanotubes (CNTs) is attributed to Sumio Iijima (1991), although some earlier reports from different laboratories also described hollow nanosized carbon tubes (Oberlin et al. 1976). CNTs are composed of carbon atoms arranged in hexagonal ring structures similar to graphite, with some five-membered or seven-membered rings providing the structure curvature (Baxendale 2003). CNTs and related carbon spheres and buckyballs belong to a broader class of carbon allotropes named fullerenes. The discovery of fullerene chemistry in 1985 is attributed to H. Kroto, R. Curl, and R. Smalley (Kroto et al. 1985), who were awarded the Nobel Prize in chemistry in 1996 for this discovery. These materials can be synthesized through discharge of arc-burned graphite rods, laser ablation, chemical vapor deposition, or high-pressure carbon monoxide (Hirsch et al. 2005). Studies have reported that CNTs are compatible with biological tissues for scaffolding purposes and that the charge carried by the nanotubes can be manipulated to control neurite outgrowth (Lovat et al. 2005). Another study also suggested that CNTs functionalized with growth factors, such as nerve growth factor or brain-derived neurotrophic factor, can stimulate growth of neurons on the nanotube scaffold (Matsumoto et al. 2007). The toxicity of CNTs remains an issue in such applications. Recent reports suggest that such materials can induce lipid peroxidation and oxidative stress, pulmonary toxicity, carcinotoxicity, as well as toxicity from synthetic impurities (Lacerda et al. 2006). Furthermore, they are hydrophobic and tend to aggregate in the body fluids.

Although the majority of early work focused on CNTs, several new techniques have been recently described which can produce nanotubes from a variety of other materials, including synthetic polymers, DNA, proteins, lipids, silicon, and glass (Martin 1994; Greiner et al. 2006; Tsai et al. 2006; Yuwono and Hartgerink 2007). These techniques include templating of nanotubes on porous templates, such as alumina or silica (Fig. 1a), on electrospun nanofibers of degradable polymers (Fig. 1b), or using self-assembled nanofibers of peptide molecules (Fig. 1c). These techniques allow for the production of various nanotube designs, including layered nanotubes, conducting nanotubes, and biopolymer nanotubes from proteins or DNA. Multilayer nanotubes with a controlled wall diameter and different materials in the layers can also be synthesized by templating (Liang et al. 2003). For example, a recent study reported a nanotube produced by a layer-based assembly of cytochrome C, poly(sodium styrene sulfonate), and poly(ethylenimine) (PEI). The enzyme incorporated in such nanotubes retained its catalytic activity, suggesting that it may be possible to use these materials to catalyze redox processes (Tian et al. 2006). Similar designs have also been used to produce nanotubes with glucose oxidase and hemoglobin (Hou et al. 2005b). As a result, novel nanomaterials are available that have decreased toxicity and increased biocompatibility compared to CNTs. For example, nanotubes made of conducting polymers, such as polypyrrole, polyaniline, poly (3-hexylthiophene), and poly(3,4-ethylenedioxythiophene) (PEDOT) can be synthesized by electrochemical polymerization on a porous surface (Xiao et al. 2007). Although these nanotubes were initially applied in electronic devices (Xiao et al. 2007), they also have significant potential in biomedical applications. A recent study by D. C. Martin and colleagues developed conductive nanotubes, which can be electrically stimulated for a controlled release

of drugs (Abidian et al. 2006). For this purpose, they generated PEDOT nanotubes with biodegradable poly(lactide-coglycolide) (PLGA) fiber cores loaded with dexamethasone. PLGA fibers with the nanotube coating substantially slowed down the release of dexamethasone compared to uncoated fibers. However, an electrical stimulation to the nanotube caused it to dilate and facilitated the drug release. This design can be used to deliver drugs to neural prosthetic devices. Furthermore, similar designs can be also used for local neurite outgrowth stimulation, neural tissue regeneration, as well as local release of drugs to other specific populations of cells. More recently the same group used PEDOT nanotubes to generate conductive polymer coatings for living neural cells (Richardson-Burns et al. 2007). In this setup, the electric conductivity of PEDOT was used to enhance the electrical activity of the tissue with a long range aim of treating CNS disorders, which show sensory and motor impairments. On a downside, this study reported that cells coated with the polymers showed increased apoptosis, increased permeability, and cytoskeletal changes. Nevertheless, future designs of nanotubes as neuronal scaffolds may provide novel modalities for treatment of neurodegenerative diseases.

