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EXPERT REVIEW

Nanotechnology-Based Drug Delivery Systems for Targeting, Imaging and Diagnosis of Neurodegenerative Diseases

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ABSTRACT Neurodegenerative disorders are becoming prevalent with the increasing age of the general population. A number of difficulties have emerged for the potential treatment of neurodegenerative diseases, as these disorders may be multi systemic in nature. Due to limitations regarding the blood brain barrier (BBB) structure, efflux pumps and metabolic enzyme expression, conventional drug delivery systems do not provide efficient therapy for neurodegenerative disorders. Nanotechnology can offer impressive improvement of the neurodegenerative disease treatment by using bio-engineered systems interacting with biological systems at a molecular level. This review focuses on the nano-enabled system applications for the treatment and diagnosis of neurodegenerative diseases, in particular Alzheimer's, Parkinson's and Prion diseases.

KEY WORDS blood brain barrier · nano-enabled delivery systems · nanotechnology · neurodegenerative diseases

INTRODUCTION

The neurodegenerative diseases (ND) are described by the progressive neuron dysfunction and deaths. The degeneration frequently affects special systems, indicating some form of selective neuronal vulnerability (1–3). ND are often associated with atrophy of the affected central or peripheral structures of the nervous system. Alzheimer's disease is the most prevalent of the neurodegenerative diseases followed by Parkinson's diseases. Lesser extent of patients are affected by amyotrophic lateral sclerosis and Huntington's diseases which have destroying consequences (2–6).

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ND is becoming prevalent with the age of the general population. These devastating disorders require expensive treatments, with more than hundreds billions US dollars annual costs. For instance, an estimated 5.4 million Americans of all ages have Alzheimer's disease in 2012 of which 13% of people are in the age group of 65 and older. Alzheimer's disease is the sixth-leading cause of death in the United States and the payments to care this disease are estimated to be $200 billion in 2012 (http://www.alz.org/downloads/facts_figures_2012.pdf).

There are a number of well described ND in terms of mechanism of the disease and disease pathology but, despite of many progresses, early diagnosis and treatment strategies are still limited (7–9). Brain drug delivery is still a challenge for the treatment of ND. One of the significant constraints for the effective therapy is the presence of the blood brain barrier (BBB) that prevents the diagnostic and therapeutic agents' delivery while it protects the brain from toxic substances (10–13). Additionally, ND may be multisystemic in nature, which causes a great deal for the potential treatment difficulties of ND. More than a single pathogenic factor, a cascade of multiple deleterious molecular and cellular events results in the specific types of neurons death in ND (8,14,15).

In the *nanotechnology* world, *nano* means a billionth. Nanotechnology deals with various structures with dimensions of a billionth of a meter (16–19). The concept led that the function of materials are dictated by the size and shape. This makes the difference between nanoscience and the other conventional technologies, which have some aspect at the nanosize range. The 1 to 100 nm size range encircles the dimensions of biomolecules such as DNA and proteins (17,20–24). Nanotechnology provides a numerous advantages to the nanotechnology-based materials due to their size related properties leading to great potentials for medicinal and pharmaceutical applications including drug-targeted therapies. Currently, 95% of all new drug candidates have poor pharmacokinetics and biopharmaceutical

properties. Thus, design of proper drug delivery systems are required to distribute the drug molecule only to the target site, without affecting healthy organs and tissues, are strongly needed. Nanotechnology as nanomedicines plays an important role by making this possible and reduce the required doses as well as increasing the safety profiles of new drug molecules (25–28). In addition to targeted drug delivery, sustained release drug profiles and intracellular sanctuary to protect therapeutics from efflux or degradation are obtained by nanomedicines.

Considering that, the use of nanotechnology based drug delivery systems for brain drug delivery by passing BBB is crucial strategy. These systems allow the transport of non-transportable drugs or diagnostic agents across the BBB by masking their physico-chemical properties through their encapsulation in these systems. Furthermore, they may reduce the leaching of the drug in the brain and decrease peripheral toxicity (8,29–31).

This review focuses on applications of nanotechnology-based drug delivery systems in the therapy, imaging and diagnosis of the most common ND, emphasizing future nanotechnological approaches.

THE BLOOD-BRAIN BARRIER

The blood–brain barrier (BBB) is the organized interface between the central nervous system and the peripheral circulation. Although astrocytes and pericytes are important components, BBB is mainly formed by the unique properties of endothelial cells: i) the tight junctions which connect adjacent endothelial cells and physically restrict solute flux between the blood and the brain, which causes the limited passive diffusion to the brain for small lipophilic compounds (optimal log P is 1–3) of molecular weights below 400 to 500 Da, ii) selective influx transport of hydrophilic compounds is permitted by transport proteins, iii) biotransformation and detoxification system is provided by metabolic barriers, and iv) insignificant pinocytotic activity (17,32–35) (Fig. 1).

BBB and enzymes such as gamma-glutamyl transpeptidase, alkaline phosphatase, aromatic acid decarboxylase and several cytochrome 450 enzymes restricts the entry of substances for maintaining the internal environment of the brain. BBB allows a selective entry of nutrients and minerals due to the presence of multiple endogenous transporters, and limits the foreign

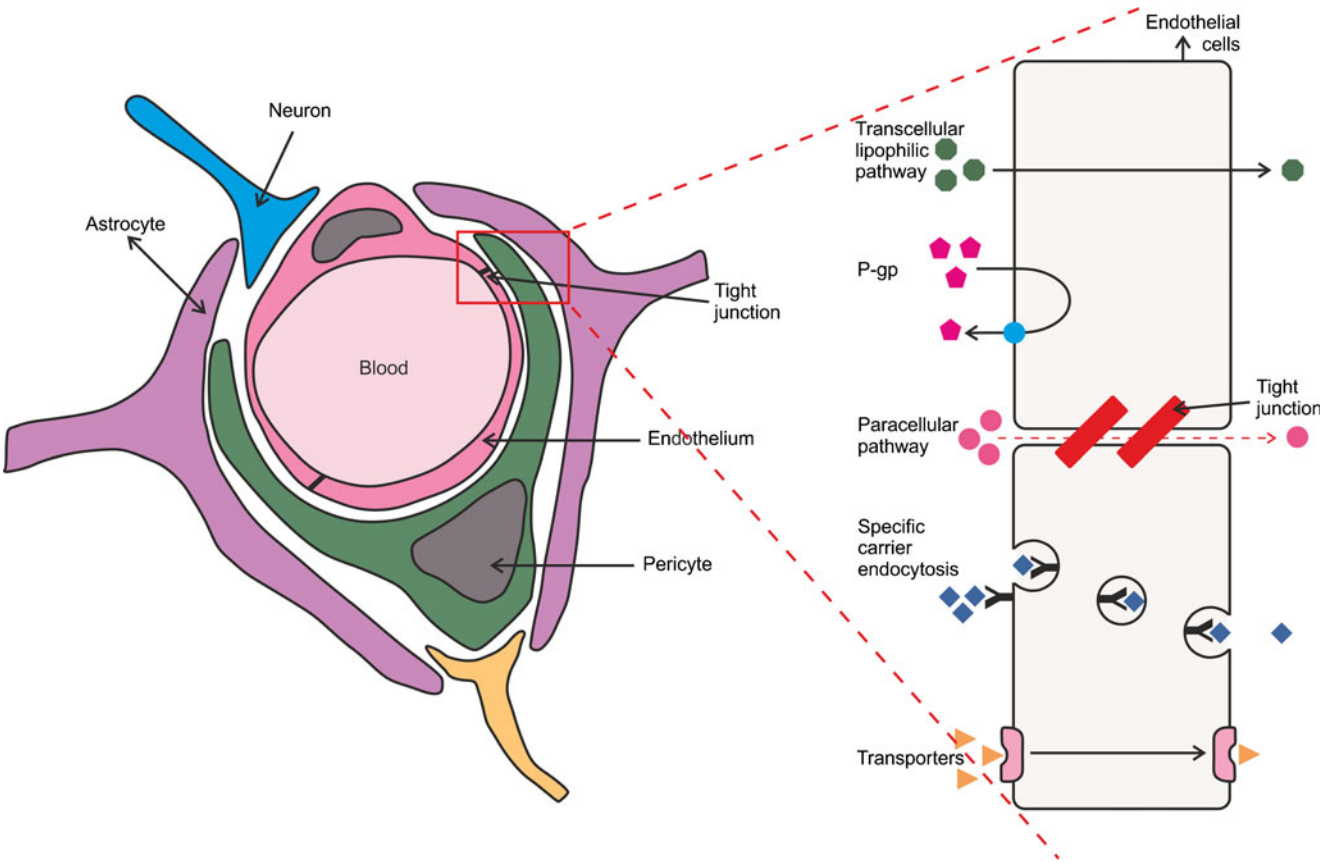

Fig. 1 Schematic diagrams of the Blood Brain Barrier (BBB) and the transport mechanisms across the BBB.

substances entry like drugs as well as neurotherapeutic and diagnostic agents. Therefore, many drugs, therapeutic and diagnostic agents are unsuccessful in treating and/or diagnosis of ND disorders since they cannot be delivered to the brain effectively (36–39). Efforts on research have focused on more effective strategies to deliver drug molecules to the brain.

Currently, three different approaches are used to bypass the blood brain barrier; invasive, pharmacological and physiological.

