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Nanomedicine: Nanotechnology, Biology, and Medicine
11 (2015) 715–729



nanomedjournal.com

Review Article

Enabling nanomaterial, nanofabrication and cellular technologies for nanoneuromedicines

Surya K. Mallapragada, PhD^{a,*}, Timothy M. Brenza, PhD^a, JoEllyn M. McMillan^b,
Balaji Narasimhan, PhD^a, Donald S. Sakaguchi, PhD^c, Anup D. Sharma^a,
Svitlana Zbarska, PhD^c, Howard E. Gendelman, MD^{b,**}

^aDepartment of Chemical and Biological Engineering, Iowa State University, Ames, IA USA

^bDepartment of Pharmacology and Experimental Neuroscience University of Nebraska Medical Center, Omaha, NE, USA

^cDepartment of Genetics, Development and Cell Biology, Iowa State University, Ames, IA USA

Received 16 September 2014; accepted 18 December 2014

Abstract

Nanoparticulate delivery systems represent an area of particular promise for nanoneuromedicines. They possess significant potential for desperately needed therapies designed to combat a range of disorders associated with aging. As such, the field was selected as the focus for the 2014 meeting of the American Society for Nanomedicine. Regenerative, protective, immune modulatory, anti-microbial and anti-inflammatory products, or imaging agents are readily encapsulated in or conjugated to nanoparticles and as such facilitate the delivery of drug payloads to specific action sites across the blood-brain barrier. Diagnostic imaging serves to precisely monitor disease onset and progression while neural stem cell replacement can regenerate damaged tissue through control of stem cell fates. These, taken together, can improve disease burden and limit systemic toxicities. Such enabling technologies serve to protect the nervous system against a broad range of degenerative, traumatic, metabolic, infectious and immune disorders.

From the Clinical Editor: Nanoneuromedicine is a branch of nanomedicine that specifically looks at the nervous system. In the clinical setting, a fundamental hurdle in nervous system disorders is due to an inherent inability of nerve cells to regenerate after damage. Nanotechnology can offer new approaches to overcome these challenges. This review describes recent developments in nanomedicine delivery systems that would affect stem cell repair and regeneration in the nervous system.

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Key words: Nanoneuromedicine; Nanotechnology; Nanoformulation; Drug development; Targeting

Nanotechnology and nanomedicine approaches involving therapeutics and diagnostics have had a huge impact on medicine notably for cancer, developmental, infectious and immune disorders.

However, until very recently, nanomedicine approaches have not been as deeply developed for the neurosciences. Nanoneuromedicines possess significant potential as opportunities abound in the

Abbreviations: AA, acrylic acid; Apo, apolipoprotein; BBB, blood-brain barrier; BCEC, brain capillary endothelial cells; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; CNT, carbon nanotubes; FDA, U.S. Food and Drug Administration; GFAP, glial fibrillary acidic protein; HGF, hepatocyte growth factor; HIV-1, human immunodeficiency virus type 1; HL, hydrophilic; HP, hydrophobic; HAS, human serum albumin; LIF, leukemia inhibitory factor; MAP-2, microtubule-associated protein-2; MMA, methyl methacrylate; MWNT, multi-walled carbon nanotubes; NPC, neural progenitor cells; NSC, neural stem cell; P3HB4HB, copolymer of 3-hydroxybutyrate and 4-hydroxybutyrate; PANi, polyaniline; PBCA, poly(butyl cyanoacrylate); PD, Parkinson's disease; PEG, polyethylene glycol; PHA, polyhydroxyalkanoate; PHB, poly(3-hydroxybutyrate); PHBHHx, copolymer of 3-hydroxybutyrate and 3-hydroxyhexanoate; PHDCA, polyhexadecylcyanoacrylate; PLA, poly(lactic acid); PLO, poly-L-ornithine; PLGA, poly(lactic-co-glycolic acid); PLLA, poly-L-lactide; PMMAAA, copolymer of MMA and AA; PNS, peripheral nervous system; PPG, poly(propylene glycol); pVA, Poly(vinyl alcohol); RES, reticuloendothelial system; SWNT, single-walled carbon nanotubes.

Funding: Funding for this work (Grant account no. W81XWH-11-1-0700) was provided by the US Army Medical Research and Material Command. University of Nebraska Medical Center research support includes individual donations from Dr. Carol Swarts and Frances and Louie Blumkin, the Vice Chancellor's office of the University of Nebraska Medical Center, ViiV Healthcare. SKM acknowledges the support of the Stanley Chair and BN acknowledges the Vlasta Klima Balloun Professorship. National Institutes of Health grants to researchers at Iowa State University and the University of Nebraska Medical Center: P01 MH64570, R01 MH104147, P01 DA028555, R01 NS036126, P01 NS031492, 2R01 NS034239, R01 AG043540, P01 NS43985, P30 MH062261, (HEG); and P20 GM103480 (TB) are gratefully acknowledged.

*Correspondence to: S. K. Mallapragada, Department of Chemical and Biological Engineering, Iowa State University, Ames, IA, USA.

**Correspondence to: H. E. Gendelman, Department of Pharmacology and Experimental Neuroscience, Omaha, NE, USA.

E-mail addresses: suryakm@iastate.edu (S.K. Mallapragada), hegendel@unmc.edu (H.E. Gendelman).

