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EXPERT REVIEW

# Nanoparticle-Mediated Brain-Specific Drug Delivery, Imaging, and Diagnosis

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**ABSTRACT** Central nervous system (CNS) diseases represent the largest and fastest-growing area of unmet medical need. Nanotechnology plays a unique instrumental role in the revolutionary development of brain-specific drug delivery, imaging, and diagnosis. With the aid of nanoparticles of high specificity and multifunctionality, such as dendrimers and quantum dots, therapeutics, imaging agents, and diagnostic molecules can be delivered to the brain across the blood-brain barrier (BBB), enabling considerable progress in the understanding, diagnosis, and treatment of CNS diseases. Nanoparticles used in the CNS for drug delivery, imaging, and diagnosis are reviewed, as well as their administration routes, toxicity, and routes to cross the BBB. Future directions and major challenges are outlined.

**KEY WORDS** BBB · diagnosis · drug delivery · imaging · nanoparticles

## INTRODUCTION

Central nervous system (CNS) diseases represent the largest and fastest growing area of unmet medical need. Over 1.5 billion people worldwide, including over 100 million people in the US, suffer from CNS diseases or disorders. The World Health Organization (WHO) predicted an increasing medical need for CNS diseases such as Alzheimer's diseases (AD), owing to world population aging and an exponential increase of these diseases in patients beyond 65 years of age. When societal costs are taken into account, including fees for health-care services and value losses in time and productivity of patients and caregivers, overall costs will be even more enormous. For instance, the societal costs of AD alone exceed $100 billion per year in the United States (1).

Finding ways to get therapeutic drugs to the CNS effectively, safely, and conveniently is becoming more important than ever. The biomedical and pharmaceutical applications of nanotechnology have greatly facilitated diagnosis and treatment of CNS diseases. A number of nanoparticulate delivery systems have been developed and demonstrated promising properties (Fig. 1). This review summarizes the nanoparticles recently developed or engineered for brain-specific drug delivery, imaging, or diagnosis. In addition, new administration routes and toxicity of brain-targeted nanoparticles and their transcytosis across the blood-brain barrier (BBB) are discussed.

## THE BBB AND TRANSPORT OF NANOPARTICLES ACROSS THE BBB

The capillary endothelial cells that line the cerebral microvessels and surrounding perivascular elements (basal lamina, pericyte, astrocyte end-feet, and interneurons) make up the BBB, separating the brain from the rest of the body with tight junctions (2,3). Because of the tight junctions, the transendothelial electrical resistance (TEER) of the BBB can be as high as 8,000 $\Omega cm^2$ (4). The structure of the BBB has been well characterized. The length of the cerebral microvessels in the human brain is approximately 650 km, and the total surface area of the human brain microvasculature is about 12 $m^3$ (5). In a gram of brain there is approximately 8 μl of capillary volume (5). The BBB

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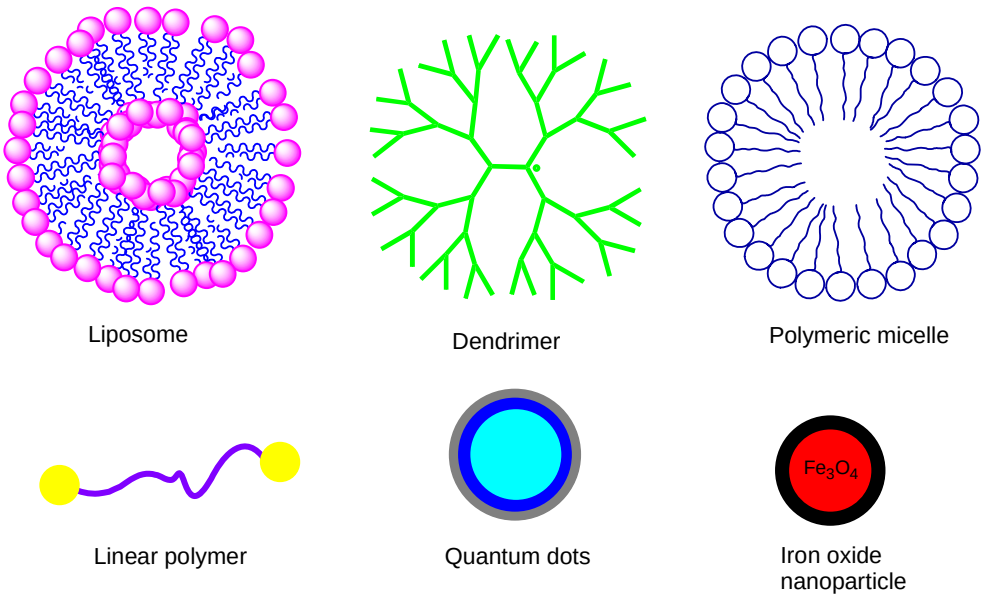


**Fig. 1** Illustration of typical nanoparticles used in brain-specific drug delivery, imaging, and diagnosis.

remains a formidable barrier to most CNS drugs, preventing them from entering the brain (6,7). The BBB allows only highly lipid-soluble molecules under a threshold of 400–600 Daltons to penetrate (8,9). More than 98% of small-molecular-weight drugs do not cross the BBB, and nearly 100% of large-molecular-weight drugs do not cross the BBB (10).

As illustrated in Fig. 2, substances can cross the BBB following paracellular aqueous pathway, transcellular lipophilic pathway, transport proteins, receptor-mediated transcytosis, or adsorptive-mediated transcytosis (3). For instance, a few transport systems are able to deliver certain endogenous water-soluble nutrients to cross the BBB, such as hexose transport protein for glucose and mannose (11,12). Some drugs can be structurally modified to mimic endogenous molecules to take advantage of these transport proteins. A wide range of CNS drugs including large-molecular-weight biological therapeutic peptides, proteins, and genes may gain entry into the brain with nanoparticles as carriers. Various mechanisms have been identified or proposed to explain the transport of nanoparticles to the brain (13). Receptor-mediated transcytosis and adsorptive transcytosis are two major routes for nanoparticle delivery across the BBB. Adsorptive-mediated transcytosis relies on the interaction of a ligand with moieties expressed at the luminal surface of cerebral endothelial cells. Cell-penetrating peptides (e.g. TAT-derived peptides) (14) and cationic proteins (e.g. albumin) are commonly used to enhance brain drug delivery via adsorptive-mediated transcytosis. This route is discussed thoroughly in a recent review (15).

Receptor-mediated transcytosis across the BBB has been explored more actively because of its high specificity. The discovery of receptors that are uniquely expressed by the CNS has opened up a new approach to effective brain drug delivery. Receptor-mediated transcytosis has been demonstrated for insulin, insulin-like growth factors (IGF-1 and IGF-2) (16), leptin (17), transferrin (18), the low-density lipoprotein receptor-related protein (LRP) (19). Certain monoclonal antibodies (mAbs) mimic peptide structure and undergo receptor-mediated transcytosis. For instance, OX26 mAb mimics transferrin and binds to transferring receptor. Nonetheless, OX26 mAb can avoid competition

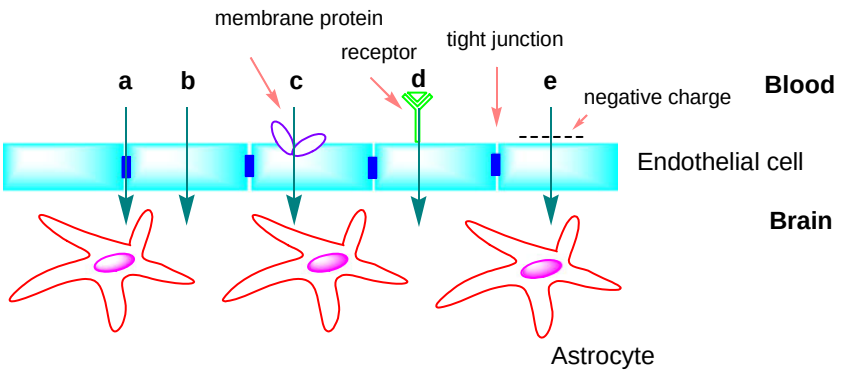


**Fig. 2** The main routes for transport of substances across the BBB. **a** Paracellular aqueous pathway, **b** transcellular lipophilic pathway, **c** transport proteins, **d** receptor-mediated transcytosis, and **e** adsorptive-mediated transcytosis.

with endogenous transferrin in the circulation system because it binds to an extracellular domain of TfR (20). Pardridge and coworkers employed the OX26 mAb as a targeting ligand to deliver therapeutic genes, proteins, and peptides to the brain with liposomes (21,22). Nowadays, OX26 mAb is commonly used to validate the targeting ability and delivery efficiency of nanoparticles used in brain drug delivery. OX26 mAb is reportedly active to rats but not in mice or other species (23). Aktas *et al.* recently designed OX26 mAb-bearing chitosan-PEG nanoparticles and confirmed that OX26 mAb is a critical functional moiety facilitating nanoparticles to cross the BBB (24). Similar to OX26 mAb, 8D3 mAb, and the R17217 mAb are also able to bind to transferrin receptors (TfRs) and can be transcytosed across the BBB (25). Ulbrich *et al.* developed human serum albumin (HSA) nanoparticles coupled to transferrin or TfR-mAbs (OX26 or R17217) for delivery of loperamide and showed efficiency in transporting the drug to the brain using mice (26), in which OX26-conjuated HSA nanoparticles, however, may require revalidation using rats. In addition to transferrin receptors, the use of lactoferrin and insulin receptors for brain targeting has also been demonstrated (27,28). Like OX26 mAb, most brain-targeting mAbs are species-specific and cannot be equally effective across species. Depending on the species studied for brain-targeted delivery, appropriate mAbs shall be used (23). For example, a fully humanized form of the mouse 83-14 anti-human insulin receptor mAb (i.e., humanized HIRMAb) (28) may be considered in constructing a brain-targeted drug delivery if it is used in humans.

