BRAIN DRUG DELIVERY SYSTEMS FOR NEURODEGENERATIVE DISORDERS

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ABSTRACT

Neurodegenerative disorders (NDs) are rapidly increasing as population ages. However, successful treatments for NDs have so far been limited and drug delivery to the brain remains one of the major challenges to overcome. There has recently been growing interest in the development of drug delivery systems (DDS) for local or systemic brain administration. DDS are able to improve the pharmacological and therapeutic properties of conventional drugs and reduce their side effects. The present review provides a concise overview of the recent advances made in the field of brain drug delivery for treating neurodegenerative disorders. Examples include polymeric micro and nanoparticles, lipidic nanoparticles, pegylated liposomes, microemulsions and nanogels that have been tested in experimental models of Parkinson's, Alzheimer's and Hungtinton's disease. Overall, the results reviewed here show that DDS have great potential for NDs treatment.

KEYWORDS: Drug delivery systems, neurodegenerative disorders, Stereotactic surgery, Blood Brain Barrier

1. INTRODUCTION

Neurodegenerative disorders (NDs) are a varied group of conditions characterized by the gradual and progressive loss of cells from the brain and spinal cord. The symptoms vary depending on the region affected and the rate of neurodegenerative change and, for most of them, the cause of the neurodegenerative change is not known. The most widely recognized NDs are Parkinson's disease (PD), Alzheimer's disease (AD) and Huntington's disease (HD). In general, NDs have a profound effect on the quality of life of those affected. A new report from the World Health Organization shows that neurological disorders, including AD, PD, stroke, headache, brain injuries, epilepsy, neuroinfections and multiple sclerosis currently affect over 1.5 billion people worldwide and this will increase substantially due to the increase in the number of people aged over 65, since the incidence rises exponentially after this age [1]. Successful treatment strategies for NDs have so far been limited and central nervous system (CNS) drug delivery is one of the most challenging problems faced in treatment for neurodegeneration.

Drug accessibility to the CNS is mostly limited by the blood-brain barrier (BBB). BBB separates the CNS from systemic circulation and restricts the selection of applicable compounds, depending on their size and endothelial permeability. The major BBB physiological functions comprise sustaining homeostasis at the brain parenchyma and protecting the brain from potentially harmful substances. The BBB is formed principally from capillary endothelial cells without fenestrations that are closely joined together by tight intercellular junctions presenting high trans-endothelial electrical resistance compared with other tissues and thereby efficiently restrict the paracellular diffusion of solutes or drugs [2]. Another barrier that limits brain drug delivery is the blood cerebrospinal fluid barrier (BCSFB) that separates the blood from the cerebrospinal fluid. The CNS also presents functional barriers in the form of influx and efflux transporter mechanisms, which are responsible for the inclusion and exclusion of solutes/therapeutics into and out of the CNS [2].

In order to cross the BBB by passive diffusion, molecules should be relatively small, present a molecular mass of < 400 Da, a log octanol/water partition coefficient between -0.5 and 6.0, be lipid-soluble, be either neutral or significantly uncharged at physiological pH 7.4, and be capable of forming < 8 H-bonds with water [2]. Unfortunately, only a few drugs fulfil these requirements and the overwhelming majority of small molecules, proteins and peptides do not cross the BBB. It has been reported that 98% of small molecules and nearly all large molecules such as recombinant proteins or gene-based medicines do not cross the BBB [3]. Therefore, several invasive and noninvasive strategies have been proposed to bypass the BBB and

increase drug delivery to the brain. Recently, Pattel et al. [4] described the methods attempted so far to get into the brain and enhance brain drug delivery. These strategies include [4]:

- Chemical delivery systems such as lipid-mediated transport designed to increase the lipid solubility of the drug, pro-drug formation which involves the transient chemical modification of biologically active species and the lock-in system that lock inactive drug precursors in the brain on arrival, preventing their exit back across the BBB.

- Biological delivery systems in which drugs are re-engineered to cross the BBB using endogenous transporters localized within the brain capillary endothelium.

- Disrupting the BBB, by tight junction modification, which causes a controlled and transient increase in the brain capillary permeability leading to an augment in the parenchymal drug concentration.

- Receptor-mediated delivery which is employed in the transport of macromolecules like peptides and proteins by conjugating the substance with ligands such as transferrin and insulin which receptors are abundant on brain microvascular endothelium. Molecular trojan horses technology, such as peptidomimetic monoclonal antibodies, uses this mechanism to convey large molecules (e.g. antibodies, recombinant proteins, nonviral gene medicines or RNA interference drugs) across the BBB. Receptor-mediated transport is an attractive transport mechanism which is of predominant interest in drug delivery.

Despite advances in CNS drug design and in disrupting the BBB many attractive drug molecules still cannot penetrate into the brain parenchyma and alternative delivery routes (e.g. intranasal drug delivery, convection-enhanced diffusion and intrathecal/intraventricular drug delivery systems) have also been studied to overcome brain barriers and achieve high drug concentrations within this organ.

Figure 1: Scanning electron microscopy of polymeric microparticles, the DDS more frequently used for local brain administration of drugs.

Figure 2: Illustration of the typical polymeric nanoparticles used for neurodegenerative disorder treatment. (A) Nanocapsules, (B) PBCA nanoparticles coated with Tween 80 to cross the BBB (C) nanospheres, (D) pegylated nanospheres, (E) nanopheres coated with ligands and/or antibodies (F) pegylated nanospheres with additional ligands and/or antibodies.

Figure 3: (A) Schematic representation of solid lipid nanoparticles, (B) pegylated solid lipid nanoparticles and (C) scanning electron microcopy of solid lipid nanoparticles.

2. DRUG DELIVERY SYSTEMS USED FOR TREATMENT OF NEURODEGENERATIVE DISEASES

During recent years there has been growing interest in the development of micro and nanosystems for brain drug delivery capable of improving the pharmacological and therapeutic properties of conventional drugs and reducing their side effects. The important factors for a delivery system to be effective are high drug loading, physical and chemical stability and a low incidence of toxicity of the carrier used. Furthermore, the in vivo fate of the carrier, the chances of scaling up the producing process and the overall cost are other considerations to be kept in mind before deciding on the suitability of the system [5]. Among DDS, polymeric (Fig 1 and 2) or lipidic (Fig 3) micro- and nanoparticles, liposomes (Fig 4), and polymeric micelles (Fig 5) seem to be the most effective in providing neuroprotection and facilitating the delivery of drugs and small molecules to the brain [6]. DDS can be categorized as either local or systemic delivery. In this section, we will review the main developments in local and systemic biomaterial-based systems for drug delivery to the brain.

Figure 4: Schematic representation of a typical drug-loaded liposome for brain drug delivery.

Figure 5: Schematic representation of a typical polymeric micelle for brain drug delivery.

2.1. Local drug delivery systems:

Local drug delivery avoids the difficulty of BBB penetration, systemic side effects and toxicity, peripheral drug inactivation and necessity for carrier surface modification [7, 8]. Moreover, local drug delivery systems expand the spectrum of drugs available for the treatment of NDs.

DDS are administered by stereotaxy in the target site. Stereotaxy, or stereotactic surgery, is a type of minimally invasive brain surgery that uses a system of three-dimensional coordinates to locate a site within the brain (Fig 6). It requires only a small incision and a hole less than 12.5 mm in diameter to be made in the skull, which is usually performed under local anesthesia. The stereotactic operation has been commonly employed in the field of neurosurgery to perform injections, implantations, stimulation and biopsies. Traditionally, frame-based techniques were the standard method used and more recently, frameless stereotaxy or neuronavigation has been introduced. One relevant aspect of stereotactic surgery is that drugs can be easily implanted in discreet, precise and functional areas of the brain, without damaging the surrounding tissue. Injections can be repeated if necessary. Most

probably, the main disadvantage of local drug delivery administration is that the dosage cannot be adjusted after brain implantation.

