



## Review article

## Nanoparticle-mediated brain drug delivery: Overcoming blood–brain barrier to treat neurodegenerative diseases



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## ABSTRACT

The blood–brain barrier (BBB) is a vital boundary between neural tissue and circulating blood. The BBB's unique and protective features control brain homeostasis as well as ion and molecule movement. Failure in maintaining any of these components results in the breakdown of this specialized multicellular structure and consequently promotes neuroinflammation and neurodegeneration. In several high incidence pathologies such as stroke, Alzheimer's (AD) and Parkinson's disease (PD) the BBB is impaired. However, even a damaged and more permeable BBB can pose serious challenges to drug delivery into the brain. The use of nanoparticle (NP) formulations able to encapsulate molecules with therapeutic value, while targeting specific transport processes in the brain vasculature, may enhance drug transport through the BBB in neurodegenerative/ischemic disorders and target relevant regions in the brain for regenerative processes. In this review, we will discuss BBB composition and characteristics and how these features are altered in pathology, namely in stroke, AD and PD. Additionally, factors influencing an efficient intravenous delivery of polymeric and inorganic NPs into the brain as well as NP-related delivery systems with the most promising functional outcomes will also be discussed.

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## Contents

1.	Introduction	35
2.	BBB, general concept and mechanisms of passage	35
3.	Other barriers and their role on neurogenic niches	36
4.	Models to study NP transport through the BBB	36
5.	NPs for drug delivery into the brain	37
5.1.	Factors that influence the passage of NPs across the BBB	37
6.	BBB modifications and noninvasive delivery of NPs in a context of brain diseases	39
6.1.	Stroke	39
6.1.1.	BBB alterations	39
6.1.2.	BBB-permeable NPs for stroke therapy	40
6.2.	Alzheimer's disease	41
6.2.1.	BBB modifications	41
6.2.2.	BBB-permeable NPs for AD therapy	42
6.3.	Parkinson's disease	42
6.3.1.	BBB modifications	42
6.3.2.	BBB-permeable NPs for PD therapy	43

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7. Future prospects . . . . .	43
Acknowledgments . . . . .	44
References . . . . .	44

## 1. Introduction

The central nervous system (CNS) barriers are essential interfaces between the CNS and the periphery. The most selective one is the blood brain barrier (BBB), being mainly composed of endothelial cells connected by tight junctions (TJs) and adherens junctions (AJs) [1]. In some brain pathologies (e.g. brain infections, neurodegenerative disorders, and stroke) the BBB is altered and becomes more permeable allowing the entry of molecules that can induce inflammatory responses and neuronal damage [2].

In parallel, increasing worldwide lifespan has led to a rise in the prevalence of stroke, Alzheimer's (AD) and Parkinson's disease (PD), having a huge impact in society and the economy [3–5]. However, the majority of current available treatments are symptomatic and unable to restore quality of life and halt or ameliorate damage. Until now the search for new therapies remains without significant improvements, and drug delivery – promising new molecules or even rehabilitating old ones – is the major challenge to be overcome.

Nanoparticles (NPs) are considered one of the most auspicious and versatile drug delivery systems into inaccessible regions like the brain, being able to provide protection to therapeutic agents while efficiently delivering them into the damaged areas. Several NP formulations have been administered intravenously in healthy animals proving their efficacy in crossing the BBB, mainly when they are modified with surfactants or ligands. With this in mind, it is important to understand BBB modifications in pathology in order to take advantage of these traits to develop new and innovative NP formulations capable of successfully targeting damaged areas of the brain.

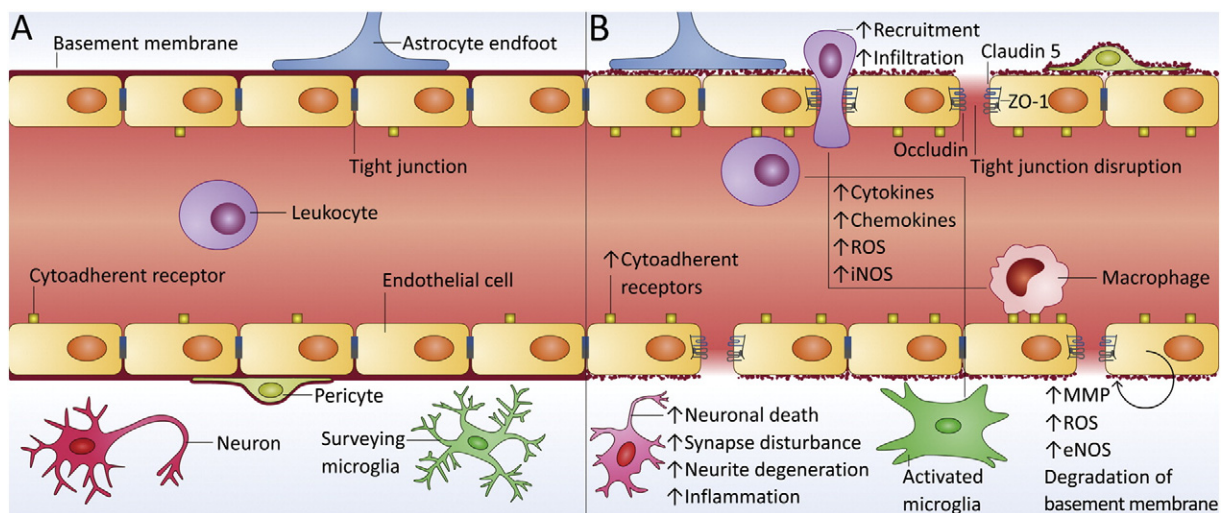
In this review, the alterations of the BBB in pathology, namely in stroke, AD and PD, will be reviewed. The principal characteristics of NPs that allow efficient brain delivery as well as targeting of the disease regions for repair will be covered.

## 2. BBB, general concept and mechanisms of passage

The CNS has developed a series of barriers to protect itself from invading pathogens, neurotoxic molecules and circulating blood cells. These structures with diverse degrees of permeability include the blood–cerebrospinal fluid (CSF) barrier, the blood–brain barrier (BBB), the blood–retinal barrier and the blood–spinal cord barrier [1]. Among these, the BBB is the most extensive and exclusive. It is mainly composed of tightly connected brain endothelial cells and a discontinuous layer of pericytes. The cellular architecture of the BBB and key alterations occurring in a pathological context are depicted in Fig. 1.

The cerebral endothelium has unique properties that allow it to maintain BBB integrity, transendothelial transport of cells and angiogenic capability to allow revascularization when needed [6,7]. To reinforce the cohesiveness of the barrier, brain endothelial cells express specific proteins, namely TJs and AJs [8]. TJs are constituted by transmembrane and cytoplasmic proteins that include claudins, occludin, junction adhesion molecules (JAMs), zonula occludens (ZO) and accessory proteins. Although there is a strong cohesive system keeping endothelial cells tightly connected, the BBB sanctions the selective passage of cells and small molecules to the brain.

The mechanism of passage between endothelial cells is named paracellular, and is utilized for ions and solutes that depend on a gradient of concentration. The passage occurring through endothelial cells is termed transcellular and the balance between paracellular–transcellular transport is decisive to define the degree of permeability in a healthy BBB [9]. The transcellular pathway occurs in most cases with passive diffusion of lipophilic molecules, which takes place through specific receptors to transport molecules such as carbon dioxide [6]. Proteins and peptides, which are hydrophilic molecules, depend on a specific type of transport to enter the brain, such as in the case of glucose transporter-1 (GLUT-1), which is responsible for glucose uptake, or specific receptors