## THERAPEUTIC NANOPARTICLES

Nanoparticles serving as drug carriers play an essential role in brain drug delivery. They can be utilized to maintain drug levels in a therapeutically desirable range and increase half-lives, solubility, stability and permeability of drugs. Importantly, they can be structurally adapted to deliver a variety of drugs, improve delivery efficiency, and reduce side effects by targeted delivery. Several major types of nanoparticles that have been widely used for construction of nanomedicines, such as liposomes, dendrimers, and polymeric micelles, have also found applications in the CNS and are summarized in Table 1.

### Liposomes

Liposomes with large drug-loading capacity are made of one or more phospholipid bilayers. Commonly, liposomes have such problems as short clearance time and low transport rate, which, however, can be overcome by incorporating PEG and targeting ligands. In addition, liposomes modified with PEG have improved structural stability against rapid release of drug molecules. Pardridge and coworkers developed a series of antibody-coupled PEGylated liposomes (immunoliposomes) for receptor-mediated delivery of various therapeutics including nucleic acids and drug molecules to the brain (21,25,28–32). Recently, Feng *et al.* used anti-EGFR antibody-carrying immunoliposomes to deliver sodium borocaptate (BSH) for boron capture neuron therapy (33). This new delivery system selectively delivered a large amount of boron to glioma cells *in vitro* and *in vivo*. In the mice treated with the control liposome without the anti-EGFR mAb, the level of BSH was low in both tumor and normal tissues. In contrast, in immunoliposome-treated mice, BSH was detected in the tumor and surrounding regions at 24 h after injection and remained a high level for another 24 h (33). The inhibition of reactive oxygen species (ROS) may be a viable strategy to treat some autoimmune demyelinative diseases, such as multiple sclerosis (MS) and various other neurodegenerative diseases. Kizelsztein *et al.* prepared sterically stabilized nanoliposomes by mixing the liposome-forming lipid, cholesterol and the lipopolymer $^{2000}$PEG-DSPE and used it to deliver tempamine (TMN), a stable radical with antioxidant and proapoptotic activities (34). This system shows efficiency in inhibiting autoimmune encephalomyelitis (EAE) in mice. The penetration of TMN-loaded liposome dose into the brain was 3–6-fold higher in EAE mice than in normal mice. This formulation also helped extend half-life of TMN in plasma to 5 h in normal mice and more than 10 h in EAE mice. In contrast, free TMN was completely cleared from plasma in 1 h (34). PEGylated liposomes were also explored to encapsulate vector/DNA plasmid complexes such as polyethylenimine/oligodeoxynucleotides (PEI/ODN) (35). As a consequence, the blood clearance of gene delivery systems, polyplexes was extended. Further, the accumulation of ODN in the brain was significantly improved once transferrin receptor-specific antibody 8D3 was non-covalently bound to the PEGylated liposome through streptavidin–biotin binding (35). Grahn and coworkers employed non-PEGylated liposomes for simultaneous encapsulation of topotecan (topoCED) and gadodiamide (gadoCED), thus enabling therapy of glioblastoma multiforme and real-time monitoring of nanoparticle distribution (36). The liposomes loaded with TPT and gadodiamide were administered by convection-enhanced delivery (CED) using an *in vivo* U87MG intracranial rodent xenograft mouse model. Desirable TPT pharmacokinetics and co-convection of gadodiamide have been demonstrated. When administered at a dose of 10 μg (0.5 mg/ml), a half-life of approximately 1 day in the brain and an area under the concentration–time curve (AUC) of 153.8 μg day/g, 28-fold over free topotecan (5.5 μg day/g)

**Table I** Brain-Specific Delivery of Representative Therapeutics by Nanoparticles

| Drug | Property/Function | Nanocarrier | Delivery means | Model (s) | Admin. route for *in vivo* | Ref. |
|---|---|---|---|---|---|---|
| Boron | Metal used in boron neuron capture technology | EGF-carrying boronated PAMAM dendrimer | Covalent conjugation | Rats | Intratumoral injection or CED | (73) |
| Ciprofloxacin | Antibiotic | Cholesterol conjugated PEG and anchored with TAT peptide | Encapsulation | Cell culture and rats | i.v. | (42) |
| Doxorubicin | Anthracycline antibiotic used for cancer treatment | OX26-conjugated PEGylated liposome | Encapsulation | Rats | i.v. | (29) |
| | | Folate-conjugated PEGylated liposome | Encapsulation | Rats | i.v. | (40) |
| | | Cysteine-cleavable PEGylated liposome conjugated with folate | Encapsulation | Rats | i.v. | (41) |
| | | Poly(ethylene glycol)-b-poly(aspartic acid) block copolymer | Covalent conjugation | Cell culture and rats | CED | (44) |
| DNA plasmid coding pGL2-control vector coding luciferase | Expressing luciferase for bioluminescence | Lactoferrin-conjugated dendrimer | Complexation | Cell culture and monolayer | | (72) |
| EGFR siRNA | Knockdown EGFR expression | Dendriworms | Complexation | Mice | i.v. or CED | (71) |
| Horseradish peroxidase | Model polypeptide | Pluronic block copolymer | Covalent conjugation through degradable disulfide links | Cell culture, monolayer, and mice | i.v. | (47) |
| N-Hexyl carbamoyl-5-fluorouracil | Prodrug of 5-fluorouracil used for cancer treatment | Polysorbate 80-coated nano-gel (cross-linked copolymeric micelles of N-isopropylacrylamide and N-vinylpyrrolidone) | Encapsulation | Rabbits | i.v. | (43) |
| Oligodeoxynucleotides (ODN) | Containing a NF-κB cis-element | PEGylated liposome | Encapsulation of PEI/ODN complexes | Cell culture and mice | i.v. | (35) |
| Ovalbumin | Model protein | Cationic liposome | Encapsulation | Rats | intranasal | (111) |
| Plasmid pEGFP-N2 | Encoding green fluorescence protein for bioluminescence | Angiopep-carrying PEGylated PAMAM dendrimer G5.0 | Complexation | Cell culture, monolayer, and mice | i.v. | (19) |
| Rivastigmine | Cholinesterase inhibitor for treatment of Alzheimer's disease and Parkinson's disease | Multi lamellar liposome | Encapsulation | Rats | intranasal | (110) |
| Sodium borocaptate | Carrying boron for boron neuron capture technology | EGFR-antibody carrying liposome | Encapsulation | Cell culture and mice | i.v. | (33) |
| Tempamine | Stable radical with antioxidant and proapoptotic activities | PEGylated Liposome | Encapsulation | Mice | i.v. | (34) |
| Topotecan | Topoisomerase I inhibitor | Non-PEGylated liposome | Encapsulation | Rats | CED | (36) |

were achieved for topoCED. A recent report described an innovative use of liposomes as a secondary vehicle for delivery of serotonin. Serotonin-encapsulated liposomes were phagocytosed by monocytes. With the permeation of monocytes across the BBB, phagocytosed serotonin was selectively delivered to the brain (37).

