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Review

Surface Functionalization of PLGA Nanoparticles to Increase Transport across the BBB for Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is a chronic neurodegenerative disorder that accounts for about 60% of all diagnosed cases of dementia worldwide. Although there are currently several drugs marketed for its treatment, none are capable of slowing down or stopping the progression of AD. The role of the blood-brain barrier (BBB) plays a key role in the design of a successful treatment for this neurodegenerative disease. Nanosized particles have been proposed as suitable drug delivery systems to overcome BBB with the purpose of increasing bioavailability of drugs in the brain. Biodegradable poly (lactic-co-glycolic acid) nanoparticles (PLGA-NPs) have been particularly regarded as promising drug delivery systems as they can be surface-tailored with functionalized molecules for site-specific targeting. In this review, a thorough discussion about the most recent functionalization strategies based on PLGA-NPs for AD and their mechanisms of action is provided, together with a description of AD pathogenesis and the role of the BBB in brain targeting.

Keywords: functionalized PLGA nanoparticles; brain delivery; blood-brain barrier; Alzheimer's disease

1. Introduction

Alzheimer's disease (AD) is recognised as a chronic neurodegenerative disease characterized by amyloid beta accumulation and brain intracellular neurofibrillary tangles [1]. Despite the several drugs approved by the Food and Drug Administration (FDA) for the treatment of AD, they are not fully effective in ameliorating the symptoms and lose effectiveness over time. We still face an unmet medical need with respect to effective treatment and management of this neurodegenerative disorder.

To design and develop a successful treatment strategy for AD, the role of the blood-brain barrier (BBB) has to be taken into account [2]. The BBB consists of a continuous layer of differentiated endothelial cells linked together by tight junctions, pericytes, nonfenestrated basal lamina and astrocytic foot processes [3]. Due to its properties, this complex barrier makes the CNS a challenging microenvironment to be reached by drug molecules through conventional approaches [2]. The BBB is thus a limiting barrier for conventional drug delivery [3].

Therefore, several innovative strategies have been proposed to enhance the transport of therapeutics through the BBB [3]. Among different types of nanosized particles, polymeric (e.g., PLGA-NPs, PLA-NPs, polymeric micelles, dendrimers) and lipid (e.g., liposomes, solid lipid nanoparticles) nanoparticles have been extensively studied to deliver therapeutic drugs and macromolecules to the brain [4].

In this area, polymeric nanoparticles can be made of biocompatible copolymers of poly(D,L-lactic-co-glycolic) acid that have low solubility in water [5,6]. Moreover, characteristics such as size, zeta potential and hydrophilicity can be controlled by surface modifications, such as surfactant coating, to enhance brain uptake [7]. In addition, ligands known to target the surface receptors on endothelial cells of the BBB (e.g., transferrin, insulin and lipoprotein receptors) can be linked to the surface of PLGA-NPs to provide targeted brain delivery and improve NP uptake [8].

PLGA is, indeed, one of the most successful polymeric materials used to produce functionalized particles, given its biodegradability and biocompatibility properties that have contributed to its approval by the FDA and EMA (1986) for parenteral administration. Besides, the loading of drugs into a polymeric matrix core of nanoparticles promotes drug protection against degradation and offers the possibility to modify the release profile both in vitro and in vivo [9].

The aim of the present work is to identify different strategies of PLGA-NPs surface functionalization to enhance their transport through the BBB for successful treatment of neurodegenerative diseases, in particular for AD. In order to undertake this objective, some secondary aims have been established, such as the thorough analysis of AD pathology highlighting the challenges encountered in the physiological properties of the BBB for drug delivery, the role of PLGA NPs and their surface targeting to increase transport through the BBB for AD treatment.

2. Materials and Methods

Different databases were used for the literature search: PubMed, SpringerLink, ScienceDirect, making use of the following key words: "functionalized/targeted PLGA nanoparticles", "Alzheimer's disease", "brain delivery" and "blood-brain barrier". Relevant studies were selected based on the year of publication (after 2010) and the different strategies used to enhance BBB crossing. A total of 13 papers on functionalized PLGA NPs for AD treatment were used. Information about NP characteristics, in vitro and in vivo models, and the most relevant findings were drawn from selected papers, and are also summarised.

3. Alzheimer's Disease

3.1. Prevalence and Incidence

Alzheimer's disease (AD) accounts for about 60% of all dementia cases worldwide, with an estimated global incidence of 24.3 million cases, which increases with the aging population [10,11] with an incidence rate that increases exponentially with age until 85 years [12,13].

Age of the onset can thus be used as criterion for the categorization of early-onset AD (EOAD) and late-onset AD (LOAD). The former accounts for about 1–6% of all cases, with ages ranging from 30 to 60–65 years old. The latter is the most common form of the disease, with an onset later than 60–65 years [11]. Both EOAD and LOAD are characterized by a progressive loss of memory and orientation together with other cognitive deficits that eventually become incapacitating, including impaired judgment and decision making, apraxia and language disturbances. In addition, these are typically followed by other neuropsychiatric symptoms such as depression, anxiety, apathy, delusions, agitation or hallucinations [14].

Moreover, in parallel with lifetime expectancy, the increasing numbers of patients diagnosed with AD constitute a global health concern with huge implications for patients and caretakers [15]. This has led to an enormous increase in research focused on under-

standing AD pathogenesis in order to discover drugs for prevention and treatment of the disease [16].

3.2. Neuropathology

The major pathological hallmarks of AD are the set-up of extracellular amyloid plaques and intraneuronal neurofibrillary tangles (NFTs) in the brain. Although plaques and tangles are also identified in cognitively normal age-matched controls, their density and distribution are significantly more severe in AD patients [17].

Amyloid plaques are mainly composed of the amyloid-β (Aβ) peptide that accumulate in the extracellular cortex [18]. The 40 amino acids-peptide is the most common form of Aβ in humans and is called Aβ40, whereas a 42-amino-acid-long fragment, called Aβ42, is less abundant. The only difference is that Aβ42 has two additional amino acid residues at the C-terminus [19]. However, Aβ42 has been associated with AD because it is more prone to aggregation than Aβ40, and thus would be deposited before Aβ40 [20], leading to the formation of these amyloid plaques.

The gene of the amyloid precursor protein (APP) that originates the Aβ peptide is located in chromosome 21 in humans with three major isoforms arising from alternative splicing [21]. However, the physiological function of APP, despite intensive research, is not yet fully disclosed.

Three distinct secretases (α, β, or γ) can induce cleavage of full-length APP, which thus undergoes different sequential proteolytic processing. In the nonamyloidogenic pathway, APP is first cleaved by α-secretase (non-neurotoxic, "normal" cleavage), releasing a large soluble ectodomain of APP (sAPP-α) into the extracellular space. Opposed to Aβ, sAPP-α plays an important role in survival and in neuronal plasticity, showing a protective effect against excitotoxicity. sAPP-α also regulates the proliferation of neural stem cells, being instrumental for early neural development [22]. On the contrary, in the amyloidogenic processing, APP is first cleaved by β-secretase (potentially neurotoxic, "abnormal" cleavage), releasing another soluble ectodomain of APP (sAPP-β) into the extracellular space.

Upon α or β-cleavage, the respective carboxyl terminal fragments (CTFs) of APP (α-CTF and β-CTF) are kept in the cell membrane and suffer γ-secretase-mediated cleavage. Subsequent to this, γ-cleavage, α-CTF and β-CTF generate p83 and Aβ, respectively, (Figure 1). It should be noted that γ-secretase cleavage occurs in the transmembrane domain, even though the exact site may vary. Indeed, major sites of γ-secretase cleavage are the Aβ 40 and 42 positions [14], resulting in the production of Aβ40 or Aβ42, i.e., two main forms of Aβ consisting, respectively, of either 40 or 42 amino acid residues.

The prevalent theory of AD pathogenesis is currently accepted to be the amyloid hypothesis, suggesting the accumulation of insoluble forms of Aβ as the primary pathological process, which results from the imbalance between Aβ production and Aβ clearance [23]. Indeed, the Aβ40/Aβ42 ratio is key in the set-up of this disease. Aβ42 is the predominant form of Aβ found in the brain parenchyma of AD patients, whereas Aβ40 is mostly found in the cerebral vasculature [24]. Since Aβ42 is the most soluble form, it has the risk of oligomerizing to form Aβ-fibrils and protofibrils responsible for the formation of amyloid plaques. Although amyloid plaques are assumed to be nontoxic, the formation of amyloid oligomers may be responsible for neurotoxicity. As a result, the amyloid cascade would lead to the clinical syndrome of AD [14].

This cascade of events includes local oxidation, inflammation, excitotoxicity (due to excessive glutamate) and tau hyperphosphorylation [14]. In this context, the formation of NFTs is considered a downstream process in which tau proteins aggregate in a soluble form, which results in neuronal dysfunction and neurodegeneration. Moreover, this progressive neuronal degeneration would result in an imbalance and shortage of several neurotransmitters (e.g., serotonin, dopamine, acetylcholine), thereby leading to the known cognitive AD deficiencies [25]. The brain changes caused by the disease are shown in Figure 2.