Invasive Approach

These physical techniques including the use of intracerebroventricular infusion, convection enhanced delivery, disruption of BBB and polymeric or microchip systems, that are mechanically breaching the BBB and deliver drug to the brain. The disadvantages of these techniques are they need hospitalization and leave the BBB damaged/open for a long period which may cause secondary infections and neurons may be damaged from undesirable entry of the blood components to the brain as well as traumatic injury due to mechanical approach.

Pharmacological Approach

This approach consists of modification of drugs to reduce the relative number of polar groups for increasing the cross of a drug through the BBB. Hydrophobic derivatives of creatine, a neuroprotective compound, which cannot pass the BBB due to its polar nature, capable of crossing the BBB has been synthesized for acute and chronic ND treatment (40). It has been reported creatinyl-glycine ethyl ester—which is one of the most effective derivatives- increases survival time of mice more than two times in hypoxia model and has neuroprotective action in brain stroke model when applied both before and after ischemia. Some disadvantages of pharmacological approach are loss of desired activity of modified drugs and extrusion of the drug outside with efflux pump P-glycoprotein (P-gp) due to increased drug lipophilicity (Fig. 1).

Physiological Approach

The essential substances for metabolism and survival of the brain such as glucose, growth hormone (GH), insulin, low density lipoprotein (LDL) are identified by specific receptors or transport mechanisms, culminating in selective transport to the brain. In this approach, drugs can be modified to resemble to the nutrient transport systems of BBB or can be conjugated to the ligands, which recognize expressed receptors at the BBB. Difficulties in drug dissociation from the ligands and possible non-specific drug-receptor interactions expressed on the BBB in addition peripheral organs are the disadvantages of these approaches. However, this approach is admitted to be a great potential of success by the scientific community (Fig. 1).

Transporter-Mediated Delivery

Specific transporters like choline and amino acid transporters expressed on the BBB may be used by peptides and small molecules to pass through this barrier. For instance, the large neutralamino acid carrier has been used to deliver L-Dopa, which is dopamine's metabolic precursor and provided clinical benefit to Parkinson's disease patients compared to dopamine itself.

Receptor-Mediated Transcytosis

Essential large molecules for the regular brain functions are carried by the specific receptors such as transferrin receptor, insulin receptor, LDL receptor and its related proteins which are highly expressed on the endothelial cells forming the BBB. There are still on going studies to identify new receptors. Liu and co-workers (41) has designed a nano-device which was based on dendrigraft poly-l-lysines (DGLs) and linked with a brain-targeted peptide RVG29 as well as the caspase-3 cleavable peptide linker (DEVD) in order to deliver diagnostic agent to the brain crossing the BBB. The authors reported that the nano-device would help to image activated caspase-3 *in vivo* and hold great promise in early diagnosis of neurodegenerative diseases.

The transferrin receptor (TfR) is highly expressed on the luminal side of brain capillary endothelium and might lead to receptor-mediated transcytosis across the BBB when activated. Thus, TfR has been frequently considered during design of brain targeting drug delivery system (42–48). *N*-Benzyloxycarbonyl-Asp(OMe)-Glu(OMe)-Val-Asp(OMe)-fluoromethyl ketone (Z-DEVD-FMK) is an irreversible inhibitor of caspase-3 which is a potent mediator of apoptosis and plays an important role in death of neurons following global or focal cerebral ischemia. Z-DEVD-FMK, a peptide, is unable to pass the BBB and cannot achieve therapeutic levels within the brain parenchyma after systemic administration. Polyethylene glycol (PEG) surface modified, Z-DEVD-FMK loaded nanoparticles have been conjugated to anti-TfR monoclonal antibody (TfRMAb) using biotin–streptavidin bonds and evaluated their efficacy on experimental focal cerebral ischemia model. It has been reported that the high dose of Z-DEVD-FMK loaded nanoparticles and conjugated with TfRMAb significantly inhibited caspase-3 activity, whereas nanoparticles not loaded with the peptide (blank) or unconjugated with TfRMAb (TfRMAb-free) were ineffective (49).

Amphotericin B (AmB) is an antifungal drug that penetrates poorly into the central nervous system. Angiopep-2 modified 1, 2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-

N-[methoxy(polyethylene glycol)-2000] (PE-PEG) based micellar system containing AmB has been designed to deliver the drug into brain crossing the BBB by means of receptor-ligand interaction between angiopep-2 and low-density lipoprotein receptor-related protein (LRP) present on the BBB. It has been reported that In terms of penetrating across the BBB than AmB loaded angiopep-2 modified micelles were found more efficient than unmodified micelles and Fungizone® (deoxycholate AmB) *in vitro* and *in vivo* and this system could be used for brain targeting (50).

Adsorptive-Mediated Transcytosis (AMT)

AMT depends on a ligand's electrostatic interaction with the luminal surface charges of endothelial cells forming the BBB. Cationic peptides and proteins initially bind to the luminal plasma membrane by interacting electrostatically with anionic sites, which triggers adsorptive endocytosis. Zhao and co-workers (51) have prepared paclitaxel and Fe_3O_4 loaded polymeric magnetic liposomes to achieve efficient therapy for CNS diseases by passing the BBB. In addition to magnetic characteristics, the liposomes have designed to possess cationic charge in order to reinforce their penetration through BBB. The results have indicated that brain paclitaxel concentration increased 2–5 folds without magnetic targeting and 5–15 folds after magnetic targeting following the injection of liposomes to rats through the caudal vein.

Bovine serum albumin conjugated fluorescein isothiocyanate (BSA-FITC) loaded cationic vesicles, which has acetylcholine (ACh) surface groups have been evaluated in terms of crossing biological barriers including the BBB. The formulations have been administered to pyridostigmine, a choline esterase inhibitor, -pretreated mice intravenously following their stability evaluation in whole serum that contains choline esterase. The study has shown that BSA-FITC loaded choline esterase-sensitive vesicles accumulated in the brain significantly while distributing into various other tissues, though free BSA-FITC was not detected in the brain (52).

Doxorubicin (DOX) - a BBB impermeable anticancer drug —loaded and cationic bovine serum albumin (CBSA) conjugated solid lipid nanoparticles (SLNs) have been prepared in order to provide efficient drug level in brain by passing the BBB. Transendothelial studies have shown that CBSA conjugated SLNs has the highest transcytosis ability to across brain capillary endothelial cells. *In vivo* studies and biodistribution data have demonstrated that following the administration of drug solution or CBSA-SLN-DOX; no DOX or efficient amount of DOX was detected in the brain, respectively, indicating targeted brain drug delivery has achieved with conjugated SLNs (53).

Recently, the most encouraging strategies are those based on nanotechnology based drug delivery systems (NBDDS) designed to interact with the BBB cells at molecular level, the existing physiological mechanisms of transport,without interfering with the normal function of the barrier itself. Receptor- and adsorptive-mediated transcytosis are the most hopeful mechanisms to facilitate the transcellular transport of NBDDS from the blood to the brain. In order to achieve this goal, NBDDS should carry, at the minimum, a surface functionalization for targeting and crossing the BBB and a prolonged half-life in blood, avoiding the reticulo-endothelial system (9).

NANOTECHNOLOGY-BASED DRUG DELIVERY STRATEGIES FOR NEURODEGENERATIVE DISEASES

NBDDS (Fig. 2) play crucial role to solve potential problems of CNS-targeted therapies such as passing the BBB, one of the biggest handicaps for these therapies. It is also possible to design NBDDS for interaction with defined cellular subsets or molecules, whereat affording treatment specificity. Moreover, multifunctional properties as bioactivity, targeting, imaging capabilities and gene delivery can be achieved simultaneously with these systems (Fig. 3). In this respect, NBDDS have been extensively applied for diagnosis, imaging and treatment purposes.

NBDDS for Alzeimer's Disease

Alzheimer's disease (AD) is one of the frequent cause of dementia affecting mainly older population. The cause of AD at the molecular level is still unknown, however there are lots of factors thought to play a significant role in its pathogenesis, such as abnormal proteins, oxidative stress and excessive metal ion accumulation in brain, and reduced acetylcholine (ACh) levels (54–60). The current methods do not seem to be capable to overcome the disease, and several factors should be considered while surpassing AD because of the multifactorial nature of Alzeimer (61).

Nanoparticles sizes vary in a range of 10 to 1,000 nm. NPs can be constructed from various materials (e.g. polymers, lipids, metals) and can host a wide range of active components, including chemotherapeutics, contrast agents, proteins and nucleic acids, for various biomedical applications (62). Liposomes are the vesicles composed of a lipid bilayer, with diameters ranging between 20 nm to micrometer size. The nanoliposome term has been defined to particularly refer to nanosize lipid vesicles. Both of them are particularly useful as efficient drug delivery systems because of their capability of passing through lipid bilayers and cell membranes, hence improving bioavailability (63).