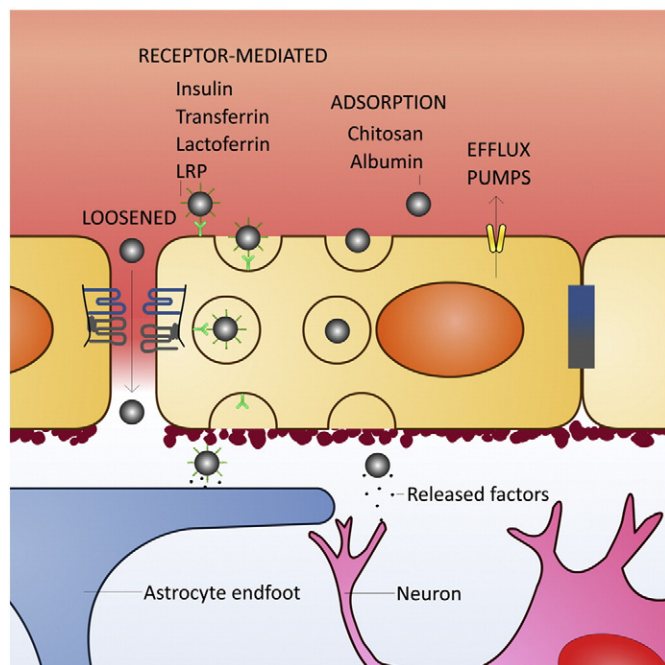


**Fig. 1.** Blood–brain barrier (BBB) composition and main alterations found in pathological conditions. A) The BBB is mainly composed of vascular endothelial cells, highly connected by adherens and tight junctions (TJs), and a sparse layer of pericytes. A basement membrane and a layer of astrocyte end-foot processes surround the endothelium. Neurons and surveying microglia are also important mediators of BBB integrity in physiological conditions. B) In pathological conditions several BBB alterations occur culminating in increased permeability. Increased matrix metalloproteinase (MMP) activity, higher reactive oxygen species (ROS) and nitric oxide levels (derived from endothelial cells – via endothelial nitric oxide synthase (eNOS) or from microglia/macrophage cells – via inducible NOS (iNOS)) along with release of cytokines and chemokines by activated microglia/macrophages lead to basement membrane degradation, TJ disruption (namely in occludin, zonula occludens (ZO)-1 and claudin 5 integrity) and an inflammatory response. Altogether these events culminate in neuroinflammation, leukocyte recruitment and brain parenchyma invasion, neuronal dysfunction and neurodegeneration.

involved in the transport of insulin or transferrin, to name a few [10]. Other forms of transport occur via the formation of cellular invaginations known as caveolae. These structures form vesicles around the molecule allowing the transport in or out of the brain. As described in the next sections, transcytosis mechanisms occurring at BBB endothelial cells are being currently explored as a way for transporting therapeutic drugs into the brain (Fig. 2). However, the delivery of several of these drugs to the brain parenchyma may also be reduced by ATP-binding cassette transporters (ABCs), which are active efflux pumps that transport possibly neurotoxic lipid-soluble molecules or other pharmaceutical drugs into the blood [11]. A deeper knowledge regarding the mechanisms of passage across this highly selective barrier will foster the development of new strategies for the delivery of neuroprotective and regenerative molecules that in normal conditions cannot pass the BBB.

### 3. Other barriers and their role on neurogenic niches

Contrary to expectations, there are regions of the CNS that actually benefit from exposure to a more permissive barrier, such as the ventricular and circumventricular areas of the brain. The ventricular system is composed by four cavities: two lateral ventricles, and a third and fourth ventricle whose choroid plexus and capillaries are responsible for the production of CSF. Other important structures are the circumventricular organs (CVOs) that line the third and fourth ventricle walls. These highly vascularized structures have fenestrated capillaries and a weak astrocytic contact that allows a direct exchange among the blood stream and the brain parenchyma.



**Fig. 2.** Blood–brain barrier (BBB) transport mechanisms for brain delivery of nanoparticles (NPs). The BBB is highly selective and has specific transport mechanisms allowing a close control of molecules/cells that enter the brain parenchyma. Loosened tight junctions (TJs) allow the cross of NPs through the BBB, either by the presence of a surfactant in NPs able to disrupt the TJs or by BBB impairment due to pathological conditions. Receptor-mediated transcytosis is the most common type of transport for NP entry into the brain. NPs can be functionalized with different types of ligands (such as insulin, transferrin, lactoferrin or antibodies against some endothelial receptors), or surfactants like polysorbate 80 (that adsorbs plasma proteins, namely apolipoprotein E enabling their binding to the lipoprotein receptor-related proteins (LRPs)). The interaction between NP ligands and respective receptors in the endothelial cell (luminal side) surface triggers plasma membrane invaginations followed by pinch free forming vesicles, which facilitates the release of the NPs in the opposite site of the membrane (parenchymal side). NPs coated with molecules such as albumin or chitosan can cross the BBB by adsorptive transcytosis. Efflux pumps may reduce the amount of NPs retained in brain parenchyma.

One of the main neurogenic niches of the adult mammalian brain, the subventricular zone (SVZ), located on the walls of the lateral ventricles, also relies on a more permissive vasculature [12]. The SVZ blood vessels have defined characteristics, namely areas that do not have contacting astrocyte endfeet or pericytes, rendering it thinner and more permeable [13]. Adjacent to the SVZ, the rostral migratory stream (RMS), a highly vascularized area, comprises a demarcated route of transit for newborn neurons in direct contact with the vasculature [14]. Interestingly, there are studies that claim that new neurons can also arise in CVO [15,16]. In a recent study, ischemic injury was induced to stimulate the proliferation of stem cells in the SVZ and CVO. An increase in both proliferation and differentiation was found not only in these areas but also along the third and fourth ventricles [17]. All of these brain areas share a common trait, a leaky BBB, which endows them with a greater ability to perceive damage and to engage in brain repair. Interestingly, recent studies have shown that intravenous injection of NPs, independently of their cargo and mechanism of transport across the BBB, accumulate at high levels in these leaky regions. For example, angiopep-conjugated poly(ethylene glycol)-copoly( $\epsilon$ -caprolactone) nanoparticles (ANG-PEG-NPs; with a mean diameter of around 90 nm) that were administered intravenously into healthy mice passed through the BBB and accumulated in the ventricles, hippocampus and cortical layer. The ability of this formulation to accumulate into specific areas can be an asset in the development of new therapeutic strategies [18]. There are, therefore, strong evidences suggesting that these brain regions which rely on a leaky BBB may provide an alternative route for NP entrance into the brain and, importantly, modulate the regenerative ability of neural stem/progenitor cells. However, it should be noted that these brain regions may mount other forms of obstruction to therapeutic drugs, by having increased enzymatic activity (i.e. enzymatic barrier) in CVO.

### 4. Models to study NP transport through the BBB

In vitro BBB models offer interesting opportunities to study the uptake, mechanism of transport, and cytotoxicity of NPs. In addition, these models allow the performance of high-throughput screenings, facilitate the manipulation of some parameters that affect BBB (e.g. hypoxia, aglycemia, toxins, among others), reduce animal testing and are less expensive than in vivo experiments. Models of BBB are mostly based on endothelial cell cultures isolated from human [19,20] or animal [21,22] sources. Nevertheless, in vitro BBB models based in stem cells are also extensively used [23,24]. Endothelial cell monolayers, produced in two-dimensions or three-dimensions, or co-cultures of endothelial cells–astrocytes, endothelial cells–pericytes, endothelial cells–pericytes–astrocytes to name a few, are some of the examples. In the last years some in vitro BBB models that recapitulate features of the BBB in stroke have been generated. For instance, Cho and colleagues developed a microfluidic platform based on rat brain endothelial cells which showed increase in reactive oxygen species (ROS) levels and decrease in ZO-1 expression upon oxygen and glucose deprivation, mimicking BBB dynamics in stroke. [25]. Additionally, in vitro BBB models reproducing aspects of PD and AD BBB properties have been also reported recently. A BBB model based in a co-culture of rat cerebral microvessel endothelial cells isolated from PD animals (chemically induced by 6-hydroxydopamine) with rat astroglial cells showed signals of BBB dysfunction (P-gp overexpression, lower transelectrical resistance) similar to the ones observed in vivo in 6-OHDA PD models, being handy for drug delivery studies in PD [26]. A novel in vitro BBB model based in the co-culture of porcine brain endothelial cells with a human neuroblastoma cell line (SH-SY5Y) transfected with a luciferase reporter vector coupled to an ADAM10-promoter (important to avoid toxic cleavage of amyloid precursor protein) showed to be a versatile tool to predict drug passage across the BBB in AD. Interestingly, this model can be easily tuned to test drug delivery in other pathologies [27]. Overall, these in vitro BBB models have been used to evaluate the

mechanism of increased permeability of endothelial cell after hypoxia, oxidative stress, but not the permeation of NPs. Besides, it is important to notice that: 1) inter-species differences in the concentration of transporters and TJs may affect the final readouts of the mechanism of transport [23,28]; 2) co-culture BBB models are superior to mono-culture ones because they show improved barrier properties, preserve better endothelial cell polarity and show increased expression of transporters and TJs [29]; and 3) most studies have been performed with cells from non-human sources and thus further advances are needed to produce human models for better prediction of NP transport. Therefore, *in vivo* models are essential to provide more insights into drug delivery across the BBB due to their higher complexity and clinical relevance. NP transport through the BBB is based mostly in *in vivo* animal models and requires quantification (e.g. inductively coupled plasma mass spectrometry (ICP-MS) and neutron activation) and/or imaging (e.g. fluorescence microscopy, transmission electron microscopy (TEM)) techniques to monitor NP transport into the brain. Gold NPs are easily quantifiable and monitored by ICP-MS and TEM [22,30–32] and have been highly used to screen NP passage across the BBB *in vivo*. NPs can be found in brain capillaries of mice and rats only 30 min after intravenous administration [33,34]. These studies are in line with *in vitro* data that report endothelial cell endocytosis as a relatively fast event with NPs being observed after 30 min in the cell endolysosomal compartment [19,35]. Sixty minutes after administration, NPs are observed across the brain tissue [34]. According to several studies using different NP formulations in mice models, the transport of NPs through the brain peaks during the first few hours (typically below 5 h), decreasing afterwards [21,22,31,32,36]. Typically, the kinetics of transport is affected by NP size and surface chemistry. The drawbacks of the current studies are that, in most cases healthy animals have been used and no systematic study has been performed with animals at different development stages or, in particular, with aged animals. Also, very few *in vivo* studies have characterized NP transport in disease animal models for stroke, AD or PD.

