Chapter 6

Multifunctional Nanoparticles for Successful Targeted Drug Delivery across the Blood-Brain Barrier

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Additional information is available at the end of the chapter

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Abstract

The blood-brain barrier (BBB) is the major problem for the treatment of brain diseases because we need to be able to deliver drugs from the vascular system into the central nervous system (CNS). There are no drug therapies for a wide range of CNS diseases and these include neurodegenerative diseases such as Alzheimer's and Parkinson's diseases and cerebral ischemia. Therefore, the focus of this chapter is to discuss how nanoparticles (NPs) can be modified to transport different drug molecules for the treatment of brain diseases. In essence, NPs' surface can be functionalized with molecules such as peptides, antibodies and RNA aptamers and these macromolecules can be attached to the receptors present at the BBB endothelial cell surface, which allows the NPs cross the barrier and subsequently deliver pharmaceuticals to the brain for the therapeutic and/or imaging of neurological disorders. In fact, part of the difficulty in finding an effective treatment for these CNS disorders is that there is not yet an efficient delivery method for drug delivery across the BBB. However, over the last several years, researches have started to understand some of the design rules to efficiently deliver NPs to the brain.

Keywords: blood-brain barrier, multifunctional nanoparticles, Alzheimer's, Parkinson's, cerebral ischemia, stroke

1. Introduction

Technological innovations, referred to as nanomedicine, is an exciting field of applications of nanotechnology to the diagnostic, treatment and/or prevention of traumatic injury or disease of the human body. This field holds the promise to deeply revolutionize the medicine to treatment and therapy areas such as imaging, drug delivery, cell therapy, tissue regeneration and development of new nanomedicine products. Due to its great importance, recent global

marketed report expects that the applications of nanotechnology in medicine could reach $528 billion by 2019 [1]. Indeed, a broad range of nanoparticles (NPs) made of various materials (e.g., polymers, dendrimers, gold, silver, lipids, metals, and virus-like particles), differing in their size, architecture and surface properties, has been initially engineered to improve parameters such as the pharmacokinetics and biodistribution of therapeutic molecules and to reduce drugs' toxicity side effects [2]. Additionally, NPs are also useful tools for body or organ imaging [3]. During the past few decades, NPs have been successfully developed as drug, gene and/or imaging delivery vehicles due to their key properties of enhancing water solubility of poorly water-soluble molecules, extending the plasma circulation time and targeting the site of disease, while avoiding nonspecific toxicity effects [4, 5].

In fact, NPs have provided remarkable progress in therapy and diagnostic imaging of several diseases. Since 1990, a high number of nanocarrier formulations have been approved by regulatory authorities for clinical use [6, 7]. There are five different applications of nanomedicine products on the market within healthcare – *in vitro* diagnostics; biomaterials; drug delivery; *in vivo* imaging and active implants [7]. Of these products, the type of NPs that exists on the market is diverse and it includes the following: (i) liposomes (e.g., Ambisome®, Albelcet®, DaunoXome®, Depocyt® and Myocet®); (ii) polymer-coated liposomes (e.g., Doxil® and Lipo-Dox®); (iii) polymeric drugs (e.g., Copaxone®); (iv) polymer-protein conjugates (e.g., Oncospar®, PEG-Intron® and Pegasys®); (v) nanoparticle containing paclitaxel (e.g., Abraxane™), (vi) antibodies (e.g., Avastin™ and Herceptin®) and (vii) antibody conjugates (e.g., Mylotarg®); (viii) aptamer conjugates (e.g., Macugen®); (viii) micelles (e.g., Estrasorb®); among others. These formulations are considered the first generation of nanomedicine, already bringing clinical benefits to patients [8].

Moreover, researches are constantly focusing on the development of NPs that can accumulate and deliver their cargo specifically at the diseased site, and these efforts are bringing important advances toward the development of NP-based targeted drug delivery systems. To increase the specificity of NPs to the targeted area, nanocarriers that can either passively or actively target the unhealthy site have been engineered. In passive targeting, the capacity of NPs to accumulate in the angiogenic site of tumors by the enhanced permeability and retention effect is explored [6, 9]. This strategy is achieved by recovering surface of NPs with some sort of coating with several compounds such as poly(ethylene glycol) (PEG) and poly(phosphoester) (PEEP) [10]. By binding PEG or PEEP to the surface of NPs, there occurs a change in the protein corona populations that adhere to the surface of NPs, reducing drastically the opsonization process of the nanocarriers thus preventing recognition by macrophages and monocytes and rapid clearance of NPs from the blood [10, 11]. Also, the accumulation and cellular uptake of NPs could be further enhanced by conjugating the NPs with molecules such as antibodies, peptides and aptamers that are able to bind to overexpressed receptor or antigens on the surface of targeted cells [12].

More recently, various researchers have been developing NPs able to perform two or more functions for the simultaneous or sequential delivery of single or multiple therapeutic active principles to the required targeted site in the body, overcoming multiple physiological barriers [13]. Multifunctional NPs often have the ability to: (i) encapsulate sufficient

amount of drug or therapeutic macromolecules for a sufficient time; (ii) increase residence time in the blood through the use of soluble polymers such as polyethylene glycol (PEG); (iii) increase their accumulation at the desired site in the body by attaching to NPs, surface macromolecules such as antibodies, RNA aptamers and peptides; (iii) respond to several intrinsic or extrinsic stimuli for "on demand" delivery such as abnormal pH, temperature or magnetic and ultrasound fields and (iv) entrap concomitantly an imaging agent to enable the real-time monitoring of their biodistribution, targeted accumulation and/or therapy efficiency [2, 13].

Despite the exciting advances in the discipline of nanotechnology-based approaches, different challenges arise in their efficacy toward the treatment of neurodegenerative diseases. One of the major obstacles that limit the application of NPs for effective delivery of drugs and diagnostic imaging agents to the central nervous system (CNS) is the presence of the blood-brain barrier (BBB) [14]. As a result, new and innovative invasive and noninvasive NPs formulations have been engineered to provide efficacy in crossing the BBB, mainly by the functionalization of NPs' surface with ligands. Invasive strategies show potential and are being explored for efficient NPs' access to the brain. Some examples of invasive strategies are: convention-enhanced delivery, intracerebral or nasal injection and use of implants. With this in mind, it is important to understand the general concept of BBB, mechanisms of transport in and out of the brain and the BBB alterations in pathology.