<http://dx.doi.org/10.1016/j.nano.2014.12.013>

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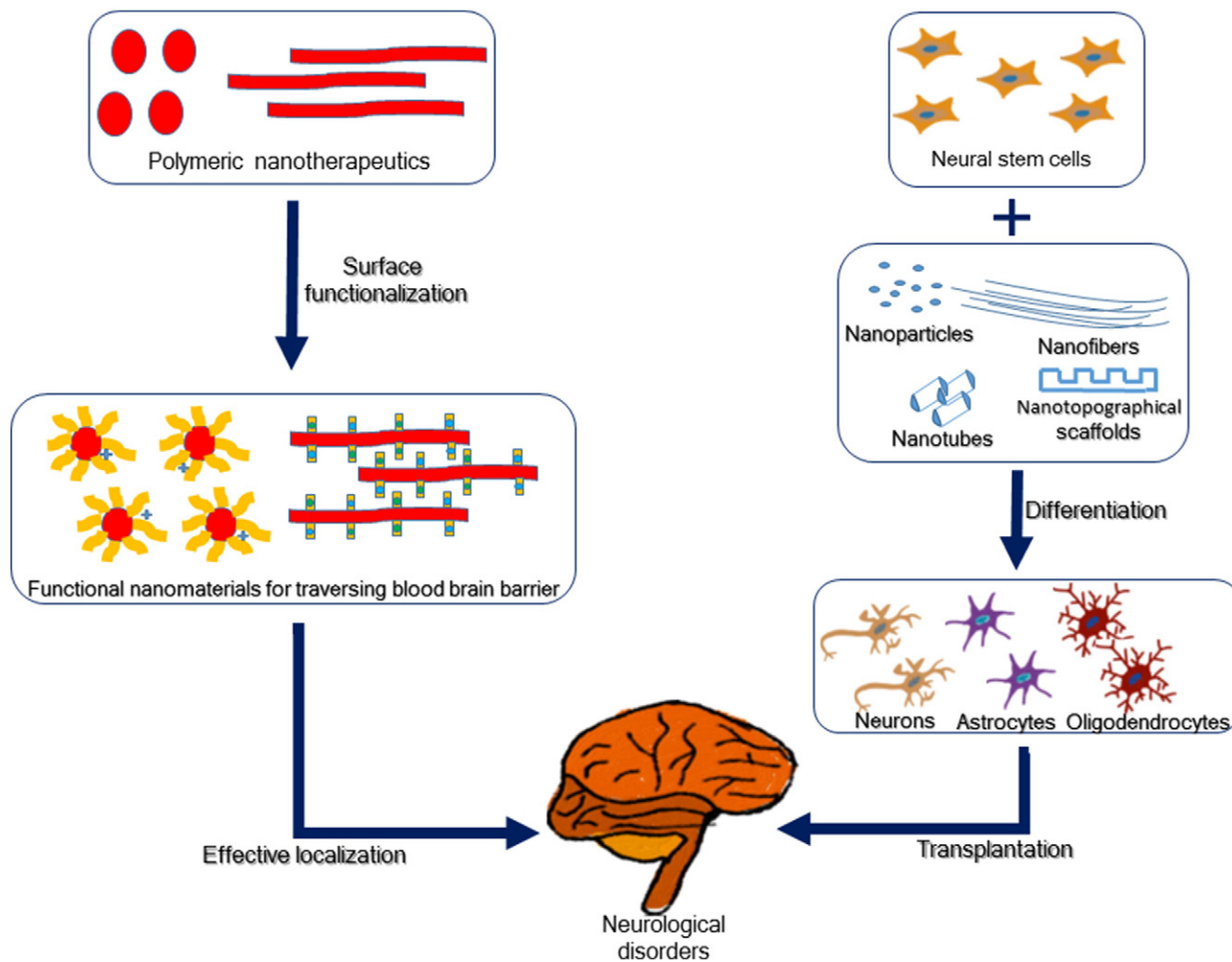


Figure 1. Nanotechnology approaches for targeted delivery of therapeutics and for control of stem cell behavior. These are outlined in box designations in their utilities to address neurological disorders.

development of desperately needed therapies and diagnostics to combat degenerative, inflammatory, infectious and genetic disorders associated with aging. This growing field was selected as the focus for the 2014 meeting of the American Society for Nanomedicine.¹

A fundamental hurdle in developing effective therapies for nervous system disorders resides in an inherent inability of nerve cells to regenerate and/or even repair modest damage incurred to the brain and spinal cord.² Nervous system injury follows a variety of insults such as stroke, trauma, developmental disorders, aging, malignancy, chemical exposures or microbial infections.^{3–7} Typical treatment options utilized, or in development, include therapeutic symptomatic management, stem cell implantation, neural tissue grafts or guidance strategies.^{8–12} Another significant challenge associated with improving nervous system function includes transport of therapeutics across the blood-brain barrier (BBB). Typically, the therapies need to be delivered to the site of the nervous system malfunction and be available long-term. This could be overcome by surgical delivery of therapies to the affected brain and spinal cord sites. Alternative approaches are site-directed drug delivery. However, in contrast to other regions of the body, the nervous system poses unique challenges to site-specific drug delivery as the BBB moderates entry of substances into the brain.¹³

Nanotechnology approaches offer several opportunities to overcome these challenges, including the ability to circulate drug for extended times and to permit functionalization with targeting moieties to promote transport across cell membranes.^{6,14} This could facilitate the use of multifunctional therapeutic, imaging and diagnostic devices, called theranostics.¹⁵

Drug targeting to specific locations is needed for enabling delivery across the BBB and for controlling the fate and behavior of the stem cells in stem cell-based therapies. This review surveys recent developments in delivery systems for nanomedicines that cross the BBB and those that affect stem cell repair or regeneration (Figure 1). These nanotechnology approaches serve as enabling technologies in the emerging field of nanoneuroscience related to applications in diagnostics, imaging and therapeutics of relevance to the nervous system.

Advances in polymer chemistry and nanoparticle delivery for central nervous system (CNS) targeting

In many cases, nervous system targeted therapies include antioxidants, anti-inflammatory agents, immunomodulatory

Table 1
Summary of polymeric nanoparticle use for enhanced CNS drug delivery.

Approach	Nanoparticle properties								Targeting		In vivo efficacy			Ref
	Core polymer	Linkage	Surface coating	Size (nm)	PDI/GSD	Z-Pot. (mV)	Payload	Encap. %	Ligand	Amt.	Admin Route	Dosage (mg/kg)	Response	
Receptor	PBCA	Acrylate	PS80	230	NA	NA	Dalargin	30 (S)	NA	NA	i.v.	7.5	42.5 (28.9) % MPE	18
Receptor	PBCA	Acrylate	PS20 PS40 PS80 C40	230	0.1	NA	Dalargin	30 (S)	NA	NA	i.v.	10 85.2 (20.7) % MPE 97.7 (4.6) % MPE 17.9 (16) % MPE	7 7.4 (25.9) % MPE	20
Receptor	PBCA	Acrylate	PS80	270	NA	NA	Doxorubicin	80 (S)	NA	NA	i.v.	5	6 µg/g	21
Receptor	PBCA	Acrylate	PS80	270	1.07	NA	Doxorubicin	70 (S)	NA	NA	i.v.	3 × 2.5	43 % IST	22
Receptor	PBCA	Acrylate	PS80	290	0.08	NA	Loperamide	47 (S)	NA	NA	i.v.	3.6	53.9 (34.2) % MPE	23
Receptor	PLGA/PVA	Ester	PS80 P188	239.9 242.4	0.187 0.211	8.2 6.0	Doxorubicin	75	NA	NA	i.v. i.v.	3 × 1.5	40 % LTS 40% LTS	27
	PLGA/HSA		P188	408.6	0.289	8.1		97					25% LTS	
	PLGA/PVA		PS80 P188	166.9 168.5	0.266 0.346	-25.0 -17.9	Loperamide	77				7	80% MPE 80% MPE	
	PLGA/HSA		PS80 P188	292.4 287.7	0.092 .077	-18.9 -17.5		82					40% MPE 50% MPE	
Receptor	PLGA/HSA	Ester	P188	468 (19)	.404 (.158)	-11.2	Doxorubicin	88.5	Lecithin	7%	i.v.	3 × 2.5	-12.1 (24.1) µg/mm ² PEC	31
Receptor	Chitosan-PEG	Ether	NA	637 (2)	NA	18 (4)	Z-DEVD-FMK	23 (1)	Transferrin Receptor Mab	NA	i.v.	1		62
Receptor	PBCA	Acrylate	NA PS80	300	0.177	NA	Dalargin	(S)	Apo B Apo E Apo B Apo E	12.5 µg/mL (S)	i.v.	7.5	15.17 (14.11) % MPE ^S 26.08 (21.43) % MPE 64.68 (25.61) % MPE 52.09 (11.22) % MPE	107
			NA				Loperamide	(S)	Apo E			3.6	52.8 (35.5) % MPE ^P	
			PS80						NA				96.7 (12.1) % MPE 96.7 (12.1) % MPE	
Receptor	PEG-PLGA (50:50)	Ester-Ether	NA	120	NA	-14	Urocortin	NA	Lactoferrin	42/ particle	i.v.	28 µg	25%	87
Adsorption	PLGA	Ester	P188	155 (26)	0.13 (0.01)	-15.2 (5.6)	Loperamide	15.1 (0.7)	g7 peptide	39 umol/g	i.v.	2.7	60% MPE	108