## 5. NPs for drug delivery into the brain

The development of new strategies to treat brain diseases is one of the most challenging and expensive market niches for pharmaceutical companies. During the process of development and discovery of new compounds for the CNS, the costs for reaching phase I clinical trials can go up to US\$100 million and around US\$1 billion before reaching the consumer [37]. Taking into consideration these numbers it is of utmost importance to be effective in the development phase. However, in recent years, only a minor number of brain-directed pharmaceuticals have reached the market (3–5%) since most of them were incapable of crossing the BBB *in vivo* [38]. Currently, advances in the field of nanomedicine have generated several platforms that improve drug transport across the BBB, namely NPs [39–42]. In this review, we will cover NPs used to transport drugs through the BBB when administered intravenously as well as the factors that influence its transportation.

NPs are colloidal carriers that can have a natural or synthetic origin and can vary from 1 to 1000 nm in size. There are other types of colloidal carriers, for example liposomes and micelles that have been extensively studied for drug delivery to the brain. Since they possess unique features that distinguish them from polymeric and inorganic NPs, this issue will not be covered in this review.

Synthetic NPs may be prepared from polymeric materials such as poly(ethylenimine) (PEI), poly(alkylcyanoacrylates), poly(amidoamine) dendrimers (PAMAM), poly( $\epsilon$ -caprolactone) (PCL), poly(lactic-co-glycolic acid) (PLGA), polyesters (poly(lactic acid) (PLA), or from inorganic materials such as gold, silicon dioxide (silica), among others (Fig. 3). These carriers can transport drugs by adsorbing, entrapping or bounding covalently to them [43–45]. Inorganic NPs offer advantages over polymeric NPs in terms of control over size and shape and simplicity of preparation and functionalization. Most importantly,

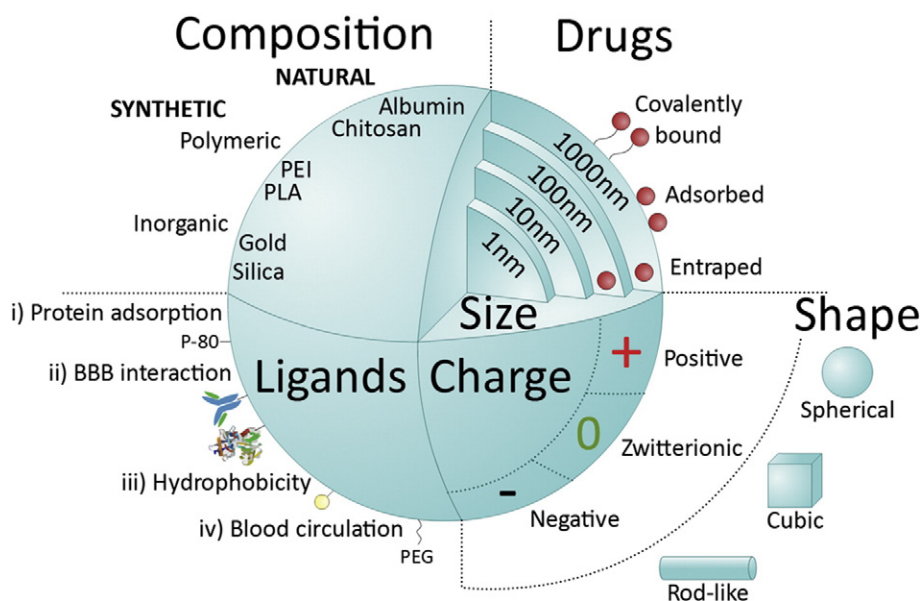
inorganic NPs are easier to track by microscopy techniques (e.g. magnetic resonance imaging (MRI), TEM) or analytic techniques (e.g. ICP-MS). However, inorganic NPs also have disadvantages because they might not be degraded (or eliminated through the kidneys) or present undesired toxicity (e.g. carbon nanotubes and fullerenes may lead to lipid peroxidation and oxygen radical formation). On the other hand, natural NPs are produced from natural polymers, such as polysaccharides (chitosan and alginate), amino acids (poly(lysine), poly(aspartic acid) (PASA)), or proteins (gelatin and albumin) [36,46]. Natural NPs have the advantage of providing biological signals to interact with specific receptors/transporters expressed by endothelial cells but they have the disadvantage of batch-to-batch variability, limited ability for controlled modification and poor tracking capacity by imaging platforms (Fig. 3).

The physico-chemical properties of NPs determine which is the passage mechanism across the BBB. The following transport mechanisms have been described (Fig. 2): (i) NPs open TJs between endothelial cells or induce local toxic effects which leads to a localized permeabilization of the BBB allowing the penetration of the drug in a free form or conjugated with the NPs [21,47]; (ii) NPs pass through endothelial cell by transcytosis [48]; (iii) NPs are transported through endothelial cells by endocytosis, their content is released into the cell cytoplasm and then exocytosed in the endothelium abluminal side [49]; or (iv) a combination of several of the mechanisms described previously. According to some studies, mechanisms ii, iii and iv are the main transport mechanisms of NPs. In case of mechanism ii, several receptors have been targeted by NPs including transferrin [50] and low-density lipoprotein receptors [51,52]. The targeting has been achieved by peptides [51,53], proteins [52] or antibodies [50] physically or chemically immobilized on top of the NPs.

NPs are exciting systems for brain drug delivery due to the possibility to modulate them in terms of shape, size, hydrophobicity, coating, chemistry and surface charge. Control over these features can enhance the ability of NPs to improve the therapeutic agent stability in circulation, to control the cargo release into the desired target site, to enhance BBB penetration efficiency and to escape the reticuloendothelial system [39,44,45]. These features will be discussed in the next section and are summarized in Fig. 3.

### 5.1. Factors that influence the passage of NPs across the BBB

There are several parameters that affect the efficiency of NP systemic circulation, BBB passage and cellular delivery. Several studies have been shown a clear inverse correlation among NP size and BBB penetration [30,54,55] (Fig. 3). In particular, most of the studies performed so far with stroke, AD or PD animal models have used NPs with diameters between 50 nm to 100 nm (see below Section 6). The shape of NPs also influences body distribution and cellular uptake [22]. The shape of NPs can vary from spherical, cubic, rod-like, among other forms (Fig. 3). Most of the studies have been performed with spherical NPs since they are relatively easy to prepare. Although, *in vitro* studies have also demonstrated that nanorods coated with specific antibodies have higher adhesion propensity than their spherical counterparts. Specifically, polystyrene NPs with a rod shape ( $501 \pm 43.6 \times 123.6 \pm 13.3$  nm) coated with an antibody against the transferrin receptor showed *in vivo* a 7-fold increase in brain accumulation when compared to their spherical NP counterpart ( $200 \pm 0.01$  nm) [56]. Zeta potential is another important parameter that affects the passage of NPs through the BBB. It has been shown that NPs with high zeta potential (high positive charge) cause immediate toxicity to the BBB [57]. Therefore, most of the NP formulations described in the literature for brain delivery have moderate (between  $-1$  to  $-15$  mV) [35,47,48,58] or high (between  $-15$  to  $-45$  mV) [22,59] negative zeta potentials. Yet, some NP formulations with moderate (up to 15 mV) or high positive zeta potential (above 15 mV) have been able to cross the BBB and in some cases are efficient brain delivery systems (Fig. 3) [21,60].