Figure 6: Stereotactic surgery in rats used to administer DDS locally into the brain.

In the CNS, the most commonly used systems for local drug delivery are poly(lactic-co-glycolic acid) (PLGA) polymeric microparticles (Fig 1). Microparticles are particles with a diameter of approximately 1 to 1000 microns. They present important advantages as drug delivery systems, including encapsulated drug protection against degradation and the possibility of controlling the drug release rate over periods of hours to months. The most widely utilized methods for preparing biodegradable microparticles are phase separation, spray drying, and solvent evaporation. PLGA, a non-toxic, biodegradable and biocompatible polymer [9, 10] approved by the FDA for its use in humans is the most widely used polymer for brain application. Local delivery of drugs with PLGA microparticles has been shown to be effective for the treatment of neurodegenerative disorders like PD, AD and HD, as will be seen in more detail in the following sections.

2.2. Systemic drug delivery systems:

Several systems such as liposomes (Fig 4), polymeric nanoparticles (Fig 2), polymeric nanogels, solid lipid nanoparticles (Fig 3) and polymeric micelles (Fig 5) have been investigated in systemic delivery [5, 11-13]. Up to now, liposomes and polymeric nanoparticles are most generally exploited for brain applications [14].

2.2.1. Polymeric nanoparticles, nanocapsules and nanospheres.

Polymeric nanoparticles (Fig 2), which include nanocapsules (Fig 2A) and nanospheres (Fig 2C), are particles ranging from 10 to 1,000 nm in diameter made of natural or synthetic polymers in which therapeutic drugs can be adsorbed, dissolved, entrapped, encapsulated or covalently linked to the particle [15]. The synthetic polymers most commonly used for brain application are PLGA, poly(alkylcyanoacrylate) and polyanhydride poly[bis(p-carboxyphenoxy)]propane-sebacic acid (PCPP-SA). Normally, when polymeric nanoparticles are administered systemically, they have poor ability to cross the BBB. Coating of polymeric nanoparticles with polysorbate has been reported to improve their brain bioavailability and pharmacokinetics [16] (Fig 2B). The mechanism by which polysorbate coated-nanoparticles achieve drug transport across the BBB, however, has so far not been totally elucidated and has attracted considerable debate.

PBCA nanoparticles coated with polysorbate 80 are thought to cross the BBB via plasma adsorption of apolipoproteins resulting in receptor-mediated endocytosis by brain capillary endothelial cells, as apolipoproteins naturally cross the BBB [16]. There are various problems associated with the use of these polymeric nanoparticles, such as residual contamination from the production process, for example by organic solvents, polymerization iniziation, large polymer aggregates, toxic monomers and toxic degradation products.

2.2.2. Pegylated targeted liposomes.

Liposomes, vesicles made by double phospholipid layers which may encapsulate aqueous solutions, have been introduced as DDS due to their structural flexibility in size, composition and bilayer fluidity as well as their ability to incorporate a large variety of both hydrophilic and hydrophobic compounds [17] (Fig 4). Since conventional liposomes do not cross the BBB, poly(ethylenglycol) (PEG) is commonly used to modify their surface reducing opsonisation in plasma and decrease its recognition and removal by the liver and spleen [17]. In addition, liposome penetration through the BBB can be enhanced by active targeting with monoclonal antibodies to glial fibrillary acidic proteins, transferring receptors or human insulin receptors [11, 18-21].

2.2.3. Polymeric nanogels.

Nanogels are networks of cross-linked polymers that often combine ionic and non-ionic polymeric chains. Such networks can incorporate charged molecules such as siRNA, oligonucleotides, DNA, proteins and low-molecular-mass drugs, which bind to oppositely charged ionic chains. Recently, Vinogradov and co-workers [22] used this strategy to encapsulate oligonucleotides for delivery across the BBB. In vivo studies suggested that the nanogel increased oligonucleotides brain uptake while decreasing uptake in the liver and spleen. The mechanisms of nanogel-mediated delivery of drugs apparently involves transcytosis across brain microvessel endothelial cells. The effect was enhanced when nanogel surface was modified with polypeptides (transferring or insulin) [22, 23].

2.2.4. Solid lipid nanoparticles (SLN).

SLN are composed of a solid lipid matrix stabilized by surfactants (Fig 3). The solid core may contain the drug dissolved or dispersed in the solid high melting fat matrix with the hydrophobic end of the phospholipid chains embedded in the fat matrix. SLN can incorporate both lipophilic and hydrophilic molecules. The carrier lipids are biodegradable and safe. They are easy to prepare, possess low cytotoxicity and good physical stability and can provide controlled drug release. SLN are taken up readily by the brain due to their lipidic nature [5]. Up to now, SLN have only been used for the delivery of antineoplastic agents in brain tumor therapy [24] and to enhance brain uptake HIV protease inhibitors such as atazanavir [25] but they have great potential to deliver drugs that promote neuroprotection, neuroregeneration

or neurorepair. Pegylation (Fig 3B) and other stealth modifications will probably be necessary to prolong their brain retention and maximize their ability to deliver neuroactive drugs into the CNS.

2.2.5. Polymeric micelles.

These micelles form spontaneously in aqueous solutions of amphiphilic block copolymers and have core-shell architecture (Fig 5). The core is composed of hydrophobic polymers blocks [poly (propylene glycol), PLGA, poly(caprolactone)] and the shell of hydrophilic polymer blocks (often PEG) [26]. They can solubilise important poorly water-soluble drugs. The surface can be functionalized to cross the BBB. Polymeric micelles show efficient delivery of DNA molecules in vitro and in vivo [27]. These systems have also been used to deliver antibiotics across the BBB [28]. Polymeric micelles are under extensive study for drug delivery to the brain.

3. DDS STRATEGIES IN PARKINSON'S DISEASE

PD is the most widely studied and the second most common neurodegenerative disorder after AD. Although the cause of PD is unknown, the pathologic manifestation involves the degeneration of the nigrostriatal dopaminergic system that causes progressive dopamine loss in the basal ganglia. The characteristic clinical manifestations include difficulty with coordinated movement, such as asymmetric resting tremor, rigidity, and bradykinesia. The current treatment, based on a dopamine replacement strategy, consists of the oral administration of the dopamine precursor L-DOPA, but its long term administration has been known for quite some time to produce severe detrimental side-effects [29, 30]. Although dopamine replacement is efficacious in the early stage of the disease, new agents that can extend the length of effective treatment or ideally reverse the degenerative process are needed. An overview of the different approaches using drug delivery systems tested so far for PD is reviewed in tables 1 and 2.

3.1. Local DDS in PD

3.1.1. Microparticles for dopamine delivery

In order to achieve high drug concentration levels confined to the region of interest and to reduce the systemic toxicity compared to intravenous administration, Arica et al., [31] proposed the sustained intrastriatal release of carbidopa/levodopa from PLA and PLGA microspheres to manage PD symptoms [31]. In this study, microspheres reduced apomorphine-induced rotational motor asymmetry in hemiparkinsonian rats. The reduction in asymmetry was detectable 1 week after the lesion, but only reached the relatively modest level of 25% eight weeks after the lesion. In a similar study, PLGA microspheres containing dopamine or norepinephrine were implanted in the parkinsonian rat striatum. Microspheres

stimulated dopaminergic fiber regrowth and rats experienced a functional recovery measured as contralateral-rotational behavior induced by apomorphine [32].

3.1.2. Microparticles for neurotrophic factor delivery

Glial cell line-derived neurotrophic factor (GDNF) was selected as the most suitable candidate for PD treatment due to its strong trophic effect on the dopaminergic system [33, 34]. Clinical trials that mechanically injected GDNF intracerebrally, while demonstrating relative safety, have been clinically disappointing to date [35-38]. The failure brought some doubt about trophic factor delivery for PD treatment. Different strategies including direct gene transfer using recombinant adeno-associated virus and PLGA microparticles were proposed to improve brain GDNF delivery [39-44].