It has been postulated that along with oxidative stress, amyloid-β (Aβ), a 39–43 amino acid peptide, plays a major role in AD pathogenesis (64,65). The neurotoxicity of Aβ may

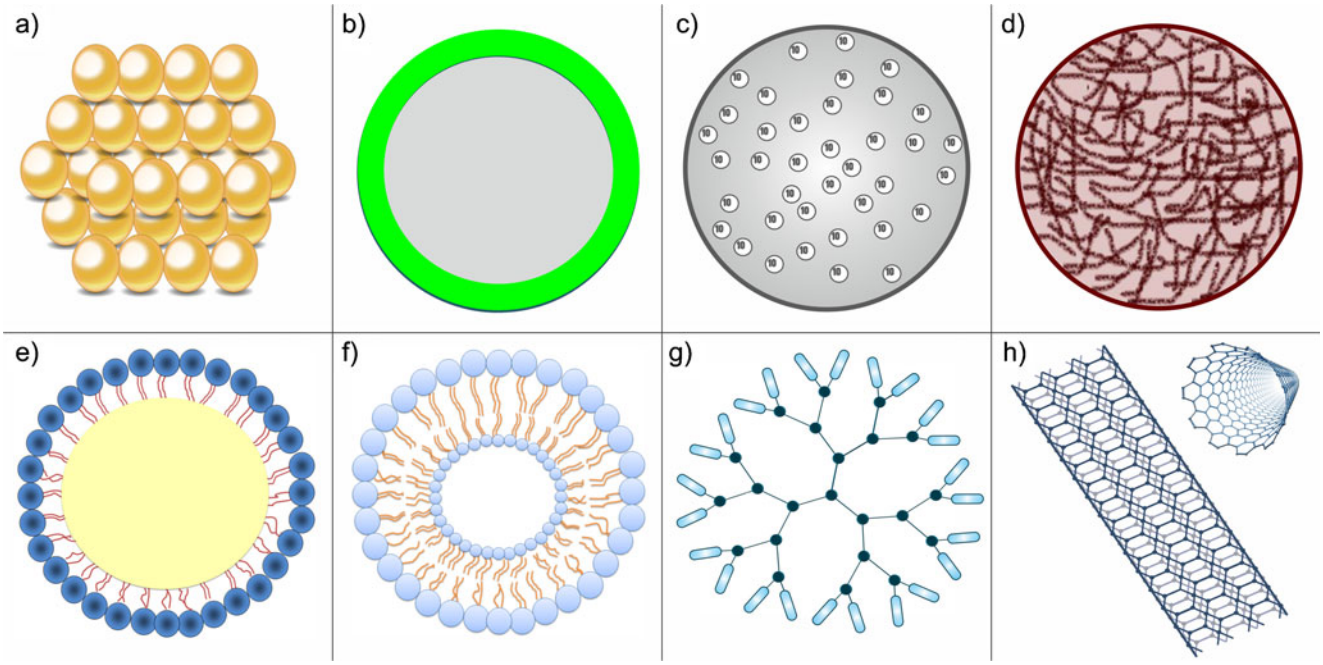

Fig. 2 Illustrative examples of selected nanotechnology based delivery systems: (**a**) gold nanoparticles, (**b**) quantum dots, (**c**) iron oxide nanoparticles, (**d**) polymeric nanoparticles, (**e**) solid lipid nanoparticles, (**f**) liposomes, (**g**) dendrimers, (**h**) carbon nanotubes

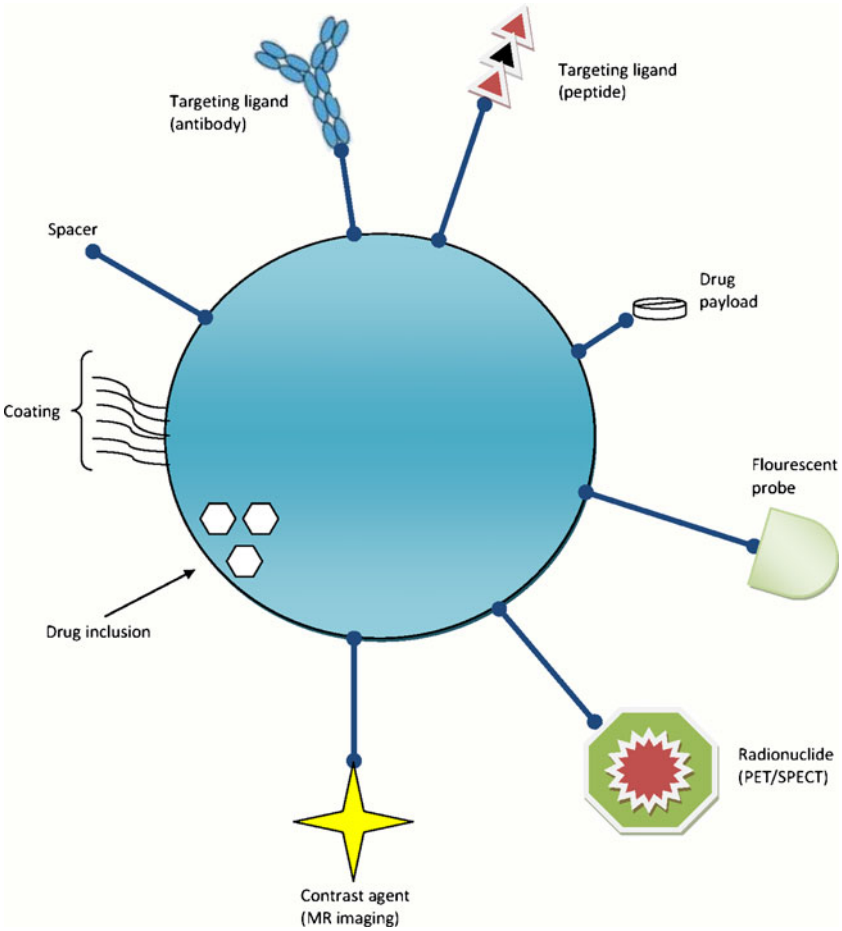

Fig. 3 Illustration of multi-functionalized nanoparticles

result due to the formation of new forms of Aβ, such as protease resistant oligomeric and fibrillar (66). One of the agents with potential to prevent and reverse Aβ aggregation are metal chelators (67–69). However, difficulty to bypass the BBB, poor absorption and toxic side effects are limitations of chelation therapy. NBDDS are useful tools to overcome these disadvantages due to their unique characteristics mentioned above (70,71). Four different chelating ligands (CuAc, EDTA, histidine and ZnAc) have been conjugated onto nanoliposome formulations using either covalent or non-covalent conjugation procedure and evaluated for particle size, zeta potential, resolubilization of Aβ(1–42) peptides, neurotoxicity analysis and *ex vivo* uptake by PC12 neuronal cells (70). It has been confirmed that EDTA, histidine or ZnAc conjugated nanoliposome formulations were suitable for resolubilization of CuAβ(1–42) and ZnAβ(1–42) aggregates *in vitro*. Moreover, protecting the neuronal cells from Aβ(1–42) aggregate-related toxicity has caused to the high survival of PC12 neuronal cells after treatment with the formulations, which indicates the potential of developed formulations to chelation therapy for AD. Correspondingly, Liu and co-workers (71) have prepared a prototype nanoparticle chelater conjugate (Nano-N2PY) and examined the inhibition of Aβ aggregate formation and Aβ- associated cytotoxicity on human cortical neuronal cells. It has been proposed that Nano-N2PY can hinder Aβ aggregate formation effectively and, thus it protects human brain cells from Aβ-related toxicity.

The use of some subfragments of amyloid-beta (Aβ) to protect neurons from AD is an alternative strategy (72–75). Because the BBB limits the influx of antigens and antibodies to the brain, and it is possible to use NBDDS to carry these subfragments to the brain. Chitosan nanoparticles loaded with intramembranous fragments of Aβ (NP-IF-A) have been prepared and administrated to Kunming male mice to examine the brain translocation of the formulations (76). It was reported that the brain uptake efficiency of the IF-A in nanoparticle formulation and in plain solution were 80.6% and 20.6%, respectively, indicating the developed formulations are able to be a carrier for IF-A. Analogously, Agyare and co-workers (77) have developed a smart nano-vehicle (SNV) which is able to pass the BBB in order to target to Aβ formed in both AD and cerebrovascular amyloid angiopathy (CAA). For this purpose, the FITC-BSA loaded chitosan nanoparticles have been prepared and coated with a biosensor, polyamine-modified anti-amyloid antibody fragment (pF(ab')24.1, molecular wt.≈80 kDa) which has excellent permeability at the BBB and has labeled brain parenchymal amyloid plaques as well as cerebrovascular amyloid (78). As control nano-vehicles (CNVs), the nanoparticles have been prepared using same core and coated with BSA. The particle sizes were between 221.6±22.5 nm–894.0 nm and 235.7±16.3 nm–956.1±23.3 nm for SNVs and CNVs, respectively. Furthermore, the zeta potential values of CNVs and SNVs have found between 12.8±3.0–37.2±5.5 mV and 32.7±2.4–41.6±2.6 mV, respectively. It was reported that the SNVs accumulation in various brain regions are significantly higher than after bolus injection of CNVs in WT (Wild type) mouse and the FITC-BSA loaded SNVs uptake in bovine brain microvascular endothelial cells (BBMECs) was twice the uptake of FITC-BSA loaded CNVs. It has been reported that SNVs were able to deliver diagnostics or therapeutics to the brain and target amyloid deposits in the cerebral vasculature of AD transgenic mice.