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(continued on next page)

Table 1 (continued)

Approach	Nanoparticle properties							Targeting		In vivo efficacy			Ref	
	Core polymer	Linkage	Surface coating	Size (nm)	PDI/GSD	Z-Pot. (mV)	Payload	Encap. %	Ligand	Amt.	Admin Route	Dosage (mg/kg)		Response
Receptor Adsorption	PEG-PLGA (25:75)	Ester-Ether	NA	132	NA	−21.42	Novel active peptide	57.52	NA	NA	i.v.	4	PPT	29
Receptor Adsorption	PLA	Ester	PVA	300 or 125 ^{TEM} or 157 ^{TEM}	1.05 or 0.14 or 1.06	−19.3 (0.5) or 2.4 (0.3)	Ritonavir	89.7	NA	NA	i.v. (d10)	45	10 µg/g	30
Cell	P407-Chitosan	Ester-Ether	PEG	148 (31)	.30 (.01)	12.1 (0.8)	β-galactosidase	>90	RVG29	1.8%	i.v.	5	~25% of dose	63
Cell	NA	NA	P407	383 or 365 or 471 or 454	NA	−10.2 or −24.6 or −21.5 or −18.3	Atazanavir or Ritonavir	100 (H)	NA or Folate or NA or Folate	NA or 40% or NA or 40%	s.c.	2 × 250	10.6 ng/g or 33 ng/g or 4.1 ng/g or 34.5 ng/g	105

Values inside parenthesis are composition for core polymer or standard deviation for numerical values.

NA – Not applicable or not analyzed in reference.

Size – Hydrodynamic diameter determined from DLS unless otherwise noted.

Encap % – percentage of drug encapsulated into core polymer. (S) – surface absorption of drug. (H) – core is homogenized drug.

i.v. – intravenous administration via tail vein.

s.c. – subcutaneous administration.

Dosage – mg drug/kg mouse unless otherwise noted. Multiple administrations are listed as number of administrations × dose of drug for each administration.

Response – Various methods utilized to evaluate response compared to either soluble drug or nanoparticle drug without targeting modification: concentration (µg/g, ng/g) given as mass of drug present per mass of brain tissue; PEC – performance equivalent to control; PPT – positive performance to test; MPE – maximal possible effect; LTS – long-term survival; IST – increased survival time.

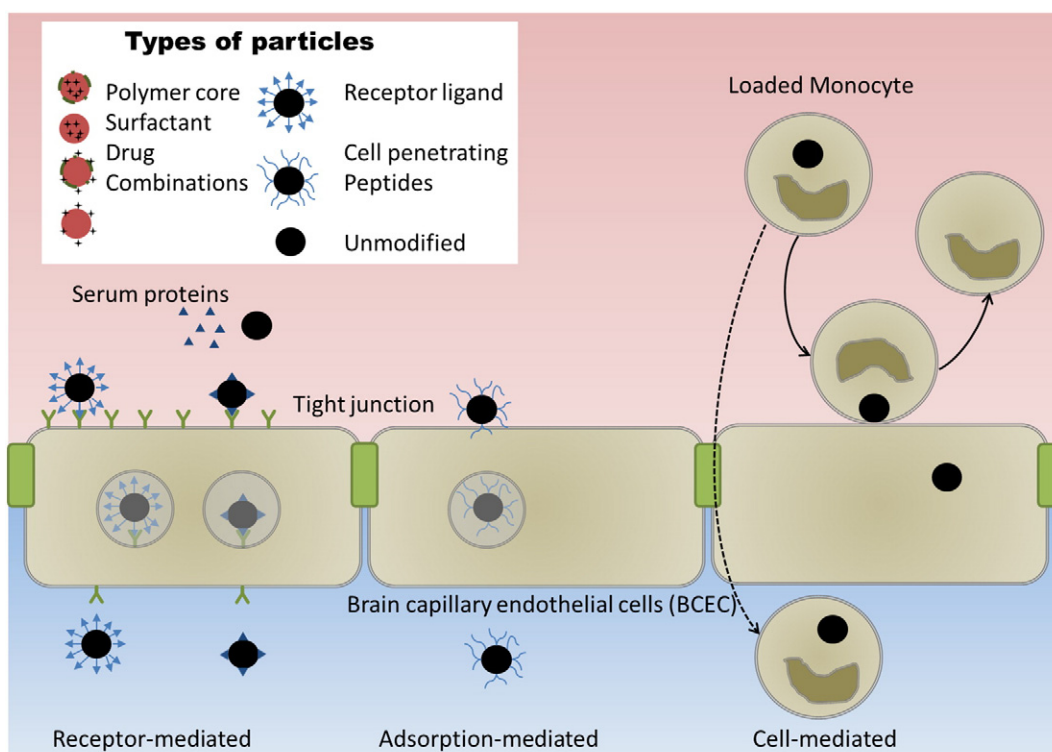


Figure 2. Strategies for nanoparticles to traverse the blood-brain barrier.

compounds, growth factors, genes, siRNA and anti-microbials. The rational design of particles for central nervous system (CNS) drug delivery should take into consideration both drug-polymer compatibility and BBB transport. Development of a CNS targeting strategy is dictated by the desired surface properties of the particulate drug carrier. In this context, particle chemistry, particle surface modification and functionalization, and drug targeting strategies are discussed.