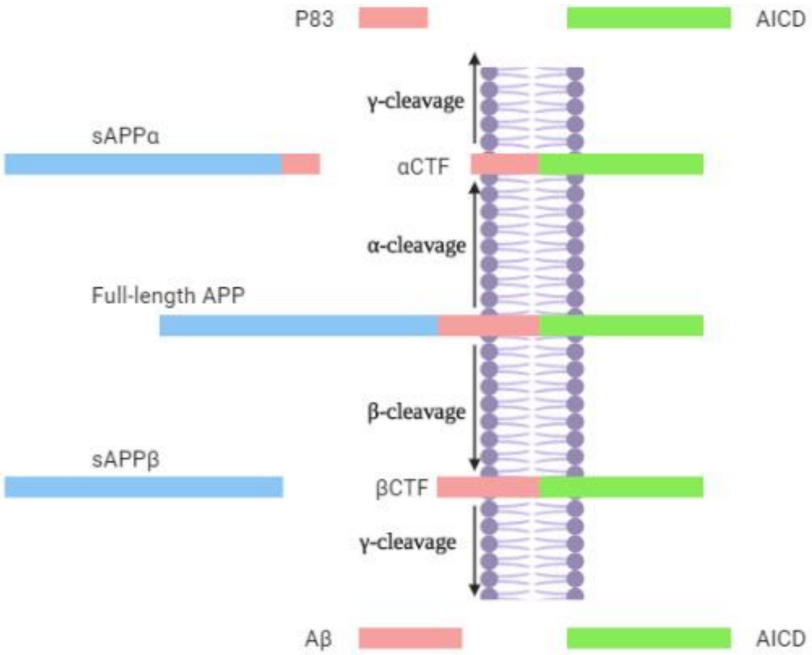

Figure 1. Schematic representation of nonamyloidogenic and amyloidogenic pathways of APP processing. APP cleavage takes place either by alpha-secretase (the nonamyloidogenic pathway) generating sAPP-alpha and C83, or by beta-secretase (the amyloidogenic pathway) generating sAPP-beta and C99 (based on [22]).

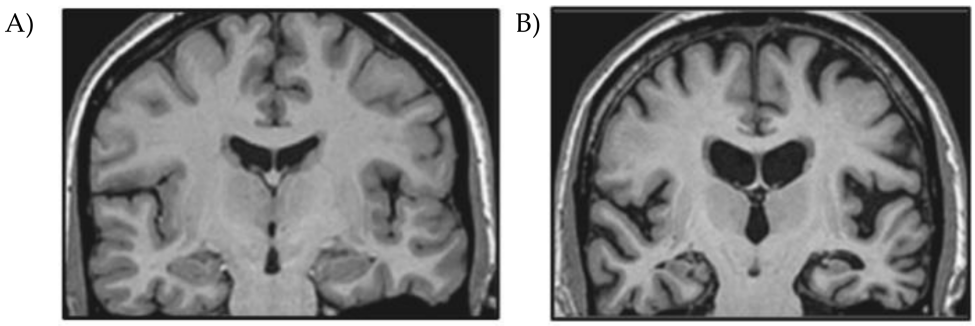

Figure 2. Differences between human brain. (**A**) Healthy brain and (**B**) AD brain (excerpted from BioRender).

Although a good deal of data, collected over decades of scientific and clinical research of this disease, still supports the role of Aβ as the primary initiator of the AD complex pathogenic cascade, an increasing number of indicators point out that while the triggering effect of Aβ seems necessary, it does not seem crucial in later stages of the disease [26]. Therefore, other hypotheses explaining AD pathogenesis have been developed.

As already mentioned, NFTs (composed of Tau protein) constitute another pathological AD hallmark. Tau is a microtubule-associated protein abundant in the neurons of the CNS that works as a scaffold protein, maintaining the stability of microtubules in axons. Under pathological conditions, tau hyperphosphorylation is increased, which results in the Tau removal from the microtubule, which causes the collapse of the microtubule and impairs neuronal axons, causing neurodegeneration [27–30]. In addition, this hyperphosphorylation generates tau aggregates that eventually form neurofibrillary tangles [29–31], leading to loss of neuronal function and resulting in apoptosis [32]. Therefore, several studies on biomarkers point out that Tau pathology is intimately related to the progression of neurodegeneration [33].

Moreover, researchers also suggest that neuroinflammation [34], cholinergic neuronal damage and oxidative stress [27] play an important role in the neuropathological progression of AD.

3.3. Treatment

Despite all the advances made since Dr. Alois Alzheimer described the first case of AD in 1907, the precise mechanisms of amyloid and tau pathology behind AD pathogenesis have still not been clearly identified. As a result, there are still no effective pharmacotherapeutic alternatives for prophylaxis, management and treatment of AD [14]. Although antidementia agents developed for the treatment of AD can be categorized as symptomatic or disease-modifying [35], none of the established treatments can fully ameliorate AD progression [36].

Some of these current symptomatic treatments include acetylcholinesterase (AChE) inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonists, such as memantine. However, all these drugs (Table 1) can only alleviate AD symptoms. Furthermore, the efficiency of the drugs varies between patients and disease stages. In addition, they possess several adverse effects such as nausea, vomiting and diarrhoea [37].

Besides, antipsychotic and antidepressant treatments are also used to ameliorate the behavioural symptoms [38]. In addition, compounds that act on the pathological substrate of the disease, namely on extracellular Aβ plaques and intracellular NFTs, are currently under research [14].

Table 1. FDA-approved drugs for AD (based on [39]).

Drug	Donepezil (Aricept®, 1996)	Rivastigmine (Exelon®, 2000)	Galantamine (Razadyne®, 2001)	Memantine (Ebixa®, 2003)
Pharmaceutical company	Pfizer, New York, USA	Novartis, Basel, Switzerland	Janssen, New Jersey, USA	Lundbeck, Valby, Denmark
Class and indication	AChE inhibitor prescribed to treat symptoms of mild-to-moderate and moderate-to-severe AD	AChE inhibitor prescribed to treat symptoms of mild-to-moderate AD	AChE inhibitor prescribed to treat symptoms of mild-to-moderate AD	NMDA receptor antagonist prescribed to treat symptoms of moderate-to-severe AD
Mechanism of action	Prevents the breakdown of ACh in the brain	Prevents the breakdown of Ach and butyrylcholine in the brain	Prevents the breakdown of ACh and stimulates nicotinic receptors to release more ACh in the brain	Blocks the toxic effects associated with excess glutamate and regulates glutamate activation
Common adverse effects	Nausea (3–19%), vomiting (3–9%), diarrhea (5–15%)	Nausea (17–47%), vomiting (13–31%), diarrhea (5–19%), loss of appetite (≥17%), weight loss (3–26%), muscle weakness	Nausea (21%), vomiting (11%), diarrhea (7%), loss of appetite (7%), weight loss	Dizziness (5–7%), headache (6%), constipation (3–5%), confusion

It is also worth noting that the limited or unsuccessful development of new therapeutic agents for AD is frequently attributed to the presence of the BBB, whose properties make the CNS one of the most complex microenvironments of the body, thus compromising the development of novel effective compounds [2].

4. The Blood-Brain-Barrier

On average, the adult human brain accounts for about 2% of body weight. Despite its relatively small size, it consumes about 20% of glucose-derived energy [40]. Indeed, the mammalian brain depend upon glucose as its main source of energy. Furthermore, neurons within the CNS have a high energy demand, requiring a continuous supply of energy substrates (mainly glucose) and nutrients from the blood [41].

Moreover, neurons communicate using several chemical (ions, neurotransmitters, neuromodulators and neuropeptides) and electrical signals (synaptic and action potentials). Therefore, an accurate regulation of the axons and synaptic ionic microenvironments is

critical for healthy brain physiology [42]; thus, reliable neural signaling is governed by the barrier layers existing between blood and neural tissue [43].

The adult brain is composed of two main interfacial barriers, namely: (i) the blood-cerebrospinal fluid barrier, which is composed of epithelial cells of the choroid plexus facing the cerebrospinal fluid [42] and (ii) the avascular arachnoid epithelium, underlying dura and enclosing CNS, also forms a barrier layer, even though its avascular nature and small surface area do not promote a significant surface extension for exchange between the blood and the CNS [44]. Besides these two barriers, the BBB represents the largest interface for blood-brain exchange, and is created by the endothelial cells that form the walls of the capillaries [42]. As a result, there is a direct interaction with the circulating blood, making the BBB the most selective physical barrier and allowing it to exert the tightest control over the immediate microenvironment of brain cells [45]. In fact, the BBB is present in all organisms with a well-developed CNS [46], and is responsible for ensuring the proper environment for neuronal network functionality and brain homeostasis. The BBB protects the brain against pathogenic agents and it regulates the influx and efflux of fluids by means of dynamic combinations of ionic, molecular, vascular and cellular factors [47].