Because of the complex and heterogeneous nature of AD, there is an increased need for the manufacturing of newer drugs such as curcumin, a plant derived compound which has potential activities beneficial for the treatment of AD due to its anti-amyloid and anti-oxidant activity. Curcumin loaded PLGA nanoparticles have been prepared and conjugated with Tet-1 protein, that has neuron affinity and reverse transportation properties, in order to provide brain targeting of the formulations. It was reported that the nanoparticles with a size range of 150 to 200 nm and the zeta potential of the formulations were changing between −30 and −20 mV. Furthermore, the anti-oxidant and anti-amyloid properties of the nanoparticles have been proven. The authors reported that the Tet- 1 peptide conjugated, targeted nanoparticles enhance the uptake by the neuronal cells and the formulations are promising in treating AD (61). With similar objective, Mourtas and co-workers (79) have designed two types of nanoliposome formulations functionalized with curcumin derivatives, which have been prepared using a conventional synthetic method or a click chemistry technique. The click chemistry technique has been used for nanoliposome surface decoration with a curcumin derivative that has the required structural properties for the antifibrillogenic activity. It has been notified that the nanoliposomes are potentially beneficial to target certain AD pathogenic markers (Aβ1-42 fibrils, 1–5 nM) for diagnostic and/or therapeutic purposes.

In another study to design newer drugs for the treatment of AD, nanoliposome formulations containining phosphatidic acid (PA) or cardiolipin (CL) have been prepared and conjugated with apolipoprotein E (ApoE) derived peptides (mApoE or dApoE) in order to provide increased drug accumulation in brain by means of targeting low-density lipoprotein-receptor (LDLr) present on capillary endothelial cells of BBB (80). It has been noted that non-functionalized nanoliposomes have not showed either membrane accumulation or cellular uptake in hCMEC/D3 cells while substantially higher uptake has been observed in peptide conjugated formulations. Furthermore, the ultracentrifugation results have indicated that the peptides functionalization of nanoliposomes containing PA or CL, maintain their ability to bind the Aβ 1–42 peptide *in vitro* and the formulations are promising for the brain targeting of the peptide.

Ethosomes are novel liposomal drug delivery systems, which contains soft phospholipid vesicles in high amount of ethanol presence. Ethosomes might be efficient sytems for transdermal drug delivery because they are able to deliver drugs to the skins deeper layers by passing the skin layers (81,82). Ligustrazine phosphate (LP), a synthetic ligustrazine product, which previously has been proved that significantly improves the hippocampal cholinergic system function, decrease oxidative damage, and thus significantly enhances the learning abilities and improves memory in D-galactose induced AD mice model (83). LP can penetrate the brain through BBB, but it undergoes extensive first pass metabolism, which causes a low oral bioavailability and a very short elimination half life after oral administration. In order to overcome the drawbacks of the oral or injection drug administration, Shi and co-workers (84) has designed and evaluated *in vitro* and *in vivo* ligustrazine phosphate (LP) loaded ethosomes for efficient treatment of AD through transdermal administration. The authors have reported that penetration ability and drug deposition in skin of LP loaded ethosome formulations were higher comparing to plain LP solution. Furthermore, it was also reported that the formulations were capable of recovering the antioxidant enzyme activity and malondialdehyde level in the brain of the amnesic rats compared to condition of the healthy rats, which has also been reflected by behavioral performance improvement.

Dendrimers are highly branched three-dimensional macromolecules constructed from the core of ABn (where $n=2$ or more) comprising a series of branches which are tree like around the core. Different linkages such as polyamines, polyamides or a mixture of both can form the dendrimer design. Due to their nanosize, ease to prepare and functionalisation and polymorphism, dendrimers are ideally suited for targeted drug delivery (25,85,86). Wasiak and co-workers (87) have synthesized two generations (3,4) of phosphorus dendrimers (CPDs) and evaluated on formation of amyloid fibril and the MAP-Tau aggregation process. It has been mentioned that besides Aβ, which plays an important role in AD pathogenesis, neurofibrillar tangles (NTFs) composed of hyperphosphorylated MAP-Tau protein aggregates are the second distinguishing feature of AD. Following the synthesis and characterization of cationic phosphorus-containing dendrimers of 3rd and 4th generation (CPDG3, CPDG4), both biophysical and biochemical methods such as circular dichroism (CD), fluorescence intensity of thioflavin T and thioflavin S, spectrofluorimetry, transmission electron microscopy (TEM) and MTT test have been carried out to evaluate the interactions between dendrimers and fragment of Aβ (Aβ1–28) and MAP-Tau protein. It has been demonstrated that CPDs modify the aggregation of both amyloid peptide Aβ 1–28 and MAP-Tau protein and the formulations are promising for regulating the fibrilization processes in AD.

NBDDS for Parkinson's Disease

Parkinson's disease (PD) is a chronic and progressive movement disorder, characterized generally by pigmented neuron degeneration in the substantia nigra, that results a decrease in nigrostriatal availability of the neurotransmitter dopamine (DA). For the treatment of PD, Levodopa (L-dopa, LDA), the levorotatory isomer of dihydroxyphenylalanine, metabolic precursor of dopamine has been on the market for a long time because DA alone is not able to cross the BBB due to its hydrophobicity and the lack of a specific transporter (88–90). LDA can cross the BBB and it is converted into DA in the basal ganglia. Despite LDA is still used in the treatment of PD (91), it is catalyzed to DA by peripheral enzyme and cause peripheral side effects, such as nausea, sleepiness and dyskinesia (92). Thus, region specific delivery of LDA to the central nervous system (CNS) is needed to overcome these side-effects and to obtain efficient therapy. NBDDS provides more opportunities for the targeted LDA delivery for PD therapy.

In the last two decades, studies have been focused on NBDDS development to provide targeted drug delivery for PD therapy. During and co-workers (93) have designed DA containing liposome formulations to obtain controlled delivery of the drug in a PD model. The formulations have provided sustained DA release *in vitro* for over 40 days and high DA levels for 25 days when they stereotactically implanted in the partially denervated corpus striatum of rats subjected to unilateral lesions of the substantia nigra. These results suggested that the DA containing liposomes have potential to deliver drug to discrete areas of the brain. Muthuprasanna and co-workers (94) have also evaluated that the efficiency of surfactant-modified DA containing liposomal preparation on haloperidol induced extra pyramidal side effects of parkinsonism in Wistar rats using actophotometer and rotorod. Authors have reported that the formulations arrested the effect of haloperidol-induced parkinsonism and they could be ideal carrier for targeting DA to brain through BBB. With similar objective, stealth liposomes containing LDA have been prepared and conjugated with chlorotoxin (ClTx), a 36-amino acid peptide, which has high potential for the targeting drugs to the brain microvascular endothelial cells (89). ClTx conjugated liposome formulations have mild positive surface charge and their particle sizes were around 100 nm. Also, ClTx conjugated formulations showed higher intracellular drug uptake in murine brain microvascular endothelial cells (BMECs) compared to unconjugated liposomes *in vitro*. Increased DA and its main metabolite, dihydroxyphenyl acetic acid (DOPAC), levels have been measured in the substantia nigra and striata of mice, compared with unconjugated formulations and plain LDA solution. The *in vivo* results have been also confirmed in rotarod test and TH immunohistochemical assay based on the 1-methyl-4-phenyl-1,2,3,6-tetrahydro pyridine (MPTP) - induced C57

mice PD model. It has been reported that ClTx conjugated liposome formulations containing LDA may be a potential active targeting system for drug delivery enhancement into the brain to achieve a better PD therapy.

In addition to liposome studies, there have been many research efforts on nanoparticles to deliver DA to brain for the treatment of PD. Administration route's effect on Levodopa methyl ester (LDME)/benserazide-loaded nanoparticles on LDA-stimulated dyskinetic rats have been investigated (88). It has been found that dyskinetic rats treated with LDME/benserazide-loaded nanoparticles have less axial, limb, orolingual (ALO) and the locomotive abnormal involuntary movement scores (AIMs) compared with dyskinetic rats treated with LDME plus benserazide. The results have indicated that LDME/benserazide-loaded nanoparticles are able to reduce the LID expression in rats. Trapani and co-workers (90) have characterized chitosan nanoparticles and investigate their brain drug delivery potential of DA. DA at two different concentrations has been incubated with the chitosan nanoparticles and X-ray Photoelectron Spectroscopy (XPS) analysis has confirmed the adsorption of DA on the outer surface of the nanoparticles (DA/CSNPs). Transport studies performed across MDCKII-MDR1 cell line have shown that DA/CSNPs caused considerable enhancement in transport compared to the control and measurement of reactive oxygen species (ROS) has indicated a low DA/CSNPs neurotoxicity 3 h after the administration. *In vivo* brain microdialysis results in rats have indicated that intraperitoneal administration of DA/CSNPs (6–12 mg/kg) stimulated a dose dependent enhancement in striatal DA output. It has been reported that DA/CSNPs were ideal delivery systems for DA transport across BBB and could be useful for PD treatment. Tristearin/tricaprin nanostructured-solid lipid nanoparticles containing bromocriptine (BRC) have been designed and evaluated for antiparkinsonian activities in 6-hydroxydopamine hemilesioned rats comparing with free BRC (95). The results have indicated that the formulations provide controlled drug release for 48 h *in vitro* and both free and encapsulated BRC decreased the time spent on the blocks in the bar test, while effect of encapsulated BRC was faster in the beginning and prolonged. The authors have concluded that nanostructured lipid carriers can be suggested as innovative approach for BRC administeration for PD therapy. Cell mediated drug delivery systems for the treatment of PD are another innovative approaches. Zhao and co-workers (96) have developed catalase nanoparticles-loaded bone marrow-derived macrophages (BMM) to attenuate neuroinflammation and produce neuro protection for PD. For this purpose, the polycomplex of catalase and Poly (ethyleneimine)-poly (ethylene glycol) (PEI-PEG) (nanozyme) has been obtained by mixing catalase and PEI-PEG, that bind each other with electrostatic interactions and form nanoparticles with an enzyme-polyion complex core and PEG corona. The BMM-carried nanozymes have been investigated for pharmacokinetic and biodistribution properties, BBB transport and inflammation targeting capability employing C57Bl/6 mice with brain inflammation caused by MPTP, 6-OHDA, or LPS intoxications. It has been confirmed that the macrophages increased nanozyme transport across the BBB and clearance and volume of distribution for macrophage carried nanozyme was decreased from cell-free formulations, suggesting nanozyme-loaded BMM works as a depot for the enzyme's prolonged circulation and release rates.