Particle chemistry

Nanoparticles can provide targeted delivery to specific areas of the nervous system by choice of appropriate sizes and chemistries. Several classes of biodegradable polymers have been studied for CNS delivery and include polyalkyl cyanoacrylates, polyesters, polyanhydrides, and polyethers. These polymers demonstrate tunable erosion profiles, easily modified surface chemistry, and sustained payload release profiles.^{16,17} The chemistries are summarized in Table 1.

Poly(alkyl cyanoacrylates)

Dalargin, a hexapeptide, adsorbed to the surface of poly(butyl cyanoacrylate) (PBCA) nanoparticles and coated with the surfactant polysorbate 80, provided the first successful delivery of a peptide administered by intravenous injection to the CNS.¹⁸ In this and related work, polysorbate 80 coating of the nanoparticle proved essential for BBB penetration. PBCA is readily biodegradable with no toxic metabolites and is rapidly cleared.¹⁹ The rate of degradation can be modified by substitution of the alkyl group, but these substitutions also affect

metabolite toxicity. This is the most well-established polymeric nanoparticle delivery system for crossing the BBB, and has been loaded with compounds that include the hexapeptide dalargin,^{18,20} doxorubicin,^{21,22} loperamide,²³ and tubocurarine.²⁴ Therapeutics predominantly were adsorbed onto the PBCA nanoparticle surface after polymerization. This decouples the release of the drug from PBCA degradation, often resulting in poor controlled release.

Polyesters

The biomedical applications of polyesters have been known for more than 40 years. Degradable polyesters were investigated for CNS delivery and include poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA).²⁵ Polyesters are commercially available and approved for human use by the U.S. Food and Drug Administration (FDA, Silver Spring, MD) making them promising candidates for use as biodegradable platforms. One of their most important properties is their low cytotoxicity attributable to their rapid degradation into metabolites that are quickly processed by cells.²⁶ Additionally, the preparation of polymers into nanoparticles is such that the therapeutic agent can be incorporated into the polymer matrix, coupling their release to polymer degradation kinetics.^{25,26} Surface modifications can be performed either by altering the polymer prior to particle formation or by conjugation to the surface post-particle formation. There are advantages and disadvantages to both methods of surface modifications. CNS delivery of drugs is enhanced by polyester core nanoparticles including, but not limited to, loperamide,^{27,28} active peptides,²⁹ ritonavir,³⁰ and doxorubicin.^{27,31}

Even through the use of polyesters for drug delivery, shortcomings remain for their general use. Notably, all polyesters undergo bulk erosion due to the stability of ester bonds,^{32,33} which often result in rapid, or burst, release of drug payloads.^{28,31,34–36} Degradation, notably, depends on the backbone chemistry. Polyglycerol adipate is more hydrophilic because of the single carbon chain compared to PLA, which has a two-carbon chain backbone and shows much slower degradation. The lactic acid component of PLGA can easily be varied between 50 and 100% and the release profiles of encapsulated payloads extended.^{36,37} The molecular weight of the polymer can be varied to marginally control the release of payload.^{35,36,38} Coating the PLGA surface with hydrophobic materials, like gelatin or chitosan³⁴, can decrease the initial drug burst and extend the release period. The rapid degradation of polyester products occurs in microenvironments with a low pH (1.5 – 3.6),^{39,40} which can be problematic when the therapeutic payload is denatured in parallel.

Polyanhydrides

Polyanhydrides possess good biocompatibility and drug delivery potential. This has fostered significant research with these materials.⁴¹ Degradable polyanhydrides were developed for CNS delivery and include sebacic acid and 1,3 bis(*p*-carboxyphenoxy)propane. Polyanhydrides specifically developed for CNS delivery included implantable wafer systems for the treatment of Alzheimer's disease^{42,43} and for brain cancer.^{44–46} These polyanhydride implants are degraded into biocompatible metabolites and are readily eliminated.⁴⁷ The design and commercialization of the Gliadel® wafer, a FDA approved implantable device for the controlled release of carmustine, is an example of a successful polyanhydride implant⁴⁸ and is inserted following surgical removal of brain tumors.

The preparation of polyanhydride nanoparticles allows for the incorporation of drugs within a polymer matrix, which enables the release of the payload with the polymer degradation. With most backbone chemistries (aliphatic or aromatic hydrocarbons), polyanhydride-based devices are surface degrading.^{32,33} By varying the degree of hydrophobicity based on backbone chemistry, polyanhydride devices can rapidly degrade (days), or very slowly degrade (over one year), and as such control drug release.^{49–51} The incorporation of moieties (e.g., ethylene glycol) within the polymer backbone shifts the degradation towards a combination of bulk and surface erosion.¹⁷ Polyanhydride particles are also affected by surface modification of the terminal carboxylic acid groups.^{52,53} The monomers released from polyanhydride degradation are not as acidic (4.2 – 6.5) as those seen during polyester degradation.^{39,40} Surface erosion, along with a wider range of pH microenvironments make polyanhydrides promising carrier materials. However, polyanhydrides are highly susceptible to hydrolytic degeneration with the half-lives of the anhydrides six orders of magnitude greater than polyesters.³³ Partially due to this hydrolytic susceptibility, they are not as translatable as the other polymer chemistries.

Polyethers

Synthetic and naturally inspired polyethers have been used in polymeric drug delivery for over 30 years.^{54–56} Poly(ethylene

glycol) (PEG) and poly(propylene glycol) (PPG) have been used as triblock pluronics ([PEG]*n*-[PPG]*m*-[PEG]*n*) together with other polymers. Those naturally derived polymers such as chitosan, a cationic polysaccharide, are promising drug delivery vehicles.^{57,58} Polyethers are not very susceptible to hydrolytic degradation since their ether bond is very stable in water. Instead, polyethers can be degraded either by enzymes, through oxidation or by dissociation prior to excretion. While there are specific enzymes for chitosan, degradation of the synthetically derived polyethers has only been reported in bacterial cultures.^{59,60} Without a biodegradation mechanism, polyether particles synthesized from synthetically-derived polyethers are inert.⁶¹ For CNS delivery, polyether particle cores can either incorporate chitosan^{62,63} or be incorporated into the backbone of other polymers to facilitate desired amphiphilicity in polyanhydrides.^{64,65}