Repeated injection induces accelerated blood clearance of PEGylated liposomes, which was observed in the studies based on rodents and rhesus monkeys (38). It was believed that the first dose of injected liposomes solicits the generation of PEG-specific IgM, which then leads to the activation of the complement system and may even cause complement activation-related pseudoallergy (39). Prolonged circulation times of PEGylated liposomes can also be compromised by ligands coupled to the PEG chains. McNeeley *et al.* found the blood circulation time of folate-carrying PEGylated liposomes was significantly reduced, counteracting the efficacy of the targeting ligand in facilitating receptor-mediated drug delivery to gliomas (40). As an alternative strategy, targeting ligand can be directly conjugated to the surface of liposome. Although long PEG chains help to prevent accelerated clearance by

the reticuloendothelial system (RES), they may impede folate receptor-mediated uptake due to their steric hindrance. To overcome the potentially shortened circulation time of liposomes by active targeting, this group recently designed a cysteine-cleavable phospholipid-PEG (i.e. DSPE-S-S-PEG5000) to mask the coupled targeting ligands from the RES clearance, enabling passive targeting to tumor. Afterward, PEG chains may be detached at the target site, resulting in exposure of folate to cells for receptor-mediated uptake (41). Their *in vivo* plasma clearance studies determined that the cleavable formulation containing 8% DSPE-S-S-PEG5000 sufficiently concealed DSPE-PEG2000-folate from the RES and achieved a similar plasma half-life of doxorubicin to that by non-targeted liposome.

## Micellar Nanoparticles

Micellar nanoparticles are made of amphiphilic polymers. In general, micellar nanoparticles have a hydrophobic inner core in aqueous phase. Hydrophobic compounds with limited transport to the brain can be physically encapsulated into micellar nanoparticles against degradation and rapid blood clearance to achieve prolonged therapeutic activities. Liu *et al.* synthesized cholesterol-terminated PEG and modified the other end of PEG with TAT peptide for aiding the transport of nanoparticles to the brain (42). TAT-PEG-cholesterol could self-assemble into micelles with a hydrophobic core of cholesterol for encapsulation of ciprofloxacin, an antibiotic against neuron inflammatory diseases. Their animal studies based on rats qualitatively confirmed that TAT-conjugated micelles were able to cross the BBB (42). Soni *et al.* prepared nanogels by crosslinking polymeric micelles composed of N-isopropylacrylamide and N-vinylpyrrolidone for delivery of poorly water-soluble N-hexylcarbamoyl-5-fluorouracil (HCFU) (43). They reported that the accumulation of HCFU in the brain was enhanced by coating drug-carrying nanogels with polysorbate 80 (43). Although they found that a large portion of HCFU-loaded nanogels are accumulated in the RES (lung, liver and spleen), coating nanogel with polysorbate 80 increased the amount of nanogel accumulated in the brain from 0.18% of the injected dose to 0.52%. Covalently coupling drugs to micellar nanoparticles has also been explored for brain drug delivery. Inoue and coworkers conjugated doxorubicin (DOX) to the aspartic acid residue of poly(ethylene glycol)-b-poly(aspartic acid) block copolymer (44). The polymeric micelles formed by the resultant DOX-conjugated poly (aspartic acid) block were delivered to the brain through CED. According to their report, micellar DOX infused by CED resulted in prolonged median survival (36 days) compared with free DOX (19.6 days) and liposomal DOX (16.6 days at the same dose (0.2 mg/ml) (44).

Interestingly, individual pluronic copolymer chains facilitate drug transport across the BBB by inhibiting drug efflux transport systems (45,46). Accordingly, molecules of interest can be covalently conjugated to amphiphilic pluronic polymer chains to gain improved penetration into the brain. For instance, the permeability of a BBB-impermeable polypeptide, horseradish peroxidase (HRP), was significantly improved after it was modified with pluronic block copolymers P85 and L121 via degradable linkages (47). The multiple-fold increase of transport of HRP was believed to be the consequence of the generation of an optimal hydrophilic-lipophilic balance of the conjugates (47).

## Dendrimers

Dendrimers have a highly branched, nanoscale architecture with very low polydispersity and high functionality, comprising a central core, internal braches, and many reactive surface groups (48–50). The number of arms and surface groups exponentially increase with generation. The presence of numerous surface groups allows for high drug payload and/or multifunctionality. As the most versatile nanoscale building blocks, dendrimers have been extensively utilized for construction of nanodevices and nanomedicines, among which PAMAM dendrimers are most investigated (51–53). Therapeutics can cross various cell membranes or biological barriers with the aid of dendrimers. Polyanionic PAMAM dendrimers showed rapid serosal transfer rates in crossing adult rat intestine *in vitro* and had low tissue deposition (54). The transport of PAMAM and surface-modified PAMAM across cell monolayer follows endocytosis-mediated cellular internalization (55). The dynamics of cellular entry of dendrimers into human lung epithelial carcinoma cells are dependent on the functional end groups and molecular mass (56). Although non-biodegradable dendrimers may potentially accumulate in lysosomes depending on their frequency and dose of administration, the precise mechanisms of dendrimer internalization and subsequent fate remain to be elucidated.

Conjugating PEG to dendrimer offers more structural advantages than the individual dendrimer and PEG components. A recent study revealed that PEG-modified dendrimers of high molecular weight and many arms have high exocytosis rate and low accumulation in endothelial cells because of their branched structures (57). Many studies, including ours, confirmed that conjugated PEG not only reduces cytotoxicity and immunogenicity of dendrimers but provides dendrimers with excellent solubility and favorable pharmacokinetic and tissue distribution (53,58,59). Amine-terminated polyamidoamine (PAMAM) dendrimers appear to be an ideal class of building blocks for developing multifunctional gene vectors, not only because of their well-defined, highly branched structures

and number of surface groups available for assembly of many different types of functional entities but also because of their inherent properties desired for gene delivery (60–70). The use of dendrimers for gene therapy of brain tumors has been demonstrated.

Angiopep is a high brain penetration peptide, which targets to the low-density lipoprotein receptor-related protein-1. Ke *et al.* coupled angiopep to PEGylated PAMAM dendrimer G5.0 via the distal end of PEG and used it to deliver pEGFP-N2 plasmid to the brain both *in vitro* and *in vivo* (19). The plasmid DNA covalently labeled with fluorescent dye, ethidium monoazide bromide (EMA), was detected in the brain of the mice treated with the PAMAM-PEG-Angiopep/DNA, while the fluorescence in the brain of the mice treated with PAMAM/DNA was not so noticeable. However, both formulations were found to accumulate in the spleen at a similar level. Multimodal dendrimer-conjugated magnetofluorescent nanoworms called dendriworms were developed recently for siRNA delivery (71). The magnetic core in dendriworms enables *in vivo* imaging, of dendriworms with magnetic resonance imaging, while PAMAM dendrimers conjugated to the magnetic core allow nucleic acid delivery and targeting. Dendriworms accumulate in the lungs and the reticuloendothelial filtration organs following systemic delivery. Dendriworms administered with CED efficiently delivered EGFR siRNA to suppress the expression of EGFR in glioblastoma tumors in a mouse model (71).

Targeted dendrimer drug delivery systems utilizing various targeting ligands have been developed. Lactoferrin, a newly explored brain-targeting ligand, was coupled to the dendrimer for targeted delivery of DNAs, and the lactoferrin-coupled dendrimer was reported to have high BBB-crossing efficiency (72). Yang *et al.* prepared epidermal growth factor (EGF)-carrying boronated PAMAM dendrimer G4.0 for neutron capture therapy of brain tumors (73). Doxorubicin was conjugated to RGD-coupled PEGylated PAMAM dendrimer via a degradable disulfide spacer for controlled release in the treatment of glioma tumors (74). Doxorubicin was loaded to cetuximab-conjugated PAMAM dendrimer in an attempt to treat EGFR-positive brain tumors, although the delivery system remains to be optimized for achieving therapeutic efficacy (75).

The size and architecture of dendritic nanoparticles affect their brain drug delivery efficiency and will be taken into account for the rational design of brain-targeted drug delivery. Dhanikula *et al.* synthesized a series of polyether-copolyester (PEPE) dendrimers having various types of branching structures (76). Their *in vitro* study showed that the architecture of dendrimers affects the cellular update of dendrimers and their permeation across the BBB on the basis of an *in vitro* model (76). Clathrin- and caveolin-mediated endocytosis were found to be mainly responsible for internalization of the synthesized PEPE dendrimers. Further, the PEPE dendrimers could cross the BBB *in vitro* in significant amounts without disrupting the tight junctions (76). Their work suggested that this new type of dendrimer can be further validated for brain drug delivery. To get drugs to brain tumors, the blood-brain tumor barrier remains another formidable challenge. It has been reported that dendrimer nanoparticles of less than approximately 11.7 to 11.9 nm are able to cross the pores of the blood-brain tumor barrier of RG-2 malignant gliomas (77).