2. BBB, general concept and the transport of drugs inside the brain

The BBB is a formidable physiological structure that acts as an effective security system for the brain, letting in circulating compounds that this organ needs, but at the same time, these cells have evolved a system of biological pumps and if these pumps recognize molecules that should not be on their way to the brain, they will be pumped right back out into the vascular system [15]. The BBB is primarily composed of brain endothelial cells, which are cells that line microvessels and capillaries in the brain, and these are highly specialized cells that are knitted together very tightly by tight junctions, so there are no gaps between the cells (**Figure 1**). In fact, endothelial cells' tight junctions control the flux of hydrophilic molecules and small lipophilic substances such as water and some gases, respectively, that go through the BBB [15, 16]. Also, the brain endothelial cells are surrounded by a structure known as basal lamina, composed of fibronectin, type IV collagen, heparin sulfate and laminin [16, 17].

Other structures such as neurons, astrocytes, pericytes and extracellular matrix components constitute the neurovascular unit that is also part of the BBB structure (**Figure 1**). Neurons are electrically excitable cells responsible for processing and transmitting information throughout the mind and body. In the nervous system, chemical and electrical signals between neurons occur via synapses, or junctions, that connect these cells [18]. Astrocytes play a key role in providing nutrients to neurons by shuttling them from the blood vessels to neurons themselves; they also help to control the ion concentration in the brain; are part of the repair process that happens after brain injury and help neurons recycle their neurotransmitters [19].

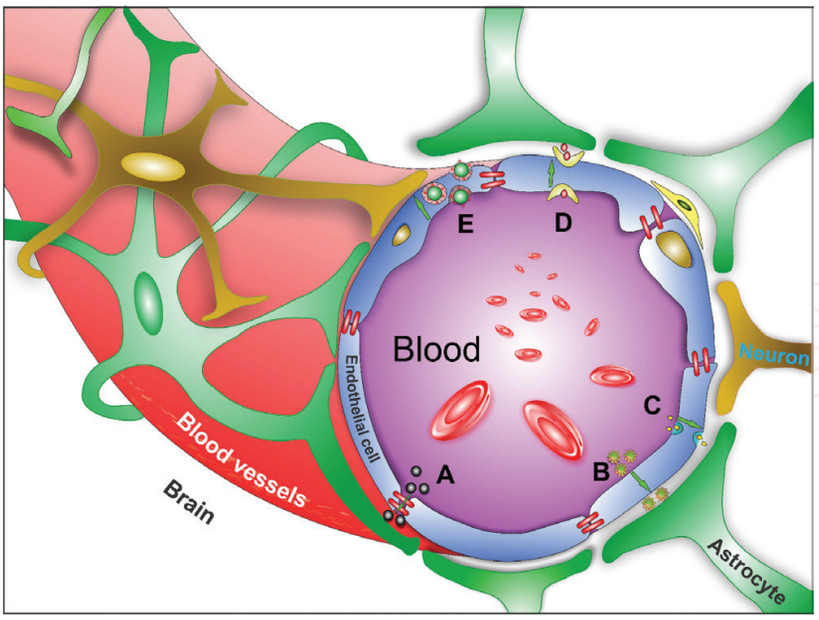

Figure 1. The Blood-Brain Barrier. Schematic cross-sectional representation of the blood brain barrier (BBB) and other components of vascular unit like neurons, astrocytes, pericytes that are essential for the health function of the CNS. Also, depicted in the picture are the BBB mechanism of passage: (A) Water soluble agents; (B) Lipid solid agents; (C) Protein transport; (D) Receptor-mediated transport, and (E) Adsorptive transcytosis.

Pericytes appear to play a key role in BBB endothelial cells barrier formation [20]. Finally, the extracellular matrix occupies 10–20% of brain volume and has a major role in its normal pathology [21]. Therefore, it is the vascular unit that controls permeability and cerebral blood flow throughout the CNS, ensuring physiological CNS functioning.

In **Figure 1**, a schematic overview of the mechanisms of transport through the BBB is depicted. Molecules that present either a high degree of lipophilicity and molecular weight smaller than 500 Da can penetrate the CNS by simple diffusion (**Figure 1A**). However, in the absence of these characteristics, other circulating molecules can cross the BBB by their interaction with specific transport proteins located at the brain endothelial surface. These proteins are classified into two main categories: (i) carrier-mediated transport and (ii) receptor-mediated transport. Carrier-mediated transport (CTM) systems are responsible for the transport of small-drug molecules or small nutrient molecules including monosaccharaides and amino acids with a molecular mass smaller than 600 Da. These molecules can cross the BBB endothelial cells via active transport mediated by specific proteins (**Figure 2C**). The diffusion of molecules from the blood to the brain may be passive or active. For example, the transport of neutral L-amino acids such as leucine, phenylalanine and tyrosine is mediated by the large neutral amino acid transport (LAT1), whereas cationic amino acid transporter (CAT1) mediates the transport of cationic amino acids such as lysine and histidine. Other examples of transporters of polar substances into the brain include the nucleoside transporter (CNT2), the glucose transporter (GLUT1) and the monocarboxylic acid transporter (MCT1) for nucleoside, glucose and carboxylic acids transport, respectively. Moreover, transporters presented at brain endothelial cells' surface are also able to expel endogenous peptides such as Tyr-Pro-Trp-Gly or a multiplicity of drugs from the CNS to the blood are to be mediated, respectively, by peptide

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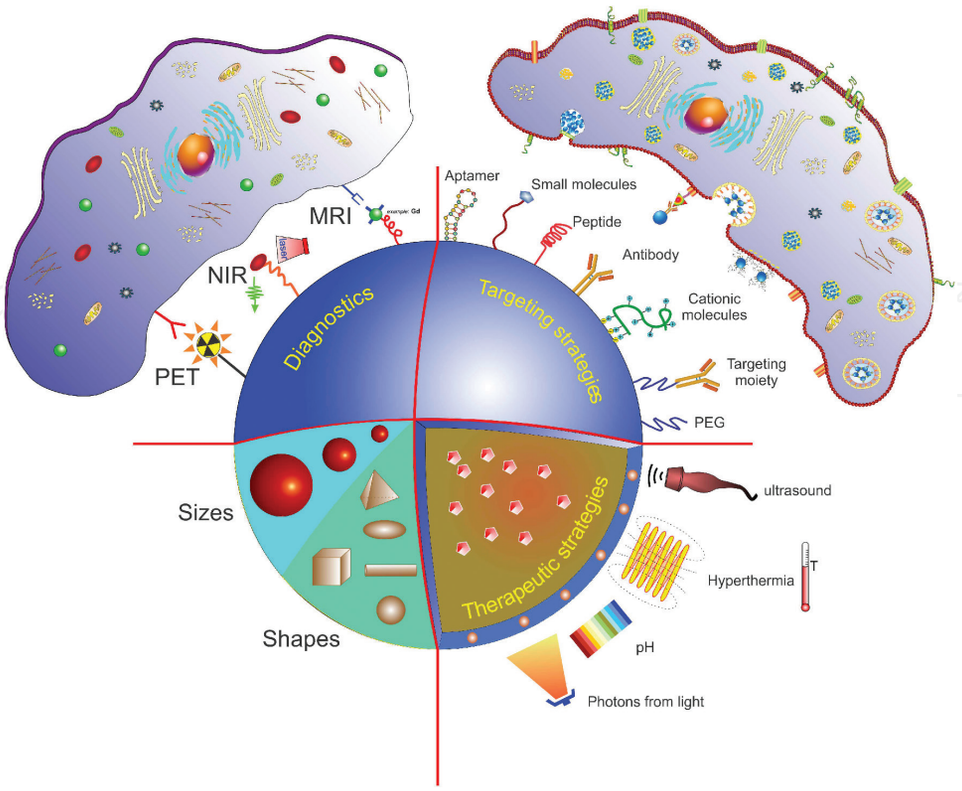