These studies may be aided by using well-known biocompatible polymers, such as poly(α-hydroxy acids)-poly(lactic acid) (PLA), poly(glycolic acid) and PLGA. For example, PLA was successfully used to produce nanomaterial scaffolds for tissue regeneration after spinalcord injury (Yang et al. 2004). In this study, scaffolds of nanoscale PLA fibers with diameters of 50~350 nm were fabricated using liquid–liquid separation in tetrahydrofuran system. The neonatal mouse cerebellar progenitor cells cultured with such scaffolds were shown to extend neurites and differentiate into mature neurons. All together, these observations suggested that nanotube and nanofiber scaffolds have potential for neuroregeneration as well as treatment of CNS trauma.

These approaches may be greatly enhanced by new possibilities of assembling nanotubes and nanofibers from biologically active molecules, such as certain peptides and proteins (Tsai et al. 2006). For instance, a well-known antibiotic, gramicidin A, has a tubular structure forming transmembrane ion-channels. Similar characteristic features have been used to design other nanotube-forming peptides. In this design, cyclic peptides with an even number of alternating D- and L-amino acids assemble in β-sheet-like structures with outward-facing side chains, which are stabilized by hydrophobic packing and a network of hydrogen bonds (Hartgerink et al. 1996). Peptide nanofibers have been examined as potential neuronal scaffolds. In one study, nanofibers of self-assembling peptide molecules (IKVAV) were shown to promote and support neuronal regeneration (Silva et al. 2004). In physiological conditions, the peptide molecules self-assemble into a dense three-dimensional network of nanofibers, which expose the neurite-promoting laminin epitope. Such networks mimic the neuron-specific extracellular matrix and induce very rapid differentiation of cells into neurons, without stimulation of the growth of astrocytes. In another publication, a nanofiber scaffold of self-assembling peptide (RADA) was shown to induce regeneration of axons with sufficient density to promote functional return of vision (Ellis-Behnke et al. 2006).

DNA and other polynucleotide molecules can also be successfully incorporated into nanotubes. For example, dual-functionalized nanotubes were developed for use either as molecular probes or DNA carriers (Jang et al. 2006). These nanotubes were fabricated in the pores of an anodic alumina oxide membrane using a pyrrole-2-carboxylic acid monomer and then functionalized by attachment of silica-NH_2 nanoparticles. To obtain a molecular probe, the free amino groups of these nanotubes were modified with pyrene acetic acid. To obtain a DNA carrier, the carboxyl groups of the nanotubes were used to conjugate DNA. Another study reported nanotubes designed with multiple layers of hybridized DNA fragments (Hou et al. 2005a). These materials were unstable, as the DNA dissociated from the nanotubes at temperatures above the DNA melting point. However, stabilization may be achieved by adding extra ‘outer

skin' layers, e.g., α,ω-diorganophosphonate Zr(IV), and the resulting materials may have potential applications in DNA delivery. One work in this direction by Li et al. described nanotubes composed of cationic dipeptides that were loaded with ssDNA and then shown to spontaneously disassemble into vesicles and enter into cells (Yan et al. 2007). C. R. Martin et al. developed 380 nm long nanotubes, which are open at one end and closed at the other, which they refer to as "nano test tubes" (Buyukserin et al. 2007). Such tubes can be loaded with drug and DNA and used as nanocontainers for drug delivery. All together, these examples suggest that nanotechnology can provide a new range of promising applications for neuroregeneration by combining tissue and cell engineering with drug- and gene-delivery approaches.