Particle surface modification and functionalization

While the route of administration can affect the bioavailability of nanotherapeutics, intravenous injection remains the preferred delivery method for evaluating CNS nanotherapeutic efficacy. When administered intravenously, nanotherapeutics first interact with the plasma in the circulation. Particle size, surface chemistry, hydrophobicity and charge are all known to greatly influence the absorption of proteins, cellular interactions and duration of circulation.^{66–68} The presence of a PEG corona on the particle surface also alters the profile of absorbed serum proteins on the surface when administered intravenously.⁶⁹ Additionally, these surface coatings have been observed to reduce clearance through the reticuloendothelial system (RES).^{68,70} Only the polysorbates result in biological effects after intravenous injection, with polysorbate 80 having the greatest efficiency.²⁰ This has also been shown to occur in other drug-PBCA nanoparticles containing doxorubicin.²¹ Coating PBCA with polysorbate 80 can facilitate delivery of particle therapeutics across the BBB, although the exact mechanism of enhancement is unclear.^{71,72} In a comparison of surfactants, multiple polysorbates (20,40,60,80), multiple poloxamers (184, 188, 388, 407, 908), Brij 35 and Cremophors (EZ, RH 40) were coated onto PBCA nanoparticles with dalargin adsorbed to the surface. The current consensus is that polysorbate 80 on the surface affects the type of serum proteins which are adsorbed, influencing transport across the BBB.⁷³ An alternative route of administration is intranasal administration that has received considerable attention in recent years.^{66–68,74,75} Properties of the core polymers, including additional modification of the particle surface either by polyethers (specifically PEG), stabilizers or surfactants are commonly used to enhance drug delivery to the CNS. Moreover, the choice of surface functionalization has significant implications on the overall effectiveness of delivery across the BBB.⁷⁶

Polyethers are used as surface modifiers to alter the surface property of the core nanoparticle for CNS therapies. The surface attachment of PEG is generally accomplished through conjugation to the core polymer either pre- or post-particle synthesis, essentially forming a block copolymer. For example, the tri-block copolymer pluronic can be used to coat the surface of

particles through adsorption as a stabilizer in emulsion particle synthesis. Drug devices and nanoparticles coated with PEG possess a steric stabilization effect in which the hydrophilic PEG opposes interactions with the host, especially phagocytosis and cellular adhesion.^{20,21,68–73,77–79} Surfactants, including pluronic, poly(vinyl alcohol) (PVA) and to a lesser extent human serum albumin (HSA), are used as stabilizers in many nanoparticle formation methods. While both PVA and HSA are biocompatible, PVA is not biodegradable.⁸⁰ The use of these stabilizers controls the size of the particles synthesized, reduce the polydispersity of the synthesized particle size, and enhance drug encapsulation efficiency. However, the inclusion of these surfactants can alter the surface properties of the nanoparticle core, which can be more important in influencing penetration of the BBB.⁷⁶ A summary of particle surface modifications and their impact on BBB penetration is shown in Table 1.

CNS nanoparticle drug targeting strategies

While particle core chemistry and surface modification can control the release of the payload and reduce RES clearance, neither directly addresses the mechanism by which they cross the BBB. One strategy to move nanoparticles across the BBB is to initiate transcytosis of the brain capillary endothelial cells (BCEC). This is accomplished by either binding a ligand for a surface expressed receptor on the circulation side of the BCEC (i.e., receptor-mediated) or by adsorbing the particle to the BCEC membrane, inducing endocytosis (i.e., adsorptive mediated). A second strategy to transverse the BBB is utilization of innate immune cells, like monocytes and macrophages that phagocytose nanoparticles with drug payload(s) and carry the drug within the cells across the BCEC (i.e., cell-mediated). A schematic of these strategies to transverse the BBB is depicted in Figure 2. While this section focuses on the use of these technologies to improve polymeric nanoparticle delivery of therapeutics across the BBB, these methods have also been applied to liposomes, solid lipid nanoparticles and inorganic nanoparticles. Recent reviews have discussed the utility of these additional delivery systems.^{25,81}

Endothelial transcytosis

Receptor-mediated. Receptors on the BCEC can be targets to improve nanoparticle uptake through receptor-mediated endothelial transcytosis and include low density lipoprotein receptor,^{69,82,83} transferrins,^{84–87} leptins,⁸⁸ epidermal growth factor,⁸⁹ diphtheria toxin,⁹⁰ and insulin.^{91,92} Use of those cellular receptors and improved BCEC transport is attained by surface modification of nanoparticles with endogenous ligands, peptides derived from the endogenous ligands and antibodies against the receptors. A summary of particle surface modifications for receptor-mediated endothelial transcytosis and their impact on BBB penetration is provided in Table 1.

Receptor-mediated endothelial transcytosis engages proteins differently. In regards to brain endothelial cells, apolipoproteins adsorbed to the surfaces of polyhexadecylcyanoacrylate (PHDCA) nanoparticles are biologically distinct from those adsorbed onto nanoparticles of PEG-PHDCA copolymers.⁶⁹ Apolipoprotein E (ApoE) and ApoB-100 adsorbed on the surface

of the PEG-PHDCA nanoparticles results in particle penetration of BCEC; whereas, the same amount of opsonizing proteins on PHDCA nanoparticles results in clearance without BCEC penetration.⁶⁹ Two limitations to receptor-mediated endothelial transcytosis are the quantity of receptors on the BCEC surface that can limit the amount of transport and the lack of specificity of expression of these receptors for BCEC, thus limiting specificity of brain delivery and particle-receptor mediated transcytosis.⁹³

Adsorption-mediated. Cell-penetrating peptides increase the delivery of nanoparticles across the BCEC by adsorption-mediated transcytosis. Examples include the human immunodeficiency virus type 1 transactivator of transcription protein,^{30,94,95} poly-arginines,⁹⁶ and Syn-B vectors.^{97,98} The herpes simplex virus type 1 peptide (gH625) has also been shown to increase the transport of polystyrene particles across the BCEC.⁹⁹ A modified opioid peptide (g7) enhances BCEC penetration of nanoparticle-encased drugs by conformationally promoting macropinocytosis.¹⁰⁰ After traversing the BCEC layer, additional modification of the cell surface with antibodies to cell specific markers was shown to enhance the specificity of g7-nanoparticle delivery.¹⁰¹ A summary of particle surface modifications for adsorption-mediated endothelial transcytosis and the *in vivo* impact on BBB penetration is outlined in Table 1.