Several studies assessed the neurorestorative effect of controlled GDNF delivery using PLGA microspheres in a PD rat model. The encapsulation of non-glycosylated or glycosylated recombinant protein purified from eukaryotic cells [45] was the main difference between these studies. This has particular importance since previous clinical trials with GDNF showed the need for glycosylated protein use to avoid the production of antibodies against GDNF for the patients [36]. All the studies using GDNF-PLGA microspheres reported an animal functional recovery together with an increase in dopaminergic innervation in the striatum due to the sprouting of the fibers spared by the lesion. Moreover, none of the animals that received the glycosylated protein developed antibodies against GDNF demonstrating the safety of glycosylated GDNF use [41].

The efficacy of GDNF-loaded microspheres has been also tested in a primate model of PD. In a first study carried out by Menei et al., [46] two macaques intoxicated with MPTP were treated by intrastriatal implantation of non-glycosylated GDNF-microspheres (in the caudate and in the putamen). One animal died of a subdural haematoma and the other showed a transitory recovery from its symptoms. This experiment led to the conclusion that the dose of GDNF used (5 µg) was insufficient and the authors highlighted the need to use microparticles containing more GDNF and allowing a controlled release for several months. Subsequent studies carried out by Ansorena et al., assessed the efficacy of glycosylated human GDNF produced in a mammalian cell line [47] to neuroprotect the nigrostiatal pathway in parkinsonian monkeys (unpublished results). Macaques received intravenously follow-up doses of the neurotoxic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) until a stable bilateral parkinsonian syndrome was achieved. hGDNF-loaded PLGA microspheres were stereotaxically implanted in the postcommissural putamen of the animals. Each monkey received a total dose of 25 □g of hGDNF divided in four injections. Motor functions and behavioral changes are currently being evaluating using a nonhuman primate parkinsonian rating scale which independently scores the main parkinsonian features present in MPTP-treated monkeys (such as the loss of spontaneous activity, bradykinesia, impairment of balance, postural tremor, and freezing, etc.)

with promising results. The integrity of the nigrostriatal dopaminergic system is being studied using positron emission tomography employing the radioligand specific for VMAT2 dihydrotetrabenazine ([11C] DTBZ), with an increase in the uptake of the ligand in the target areas. Tyrosin hydroxylase immunohistochemical studies reflect an increase in the reinnervation of the putamen of these animals.

3.1.3. PLGA-based scaffolds for stem cell therapy

PLGA microparticles can be functionalized for its use as scaffolds for cell therapy. Cell transplantation is a potential therapy for central nervous disorders such as PD. However, cell therapy is confronted with cell engraftment and delivery issues. In general, cells are actually implanted into scaffolds to improve their survival and differentiation. Within this line, Pharmacologically Active Microcarriers (PAMs) were developed. PAMs are PLGA microparticles with a biomimetic surface, which release a growth factor in a sustained and controlled manner and convey cells on their surface. PLGA microparticles provide a 3D microenvironment in vivo. Moreover, the growth factor delivery combined with the biomimetic surface act synergistically to stimulate cell survival and/or differentiation. PAMs, like PLGA microparticles, can easily be administered by stereotaxic surgery into the brain region of interest. The efficacy of this unique and simple device for cell and protein-delivery in neuroprotection and tissue repair for the treatment of neurodegenerative disorders has been validated in a PD rat model. The proof of concept was obtained with a neuronal cell line (PC12 cells) transported by NGF-releasing PAMs [48] and then using GDNF-releasing PAMs conveying embryonic ventral mesencephalon dopaminergic cells [49].

3.1.4. Other DDS administered locally

Pillay et al. [50] proposed the intracranial nano-enabled scaffold divide (NESD) for the site-specific delivery of dopamine. The NESD is composed of a binary crosslinked alginate scaffold embedding stable dopamine-loaded cellulose acetate phthalate nanoparticles. The NESD was implanted at the frontal lobe parenchyma and improved dopamine delivery to the brain. Moreover, dopamine concentrations in the plasma were minimal and therefore the peripheral conversion to dopamine that leads to numerous side-effects would be reduced.

3.2. Systemic DDS in PD

3.2.1. Nanoparticles for growth factor delivery

The antiparkinsonian effect of nerve growth factor (NGF) adsorbed on PBCA nanoparticles coated with polysorbate-80 was assessed by Kurakhmaeva et al., [51, 52]. This formulation demonstrated reduction of the basic PD symptoms (oligokinesia, rigidity, tremor) and efficient NGF transport across the BBB was confirmed by direct measurement of NGF concentrations in the murine brain.

3.2.2. Nanoparticles for antioxidant delivery

Carrol et al., [53] proposed the use of Tempol-loaded PLGA nanoparticles conjugated with a transferring receptor monoclonal antibody (OX 26) for treating PD or AD. Receptor-mediated nanoparticles showed a particle size (80-110 nm) suitable for BBB permeation and in vitro results suggested that transferring-conjugated nanoparticles containing antioxidants may be useful in the treatment of neurodegenerative diseases [53].

3.2.3. Nanoparticles for gene therapy

Gene therapy using viral vectors to deliver proteins of interest to the brain is one more strategy used for PD treatment [54]. Clinical trials using adeno-associated virus to deliver AADC or nerturin to the striatum and GAD to the STN are currently under way. However, a concern for gene therapy is the vector safety, as there is a risk of excessive immune response as well as insertional mutagenesis. Thus, the development of non-viral vectors is attractive due to the potential for improved safety, reduced immunogenicity, ease of manufacturing and scale-up, and the ability to accommodate larger DNA plasmids compared to viral vectors [55].

Within this line, researchers are assessing the feasibility of using nanotechnology to condense DNA plasmids into nanoparticles and deliver them to the brain as a means to halt or prevent the neurodegenerative process [55]. The neuroprotective effect of Lactoferrin (Lf)-modified nanoparticles encapsulating GDNF gene via a regimen of multiple dosing intravenous administration was examined in 2 different rat PD models. Lf-modified nanoparticles were used due to their brain-targeting and BBB-crossing ability. Nanoparticles improved locomotor activity, reduced dopaminergic neuronal loss, and enhanced monoamine neurotransmitter levels in PD rats [56-58]. In another study, plasmid DNA encoding for GDNF was compacted into DNA nanoparticles (DNPs) by 10 kDa polyethylene glycol (PEG)-substituted lysine 30-mers (CK(30)PEG10k) and then injected intrastrially in rats lesioned with 6-hydroxydopamine. One week later, animals received fetal dopamine neurons. Data analysis for protein, morphological, and behavioral measures suggested that compacted pGDNF DNPs injected into the striatum can result in transfected cells overexpressing GDNF protein at levels that provide neurotrophic support for grafted embryonic dopamine neurons [59]. Finally, the ability of amino-functionalized organically modified silica (ORMOSIL) nanoparticles to bind and protect plasmid DNA from enzymatic digestion and to effect cell transfection was tested in vitro [60, 61].

3.2.4. Pegylated immune liposomes for gene therapy

This study proposes PD treatment using dual gene therapy that seeks both to replace striatal TH gene expression with TH gene therapy, and to halt or reverse nigro-striatal tract neurodegeneration with neurotrophin gene therapy using pegylated immune liposomes able to cross the BBB [62]. The liposomes are targeted across the BBB via attachment to the tips of 1-2% of the PEG strands of a receptor-specific monoclonal antibody (mAb) directed at a BBB

receptor, such as the insulin receptor or transferrin receptor (TfR). Owing to the expression of the insulin receptor or the TfR on both the BBB and the neuronal plasma membrane, the PIL is able to reach the neuronal nuclear compartment from the circulation. This strategy was tested in the 6-hydroxydopamine PD rat model, and striatal tyrosine hydroxylase (TH) activity was completely normalized after an intravenous administration of TfRmAb-targeted PILs carrying a TH expression plasmid.