## Other Polymeric Nanoparticles

Although the mechanism of polysorbate 80-mediated drug delivery to the brain remains controversial, polysorbate-triggered endocytosis was supported (13). Particularly, polysorbate 80-coated nanoparticles may selectively adsorb plasma proteins such as apolipoproteins E and B, which promote receptor-mediated endocytosis across the BBB (78). Poly(butyl cyanoacrylate) (PBCA) nanoparticles coated with polysorbate 80 have been used to deliver a variety of drugs such as loperamide and tacrine to the brain (79,80). However, toxicity-induced non-specific opening of the tight junctions of the BBB by PBCA was also proposed (81). PBCA nanoparticles coated with polysorbate 80 improved the transport of nerve growth factor (NGF) to the brain across the BBB (82). Kurakhmaeva *et al.* observed a significant reduction of the basic symptoms of Parkinsonism in rats by this formulation, which suggested a new delivery system for the efficient transport of NGF and NGF-based treatment of neurodegenerative diseases (82). Biodegradable poly(lactic acid)-b-poly(ethylene glycol) (PLA-b-PEG) nanoparticles coated with polysorbate 80 were found to be efficient in increasing the concentration of amphotericin B in the brain in mice (83). PLGA coated with polysorbate 80 or poloxamer 188 (pluronic F-68) improved delivery of doxorubicin and loperamide to the brain and resulted in pronounced pharmacological effects (78).

Cationic proteins such as albumin and chitosan have been explored as carriers for brain drug delivery. Albumin nanoparticles covalently bound with apolipoprotein E target apolipoprotein receptors at the BBB and enter into the brain through adsorptive-mediated transcytosis (84). Xu *et al.* designed an albumin-conjugated poly(ethylene glycol)–poly(D, L-lactide-co-glycolide) (PEG–PLGA) nanoparticulate delivery system and explored optimal formulation parameters on the basis of 6-coumarin as a fluorescent probe (85). Their biodistribution studies showed that the optimized nanoparticles enabled efficient uptake by the brain without necessarily eliciting enhanced uptake by other tissues (85).

## NANOPARTICLES IN BRAIN IMAGING AND DIAGNOSIS

The utility of nanoparticles has boosted the development of new imaging and diagnostic agents for assessment of brain function and diagnosis of CNS disorders and diseases using structural imaging techniques, such as magnetic resonance imaging (MRI), functional MRI (fMRI), computed axial tomography (CAT), positron emission tomography (PET), magnetoencephalography (MEG), and optical imaging. Nanoparticles in brain imaging can be divided into two major categories: nanosized imaging agents such as superparamagnetic iron oxide (SPIO) nanoparticles (86–89) and trimetallic nitride endohedral metallofullerene nanoparticles (90), and nanocarriers functionalized with imaging agents such as gadolinium, while being capable of carrying other functional entities if necessary. Theoretically, nanoparticles used in drug delivery can also be applied to deliver imaging agents and diagnostic molecules. The increasing attention has been paid to the utility of nanoparticles for fulfilling multiple functions including therapy, imaging, and diagnosis. Nanoparticles, such as quantum dots (QDs) and dendrimers, with high structural adaptability and large carrier capacity for accommodation of various functional entities have been investigated most for brain imaging and diagnosis.

### Quantum Dots

QDs are composed of a metalloid crystalline core and a shell that shields the core. With high brightness, long-term photostability, and size-tunable narrow emission spectra, QDs have emerged as a revolutionary imaging technology and have found diverse biomedical applications including some in the CNS. Arndt-Jovin *et al.* applied QDs coupled to EGF or anti-EGFR (Her1) monoclonal antibodies (MAbs) (mouse MAb 528, H-11, and H199.12) to map human glioblastoma multiforme with fluorescence microscopy (91). Several human glioblastoma-derived cell lines G120, G112, G44, G28, and U87 were tested *in vitro* and in an orthotopic glioma model. They found QDs without EGF were not taken up in any test cell lines. In contrast, QDs coupled to EGF could be taken up, and the level of their uptake varied, consistent with the wild-type and mutant EGFR expression in the cell lines. Glioblastomas often express mutations and upregulated Her1. Therefore, QDs coupled to anti-EGFR MAbs can be used to stain additional classes of glioblastomas as demonstrated in this work (91). Their orthotopic glioma model showed that tumors derived from the G-28 cell line could significantly take up QD-EGF after it was transplanted into mouse brains (91). A study by Wang *et al.* also confirmed the feasibility of QDs conjugated with anti-EGFR antibodies in intraoperative diagnosis of brain tumors (92).

QDs have also been explored as therapeutic carriers. Recently, QDs were used to deliver MMP 9-siRNA to brain microvascular endothelial cells (93). According to the *in vitro* studies, the inhibition of MMP-9 expression resulted in the enhanced expression of extracellular matrix proteins including collagens I, IV, and V, and hence a decrease in endothelial permeability, which can potentially fortify the BBB against invasion of inflammatory cells (93). It was reported that TAT-conjugated Qdots are able to cross the BBB and reach the brain parenchyma following intra-arterial administration (94). Transferrin-conjugated QDs can efficiently transmigrate across an *in vitro* BBB (95). To enhance the stability of QDs in aqueous media, Gao and coworkers designed a new platform, in which QDs were encapsulated into poly(ethylene glycol)-poly(lactic acid) nanoparticles functionalized with wheat germ agglutinin (96). This new platform shows both brain targeting and imaging properties following intranasal administration (95).

### Dendritic Nanoparticles

In addition to the extensive studies of dendrimers in drug delivery, developing dendritic nanoparticle-mediated brain imaging and diagnosis has attracted considerable attention. Bertin *et al.* developed a hydrophilic dendritic manganese (Mn(II)) contrast agent, derived from diethylenetriamine pentaacetic acid (97). They found that the complexed dendritic ligand/manganese possessed increased hydrophilicity, which would help increase the effectiveness of the contrast as it is dependent on the paramagnetic species concentration. Following intraperitoneal injection (i.p.), a contrast increase in the rat brain on both the T1- and T2-weighted images was observed. Since the uptake of Mn(II) by a neuron can reveal its physiological activity and function, this new contrast agent can be applied to probe neurological diseases such as Alzheimer's and Parkinson's diseases despite the relative instability of manganese complexes *in vivo* (97). Because of their well-defined structures and sizes, PAMAM dendrimers were investigated to determine how nanoparticle size affects particle accumulation in malignant glioma cells and transvascular delivery of nanoparticle-based chemotherapeutics across the blood-brain tumor barrier (77). Particularly, Sarin and coworkers functionalized PAMAM dendrimers generations 1 through 8 with gadolinium and rhodamine B for both magnetic resonance and fluorescence imaging. They found that following intravenous injection, the functionalized dendrimers with a diameter of less than approximately 11.7 to 11.9 nm were able to traverse the pores of the blood-brain tumor barrier of RG-2 malignant gliomas (77). This finding can serve as a guide for future nanoparticle-based chemotherapy design.

### Iron Oxide Nanoparticles

Superparamagnetic iron oxide nanoparticles have been explored for various biomedical applications such as magnetic drug targeting and enhanced magnetic resonance imaging. Skaat and Margel designed fluorescent-magnetic iron oxide nanoparticles bearing rhodamine or congo red for amyloid-b (Ab) fibril detection (98). The synthesized multimodal imaging nanoparticles were able to detect Aβ40 fibrils, the main constituent of amyloid plaques in Alzheimer's disease (AD). This new platform shows promise for the early diagnosis of AD (98). Macrophages invade the CNS early during multiple sclerosis (MS). Tracking macrophage infiltration would shed light on the early pathophysiology of MS. Tysiak and coworkers applied very small superparamagnetic iron oxide particles (VSOP) to detect subtle macrophage infiltration into active neuroinflammatory plaques in murine experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS) (99). One advantage of the application of VSOP is that VSOP has the capability of visualizing subtle macrophage infiltration into active neuroinflammatory plaques (99). Veiseh *et al.* designed a nanoprobe (NPCP-CTX-Cy5.5) comprised of an iron oxide nanoparticle coated with a PEGylated chitosan, to which a targeting ligand (chlorotoxin (CTX)) and a near-IR fluorophore (NIRF, Cy.5.5) were conjugated (100). CTX was used as a tumor-targeting ligand because it selectively binds to a variety of cancers including glioma, medulloblastoma, prostate cancer, sarcoma, and intestinal cancer (101). Their studies showed that this nanoprobe, administered via tail vein injection crossed the BBB without compromising the integrity of the BBB and specifically accumulated in brain tumors with sustained retention (100). The combination of MRI detectability and NIRF illumination allows this probe to be used flexibly for preoperative diagnostics, tumor resection, and postoperative assessment with MR and optical imaging.