Nanomaterials for neuroprotection

Neuroprotection is an effect that may result in salvage, recovery, or regeneration of the nervous system. A promising neuroprotective strategy involves mitigating oxidative stress, which is believed to be a key neuropathological process that contributes to CNS ischemia, trauma, and degenerative disorders (Metodiewa and Koska 2000) Hence, some recent studies focused on using nanomaterials having antioxidant properties to eliminate reactive oxygen species (ROS) in the brain (Blass 2003). For example, nanoparticles composed of cerium and yttrium oxides (CeO_2 and Y_2O_3) can act as direct antioxidants and inhibit the ROS production pathway. Neuroprotective effect of these nanoparticles were shown using HT22 hippocampal neuronal cell line (Schubert et al. 2006). Separately, many recent studies focused on potential neuroprotective effects of fullerenes. Fullerenes are composed of three-dimensional arrays of evenly spaced carbon atoms with extensive interconnecting double bonds. As a result, water-soluble derivatives of fullerenes display high reactivity with respect to oxygen free radicals and possess antioxidant and free-radical scavenger properties. Specifically, polyhydroxylated C_{60} fullerenol was shown to inhibit glutamate receptors, which can further induce elevation of intracellular calcium and lead to reduction of neuronal toxicity (Jin et al. 2000). Furthermore, a water-soluble carboxyfullerene, malonic acid C_{60} derivative exhibited neuroprotective effects in vitro by limiting excitotoxicity (Dugan et al. 1997) and apoptosis of cultured cortical neurons and in vivo by delaying the onset of motor degeneration in a mouse model of familial amyotrophic lateral sclerosis (Dugan et al. 2001). In another study, fullerene C_{60} core was modified with a layer of malonate ester dendrimers, which increased solubility and reactive surface area of the nanoparticles. The resulting core-shell material possessed even more pronounced antioxidant activity than fullerenol (Hirsch 2003).

However, as already mentioned above, toxicity of fullerenes hinders their application in clinical neuroscience. In particular, ability of fullerenes to induce lipid peroxidation has caused serious concern. A recent study of largemouth bass exposure to pure unmodified fullerenes showed that C_{60} fullerenes localized to the brain and led to an increase in lipid peroxidation, as well as a decrease of glutathione in the gill (Oberdorster 2004). Part of the problem may be related to hydrophobicity of fullerenes and their propensity to aggregate and interact with the cell membranes. There are indications that these problems may be mitigated by surface modifications of fullerenes. For example, some modifications that decrease surface hydrophobicity and increase solubility of the CNTs were also shown to decrease CNTs cytotoxicity (Sayes et al. 2006). Furthermore, there are reports suggesting that ability of fullerenes to generate free radicals may be due to metal ion impurities that can be removed from fullerene samples. One work concluded that the free-radical generation by commercial single-walled CNTs (SWCNTs) can be abolished by separating SWCNTs from iron impurities (Pulskamp et al. 2007). It was also shown that simple filtering of SWCNTs can decrease their toxicity with respect to smooth muscle cells (SMC), as measured by SMC growth inhibition (Raja et al. 2007). Another study evaluated the free-radical generation and scavenging activities of purified multi-walled carbon nanotubes (MWCNTs; Fenoglio et al. 2006). It was found that purified MWCNTs do not produce free radicals but display scavenging activity towards

hydroxyl radicals and superoxide radicals. Hence, purification and chemical modification of fullerenes aimed to increase solubility and decrease toxicity will be needed for their successful application in medicine and clinical neuroscience.

Nanomaterials for drug delivery across the blood–brain barrier

The BBB is the most restrictive barrier in the body, which prevents most small molecules and nearly all macromolecules from entering the CNS (de Boer and Gaillard 2007). Current strategies used for drug delivery to the brain include invasive delivery, temporary disruption of the BBB, as well as the use of drug-delivery systems. While direct injection in selected cases can be an effective invasive modality for local delivery (e.g., in some tumors), it is not efficient for brain metastasis or neurodegenerative diseases, which require therapeutic agents to be widely spread in the brain (de Boer and Gaillard 2007). Reversible opening of the BBB by an osmotic or chemical method allows therapeutic agents to enter the brain (Fortin 2003). However, this approach can also result in significant damage to the brain. In comparison, selective delivery of diagnostic and therapeutic molecules to the whole brain through the vascular route using drug-delivery system is much less invasive and is potentially safe. This strategy has been receiving significant attention with remarkable development of nanomedicine. Recent advances in using polymers and nanotechnology for effective drug delivery to the brain have been comprehensively reviewed elsewhere (Kabanov and Gendelman 2007) This section presents a brief overview of these technologies including liposomes, nanoparticles, polymeric micelles, nanogels, and dendrimers for CNS drug delivery.