Figure 2. Schematic representation of a drug-loaded, multifunctional, stimuli-responsive NP. The structure of a nanocarrier allows the incorporation of one or multiple therapeutic molecules. These NPs can be found in different sizes and shapes. Increased blood circulation time can be achieved with soluble polymers such as polyethylene glycol (PEG). Nonspecifically target the intended site of action can be achieved by exploring, for example, leaky vessels of tumors. NPs can be actively targeted via the attachment of targeted-specific ligands such as antibody, antibody fragments, aptamers and peptides at their surface. Depending on the kind of application of NPs, various compounds can be added to turn the nanocarrier into a responsive device to a specific stimuli such as temperature, pH or magnetic and ultrasound fields. Imaging or contrast agents such as magnetic resonance imaging (MRI) , near infrared (NIR) and/ or polyethylene terephthalate (PET) compounds can also be incorporated into a single platform to enable imaging and releasing of drugs from NPs.

transport system-1 or P-glycoprotein, via active efflux transport (AET). In fact, if a drug is a substrate of any AET protein, multidrug resistance occurs, and this phenomenon is a great obstacle for therapeutic drug delivery to the CNS.

Chemotherapy agents, natural, synthesized or recombinant peptides and proteins, nucleic acids, monoclonal antibodies and other pharmaceutical breakthroughs do not readily cross the BBB (**Figure 2D**). Nonetheless, there are some specific proteins that the brain needs to function correctly, so they can access the brain by attaching to receptors, which are transported across the barrier and subsequently release into the brain. This mechanism of transport is known as receptor-mediated transport (RMT) and the internalization of these relatively large compounds is done via endocytosis (**Figure 2E**). It is the most studied transport mechanism for drug delivery, since receptor-specific ligands such as peptides and antibodies against receptors that are expressed on brain endothelial cells surface can be attached to the surface of nanoparticles or drugs, enabling their accumulation and internalization by cells of vascular side and, consequently, being transported into the brain. In addition, adsorptive-mediated

transport (AMT) is a kind of transport where endocytosis is induced by the binding of cationic substances to the negatively charged plasma membrane of brain endothelial cells interaction. Therefore, due to the electrostatic interaction between the negatively charged membranes, the cationic therapeutic compound takes the AMT to enter the CNS.

This becomes a problem when treating diseases of the brain because we need to be able to deliver drugs from the vascular system into the CNS [22]. Unfortunately, at the moment, there are no drug therapies for a wide range of CNS diseases, and these include neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) and cerebral ischemia (or stroke). Therefore, the objective of this chapter is to discuss how NPs can be modified to transport thousands of different drug molecules for the treatment of brain diseases. In essence, NPs for drug delivery into the brain is a method for transporting drug molecules across the BBB using nanocarriers. NPs, surface can be functionalized with molecules such as peptides, antibodies and RNA aptamers, and these macromolecules can be attached to the receptors present at the BBB endothelial cell surface, which allow the NPs across the barrier and subsequently deliver pharmaceuticals to the brain for the therapeutic and/or imaging of neurological disorders [14]. In fact, part of the difficulty in finding an effective treatment for these CNS disorders is that there is not yet an efficient delivery method for drug delivery across the BBB. However, over the last several years, researches have started to understand some of the design rules for efficient delivery of NPs to the brain.

3. *In vitro* approaches to study NPs' transport through the BBB

As mentioned earlier, the BBB is a selective and dynamic barrier restricting the passage of a huge variety of compounds across this barrier, which is essential for the maintenance of homeostasis and functionality of the CNS. Therefore, the BBB is considered the major obstacle for the use of NPs as delivery systems to brain diseases. As shown in **Figure 2**, endothelial cells of the cerebral microvasculature are associated with perivascular cells form the BBB. The functional interaction between endothelial and perivascular cells and their response to injury have led to the concept of the neurovascular unit [15, 17]. Studying the mechanisms of uptake, transport and cytotoxicity of NPs through the BBB is an extremely challenging task *in vivo* because of the technical limitation to access the interface between the vascular system and the brain, since it is estimated that the brain capillary length is about 650 km [23]. To overcome this problem, *in vitro* BBB models have been built to reproduce as precise as possible the major BBB features, allowing investigation of cellular and molecular mechanisms that occur in the barrier; prediction of the transport of compounds across the BBB and performing high-throughput platform to test NPs transport through the barrier for the effective treatment of brain diseases. For example, it was observed that NPs can reach the capillaries into the brain of rats or mice 30 m after intravenous injection and, up to 5 h after NPs administration, they could go through the barrier, decreasing afterward [24–29]. These studies of NPs across the brain tissue are in line with *in vitro* BBB models data reported [30, 31]. In addition, it was observed that *in vitro* BBB model facilitates the manipulation of some parameters that affects

the barrier such as aglycemia, hypoxia, among others [32]. For decades, two-dimensional or three-dimensional *in vitro* models of BBB have been developed by cultivating either as a monolayer or in cocultivation with mouse brain microvascular endothelial cells and murine or human endothelial cells with pericytes or astrocytes or glial tissue among others in a way that mimics the barrier under physiological or pathological conditions such as Alzheimer's and Parkinson's diseases or stroke [33–37]. Models of BBB based on stem cells are also reported in the literature [38, 39]. Moreover, by taking permeability measurements on the cultured cells, it is possible to test the physiological relevance of the developed model. In addition, experiments such as gene expression analysis using real-time polymerase chain reaction (PCR), permeability analysis [40, 41] and immunocytochemistry can also be used to validate the BBB model obtained. Although we are still not able to make this platform available both in academic and industry setting, this kind of technology has been showing the importance of considering *in vitro* data together with *in vivo* studies to understand the transport process of NPs into the brain.