Transdermal nanoemulsion gel formulations containing ropinirole have been designed for the efficient treatment of PD by means of overcoming disadvantages of the drug such as poor oral bioavailability and high dosing frequency (97). The pharmacokinetic, biochemical and physical assessment of the formulations have been carried out in rats induced with Parkinson lesioned brain by 6-OHDA. Results have shown that the formulations have been non-toxic and non-irritant to the skin and greater extent of drug absorption has been obtained from nanoemulsion gel formulations compare with oral marketed tablet (Ropitor®) and conventional gel of ropinirole. Furthermore, biochemical studies have indicated that in case of Parkinson induced rats, ropinirole nanoemulsion gel has higher activity compared with conventional tablets.

Nano-enabled scaffold device (NESD) for the targeted DA delivery, has been designed to reduce the undesired effects of conventional PD therapy. NESD has been prepared by embedding of DA loaded-cellulose acetate phthalate (CAP) nanoparticles to crosslinked alginate scaffold. The results have confirmed that increased levels and controlled delivery of DA in the cerebrospinal fluid of the Sprague–Dawley rat model obtained with NESD compared to CAP nanoparticles. It has been suggested that nanotechnology and coupling polymeric scaffold science leads to an attractive platform (NESD) for the chronic management of PD (98).

The effect of generation 3,4 and 5 PAMAM dendrimers on the fibrillation of α-synuclein, a140 amino acid protein, which plays the central role in PD, has been evaluated *in vitro* by SANS, fluorescence, CD spectroscopy, and TEM. The authors have stated that PAMAM dendrimers are responsible for inhibition of fibrillation of α-synuclein and according to this both with generation number and PAMAM concentration were increased. This effect can offer possibilities for a preventative approach to the of neurological disorder management (99).

Multifunctional NBDDS have been described for both imaging and therapeutic purposes for PD. Highly fluorescent core/shell CdSe/CdS quantum rods (QRs) have been synthesized and made water-soluble and highly biocompatible by intercalating an amphiphilic polymer and conjugating diamino PEG molecules. Also, galactose has been introduced through a reductive amination step, while succinyl DA has been subsequently coupled via an ester bond to the

carbohydrate (glyco-QRs). The results have indicated that glyco-QRs have been recognized by KB cells through the GLUT-1 transporter on the outer cellular membrane and internalized through an endocytic pathway, suggesting they have potential application in biodiagnostic devices or *in vivo* cellular imaging of PD. Furthermore, the ability of the formulations to release the DA has been demonstrated, indicating their therapeutic potentials for PD (100). With similar approach, the lactoferrin (Lf) conjugated polyethylene glycolpolylactide-polyglycolide (PEG-PLGA) nanoparticles (Lf-NP) have been designed and loaded with a fluorescent probe coumarin-6 or urocortin, for diagnostic or therapeutic application, respectively, in PD. The results have revealed that coumarin-6 loaded Lf-NP (C-Lf-NP) showed more significant accumulation in bEnd.3 cells than that of unconjugated nanoparticles and enhanced uptake of C-Lf-NP was using a clathrin mediated endocytosis processes. Also, after the C-Lf-NP injection in mouse caudal vein, the coronal section of the brain has evidenced that C-Lf-NP was accumulating higher in the cortex, striatum region and substantia nigra than that of NP and the AUC and Cmax of coumarin-6 in the brain by C-Lf-NP was about 2.49 and 2.36 folds comparing to that by NP, respectively. Analogously, the action, immunohistochemistry and transmitter contents results indicated that i.v. injection of urocortin loaded Lf-NP (U-Lf-NP) was able to reduce the 6-OHDA dependent striatum lesion. The authors have reported that Lf-NP was a promising delivery system for brain targeting of diagnostic and therapeutic agents for PD therapy (101).

NBDDS for Prion Diseases

Prion diseases (PRD) are fatal neurodegenerative disorders, which arise from conformational transformation from the conventional cellular form of prion proteins (PrPC) to the infectious scrapie isoform (PrPSc). PrPSc spontaneously form fibrils-amyloid-like structures and the accumulation of these structures cause neurological dysfunction concomitantly neuronal vacuolation and astrocytic gliosis, leading eventually to death (102–105).

Recently, dendrimers and nanoparticles have gained increasing attention for the treatment of PRD. Klajnert and co-workers (105) have investigated that *in vitro* the effect of cationic phosphorous dendrimers on the prion peptide PrP 185–208's the aggregation process by a spectrofluorometric assay and FTIR. The results have revealed that phosphorous dendrimers were capable of interfering with PrP 185–208's aggregation process by either reducing the formation speed of aggregates or by lowering the quantity of amyloid fibrils. Similarly, PAMAM-G3 (generation 3) dendrimers have been evaluated for their effect on the aggregation of ADs peptide Aβ1–28 and the prion protein Prp185–208's segment. It has been reported that dendrimers has an effect on the aggregation properties of both peptides and it is beneficial to investigate their potential applications as anti-amyloidogenic agents in neurodegenerative disorders such as PRD.

Magnetic γ-Fe_2O_3/poly(2,2,3,3,4,4,4-heptafluorobutylacrylate) (γ-Fe2O3/PHFBA) core–shell nanoparticles have been examined for their effect on fibril formation of insulin, a model amyloidogenic protein. The results demonstrated that γ-Fe2O3/PHFBA inhibits the insulin fibrillation process significantly. The authors have suggested that this formulation could be promising for neurodegenerative disorders including PRD (106). Sousa and co-workers (107) have evaluated the *in vivo* distribution of polyelectrolyte multilayer coated gold nanoparticles. It has been reported that the nanoparticles passed through the BBB and cumulated in brain, which makes the system attractive for diagnostic purposes in the detection of PRD. Calvo and co-workers (108) have been designed PEGylated polycyanoacrylate nanoparticles (PEG-PHDCA) as drug carrier in experimental model of PRD. The results have indicated that PEG-PHDCA provided a higher uptake by both the brain and the spleen, that were the target tissues of prion proteins compared to non-PEGylated formulations.

Neurotoxicity of NBDDS

The field of nanotechnology and its applications in medicine have experienced unprecedented growth during the last few years, however, due lack of toxicology data on this field, concerns have been expressed about their potential hazard for human health, in particular their possible toxic effects on CNS (109–111).

Majority of the data in the literature indicated that the toxicity of NBDDS is related to many parsameters including chemical composition, size, shape, surface area, surface charge, and others (9,111,112).

PC12 cells are the most widely used cell model for nanoparticle neurotoxicity studies (113–117). Wang and co-workers (113) have investigated the neurotoxic effect of two types of commercially available single-walled carbon nanotubes (SWCNTs) on PC12 cells. The results have shown that the decreased PC12 cells viability and disturbed cell cycle were observed. Furthermore, reduced mitochondrial membrane potential (MMP), stimulated the formation of ROS, increased lipid peroxide level and decreased superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and the content of glutathione (GSH) activities have been noted. Similarly, Pisanic and co-workers (117) have revealed that exposure to anionic magnetic nanoparticles with increasing concentrations, in the range of 0.15 to 15 nm of iron, led to a dose dependent downward viability of PC12 cells using MTT assay. Hussain and co-workers (115) have suggested that the exposure of PC12 cells to manganese oxide nanoparticles can deplete DA, dihydroxyphenylacetic acid

and homovanillic acid while Ag nanoparticles (15 nm) could cause cell withdrawal and non-uniform membrane borders.

The neurotoxicity of ZnO nanoparticles with different dimensions in mouse neural stem cells (NSCs) has been examined (114). It has been stated that apoptotic cells were seen and assayed by confocal microscopy, TEM examination, flow cytometry and ZnO based systems should be carefully evaluated to obstruct the unintended environmental and health impacts.

The potential toxicity of aluminum oxide (alumina) nanoparticles has been evaluated in ICR strained mice focusing on potential neurobehavioral defects and the possible mechanisms. The results have revealed that the nanoparticles damaged neurobehavioral functions such as prolonged escape latency, spending less time in the target quadrant and reduced number of platform passage and they caused the cell necrosis and apoptosis. The authors have reported that surface chemical properties and nanoscale dimensions of the formulations might be significantly contributing to neurotoxicity and could be more toxic to the cerebrum than those of nano-carbon nanoparticles with the same size and alumina microparticles with the same surface properties (118).