Liposomes are, perhaps, the earliest type of nanomaterial developed for drug delivery (Maurer et al. 2001). They are vesicles composed of one (unilamellar) or several (multilamellar) lipid bilayers surrounding internal aqueous compartments. Relatively large amounts of drug molecules can be incorporated into the aqueous compartment (water-soluble compounds) or lipid bilayers (lipophilic compounds). Conventional liposomes are rapidly cleared from circulation by the reticuloendothelial system (RES). Extended circulation time can be accomplished by decreasing the particle size (<100 nm) and by liposome-surface modification with polyethylene glycol (PEG). To target PEGylated liposomes to the brain, they can be additionally modified (vectorized) with monoclonal antibodies to glial fibrillary acidic proteins (Pardridge 1999; Chekhonin et al. 2005), transferrin receptors (OX26), or human insulin receptors (83–14 Mab; Pardridge 1999). Such immunoliposome constructs were successfully used to deliver small drugs, Daunomycin and Digoxin, as well as DNA to the brain (Shi et al. 2001).

Nanoparticles (NPs) are solid colloidal particles often made of insoluble (bio) degradable polymers. As carriers for drug delivery to the brain, NPs need to be small (<100 nm) and stabile in the blood, as well as avoid the RES, neutrophil activation, platelet aggregation, and inflammation (Lockman et al. 2002). Surfactant-coated polybutylcyanoacrylate NPs were shown to successfully deliver analgesics (Dalargin, Loperamide), anti-cancer agents (Doxorubicin), and anticonvulsants (NMDA receptor antagonist, MRZ 2/576) across the BBB (Kreuter et al. 2003). In another work, NPs conjugated with the metal chelator, Desferioxamine, were reported to cross the BBB and reduce the metal load in neuronal tissue (Cui et al. 2005). These results would be of significance for mitigating the harmful effects of oxidative damage in AD and other neurodegenerative diseases. Overall, the mechanism of entry of NPs as well as liposomes into the brain remains unclear and, in some cases, may include disruption of the tight junctions of brain microvessel endothelial cells forming the BBB.

Polymeric micelles are another type of nanomaterial that have attracted considerable attention as carriers for drug delivery (Kataoka et al. 2001; Kabanov and Alakhov 2002; Kwon 2003;

Allen and Cullis 2004; Torchilin 2004; Aliabadi and Lavasanifar 2006) and diagnostic imaging agents (Torchilin 2002). These micelles form spontaneously in aqueous solutions of amphiphilic block copolymers and have core-shell architecture. The core is composed of hydrophobic polymer blocks [e.g., poly(propylene glycol) (PPG), poly($_{D,L}$-lactide), poly (caprolactone), etc.] and a shell of hydrophilic polymer blocks (often PEG). The size of polymeric micelles usually varies from ca. 10 to 100 nm. Their core can incorporate considerable amounts (up to 20–30% weight) of water-insoluble drugs preventing premature drug release and degradation. The shell stabilizes micelles in dispersion and masks the drug from interactions with serum proteins and untargeted cells. After reaching target cells, the drug is released from the micelle via diffusion. Several clinical trials are completed or are underway to evaluate polymeric micelles for delivery of anti-cancer drugs (Danson et al. 2004; Kim et al. 2004; Matsumura et al. 2004; Armstrong et al. 2006; Matsumura 2006).

One study has shown that polymeric micelles of Pluronic block copolymers, after conjugation with an antibody against α_2-glycoprotein or insulin, showed increased delivery of a drug (haloperidol) or a fluorescent probe to the brain in vivo (Kabanov et al. 1989). Pluronic block copolymers contain two hydrophilic PEG and one hydrophobic PPG blocks (PEG-PPG-PEG). They were shown to bind with the membranes of brain microvessel endothelial cells and to inhibit a drug efflux transport protein, Pglycoprotein (Pgp) that severely restricts transport of many drugs to the brain (Batrakova et al. 1998, 2001) As a result of formulating Pgp-dependent drugs with Pluronic, the bioavailability of such drugs to the brain was increased. Another recent work used Pluronic molecules capable of crossing the membranes of brain microvessel endothelial cells to increase delivery of proteins to the brain (Batrakova et al. 2005). It was shown that by conjugating Pluronic molecules with a model protein, horseradish peroxidase (HRP), the transport of HRP across the BBB in vitro and in vivo was considerably enhanced. All together, significant promise has been achieved by utilizing Pluronic block copolymers and polymeric micelle systems for CNS drug delivery, and one should expect further research and development in this direction.