## ADMINISTRATION ROUTES

The administration routes used to get drugs to the brain include osmotic and chemical opening of the BBB (102), intracerebral injection or implantation (103), intravenous (IV) injection (104), intranasal delivery (105), etc. The drug administration strategies can be classified into two major categories: local drug delivery and systemic administration. Local drug delivery offers effective therapeutic concentrations in the brain, and drug reservoir implants can be directly placed in the brain to achieve controlled release for an extended period of treatment. However, local drug delivery requires surgeries or invasive ways to place or remove drugs or drug reservoir implants. More surgeries are needed if the treatment requires multiple doses. Convection-enhanced delivery (CED) has been demonstrated to be an innovative way for local brain drug delivery. CED depends on a pressure gradient or bulk flow, which helps to enhance the diffusion and distribution of infusates within specific regions of the CNS. CED is particularly beneficial in the delivery of anticancer drugs to the brain because it bypasses the BBB, resulting in improved therapeutic efficacy and avoidance of systemic toxicity. Delivery of nanoparticles to the brain by CED has been demonstrated (106,107). More recently, Huynh and coworkers successfully applied a retro-convection-enhanced delivery (R-CED) to improve the entry of intravenously administered therapeutics within solid brain tumors (108). By withdrawing brain interstitial fluid, they observed a two-fold increase in the extravasation of intravenous Evans blue (EB) albumin in normal brain and in an orthotopic 9 L glioma tumor. Impressively, they observed a five-fold increase in the extravasation of 70 nm fluorescent liposomes in the 9 L tumor (108). Their studies showed the feasibility of applying R-CED to increase the distribution of systemically administered drugs in both the normal tissue-tumor margin as well as in the central tumor core.

A majority of CNS drugs and polymer-based brain drug delivery systems containing biomacromolecules (e.g., genes, proteins, antibodies, or peptides) are administered systemically, most likely via intravenous (i.v.) injection because of the ease of application and avoidance of the first pass effect (109). Following systemic administration, nanoparticles can follow either receptor-mediated transcytosis or adsorptive-mediated transcytosis to get into the brain. Intranasal drug administration has been investigated recently as an alternative to i.v. administration as it allows direct access of drugs to the brain by circumventing the BBB (105). A liposomal formulation following intranasal administration has been used to deliver rivastigmine (110). As a result, rivastigmine achieved a higher drug concentration and a longer half-life in the brain as compared to the intranasal free drug and the oral route (110). This route offers a new means for the long-term non-invasive management of chronic neurological disorders and diseases such as Alzheimer's disease. Delivery of proteins to the brain by cationic liposomes via intranasal route has been demonstrated (111).

## TOXICITY

A systematic evaluation of nanoparticle toxicokinetics in terms of adsorption, distribution, metabolism, and excretion is demanded in order to ease the safety concerns of those nanoparticles to be used clinically. A number of

factors can potentially influence nanoparticle toxicokinetics, such as size and surface properties of nanoparticles, building blocks of nanoparticles, administration routes, and so on (112,113). Particularly, the toxicokinetics of nanoparticles used in the CNS must be understood, as they may affect the integrity of the BBB and cause acute or long-term toxic effects to the CNS. Given that the application of nanoparticles to the CNS is at a nascent stage, *in vivo* and clinical data of the toxic effects of most nanoparticles on the CNS are still scarce, hence calling for considerable attention to the systematic toxicity study of those nanoparticles intended for brain-specific drug delivery, imaging, and diagnosis. A few types of nanoparticles are discussed below.

Liposomes are generally considered nontoxic because they are made from naturally occurring lipids. A recent study by Bauer and coworkers utilized organotypic cultures derived from embryonic ventral mesencephalon to study the toxicity of a liposomal gene delivery vehicle (lipofectamine) in comparison to a non-liposomal (effectene) transfection reagent by monitoring cell membrane damage in terms of cellular uptake of propidium iodide and release of cytosolic lactate dehydrogenase to the culture medium (114). They observed cell membrane damage 24 h after transfection with lipofectamine. The method they developed can be used to test transfection-associated toxicity of different reagents prior to *in vivo* applications into the striatonigral system in the brain.

Nanoparticle toxicity may induce tight junction opening of the BBB. An *in vitro* study by Olivier and coworkers showed that PBCA nanoparticle alone at 10 μg/ml induced a permeabilization of the BBB model, which was presumably attributed to the toxicity of the carrier (81). However, the *in vivo* data is yet to be obtained for the further confirmation of its toxicity-induced permeabilization of the BBB. PAMAM dendrimer toxicity is dose- and generation-dependent *in vitro* (58). Low generation amine-terminated PAMAM dendrimers are less toxic than high generations. QD toxicity is dependent on factors derived from both their inherent physicochemical properties and environmental conditions, such as particle size, charge, concentration, outer coating bioactivity, and mechanical stability (115). Muldoon *et al*. studied the neurotoxicity of the clinical iron oxide agents ferumoxtran-10, ferumoxides, and ferumoxytol, and the laboratory preparation monocrystalline iron oxide nanocompound (MION-46) in rat brain. They demonstrated the safety of those clinical iron oxide agents, as no pathological brain cell or myelin changes were detected after delivery of the clinical iron oxide agents to normal brains (116). PEGylation has been commonly employed to reduce nanoparticle toxicity. PEGylated nanoparticles have favorable pharmacokinetic (e.g., prolonged half-lives) and tissue distribution with reduced accumulation of side effects. Liposomes can also be stabilized through modification with PEG against leakage releasing drug before reaching the targeted site (20,29). It has been documented that PEGylated PAMAM dendrimers have significantly reduced toxicity (59).

## FUTURE DIRECTIONS AND MAJOR CHALLENGES

Rapid advances in the molecular neurosciences have revolutionized brain-specific drug delivery, imaging, and diagnosis with the implementation of nanotechnology. To meet increasing needs for CNS disease treatment, exploring new nanostructured carriers of high drug-loading capacity and brain-targeting ability is important in order to improve treatment efficacy and diagnosis accuracy. A clinically acceptable brain drug delivery system has to undergo multiple phases of studies and trials. Testing of several important delivery properties of a new brain drug delivery system, including targeting ability and delivery efficiency of the delivery system across the BBB at an early stage, is critical for further development of this new delivery system. The other advantages using this *in vitro* model are apparent: cost-effective, simple setup, and well-defined conditions with excellent repeatability. To this end, an *in vitro* model will be developed or applied to examine brain-specific drug delivery systems and collect pilot data. Among many *in vitro* models, a dynamic *in vitro* (DIV)-BBB model developed by Janigro and coworkers represents one that exhibits many BBB characteristics: matching ratio of luminal to ablumenal volume found *in vivo*, requiring fewer cells, and resulting in more accurate pharmacokinetic and toxicological results (117). One future direction can be focused on the validation of this novel DIV-BBB model in assessment of brain drug delivery systems. Another important direction is to engineer those nanoparticles to be safe and enable long-term use without accumulation with adverse affects. Meanwhile, searching non-invasive and safe alternative drug administration to substitute i.v. administration is highly demanded because i.v. injection has poor patient compliance. Further, with the significant increase in the number of CNS drug prescriptions worldwide as predicted, the societal burden of health-care services and the risk of cross-contamination of i.v. injections will be high. New drug administration routes for CNS drugs or delivery systems that are conventionally administered intravenously need to be developed without compromising the efficacy of intravenous administration. To date, no single delivery strategy can provide a conclusive solution to all the problems associated with brain drug delivery. Developing innovative administration routes is as important as developing new delivery systems. It is the goal to develop an effective brain drug delivery system as well as a non-invasive, safe, low-cost route to administer this delivery system with the ease of application,

hence increasing treatment efficacy and patient compliance and reducing societal service burden.

## CONCLUSIONS

Nanotechnology plays a unique instrumental role in the revolutionary development of drug delivery, imaging, and diagnosis. A variety of nanoparticles have been developed and engineered for specific applications in the CNS. With the aid of nanomaterials of high specificity and multi-functionality, therapeutic, imaging, and diagnostic molecules can be delivered to the brain across the BBB, enabling considerable progress in the fundamental understanding, diagnosis, and treatment of CNS disorders and diseases. Because of the inherent complexity of the CNS and the safety concerns of nanomaterials, nanoparticle-mediated technologies that have shown great promises should be further scrutinized prior to their clinical applications.