Wu and co-workers (119) have been investigated the potential neurotoxic effects of silica nanoparticles (SiO2-NPs) which were increasingly being used in drug delivery, diagnosis and imaging for CNS. They found that after intranasal administration, SiO2-NPs penetrated into the brain and especially accumulated in the striatum and the formulations reduced cell viability, triggered oxidative stress, induced lactate dehydrogenase levels, disturbed cell cycle, induced apoptosis, and activated the p53-mediated signaling pathway. It was reported that the formulations have neurotoxicity potential and have possible risk for neurodegenerative disorders.

There are another reports present in literature concerning the potential neurotoxicity of NBDDS (9,111,120–123). For this reason, NBDDS should be carefully designed and evaluated for brain-specific drug delivery, and diagnostics. NBDDS should be safe, have good biocompatibility, low immunogenicity and be biodegradable. According to these prerequisites, most of the NBDDS cannot be translated into clinic. Although many promising aspects *in vitro* result through the NBDDS, nanotechnology does not meet the expectations of the clinical arena. By this time, only one phase I clinical trial involving nanotechnology in CNS therapeutics has been initiated; this trial, to investigate the use of nanoliposomal irinotecan for the treatment of recurrent glioma, is still recruiting patients (30). In addition to toxicological concerns mentioned above, lack of standardization of NBDDS causes another barrier to the undertaking of nanotechnology-based clinical trials. Many different physical and chemical properties of NBDDS, such as size, charge and surface modifications, affect both their efficacy and toxicity.

In order to overcome obstacles to clinical translation of NBDDS, it is very important for neurosurgeons, neurologists, and neuroscientists to participate and contribute to the scientific process alongside physical science and engineering colleagues.

FUTURE DIRECTIONS AND CONCLUSION

Nowadays, NDs are one of the major health problems in the world, and an increase in the number of ND is expected in the future due to the extended lifetime and the aging of the population. Thus, an immediate development is needed for novel therapeutics for neurodegenerative diseases that affect the disease progression, rather than simply treating the symptoms. Nanotechnology shows great promise in the therapy of neurodegenerative disorders such as AD, PD and PRD by supporting and promoting functional regeneration of damaged neurons, providing neuroprotection, and simplifying the delivery of drugs, growth factors, genes and cells across the BBB. It may contribute significantly toward the delivery of the diagnostics for the diagnosis and monitoring of neurodegenerative disorders.

The size, shape, geometry, charge, structure and composition of NBDDS can be controlled easily, which will secure their future development and success. Challenges towards the future of these systems contain developing technologies for their physical, chemical and biological evaluations; the possible toxicity and immunological reactions; lack of scalability; batch-to-batch variation and the need for case-by case evaluation.

An attractive challenge will be the use of NBDDS for integrated therapy and diagnosis strategies (theranostics), which provide targeted delivery of drugs and genes, magnetic properties, tissue engineering and cell tracking, for their unique ability to be guided by an outer magnetic field. Furthermore, more research will be required in NBDDS to allow the clinical applications in neurodegenerative disorders.

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REFERENCES

1. Ellison D, Love S, Chimelli L, Harding BN, Lowe J, Vinters HV. Neuropathology: A reference text of CNS pathology. London: Mosby; 2004.
2. Beal MF, Lang AE, Ludolph AC. Neudegenerative diseases: neurobiology, pathogenesis and therapeutics. Cambridge: Cambridge University Press; 2005.

3. Cacciatore I, Baldassarre L, Fornasari E, Mollica A, Pinnen F. Recent advances in the treatment of neurodegenerative diseases based on GSH delivery systems. Oxide Med Cell Longev. 2012. doi:10.1155/2012/240146.
4. Whalley K. Neurodegenerative disease: undoing aggregation. Nat Rev Neurosci. 2008;9:83.
5. Bartus RT. On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. Exp Neurol. 2000;163(2): 495–529.
6. Burridge S. Neurodegenerative diseases: novel route to neuroprotection. Nat Rev Drug Discov. 2012;11:906–7.
7. Waldmeier PC, Tatton WG. Interrupting apoptosis in neurodegenerative disease: potential for effective therapy? Drug Discov Today. 2004;9(5):210–8.
8. Fernandes C, Soni U, Patravale V. Nano-interventions for neurodegenerative disorders. Pharmacol Res. 2010;62:166–78.
9. Re F, Gregori M, Masserini M. Nanotechnology for neurodegenerative disorders. Nanomed Nanotech Biol Med. 2012; (Suppl 1): S51-S58.
10. Modi G, Pillay V, Choonara YE. Advances in the treatment of neurodegenerative disorders employing nanotechnology. Ann N Y Acad Sci. 2010;1184:154–72.
11. Nowacek A, Gendelman E. NanoART, neuroAIDS and CNS drug delivery. Nanomedicine (Lond). 2009;4(5):557–74.
12. Modi G, Pillay V, Choonara YE, Ndesendo VM, du Toit LC, Naidoo D. Nanotechnological applications for the treatment of neurodegenerative disorders. Prog Neurobiol. 2009;88(4):272–85.
13. Barchet T, Amiji MM. Challenges and opportunities in CNS delivery of therapeutics for neurodegenerative diseases. Expert Opin Drug Deliv. 2009;6(3):211–25.
14. Spuch C, Navarro C. Liposomes for targeted delivery of active agents against neurodegenerative diseases (Alzheimer's Disease and Parkinson's Disease). J Drug Deliv. 2011;2011:1–12.
15. Awad RA. Neurogenic bowel dysfunction in patients with spinal cord injury, myelomeningocele, multiple sclerosis and Parkinson's disease. World J Gastroenterol. 2011;17(46):5035–48.
16. Poole CP, Owens FJ. Introduction to nanotechnology. New Jersey: Wiley; 2003.
17. Vo-Dinh T. Nanotechnology in biology and medicine: methods, devices, and applications. Boca Raton: CRC Press Taylor & Francis Group; 2007.
18. Cho Y, Borgens RB. Polymer and nano-technology applications for repair and reconstruction of the central nervous system. Exp Neurol. 2012;233(1):126–44.
19. Mahmood M, Casciano D, Xu Y, Biris AS. Engineered nanostructural materials for application in cancer biology and medicine. J Appl Toxicol. 2012;32(1):10–9.
20. Leucuta SE. Nanotechnology for delivery of drugs and biomedical applications. Curr Clin Pharmacol. 2010;5(4):257–80.
21. Silva GA. Nanotechnology approaches to crossing the blood–brain barrier and drug delivery to the CNS. BMC Neurosci. 2008;9 Suppl 3:S1–4.
22. Jain KK. Nanomedicine: application of nanobiotechnology in medical practice. Med Princ Pract. 2008;17(2):89–101.
23. Liu Y, Tan J, Thomas A, Ou-Yang D, Muzykantov VR. The shape of things to come: importance of design in nanotechnology for drug delivery. Ther Deliv. 2012;3(2):181–94.
24. Singh S. Nanomedicine-nanoscale drugs and delivery systems. J Nanosci Nanotechnol. 2010;10(12):7906–18.
25. Koo OM, Rubinstein I, Onyuksel H. Role of nanotechnology in targeted drug delivery and imaging:a concise review. Nanomed Nanotech Biol Med. 2005;1:193–212.
26. Manish G, Sharma V. Targeted drug delivery system: a review. Res J Chem Sci. 2011;1(2):135–8.
27. Petrak K. Nanotechnology and site-targeted drug delivery. J Biomater Sci Polym Ed. 2006;17(11):1209–19.
28. Paulo CS, Pires das Neves R, Ferreira LS. Nanoparticles for intracellular-targeted drug delivery. Nanotechnology. 2011;22(49): 1–11.
29. Gagliardi M, Bardi G, Bifone A. Polymeric nanocarriers for controlled and enhanced delivery of therapeutic agents to the CNS. Ther Deliv. 2012;3(7):875–87.
30. Srikanth M, Kessler JA. Nanotechnology-novel therapeutics for CNS disorders. Nat Rev Neurol. 2012;8(6):307–18.
31. De Rosa G, Salzano G, Caraglia M, Abbruzzese A. Nanotechnologies: a strategy to overcome blood–brain barrier. Curr Drug Metab. 2012;13(1):61–9.
32. Mangas-Sanjuan V, González-Alvarez M, Gonzalez-Alvarez I, Bermejo M. Drug penetration across the blood–brain barrier: an overview. Ther Deliv. 2010;1(4):535–62.
33. Tucker IG, Yang L, Mujoo H. Delivery of drugs to the brain via the blood brain barrier using colloidal carriers. J Microencapsul. 2012;29(5):475–86.
34. Urquhart BL, Kim RB. Blood–brain barrier transporters and response to CNS-active drugs. Eur J Clin Pharmacol. 2009;65(11):1063–70.
35. Geldenhuys WJ, Allen DD, Bloomquist JR. Novel models for assessing blood–brain barrier drug permeation. Expert Opin Drug Metab Toxicol. 2012;8(6):647–53.
36. Orthmann A, Fichtner I, Zeisig R. Improving the transport of chemotherapeutic drugs across the blood–brain barrier. Expert Rev Clin Pharmacol. 2011;4(4):477–90.
37. Potschka H. Targeting the brain–surmounting or bypassing the blood–brain barrier. Handb Exp Pharmacol. 2010;197:411–31.
38. Pardridge WM. Biopharmaceutical drug targeting to the brain. J Drug Target. 2010;18(3):157–67.
39. Alam MI, Beg S, Samad A, Baboota S, Kohli K, Ali J, *et al.* Strategy for effective brain drug delivery. Eur J Pharm Sci. 2010;40(5):385–403.
40. Burov S, Leko M, Dorosh M, Dobrodumov A, Veselkina O. Creatinyl amino acids: new hybrid compounds with neuroprotective activity. J Pept Sci. 2011;17(9):620–6.
41. Liu Y, Hu Y, Guo Y, Ma H, Li J, Jiang C. Targeted imaging of activated caspase-3 in the central nervous system by a dual functional nano-device. J Control Release. 2012;163(2):203–10.
42. Prades R, Guerrero S, Araya E, Molina C, Salas E, Zurita E, *et al.* Delivery of gold nanoparticles to the brain by conjugation with a peptide that recognizes the transferrin receptor. Biomaterials. 2012;33(29):7194–205.
43. 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(2):251–6.
44. Zhang P, Hu L, Yin Q, Zhang Z, Feng L, Li Y. Transferrin-conjugated polyphosphoester hybrid micelle loading paclitaxel for brain-targeting delivery: synthesis, preparation and in vivo evaluation. J Control Release. 2012;159(3):429–34.
45. Chang J, Paillard A, Passirani C, Morille M, Benoit JP, Betbeder D, *et al.* Transferrin adsorption onto PLGA nanoparticles governs their interaction with biological systems from blood circulation to brain cancer cells. Pharm Res. 2012;29(6):1495–505.
46. Kuo YC, Lin PI, Wang CC. Targeting nevirapine delivery across human brain microvascular endothelial cells using transferrin-grafted poly(lactide-co-glycolide) nanoparticles. Nanomedicine (Lond). 2011;6(6):1011–26.
47. Prabhakar K, Afzal SM, Kumar PU, Rajanna A, Kishan V. Brain delivery of transferrin coupled indinavir submicron lipid emulsions–pharmacokinetics and tissue distribution. Colloids Surf B Biointerfaces. 2011;86(2):305–13.