4. Other barriers that limit effective drug delivery into the brain

In addition, the BBB is not the only physiological barrier for drug delivery to the brain. If we consider the anatomic aspects of our body, the brain and the spinal cord are completely cushioned and protected by the cerebrospinal fluid (CSF) [42, 43]. This fluid is also responsible for carrying nutrients to and waste products away from the brain. The great majority of CSF is produced within ventricular areas of the brain, as a result of the specialized tissue known as choroid plexus. The choroid plexus is located in each of the four ventricles within the brain area: two lateral ventricles, and the third and fourth ventricles. Here, it is important to clarify that the cells of the choroid plexus do not produce the CSF; instead, this fluid is a filtrate of the blood that is performed by the highly specialized cells of choroid plexus known as cuboidal epithelial cells. Cuboidal epithelial cells are exactly located between the capillary and the ventricle. As all capillaries present within the brain, the capillaries of the choroid plexus have a wall formed by single cells responsible for ready transportation of ions and molecules to and from the choroid plexus capillary. Tight-gap junctions hold the choroid plexus epithelial cells together. These gap junctions prevent substances from entering or leaving the CSF; thus, the choroid plexus acts as a blood-CSF barrier. Lastly, although the CSF originates in the ventricles, this fluid flows through to the ventricles and then surrounds the brain and the spinal cord.

5. NPs for brain drug delivery

Over the last several years, researches have engineered a variety of NPs that can potentially deliver therapies and/or imaging agents directly into the brain [14, 42, 44–48]. It is really challenging to get these nanoparticles across the BBB to treat a CNS disease in sufficient amount and without causing major side effects on healthy brain cells. NPs are available in many sizes

and shapes and they can have a positive, negative or neutral surface charge (**Figures 2**, **3**). Their core can be made of a variety of materials such as biological, synthetic or energy receptive. NPs can also be coated with specialized molecules that allow them to interact with their environment. NPs can also be loaded with therapeutic molecules that are released in a controlled way and, at the same time, retain the drug stability and prevent them from degradation once in the blood. Therefore, for an efficient drug delivery into the CNS, it is very important to engineer NPs with the following properties: (i) small size (NP diameter should be smaller than 100 nm); (ii) biocompatible, biodegradable, nontoxic and noninflammatory; (iii) prolonged circulation time in the body; (iv) stable in the plasma; (v) protect the cargo such as small molecules, peptides [43, 49–51], proteins or nucleic acids from degradation; (vi) targetability to the BBB and (vii) controlled drug release [44].

One of the most important and challenging characteristics in engineering NPs is their functionalization. Active targeting of NPs can be achieved by attaching onto their surface, in a highly controlled way, specific molecules such as monoclonal antibody, RNA aptamers, transferrin, lactoferrin and peptides (**Figure 2**). An example of such active NP is the extensive

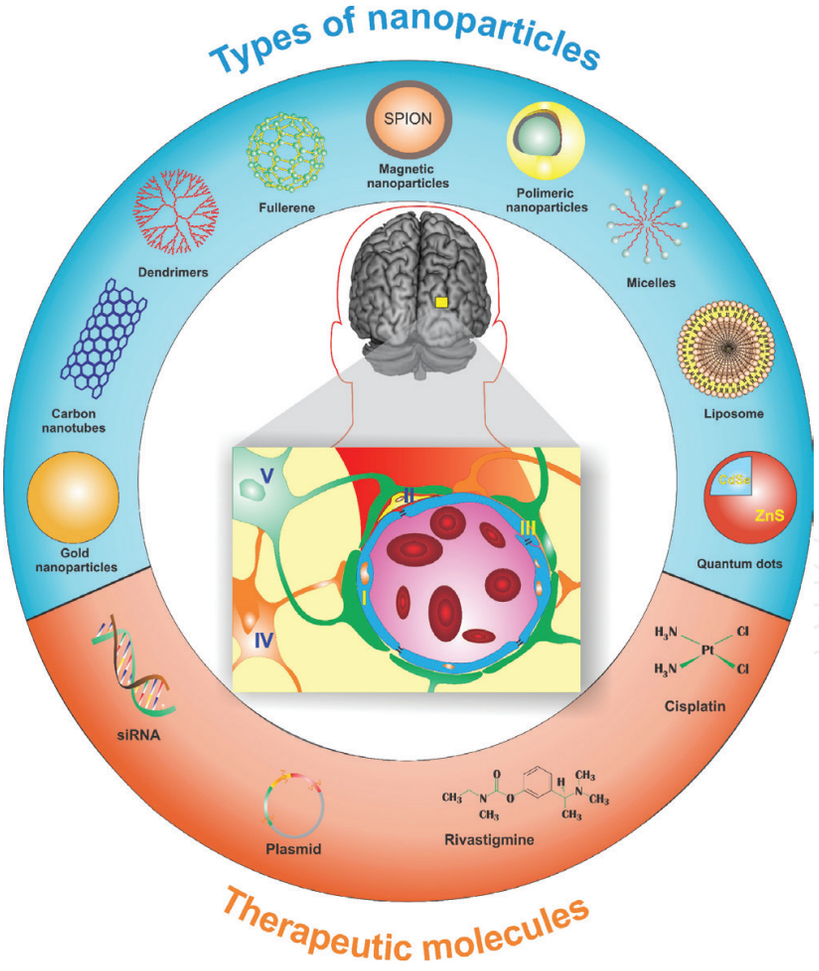

Figure 3. Types of nanoparticles for brain delivery. Enhancing brain drug delivery with the use of several nanocarriers, able to carry the most diverse kind of molecules.

use of cell penetrating peptides such as SynB vectors, Tat and penetratin that were successfully used to target the BBB [52–55]. In fact, a huge variety of molecules that increased targeting strategies to the BBB such as growth factors (e.g., vascular endothelial growth factor; epidermal growth factor) [56], albumin [57], insulin [58], lactoferrin [59], transferrin [60], angiopep-2 [61] and biotin-binding proteins has been reported [62]. Targetability is usually associated with nanoparticles with prolonged circulation time. This characteristic is achieved by coating the surface of NPs with hydrophilic polymers such a polyethylene glycol (PEG), poly(acryloylmorpholine), poly-N-vinylpyrrolidones, polyvinyl alcohol and poly[N-(2-hydroxypropyl) methacrylamide] [63] (**Figures 2**, **3**). Among these polymers, PEG is still the most useful polymer in obtaining long circulating NP. The attachment of polymers onto the surface of NPs works by preventing NPs interaction with opsonins present at the plasma and, in this way, impeding their capture and subsequent clearance from the body. However, it was observed that the blood clearance phenomenon is accelerated after repetitive administration of clinically used PEGylated NPs due to the induction of production of antibodies (the NPs used in these studies were PEGylated liposomes) [64, 65]. Moreover, PEGylated NPs are particularly useful for neurological disease treatment, since the long-circulating NPs into the brain by diverse mechanisms were observed. Nevertheless, for brain tumors, reliance on the enhanced permeability and retention (EPR) effect for drug delivery strategies faces several challenges, since the accumulation of NPs at the tumor site is very low [66].

