HHS Public Access

Author manuscript

Mater Today (Kidlington). Author manuscript; available in PMC 2021 July 01.

Published in final edited form as:

Mater Today (Kidlington). 2020 ; 37: 112–125. doi:10.1016/j.mattod.2020.02.001.

Overcoming blood-brain barrier transport: Advances in nanoparticle-based drug delivery strategies

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Abstract

The Blood-Brain Barrier (BBB), a unique structure in the central nervous system (CNS), protects the brain from bloodborne pathogens by its excellent barrier properties. Nevertheless, this barrier limits therapeutic efficacy and becomes one of the biggest challenges in new drug development for neurodegenerative disease and brain cancer. Recent breakthroughs in nanotechnology have resulted in various nanoparticles (NPs) as drug carriers to cross the BBB by different methods. This review presents the current understanding of advanced NP-mediated non-invasive drug delivery for the treatment of neurological disorders. Herein, the complex compositions and special characteristics of BBB are elucidated exhaustively. Moreover, versatile drug nanocarriers with their recent applications and their pathways on different drug delivery strategies to overcome the formidable BBB obstacle are briefly discussed. In terms of significance, this paper provides a general understanding of how various properties of nanoparticles aid in drug delivery through BBB and usher the development of novel nanotechnology-based nanomaterials for cerebral disease therapies.

Keywords

blood-brain barrier; nanoparticle; drug delivery; transcytosis

1. Introduction

The term “blood-brain barrier” was first introduced by Lewandowsky and his co-workers based on the observations that the intravenous injection of dyes (e.g., Prussian blue, trypan blue) or chemicals (e.g., cholic acids, sodium ferrocyanide) has little or no pharmacological effects on the central nervous system (CNS), whereas intraventricular injection of the same substances has significant neurological symptoms [1, 2]. CNS endothelial cells along with astrocytes and pericytes constitute the primary components of BBB. The barrier properties of BBB are maintained and regulated by the dynamic and continuous crosstalk among the cellular elements of the neurovascular unit. Usually, BBB acts as a protective layer that shields the brain by preventing its direct exposure to the bloodborne pathogens, and it

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maintains the homeostatic regulation of the brain microenvironment [3, 4]. Besides, BBB is also responsible for regulating the influx and efflux of ion, macromolecules, and nutrients [5].

An intact BBB is essential for the proper function of the brain. However, BBB would restrict therapeutic efficacy and present formidable challenges in developing new drugs for treating neurodegenerative diseases and brain cancer. Most importantly, brain diseases have severely affected human health nowadays. Millions of people throughout the world are suffered from neurodegenerative diseases such as Alzheimer's, Parkinson's, Lewy body dementia, frontotemporal dementia, amyotrophic lateral sclerosis, Huntington disease, and prion diseases [6–9]. In the US alone, one in every 60 Americans have Alzheimer's disease, and at least 500,000 Americans are living with Parkinson's disease. Treatment of these patients requires delivery of the therapeutics to the targeted location of the brain. However, the highly selective nature of BBB excludes all large-molecule therapeutics and more than 98% of all small-molecule drugs to reach the brain [10]. Therefore, it is urgent to address these bottlenecks via developing some new drug delivery approaches that can effectively deliver therapeutics to the brain without affecting the normal structures and functions of BBB.

In the last few decades, several therapeutic delivery strategies have been demonstrated to transport drug molecules across the BBB [11]. Among them, tight junction modulation by physical or chemical stimuli [11, 12] and drug molecule modification [13, 14] have shown some potential. Modulating tight junctions with various physical or chemical stimuli can potentially enhance the effectiveness of the drug transport process, but high concentrations of these stimuli can adversely affect the brain function [15]. Although modifications of drug molecules by lipidation are an effective way to cross BBB for passive penetration of therapeutics, the strategy is only suitable for small drug molecules (below 500 Da), limiting its wide-range of availability and usage [16]. Moreover, the Trojan horse strategy for transporting drugs through BBB is very challenging because of the highly selective nature of BBB [14]. Furthermore, due to the presence of P-glycoprotein (commonly referred to as multidrug resistance protein) at the luminal plasma membrane, drugs may return to the blood side by the ATP-dependent efflux pump even after successful penetration of drug molecules into BBB endothelium [17].

In recent years, the design of a noninvasive approach for the delivery of therapeutics and macromolecules to the brain has been at the forefront of research [18–22]. Most recently, with the advent of nanotechnology, various kinds of nanomaterials (Fig.1A) have been considered as promising carriers owing to their unique advantages such as small size, high drug-loading capacity, excellent stability, easy-to-design, biodegradability, and biocompatibility [23, 24]. Nano-carrier based transport techniques have become new dawn for drug delivery across BBB without disrupting its structure or functionalities. Fig. 1B summaries the number of research articles published in each year for drug, gene or therapeutic delivery across BBB utilizing the nanoparticle (NP) approach. This exponential growth of studies in this field indicates that the NP-based drug delivery across BBB is not only an emerging research topic, but also possesses huge application potential.

In this review, we primarily provide a comprehensive overview of the development and use of various NP-based drug delivery systems across the BBB. While several reviews have previously been published on strategies of NP-mediated brain drug delivery, the specific BBB features, role of NPs and their detailed working conditions have rarely been identified [23, 25]. We, therefore, focus on the distinct roles played by NPs on drug delivery across BBB, current successes/achievements of NP-based drug delivery, and future prospects NP-based technology to treat neurodegenerative diseases. Moreover, the recent understanding of BBB structure, drugs for brain diseases and different drug loading methods are also summarized.