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## REFERENCES

1. Johnson N, Davis T, Bosanquet N. The epidemic of Alzheimer's disease. How can we manage the costs? Pharmacoeconomics. 2000;18:215–23.
2. Pardridge WM. Blood-brain barrier biology and methodology. J Neurovirol. 1999;5:556–69.
3. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci. 2006;7:41–53.
4. Smith QR, Rapoport SI. Cerebrovascular permeability coefficients to sodium, potassium, and chloride. J Neurochem. 1986;46:1732–42.
5. Pardridge WM, Buciak JL, Friden PM. Selective transport of an anti-transferrin receptor antibody through the blood-brain barrier *in vivo*. J Pharmacol Exp Ther. 1991;259:66–70.
6. Goldstein GW, Betz AL. The blood-brain barrier. Sci Am. 1986;255:74–83.
7. Schlosshauer B. The blood-brain barrier: morphology, molecules, and neurothelin. Bioessays. 1993;15:341–6.
8. Pardridge WM. The blood-brain barrier: bottleneck in brain drug development. NeuroRx. 2005;2:3–14.
9. Levin VA. Relationship of octanol/water partition coefficient and molecular weight to rat brain capillary permeability. J Med Chem. 1980;23:682–4.
10. Pardridge WM. Drug and gene delivery to the brain: the vascular route. Neuron. 2002;36:555–8.
11. Cornford EM, Young D, Paxton JW, Hyman S, Farrell CL, Elliott RB. Blood-brain glucose transfer in the mouse. Neurochem Res. 1993;18:591–7.
12. Mooradian AD, Morin AM. Brain uptake of glucose in diabetes mellitus: the role of glucose transporters. Am J Med Sci. 1991;301:173–7.
13. Kreuter J. Nanoparticulate systems for brain delivery of drugs. Adv Drug Deliv Rev. 2001;47:65–81.
14. Gupta B, Levchenko TS, Torchilin VP. TAT peptide-modified liposomes provide enhanced gene delivery to intracranial human brain tumor xenografts in nude mice. Oncol Res. 2007;16:351–9.
15. Herve F, Ghinea N, Scherrmann JM. CNS delivery via adsorptive transcytosis. AAPS J. 2008;10:455–72.
16. Reinhardt RR, Bondy CA. Insulin-like growth factors cross the blood-brain barrier. Endocrinology. 1994;135:1753–61.
17. Golden PL, Maccagnan TJ, Pardridge WM. Human blood-brain barrier leptin receptor. Binding and endocytosis in isolated human brain microvessels. J Clin Invest. 1997;99:14–8.
18. Fishman JB, Rubin JB, Handrahan JV, Connor JR, Fine RE. Receptor-mediated transcytosis of transferrin across the blood-brain barrier. J Neurosci Res. 1987;18:299–304.
19. Ke W, Shao K, Huang R, Han L, Liu Y, Li J, Kuang Y, Ye L, Lou J, Jiang C. Gene delivery targeted to the brain using an Angiopep-conjugated polyethyleneglycol-modified polyamidoamine dendrimer. Biomaterials. 2009;30:6976–85.
20. Shi N, Boado RJ, Pardridge WM. Receptor-mediated gene targeting to tissues in vivo following intravenous administration of pegylated immunoliposomes. Pharm Res. 2001;18:1091–5.
21. Zhang Y, Pardridge WM. Conjugation of brain-derived neurotrophic factor to a blood-brain barrier drug targeting system enables neuroprotection in regional brain ischemia following intravenous injection of the neurotrophin. Brain Research. 2001;889:49–56.
22. Shi N, Pardridge WM. Noninvasive gene targeting to the brain. Proc Natl Acad Sci U S A. 2000;97:7567–72.
23. Pardridge WM. Re-engineering biopharmaceuticals for delivery to brain with molecular Trojan horses. Bioconjug Chem. 2008;19:1327–38.
24. Aktas Y, Yemisci M, Andrieux K, Gursoy RN, Alonso MJ, Fernandez-Megia E *et al.* Development and brain delivery of chitosan-PEG nanoparticles functionalized with the monoclonal antibody OX26. Bioconjug Chem. 2005;16:1503–11.
25. Lee HJ, Engelhardt B, Lesley J, Bickel U, Pardridge WM. Targeting rat anti-mouse transferrin receptor monoclonal antibodies through blood-brain barrier in mouse. J Pharmacol Exp Ther. 2000;292:1048–52.
26. Ulbrich K, Hekmatara T, Herbert E, Kreuter J. Transferrin- and transferrin-receptor-antibody-modified nanoparticles enable drug delivery across the blood-brain barrier (BBB). Eur J Pharm Biopharm. 2009;71:251–6.
27. R-q Huang, W-l Ke, Y-h Qu, J-h Zhu, Y-y Pei, Jiang C. Characterization of lactoferrin receptor in brain endothelial capillary cells and mouse brain. J Biomed Sci (Dordrecht, Netherlands). 2007;14:121–8.
28. Boado RJ, Zhang Y, Zhang Y, Pardridge WM. Humanization of anti-human insulin receptor antibody for drug targeting across the human blood-brain barrier. Biotechnol Bioeng. 2006; 96:381–91.
29. Huwyler J, Wu D, Pardridge WM. Brain drug delivery of small molecules using immunoliposomes. Proc Natl Acad Sci U S A. 1996;93:14164–9.
30. Pardridge WM, Boado RJ, Kang YS. Vector-mediated delivery of a polyamide ("peptide") nucleic acid analogue through the blood-brain barrier *in vivo*. Proc Natl Acad Sci U S A. 1995;92:5592–6.
31. Pardridge WM. Vector-mediated drug delivery to the brain. Adv Drug Delivery Rev. 1999;36:299–321.
32. Olivier JC, Huertas R, Lee HJ, Calon F, Pardridge WM. Synthesis of pegylated immunonanoparticles. Pharm Res. 2002;19:1137–43.
33. Feng B, Tomizawa K, Michiue H, Miyatake S, Han XJ, Fujimura A *et al.* Delivery of sodium borocaptate to glioma cells