**Fig. 3.** Main nanoparticle (NP) features influencing systemic delivery and blood brain barrier (BBB) passage. NPs can be classified into natural, when molecules such as proteins (albumin), polysaccharides, chitosan, among others are used, or synthetic. Synthetic NPs can be made of very common polymers such as poly(lactic-co-glycolic acid) (PLGA), poly(ethylenimine) (PEI), polyesters (poly(lactic acid) (PLA), or from inorganic agents like gold, silica or alumina. NPs can vary in their size (1–1000 nm) and are able to deliver drugs into cells by entrapping, adsorbing or covalently bounding them. NPs can assume different shapes (spherical, cubic, and rod-like) and charges (negative, zwitterionic, and positive); negatively charged spheres are widely used in intravenous applications. Another important feature of NPs is the possibility of functionalization with different types of ligands. Ligands are distributed into four major categories: i) capable of mediating protein adsorption (e.g. poly(sorbate) 80 (P-80)); ii) able to interact directly with the BBB (e.g. transferrin proteins, antibody or peptides); iii) capable of increasing hydrophobicity (e.g. amphiphilic peptides); and iv) able to improve blood circulation (e.g. poly(ethylene glycol) (PEG)).

A number of ligands have been conjugated to NPs to facilitate BBB penetration. Such molecules can be grouped into four different types (Fig. 3): (i) ligands that mediate the adsorption of proteins from the bloodstream that interact directly with BBB receptors or transporters [61]; (ii) ligands that have direct interaction per se with BBB receptors or transporters [20,31,48], (iii) ligands that increase charge and hydrophobicity [32] and (iv) ligands that improve blood circulation time (e.g. PEG) [47]. In the first case, we can include poly(sorbate 80) (also known as Tween 80) that can adsorb apolipoprotein E and/or A-I. The surfactant allows the anchoring of apolipoproteins whose interaction with lipoprotein receptors expressed in the brain endothelium enables the crossing of the BBB. In the second case, we can include several targeting ligands such as the ones for transferrin receptor (transferrin peptide, transferrin protein or antibody against transferrin) [48,62,63], insulin receptor [31,64], glucose transporter [20], among others (Fig. 2). In the third case, NPs have been coated with amphiphilic peptides to facilitate the uptake by BBB endothelial cells. In addition, the number of ligands as well as their receptor affinity has an important impact in the transport of NPs across the BBB (avidity). Ligand density depends on both the NP surface area and the ligand size. Typically, the ligand affinity to its receptor is reduced when conjugated to NPs. NP avidity and selectivity increases when multiple targeting ligands are conjugated [47,65]. However, NP avidity must be modulated for effective BBB transcytosis. High avidity will impede NPs bound to the receptor to be released into the brain parenchyma. It has been shown that gold NPs conjugated with high concentrations of transferrin (100–200 molecules of transferrin *per* NP) stay bound to brain endothelial cells. In contrast, gold NPs conjugated with low concentrations of transferrin (20–30 molecules of transferrin *per* NP) can interact effectively with the receptor, undergo transcytosis and be released into brain parenchyma [48].

When NPs enter a physiological environment there is a rapid adsorption of proteins from the bloodstream to the NP surface forming a protein coating – the “protein corona” [66,67]. Over 70 different serum proteins have been reported to adsorb to the surface of gold NPs [66]. The protein corona may alter the surface chemistry of the NPs along with its aggregation state. Very often it also accelerates

blood clearance of the NPs through the reticuloendothelial system localized mostly in the spleen and liver [39,68], which may decrease the NP dose available for accumulation in the brain as well as induce inflammation. The most common way to overcome this issue is to use molecules with the capacity to minimize surface fouling in order to maintain performance and safety of materials. In this sense, antifouling properties can be enhanced by using PEG-coated NPs. PEGylated NPs present minimal surface charge leading to lower NP opsonization and lower reticuloendothelial system uptake [69]. Grafting NPs with PEG (5 kDa; between 0.16 and 0.64 PEG molecules *per* nm<sup>2</sup>) decreases protein adsorption and slows down the clearance of the nanomaterials [66,70]. Moreover, due to its improved blood circulation time, PEGylated NPs accumulate more efficiently in the brain [65,71]. For instance, polystyrene NPs (below 200 nm) coated with PEG (5 kDa; 9 PEG molecules *per* 100 nm<sup>2</sup>) are able to cross the BBB. Additionally, PLGA NPs (ca. 78 nm) coated with PEG are also able to rapidly penetrate rat brain tissue *ex vivo*, in contrast with uncoated NPs [71].

In summary, several parameters influence the transport of NPs through the BBB at different extents. The characterization of the NP is highly variable and some aspects such as ligand density and its impact in NP transport through the BBB are not well studied. So far, NPs conjugated with ligands able to interact with BBB receptors at a relatively low density (low avidity) have the best performance. Yet, it is important to note that the best formulations administered intravenously deliver up to 5% of the initial dose effectively across the brain. NP brain delivery improvement might require systems that target and cross more efficiently the BBB but also systems that are slowly clear from the bloodstream. Regarding this last issue, the charge and the morphology of the NP have a very important effect in the clearance. Neutral and zwitterionic NPs have a longer circulation time after intravenous administration, in contrast to negatively and positively charged NPs [72]. In addition, short-rod NPs are preferentially retained in the liver and present a rapid clearance rate, while long-rod NPs are caught in the spleen and have a lower clearance rate. If the surface is modified with PEG, retention increases in lung for both formulations [58]. In the section below we discuss how NP parameters influence the targeting and transport of the NPs across the BBB in animal models of stroke, AD and PD.

## 6. BBB modifications and noninvasive delivery of NPs in a context of brain diseases

The BBB is responsible for brain homeostasis and protection. Impairment of this structure, observed in neurodegenerative disorders, leads to inflammation perpetuation and neurodegeneration (Fig. 1) [2]. In some cases, it is clear that BBB breakdown is a consequence of a specific event such as traumatic brain injury or ischemic stroke [73]. In other cases, especially in chronic neurodegenerative conditions like AD and PD, it remains unclear if it is a downstream process or if it plays a role in disease onset and development [74,75]. Importantly, these modifications should be considered as an opportunity to design more efficient delivery systems and launch novel promising noninvasive therapeutic approaches for brain disorders. In the next sections, we will review the most relevant NP-related delivery systems *in vivo* that can be administered intravenously and have promising functional recovery outcomes in stroke, AD and PD pathologies (Figs. 4B, 5B, 6B).

### 6.1. Stroke

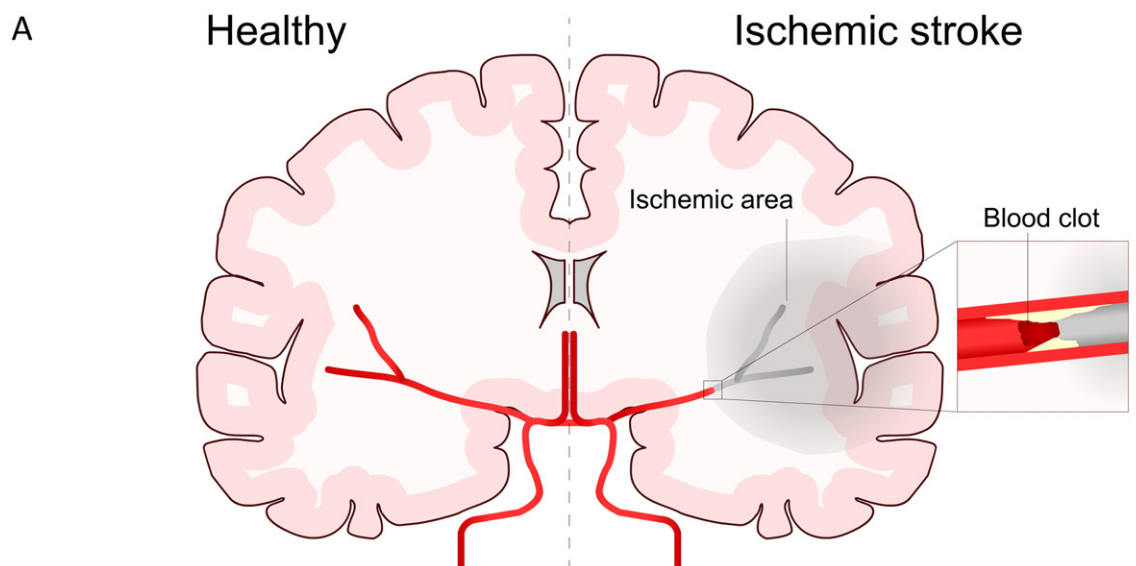
#### 6.1.1. BBB alterations

Stroke is the most costly and long term disabling condition in adulthood worldwide affecting approximately 800,000 people *per year* [3]. During a stroke episode the brain is deprived of blood supply, by a

bleeding vessel (hemorrhagic stroke) or by occlusion of a vessel due to a blood clot (ischemic stroke) (Fig. 4A) [76]. In both cases there is a deprivation of oxygen and nutrients, resulting in brain cell death that can culminate in the loss of neurological functions and ultimately in patient death.