Due to their ability to carry hydrophilic, hydrophobic and/or lipophilic compounds and high specificity, the use of NPs provides a very efficient platform for drug delivery into the CNS. The most popular nanocarrier studied for brain drug delivery is liposomes and several liposomal formulations are clinically available or tested at different clinical trial phases [14]. Liposomes are spherical concentric vesicles, consisting of at least one lipid bilayer, enclosing an aqueous compartment. This nanocarrier has been employed for therapeutically active compounds delivery soon after its discovery by Bangham in the early 1960s. This NP has been successfully engineered for a variety of brain neurodegenerative disorders and brain tumors. For a detailed overview of liposome-based strategies to drug delivery across the BBB, we refer the reader to Vieira and Gamarra's article [14].

One of the breakthroughs of nanoparticles formulations is to target the nanocarriers to deliver their cargo into the brain. The brain endothelial cells contain several targets as discussed earlier that are explored on the studies of nanoparticles for brain delivery. Each of these targets could be specific for a brain disease or brain diseases. For example, transferrin has been described as the BBB-targeting ligand in studies of nanoparticle formulations [67, 68]. Transferrin is a glycoprotein (80 kDa) that binds to the transferrin receptor and is taken across the BBB via Receptor mediated endocytosis (RME). Indeed, these studies demonstrated that transferrin conjugated to liposomes exhibited a significant increase in the concentration of therapeutic molecules delivered by NPs into the brain when compared to the administration of the drug alone. In addition, broad ranges of nanocarriers with different shapes, sizes and surface properties have been developed for the transport of therapeutic or imaging molecules across the BBB. These also include carbon nanotubes [69, 70], micelles [71], dendrimers [72, 73], nanofibers [74, 75], polymer [46, 76], gold [77] and iron oxide nanoparticles [78] NPs (**Figure 3**).

Although nanotechnology-based strategies to get into the brain have shown progress in animal models, the translation of passive- and active-targeting delivery strategies into clinical studies is still questionable. This might be due to the random nature of receptor-ligand interactions and/or ineffective release of drug from the nanocarrier at the targeted site [79]. Therefore, the development of multifunctional nanoparticles is becoming possible due to the engineering of stimuli-responsive systems that are able to control the release of their cargo and drug distribution in response to specific stimuli such as magnetic field, light, changes in pH, variations in temperature, among others (**Figure 2**).

6. NPs in context of brain neurological diseases

Getting NPs into CNS is not an easy task. As discussed earlier, the BBB is the main structure responsible for brain protection and homeostasis. In addition, it is important to mention that in neurological diseases, several impairments of this structure occur, leading to the perpetuation of the inflammatory cycle that damages neuronal cells and neurodegeneration [80] Moreover, the BBB breakdown can occur, which is clearly a consequence of an ischemic stroke that occurred [81] due to an obstruction within a blood vessel that supplies the brain with oxygen and several nutrients, leading to brain cell death.

In other cases, especially in chronic neurological diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD), it remains unclear how these diseases promote the BBB impairment [32]. Importantly, these modifications in the BBB structure should be taken into consideration when you are planning the engineering of effective multifunctional NPs for brain delivery. In this context, NPs have been designed to cross the BBB and this new technology has some applications so far in the treatment of Alzheimer's and Parkinson's diseases, stroke and brain tumors, which are discussed in the following section.

6.1. Stroke

Stroke is a serious disease that occurs when some or all of the blood supply to a part of the brain is restricted or cut off and, therefore, this can lead to disability, brain injury or death. Thus, the loss of oxygen and nutrients provided by the blood causing the loss of brain function is a stroke also known as cerebral ischemia. There are two ways to disturb the blood supply to the brain (**Figure 4**). The most common type occurs when there is a stoppage of blood flow to a part of the brain due to a blood clot. This cause of strokes accounts for 85% of all cases and it is known as ischemic stroke. The second cause of stroke, that is not as common as the ischemic stroke, but still very serious, happens when one of the blood vessels that is a part of the cerebral circulation supplying the brain ruptures. This kind of stroke is called a hemorrhagic stroke. In addition, "hemorrhagic" refers to a sudden torrential bleeding outburst. However, regardless of whether it is an ischemic stroke or a hemorrhagic stroke, the brain cells start to malfunction after some minutes due to the lack of oxygen and nutrients owing to improper blood flow or improper blood supply.