4.1. Structure

Although endothelial cells of the CNS vasculature form the main barrier against the entry of xenobiotics in the brain, these cells do not function independently from others, but rather act as modules within the multicellular neurovascular unit. In fact, circulating immune cells, neurons, microglia, pericytes and astrocytes are intimately linked with the endothelium and play supporting roles in maintaining and functioning this barrier [43,48,49] due to an intricate network of molecular crosstalk between them [45] (Figure 3).

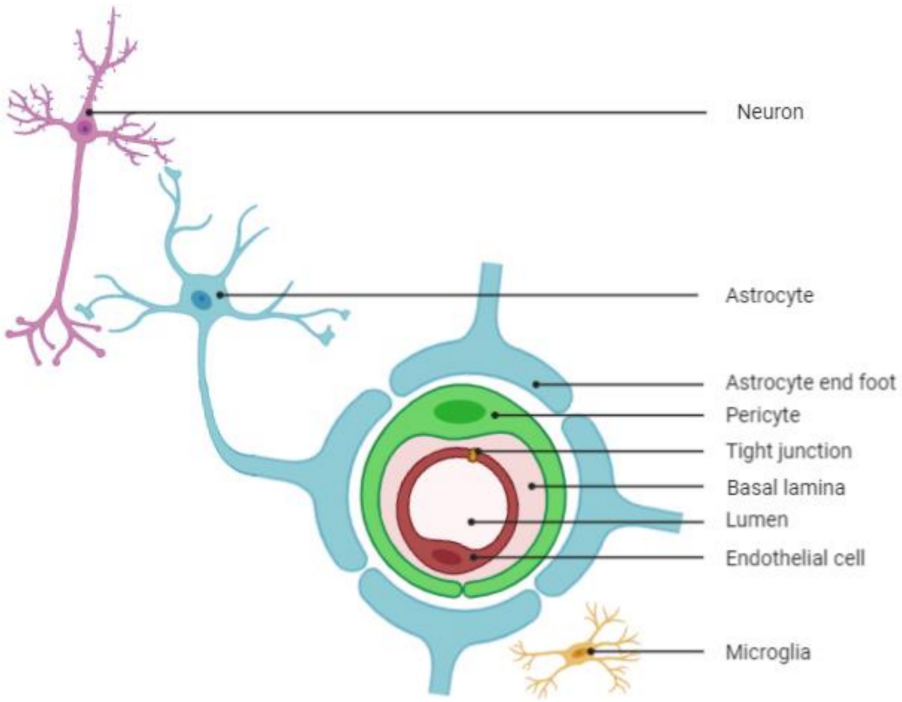

Figure 3. Schematic representation of the cellular networks of the BBB. The paracellular pathway is governed by the tight junctions created by the brain endothelial cells. Foot processes from astrocytes form a complex network surrounding the capillaries and provide links to neurons. Pericytes are distributed discontinuously along the length of the brain capillaries and partially surround the endothelium. Microglia are CNS-resident immune cells (based on [50]).

Indeed, in addition to the CNS endothelial cells, several functional layers exist between the blood and brain. This is the case of the basement membrane, which completely covers the capillaries and is made of laminin, fibronectin and type IV collagen, and contains pericytes; astrocytes also surround this basement membrane of the BBB. Each of these layers may contribute to solute movement through BBB [51].

However, the endothelial cells lining the cerebral blood vessels still represent the main anatomical structure of the BBB and can be distinguished, both functionally and morphologically, from the peripheral endothelial cells. For example, they have a high concentration of mitochondria, which is an indication of a high energy expenditure [52].

Moreover, endothelial cells from the CNS composing the tight junctions, greatly limit paracellular permeability [53–55]. They also display low rates of transcytosis when compared to peripheral endothelial cells, effectively reducing vesicle-mediated transcellular transport [56].

As a result of this tight paracellular and transcellular barrier, endothelial cells are polarized, presenting distinct abluminal and luminal surfaces with efflux and influx transporters and receptors, which together control the movement between blood and brain [52].

In addition, these endothelial cells have a net negative surface charge, repelling negatively charged compounds, and they also have no fenestrations (small transcellular pores that allow free diffusion), thus preventing fast exchange of molecules. Endothelial cells also have very low levels of leucocyte adhesion molecules, restricting the number of immune cells that can indeed enter the CNS [52].

4.2. Transport across the BBB

The BBB plays a role not only as a barrier to cells and solutes, but also as a carrier for selective drug molecules. Potential routes for the transport and permeation of small molecules and biomacromolecules across the BBB do exist (Figure 4), primarily through either paracellular or transcellular transport [57]. On the one hand, small hydrophilic molecules can cross the BBB and reach the brain through an aqueous pathway via paracellular transport. On the other hand, small lipophilic compounds enter the brain tissue through transcellular diffusion, a nonsaturable pathway, which could be optimized by modifying the physicochemical properties of the drug.

There is a correlation between the lipid solubility of a drug and the rate at which it enters the CNS. Factors such as molecular weight (<400–500 Da) and hydrogen bonding capacity (<8–10 hydrogen bonds) compromise drug access to the brain [58]. However, there are several other examples of drugs entering the CNS that are not affected by these properties [50].

Moreover, tight junctions limiting paracellular permeability potentially isolate the brain from many ionic nutrients, such as glucose and amino acids which are needed for brain metabolism and, therefore, the BBB endothelium also contains many specific solute transporters to allow carrier-mediated transport (CMT) of these substances. Indeed, the endothelial cells present in the BBB express transport proteins on their surface for a wide range of molecules, thereby mediating their brain influx and efflux [42].

Regarding large molecular weight solutes such as selective peptides, proteins and larger macromolecules, the mechanisms for crossing the BBB and entering the CNS consist of binding to specific receptors on the cell surface of endothelial cells via endocytosis. This process of endocytosis can be either through receptor-mediated transcytosis (RMT), providing the main route to which these large molecular weight solutes cross the BBB and enter the brain, or through adsorptive-mediated endocytosis (AMT) or pinocytosis [57].

In summary, the movement of molecules and drugs through the BBB is either passive or active. The former is driven by a concentration gradient from plasma to brain, with more lipophilic compounds entering more easily; the latter being facilitated by active transporters in the endothelial cell membranes [42]. However, the BBB is an obstacle for drug delivery to the brain. Therefore, increasing efforts are currently ongoing to overcome the limitations encountered in the BBB for delivery of therapeutics [52].

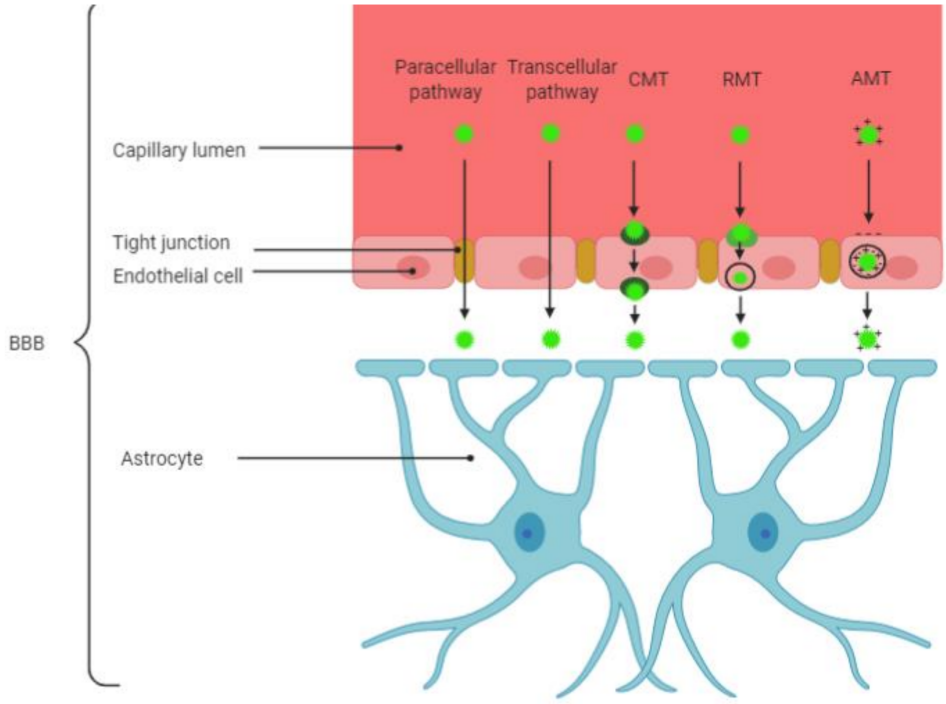

Figure 4. Schematic representation of potential routes of transport and permeation across the BBB (based on [47]).

5. Strategies to Enhance the Delivery of Therapeutic Agents across the BBB

The delivery of drugs to the brain is mandatory for the treatment of brain-associated diseases, but it is compromised by the presence of the BBB [59].