The development of several animal models of stroke [77] was essential to shed light into the cellular and molecular mechanisms that occur in a stroke episode. The middle cerebral artery occlusion (MCAO) model, that can be transient or permanent, is the gold standard for the brain ischemic animal models of stroke. Nevertheless, other models such as the photothrombosis model are starting to be more widely used due to its less invasiveness and high reproducibility.

During the ischemic stroke the BBB opens for a short period (minutes to hours), followed by a refractory interval and then, it reopens for an extended time (hours to days) [78–80]. The restitution of the blood supply (reperfusion) is essential to limit cerebral injury, but this process can also exacerbate damage (appropriately termed reperfusion injury). Specifically, it contributes to the latter reopening of BBB which is attributed to endothelium activation, ROS production, leukocyte recruitment, cytokine production and edema formation [73,81]. BBB dysfunction developed during ischemic stroke is mainly associated with loss and disruption of TJs [82]. TJs are degraded by matrix metalloproteinases (MMPs), which are widely involved in tissue remodeling [79,83]. MMPs also contribute to BBB extracellular matrix degradation,

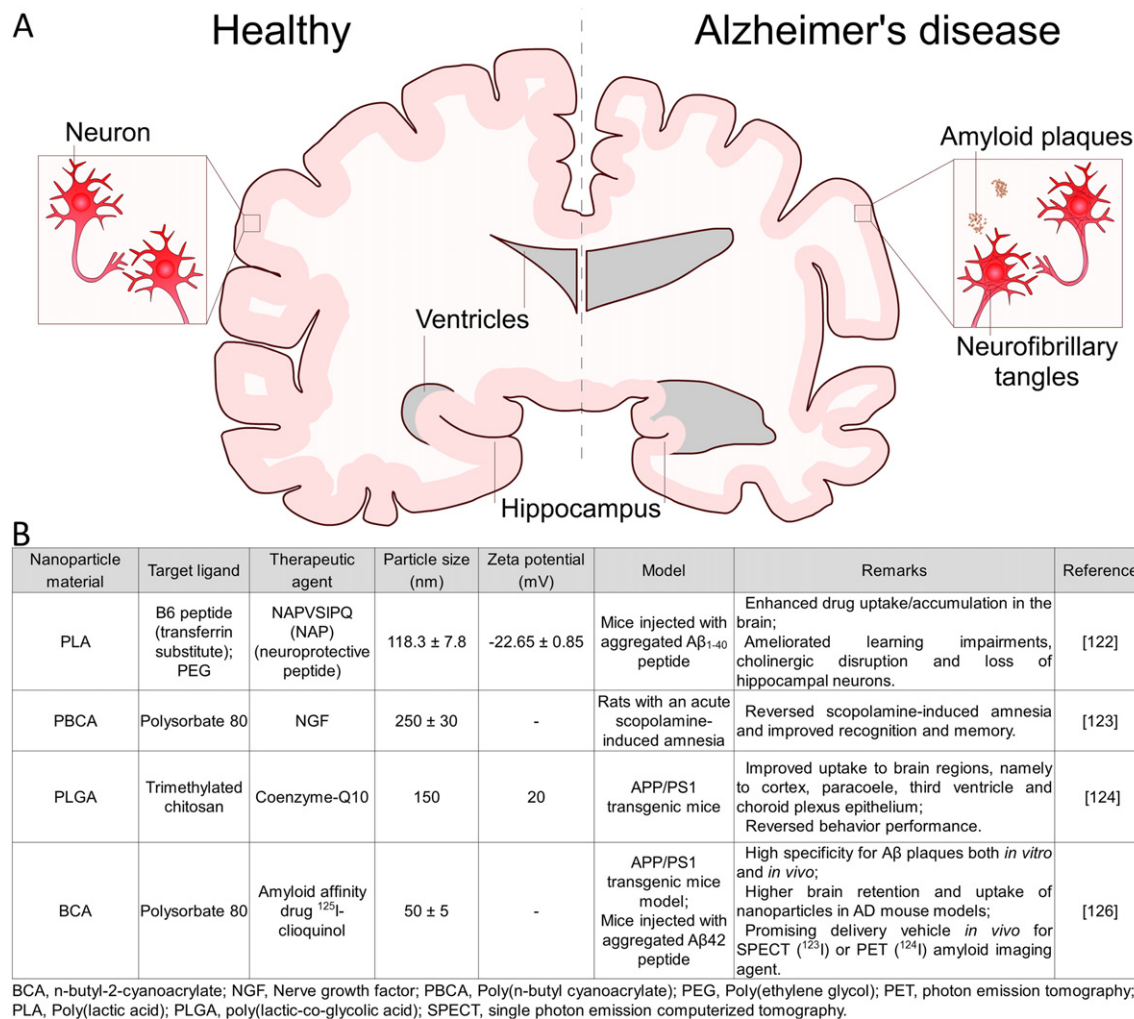


**B**

Nanoparticle material	Target ligand	Therapeutic agent	Particle size (nm)	Zeta potential (mV)	Model	Remarks	Reference
Chitosan	Transferrin receptor Antibody; PEG	Z-DEVD-FMK (caspase-3 inhibitor)	650 ± 2	20 ± 4	Mouse 2 h MCAO and 24 h reperfusion	Decreased infarct volume, neurological deficit and caspase-3 activity.	[36]
Chitosan	Transferrin receptor antibody	Z-DEVD-FMK and bFGF	747 ± 42	-	Mouse 2 h MCAO and 24 h reperfusion	Decreased infarct volume; Increased motor function deficit scores; 3h therapeutic window.	[50]
Cationic Bovine Serum Albumin	PEG	Tanshinone IIA	118 ± 14	-17.8 ± 1.6	Rat 2 h MCAO and 24 h reperfusion	Decreased infarct volume, neurological deficit, neutrophil infiltration and neuronal apoptosis; Modulates inflammatory responses and neuronal signaling.	[95] [96]
Lipidic (Squalene)	-	Adenosine	120	-25 ± 4	Mouse 2 h MCAO and 22 h reperfusion; 24 h of permanent MCAO	Decreased infarct volume; Increased neurological deficit scores.	[100]

bFGF, Basic fibroblast growth factor; DEVD-FMK, N-benzyloxycarbonyl-Asp(OMe)-Glu(OMe)-Val-Asp(OMe)-fluoromethyl ketone; MCAO, Middle cerebral artery occlusion; PEG, Poly(ethylene glycol).

**Fig. 4.** Schematic representation of the main event causing ischemic stroke and summary of the most promising nanoparticle (NP)-based strategies used in pre-clinical studies for ischemic stroke. A) Ischemic stroke occurs when there is a blood flow interruption caused by a clot culminating in oxygen and nutrient depletion and consequently in neuroinflammation and neurodegeneration. B) Description of the therapeutic approaches and main conclusions obtained in studies using intravenously-administered NPs in stroke pre-clinical models.



**Fig. 5.** Main pathologic hallmarks found in Alzheimer's disease (AD) brain and nanoparticle (NP) delivery systems used in pre-clinical animal models of AD. A) The main characteristics of AD are the presence of amyloid plaques and neurofibrillary tangles in neurons, which culminates in severe neurodegeneration, shrinkage of the cerebral cortices and hippocampus, and enlargement of the ventricles. B) Description of the therapeutic approaches and main conclusions obtained in studies using intravenously-administered NPs in pre-clinical models of AD.

namely type IV collagen, further increasing BBB permeability [84]. In particular, increased levels of MMP-9 are correlated with higher BBB permeability and disease severity in stroke patients and in stroke animal models [85,86]. In turn, in ischemia reperfusion the increased levels of nitric oxide (NO) activate MMP-9 and MMP-2 promoting a leaky BBB [87]. MMP activity may also be stimulated by vascular endothelial growth factor (VEGF), an angiogenic factor. Accordingly, endothelial cells treated with VEGF showed reduction in both transepithelial electrical resistance and claudin-5 and occluding expression, while *in vivo* inhibitors of VEGF were able to reduce BBB permeability and infarct volume in hypoxia models [88].