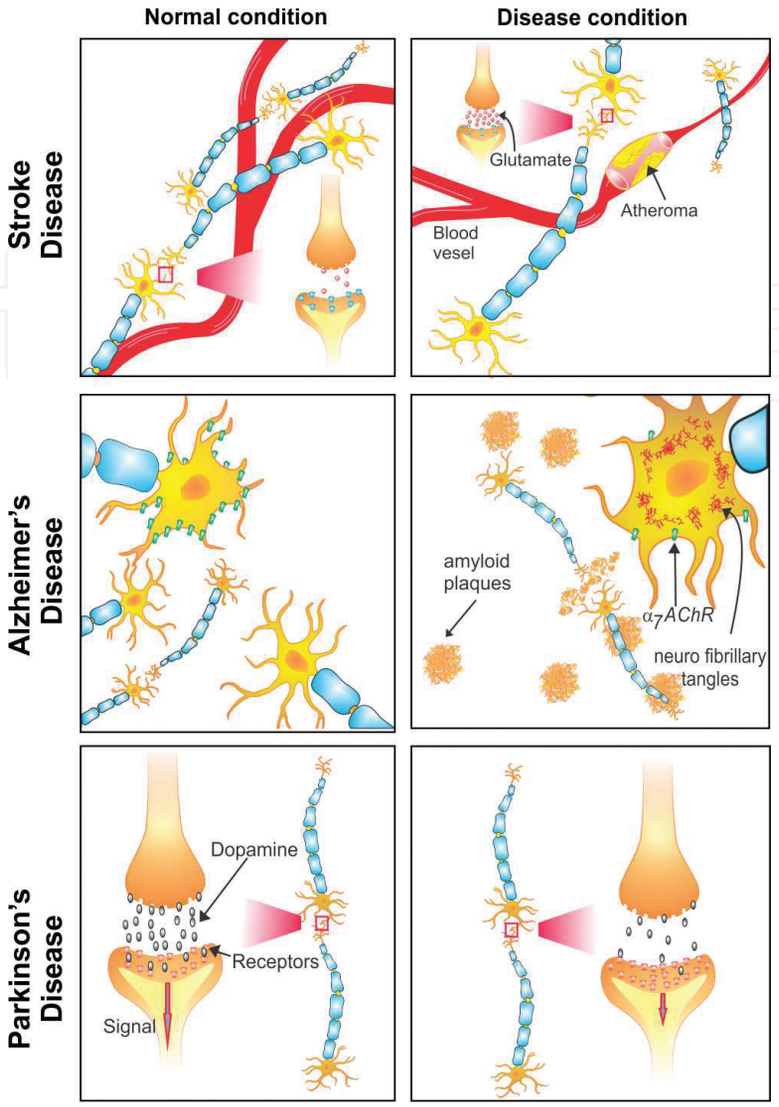

Figure 4. Schematic representation of the main event causing ischemic stroke, Alzheimer's and Parkinson's disease. (Adapted from Alvarim et al. [82]) ischemic stroke is caused by the interruption of the blood flow, depriving the brain of oxygen and some nutrients. For Alzheimer's disease, the main characteristic of this disease is the presence of neurofibrillary tangles and amyloid plaques in the brain, leading to shrinkage of some structures of the brain such as the hippocampus and the enlargement of the brain ventricles, resulting in neurodegeneration. In Parkinson's disease, substantia nigra dopamine neurons are lost.

There is also a related condition called the transient ischemic attack (TIA), also known as a mini-stroke. It is essentially a temporary interruption of blood flow to a part of the brain often lasting between 30 m and several hours. Therefore, the symptoms of TIA and stroke are similar. However, the difference is that a TIA does not actually destroy brain cells and it does not cause permanent disability. Nevertheless, it is often a warning signal that an individual is at risk of having a stroke in the near future. One of the key differences is that a TIA will resolve, whereas, if an individual has a stroke, he/she may not gain normal functioning again for weeks or months, or maybe even for the rest of his/her life.

Nevertheless, the cellular and molecular mechanisms of a stroke episode have been very well known due to the development of several experimental animal models of ischemic stroke [83]. From the studies with these animal models, it was observed that during ischemic stroke, first occurs the opening of the BBB for a short time period. Then, occurs a refractory interval, followed by a reopening of the BBB, but this time for a long period [84] The reopening of the BBB is the step responsible for the activation of the endothelium, leukocyte recruitment, reactive oxygen species (ROS) and cytokine productions and edema formation [85], leading to an inflammatory response and the BBB breakdown and cell death upon stroke [86]. Moreover, dysregulation of tight-junction proteins is also observed during ischemic stroke, due to their degradation by matrix metalloproteinases, which are involved in the process of BBB extracellular degradation, leading to an increase in the permeability of the brain structure [32].

Therefore, besides the BBB itself being an excellent target for itself for treating ischemic stroke, the design of effective drug delivery systems has also to take into consideration the cellular and molecular mechanisms described earlier. One of the strategies described in the literature to overcome neuronal tissue damage after a stroke event is the use of multifunctional NPs to deliver neuroprotective drugs into the brain, since the majority of neuroprotective drugs do not cross the BBB in their free form. For example, the inactive caspase-3 activation in the brain cells likely decreases the probability of brain cell damage after a stroke event. Although it was shown that some peptide inhibitors of caspase are effective compounds in promoting neuroprotection, they cannot readily cross the BBB. For this purpose, a positively charged NP of chitosan conjugated with transferrin receptor was designed to deliver the relatively specific caspase-3 inhibitor N-benzyloxycarbonyl-Asp(OMe)-Glu(OMe)-Val-Asp(OMe)-fluoromethyl ketone (Z-DEVD-FMK) across the BBB [36]. By clearly reducing the caspase 3-activity *in vivo*, this formulation was also readily transported across the BBB (less than 1 h) and it decreased neurological deficits and the infarcted area, proving to be a very promising formulation [27].

In the same way, several other compounds have been described to provide neuroprotection and to prevent neurodegeneration. One of these compounds is the Tanshinone IIA that has demonstrated neuroprotective effects against ischemic injury [87]. However, its use in the treatment of this disease is limited due to the compound's low solubility in aqueous medium, short-half circulation in the plasma and inability to cross the BBB. To overcome these limitations, Tanshinone IIA was successfully conjugated to PEGylated-cationic bovine serum albumin. These nanoparticles were able to cross the BBB *in vivo*, as a significant decreasing in the infarcted volume was observed. In addition, a reduction in the neutrophil infiltration and neuronal apoptosis was observed [88]. The authors also explored the molecular mechanisms by which this formulation conferred neuroprotection. It seems that the mechanism of action of this formulation in the brain is related to the down-regulation of pro-inflammatory cytokines (mainly IL-8 and TNF-α), to the up-regulation of anti-inflammatory cytokines (mainly transforming growth factor-β1 and IL-10) and to the reduction or inhibition of mRNA and proteins (mainly GFAP, MMP-9, COX-2, p38MAPK, ERK1/2 and JNK) [88].

As a last example, adenosine is also a powerful molecule that has demonstrated neuroprotection to the brain after an ischemic stroke event. This molecule also presents as limitations short-life time in the plasma and inability to cross the BBB. Here, adenosine was conjugated to

the lipid-squalene and, then, yielded negatively charged NPs, showing promising results [89]. *In vivo* experiments showed that this formulation was able to extend adenosine circulation time in the plasma, interact with the neurovascular unit, enhance animal neurological deficit scores and decrease the size of the infarcted area [89].

6.2. Alzheimer's disease

It has been estimated that AD, only in the USA, affects over 5.5 million people. Moreover, Alzheimer's AD is the most common cause of dementia [90]. Dementia is a serious brain disease that has as major symptoms deterioration in memory, behavior and thinking. In 2015, dementia affected 47.5 million people worldwide. Most were over the age of 60. The United States data related to AD account that this disease was the sixth highest cause of death in 2005. It was also observed that 1 in 3 seniors who died had AD or other kinds of dementia. It is also expected that the number of people with Alzheimer's will grow as the population of those over the age of 65 rises. In 2015, Alzheimer's disease and other dementia cost the nation $226 billion and by 2050 this may rise to $1.1 trillion [90].