Despite the multiple BBB crossing pathways, approximately 98% of small molecules, and most large molecules (e.g., antibodies, recombinant proteins and peptides, viral vectors), are unable to reach the brain through the BBB [60,61], resulting in a very low bioavailability in the brain [62].

All this has slowed down the exploitation of immunotherapy in brain diseases. In fact, intravenously administered antibodies rapidly enter the brain and subsequently are quickly expelled from it. Thus, only about 0.1% of therapeutic antibodies reach the targeting site, resulting in a demand of significantly higher antibody concentrations in systemic circulation, which, in turn, is associated with an increased risk of systemic toxicity [63].

A common noninvasive strategy to enhance the BBB permeation of small drugs is to increase the lipophilic character of the molecule by chemical modification [45]. However, this approach may promote a faster clearance of the drug from the circulatory system through efflux transporters, thereby compromising the drug distribution and effectiveness. Thus, structural modification of the drug to increase its affinity to endogenous transport proteins on the cerebellar endothelium has been proposed [64].

In addition to drug modification, increase of lipophilicity, or reduction of the molecular size, may also contribute to increase BBB permeation by focusing on the reduction of efflux transport, thus enhancing the transcellular diffusion permeability or disrupting the tight junction complexes [47]. Furthermore, the use of nanoparticles and other molecular Trojan horses may also be exploited to enhance the delivery of drug molecules across the BBB [65].

5.1. Nanotechnological Tools to Overcome the BBB

Multidisciplinary efforts are being made combining chemistry, physics, engineering, and biology to create effective delivery systems able to cross the BBB with the aim to diagnose and/or treat brain diseases [66].

This is especially relevant to overcome the limitations encountered with currently available strategies to deliver drugs into the CNS through the BBB [47], in particular for

the delivery of peptides, recombinant proteins, vaccines and nucleotides [67,68]. The most commonly used delivery systems are liposomes, micelles, dendrimers and micro and nanoparticles [69], which can be designed for reduced size, biodegradability and biocompatibility, prolonged blood half-life and no toxicity, making these drug delivery systems very attractive [70]. These properties have been associated with reduced side effects, improved site-specific targeting capacity and better patient compliance [71].

5.2. Polymeric Nanoparticles

Polymeric nanoparticles (NPs) are particles of nanometric size (1–1000 nm) composed of a solid core, and have been widely exploited to cross the BBB due to their properties for drug delivery, such as high loading capacity, high stability, controlled drug release and targeting efficiency [5].

Polymeric NPs can be produced using either synthetic or natural polymers (more limited in terms of their synthesis and processing). According to their morphology, polymeric NPs are classified in two distinct categories, namely, nanospheres and nanocapsules (Figure 5). In nanospheres, the drug is molecularly dispersed throughout the polymeric network or is placed onto the surface of the polymeric core, while in the case of nanocapsules, the drug molecules are solubilized in an oil or aqueous core which is surrounded by a polymeric layer [59].

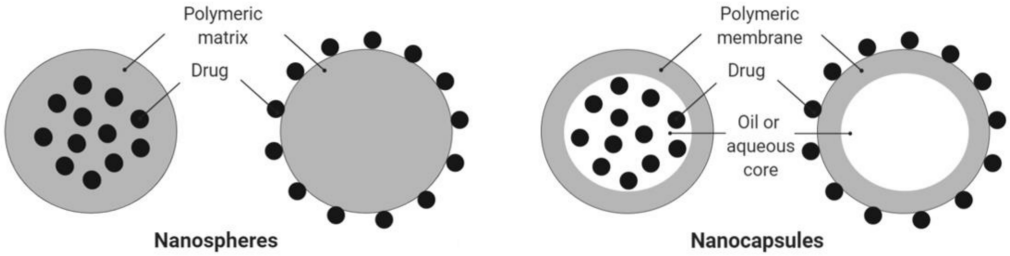

Figure 5. Schematic representation of nanospheres and nanocapsules with the drug entrapped or adsorbed onto the surface of nanoparticles (based on [72]).

Moreover, the properties of these NPs can be tailored by the introduction of third party components [73] in order to increase their half-life, to reach the BBB more easily, and to increase drug bioavailability into the brain for the treatment of neurological disorders such as AD [69].

The selection of the type of polymer for the production of such NPs is based on several criteria and factors, namely, the desired size for a particular application, physicochemical properties of the drug to be loaded within the polymeric core, surface characteristics and required functionality, degree of biodegradability and biocompatibility, and drug release profile of the final formulation [74]. Several natural and synthetic polymers have been used to prepare NPs for brain delivery [75].

Poly (butyl cyanoacrylate) (PBCA) NPs were the first polymer-based DDS used to deliver drugs to the CNS [76]. Unfortunately, despite many advantages they possess, such as biocompatibility and biodegradability, PBCA NPs have not yet been launched in clinical use. One of the major limitations of these particles is their poor drug loading capacity, in particular for hydrophobic molecules. Besides, the burst release of the drug from PBCA-NPs has also been pointed out as a shortcoming in most of the in vitro release studies describing the use of PBCA-NPs [77]. Moreover, it has been seen recently that PBCA NPs could present potential toxicity, which could be attributed to faster degradation and a more rapid release of degradation products [78]. However, other authors have demonstrated that they can induce oxidative stress, ferroptosis and necrosis [79].

Alternatively, several types of polymeric NPs with high positive charge have been reported to cross the BBB due to their electrostatic interaction with brain endothelial cells (negatively charged). Chitosan (CS) is a naturally occurring polysaccharide that possesses

high biodegradability, low toxicity and good biocompatibility, which has the ability to efficiently form NPs [80]. However, it shows low solubility in neutral and alkaline pH, thereby requiring production methods adapted to the physicochemical properties of the drug in question, such as the careful choice of a specific chitosan (e.g., molecular weight and degree of acetylation) and possible chemical modification [81].

Polyesters (e.g., poly (lactic acid) (PLA), poly (glycolic acid) (PGA), poly(lactic-co-glycolic acid) (PLGA)) have also been widely used for parenteral administration of drugs [75].

5.3. PLGA Nanoparticles

PLGA nanoparticles are associated with high cost of production and difficulty in scaling up [82]. Most recent studies have focused on the use of PLGA as a material for the synthesis of NPs to encapsulate a wide variety of drugs for the treatment of neurological disorders, including AD. Several in vitro studies have showed that the use of these polymeric NPs enhanced bioavailability in the brain, with reduced inflammation, oxidative stress and plaque load [83].

Indeed, PLGA is one of the most widely used biodegradable copolymers because the cleavage of polymer chains by hydrolysis results in free lactic acid (LA) and glycolic acid (GA) [84] as showed in Figure 6. Given the fact that these two metabolite monomers are endogenous, and easily eliminated from the body via the Krebs cycle in the form of H_2O and CO_2, a minimal systemic toxicity is associated with the use of PLGA for drug delivery [9]. Formulations composed of PLGA and its related homopolymers (i.e., poly (lactic acid) (PLA) and poly (glycolic acid) (PGA)) have been approved by the FDA and EMA for medical applications [85].

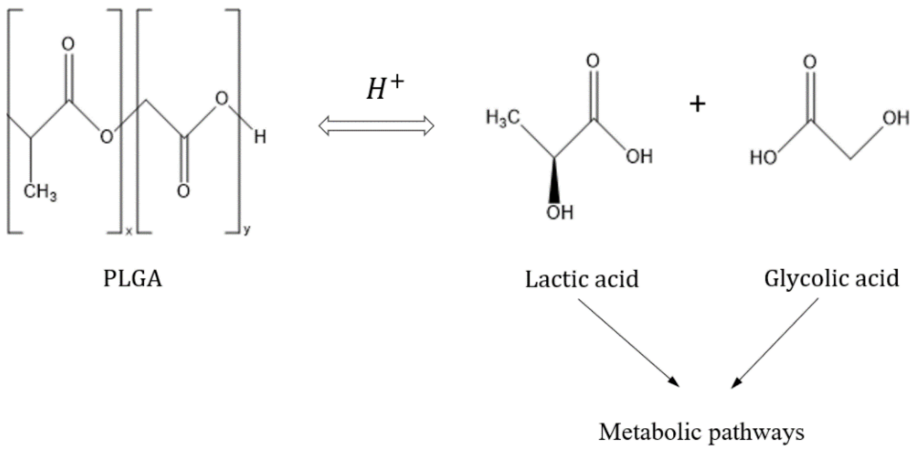

Figure 6. Hydrolysis of PLGA NPs (based on [5]).

PLGA NPs undergo degradation via hydrolysis of ester bonds to monomeric anions (lactate and glycolate). While L-lactate is converted into CO_2, which is then excreted through the lungs and converted to pyruvate entering the Krebs cycle, D-lactate is not metabolized before excretion. Glycolate is either directly excreted through the renal system or oxidized into glyoxylate, which is then converted into glycine, serine and pyruvate [73]. These can also enter the Krebs cycle where they are metabolized into CO_2 and H_2O [9,84].