3.2.5. Liposomes for dopamine delivery

Di Stefano et al., [63] reported the use of dopamine prodrugs encapsulated in unilamellar liposomes of dimiristoylphosphatidylcholine (DMPC) and cholesterol (CHOL) as a method for the controlled delivery of antiparkinson agents. Liposomes were administered intraperitoneally. Results showed that liposomes improved the release of dopamine in the rat brain, demonstrating the potential of these formulations [63].

4. DDS STRATEGIES IN ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a progressive neurodegenerative disorder of the brain associated with the most common form of dementia in the elderly, and accounting for around 50-60% of dementia in any age group [64]. It is characterized by memory loss and impairment in reasoning, judgment, and language. Clinically, it is defined by the association of cognitive impairment, the behavioural characteristics of dementia and the histopathological hallmarks of deposition of extracellular amyloid plaques containing amyloid beta (A□) peptide [65] and formation of intraneuronal neurofibrillary tangles [66], as well as neuronal degeneration. The cause of AD is unknown, and only a small number of cases (<1%) have a known genetic causes [67], age being the principal risk factor. Thus, AD affects 10% of people over the age of 65 and 50% of people over the age of 85 [68]. Different approaches using DDS have been developed to overcome the difficulties associated with systemic drug administration, as seen in tables 1 and 2.

4.1. Local DDS in AD

4.1.1. Microparticles for bethanecol delivery

Bethanechol, an acetylcholinesterase-resistant cholinomimetic, was encapsulated in microparticles formed of polyanhydride sebacic acid copolymer. These microparticles were tested in an animal model of AD based on the bilateral lesion in the fimbria-fornix. The fimbria-fornix constitutes a major afferent and efferent fiber tract connecting the hippocampus with the diencephalon, forebrain, striatum, and prefrontal cortex. Its unilateral or bilateral section induces a similar hippocampal cholinergic denervation that results in spatial memory deficits in the animals. Following intrahippocampal implantation in both hemispheres of the rats, spatial memory was assessed by radial-maze performance, and a significant improvement within 10

days after the implantation was displayed in treated rats. This improvement persisted for the duration of the experiment (40 days) [69].

4.1.2. Monolithic implantable devices for neurotrophic factor delivery

Nerve growth factor (NGF) is a neurotrophic factor described for the treatment of AD, as it promotes survival and the maintenance of the phenotype of these cholinergic neurons, evidenced by increased cholinergic activity [70, 71]. The problem of this neurotrophic factor, as with many other neurotrophic factors, is that it is a labile molecule with a short half-life, does not cross the BBB when administered systemically [72] and therefore needs to be administered directly to their target in the brain [73], and needs to be continuously administered since non-clinical studies have found that when NGF is withdrawn, its effects are not maintained [74, 75].

NGF was used as a model for the encapsulation of neurotrophic factors in polymeric DDS and its implantation in the target areas of the brain, and much effort was made by different research groups in the development and optimization of methods for the encapsulation of the protein [76]. The first work focused on the entrapment of NGF in monolithic implantable devices [77-79]. One of these devices was tested in an animal model of AD, based on the unilateral transection of the fimbria-fornix pathway in rodents. NGF-loaded rods, made of ethylene vinyl acetate copolymer (EVAc) and fabricated by a melt-extrusion process, were intraventriculary implanted into the ipsilateral lateral ventricle of the lesion. The rods reduced the loss of choline acetyltransferase positive neurons by 46% in the medial septum and the vertical limb of the diagonal band of Broca in the treated rats [80].

4.1.3. Microparticles for neurotrophic factor delivery

Several groups started to encapsulate NGF in PLGA or PLA microspheres, and studied the effect of these systems in several in vitro and in vivo models [81-85]. The group of Benoit developed PLGA-NGF loaded microspheres prepared by the w/o/w double emulsion technique, incorporating human serum albumine and polyethylene glycol 400 into the internal aqueous phase as stabilizing and protecting factors [86-88]. The microparticles presented a mean particle size of 27 □m, and an in vitro controlled release during 6 months. Using an unilateral transaction model of the fornix-fimbria, microparticles were implanted by stereotaxic surgery near the septal cholinergic neurons, as the NGF diffusion in the brain tissue from polymeric devices is documented to be limited to 2-3 mm [79]. The percent of cholinergic neurons was increased from 31% and 27% at two and six weeks respectively in non-treated animals, to 66% and 61% in NGF-treated animals in the same period of time, when compared to the contralateral intact side, without any evidence of neural toxicity [89]. Recently, Gu et al. [90] implanted microparticles into the basal forebrain of rats with the same animal model of AD. NGF loaded PLGA microparticles were formulated using the same technique and including BSA and PEG in the formulation [91]. Four weeks after implantation, immunohistochemical analysis showed that rhNGF-loaded microspheres had a significant effect on the survival of

axotomized cholinergic neurons in the medial septum and vertical diagonal branch (an improvement of around 45% compared with the non treated control group). More importantly, Y-maze tests showed that the NGF-loaded microspheres significantly improved the spatial learning and memory ability of treated rats.

4.2. Systemic DDS in AD

4.2.1. Microemulsions for tacrine delivery

Tacrine is one of the drugs approved by the FDA for the treatment of AD. It was the first acetylcholinestearse inhibitor licensed for the treatment of AD [92]. Although the precise mechanism of action is unknown, it is postulated to exert its effect by enhancing cholinergic functions. The drug presents a short biologic half-life of 2-3 h, with an absolute bioavailability of about 17% ±13%, probably due to very high first-pass metabolism. It presents gastrointestinal, cholinergic, and hepatic adverse affects, associated with high doses of the drug. Several studies have been published using different polymers and strategies to deliver this drug in controlled delivery systems, to minimize the side effects associated with the drug. Jogani et al., [93] prepared and characterized microemulsions of tacrine by the titration method. Biodistribution of the drug was evaluated after intranasal and intravenous administration of the formulation prepared, as well as a solution of tacrine, obtaining the pharmacokinetic parameters and drug targeting efficiency. Drug localization in the brain was also confirmed by gamma scintigraphy in rabbits, suggesting direct nose-to-brain transport. Afterwards, mucoadhesive microemulsion of tacrine was developed, and the brain bioavailability of tacrine after its intranasal administration was found to be 2-fold higher compared with intranasal administration of a solution of tacrine. In scopolamine-induced amnesic mice, the faster and larger extent of transport of tacrine into the brain and the fastest regain of memory loss were obtained with intranasal administration of the drug using this mucoadhesive microemulsion.

4.2.2. Microparticles for tacrine delivery

Recently, a study using magnetic chitosan microparticles prepared by emulsion cross linking to deliver this drug into the brain has been depicted [94]. The process yield was around 70%, and the drug loading varied from 4.5±0.3% to 9.2±0.2% (w/w). The release of tacrine was diffusion

controlled, presenting a biphasic release pattern, and had a cumulative percentage release around 78% for 24h. For the animal testing, microparticles were injected intravenously in the tail of the rats. In order to produce a depot in the target organ, a suitable magnet was kept (8000 Gaussauss/cm strength) on the head. It has been suggested that at the arterio-capillary blood flow rate of 0.005-0.1 cm/s, 20% (w/w) magnetite is sufficient to achieve 100% retention of the magnetic carrier using 8000 Gauss magnet [95], and these microparticles presented a magnetite content of 30% (w/w). There was a reduction in the accumulation of tacrine in the non-targeted organs liver, spleen and kidneys, when it was administered in the form of magnetic chitosan microparticles, compared to the free drug tacrine. By contrast, in the brain, the use of the magnetic microparticles significantly increased the tacrine uptake (a 5.38-fold increase) compared to the free drug.