Cell-mediated transcytosis

Cell-mediated transcytosis was first demonstrated utilizing serotonin-carrying liposomes in monocyte-macrophages.¹⁰² Such a transport method was also used in the delivery of antiretrovirals¹⁰³ and catalase.¹⁰⁴ Crystalline antiretroviral drug nanoparticles were rapidly taken up into human monocyte derived macrophages and subsequently transferred to BCEC.¹⁰⁵ Experiments performed with catalase-loaded polymeric nanoparticles also showed enhanced brain delivery when the nanoparticles were pre-loaded into macrophages.¹⁰⁶

For this method of delivery, the liposomes were not modified to be hydrophilic, neutrally charged or an ultra-small size, but rather to make the particles more amenable to phagocytosis. Similarly, folate surface modification of nanoformulated antiretroviral therapy particles to engage the folate receptor on macrophages resulted in transfer of more nanoparticles to BCECs *in vitro* and corresponded to pharmacodynamic benefits.¹⁰⁵ A summary of the use of particles for cell-mediated transcytosis and their *in vivo* impact on BBB penetration are shown in Table 1.

Neural stem and neural progenitor cells

Neural stem cells and neural progenitor cells can play key roles in addressing neurodegenerative disorders. Studies demonstrating the importance of nanoscale materials and features that help to regulate neural stem cell (NSC) adhesion, proliferation and differentiation into specific neural lineages are discussed. Although details defining mechanisms regulating NSC behaviors with nanomaterials remain to be clearly elucidated, a number of significant advances have been achieved

that can be used for targeting specific neural tissue sites for delivery as well as nanotechnological approaches to control stem cell differentiation and behavior. Applications of nanotechnologies to address neurodegenerative disorders and infections of the nervous system have been developed. In addition to delivery of bioactive molecules, the use of NSC and neural progenitor cells (NPC) synergistically with nanotechnological approaches offers a novel opportunity to address treatments for nervous system disorders and may serve as mechanism(s) for repairing deficits after injury. This requires the development of methods for controlling the development and differentiation of these cells in ways that are relevant for their use in cell transplants or within implants to be used in a variety of CNS or peripheral nervous system (PNS) targets.

In a developing embryo, NSC can differentiate into all of the specialized cell types of the CNS and PNS. Of particular interest is the potential for use of human NSC in regenerative medicine to treat a range of conditions including spinal cord injury, Parkinson's disease (PD), amyotrophic lateral sclerosis and blindness. Moreover, the ability to use NSC as "off-the-shelf" cellular targets to improve drug design and validation for screening is of intense interest to pharmaceutical companies. NSC are also being studied to improve our fundamental knowledge of developmental principles as well as to improve our understanding of CNS birth disorders.

NSCs are multipotential progenitors of neurons and glia that have been isolated from the CNS. Like NSC, NPC have the capacity to differentiate into specific types of cells though they are somewhat more specified in their differentiation capacities.¹⁰⁹ NSC and NPC offer several advantages for CNS repair. NPCs can proliferate in culture and can survive following transplantation into the brain, spinal cord and eye, which is being used as a basis for therapeutic approaches. Here they integrate and stably express foreign genes, or help replace damaged or diseased cells.¹⁰⁹ They can be clonally expanded, providing a renewable supply of transplantable material. They can also be engineered to express exogenous genes for neurotransmitters and neurotrophic factors that can help neuron survival. Thus, it is possible that NPC may also be capable of integrating into host neural circuitry and/or supply trophic factors to enable cell survival and recovery.

NPC have been isolated from various regions of the CNS, such as the cortex, hippocampus, subventricular zone, spinal cord ependyma and retina.¹¹⁰⁻¹¹⁴ Considerable effort has been devoted to elucidate the stem cell microenvironment, or "niche", controlling cell fate.¹¹⁵ A number of studies demonstrated that differentiating NPC are regulated not only via intrinsic genetic control, but also in large part by direct cell-to-cell contact and cell-to-extracellular matrix interactions, topographical control as well as by soluble factors.¹¹⁶⁻¹²² Such interactions can involve a complex "cocktail" of these signaling proteins.¹²³

An important strategy to regulate NPC is to manipulate the microenvironment. Micro- and nanotechnology approaches have considerable potential to mimic the microenvironment in which NPC integrate at the site of injury or neurodegeneration.¹²⁴⁻¹²⁷ Nanomaterials have unique biomimetic characteristics and can manipulate biological and mechanical properties of this microenvironment.¹²⁷ This can have profound influences on

neural stem cell differentiation and functional integration.¹²⁴⁻¹²⁶ Different nanomaterial preparations including nanoparticles, nanofibers, nanotubes and nanotopographical scaffolds can be fabricated and applied to address critical requirements for cell control in repair and can best affect the microenvironment of the CNS.

Nanoparticles are commonly used for stem cell imaging and tracking; intracellular drug/trophic factor/plasmid DNA carriers to control stem cell proliferation and differentiation; and as biosensors to monitor intracellular levels of relevant biomolecules/enzymes.¹²⁸⁻¹³² Nanofibers and nanotopographical scaffolds have been used to direct cell fate during differentiation because they can be designed to mimic the microenvironment. Nanotubes are mostly used in tissue engineering due to their mechanical, electrical and thermal properties. Each of these nanoengineered systems can have a broad range of applications for cell therapy to address a variety of neurodegenerative disorders.

Nanofibers

Nanofibers are an important tool in the field of tissue engineering as they can closely mimic the extracellular matrix architecture, and thus specifically be used to maximize cell-substrate interactions.¹³³ Other benefits of nanofibers include drug delivery and tissue engineering scaffolds. In neural tissue engineering, they can act as a guidance cue for various cell types and sprouting axons. A number of studies have characterized the innate properties of biopolymeric nanofibers towards survival, proliferation and differentiation of NPC as an initial step before use for tissue engineering scaffold constructs. Common methods for fabricating nanofibers involve electrospinning, phase separation and self-assembly.^{133,134} Electrospinning (or electro-spraying) is a widely used method for creating nanofibers ranging from 50 nm to 1000 nm.¹³³ NPC have been shown to selectively differentiate into various neuronal and glial cell types depending on the varying tunable properties of nanofibers. Graphene oxide has been shown to promote the growth and differentiation of adult stem cells and when coated in varying amounts on polycaprolactone nanofibers caused differing expression of neural markers in differentiated adult hippocampal NSC.¹³⁵ Coating with high concentrations of graphene oxide resulted in differentiation to myelinating oligodendrocytes and also an increased expression of various molecules responsible for enhancing differentiation for oligodendrocytes during development. Retinoic acid induced differentiation for adult NSC and resulted in expression of neural differentiation markers when cells were cultured on nanofibers.¹³⁶ Polysaccharide chitosan-derived nanofibers enhance both proliferation and differentiation of neurons and human NSC as compared to another polysaccharide, cellulose acetate.¹³⁷ Ren et al fabricated nanofibers of varying diameters and alignment using polyether sulfone and optimized the differentiation of human pluripotent stem cell-derived neural crest stem cells towards a Schwann cell lineage.¹³¹ Electrical stimulation of NSC resulted in increased neurite outgrowth when cultured on electrospun nanofibers of poly-L-lactide (PLLA) blended with polyaniline (PANi) (PLLA/PANi nanofibers).¹³⁸