PLGA is typically produced by a catalysed ring-opening copolymerization of lactic acid and glycolic acid [86]. As a copolymer of both, PLGA inherits the intrinsic properties of these monomers [73]. However, it is essential to understand the effects of the lactate/glycolate acids ratio and the molecular weight of PLGA copolymers on the degradation behaviour and the kinetics of drug delivery.

The most important influencing factor for controlling the degradation rates of the PLGA matrix is the ratio between lactate/glycolate acids in the copolymer. Whereas an increase in the lactate/glycolate ratio results in higher hydrophobicity, leading to lower

degradation and slower drug release kinetics [87], a higher content of glycolate contributes to increase the hydrophilic character of PLGA being more amorphous and thus leading to much faster degradation and drug release [88].

Since the lactate/glycolate ratio can be easily changed during synthesis, several publications discuss its impact on release kinetics and degradation [89–92]. It should be noted that most of the studied PLGA NPs are prepared with lactate/glycolate ratios of 50/50 or higher because PLGA 50/50 constitutes a suitable choice for medium-rapid drug release if only the effect of lactate/glycolate ratio is considered [88].

In fact, the final molecular weight of PLGA also significantly influences the degradation and drug release kinetics of the resulting nanoparticles [73]. It has been seen that a lower molecular weight leads to higher degradation and shorter drug release rates because a decrease results in a less hydrophobic polymer, which increases the water absorption rate, hydrolysis, and polymeric erosion [93]. On the other hand, a lower molecular weight promotes drug diffusion, thereby accelerating drug release kinetics [88,94].

6. PLGA Nanoparticles Functionalization

PLGA NPs can be produced by several different methods, such as nanoprecipitation or solvent displacement, emulsification-evaporation, solvent diffusion, or by phase-inversion. The resulting particles may have sizes ranging between 10 and 1000 nm [95]. For the loading of hydrophilic molecules, nanoprecipitation and emulsification-evaporation are the selected approaches [96].

In addition to the intrinsic properties of the prepared PLGA NPs, surface modification strategies to overcome the BBB and deliver compounds into the brain play an important role. Indeed, nonsurface-modified PLGA NPs have shown some limiting features such as the negative surface charge, hydrophobic nature, and no targeting capacity to reach BBB [97]. These properties compromise the half-life of particles in the blood circulation time and the extent to which they are taken up by target cells [98]. Therefore, functionalization of the NP surface using specific proteins, peptides or monoclonal antibodies is needed (see Figure 7). Table 2 summarizes the selected papers that will are explained below.

Surface functionalization strategies can be categorized according to the surface modifications onto PLGA NPs [97].

- Pretranscytosis strategies: NPs remain for a greater time in the blood circulation by increasing their surface hydrophilicity, escaping from macrophages and from the reticuloendothelial system.
- BBB transcytosis strategies that recognize the CNS endothelial cells and enhance passage across the BBB.
- Post-transcytosis strategies: NPs are surface-tailored with specific targeting moieties to achieve targeting of the impaired nervous system cells.

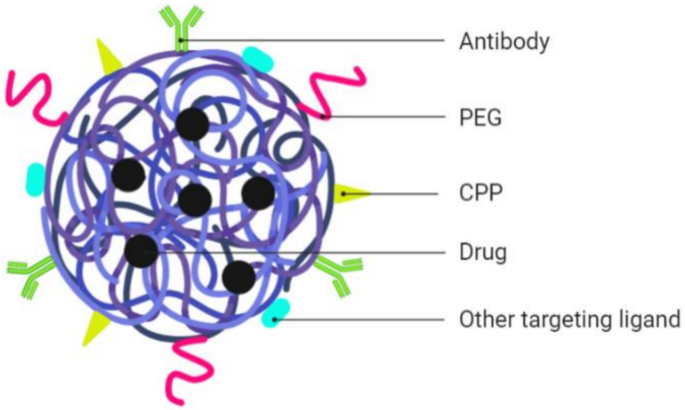

Figure 7. Schematic representation of a functionalized NP.

Table 2. Functionalized PLGA NPs prepared to treat AD.

Encapsulated Drugs	Drug Delivery System	Targeting Vectors	Physicochemical Characteristics	Elevated Model	Results	References
-	PLGA-PEG	Angiopep-2	~170 nm ~−25 mV	C57BL/6 mice	Clear accumulation of NPs in brain parenchyma	[99]
Co-Q10	PLGA	TMC	146.7 ± 5.1 nm 21.0 ± 2.9 mV	APP/PS1 mice	Negligible cytotoxicity, increased BBB permeability and reached the brain parenchyma, great reduction of memory impairment	[100]
6-coumarin	PLGA-PEG	Pep-TGN	~100 nm −18 to −24 mV	bEnd.3 cells and nude mice	Enhanced cellular uptake, enhanced brain accumulation	[101]
6-coumarin	PLGA	P80/P188/CS	396.2 ± 14.4 nm (P80) 231.7 ± 9.1 nm (P188) 252.2 ± 7.3 nm (CS) 20.3 mV (P80) 19.0 mV (P188) 6.1 mV (CS)	Wister rats	Prolonged blood circulation with P80, increased cellular uptake with CS, higher brain distribution with P80	[102]
6-coumarin	PLGA	DMAB	50–300 nm ~57 mV	Caco-2 and HT-29 cells	Increased cellular uptake, size-dependent and with an optimal particle size of 100 nm	[103]
Curcumin	PLGA	Tet-1 peptide	150–200 nm −30 to −20 mV	GI-1 glioma cells without in vivo data	Noncytotoxic, increased neuronal uptake, stopped the aggregation of amyloid plaques and disrupted the aggregates already formed	[104]
Curcumin	PLGA	g7 peptide	204 ± 10 nm −13 ± 2 mV	Primary hippocampal rat neurons without in vivo data	No apparent toxicity, increased cellular affinity for neuronal cells, significant decrease of Aβ aggregates	[83]
Curcumin	PLGA-PEG	GSH	158.7 ± 12.8 nm −4.5 ± 0.35 mV	SK-N-SH cells without in vivo data	Increased cellular uptake, improved cellular trafficking	[105]
Curcumin	PLGA-PEG	B6 peptide	50–250 nm 3.83 ± 0.89 mV	HT22 cells and APPswe/PS1dE9 mice	Biocompatible and relatively low toxicity, increased cellular uptake, remarkable improvement of cognitive impairment	[106]
Curcumin and S1 peptide	PLGA-PEG	CRT peptide	139.8 nm −25.7 mV	bEnd.3 cells and APP/PS1dE9 mice	Increased cellular uptake, significant improvement of spatial memory and recognition	[107]
Huperzine A	PLGA, TMC	Lactoferrin	153.2 ± 13.7 nm +35.6 ± 5.2 mV 73.8% ± 5.7%	SH-SY5Y cells, without in vivo data	Increased cellular uptake, improved drug delivery to the brain	[108]
iAβ5 peptide	PLGA	OX26 and DE2B4 antibodies	166 ± 2 nm −13 ± 1 mV	Porcine brain capillary endothelial cells without in vivo data	Nontoxic, increased cellular uptake	[109]
Memantine	PLGA-PEG	-	152.6 ± 0.5 nm −22.4 ± 0.5 mV	bEnd.3 cells, astrocytes and transgenic APPswe/PS1dE9 mice	Noncytotoxic, enhanced decrease of memory impairment, reduction of β-amyloid plaques and respective inflammation	[110]

6.1. Pretranscytosis Circulation

6.1.1. Size Modification

Size modification is a crucial point for approaches intended to enhance pretranscytosis circulation of NPs for drug delivery, that is, NP stabilization in the blood circulation and escaping the reticuloendothelial system. In fact, NPs with mean diameters of 100 nm or less are excreted faster, whereas NPs with mean diameters over 200 nm may be sequestrated by the liver or spleen and thus exhibit a more rapid rate of clearance due to a more efficient uptake by phagocytes [111]. Moreover, NPs under 200 nm are compatible with systemic administration, and might be able to get through the BBB without much difficulty [112]. Therefore, NPs with diameters in this range (100–200 nm) would be optimal for in vivo applications [113].

The control of the PLGA NP size during synthesis can be carried out by modifying surfactant or polymer concentrations, molecular weight of the polymer, solvent type, and/or phase ratio. The most frequently used surfactants include polysorbates (e.g., Tween 20, Tween 80) and poloxamers (e.g., Pluronic F127, Pluronic F68), poly(vinyl alcohol)s (PVA, e.g., Mowiol X-88 and X-98) [114,115]. Different organic solvents such as ethyl acetate, propylene carbonate, acetone and dichloromethane [116] allow production of smaller PLGA NPs.

Consequently, careful tuning of these features has enabled significant advances in designing novel formulations for improved drug encapsulation parameters [117], particle stability, pharmacokinetics and release profiles [118].