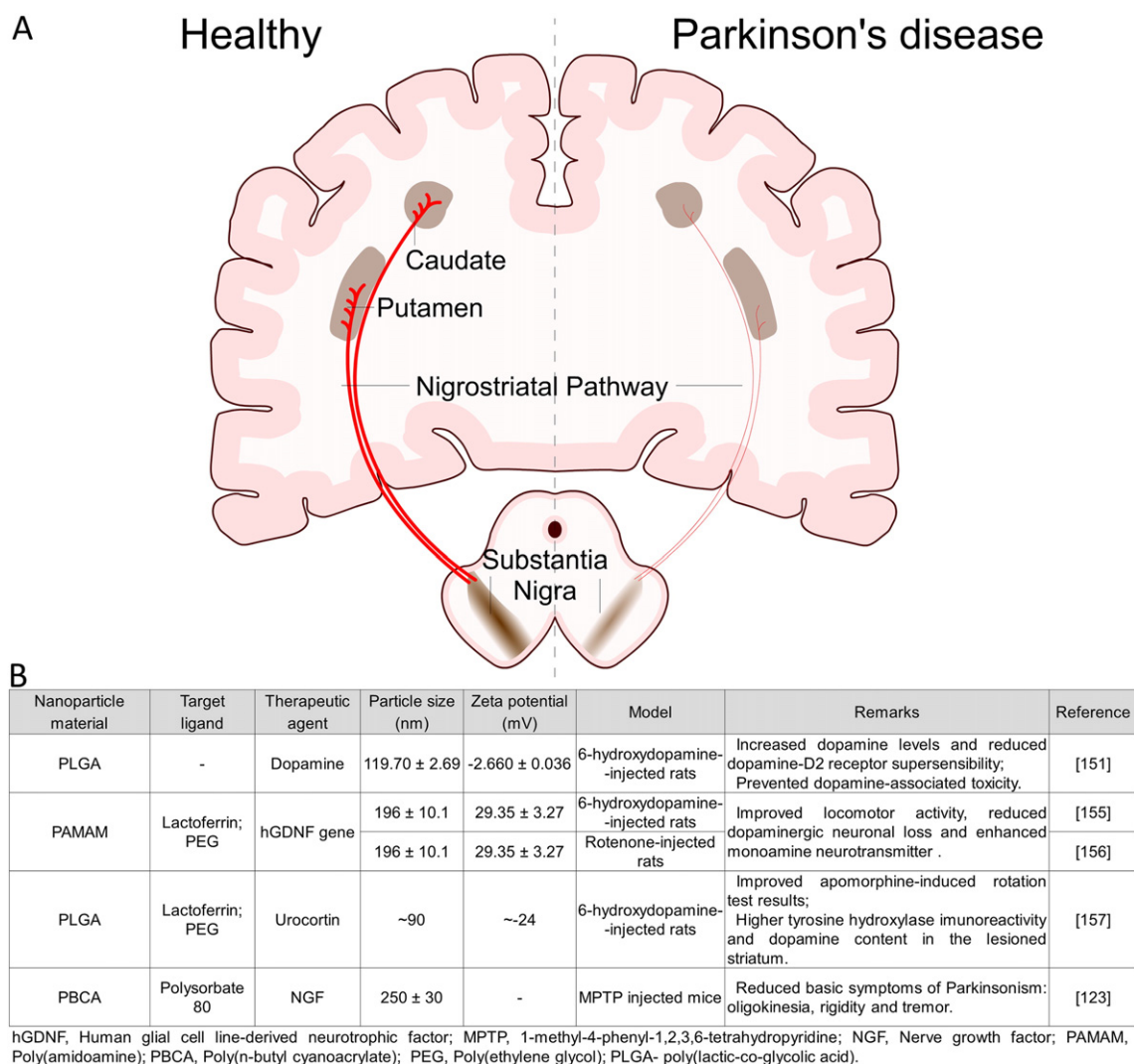
The inflammatory response, particularly through the activation of microglial cells and infiltration and activation of peripheral leukocytes is also responsible for BBB breakdown and cell death upon stroke [89]. Microglia, the brain's first line of defense, become activated and release NO and produce ROS, cytokines (e.g. tumor necrosis factor- $\alpha$  (TNF)- $\alpha$ , interleukin-1beta (IL-1 $\beta$ ) and IL-6) and chemokines (e.g. macrophage inflammatory proteins-1alpha (MIP-1 $\alpha$ )/CCL3, monocyte chemoattractant protein-1 (MCP-1)/CCL-2 and chemokine (C-X-C motif) ligand-1 (CXCL-1)). These inflammatory modulators stimulate endothelial cells and activate the nuclear factor (NF)- $\kappa$ B pathway promoting the expression of adhesion molecules such as vascular cell adhesion protein (VCAM), intercellular adhesion molecule-1 (ICAM-1) and P-selectin [89]. These events culminate in the recruitment

and brain parenchyma invasion of peripheral leukocytes enhancing and perpetuating the inflammatory cascade [90].

Drug delivery in cases of stroke should take into consideration that TJs are compromised and that there is an initial and late opening of the BBB. The leaky BBB and/or the expression of some receptors onto the luminal side of endothelial cells may stand for an opportunity to increase the rates of NPs bypassing the BBB. As so, BBB can be by itself a promising target for improving drug delivery into the ischemic brain.

#### 6.1.2. BBB-permeable NPs for stroke therapy

To overcome stroke-induced neuronal tissue damage, NPs have been used to deliver neuroprotective drugs that in their free form cannot pass the BBB, or do so in very low amounts, and are rapidly cleared by the reticuloendothelial system [91]. For example, chitosan NPs (with a diameter of ca. 650 nm; Zeta potential: +20 mV) conjugated with transferrin receptor antibody and loaded with a specific caspase-3 inhibitor (Z-DEVD-FMK), showed promising results. This formulation was able to cross the BBB (peak levels after 75 min), and decreased infarct volume (between 40 and 45%) and neurological deficits induced by ischemia in a MCAO mice model of stroke. It was also able to reduce caspase-3 activity [36]. Moreover, these NPs were able to further decrease the infarct volume and to improve the motor function deficit scores of MCAO mice when loaded with both Z-DEVD-FMK and basic fibroblast growth factor (bFGF) providing a 3 h therapeutic window [50]. Besides bFGF and Z-



**Fig. 6.** Parkinson's disease (PD) hallmarks and nanoparticle (NP) delivery systems used in pre-clinical animal models of PD. A) The PD brain is characterized by a selective loss of *substantia nigra* dopaminergic neurons culminating in striatal dopamine depletion due to the degeneration of dopaminergic nigrostriatal pathway. B) Summary of the most relevant therapeutic approaches used in pre-clinical studies of PD based in a systemic delivery of NPs.

DEVD-FMK, several other drugs have been proposed to induce neuroprotection and neuroregeneration. Tanshinone IIA, a phenanthrene-quinone derivative, has been proposed as a promising drug against oxidative stress in neurological disorders, namely in the prevention of ischemic injury [92,93], however, like many other molecules, it has a short half-life in circulation, poor solubility and low BBB permeation [94]. To overcome these limitations, cationic bovine serum albumin-conjugated tanshinone IIA PEGylated NPs (with a diameter of ca. 118 nm; Zeta potential:  $-18$  mV) were developed and injected in a MCAO rat model. This approach resulted in ameliorated infarct volume (decrease of approximately 70%), reduced neurological deficit, neutrophil infiltration and neuronal apoptosis after an intravenous injection at the time of reperfusion. Moreover, these NPs induced neuroprotective effects through the modulation of inflammatory processes and neuronal signaling pathways, by down-regulating pro-inflammatory cytokines, like IL-8 and TNF- $\alpha$  and up-regulating anti-inflammatory cytokines, such as IL-10 and transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) in the rat ischemic brain. NP administration also resulted in lower mRNA and protein levels of glial fibrillary acidic protein (GFAP), MMP-9, cyclo-oxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). Reduced mRNA and protein phosphorylation levels of p38 mitogen-activated protein kinase (p38MAPK), c-Jun N-terminal kinase (JNK) and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2)

were also observed [95,96]. Another molecule with a significant potential for neuroprotection is adenosine [97]. However, the inability to cross the BBB together with moderate toxicity and short half-life [98,99] hamper the brain delivery of adenosine per se. To bypass these limitations an injectable NP formulation (with a diameter of 120 nm; Zeta potential:  $-25$  mV) obtained by the conjugation of adenosine with squalene was developed [100]. Injection of this formulation prior to stroke-induction by MCAO decreased the infarct area and enhanced neurological deficit scores. The above strategies are summarized in Fig. 4B.