AD was named after the German physician Dr. Alois Alzheimer who presented a case history before an important medical meeting. In 1901, he was closely following a 51-year-old woman patient with a mental disorder, the manifestations of which were language problems and memory loss. After her death, Dr. Alzheimer took a serious examination of her brain and found the presence of plaques and tangles that today characterize AD [91]. This disease accounts for about 60–80% of the dementia cases. In most cases, Alzheimer's clinical manifestations first appear after the age of 65. However, Alzheimer's disease is not considered normal aging although the greatest risk factor of developing the disease is increased age. This is actually the greatest known risk factor for developing AD. However, as mentioned earlier, Alzheimer's is not a normal part of aging. It was observed that a greater proportion of patients over 85 years have AD compared to those over 65 years as AD is more likely to affect older individuals. Dominant genes that are transmitted through generations cause less than 5% of Alzheimer's. However, family risk is the second biggest factor for the development of AD after a certain age. In these families, individuals usually present symptoms of Alzheimer's before the age of 65 and these symptoms sometimes appear in their 30s. This form of AD that is hereditary and marked by Alzheimer's symptoms at an early age is called early-onset familial Alzheimer's disease (EOFAD). To date, mutations in presenilin (PS1 on chromosome 14 and PS2 on chromosome 1) and the amyloid precursor protein gene (APP) on chromosome 21 have been associated with EOFAD. All these three gene mutations (PS1/PS2/APP) affect the pathway in amyloid precursor protein synthesis, which leads to the increase of production of Aβ, creating plaques in the brain [92]. Additionally, there are certain genes such as apoE gene on chromosome 19 that increase the susceptibility to AD. There are three forms of the apoE gene: APOE2, APOE3 and APOE4, the last one being the one associated with a high risk for developing AD. Actually, an individual with two copies of this gene is at three to eight more risk than people with one copy of this gene.

The human brain contains about 100 billion neurons that communicate to one another via synapses, when a burst of chemicals called neurotransmitters are released [93]. The neurotransmitters are synthesized into the synaptic gap. Then, neurotransmitters move across

these synaptic gaps between neurons and bind to receptor sites on the dendrites of the next neuron. Unfortunately, neurons are the type of cells affected by AD. To date, scientists still do not know exactly the causes of AD and how this process begins. However, according to recent studies, it appears to be likely that astrocytes' activation contributes to the neuroinflammatory component responsible for the damage of neurons decades before the issue becomes obvious [94].

Abnormal structures called β-amyloid plaques and neurofibrillary tangles are classical biological hallmarks of the disease [91]. The formation of extracellular β-amyloid plaques occurs when amyloid precursor protein in the neuron cell membrane is cleaved at different positions, releasing small fragments called amyloid β (Aβ) that are highly toxic to the neurons and also interfere with the function of the brain cells [95]. Neurofibrillary tangles, on the other hand, are aggregates of hyperphosphorylate of a microtubule-associated protein known as tau. Tau protein, which in normal cells is responsible for helping nerve cells transport nutrients and maintain their proper shape, is altered in AD and, as a consequence, the transport of nutrients and other essential supplies into the neuron is affected, causing its death. At the same time, the health neurons start working less effectively. After some time, these neurons start losing their capacity to function and communicate to one another and, eventually, they die. Then, the harm may spread to structures in the brain such as hippocampus and entorhinal cortex, which are crucial areas of the brain responsible for forming new memories, thus causing memory loss. As neurons continue to die, affected areas of the brain begin to shrinks and brain functions are lost (**Figure 4**).

The BBB impairment in AD has been controversial [96]. However, several studies carried out in AD patients or AD animal models have been suggesting that the cause of cerebrovascular alterations in the BBB of the diseased brains is the accumulation of Aβ peptide [97–99]. Nevertheless, there are also studies suggesting that the BBB impairment is the cause of neurodegeneration, since the dysfunction of the brain structure in AD animal models was observed before Aβ aggregates were accumulated [100]. In any case, both hypotheses for the dysfunction of the BBB consider as a secondary event the tauopathies. However, a study reported that the tau filaments alone are able to start the disruption of the BBB and when it was deregulated, the BBB integrity was recovered [101].

Currently, there are no drug treatments that can cure AD. For this reason, approaches for treating AD are focused more on therapeutic interventions that alleviate symptoms, slow down or delay the progression of the disease, improving the patient's quality of life. To date, there are two types of medications for Alzheimer's treatment: acetylcholinesterase inhibitors (Aricept, Reminyl, Exelon and Cognex) and N-Methyl-D-aspartate (NMDA) receptor antagonist (Namenda). Nevertheless, the administration of these therapeutic molecules is associated with severe side effects. Thus, it would be desirable to develop drugs that can efficiently deliver these drugs into the brain. Moreover, there are also several studies showing that neuroprotective peptides might be an excellent compound for AD therapy, since they have shown to be able to break down and degrade Aβ plaques.

Multifunctional NPs are a good option to carry these peptides, since nanocarriers can protect them from degrading into the plasma by proteolytic enzymes and increase their stability in the

serum. For example, PEG-PLA NPs were able to protect the neuroprotective peptide NAPVSIPQ from degradation. However, just NPs modified with B6 peptide (similar to transferrin) were able to cross the blood-brain barrier in mice and successfully deliver the neuroprotective peptide into the brain [102]. Moreover, it was observed that the treatment with this formulation improved cholinergic function and ameliorated spatial learning of AD mouse model [102]. In the same way, the nerve growth factor (NGF) has also been explored as a good drug for treating AD, although it is not able to cross the BBB. For this purpose, NGF was encapsulated into PBCA NPs, decorated with polysorbate 80 [103]. These NPs presented very promising results, since they were able to reach the mice brain parenchyma in less than 1 h after administration and these nanocarriers also proved to be able to improve recognition and memory of mice and to reduce by almost 40% the PD symptoms such as rigidity, tremor and oligokinesia in animal models [103]. Coenzyme Q10, a powerful antioxidant macromolecule, has also been explored at AD therapy. In this way, this coenzyme was encapsulated within PLGA NPs decorated with trimethylates chitosan. The results showed that these nanoparticles were able to cross the BBB and accumulate in the choroid plexus, ventricles and cortex. Moreover, the authors also observed an improvement in the cognitive and spatial memory performance of AD mice models and a significant reduction of senile plaques and levels of ROS [104].

6.3. Parkinson's disease

After Alzheimer's, PD is the second most common disease in terms of neurodegenerative diseases. As the aging population increases, the number of people with this disease is expected to rise. It affects 0.3% and 1% of the population worldwide over the age of 40 years and 65 years, respectively. Pathologically, PD is characterized by progressive loss of muscle control, which leads to tremor of hands, bradykinesia, rigidity and postural instability [105] Motor impairment in PD can also cause hypomimia, which is the decreased degree of facial expression. Dysphagia and hypophonia, which are disruption of the swallowing process and lack of coordination in the vocal musculature, are also common features in PD. Other symptoms also include ophthalmologic complaints such as blurred vision and gate. It is important to mention that all these signs and symptoms are a result of affected areas that occur within the brain, especially in an area known as basal ganglia [105].