6.1.2. Hydrophilic Surface

Due to their hydrophobic nature, PLGA NPs are recognized as foreign substances by the cells of the mononuclear phagocyte system, which are responsible for the clearance of particles from the blood stream [5]. As a result, PLGA NPs experience high rates of opsonization and thus their accumulation in target cells and tissues is reduced. Therefore, in order to reduce PLGA NPs immunogenicity, several strategies to create a hydrophilic surroundings of these NPs have been developed [97]. Examples of PLGA NPs functionalization to provide such hydrophilic coatings include:

- Polyethylene glycol (PEG): in 2007, Tang et al. [119] demonstrated that PEG-based NPs present a tightly packed composite for a longer circulation time in plasma.
- Human serum albumin (HSA): which is the most abundant native protein in the human body and shows several advantages including high availability, biodegradability, and low toxicity and immunogenicity [120]. In 2015, Esfandyari-Manesh et al. [121] decorated PLGA NPs with HSA, which resulted in a longer blood circulation of NPs.
- Polyethylene oxide (PEO): in contact with an aqueous environment, highly hydrated and flexible PEO chains form dense "conformational clouds" over the particle surface, impairing interactions with approaching opsonins as well as phagocytic cells [122]. In 1999, De Jaeghere et al. [123] demonstrated a clear relationship between PEO content and the decrease of uptake by the MPS cells.
- Poloxamers and poloxamines, also known as Pluronic® and Tetronic® macromolecules, respectively [124]: they strongly adsorb onto the surface of hydrophobic nanospheres, protecting them from quick engulfing by macrophages [125].
- Polysorbate 80, d-α-tocopheryl PEG 1000 succinate (TPGS) and polysaccharides like dextran [74,126].

PEG is the most commonly used polymer used to create a hydrophilic shell surrounding NPs by grafting the PEG chains onto the surface of NPs and thus hindering biomacromolecules from penetrating into the polymer layer by stearic stabilization, and by binding to the underlying core via hydrophobic or electrostatic interactions [127]. As a result, PEG coatings on NPs increase the hydrophilicity and solubility of the formulation, shielding the surface from aggregation, opsonization and phagocytosis by the reticuloendothelial system, thereby prolonging blood circulation time [128].

Indeed, Sánchez-López et al. [110] developed memantine-PEG-PLGA NPs formulation for the treatment of AD. The resulting NPs had a mean particle size below 200 nm and were able to overcome the BBB both in vitro and in vivo, reaching the hippocampus and increasing drug concentration at the target site, and thereby enhancing memantine's effects against AD. This is consistent with what other authors observed when PEGylating polymeric NPs. For instance, Liu et al. [129] also developed PEGylated NPs with an average size smaller than 200 nm and demonstrated that they were able to effectively overcome the BBB. Moreover, Tobío et al. [130] prepared PEGylated NPs with a mean size of about 160 nm and demonstrated that PEG entrapment provided additional protection against enzyme-induced aggregation and degradation in simulated gastrointestinal fluids in vitro.

In addition, mice treated with these NPs revealed a decrease in memory impairment when compared to mice treated with the free drug solution. Moreover, histological studies confirmed that memantine-PEG–PLGA NPs reduced β-amyloid plaques and the associated inflammation characteristic of AD. Therefore, the loading of memantine into these particles could be a promising alternative to improve the treatment of AD patients [110].

To escape from interaction with serum proteins and with mononuclear phagocyte system cells, the PEG chains surrounding nanoparticles should provide a sufficiently thick layer for steric hindrance. As the molecular weight of grafted PEG chains is directly proportional to the length of the polymeric chain, that parameter is instrumental for effective surface shielding [128]. A PEG molecular weight of 2 kDa or greater is commonly required to shield the NP surface from protein adsorption and to reduce NP recognition by the MPS, thus increasing the blood half-life of the NPs. This may be due, in part, to the increased chain flexibility of higher molecular weight PEG chains [113].

However, despite improved pharmacokinetics, PEGylation may also induce degradation of PLGA NPs and promote drug leakage or faster release [73]. This is due to the hydrophilic nature of PEG chains, which promote water absorption and stimulate the decomposition of PLGA chains [131].

6.2. BBB Transcytosis

6.2.1. Cell Penetrating Peptides

Cell-penetrating peptides (CPPs), also known as membrane translocation sequences or protein transduction domains, are short cationic or amphipathic peptides with specific conserved sequences (between 7 and 30 amino acid residues) that have been widely used in drug delivery for their capacity of transporting cargoes into cells [132]. Indeed, they can traverse cell membranes and penetrate the BBB, and thus facilitate cellular uptake. However, their lack of specificity greatly restricts their application as pharmaceutical tools (unwanted peripheral effects may result), and hence methods of targeting CPPs are being investigated [97].

The incorporation of reactive maleimide groups on PLGA NPs allows efficient conjugation of CPPs to the surface of these PLGA NPs (Figure 8). Indeed, the maleimide-thiol reaction is frequently used for functionalization of particles because of its selectivity towards thiol groups at physiological pH, the formation of a thioether bond that is relatively stable, and the high reactivity of maleimide under mild conditions (i.e., at room temperature and using aqueous buffers) [133]. Besides, the thiol group of cysteine residues naturally present in peptides and proteins (or easily introduced in these molecules) facilitates this reaction [134].

Despite their wide application, there are still some limitations regarding the stability of maleimide-based linkers [135]. Indeed, it has been demonstrated that under certain conditions the Michael addition reaction is reversible [136–138]. Thiosuccinimides linkages are susceptible to undergo a retro-Michael reaction (deconjugation), which cleaves the thioether bond and thus reverts succinimide to maleimide and free thiol [139]. This leads to a lower conjugation efficacy and considerably decreased in vivo stability [140,141], since the released maleimide may react with other thiol-reactive species, and the released thiol may react with other compounds in vivo [142].

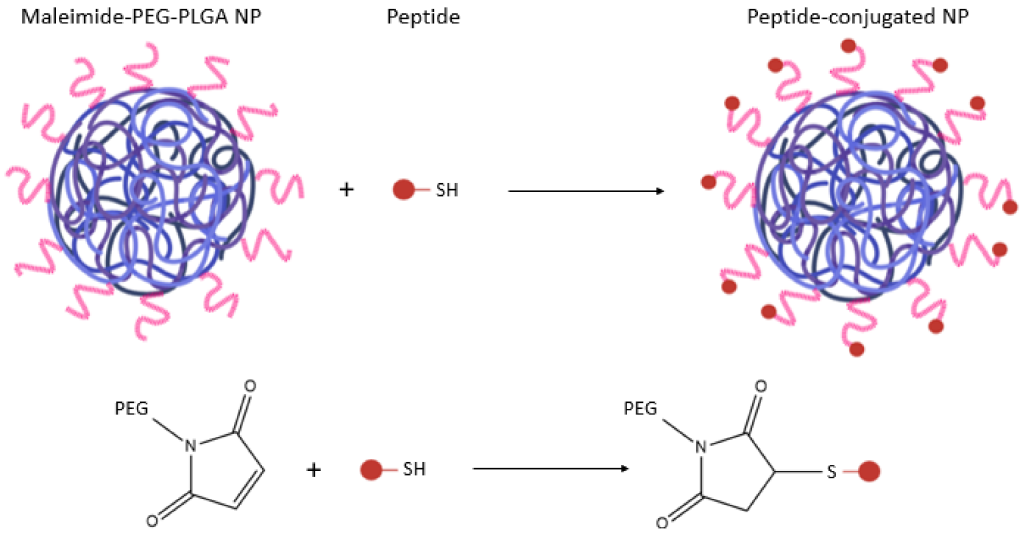

Figure 8. Conjugation of peptides to the surface of PLGA NPs through the maleimide-thiol reaction (based on [134]).

EDC-NHS coupling can also be used to conjugate peptides to the NPs. Upon exposure of EDC/NHS to the carboxyl groups, reactive NHS esters are expected to be formed [143]. Therefore, when the peptide primary amine group comes into contact with the ester, a covalent amide bond is formed [144,145], allowing for efficient peptide conjugation.

Angiopep-2 (Ang-2) is a promising ligand for targeted delivery to the brain. This peptide, composed of 19 amino acids derived from proteins known to interact with the LDL receptor related protein family, was identified and designed in 2008 [146]. Indeed, Ang-2 binds to LRP1, which is widely expressed throughout the CNS (by CNS endothelial cells, neuroblasts, microglia, astrocytes and neurons) [147–150]. Moreover, this ligand is able to activate receptor-mediated transcytosis and cross the BBB due to the existence of a corresponding receptor of Ang-2 expressed on the brain endothelial cells. Therefore, this brain-targeting peptide has been conjugated to numerous nanocarrier types in order to improve BBB crossing, including PLGA NPs [151]. Indeed, PLGA-PEG-Ang-2 NPs prepared by Hoyos-Ceballos et al. [99] had sizes lower than 200 nm, were compatible with systemic administration, and enabled possible BBB crossing. In vivo analysis confirmed a clear brain accumulation in different areas (i.e., hippocampus and cortex), unlike nonmodified NPs and modified NPs used as controls, which were unable to overcome the BBB and enter the brain [99]. Consequently, these formulations show a promising brain drug delivery system and could be used as carriers of different drugs to the CNS, thus increasing the alternatives for the treatment of brain diseases like AD [99]. However, additional studies are needed to clarify the mechanism involved in the entry of Ang–2 NP into the brain or the cells [99].