## 6.2. Alzheimer's disease

### 6.2.1. BBB modifications

AD affects over 5 million people only in the USA and it is the major cause of dementia worldwide [4]. The main characteristic of AD is memory loss. Brain atrophy, accumulation of amyloid-beta peptide (A $\beta$ ) (senile plaques), presence of hyperphosphorylated tau filaments (neurofibrillary tangles) and cerebrovascular changes that culminate in cerebral amyloid angiopathy (CAA) are hallmarks of the pathology (Fig. 5A) [75,101].

Currently, none of the available animal models truly replicate the AD neuropathology spectrum. Nevertheless, transgenic models have been



of great value to better understand the pathology although they rely on mutations from early-onset familial forms of human AD (5% of the AD total cases). Rodents models infused with A $\beta$  sequences are also highly used for understanding the physiopathology of AD and drug development due to their simplicity and affordability. Many other AD animal models have been developed over the years [102].

BBB dysfunction in AD has been investigated in the past decades and remains a controversial subject among the scientific community [103]. Several studies conducted in both AD patients and in AD animal models suggest that cerebrovascular alterations result from the accumulation of A $\beta$  peptide [104–106]. In healthy conditions, P-glycoprotein (P-gp) and low-density lipoprotein receptor-related protein (LRP) are responsible for A $\beta$  clearance while the receptor for advanced glycation end products (RAGE) controls the A $\beta$  influx to the brain [107]. Importantly, higher amounts of A $\beta$  found in AD induce upregulation of RAGE expression, generating a positive feedback loop which further exacerbates brain accumulation of A $\beta$  and also activates several inflammatory and oxidative cascades [108]. A $\beta$ -RAGE interaction also triggers TJ disruption via intracellular Ca<sup>2+</sup>-calcineurin signaling and MMP-2 and -9 secretion [109,110]. Moreover, mice deficient in apolipoprotein isoform 4 (ApoE4), the main genetic risk factor for the development of sporadic AD, present vascular atrophy and higher levels of RAGE [111], which culminate in reduced A $\beta$  clearance and a compromised BBB [112].

A reduced activity of efflux transporters of BBB endothelial cells, located on the apical side, may also account for A $\beta$  accumulation in the brain parenchyma. In particular, downregulation of LRP [113] as well as a decreased activity of P-gp [114] were reported in patients and AD animal models, further strengthening this hypothesis. Microglia and astrocytes are also main players in controlling the levels of A $\beta$  load and boosting A $\beta$  production when activated [115]. Moreover, secretion of TNF- $\alpha$  by activated microglia enhances the adhesion and transendothelial migration of T cells [116]. In parallel with A $\beta$ -induced toxicity, these events activate a cycle of inflammation and continuous tissue damage.

Another line of research suggests the dysfunction of the BBB as the cause of neurodegeneration. In mouse models of AD, BBB impairment was observed before A $\beta$  accumulation [117]. Similarly, AD patients also showed diminished blood flow and glucose uptake by the BBB prior to brain atrophy and neurodegenerative changes [118]. This impaired metabolic demand may be associated with decreased GLUT-1 expression [119,120].

Overall, both hypotheses consider tauopathies as a secondary event. However, it was shown that tau alone is able to initiate BBB breakdown and its downregulation promotes recovery of BBB integrity in a transgenic mouse model [121].

### 6.2.2. BBB-permeable NPs for AD therapy

The search for new drug candidates to fight AD pathology has shown that neuroprotective peptides may be a good investment therapy-wise. They can act in a variety of ways by breaking down A $\beta$  plaque formation, degrading A $\beta$  toxic peptide and modulating some enzymes as secretases. For instance, PEG-PLA NPs (with a diameter of ca. 120 nm; Zeta potential: -23 mV) modified with B6 peptide (a transferrin substitute) and loaded with the neuroprotective peptide NAPVSIPQ (NAP) were able to accumulate in the brain of mice (peak levels between 0.5 and 12 h) compared to NPs without B6. Peripheral accumulation of both formulations was found in the liver, lung and spleen with higher levels of accumulation for NPs without B6. The administration of the B6/NAP PEG-PLA formulation in a mouse model of AD, obtained by bilateral stereotaxic injection of A $\beta$ <sub>1–40</sub>, ameliorated spatial learning deficit and improved cholinergic function, likely due to a decrease of acetylcholinesterase (acetylcholine degrading enzyme) and an increase of choline acetyltransferase (acetylcholine synthesizing enzyme) activity [122]. Another molecule of interest for brain-targeted therapies is the nerve growth factor (NGF). In the basal forebrain this molecule is vital for central cholinergic neuron survival. However, NGF is not able

to cross the BBB rendering a major obstacle for its use in noninvasive AD therapy. NGF adsorbed on PBCA nanoparticles coated with polysorbate 80 (with a diameter of ca. 250 nm) was administered in outbred C57BL/6 mice and reached the brain parenchyma in significantly higher amounts at 45 min after administration. Its therapeutic potential in age-related neurodegenerative disorders was shown by the ability to reverse amnesia in an acute scopolamine-induced amnesia model (a proposed paradigm for AD) and to improve recognition and memory as well as to reduce some PD symptoms (rigidity, oligokinesia and tremor) by approximately 40% in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced model of PD [123].

Coenzyme Q10, the prevalent form of coenzyme Q in humans, is a cofactor involved in mitochondrial oxidative phosphorylation cascade, acting as a powerful antioxidant. There is, therefore, a strong rationale for its therapeutic use in several neurodegenerative diseases, including AD. In this sense, coenzyme Q10-loaded PLGA NPs modified with trimethylated chitosan (with a diameter of ca. 150 nm; Zeta potential: +20 mV) were able to improve cognitive and spatial memory performances in an APP/PS1 transgenic mouse model of AD. A relevant brain uptake of this formulation was observed in the ventricles, choroid plexus and cortex, probably due to adsorptive-mediated transcytosis. No brain uptake was observed when void NPs were used. Moreover, a reduction of senile plaques and oxidative stress levels obtained by assays using malondialdehyde (MDA), glutathione (GSH)-peroxydase and catalase activity was also demonstrated with trimethylated PLGA NPs, proving the potential of this formulation as a new AD therapeutic strategy [124].

NPs can be used not only as vehicles to deliver therapeutic agents but also as imaging agents, or both. These so called theranostic agents confer diagnosis and therapy and normally take advantage of nanosystems that are by themselves imaging agents, such as iron oxide, gold and silica NPs, carbon nanotubes and quantum dots [125]. Polymeric n-butyl-2-cyanoacrylate (BCA) NPs encapsulated with radio-labeled <sup>125</sup>I-clioquinol (CQ, an amyloid affinity drug) and coated with 1% Tween 80 surfactant (with a diameter of ca. 50 nm) revealed to be promising for AD diagnosis. These NPs showed high affinity for A $\beta$  plaques in vitro and in vivo, being also able to label A $\beta$  plaques from frontal cortical sections of AD human post-mortem tissue. <sup>125</sup>I-CQ-PBCA NPs were able to penetrate the brain parenchyma and its retention was significantly higher in two AD mouse models, the APP/PS1 transgenic mice and mice injected with aggregated A $\beta$ 42 peptide, than in healthy controls. <sup>125</sup>I-CQ-PBCA NP brain uptake and retention in AD mouse brain was also higher than that observed with free <sup>125</sup>I-CQ with a peak at 90 min after administration. Therefore, radiolabeled CQ-PBCA NPs can possibly be used as a promising carrier and amyloid imaging agent in vivo using non-invasive methods, single photon emission tomography (SPECT) (<sup>123</sup>I) or PET (<sup>124</sup>I) [126]. A summary of the abovementioned strategies is found in Fig. 5B.

## 6.3. Parkinson's disease

### 6.3.1. BBB modifications

PD is a neurodegenerative disease that affects approximately 10 million people throughout the world [5]. PD is characterized not only by the selective degeneration of dopaminergic neurons in the *substantia nigra* (SN) that culminates in the depletion of dopamine (DA) in the striatum (Fig. 6A), but also by the existence of  $\alpha$ -synuclein and protein inclusions in neurons termed Lewy bodies [127].

PD models are based on the administration of toxins such as 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, paraquat, to name a few, that cause the selective death of dopaminergic neurons. Genetic models are highly used to modeling familial PD, but have also been essential to shed some light on more common PD mechanisms. These animal models and others are intensively discussed in Jamag et al. [128].