Polyion complex micelles (also termed “block ionomer complexes”) are novel nanosystems for incorporation of charged molecules. They are formed as a result of the reaction of double hydrophilic block copolymers containing ionic and nonionic blocks with macromolecules of opposite charge including oligonucleotides, plasmid DNA and proteins (Kabanov et al. 1995; Harada and Kataoka 1999a, b; Nguyen et al. 2000; Zhang et al. 2003; Jaturanpinyo et al. 2004), or surfactants of opposite charge (Bronich et al. 1997, 1998, 1999, 2000; Solomatin et al. 2003, 2004). For example, block ionomer complexes were prepared by reacting trypsin or lysozyme (that are positively charged under physiological conditions) with an anionic block copolymer, PEG-poly(α,β-aspartic acid) (Harada and Kataoka 1999b; Jaturanpinyo et al. 2004). Such complexes spontaneously assemble into nanosized particles having core-shell architecture (Fig. 2). The core contains polyion complexes of a biomacromolecule and an ionic block of the copolymer. The shell is formed by the nonionic block. In the case of surfactant-based complexes, the core is composed of mutually neutralized surfactant ions and polyion chains. It contains hydrophobic domains of surfactant tail groups and can additionally incorporate water-insoluble drugs (Bronich et al. 1999; Oh et al. 2006). Depending on surfactant and block copolymer architectures, the complexes assume different morphologies including vesicles and micelles of different shapes (Bronich et al. 1998; Solomatin et al. 2007). These nanomaterials are versatile and can incorporate solutes of different structures with a high loading capacity. Furthermore, they can release solutes upon change of environmental conditions such as pH (acidification), concentration, and chemical structure of elementary salt (Solomatin et al. 2003; Oh et al. 2006). These nanomaterials were shown to efficiently deliver DNA molecules in vitro and in vivo (Roy et al. 1999; Nguyen et al. 2000; Harada-Shiba et al. 2002; Junghans et al. 2005), and were used to stabilize enzymes for cell-mediated drug delivery discussed in the next section.

Nanogels are another novel class of nanomaterials proposed in our laboratories for drug delivery (Vinogradov et al. 2002). They represent hydrogels of cross-linked polymer networks that often combine ionic and nonionic chains. Such networks can incorporate charged molecules such as siRNA, DNA, oligonucleotides, and low-molecular-mass compounds, which bind to oppositely charged ionic chains. Nanogels were shown to enhance transport of incorporated oligonucleotides to the brain in vitro and in vivo (Vinogradov et al. 2004). The mechanism of nanogel-mediated delivery of oligonucleotides apparently involves transcytosis across brain microvessel endothelial cells. The permeability of oligonucleotides with nanogels was enhanced when the nanogel surface was modified with polypeptides (transferrin or insulin) that bind receptors at the luminal side of the brain microvessel endothelial cells and transport to their abluminal side. Nanogels are currently being investigated for CNS delivery of various low-molecular-mass compounds and biomacromolecules including nucleoside analogs and plasmid DNA (Vinogradov 2006).