4.2.3. Nanoparticles for rivastigmine delivery

This research group had previously used another strategy to target DDS into the brain for the treatment of AD. In this case, poly(n-butylcyanoacrylate) nanoparticles were prepared by emulsion polymerization method and coated with 1% polysorbate 80 to target deliver rivastigmine into the brain [96]. Rivastigmine is an established noncompetitive and reversible inhibitor of both acetylcholinesterase and butyryl cholinesterase [97], indicated for the treatment of mild to moderate dementia of the Alzheimer's type. Its therapeutic effect is thought to be exerted by increasing levels of acetylcholine in the brain via reversible inhibition of its hydrolysis, thereby enhancing cholinergic function [98]. Orally administered, rivastigmine tends to present gastrointestinal adverse effects, most probably related to large fluctuations in plasma and central nervous system levels [99]. After the in vitro characterization of the nanoparticles, the drug was administered intravenously in rats as a free drug, bound to nanoparticles and also bound to polysorbate 80-coated nanoparticles, and the concentration of drug was calculated in the brain, liver, lungs, spleen and kidneys. Coating of nanoparticles with polysorbate 80 reduced the accumulation of rivastigmine in the liver and spleen, and increased the accumulation of the drug in the kidney. More importantly, a significant increase of over 3.82-fold in rivastigmine uptake was observed in the brain in the case of poly(n-butylcyanoacrylate) nanoparticles coated with 1% polysorbate 80 compared to the free drug. Apparently, these coated nanoparticles adsorb apolipoprotein B and/or E after injection into the blood stream. The polysorbate acts mainly as an anchor for the apolipoprotein-overcoated nanoparticles, and thus would mimic lipoprotein particles and could interact with, and then be taken up by the brain capillary endothelial cells via receptor-mediated endocytosis [100].

4.2.4. Single-walled carbon nanotubes for acetylcholine delivery

Yang et al, [101] have recently used the administration of acetylcholine in an animal model of AD to test the efficacy of employing single-walled carbon nanotubes (SWCNTs) as drug delivery carriers to the central nervous system. These devices entered the brain via nerve axons, presented a chemical composition of more than 99% of carbon and a length of about 50-300nm. SWCNT loaded with acetylcholine were administered by gastrogavage as a suspension,

in mice that had received an intraperitoneal single-dose injection of kainic acid, to develop the AD model. AD mice treated with acetylcholine-SWCNT recovered their learning and memory ability to the normal levels in the step test, shuttle test, and Morris water maze test, in contrast to free drug group or SWCNT alone. These positive effects showed good dose-effect relations in all three of the tests. On the other hand, this study demonstrated that lysosomes are the pharmacological target organelles for SWCNTs, and that mitochondria are the target organelles for their cytotoxicity. There are differences in doses for SWCNTs to target these two kinds of organelles, which is the key to the safe use of SWCNT as drug carriers. The gastrointestinally absorbed SWCNTs were lysosomotropic but at large doses also entered into the mitochondria. SWCNT administration resulted in collapse of mitochondrial membrane potentials, giving rise to overproduction of reactive oxygen species, leading to damage of mitochondria, which was followed by lysosomal and cellular injury.

4.2.5. Micro and nanoparticles for human Aβ delivery

DDS have been used to prevent the deposition of Aβ or its aggregation in plaques and to hasten clearance, by the development of vaccines that induce an immune response against A□□□In the case of immunotheraphy and polymeric systems, PLGA microparticles encapsulating the human Aβ(1-42) were prepared using a modification of the water-in-oil-in-water (w/o/w) double emulsion solvent evaporation technique [102]. Swiss Webster mice were injected by the sub-cutaneous or intra-peritoneal routes with 3–33 □g Aβ. A dose-dependent antibody response was induced, and serum titers were present 6 weeks after a total of four immunizations. Responses to the encapsulated protein were significantly increased by 5-8 fold in both routes over those seen with the same dose of free peptide in PBS, confirming the adjuvancy of the PLGA microparticles. Better results were obtained with the intra-peritoneal route, most likely due to a higher density of dendritic cells in the peritoneal cavity. In a similar study with the same aim of preventing the Aβ plaque deposition and enhancing its degradation in the brain, Rajkannan et al., [103] encapsulated separately the peptides B-cell epitope Aβ (1–12), T-cell epitope Aβ (29–40) and full-length Aβ (1–42) in PLGA microparticles using the same polymer and method for obtaining microparticles, but administering them by the oral route. The oral route of immunization is more attractive than systemic or nasal routes because of its ease of delivery, improved compliance, and reduced possibility of side effects [104]. The microparticles presented a size ranging from 2 to 12 □m, appropriate for optimal phagocytosis, in order to present the vaccine in the site of mucosal immune stimulation [105] and they exhibited a slow, controlled release of Aβ□peptides in vitro over the course of 15 weeks. In vitro degradation studies demonstrated that the microparticles maintained their surface integrity up to week 8, when their surface collapsed, and was eroded completely by week 16. The oral immunization in mice with the microparticles elicited a stronger immune response compared to the administration of the free Aβ peptides, by inducing anti- Aβ antibodies for prolonged time (24 weeks). The antibody levels elicited by Aβ(29–40) looked similar to Aβ(1–42) and both remained higher than the antibody levels induced by Aβ(1–12) loaded microparticles. The changes in the serum levels of anti- Aβ antibodies were related to the kind of release of the peptides from the microparticles (after the release of the peptides due to burst effect, there was a prolonged period of slow release

due to the diffusion of peptides from the microparticles, followed by a final rapid release after the complete erosion of the microparticles). This pulsatile release behavior of the microparticles prepared with PLGA mimics the traditional booster immunization regimes [81]. A recent paper describing immunotherapy against Aβ using drug delivery systems involved the encapsulation of the protein in chitosan nanoparticles, prepared by mechanical stirring emulsification methods combined with chemical crosslinking [106]. After the physico-chemical characterization of the nanoparticles, which presented a uniform particle size distribution in the range of 15.23±10.97 nm, they were administered in mice by intraperitoneal injection. Fluorescence microscopy studies revealed that chitosan nanoparticles allowed permeation of the BBB for Aβ, as the brain uptake efficiency of the antigen encapsulated was 80.6%, and the uptake efficiency of the antigen alone was only 20.6%. ELISA test demonstrated that the vaccine had favourable immunogenicity.

4.2.6. Liposomes for Aβ delivery

In the case of liposomes, palmitoylated Aβ(1–16) peptides were anchored in the liposome bilayer containing monophosphoryl lipid A in order to enhance its immunogenicity. These liposomes were administered in three different strains of mice by the intra-peritoneal route [107]. A significant immune response was observed in the liposomes/Aβ(1–16) vaccinated BALB/c mice after the third inoculation. The titers of the elicited anti Aβ(1–42) antibodies were around 1:5,000 10 weeks after the first inoculation. Moreover, these sera were able to solubilize in vitro up to 80% of preformed Aβ(1–42) aggregates. Control animals had negligible titers to Aβ(1–42). Sera of C57BL/6 mice which had received palmitoylated Aβ(1–16) reached titers up to 1:10,000 against Aβ(1–42) whereas no antibodies against Aβ(1–42) were found in sera from control mice. In NORBA mice, which overexpress human amyloid precursor protein (APP) resulting in amyloid plaque deposits on their pancreases, vaccinated 8-week-old NORBA mice did not develop amyloid plaques on their pancreases over a period of 7 months, whereas nonvaccinated NORBA mice develop plaques within 45–60 days after birth. In the case of older transgenic animals, cryosections from pancreases of 9- and 15-month-old vaccinated NORBA mice showed a significantly reduction of less than 50% of deposition of plaques, compared to unvaccinated animals of the same age. Some years later, the same author tested two different vaccines using epitopes of the Aβ protein embedded within a liposome membrane; the tetra-palmitoylated amyloid (1-15) peptide, and the amyloid (1-16) peptide linked to a polyethyleneglycol spacer at each end. Both vaccines were administered also by the intra-peritoneal route into APPxPS-1 double transgenic mice, eliciting a fast immune response [108]. The association with liposomes induced some changes in the immunogenic structures that caused different immunogenicities. The immune response obtained differed in the titer, subclasses and in their conformational specificity. The best results were obtained with the tetra-palmitoylated amyloid (1-15) peptide, which presented a predominantly □-sheet conformation. The immune response elicited with this vaccine was based on the production of IgG class immunoglobulins, and restored the memory defect of the mice as seen with the object recognition test. Mice immunized with this vaccine recognized and remembered the original object for at least 3 h, which was similar to healthy mice matched for age, gender, and genetic background.