using immunoliposome conjugated with anti-EGFR antibodies by ZZ-His. Biomaterials. 2009;30:1746–55.
34. Kizelsztein P, Ovadia H, Garbuzenko O, Sigal A, Barenholz Y. Pegylated nanoliposomes remote-loaded with the antioxidant tempamine ameliorate experimental autoimmune encephalomyelitis. J Neuroimmunol. 2009;213:20–5.
35. Ko YT, Bhattacharya R, Bickel U. Liposome encapsulated polyethylenimine/ODN polyplexes for brain targeting. J Control Release. 2009;133:230–7.
36. Grahn AY, Bankiewicz KS, Dugich-Djordjevic M, Bringas JR, Hadaczek P, Johnson GA *et al.* Non-PEGylated liposomes for convection-enhanced delivery of topotecan and gadodiamide in malignant glioma: initial experience. J Neurooncol. 2009; 95:185–97.
37. Afergan E, Epstein H, Dahan R, Koroukhov N, Rohekar K, Danenberg HD *et al.* Delivery of serotonin to the brain by monocytes following phagocytosis of liposomes. J Control Release. 2008;132:84–90.
38. Ishida T, Atobe K, Wang X, Kiwada H. Accelerated blood clearance of PEGylated liposomes upon repeated injections: effect of doxorubicin-encapsulation and high-dose first injection. J Control Release. 2006;115:251–8.
39. Szebeni J, Baranyi L, Savay S, Milosevits J, Bunger R, Laverman P *et al.* Role of complement activation in hypersensitivity reactions to doxil and hynic PEG liposomes: experimental and clinical studies. J Liposome Res. 2002;12:165–72.
40. McNeeley KM, Annapragada A, Bellamkonda RV. Decreased circulation time offsets increased efficacy of PEGylated nanocarriers targeting folate receptors of glioma. Nanotechnology. 2007;18:385101 (11pp).
41. McNeeley KM, Karathanasis E, Annapragada AV, Bellamkonda RV. Masking and triggered unmasking of targeting ligands on nanocarriers to improve drug delivery to brain tumors. Biomaterials. 2009;30:3986–95.
42. Liu L, Guo K, Lu J, Venkatraman SS, Luo D, Ng KC *et al.* Biologically active core/shell nanoparticles self-assembled from cholesterol-terminated PEG-TAT for drug delivery across the blood-brain barrier. Biomaterials. 2008;29:1509–17.
43. Soni S, Babbar AK, Sharma RK, Maitra A. Delivery of hydrophobised 5-fluorouracil derivative to brain tissue through intravenous route using surface modified nanogels. J Drug Target. 2006;14:87–95.
44. Inoue T, Yamashita Y, Nishihara M, Sugiyama S, Sonoda Y, Kumabe T *et al.* Therapeutic efficacy of a polymeric micellar doxorubicin infused by convection-enhanced delivery against intracranial 9 L brain tumor models. Neuro Oncol. 2009; 11:151–7.
45. Alakhov V, Moskaleva E, Batrakova EV, Kabanov AV. Hypersensitization of multidrug resistant human ovarian carcinoma cells by pluronic P85 block copolymer. Bioconjug Chem. 1996;7:209–16.
46. Miller DW, Batrakova EV, Waltner TO, Alakhov V, Kabanov AV. Interactions of pluronic block copolymers with brain microvessel endothelial cells: evidence of two potential pathways for drug absorption. Bioconjug Chem. 1997;8:649–57.
47. Batrakova EV, Vinogradov SV, Robinson SM, Niehoff ML, Banks WA, Kabanov AV. Polypeptide point modifications with fatty acid and amphiphilic block copolymers for enhanced brain delivery. Bioconjug Chem. 2005;16:793–802.
48. Tomalia DA, Baker H, Dewald J, Hall M, Kallos G, Martin S *et al.* A new class of polymers: starburst-dendritic macromolecules. Polym J (Tokyo). 1985;17:117–32.
49. Esfand R, Tomalia DA. Poly(amidoamine) (PAMAM) dendrimers: from biomimicry to drug delivery and biomedical applications. Drug Discov Today. 2001;6:427–36.
50. Newkome GR, Yao Z, Baker GR, Gupta VK. Micelles. Part 1. Cascade molecules: a new approach to micelles. A [27]-arborol. J Org Chem. 1985;50:2003–4.
51. Kailasan A, Yuan Q, Yang H. Synthesis and characterization of thermoresponsive polyamidoamine-polyethylene glycol-poly (D, L-lactide) (PAMAM-PEG-PDLLA) core-shell nanoparticles. Acta Biomaterialia. 2010;6:1131–9.
52. Sarkar K, Yang H. Encapsulation and extended release of anticancer anastrozole by stealth nanoparticles. Drug Deliv. 2008;15:343–6.
53. Yang H, Lopina ST. Stealth dendrimers for antiarrhythmic quinidine delivery. J Mater Sci Mater Med. 2007;18:2061–5.
54. Wiwattanapatapee R, Carreno-Gomez B, Malik N, Duncan R. Anionic PAMAM dendrimers rapidly cross adult rat intestine in vitro: a potential oral delivery system? Pharm Res. 2000;17:991–8.
55. Jevprasesphant R, Penny J, Attwood D, D'Emanuele A. Transport of dendrimer nanocarriers through epithelial cells via the transcellular route. J Control Release. 2004;97:259–67.
56. Kannan S, Kolhe P, Raykova V, Glibatec M, Kannan RM, Lieh-Lai M *et al.* Dynamics of cellular entry and drug delivery by dendritic polymers into human lung epithelial carcinoma cells. J Biomater Sci Polym Ed. 2004;15:311–30.
57. Xyloyannis M, Padilla De Jesus OL, Frechet JMJ, Duncan R. PEG-dendron architecture influences endocytic capture and intercellular trafficking. Proc Int Symp Control Release Bioact Mater. 2003;30:149.
58. Roberts JC, Bhalgat MK, Zera RT. Preliminary biological evaluation of polyamidoamine (PAMAM) starburst dendrimers. J Biomed Mater Res. 1996;30:53–65.
59. Yang H, Lopina ST, DiPersio LP, Schmidt SP. Stealth dendrimers for drug delivery: correlation between PEGylation, cytocompatibility, and drug payload. J Mater Sci Mater Med. 2008;19:1991–7.
60. Eichman JD, Bielinska AU, Kukowska-Latallo JF, Baker Jr JR. The use of PAMAM dendrimers in the efficient transfer of genetic material into cells. Pharm Sci Technol Today. 2000;3:232–45.
61. Liu M, Frechet JMJ. Designing dendrimers for drug delivery. Pharm Sci Technol Today. 1999;2:393–401.
62. Bielinska AU, Yen A, Wu HL, Zahos KM, Sun R, Weiner ND *et al.* Application of membrane-based dendrimer/DNA complexes for solid phase transfection *in vitro* and *in vivo*. Biomaterials. 2000;21:877–87.
63. Schatzlein AG, Zinselmeyer BH, Elouzi A, Dufes C, Chim YTA, Roberts CJ *et al.* Preferential liver gene expression with polypropylenimine dendrimers. J Control Release. 2005;101:247–58.
64. Haensler J, Szoka Jr FC. Polyamidoamine cascade polymers mediate efficient transfection of cells in culture. Bioconjug Chem. 1993;4:372–9.
65. Bielinska AU, Kukowska-Latallo JF, Baker Jr JR. The interaction of plasmid DNA with polyamidoamine dendrimers: mechanism of complex formation and analysis of alterations induced in nuclease sensitivity and transcriptional activity of the complexed DNA. Biochim Biophys Acta. 1997;1353:180–90.
66. Braun CS, Vetro JA, Tomalia DA, Koe GS, Koe JG, Middaugh CR. Structure/function relationships of polyamidoamine/DNA dendrimers as gene delivery vehicles. J Pharm Sci. 2005;94:423–36.
67. Yoo H, Juliano RL. Enhanced delivery of antisense oligonucleotides with fluorophore-conjugated PAMAM dendrimers. Nucleic Acids Res. 2000;28:4225–31.
68. Takahashi T, Harada A, Emi N, Kono K. Preparation of efficient gene carriers using a polyamidoamine dendron-bearing lipid: improvement of serum resistance. Bioconjug Chem. 2005; 16:1160–5.