Finally, many studies focused on using dendrimers as carriers of small drugs and biomacromolecules. Dendrimers are repeatedly branched polymer molecules containing cascade of branches grown from one or several cores. They contain three architectural domains: (1) the core, to which the branches are attached, (2) the shell of the branches surrounding the core, and (3) the multivalent surface formed by the branches termini (Svenson and Tomalia 2005). Compared to most other nanomaterials described in this section, dendrimers have a smaller size and lower polydispersity (Jean 2003). A typical dendrimer molecule, for example poly(amidoamine) (PAMAM) dendrimer, has a diameter ranging from 1.5 to 14.5 nm (Hahn et al. 2002). Various solutes can be entrapped within the dendrimer interior cavities (“dendritic boxes”) during the dendrimer synthesis (Fig. 3a). In this case, a dense shell can prevent diffusion of the solutes from the interior, even after prolonged heating, solvent extraction, or sonication (D'Emanuele and Attwood 2005). Dendritic boxes may be suitable for drug delivery if a shell can be degraded under physiological conditions resulting in drug release. Dendritic structures can also be used as building blocks for attachment of various functional moieties to the surface. For example, hydrophobic and hydrophilic polymer blocks can be grafted to the surface resulting in formation of unimolecular micelles (Fig. 3b; Liu et al. 2000; Wang et al. 2005; Tsai et al. 2006; Yang and Kao 2006). Unlike conventional micelles, such unimolecular micelles are stable upon dilution and do not disintegrate during circulation in the body.

Several studies evaluated dendrimers for CNS intratumoral delivery of dendrimer conjugates with anti-cancer agents to treat glioma (Wu et al. 2006; Yang et al. 2006). Transport of dendrimers across cell barriers was evaluated using intestinal epithelial cells (Caco-2; Kitchens et al. 2005). Notably, the generation and surface properties of dendrimers were found to be very important. Cationic dendrimers were generally more toxic and disrupted the tight junctions. These effects increased as dendrimer generation and, consequently, net surface area increased. Surface modification of dendrimers with carboxylic groups greatly decreased the toxicity, although the modified dendrimers still opened tight junctions. It is also well known that the fourth- and fifth-generation PAMAM dendrimers can bind DNA and enhance DNA delivery into a cell (Haensler and Szoka 1993). It has recently been demonstrated that Starburst™ PAMAM dendrimer/DNA complexes can also penetrate the BBB resulting in enhanced expression of a reporter gene in the brain (Ding et al. 2005). Gene transfer into brain capillary cells have been also shown using a transferrin-conjugated PEG-modified PAMAM dendrimer (Huang et al. 2007). A widespread expression of an exogenous gene in mouse brain was observed after i.v. administration of complexes of this dendrimer and DNA, suggesting potential use of such materials as nonviral vectors for CNS gene delivery.

Clearly, the use of polymers and nanomaterials for drug delivery to the brain is still in its infancy. However, one should expect new developments in this area, which ultimately can revolutionize the diagnosis and therapy of neurodegenerative disorders. Notably, several

nanoscale drugs ("nanomedicines"), such as liposomal Doxorubicin, Doxil (Gabizon et al. 2006) or albumin-bound Paclitaxel, Abraxane (Gradishar 2006) have been used in clinic already for treatment of cancer and other diseases. Others, such as Doxorubicin incorporated in polymer micelles, SP1049C (Danson et al. 2004; Armstrong et al. 2006) and NK911 (Matsumura et al. 2004), are in clinical trials. Most importantly, such studies have demonstrated that nano-materials can be safely administered in the human body. Clearly, some of the approaches developed in these earlier studies can and will be extended to develop efficient therapeutic modalities for neurodegenerative disorders.

Cells and nanomaterial hybrids for clinical neuroscience

In addition to use of nanomaterials as cell and tissue scaffolds, which in essence, places nanomaterial outside of the cell, nanomaterials can be placed inside of the cells, resulting in nanomaterial–cell hybrids, which have a variety of possible applications in medicine. One application uses immune cells as carriers for nanomaterial delivery in the body. For example, macrophages and microglia as well as other mononuclear phagocytes can endocytose colloidal nanomaterials, for example, liposomes or nanosuspensions, and subsequently carry and release the drug to site of tissue injury, infection, or disease (Daleke et al. 1990; Jain et al. 2003; Gorantla et al. 2006). Recent works by H. E. Gendelman and colleagues demonstrate that bone-marrow-derived monocytes (BMM) can be used as carriers of nanoformulated drugs, both in the periphery and across the BBB (Dou et al. 2006; Dou et al. 2007). In these studies, an anti-retroviral drug, Indinavir, was fabricated into nano-suspensions, which were then internalized into BMM. The acidic environment within the lysosomes allowed Indinavir to be steadily released from the nanosuspensions and outside the macrophages. After a single intravenous administration of BMM-laden Indinavir nanoparticles, a robust of BMM and drug distribution was observed in lung, liver, and spleen. Tissue and sera Indinavir levels were high and lasted for 2 weeks. Indinavir nanosuspension BMM administered to Human immunodeficiency virus type 1 (HIV-1)-challenged humanized mice revealed reduced numbers of virus-infected cells in plasma, lymph nodes, spleen, liver, and lung, as well as, $CD4^+$ T-cell protection. These data demonstrate that a single dose of Indinavir nanosuspension, using BMM as a carrier, is effective and leads the way towards consideration for human testing.