PLGA-PEG NPs loaded with both S1 peptide (PQVGHL) and curcumin to target the damaging factors in AD development, and conjugated with the brain-targeting peptide CRT, were prepared [107]. CRT is used to improve BBB penetration of drugs, since it is an iron-mimic peptide that targets the protein complex of transferrin and the transferrin receptor (TfR). The average NP size was around 130 nm, which was acceptable for BBB penetration. Consistent with previous reports [152,153], the S1 and curcumin loaded NPs crossed the BBB with low efficiency, whereas the CRT-NP-S1+Cur NPs had remarkably improved cellular uptake and BBB penetration ability in vitro, which suggests that the CRT peptide increases permeation of the BBB to PLGA NPs. According to these results, the higher number of CRT-NPs in the brain, the more they penetrate, indicting the contribution of CRT to increasing the penetration of PLGA NPs. The results of the behavioural tests demonstrated that these NPs significantly improved the spatial memory and recognition in AD mice, in addition to remarkably decreasing the Aβ deposit burden. Therefore,

compared to other PLGA NPs, CRT peptide-modified PLGA NPs codelivering S1 and curcumin showed potential advances for the treatment of AD mice [107].

Following the same line, B6 peptide (CGHKAKGPRK) is also considered as a promising candidate for enhancing drug delivery into the CNS due to its ability to target TfR as a substitute for the transferrin protein [154–156]. In fact, it is derived from a phage display library, and has previously shown high permeability across the BBB [157] and higher accumulation in brain capillary endothelial cells [158]. Thus, it is suggested that it would also improve curcumin bioavailability in the brain. Therefore, Fan et al. [106] prepared PLGA-PEG NPs loaded with curcumin and conjugated with the B6 peptide, obtaining NPs with a size less than 150 nm. These PLGA-PEG-B6/Cur NPs significantly increased curcumin cellular uptake in vitro and could remarkably improve cognitive impairment in APP/PS1 mice, indicating that these NPs can profoundly improve curcumin delivery efficiency to the brain. Moreover, ex vivo studies demonstrated that they could reduce hippocampal β-amyloid formation and deposits, as well as tau hyperphosphorylation. Thus, these NPs may serve as an interesting strategy for the treatment of AD [106].

Another strategy to overcome the hydrophobic nature and nonsolubility in water of curcumin is conjugating PLGA NPs with a targeting Tet-1 peptide moiety. Tet-1 is a 12- amino acid peptide (HLNILSTLWKYR) identified through phage display, which has the binding features of tetanus toxin [159], thus presenting affinity to neurons, and could target cargos to the brain, bypassing the BBB [160]. For this reason, Mathew et al. [104] synthesized water-soluble PLGA coated-curcumin NPs and coupled these NPs with Tet-1, resulting in NPs with a size between 150-200 nm. Surface modification with Tet-1 peptide increased neuronal targeting efficiency in vitro. Indeed, enhanced neuronal uptake of curcumin-PLGA NPs was observed when compared to nontargeted curcumin-PLGA NPs. Therefore, these results indicate that Tet-1-targeted PLGA-coated curcumin NPs can be additional tools for the treatment of neurological diseases, in particular AD, with respect to its antiamyloid and antioxidant activities [104]. However, this is a preliminary in vitro study that needs more detailed in vivo investigations to draw further conclusions.

Li et al. [101] employed another phage display peptide library with the purpose of isolating peptides that may be exploited to target delivery systems to the BBB and finally selected a 12-amino-acid-peptide (denoted as Pep TGN). They covalently conjugated Pep TGN (TGNYKALHPHNG) onto the surface of PEG-PLGA based NPs. The surface-modified PLGA NPs with Pep TGN had sizes about 100 nm and resulted in an improved delivery of NPs across the BBB and brain targeting. This led to significant higher cellular uptake in vitro and enhanced in vivo brain accumulation, rather than liver and spleen accumulation, showing powerful brain selectivity of Pep TGN. Therefore, Pep TGN modified NPs might be a good strategy to targeted drug delivery across the BBB [101]. Indeed, the ability of TGN to facilitate the delivery of NPs to the brain has been further demonstrated [161,162], confirming that significantly higher cellular uptake and brain distribution occurred with TGN-modified NPs compared to naked NPs.

Moreover, peptides such as g7, that are similar to synthetic opioid peptides [163], can also be employed to deliver drugs into the CNS. In fact, it was previously demonstrated that PLGA-NPs conjugated with g7 are able to efficiently cross the BBB without damage [164]. Therefore, Barbara et al. [83] designed and engineered curcumin-encapsulated PLGA NPs bound to g7 (Cur-NP-g7). The resulting NPs had a particle size around 200–250 nm, favourable for systemic administration. The authors demonstrated that Cur-NP-g7 could increase the cellular affinity of the active neuronal cells and be internalized by hippocampal neurons. Besides, they determined the effect of Cur-NP-g7 on the aggregation of Aβ, which showed an important decrease of Aβ. Thus, brain delivery of curcumin using BBB-crossing PLGA-g7 NPs is a promising alternative in the treatment of AD. However, this study has been verified by an in vitro cellular model and would need a complete in vivo study in AD animal models so that further conclusions could be drawn.

6.2.2. Receptor-Mediated Transcytosis

CNS endothelial cells express receptors, such as TfR, low-density lipoprotein receptor (LDLR), insulin-like growth factor receptor, insulin receptor, diphtheria toxin receptor, scavenger receptor class B type and nicotinic acetylcholine receptor (nAChR), onto their surfaces. Thus, delivery systems can be decorated with targeting ligands that specifically recognise these receptors to mediate drug penetration to the brain [165].

Polysorbate 80 (P80, also known as Tween 80) is particularly interesting for brain delivery, since PBCA NPs coated with P80 have already facilitated brain delivery of several drugs that were unable to cross the BBB in a free form [166]. Poloxamer 188 (P188, also known as Pluronic F-68) was also found to be effective as an NP coating material for brain targeting [97]. This effect is attributed to the enhanced adsorption of certain plasma proteins (especially the apolipoproteins E, A-I and B) onto the NPs.

Indeed, after being exposed to serum or plasma, various proteins quickly adsorb onto foreign NPs [167]. This effect is known as differential protein adsorption, and was first described in 1989 [168]. The postulated hypothesis is that the adsorption of apolipoproteins onto P80/P188-coated NPs is responsible for the subsequent interaction with their respective receptors expressed by the endothelial cells forming the BBB, and thus promotes receptor-mediated endocytosis of NPs, facilitating their delivery into the brain [169]. Therefore, all these nanoparticle DDS appear to act like Trojan horses that would transport the drugs into the brain endothelial cells and, in this way, represent a novel platform technology for the treatment of neurodegenerative diseases [170].

Moreover, the coating of particles with P80 causes a rearrangement of the proteins composing the tight junctions at the BBB which results in the increase of paracellular crossing of NPs into the brain [171,172]. Another reason is attributed to the role of P80 in blocking the efflux system, reducing the pump-off effect of the P-glycoprotein, and thereby achieving a high drug concentration in the brain [173].

Tahara et al. [102] studied different surface-modified PLGA NPs formulations based on P80, P188 and CS for targeting CNS diseases. The resulting PLGA NPs had sizes ranging from 250 to 400 nm depending on the type of surface modifier used, and after systemic administration, NP concentration in the brain increased with the surface modification of the particles, in particular, CS and P80 PLGA NP. However, CS-PLGA NPs were only adsorbed on endothelial cells and not transferred into brain tissue, whereas a higher brain distribution was seen with P80-PLGA NPs, which were seen in the parenchyma beyond the cerebral blood vessel endothelial cells. This suggested that P80-PLGA NPs could not only adsorb to the endothelial membrane of cerebral blood vessels but could also be internalized by endothelial cells and cross the BBB. Moreover, P80-PLGA NPs exhibited prolonged circulation in the blood compared to the other NPs evaluated, which might be a reason for the increased brain distribution by avoiding uptake by RES. Therefore, P80-PLGA NPs have high potential as effective drug carriers for CNS delivery [102].

This was further confirmed by Fornaguera et al. [174]. Indeed, they demonstrated the capability of galantamine-PLGA NPs to cross the BBB because of the permeabilizing and targeting effect of P80, leading to a formulation with interesting properties to be used as advanced DDS for the symptomatic treatment of AD.

As already mentioned, receptors that are highly expressed on CNS endothelial cells include TfR. Lactoferrin (Lf) is a naturally occurring iron-binding glycoprotein belonging to the transferrin family. Its receptor (LfR) is highly expressed in brain endothelial cells and in neurons, being especially overexpressed in capillaries and neurons associated with age-related neurodegenerative diseases [175,176]. This fact makes Lf a promising targeting molecule for the treatment of AD.