48. Jain A, Chasoo G, Singh SK, Saxena AK, Jain SK. Transferrin-appended PEGylated nanoparticles for temozolomide delivery to brain: in vitro characterisation. J Microencapsul. 2011;28(1):21–8.
49. Yemişci M, Gürsoy-Özdemir Y, Caban S, Bodur E, Capan Y, Dalkara T. Transport of a caspase inhibitor across the blood–brain barrier by chitosan nanoparticles. Methods Enzymol. 2012;508:253–69.
50. Shao K, Huang R, Li J, Han L, Ye L, Lou J, *et al.* Angiopep-2 modified PE-PEG based polymeric micelles for amphotericin B delivery targeted to the brain. J Control Release. 2010;147(1):118–26.
51. Zhao M, Chang J, Fu X, Liang C, Liang S, Yan R, *et al.* Nano-sized cationic polymeric magnetic liposomes significantly improves drug delivery to the brain in rats. J Drug Target. 2012;20(5):416–21.
52. Dakwar GR, Abu Hammad I, Popov M, Linder C, Grinberg S, Heldman E, *et al.* Delivery of proteins to the brain by bolaamphiphilic nano-sized vesicles. J Control Release. 2012;160(2):315–21.
53. Agarwal A, Agrawal H, Tiwari S, Jain S, Agrawal GP. Cationic ligand appended nanoconstructs: a prospective strategy for brain targeting. Int J Pharm. 2011;421(1):189–201.
54. Di Carlo M, Giacomazza D, San Biagio PL. Alzheimer's disease: biological aspects, therapeutic perspectives and diagnostic tools. J Phys Condens Matter. 2012;24(24):1–17.
55. Eskici G, Axelsen PH. Copper and oxidative stress in the pathogenesis of Alzheimer's disease. Biochemistry. 2012;51(32):6289–311.
56. Huang Y, Mucke L. Alzheimer mechanisms and therapeutic strategies. Cell. 2012;148(6):1204–22.
57. Mohamed T, Rao PP. Alzheimer's disease: emerging trends in small molecule therapies. Curr Med Chem. 2011;18(28):4299–320.
58. Loef M, Walach H. Copper and iron in Alzheimer's disease: a systematic review and its dietary implications. Br J Nutr. 2012;107(1):7–19.
59. Galimberti D, Scarpini E. Progress in Alzheimer's disease. J Neurol. 2012;259(2):201–11.
60. Di Stefano A, Lannitelli A, Laserra S, Sozio P. Drug delivery strategies for Alzheimer's disease treatment. Expert Opin Drug Deliv. 2011;8(5):581–603.
61. Mathew A, Fukuda T, Nagaoka Y, Hasumura T, Morimoto H, Yoshida Y, *et al.* Curcumin loaded-PLGA nanoparticles conjugated with Tet-1 peptide for potential use in Alzheimer's disease. PLoS One. 2012;7(3):1–10.
62. Elsabahy M, Wooley KL. Design of polymeric nanoparticles for biomedical delivery applications. Chem Soc Rev. 2012;41(7):2545–61.
63. Mozafari MR. Nanoliposomes: preparation and analysis. Methods Mol Biol. 2010;605:29–50.
64. Hardy J. Alzheimer's disease: the amyloid cascade hypothesis: an update and reappraisal. J Alzheimers Dis. 2006;9:151–3.
65. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science. 1992;256:184–5.
66. Selkoe DJ. The molecular pathology of Alzheimer's disease. Neuron. 1991;6:487–98.
67. Crouch PJ, Barnham KJ, Bush AI, White AR. Therapeutic treatments for Alzheimer's disease based on metal bioavailability. Drug News Perspect. 2006;19:469–74.
68. Liu G, Garrett MR, Men P, Zhu X, Perry G, Smith MA. Nanoparticle and other metal chelation therapeutics in Alzheimer disease. Biochim Biophys Acta. 2005;1741:246–52.
69. Smith MA. Oxidative stress and iron imbalance in Alzheimer disease: howrust became the fuss! J Alzheimers Dis. 2006;9:305–8.
70. Mufamadi MS, Choonara YE, Kumar P, Modi G, Naidoo D, Ndesendo VM, *et al.* Surface-engineered nanoliposomes by chelating ligands for modulating the neurotoxicity associated with β-Amyloid aggregates of Alzheimer's disease. Pharm Res. 2012;29(11):3075–89.
71. Liu G, Men P, Kudo W, Perry G, Smith MA. Nanoparticle-chelator conjugates as inhibitors of amyloid-beta aggregation and neurotoxicity: a novel therapeutic approach for Alzheimer disease. Neurosci Lett. 2009;455(3):187–90.
72. Lopez OL, Rabin BS, Huff FJ, Rezek D, Reinmuth OM. Serum autoantibodies in patients with Alzheimer's disease and vascular dementia and in nondemented control subjects. Stroke. 1992;23:1078–83.
73. Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, *et al.* A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature. 2000;408(6815):982–5.
74. Soto C. Plaque busters: strategies to inhibit amyloid formation in Alzheimer's disease. Mol Med Today. 1999;5:343–50.
75. Fradinger EA, Monien BH, Urbanc B, Lomakin A, Tan M, Li H, *et al.* C-terminal peptides coassemble into Abeta42 oligomers and protect neurons against Abeta42-induced neurotoxicity. Proc Natl Acad Sci U S A. 2008;105:14175–80.
76. Songjiang Z, Lixiang W. Amyloid-beta associated with chitosan nano-carrier has favorable immunogenicity and permeates the BBB. AAPS PharmSciTech. 2009;10(3):900–5.
77. Agyare EK, Curran GL, Ramakrishnan M, Yu CC, Poduslo JF, Kandimalla KK. Development of a smart nano-vehicle to target cerebrovascular amyloid deposits and brain parenchymal plaques observed in Alzheimer's disease and cerebral amyloid angiopathy. Pharm Res. 2008;25(11):2674–84.
78. Poduslo JF, Ramakrishnan M, Holasek SS, Ramirez-Alvarado M, Kandimalla KK, Gilles EJ, *et al.* In vivo targeting of antibody fragments to the nervous system for Alzheimer's disease immunotherapy and molecular imaging of amyloid plaques. J Neurochem. 2007;102:420–33.
79. Mourtas S, Canovi M, Zona C, Aurilia D, Niarakis A, La Ferla B, *et al.* Curcumin-decorated nanoliposomes with very high affinity for amyloid-β1-42 peptide. Biomaterials. 2011;32(6):1635–45.
80. Re F, Cambianica I, Sesana S, Salvati E, Cagnotto A, Salmona M, *et al.* Functionalization with ApoE-derived peptides enhances the interaction with brain capillary endothelial cells of nanoliposomes binding amyloid-beta peptide. J Biotechnol. 2010;156(4):341–6.
81. Fang YP, Tsai YH, Wu PC, Huang YB. Comparison of 5-aminolevulinic acid-encapsulated liposome versus ethosome for skin delivery for photodynamic therapy. Int J Pharm. 2008;356:144–52.
82. Mishra D, Mishra PK, Dabadghao S, Dubey V, Nahar M, Jain NK. Comparative evaluation of hepatitis B surface antigen-loaded elastic liposomes and ethosomes for human dendritic cell uptake and immune response. Nanomedicine. 2010;6:110–8.
83. Zhao L, Wei MJ, He M, Jin WB, Zhao HS, Yao WF. The effects of tetramethylpyrazine on learning and memory abilities of mice with Alzheimer disease and its possible mechanism. Chin Pharmacol Bull. 2008;24:1088–92.
84. Shi J, Wang Y, Luo G. Ligustrazine phosphate ethosomes for treatment of Alzheimer's disease, in vitro and in animal model studies. AAPS PharmSciTech. 2012;13(2):485–92.
85. Beg S, Samad A, Alam MI, Nazish I. Dendrimers as novel systems for delivery of neuropharmaceuticals to the brain. CNS Neurol Disord Drug Targets. 2011;10(5):576–88.
86. Sharma A, Gautam SP, Gupta AK. Surface modified dendrimers: synthesis and characterization for cancer targeted drug delivery. Bioorg Med Chem. 2011;19(11):3341–6.
87. Wasiak T, Ionov M, Nieznanski K, Nieznanska H, Klementieva O, Granell M, *et al.* Phosphorus dendrimers affect Alzheimer's (Aβ1-28) peptide and MAP-Tau protein aggregation. Mol Pharm. 2012;9(3):458–69.
88. Yang X, Zheng R, Cai Y, Liao M, Yuan W, Liu Z. Controlled-release levodopa methyl ester/benserazide-loaded nanoparticles ameliorate levodopa-induced dyskinesia in rats. Int J Nanomedicine. 2012;7:2077–86.
89. Xiang Y, Wu Q, Liang L, Wang X, Wang J, Zhang X, *et al.* Chlorotoxin-modified stealth liposomes encapsulating levodopa for the targeting delivery against Parkinson's disease in the MPTP-induced mice model. J Drug Target. 2012;20(1):67–75.