Entry of immune cells into the brain occurs as a consequence of the establishment of a chemokine gradient induced through neuroinflammatory responses (Kadiu et al. 2005). Moreover, the ability of BMM to cross BBB was also investigated (Lawson et al. 1992; Kurkowska-Jastrzebska et al. 1999a, b; Streit et al. 1999; Male and Rezaie 2001; Simard and Rivest 2004) In particular, it was demonstrated that monocytes infiltrate the brain in the 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD (Kurkowska-Jastrzebska et al. 1999a, b; Kokovay and Cunningham 2005).

Based on this, our laboratories developed cell mediated delivery of catalase nanozyme to the brain to mitigate production of ROS and induce neuroprotection (Batrakova et al. 2007). To preclude BMM-mediated enzyme degradation, catalase was packaged into a block ionomer complex with a cationic block copolymer, PEI-PEG. The self-assembled catalase/PEI-PEG complexes, were ca. 60 to 100 nm in size, stable in pH and ionic strength, and retained antioxidant activities. Nanozyme particles were rapidly (40–60 min) taken up by BMM, and released in active form for greater than 24 hours. The released enzyme decomposed microglial hydrogen peroxide. Following adoptive transfer of nanozyme-loaded BMM to MPTP-intoxicated mice, ca. 0.6% of the injected dose was found in the brain. Hence, nanozymes coupled with cell-delivery strategies can reduce oxidative stress in laboratory and animal models of PD. Parallel efforts are using the same system for delivery of growth factors and other anti-inflammatory nanomedicines in divergent neurodegenerative diseases. These diseases would collectively benefit from immunomodulation, neurotrophic factors such as glial

derived neurotrophic factor, or brain-derived neurotrophic factor, and in the case of HIV-1 disease, anti-retroviral therapy (Kabanov and Gendelman 2007).

Conclusion

Although applications of nanotechnologies in clinical neuroscience are only in the early stages of development, the possibilities offered by using these nanomaterials for treatment and diagnosis of CNS disorders are outstanding. As presented in this review, various nanomaterials and nanodevices can be used in neural regeneration, neuroprotection, and targeted delivery of drugs and macromolecules across the BBB. These systems have significant potential for clinical applications. All together, these works lay an important foundation for future studies into improving diagnosis and therapy of human disease. With the threat of significant increases in the prevalence and incidence of human neurodegenerative disorders, these advances are very much needed.

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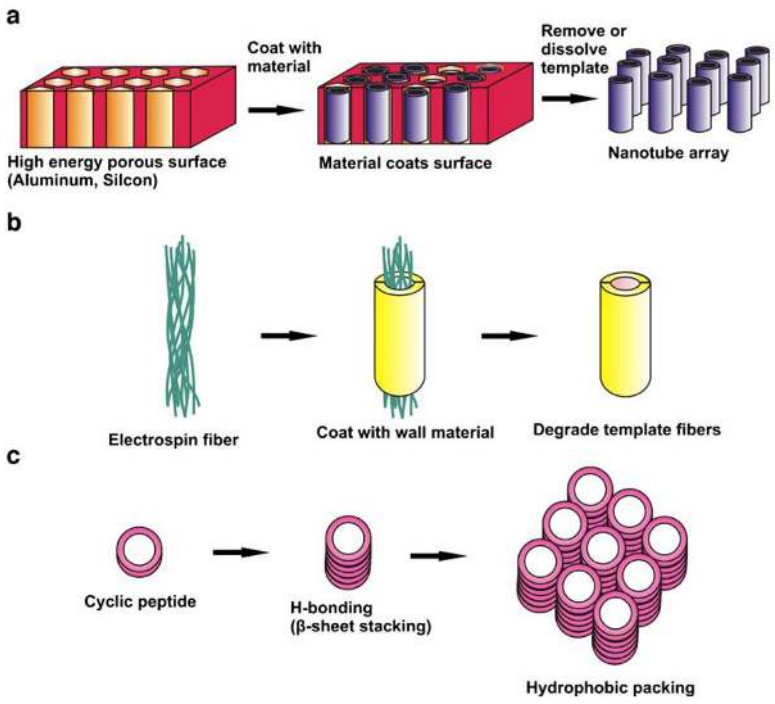