4.2.7. Nanogels composed of a polysaccharide pullulan backbone with hydrophobic cholesterol moieties as artificial chaperones.

The inhibition of Aβ aggregation using biocompatible nanogels as artificial chaperones has been suggested as a novel promising strategy for AD therapy. Ikeda et al., [109] used biocompatible nanogels composed of a polysaccharide pullulan backbone with hydrophobic cholesterol moieties (cholesterol-bearing pullulan, CHP) as artificial chaperones to prevent the formation of the Aβ fibrils. The CHP nanogels incorporated up to 6–8 Aβ (1–42) molecules per particle and induced a change in the conformation of Aβ from a random coil to □-helix- or □-sheet-rich structure. The aggregation of Aβ (1–42) was prevented. The dissociation of the nanogels caused by adding of methyl-□-cyclodextrin released monomeric Aβ molecules. Nanogels including an amino-group (CHPNH2) with positive charges under physiological conditions had a more potent inhibitory effect than CHP-nanogels, suggesting the importance of electrostatic interactions between CHPNH2 and Aβ for inhibiting the formation of fibrils. In addition, cell viability assays confirmed that CHPNH2 nanogels were able to protect PC12 cells from Aβ toxicity. Nanogels can also be applied to immunotherapy. In fact, CHPNH2-nanogels have greater cellular uptake efficiency than the cationic liposomes widely used in drug delivery systems [110]. Another advantage of these nanogels is that as they can control the conformation of Aβ, nanogel-Aβ complexes could be applicable to conformation of specific vaccination.

Apart from the delivery of the therapeutic molecules described in this review, DDS have also been employed for the delivery of antioxidant products and as vectors for the detection of senile Aβ plaques in the diagnosis of AD, as reviewed by Modi et al., [27, 111].

5. DDS STRATEGIES IN HUNGTINGTON'S DISEASE

Huntington's disease (HD) is an autosomal dominant, inherited, neurodegenerative disease, characterized by progressive motor, cognitive and behavioral symptoms. Its core pathology involves degeneration of the basal ganglia, in particular, the caudate and putamen. GABAergic medium-sized spiny neurons are preferentially affected. It is caused by an unstable cysteine–adenosine–guanine expansion within exon 1 located on the IT15 locus of chromosome 4 [112], with the production of the abnormal mutant protein huntingtin; the function of this protein, and consequently its role in the onset and progression of the disease are not known. At the present time, there are no effective treatments to slow or halt either the neurodegeneration or behavioral changes in HD. The only treatment options available in HD are symptomatic, and they do not increase patient survival or substantially improve quality of life. To our knowledge, only local DDS have so far been used in HD (Table 1).

5.1. Local DDS in HD

5.1.1 Microparticles for neurotrophic factor delivery (NGF and CNTF)

As seen with other neurodegenerative diseases, trophic factors may slow the progression of HD disease, or prevent the neural degeneration. NGF is one those neurotrophic factors that have been described in preventing the neuropathological and behavioral sequelae resulting from intrastriatal injections of excitotoxins, including quinolinic acid [113-118]. The intraestriatal infusion of the NMDA receptor agonist quinolinic acid [119, 120] represents a well known animal model of the disease. Based on the preceding experience of the research group in encapsulating NGF in PLGA, Menei et al., [121] developed NGF releasing microparticles that presented a mean particle size around 25 µm, and allowed an in vitro controlled release of bioactive NGF during 5 weeks. They were stereotaxically implanted into the intact rat striatum 7 days prior to infusing the neurotoxic quinolinic acid. In vivo studies confirmed that NGF was still detected in the microspheres 2.5 months after the implantation, the microparticles being totally degraded at 3 months. After striatal quinolinic acid infusion, the lesion size in the group treated with NGF-releasing microspheres was reduced by 40% when compared with the non-treated control group. Noticeable neuronal protection was presented as well within the lesioned area in the animals containing NGF-releasing microparticles. This protection principally involved the cholinergic interneurons, but also somatostatin/neuropeptide Y interneurons and GABAergic striatofuge neurons.

Ciliary neurotrophic factor (CNTF) is a multifunctional cytokine that can regulate the survival and the differentiation of many types of developing and adult neurons. CNTF administration prevents the loss of cholinergic [122], dopaminergic [123], GABA-ergic [124], and thalamocortical [125] neurons. Its encapsulation has been successfully reported in chitosan, alginate, and copolymers in various proportions. The combination of chitosan with copolymerized lactic and glycolic acid presented long-term secretion (up to 24 days) of biologically active CNTF [126]. Using a polymeric device, BHK cells that were genetically modified to secrete CNTF were encapsulated in poly (acrylonitrile-co-vinyl chloride) particles and implanted unilaterally into the rat lateral ventricle, 8–12 days before quinolinic acid injections into the ipsilateral rat striatum. These particles completely protected cholinergic neurons and 90% of glutamic acid decarboxylase-immunoreactive (GAD-ir) neurons. and also reduced apomorphine-induced rotation behavior [127], demonstrating that CNTF can protect against excitotoxic injury. On the basis of these promising results, and using the same strategy, these polymer capsules containing BHK-CNFT secreting cells were grafted unilaterally into the striatum of Rhesus monkeys [128]. Two capsules were placed in the putamen and one in the caudate nucleus. One week later, a quinolinic acid injection was placed in the putamen and caudate proximal to the capsule implants. Although all animals had significant lesions, there was a 3- and a 7-fold increase in GAD-ir neurons in the caudate and putamen, respectively, in CNTF-grafted animals relative to non-treated controls. Similarly, there was a 2.5- and a 4-fold increase in cholinergic neuron in the caudate and putamen, respectively, in CNTF-grafted animals. On the basis of these results, a phase I clinical trial was developed implanting unilaterally the device consisting of a semipermeable membrane encapsulating a BHK cell line engineered to synthesize CNTF, in six patients suffering from HD [129]. The capsule was retrieved and exchanged for a new one every 6 months, over a total period of 2 years. No sign of CNTF-induced toxicity was observed and improvements in electrophysiological results were observed. Heterogeneous cell survival and reduction in CNTF secretion in the retrieved

capsules, however, stress the need to improve the technique, or to develop alternative CNTF-DDS.

5.1.2. Miniature-sized implants for neurotrophic factor delivery

The brain-derived neurotropic factor (BDNF) has been incorporated in PLGA and chitosan microspheres, and biological assays have confirmed that the release factor remained biologically active in vitro [130]. Koennings et al., [131] encapsulated this protein as a potential therapeutic for HD, in miniature-sized implants of glyceryl tripalmitate, developed by the PEG co-lyophilization method. The implants presented a diameter of 1 mm, 0.8 mm height and 1 mg weight, and a controlled release at 37°C of 60% of the protein after one month. The in vivo evaluation of the lipid cylinders in the striatum of rat brains revealed a biocompatibility similar to silicone reference cylinders.