90. Trapani A, De Giglio E, Cafagna D, Denora N, Agrimi G, Cassano T, *et al.* Characterization and evaluation of chitosan nanoparticles for dopamine brain delivery. Int J Pharm. 2011;419(1–2):296–307.
91. Fahn S. Levodopa in the treatment of Parkinson's disease. J Neural Transm Suppl. 2006;71:1–15.
92. Black KJ, Carl JL, Hartlein JM, Warren SL, Hershey T, Perlmutter JS. Rapid intravenous loading of levodopa for human research: clinical results. J Neurosci Methods. 2003;127(1):19–29.
93. During MJ, Freese A, Deutch AY, Kibat PG, Sabel BA, Langer R, *et al.* Biochemical and behavioral recovery in a rodent model of Parkinson's disease following stereotactic implantation of dopamine-containing liposomes. Exp Neurol. 1992;115(2):193–9.
94. Muthuprasanna P, Manisha M, Suriaprabha K, Srinivasa Rao T, Anbu J. Formulation and psychopharmacological evaluation of surfactant modified liposome for parkinsonism disease. Asian J Pharm Clin Res. 2010;3(1):46–54.
95. Esposito E, Fantin M, Marti M, Drechsler M, Paccamiccio L, Mariani P, *et al.* Solid lipid nanoparticles as delivery systems for bromocriptine. Pharm Res. 2008;25(7):1521–30.
96. Zhao Y, Haney MJ, Klyachko NL, Li S, Booth SL, Higginbotham SM, *et al.* Polyelectrolyte complex optimization for macrophage delivery of redox enzyme nanoparticles. Nanomedicine (Lond). 2011;6(1):25–42.
97. Azeem A, Talegaonkar S, Negi LM, Ahmad FJ, Khar RK, Iqbal Z. Oil based nanocarrier system for transdermal delivery of ropinirole: a mechanistic, pharmacokinetic and biochemical investigation. Int J Pharm. 2012;422(1–2):436–44.
98. Pillay S, Pillay V, Choonara YE, Naidoo D, Khan RA, du Toit LC, *et al.* Design, biometric simulation and optimization of a nano-enabled scaffold device for enhanced delivery of dopamine to the brain. Int J Pharm. 2009;382(1–2):277–90.
99. Rekas A, Lo V, Gadd GE, Cappai R, Yun SI. PAMAM dendrimers as potential agents against fibrillation of alpha-synuclein, a Parkinson's disease-related protein. Macromol Biosci. 2009;9(3):230–8.
100. Malvindi MA, Di Corato R, Curcio A, Melisi D, Rimoli MG, Tortiglione C, *et al.* Multiple functionalization of fluorescent nanoparticles for specific biolabeling and drug delivery of dopamine. Nanoscale. 2011;3(12):5110–9.
101. Hu K, Shi Y, Jiang W, Han J, Huang S, Jiang X. Lactoferrin conjugated PEG-PLGA nanoparticles for brain delivery: preparation, characterization and efficacy in Parkinson's disease. Int J Pharm. 2011;415(1–2):273–83.
102. Marandi Y, Farahi N, Sadeghi A, Sadeghi-Hashjin G. Prion diseases - current theories and potential therapies: a brief review. Folia Neuropathol. 2012;50(1):46–9.
103. Imran M, Mahmood S. An overview of human prion diseases. Virol J. 2011;8:1–9.
104. Lloyd S, Mead S, Collinge J. Genetics of prion disease. Top Curr Chem. 2011;305:1–22.
105. Klajnert B, Cortijo-Arellano M, Bryszewska M, Cladera J. Influence of heparin and dendrimers on the aggregation of two amyloid peptides related to Alzheimer's and prion diseases. Biochem Biophys Res Commun. 2006;339(2):577–82.
106. Skaat H, Belfort G, Margel S. Synthesis and characterization of fluorinated magnetic core-shell nanoparticles for inhibition of insulin amyloid fibril formation. Nanotechnology. 2009;20(22):1–9.
107. Sousa F, Mandal S, Garrovo C, Astolfo A, Bonifacio A, Latawiec D, *et al.* Functionalized gold nanoparticles: a detailed in vivo multimodal microscopic brain distribution study. Nanoscale. 2010;2(12):2826–34.
108. Calvo P, Gouritin B, Brigger I, Lasmezas C, Deslys J, Williams A, *et al.* PEGylated polycyanoacrylate nanoparticles as vector for drug delivery in prion diseases. J Neurosci Methods. 2001;111(2):151–5.
109. Kim HR, Andrieux K, Gil S, Taverna M, Chacun H, Desmaële D, *et al.* Translocation of poly(ethylene glycolco- hexadecyl)cyanoacrylate nanoparticles into rat brain endothelial cells:role of apolipoproteins in receptor-mediated endocytosis. Biomacromolecules. 2007;8:793–9.
110. Ulbrich K, Hekmatara T, Herbert E, Kreuter J. Transferrin- and transferrinreceptor-antibody-modified nanoparticles enable drug delivery across the blood–brain barrier (BBB). Eur J Pharm Biopharm. 2009;71:251–6.
111. Monteiro-Riviere NA, Lang TC. Nanotoxicology: characterization, dosing and health effects. New York: Informa Health Care USA, Inc; 2007.
112. Jia G, Wang H, Yan L, Wang X, Pei R, Yan T, *et al.* Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene. Environ Sci Technol. 2005;39(5):1378–83.
113. Wang J, Sun P, Bao Y, Liu J, An L. Cytotoxicity of single-walled carbon nanotubes on PC12 cells. Toxicol In Vitro. 2011;25(1):242–50.
114. Deng X, Luan Q, Chen W, Wang Y, Wu M, Zhang H, *et al.* Nanosized zinc oxide particles induce neural stem cell apoptosis. Nanotechnology. 2009;20(11):1–7.
115. Hussain SM, Javorina AK, Schrand AM, Duhart HM, Ali SF, Schlager JJ. The interaction of manganese nanoparticles with PC-12 cells induces dopamine depletion. Toxicol Sci. 2006;92(2):456–63.
116. Long TC, Saleh N, Tilton RD, Lowry GV, Veronesi B. Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity. Environ Sci Technol. 2006;40(14):4346–52.
117. Pisanic 2nd TR, Blackwell JD, Shubayev VI, Fiñones RR, Jin S. Nanotoxicity of iron oxide nanoparticle internalization in growing neurons. Biomaterials. 2007;28(16):2572–81.
118. Zhang QL, Li MQ, Ji JW, Gao FP, Bai R, Chen CY, *et al.* In vivo toxicity of nano-alumina on mice neurobehavioral profiles and the potential mechanisms. Int J Immunopathol Pharmacol. 2011;24(1 Suppl):23S–9S.
119. Wu J, Wang C, Sun J, Xue Y. Neurotoxicity of silica nanoparticles: brain localization and dopaminergic neurons damage pathways. ACS Nano. 2011;5(6):4476–89.
120. Win-Shwe TT, Fujimaki H. Nanoparticles and neurotoxicity. Int J Mol Sci. 2011;12:6267–80.
121. Thassu D, Deleers M, Pathak Y. Nanoparticulate Drug Delivery Systems. New York: Informa Healthcare USA, Inc.; 2007.
122. Sharma HS, Sharma A. Recent Perspectives on Nanoneuroprotection & Nanoneurotoxicity. CNS Neurol Disord Drug Target. 2012;11(1):1–2.
123. Hu YL, Gao JQ. Potential neurotoxicity of nanoparticles. Int J Pharm. 2010;94(1–2):115–21.