Xu et al¹³⁹ examined the efficacy of polyhydroxyalkanoate (PHA) nanofiber matrices by using three different types of PHA; poly(3-hydroxybutyrate) (PHB), copolymer of 3-hydroxybutyrate and 4-hydroxybutyrate (P3HB4HB), and copolymer of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx). All three PHAs in 2- or 3D matrices supported the growth and differentiation of NSC, but PHBHHx produced the most efficient NSC neuronal differentiation. Additionally, 3D had a greater advantage over 2D matrices in regards to NSC attachment and neurite formation. Immobilization of bioactive molecules on nanofibers has been tested for culturing NSC. Collagen was immobilized on nanofibers of copolymer of methyl methacrylate (MMA) and acrylic acid (AA) (PMMAAA) by an N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC)/N-hydroxysuccinimide (NHS) activation process.¹⁴⁰ These collagen-immobilized nanofibers enhanced the attachment and viability of the cultured NSC. Coupling brain-derived neurotrophic factor (BDNF) to nanofibers is more effective in enhancing NSC proliferation and directing their differentiation toward neuronal and oligodendrocyte fates compared to soluble BDNF.¹⁴¹ Silva et al¹⁴² encapsulated murine NPC within a three-dimensional network of nanofibers formed by self-assembly of isoleucine-lysine-valine-alanine-valine-containing amphiphilic peptides. These nanofibers induced a selective differentiation of NSC towards neurons and reduced differentiation towards an astrocyte fate. In addition to NPC differentiation, nanofibers can differentiate embryonic stem cells to neurons.¹⁴³⁻¹⁴⁵

Nanoparticles

Nanoparticles, typically in the range of 1 to 100 nm are capable of acting as a whole unit in terms of size-related intensive properties. Properties of nanoparticles vary significantly compared to properties of bulk material due to the high surface area to volume ratio. In tissue engineering, nanoparticles are used for delivering therapeutic molecules such as drugs, antibiotics, growth factors, cytokines and other factors that can influence differentiation of stem cells.¹⁴⁶⁻¹⁵¹ Magnetic nanoparticles have also been used for manipulating cellular function by using an applied external magnetic field.^{152,153} Both natural and synthetic polymers can be used for nanoparticle fabrication and encapsulating bioactive molecules. Magnetic nanoparticles made up of iron oxide and conjugated with CD133 were successfully used for isolation of NSC from ependymal cells of adult rats.¹⁵⁴ All rats remained alive and healthy after the procedure and cells extracted were found to be capable of neuronal differentiation.

For cancer treatment that targets glioblastoma, a population of NSC displaying tumor-tropic migratory capabilities of NSC were loaded with pH sensitive doxorubicin-loaded mesoporous silica nanoparticles and used as self-destructive carriers.¹⁵⁵ Nanoparticles have also been used for cell tracking. NSC loaded with iron oxide nanoparticles can be transplanted and subsequently tracked using noninvasive magnetic resonance imaging.^{128,131,156} Retinoic acid (RA) loaded nanoparticles using polyethylenimine (polycation) complexed with RA and dextran sulfate (polyanion) were used for controlling the differentiation of subventricular zone NSC by intracellular delivery of RA.¹⁵⁷ A similar strategy was used for

controlling mobilization and migration of human NSC by using hepatocyte growth factor (HGF) and leukemia inhibitory factor (LIF) loaded PLGA nanoparticles.¹⁵⁸ Titanium dioxide (TiO₂) coated with silicon dioxide (SiO₂) selectively differentiate mouse NSC toward a neuronal phenotype. This can occur by altering nine different proteins involved in signaling, molecular chaperones, cytoskeleton and nucleoproteins.¹⁵⁹

Nanotubes

Nanotubes are tubular structures with diameters of a nanometer scale (~1-50 nm). They are considered to have a very large length to diameter ratio for any material. Carbon nanotubes (CNT) are most commonly used for tissue engineering¹⁶⁰⁻¹⁶² because of their various electronic, thermal and mechanical properties. CNT are characterized into single-walled carbon nanotubes (SWNT) and multi-walled carbon nanotubes (MWNT) depending on the number of tubes (single/concentric). Arc discharge, laser ablation, chemical vapor deposition, liquid pyrolysis and ball milling are the methods generally used for CNT fabrication.^{160,163,164} A rope like structure with a diameter of 1 mm and length of 1.5 cm was created using CNTs fabricated by chemical vapor deposition.¹⁶⁵ Electrical stimulation of NSC plated on these ropes caused them to differentiate into neurons at earlier stages compared to NSC growing on control conditions. Electrical stimulation promoted neuronal maturation and enhanced the speed of neurite outgrowth. In a different study, subventricular zone NSC were transplanted at the site of stroke in a rat model along with hydrophilic (HL) or hydrophobic (HP) CNT.¹⁶⁶ HP-CNT reduced infarct cyst volume and increased expression of nestin, an NSC marker. Cell proliferation was increased with improved behavioral outcomes. Both HP-CNT and HL-CNT increased expression of microtubule-associated protein-2 (MAP-2) and reduced expression of glial fibrillary acidic protein (GFAP) suggesting that NSC differentiated towards a neuronal fate when transplanted along with these CNT. Park et al¹⁶⁷ created CNT patterns by creating a monolayer of CNT followed by selective adsorption of laminin on the CNT patterns. Human NSC grew selectively on these patterns and exhibited significantly different outgrowth behaviors. Mouse embryonic NSC from cortex were shown to differentiate into neurons, astrocytes and oligodendrocytes on glass coverslips coated with layer-by-layer assembled SWNT-polyelectrolyte multilayer thin films.¹⁶⁸ Differentiation (neurite outgrowth and expression of neural markers) was comparable to NSC grown on glass coverslips coated with a poly-L-ornithine (PLO) substrate.