It was initially thought that BBB remained intact during PD development [129]. However, studies tracking drugs like benzerazide and [<sup>11</sup>C] verapamil, unable to cross BBB in normal situations, demonstrated that these drugs were increased in the brain of PD patients and rat PD models, likely due to the reduction of P-gp expression levels [130]. The albumin ratios in the brain of PD patients and age-matched controls also showed a correlation between the pathology course and the progressive BBB impairment [131]. Cerebral blood flow deficiencies and vascular alterations associated to BBB integrity loss were also found in striatum and SN of patients with PD [132–134]. A higher amount of blood vessels in the periphery of damaged dopaminergic neurons in the SN of monkeys was correlated with an increase in VEGF expression [135]. VEGF injected into the SN of rats was also able to disrupt the BBB and resulted in a consequent loss of dopaminergic neurons and strong inflammation [136]. Similarly to what was previously stated regarding AD, MMP activation is also essential in PD-associated BBB breakdown. In particular, MMP-3 promotes dopaminergic degeneration, barrier impairment and immune cell infiltration into the brain of MPTP-intoxicated mice [137].

Inflammatory events including microgliosis, astrocytosis and infiltration of T-leukocytes found in the midbrain of PD patients and rat models are also intimately related with dopaminergic neuronal loss and BBB permeability [138–141]. A higher release of pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$  and interferon- $\gamma$ ) and the production of ROS and NO by activated astrocytes and microglia, during PD, is associated with BBB impairment [141–143].

Alpha-synuclein deposition is one of the causes of PD and downregulation of the P-gp may contribute to  $\alpha$ -synuclein brain accumulation [144]. Of note, deposition of  $\alpha$ -synuclein promotes an increase in BBB dysfunction in mice injected with lipopolysaccharide [145]. In addition, increased content of metals like iron and manganese were also found in brain lesioned regions in PD patients and animal models [146]. In fact, lactoferrin receptor levels are increased in SN dopaminergic neurons of both animal models and PD patients and are potentially implicated in neuronal iron uptake contributing to dopaminergic neuronal degeneration. The upregulation of lactoferrin receptors was also found on the blood–brain vasculature and was correlated with BBB changes in PD [147–149]. Therefore, it is of the utmost importance to understand whether hallmarks of PD pathogenesis can be responsible for triggering vascular impairment to streamline the most effective BBB-directed therapy for PD. One example would be taking advantage of lactoferrin receptors not only as a way to improve drug loaded NP uptake into the brain but also as a therapeutic target in order to reduce the disease's progression.

### 6.3.2. BBB-permeable NPs for PD therapy

Dopamine replacement therapies are currently the most used forms of PD treatment, since they are able to improve motor symptoms. Nevertheless, effects on behavior and cognition are still controversial [150]. In line with this, Pahuja and colleagues have developed dopamine-loaded PLGA nanoparticles (DA NPs) (with a diameter of ca. 120 nm; Zeta potential:  $-3$  mV) that were able to improve animal behavior, namely by reducing amphetamine-induced rotation, without showing any signs of heart-related alterations or abrupt changes in the brain or in the periphery. DA NPs were able to cross the BBB mainly in the SN and striatum (PD-altered regions) of 6-hydroxydopamine (6-OHDA) rats and their presence near and inside neurons and astrocytes was also confirmed. Moreover, a slow and controlled release of DA by NPs as well as a reduced plasma clearance and autoxidation were detected 6 h after injection. DA NPs also increased the levels of DA and its metabolites homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) almost to levels similar to control mice while reducing dopamine-D2 receptor hypersensitivity to control levels. In conclusion, this study shows that DA NPs can prevent toxicity associated to bulk dopamine and can provide a novel strategy to fight PD [151].

As discussed previously, increased expression of lactoferrin and its receptors in the SN and striatal dopaminergic neurons and BBB endothelial cells of PD patients [147,148], the most susceptible regions for dopaminergic degeneration, may aid the specific uptake of NPs at lesioned regions. This suggests that the delivery system efficacy under PD conditions might be enhanced by attaching lactoferrin to NPs. Moreover, lactoferrin may protect and exert an antioxidative effect on dopaminergic neurons by chelating the increased levels of iron present in the SN and striatum of PD patients and animal models [152,153], suggesting that lactoferrin may have a dual effect, acting as a ligand to promote receptor-mediated BBB transcytosis in the lesioned dopaminergic regions and by inducing repair of the same regions. It was further demonstrated that lactoferrin has higher uptake efficacy compared to transferrin and OX-26 (an anti-transferrin-receptor antibody) (with a diameter of ca. 158/159 nm; Zeta potential:  $-11/-9$  mV, respectively) [154]. Based on these assumptions, Huang and collaborators developed a PAMAM and PEG NP (with a diameter of ca. 200 nm; Zeta potential:  $+30$  mV) modified by lactoferrin and loaded with a plasmid for the human glial cell line-derived neurotrophic factor gene (hGDNF). GDNF is considered the golden standard neurotrophic factor for PD therapy but it is unable to cross the BBB. This nanoformulation functionalized to deliver GDNF was able to cross the BBB and exert a neuroprotective effect on dopaminergic neurons as well as an improvement of the locomotor activity in both experimental protocols. Yet, these effects were more robust when the formulation was administered more than once. In addition, functional dopaminergic recovery was achieved as shown by the increased levels of monoamine neurotransmitters in both the 6-OHDA and rotenone-induced PD rat models [155,156].

Urocortin, a corticotrophin releasing hormone-related peptide, is also a promising target to protect dopaminergic neurons but it is unable to cross the BBB like many other molecules. PEG-PLGA NPs functionalized with lactoferrin (with a diameter of ca. 90 nm; Zeta potential:  $-24$  mV) for the delivery of urocortin to the brain were found in cortex, SN and striatum regions 1 h after injection while NPs without lactoferrin were barely observed. This formulation was able to protect dopaminergic neurons, improve locomotor functional deficits (evaluated by the apomorphine-induced rotation test) and increase the levels of DA and its metabolites HVA and DOPAC without an excessive toxicity (detected by cluster of differentiation (CD)68 immunoreactivity) [157]. These NP-based delivery strategies for PD are summarized in Fig. 6B.

## 7. Future prospects

The BBB is very important in the maintenance of CNS normal function and its disruption is related with progression of several brain pathologies. Understanding the mechanism(s) underlying its regulation in the healthy and disease brain is essential to a better knowledge of disease pathophysiology. Although it remains unclear what causes BBB dysfunction, either loss of CNS maintenance signals or breakdown signals from the pathological state, we now have a vast knowledge on physical and molecular alterations beyond the BBB breakdown in pathology. Taking advantage of the current knowledge on BBB impairment, which is related to higher expression of specific receptors in endothelial cells from brain capillaries such as RAGE in AD or lactoferrin in PD, it is feasible to design a strategy for more effective drug delivery into the lesioned brain. Nanocarriers are small agents with the capability of enclosing drugs conferring them protection, increasing their circulation time and providing a temporally and spatially controlled release of their cargo into the lesion site after intravenous injection. Some guiding principles to enhance the transport of NP formulations through the BBB have been recently recognized. For example, the use of certain ligands on the NP surface, ligand density and NP shape, among other aspects, have been highly scrutinized in the past decade. In addition, some of the principles in the recent successes of antibody transport across the BBB [158–160] might inspire NP bioengineers to design new formulations with enhanced properties. Additionally, an increasing number of

studies are reporting a restorative effect of NPs on animal models of neurological disorders (e.g. stroke, AD and PD). Further research is necessary to clarify the differences in NP transport in healthy and disease animal models, always bearing in mind the limitations of an experimental model which cannot fully mimic a given human disease. Although it is known that BBB properties are substantially altered in PD, AD or stroke in vivo models, no systematic studies have been performed to elucidate how NP physico-chemical properties affect NP transport and brain localization. To the best of our knowledge there is no NP formulation being currently tested in clinical trials for stroke, AD or PD treatment. However, we may speculate that it is only a matter of time before NPs developed in pre-clinical tests will be evaluated in future clinical assays.

Addressing safety issues is also very important to move this research area to potential clinical therapies. It is important to note that the most effective NP formulations for brain delivery still accumulate significantly in other regions of the body, such as liver, spleen, kidney among other organs/tissues, before being eliminated. Thus, it is important to design nanoformulations that only after reaching the brain are remotely triggered to release the drug [161] instead of doing so in other places of the body. It is expected that future developments in triggerable nanoformulations will facilitate the clinical translation of NPs in the area of regenerative medicine. Another important issue that deserves further investigation is the development of nanoparticles that can target specific brain cells. For instance, in the setting of neurodegenerative disorders the targeting to specific brain cells such as dopaminergic neurons (main target in PD), microglia (neuroinflammation), or neural stem cells (neuronal repair) might enhance its potential therapeutic value.

In sum, the development of new platforms that are able to exploit BBB alterations occurring in these disorders, in combination with promising therapeutic and/or imaging agents, is essential to develop more efficient non-evasive and brain-directed therapies able to reach the clinic.

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