Thus, PD is a result of problems that occur within the basal ganglia. The basal ganglia is a collection of nuclei located deep beneath the cerebral cortex and it is responsible for the correct execution of voluntary muscle movements and learned movement patterns. The components of basal ganglia are caudate nucleus and putamen (dorsal striatum); nucleus accumbens and olfactory tubercule (ventral striatum), ventral pallidum, globus pallidus, subthalamic nucleus and substantia nigra. In PD, the basal ganglia is disrupted, causing degeneration of dopaminergic neurons located at the substantia nigra. Essentially, it is considered a disease of the basal ganglia because what happens is that when the cerebral cortex wants to initiate a movement, the basal ganglia receives these signals and sends it back the motor cortex via the thalamus. Through various pathways, the substantia nigra is connected with nuclei in basal ganglia. The basal ganglia plays an essential role in integrating multiple input signals to modulate the output of the motor cortex. Inhibitory or excitatory connections can occur in this process. Thus, the loss of dopamine from substantia nigra in this process underlies the symptoms described earlier [105].

At the beginning, researches believed that the BBB did not suffer any kind of alteration during the disease development [106]. Surprisingly, tracking compounds such as [^{11}C]-verapamil and benserazide in the brain of PD patients or PD animal models, it was observed that the concentration of these compounds in the brain was increased, what does not happen in the brain of health patients or animals since these drugs are not able to cross the BBB [107]. In addition to this, a good correlation between the albumin ratio and progressive BB integrity loss in the brain of patients with PD was observed [107]. Moreover, other signals of the BBB impairment such as vascular alterations and blood flow deficiencies were reported [108]. Most important, the increased expression of vascular endothelial growth factor (VEGF) was directly correlated with the high amount of blood vessels presented in the damaged dopaminergic neurons in the brain of monkeys [109]. Later, it was observed that the injection of VEGF into the substantia nigra in the brain of rats disrupted the BBB, leading to a strong inflammation response and loss of dopaminergic neurons [110]. Lastly, alpha-synuclein aggregates are the central hallmark of PD and their accumulation seems to be correlated with the downregulation of the P-glycoprotein (**Figure 4**) [111]. Moreover, higher concentration of some metals like iron was found in the brain of PD patients and PD animal models because of the higher levels of lactoferrin receptor in the substantia nigra dopaminergic neurons of the diseased brain [32].

Currently, there is no cure for PD. However, there are drugs that work to decrease and relieve the symptoms of PD and maintain the quality of life of the patient. The most effective treatment for PD is the use of the drug levodopa—also called L-dopa—or dopamine that is both able to restore or increase the concentration of dopamine in the basal ganglia. But here, we want to discuss an article that explored the increased expression of lactoferrin in some region of the brain in PD patients [112]. In this way, a PAMAM and PEG NPs were developed, coated with lactoferrin to the delivery of a plasmid of human glial cell line-derived neurotrophic factor plasmid (GDNF), since GDNF is a promising factor in treating PD, but as all plasmids are unable to cross the BBB. These multifunctional nanoparticles were able to not only cross the brain barrier but also effectively deliver the plasmid into the brain, since a neuroprotective effect on dopaminergic neurons and improvement of locomotor activities in AD animal models was observed [113]. Another example is the encapsulation of urocortin (hormone-related peptide) in PEGylated-PLGA NPs covered by lactoferrin. From the results presented, this formulation was able to quickly cross the BBB and to promote protection to the dopaminergic neurons and improve locomotor functional deficits [114].

7. Conclusions

Nanotechnology in the field of medicine has brought a variety of new ways to treat and/or detect diseases [13, 115, 116]. Currently, engineered pharmaceutical NPs demonstrated abilities such as long blood circulation time in the body fluids for their accumulation at disease sites with leaky vasculature [117]; specific targeted drug delivery to the pathological area due to the surface functionalization of NPs with ligands such as antibodies [118]; contrast properties due to their unique capacity of carrying contrast agents allowing their tracking *in vivo* [119]; drug delivery from the particles responsive to a specific stimuli [79] and others. The tremendous advances in nanomedicine during the past decade have significantly advanced on the engineering of nanoparticles that

combine several of these characteristics, known as multifunctional NPs. Long-circulating and target-specific NPs capable of prolonged circulation time in the blood and targeted delivery of drug to the brain and *in vivo* imaging represent one example of a multifunctional nanocarrier [44].

Moreover, we hope this chapter was a bridge between nanotechnology and central nervous systems disorders, since multifunctional NPs have a great potential in the treatment of neurological disorders in the near future [14, 44, 120]. However, as discussed, the BBB is one of the major obstacles to the delivery of drugs into the brain and, consequently, for the treatment of neurological diseases [48, 121]. The BBB is composed of very tightly connected endothelial cells and a variety of transporters [15, 17, 120, 122]. This results in a highly selective permeability barrier that separates the circulating blood from the cerebral parenchyma, thus limiting the entry of drugs into the brain. As discussed earlier, several multifunctional NPs for delivering therapeutic and/or imaging molecules into the brain have been developed [44, 47, 123]. Thus, this part of the chapter was organized in a way to carry the reader through the fundamentals of common neurological diseases such as Alzheimer's, Parkinson's and cerebral ischemia and their potential treatments with these kinds of NPs [44, 68, 88, 102, 124–139]. The purpose was to analyze some of the major scientific data indexed in PubMed, Web of Science and Scopus to explore different approaches engineered to transport and deliver imaging or therapeutic molecules to the brain by using multifunctional NPs technology. In this way, our gathered data on different strategies for the delivery of drugs across the BBB using multifunctional NPs were reviewed, discussed and grouped in self-explanatory figures. Results of our analysis from some research articles on our search showed that several strategies have been used to deliver several therapeutic compounds to the brain by these NPs. Functionalization of the surface of these NPs by covalent ligation of macromolecules such as antibodies, RNA aptamers as well peptides is an effective method for receptor targeting nanocarriers, which allows their BBB-penetration and the efficient delivery of their cargo specifically to the disease site. Additionally, methods for the development of multifunctional NPs that can respond to external stimuli were employed, concluding that the development of multifunctional NPs for treating neurological disorders still is at its infancy, although these systems have a huge chance to revolutionize the ways that brain diseases are treated.

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