5.1.3. Alginate hydrogel systems for VEGF sustained release delivery

The administration of another peptide, the vascular endothelial growth factor (VEGF), has been postulated as a possible alternative for the treatment of HD. The neuroprotective properties of this well-known pro-angiogenic factor have been shown in animal models of central nervous system diseases. This protein has been incorporated in alginate hydrogel sustained release delivery systems. These gels provide continuous dose-controlled release of bioactive VEGF for over 2 weeks [132, 133]. These gels were stereotaxically injected into the striatum of adult rats, 3 days before the administration of the quinolinic acid into the ipsilateral striatum [134]. Stereological cell counts of NeuN-positive neurons throughout the striatum demonstrated that VEGF delivery hydrogels significantly protected against the loss of striatal neurons induced by the neurotoxin, as the lesion size was reduced 5-fold relative to non-treated animals. Moreover, behavioral testing of forelimb function confirmed the potent beneficial effects of the system. Thus, two weeks after the injection of the toxin, a significant improvement of 45% was observed in the placing test between the rats treated with the hydrogel versus the non-treated control animals, and a significant enhancement of 22% respectively, in the cylinder test.

6. CONCLUDING REMARKS AND FUTURE DIRECTIONS

This article reviews the major advances achieved in the field of DDS for treating NDs including local and systemic administration. As can be seen from the studies described in the present paper, NDs still represent one of the most formidable challenges to the drug delivery community. Although a breakthrough treatment has been promised repeatedly in the last 30 years, an effective drug has yet to arrive. The new DDS described here would probably open possibilities that extend beyond symptomatic relief to include neuroprotection and neurorepair, improving the quality of life of patients suffering NDs. But before that can happen, several issues may need to be addressed, such as how to target DDS to achieve high

drug concentrations in the specific brain nucleus affected and how to improve in vivo imaging to follow the trajectories that DDS take inside the brain when they are administered systemically. Further studies focused on the characterization of DDS biodistribution, organ accumulation, degradation and toxicology after systemic administration are necessary. Despite considerable progress in the brain targeted delivery field, more work needs to be done before DDS become a therapeutic option for NDs patients.

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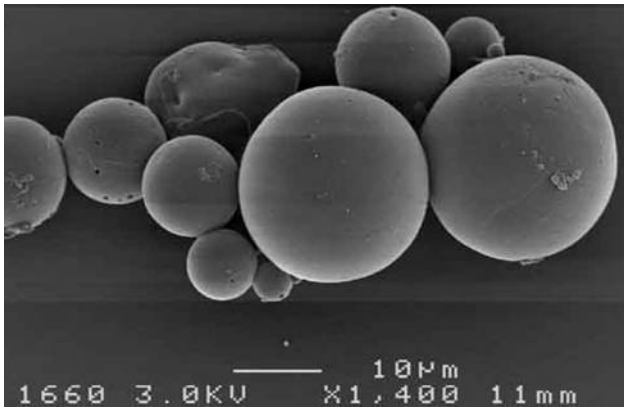

Figure 1

Figure 1: Scanning electron microscopy of polymeric microparticles, the DDS most frequently used for local brain administration of drugs

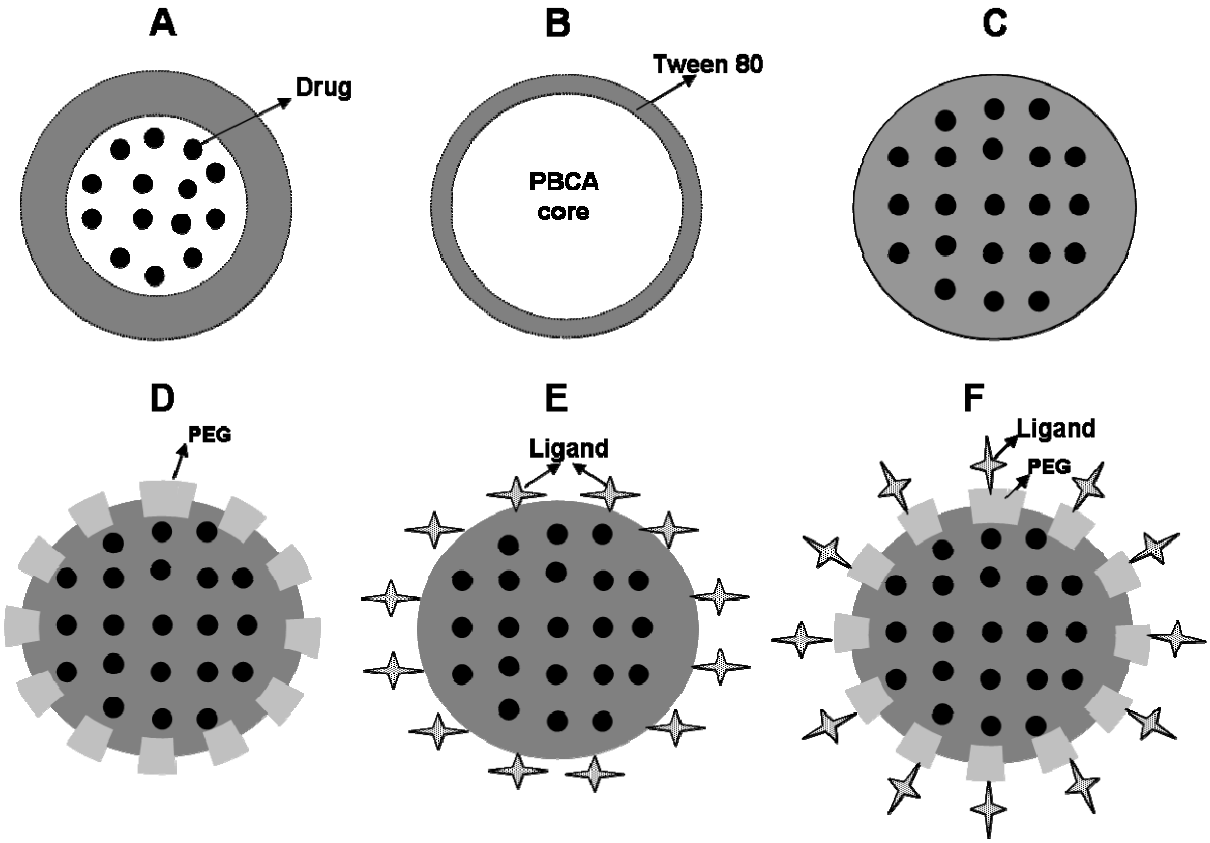

Figure 2

Figure 2: Illustration of the typical polymeric nanoparticles used for neurodegenerative disorder treatment. (A) Nanocapsules, (B) PBCA nanoparticles coated with Tween 80 to cross the BBB (C) nanospheres, (D) pegylated nanospheres, (E) nanopheres coated with ligands and/or antibodies (F) pegylated nanospheres with additional ligands and/or antibodies.

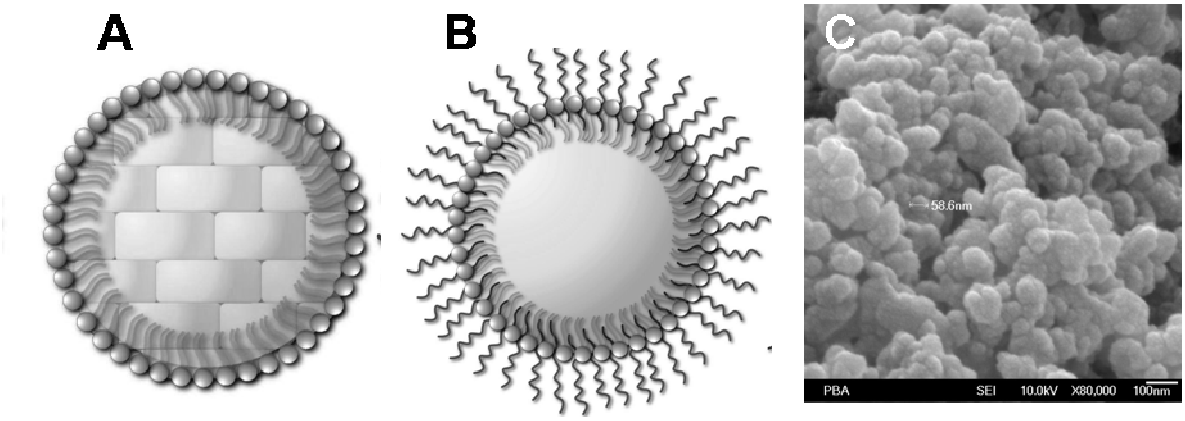

Figure 3

Figure 3: (A) Schematic representation of solid lipid nanoparticles, (B) pegylated solid lipid nanoparticles and (C) scanning electron microcopy of solid lipid nanoparticles