69. Sonawane ND, Szoka Jr FC, Verkman AS. Chloride accumulation and swelling in endosomes enhances DNA transfer by polyamine-DNA polyplexes. J Biol Chem. 2003;278:44826–31.
70. Zhang XQ, Intra J, Salem AK. Conjugation of polyamidoamine dendrimers on biodegradable microparticles for nonviral gene delivery. Bioconjug Chem. 2007;18:2068–76.
71. Agrawal A, Min DH, Singh N, Zhu H, Birjiniuk A, von Maltzahn G *et al.* Functional delivery of siRNA in mice using dendriworms. ACS Nano. 2009;3:2495–504.
72. Huang R, Ke W, Han L, Liu Y, Shao K, Ye L *et al.* Brain-targeting mechanisms of lactoferrin-modified DNA-loaded nanoparticles. J Cereb Blood Flow Metab. 2009;29(12):1914–23.
73. Yang W, Barth RF, Wu G, Huo T, Tjarks W, Ciesielski M *et al.* Convection enhanced delivery of boronated EGF as a molecular targeting agent for neutron capture therapy of brain tumors. J Neurooncol. 2009;95:355–65.
74. Kaneshiro TL, Lu ZR. Targeted intracellular codelivery of chemotherapeutics and nucleic acid with a well-defined dendrimer-based nanoglobular carrier. Biomaterials. 2009;30:5660–6.
75. Wu G, Barth RF, Yang W, Kawabata S, Zhang L, Green-Church K. Targeted delivery of methotrexate to epidermal growth factor receptor-positive brain tumors by means of cetuximab (IMC-C225) dendrimer bioconjugates. Mol Cancer Ther. 2006;5:52–9.
76. Dhanikula RS, Hildgen P. Synthesis and evaluation of novel dendrimers with a hydrophilic interior as nanocarriers for drug delivery. Bioconjug Chem. 2006;17:29–41.
77. Sarin H, Kanevsky AS, Wu H, Brimacombe KR, Fung SH, Sousa AA *et al.* Effective transvascular delivery of nanoparticles across the blood-brain tumor barrier into malignant glioma cells. J Transl Med. 2008;6:80.
78. Gelperina S, Maksimenko O, Khalansky A, Vanchugova L, Shipulo E, Abbasova K *et al.* Drug delivery to the brain using surfactant-coated poly(lactide-co-glycolide) nanoparticles: influence of the formulation parameters. Eur J Pharm Biopharm. 2009;74(2):157–63.
79. Alyautdin RN, Petrov VE, Langer K, Berthold A, Kharkevich DA, Kreuter J. Delivery of loperamide across the blood-brain barrier with polysorbate 80-coated polybutylcyanoacrylate nanoparticles. Pharm Res. 1997;14:325–8.
80. Wilson B, Samanta MK, Santhi K, Kumar KP, Paramakrishnan N, Suresh B. Targeted delivery of tacrine into the brain with polysorbate 80-coated poly(n-butylcyanoacrylate) nanoparticles. Eur J Pharm Biopharm. 2008;70:75–84.
81. Olivier JC, Fenart L, Chauvet R, Pariat C, Cecchelli R, Couet W. Indirect evidence that drug brain targeting using polysorbate 80-coated polybutylcyanoacrylate nanoparticles is related to toxicity. Pharm Res. 1999;16:1836–1842.
82. Kurakhmaeva KB, Djindjikhashvili IA, Petrov VE, Balabanyan VU, Voronina TA, Trofimov SS *et al.* Brain targeting of nerve growth factor using poly(butyl cyanoacrylate) nanoparticles. J Drug Target. 2009;17:564–74.
83. Ren T, Xu N, Cao C, Yuan W, Yu X, Chen J *et al.* Preparation and therapeutic efficacy of polysorbate-80-coated amphotericin B/PLA-b-PEG nanoparticles. J Biomater Sci Polym Ed. 2009;20:1369–80.
84. Zensi A, Begley D, Pontikis C, Legros C, Mihoreanu L, Wagner S *et al.* Albumin nanoparticles targeted with Apo E enter the CNS by transcytosis and are delivered to neurones. J Control Release. 2009;137:78–86.
85. Xu F, Lu W, Wu H, Fan L, Gao X, Jiang X. Brain delivery and systemic effect of cationic albumin conjugated PLGA nanoparticles. J Drug Target. 2009;17:423–34.
86. Kremer S, Pinel S, Vedrine PO, Bressenot A, Robert P, Bracard S *et al.* Ferumoxtran-10 enhancement in orthotopic xenograft models of human brain tumors: an indirect marker of tumor proliferation? J Neurooncol. 2007;83:111–9.
87. Manninger SP, Muldoon LL, Nesbit G, Murillo T, Jacobs PM, Neuwelt EA. An exploratory study of ferumoxtran-10 nanoparticles as a blood-brain barrier imaging agent targeting phagocytic cells in CNS inflammatory lesions. AJNR Am J Neuroradiol. 2005;26:2290–300.
88. Neuwelt EA, Varallyay CG, Manninger S, Solymosi D, Haluska M, Hunt MA *et al.* The potential of ferumoxytol nanoparticle magnetic resonance imaging, perfusion, and angiography in central nervous system malignancy: a pilot study. Neurosurgery. 2007;60:601–11. discussion 611-2.
89. Bourrinet P, Bengele HH, Bonnemain B, Dencausse A, Idee JM, Jacobs PM *et al.* Preclinical safety and pharmacokinetic profile of ferumoxtran-10, an ultrasmall superparamagnetic iron oxide magnetic resonance contrast agent. Invest Radiol. 2006;41:313–24.
90. Fatouros PP, Corwin FD, Chen ZJ, Broaddus WC, Tatum JL, Kettenmann B *et al. In vitro* and *in vivo* imaging studies of a new endohedral metallofullerene nanoparticle. Radiology. 2006; 240:756–64.
91. Arndt-Jovin DJ, Kantelhardt SR, Caarls W, de Vries AH, Giese A, Jovin Ast TM. Tumor-targeted quantum dots can help surgeons find tumor boundaries. IEEE Trans Nanobioscience. 2009;8:65–71.
92. Wang J, Yong WH, Sun Y, Vernier PT, Koeffler HP, Gundersen MA *et al.* Receptor-targeted quantum dots: fluorescent probes for brain tumor diagnosis. J Biomed Opt. 2007;12:044021.
93. Bonoiu A, Mahajan SD, Ye L, Kumar R, Ding H, Yong KT *et al.* MMP-9 gene silencing by a quantum dot-siRNA nanoplex delivery to maintain the integrity of the blood brain barrier. Brain Res. 2009;1282:142–55.
94. Santra S, Yang H, Stanley JT, Holloway PH, Moudgil BM, Walter G *et al.* Rapid and effective labeling of brain tissue using TAT-conjugated CdS:Mn/ZnS quantum dots. Chem Commun. 2005;25:3144–46.
95. Xu G, Yong KT, Roy I, Mahajan SD, Ding H, Schwartz SA *et al.* Bioconjugated quantum rods as targeted probes for efficient transmigration across an *in vitro* blood-brain barrier. Bioconjug Chem. 2008;19:1179–85.
96. Gao X, Chen J, Wu B, Chen H, Jiang X. Quantum dots bearing lectin-functionalized nanoparticles as a platform for *in vivo* brain imaging. Bioconjug Chem. 2008;19:2189–95.
97. Bertin A, Steibel J, Michou-Gallani AI, Gallani JL, Felder-Flesch D. Development of a dendritic manganese-enhanced magnetic resonance imaging (MEMRI) contrast agent: synthesis, toxicity (*in vitro*) and relaxivity (*in vitro*, *in vivo*) studies. Bioconjug Chem. 2009;20:760–7.
98. Skaat H, Margel S. Synthesis of fluorescent-maghemite nanoparticles as multimodal imaging agents for amyloid-beta fibrils detection and removal by a magnetic field. Biochem Biophys Res Commun. 2009;386:645–9.
99. Tysiak E, Asbach P, Aktas O, Waiczies H, Smyth M, Schnorr J *et al.* Beyond blood brain barrier breakdown—*in vivo* detection of occult neuroinflammatory foci by magnetic nanoparticles in high field MRI. J Neuroinflammation. 2009;6:20.
100. Veiseh O, Sun C, Fang C, Bhattarai N, Gunn J, Kievit F *et al.* Specific targeting of brain tumors with an optical/magnetic resonance imaging nanoprobe across the blood-brain barrier. Cancer Res. 2009;69:6200–7.
101. Veiseh M, Gabikian P, Bahrami SB, Veiseh O, Zhang M, Hackman RC *et al.* Tumor paint: a chlorotoxin:Cy5.5 bioconjugate for intraoperative visualization of cancer foci. Cancer Res. 2007;67:6882–8.
102. Rapoport SI. Microinfarction: osmotic BBB opening or microcrystals in infusate? J Neurosurg. 1991;74:685.

103. Broaddus WC, Liu Y, Steele LL, Gillies GT, Lin PS, Loudon WG *et al.* Enhanced radiosensitivity of malignant glioma cells after adenoviral p53 transduction. J Neurosurg. 1999;91:997–1004.
104. Song BW, Vinters HV, Wu D, Pardridge WM. Enhanced neuroprotective effects of basic fibroblast growth factor in regional brain ischemia after conjugation to a blood-brain barrier delivery vector. J Pharmacol Exp Ther. 2002;301:605–10.
105. Lerner EN, van Zanten EH, Stewart GR. Enhanced delivery of octreotide to the brain via transnasal iontophoretic administration. J Drug Target. 2004;12:273–80.
106. Krauze MT, Vandenberg SR, Yamashita Y, Saito R, Forsayeth J, Noble C *et al.* Safety of real-time convection-enhanced delivery of liposomes to primate brain: a long-term retrospective. Exp Neurol. 2008;210:638–44.
107. Saito R, Krauze MT, Bringas JR, Noble C, McKnight TR, Jackson P *et al.* Gadolinium-loaded liposomes allow for real-time magnetic resonance imaging of convection-enhanced delivery in the primate brain. Exp Neurol. 2005;196:381–9.
108. Huynh GH, Ozawa T, Deen DF, Tihan T, Szoka Jr FC. Retro-convection enhanced delivery to increase blood to brain transfer of macromolecules. Brain Res. 2007;1128:181–90.
109. Sarin H. Recent progress towards development of effective systemic chemotherapy for the treatment of malignant brain tumors. J Transl Med. 2009;7:77.
110. Arumugam K, Subramanian GS, Mallayasamy SR, Averineni RK, Reddy MS, Udupa N. A study of rivastigmine liposomes for delivery into the brain through intranasal route. Acta Pharm. 2008;58:287–97.
111. Migliore MM, Vyas TK, Campbell RB, Amiji MM, Waszczak BL. Brain delivery of proteins by the intranasal route of administration: a comparison of cationic liposomes *versus* aqueous solution formulations. J Pharm Sci. 2009;99(4):1745–61.
112. Zhao Y, Meng H, Chen Z, Zhao F, Chai Z. Biological activities of nanomaterials/nanoparticles. In: Zhao YZ, Nalwa HS, editors. Nanotoxicology. California, USA: American Scientific Publishers. 2007;1–27.
113. Powers KW, Palazuelos M, Moudgil BM, Roberts SM. Characterization of the size, shape, and state of dispersion of nanoparticles for toxicological studies. Nanotoxicology. 2007;1:42–51.
114. Bauer M, Kristensen BW, Meyer M, Gasser T, Widmer HR, Zimmer J *et al.* Toxic effects of lipid-mediated gene transfer in ventral mesencephalic explant cultures. Basic Clin Pharmacol Toxicol. 2006;98:395–400.
115. Hardman R. A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. Environ Health Perspect. 2006;114:165–72.
116. Muldoon LL, Sandor M, Pinkston KE, Neuwelt EA. Imaging, distribution, and toxicity of superparamagnetic iron oxide magnetic resonance nanoparticles in the rat brain and intracerebral tumor. Neurosurgery. 2005;57:785–96.
117. Stanness KA, Guatteo E, Janigro D. A dynamic model of the blood-brain barrier "*in vitro*". Neurotoxicology. 1996;17:481–96.