Nanotopographical scaffolds

Along with various biochemical cues, topographical cues have also been shown to alter growth, proliferation and differentiation of NSC.^{169,170} The morphology, alignment, focal adhesion assembly and differentiation of human NSC (towards neurons and astrocytes) was affected by fibronectin-coated polyurethane acrylate substrates with diverse nanoscale shapes (groove and pillar) and dimensions (ranging from 300 to 1500 nm groove width and pillar gap).¹⁷¹

Other promising nanomaterials

Various types of hybrid nanomaterials have been synthesized recently for imaging, therapeutic and biomedical applications. Hybrid nanomaterials are a combination of inorganic and organic nanomaterials, such that they not only exhibit the advantageous properties of the two materials involved but can also exhibit additional advantages of their own.^{172,173} These hybrid nanomaterials include technologies such as Nanoscale Metal–Organic Frameworks (NMOFs),^{174–176} functionalized nanotubes and nanogels. Lin and co-workers used a mesoporous silica-based nanoparticle system and cadmium sulfide nanocrystals as removable caps for controlled release of drugs and neurotransmitters.¹⁷⁷ This particle system was found to be biocompatible and was used to investigate neurochemical interactions in astrocytes. Liposomes are closed bilayer phospholipid systems used for better entrapment and delivery of therapeutic drugs.¹⁷⁸ Liposomes can also be used for virus free transfection to generate induced pluripotent cells,¹⁷⁹ gene delivery to mesenchymal stem cells,¹⁸⁰ targeting peripheral neurons and Schwann cells for enhanced uptake¹⁸¹ and targeting the CNS.^{182,183} Similarly, dendrimers are macromolecules of nanoscale dimensions with a central core, branched intermediate structure and then exterior functional groups. Combinations of various properties of these hierarchical components make dendrimers very promising candidates for drug delivery systems.^{184,185} Moreover, because of their anti-amyloidogenic potential, they have also been used for the treatment of various neurological disorders such as Alzheimer's, PD and prion diseases.^{186,187}

Altogether, nanomaterials and nanodevices have shown considerable promise in mimicking the nervous system's microenvironment, and thus can be used as effective tools for controlling NSC growth and differentiation. Functionalized nanoparticles using sugars and proteins by applying different bioconjugation techniques can resemble pathogens and target specific cell types. Cell targeting of nanoparticles during nervous system injury allows differentiation of stem cells into specific neurons or glia for controlling therapeutic cellular interventions. This approach may also reduce nonspecific deleterious bystander effects to the surrounding cells. Also, nanoscale patterning of proteins can be used for stimulating cells at the subcellular level that can affect cell migration, differentiation and proliferation. In the field of neural regeneration research, nanoscale patterning of a conduit with various neurotrophic factors can function as a guidance cue for regenerating axons. Aligned nanofibers have already been used for selective differentiation and alignment of NSC. Use of neurotrophic factor releasing nano- and micro-particles reflects a strategy for neuroprotection and neuroregeneration following spinal cord or other types of nerve injuries or neurodegeneration.

Although nanotechnology has produced positive results, a degree of caution is necessary, especially with respect to use of nanotechnology for NSC differentiation. Nanoparticles and nanotubes were found to be cytotoxic in some studies and decreased the proliferation of NSC. It has also been speculated that in some cases, immune cells may not be capable of recognizing nanoparticles all the time, and nanoparticles can pass

unaided through the BBB itself. Other challenges associated with nanotechnology would be reducing the high cost associated with fabrication, scaling up production, improving the specificity for targeted cells and finally reducing the side effects that nanodevices may have on cells and other tissues.

Clinical applications

Nanotechnology is now considered as a potent tool to overcome various clinical challenges such as tissue engineering, drug delivery, imaging, diagnostics and therapeutics. While nanofibers are used for fabricating scaffolds to mimic a tissue microenvironment, and thus used for nerve, bone and other types of tissue engineering, nanoparticles have been used chiefly as drug delivery vehicles to control delivery of therapeutic agents at sites of injury and inflammation. Before using them for clinical applications, we need to understand the stability of these nanocarriers. Polymeric micelles have been known to improve the stability of hydrophobic drugs by encapsulating them inside or near to the hydrophobic core of the micelle. Hydrophilic chains on the outside help in enhancing *in vivo* compatibility and interaction of the micelles with tissues. Some important parameters that affect the stability of micellar carriers include lengths of the hydrophobic and hydrophilic blocks, chemical nature and molecular weight of hydrophilic blocks, physical state (amorphous or crystalline) of core forming polymer, pH sensitivity, interaction of micelle with serum proteins, thermodynamic stability above critical micelle concentration (CMC) and kinetic stability below CMC by having a stiff core.^{188,189} These nanodevices have also been used as tools to augment stem cell differentiation *ex vivo*. Furthermore, imaging the molecules of interest *in vivo* has become much simpler with the improvement in technologies associated with functionalizing nanoparticles. In addition, the use of nanoscale devices in clinical trials is on a constant rise since the approval of Doxil by the US FDA, the first FDA approved nanodrug.^{81,190–192} As of January 2012, of the 789 ongoing clinical trials, 25 involved nanodevices and 122 involved nanotherapeutics.¹⁹¹ By taking into account various peer reviewed publications, Weissig et al concluded that there are 43 nanopharmaceutical drugs that have been approved by the FDA (or equivalent foreign agencies).¹⁹¹ A high percentage of clinical trials (~72% out of 6242 entries) involving nanodrugs were found to be related to cancer treatments.¹⁹⁰ Nanoengineering as a manufacturing process and the necessity of nanomaterials for enhancing the therapeutic effect or enhancing functionality of existing drugs are the two main criteria for considering a drug as a nanopharmaceutical.¹⁹¹

Conclusions and outlook

As summarized above, nanotechnology and nanomedicine approaches serve as enabling technologies to overcome significant challenges associated with diagnosis, imaging and therapies to address malfunction of the nervous system. As described here, nanoscale systems with appropriate chemistries and functionalization can be extremely promising for safe, effective, targeted and site-specific, and sustained delivery of bioactive agents for imaging and to treat disorders of the nervous

system. Nanomedicine offers new ways for therapeutics and imaging agents to traverse the BBB. A combination of delivery and stem cell-based therapies can significantly impact neuroregeneration. Future studies will continue to investigate strategies using nanotechnology to engineer scaffolds with various materials that can be used to regulate NSC fate decisions. Outcomes from these types of investigations are likely to provide important new information in designing and fabricating a 3D biomimetic neural stem cell niche. These enabling nanotechnologies can significantly impact diagnosis and therapies of nervous system disorders, which is outlined in detail in the accompanying review on clinical applications of nanoneuromedicine.

Acknowledgment

We thank Robin Taylor for outstanding editorial and graphic support.

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