Therefore, Meng et al. [108] prepared Lf-PLGA NPs loaded with Huperzine A (HupA), a reversible AChE inhibitor which enhances memory in animal models [177,178]. The resulting NPs had an average size below 200 nm and Lf-surface modification increased cellular uptake of NPs through RMT, leading to improved brain delivery. Therefore, brain accumulation of Lf-TMC NPs was higher than nontargeted analogues, especially in

the memory-related hippocampus. This outcome suggests that Lf-PLGA NPs may be a promising approach for the delivery of HupA in AD [108]. However, future studies are required to continue evaluating their therapeutic efficacy in animal models of AD, and it should be noted that the use of large proteins such as lactoferrin protein can result in problems like synthesis procedure, stability and immunological response [157].

6.2.3. Carrier-Mediated Transport

Besides receptors, active transport in the BBB may also be exploited for brain targeting. The glutathione (GSH) transporter is highly expressed at the BBB [97]. This endogenous tripeptide thiol acts as an antioxidant and helps to protect cells from ROS [179].

Since there is a large number of GSH transporters at the BBB, GSH conjugated onto the PLGA NP surface is expected to bind to these transporters and increase the number of NPs at the BBB interface [179]. However, although the mechanism of GSH transportation through the brain cells was proven to be performed through a specific mechanism [180], the exact molecular mechanism remains to be elucidated.

Paka et al. [105] developed GSH-functionalized PLGA-PEG NPs to be loaded with curcumin. The resulting NPs, of mean size between 149 to 180 nm and coated with both PEG and GSH, increased improved drug uptake in vitro. Moreover, the internalized curcumin was found localized in almost every cell and their components, meaning that the GSH surface-modification allowed better cellular trafficking of the formulation. Novel insights into the development of effective delivery systems able to escape lysosomal degradation were described, therefore increasing the therapeutic effect of drugs useful for the treatment of AD [105]. However, in vivo experiments are still required to draw more general conclusions.

6.2.4. Adsorption-Mediated Transcytosis

In addition to particle size, surface charge (i.e., zeta potential) is also expected to affect NP cellular uptake and cytotoxicity. Therefore, nanoparticles with a positive surface charge are more suitable for cellular uptake. The surface of CNS endothelial cells shows a negative charge and thus, attracts positively charged nanoparticles to interact with the BBB through adsorption-mediated endocytosis [181].

In fact, while receptor-mediated transcytosis requires the initial binding of a ligand to the membrane of the BBB endothelial cells, absorptive-mediated transcytosis relies on nonspecific charge-based interactions [100]. Consequently, poor selectivity of absorptive-mediated transcytosis is predominant, since it can be easily initiated by polycationic compounds binding the negative charges onto the membrane.

Cationic surfactant coating is believed to render a positive charge to NPs, thus improving their interaction with cells and tissues [97]. For instance, dioctadecyldimethylammonium bromide (DODAB), a quaternary ammonium surfactant, can drastically alter the negative charge of PLGA NPs by preferential adsorption [182]. In the same way, didodecyldimethylammonium bromide (DMAB), another quaternary ammonium compound, is also used for nanoparticle stabilization. Peetla et al. [183] reported that DMAB enabled the interaction of NPs with a cell membrane model in a proportional fashion to their cellular uptake in vitro. Therefore, Xu et al. [103] prepared DMAB-PLGA NPs loaded with coumarin-6 and observed that surface modification with DMAB notably improved cellular uptake in vitro, which was size-dependent with an optimal particle size of 100 nm. Indeed, DMAB-modified NPs showed smaller sizes and higher zeta potentials than the PVA-coated NPs, meaning that the DMAB-coated nanoparticles could be entrapped by the cells more easily, increasing cellular uptake.

In addition to cationic surfactants, cationic polymers such as chitosan (CS) can be employed. As previously mentioned, CS is a natural cationic polymer which has been demonstrated to promote AMT, thereby enhancing NP cellular uptake. Indeed, Tahara et al. [102] investigated CS as a surface-modifying agent to improve PLGA NPs brain delivery. NP surface modification with CS increased NP concentrations in the brain compared to un-

modified NPs. Moreover, CS-PLGA NPs were absorbed onto the cerebral blood vessel by adhesion to endothelial cells by means of electrostatic interaction with cell membranes, thereby enhancing cellular uptake of CS-PLGA NPs, whereas unabsorbed particles were eliminated rapidly from blood circulation due to uptake by the RES. Therefore, PLGA NPs surface-modified with CS may play an interesting role for CNS drug delivery.

Trimethylated chitosan (TMC) is a quaternized CS derivative that is positively charged under physiological conditions [184]. As a cationic ligand, TMC facilitates NP active transport via absorptive-mediated transcytosis. Thus, TMC-modified NPs can be exploited for delivery to the brain [185,186]. Wang et al. [100] prepared PLGA-NPs and then employed covalent binding to attach TMC to the surface of PLGA–NPs and form TMC/PLGA-NPs. The obtained particles showed a mean diameter of 150 nm and were distributed in the periventricular region of the cortex and the third ventricle extensively, while no brain uptake of unmodified PLGA–NPs was seen, showing that positively charged TMC contributed to the electrostatic interaction with the anionic binding sites of the bran capillaries. This triggered the absorptive-mediated transcytosis pathway, followed by the uptake of NPs through the BBB, reaching the brain parenchyma. The increased cellular uptake and transport into the brain after surface modification with TMC was further demonstrated by Meng et al. [108]. Moreover, TMC formed a hydrophilic surrounding, which also contributed to this enhancement and avoided uptake by the mononuclear phagocytic system. As a result, behavioural tests conducted in mice showed that these NPs greatly reduced memory impairment by restoring it to a normal level. Besides, the senile plaque and biochemical parameter tests confirmed the brain-targeted effects of TMC/PLGA–NPs. Taken together, these results indicate that TMC surface-modified NPs are able to cross the BBB and could be a promising strategy for brain targeting with low toxicity [100].

6.3. Post-Transcytosis NP-Brain Interaction

As PLGA NPs are able to reach the brain, targeted delivery systems are needed so that NPs can enter the brain cells [97]. As already mentioned, CNS endothelial cells overexpress several receptors, including TfRs, and thus, another promising strategy to improve drug transport to the brain is using monoclonal antibodies (mAbs) to target TfRs. Indeed, TfRs are overexpressed in the brain capillary endothelium and have been demonstrated to undergo endocytosis (receptor mediated transcytosis) through the BBB [187]. Thus, antitransferrin receptor monoclonal antibodies such as OX26 are being used for BBB crossing.

In fact, OX26 ability to recognize and bind to cells that express the TfR, such as the BBB endothelial cells, has been well described [188]. Loureiro et al. [109] proposed PEG-PLGA NPs functionalized with two mAbs to deliver encapsulated antiamyloid iAβ5 peptide into the brain for AD treatment. On the one hand, PEG-PLGA NPs were conjugated with OX26 mAb to bind to the TfR and cross the BBB; they were conjugated with DE2B4 mAb to bind to the Aβ peptide, the major constituent of AD plaques, thereby acting as a targeting ligand.

The resulting PLGA NPs, of mean size of 150–170 nm, were compatible with the parenteral route. The in vitro uptake of PEGylated-PLGA NPs (without mAbs attached) was significantly lower when compared with the uptake of the immune NPs, thereby confirming OX26 ability to increase the cellular uptake of NPs. Furthermore, PLGA NP cellular uptake increases with the density of surface-immobilized antibody [189], explaining the increased cellular uptake of NPs from 8% (with OX26) to 14% (with OX26 and DE2B4). Thus, the formulation of PLGA NPs conjugated with these two antibodies is a promising system to protect antiamyloid peptides from proteolytic degradation and to increase their uptake in the brain. However, the mechanism of internalization of PLGA immune NPs by brain capillary endothelial cells needs to be elucidated, and future work is required to confirm that these NPs are efficient for the treatment of AD in transgenic models. Moreover, as already mentioned, the usage of large proteins such as TfR antibodies can result in problems like synthesis procedure, stability and immunological response [157].

7. Conclusions

Although much progress has been made towards the understanding of AD pathophysiology, there is still no clinically accepted treatment to cure or halt its progression. Moreover, the structure of the BBB is a major obstacle to the delivery of drugs into the brain for the treatment of CNS diseases like AD. Therefore, nanotechnology-based DDS such as PLGA NPs have emerged and are under investigation. Indeed, the use of PLGA NPs appears to be a promising direction for the treatment of neurodegenerative diseases, since several strategies to enhance the transport of NPs through the BBB have been developed. Indeed, some of the latest approaches of PLGA NPs surface modification were discussed in this review article, and in most cases, a single functionalization strategy was not enough. PLGA NPs composition needs to be optimized, choosing appropriate components to obtain PLGA formulations able to achieve BBB crossing and precise targeting, so that these formulations can play a vital role in AD therapy.

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