Fig. 1.

Nanotube synthesis. **a** Template synthesis on a porous surface; **b** template synthesis on electrospun biodegradable nanofibers; **c** peptide nanotube self-assembly

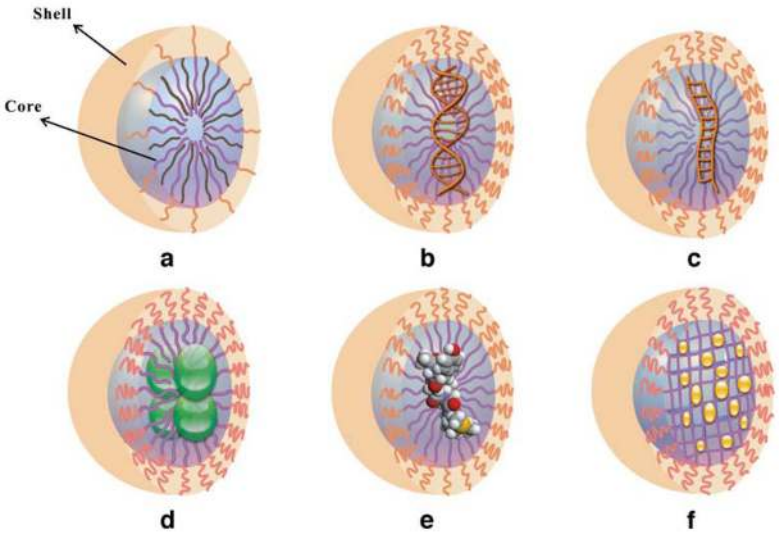

Fig. 2.

Block ionomer complexes. Block ionomer complexes contain hydrophilic shell and hydrophobic core. The hydrophilic shell is formed by the nonionic PEG. The core is formed by neutralized polyions of opposite charge such as complexes of synthetic polyions (**a**), polycation and plasmid DNA (**b**), polycation and siRNA (**c**), polycation (or polyanion) and protein (**d**), or peptide (**e**). The ionic chains in the core can be further cross-linked to form polymer networks that can incorporate small polypeptides, or charged hydrophobic drug molecules (**f**)

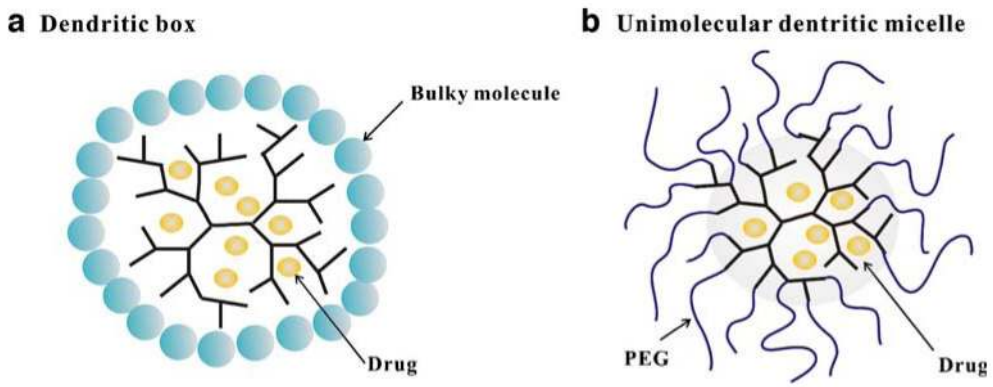

Fig. 3.
Dendrimer structures. **a** Dendritic box **b** unimolecular micelle