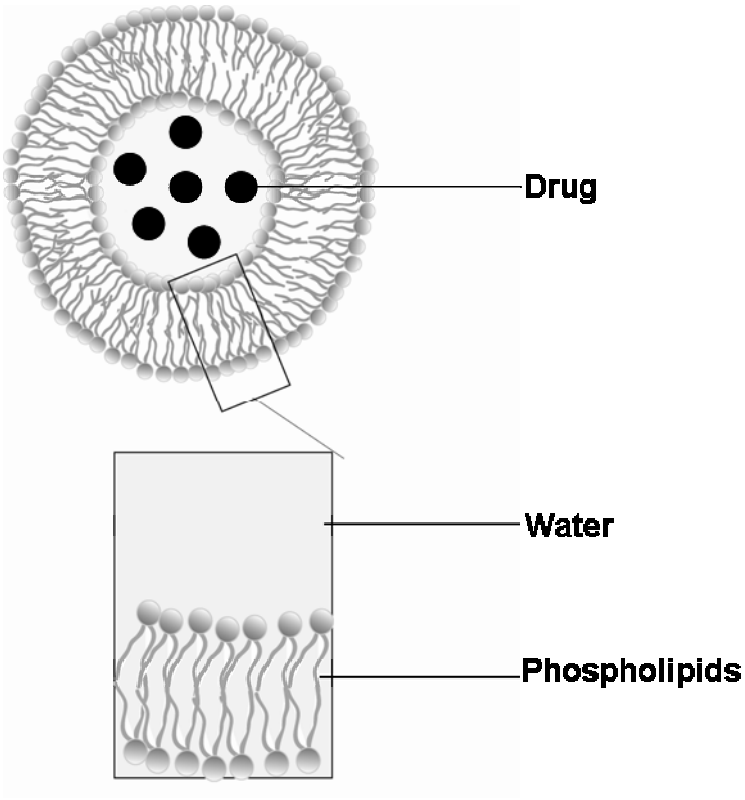

Figure 4

Figure 4: Schematic representation of a typical drug-loaded liposome for brain drug delivery

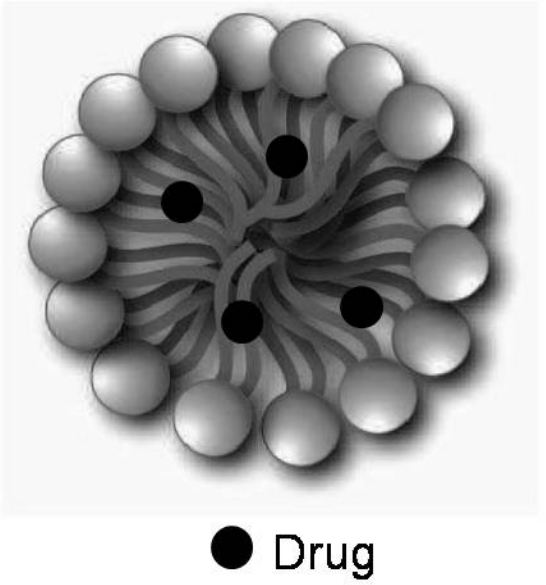

Figure 5

Figure 5: Schematic representation of a typical polymeric micelle for brain drug delivery

Figure 6

Figure 6: Stereotactic surgery in rats used to administer DDS locally into the brain.

Table 1. Overview of DDS administered locally for neurodegenerative disorder treatment

Disease	DDS	Drug	*In vitro*	*In vivo*	Ref
PD	PLGA microparticles	Carbidopa/levodopa	+	+	[31]
PD	PLGA microspheres	Dopamine or norepinephrine	+	+	[32]
PD	PLGA microspheres	Non-glycosylated GDNF	+	+	[42, 43]
PD	PLGA microspheres	Glycosylated GDNF	+	+	[41, 44]
PD	PLGA-based scaffolds for stem cell therapy	NGF and GDNF	+	+	[48, 49]
PD	NESD	Dopamine	+	+	[50]
AD	Polyanhydride sebacic acid copolymer microparticles	Bethanecol	+	+	[69]
AD	Rods of Ethylene vinyl acetate copolymer (EVAc)	NGF	+	+	[80]
AD	PLGA microparticles	NGF	+	-	[86]
AD	PLGA microparticles	NGF	+	-	[87]
AD	PLGA microparticles	NGF	+	-	[88]
AD	PLGA microparticles	NGF	-	+	[89]
AD	PLGA microparticles	NGF	+	-	[91]
AD	PLGA microparticles	NGF	-	+	[90]
HD	PLGA microparticles	NGF	+	+	[121]
HD	Chitosan, PLGA, and alginates microparticles	CNTF	+	-	[126]
HD	Particles encapsulating BHK cells of poly (acrylonitrile-co-vinyl chloride)	CNTF	-	+	[127]
HD	Particles encapsulating BHK cells of poly (acrylonitrile-co-vinyl chloride))	CNTF	-	+	[128]
HD	Particles encapsulating BHK cells of poly (acrylonitrile-co-vinyl chloride)	CNTF	-	+	[129]
HD	Chitosan/PLGA	BDNF	+	-	[130]
HD	Protein-loaded lipid implants of PEG and glyceryl tripalmitate (1 mm diameter, 0.8 mm height)	BDNF	+	+	[131]
HD	Hydrogels (Alginate)	VEGF	-	+	[134]

Table 2. Overview of DDS administered systemically for neurodegenerative disorder treatment

Disease	DDS	Drug	*In vitro*	*In vivo*	Ref
PD	PBCA nanoparticles	NGF	+	+	[51, 52]
PD	PLGA nanoparticles conjugated with a transferring antibody (OX26)	Tempol	+	+	[53]
PD	Polyethylenimine/DNA complexes	Plasmids (yellow fluorescent protein-encoding plasmid and salmon DNA)	+	-	[55]
PD	Lactoferrin-modified nanoparticles	GDNF gene	+	+	[56-58]
PD	Compacted DNA nanoparticles	GDNF gene	+	+	[59]
PD	Amino-functionalized organically modified silica (ORMOSIL)	Plasmid DNA	+	-	[60, 61]
PD	Pegylated immune liposomes for gene therapy	TH expression plasmid	+	+	[62]
PD	Liposomes	Dopamine	+	+	[6]
AD	Microemulsion	Tacrine	+	+	[93]
AD	Chitosan microparticles	Tacrine	+	+	[94]
AD	Poly (n-butylcyanocrylate) nanoparticles	Rivastigmine	+	+	[96]
AD	Single-walled carbon nanotubes	Acetylcholine	+	+	[101]
AD	PLGA microparticles	Aβ(1-42) peptide	+	+	[102]
AD	PLGA microparticles	Aβ(1-42) Aβ (1–12) Aβ (29–40) peptides	+	+	[103]
AD	Chitosan nanoparticles	Aβ(1-42) peptide	+	+	[106]
AD	Liposomes	Palmitoylated Aβ(1–16) peptides	+	+	[107]
AD	Liposomes	Tetra-palmitoylated Aβ (1-15) peptide, Aβ (1-16) peptide linked to a polyethyleneglycol	+	+	[108]
AD	Nanogels (cholesterol-bearing pullulan)	Artificial chaperones	+	-	[109]