2. BBB structure and transport routes

2.1 Neurovascular unit

The BBB exists in all organisms with a well-developed CNS, and it is primarily composed of microvasculature endothelial cells, astrocytes, and pericytes. Besides these three cells, some other components, such as smooth muscles, basement membrane, microglia, and neurons, also play roles in terms of immune function (Fig. 2A) [26]. Together with the endothelial cells, these associating cells maintain an intact BBB to ensure proper functionality of the central nervous system and usually referred to as a neurovascular unit [27].

2.1.1 Endothelial Cells—Endothelial cells are the basic building blocks of the BBB endothelium, which form a thin layer by connecting each other through extremely tight junction. Due to the tight junction, the connection between endothelial cells at BBB is ~50–100 times tighter than endothelial cells at the peripheral micro-vessel wall [28–30]. As a consequence, the intercellular junctions between the BBB endothelial cells have no fenestration even when treated with a vascular endothelial growth factor [31]. Moreover, unlike endothelial cells in the rest of the body, BBB endothelial cells have very few pinocytotic vesicles [32]. Because of these special properties, ions or small molecules (e.g. iron or glucose) [33,34] are transported across BBB by an enzyme assisted process, and this behavior is usually known as active transport. This active transport of nutrients from the blood to the brain requires greater energy potential than the diffusive transport occurring in the endothelium of other body parts. The BBB endothelial cells have five to six times more mitochondria per capillary section than that of skeletal muscle capillaries [35], and it has been thought that these excess mitochondria provide the required energy for active transport across BBB. Besides the physical barrier, BBB endothelial cells offer an enzymatic barrier due to the presence of proteolytic enzymes including γ-glutamyl transpeptidase, alkaline phosphatase, and aromatic acid decarboxylase [27]. This enzymatic barrier has the capability to break down the neuroactive bloodborne solutes and drugs.

2.1.2 Pericytes—The term pericyte originates from its early anatomical descriptions (‘peri’ means around and ‘cyto’ means cell) which reflects its peri-endothelial location at the basal side of the microvessels [36,37]. Pericytes are contractile mural cells that partially wrap around the BBB endothelial cells [38,39]. The primary function of pericytes is to form two basal laminas (BL1 and BL2) together with the smooth muscle. The BL1 is the distinct

extracellular space between endothelial cells and pericytes, whereas BL2 is the extracellular matrix between pericytes and the glial end-feet bounding the brain parenchyma [5]. Moreover, the covered pericytes around endothelium determine the permeability of the BBB and control the BBB functions [40,41]. For example, the permeability of the BBB to a variety of molecules will increase with the deficiency in pericyte coverage [42]. Besides these aforementioned functions, several other functional aspects of BBB, such as the strengthening of tight junctions, BBB-specific gene expression, vesicle trafficking, and polarization of astrocytes end-feet, are also regulated by pericytes [40,42]. Thus, the interactions between pericytes and endothelial cells are critical for BBB regulation. Disruption of these interactions may lead to BBB dysfunction and neuroinflammation during CNS injury and disease.

2.1.3 Astrocytes—Astrocytes are a sub-type star-shaped glial cells in the central nervous system. Their end-feet form a complex network surrounding the endothelial cells and basal lamina, which link up the endothelial cells with microglia and neurons [5,43]. This complex end-feet network of astrocyte is indispensable for the proper BBB properties and functions. Evidence showed that brain endothelial cells cocultured with astrocytes are less vulnerable under different pathologic conditions [44]. Moreover, astrocytes also can increase the level of tight junction proteins by expressing pentraxin 3 and inhibit the differentiation of pericytes by binding with integrin α2 receptor via brain-deriving specific basement membrane protein (Laminin) [45,46]. Both functions are essential for maintaining BBB integrity and low permeability. Experimental results show that the cocultivation of brain endothelial cells with astrocytes elevates the anti-oxidative activity of the BBB, which is critical to protect the BBB against the oxidative stress [47]. In addition to their role in the BBB formation, astrocytes are also responsible for scaffolding, injury protection, homeostasis, and clearing of synapses, and they are considered as the primary workhorse of the CNS [48].

2.1.4 Other components of BBB—Two other important components of BBB are basement membranes and microglia. Basement membranes compose of complex extracellular matrix proteins that can provide support for endothelial cells and hence separate themselves from the underlying tissue [49]. In CNS, the vascular basement membrane wraps the smooth muscle and pericytes, and separates the endothelial cells from neurons and glial cells [50]. These properties contribute to vessel formation and guarantee the integrity of BBB [51].

Microglia are monocyte lineage cells located throughout the brain and spinal cord, and consist of approximately 5–20% of the total glial cell population in the brain parenchyma [52]. As the resident macrophage cells, they usually perform two main functions: immune defense and CNS maintenance [53]. Furthermore, increasing evidences indicate that activated microglia can modulate the expression of tight junctions, which increases the integrity and function of BBB [54]. Thus, the barrier properties of the BBB are maintained and regulated by the dynamic and continuous crosstalk among the cellular elements of the neurovascular unit.

2.2 Junctions at the blood-brain barrier

The extremely tight connection between two neighboring endothelial cells is facilitated by three distinct types of inter-endothelial cell junctions: tight junction, adherens junction and newly identified gap junction (Fig. 2B).

2.2.1 Tight junction—Several transmembrane and cytoplasmic proteins form the tight junction. The transmembrane proteins include junction adhesion molecules (JAMs), claudins, and occludins; whereas cytoplasmic proteins include zonula occludins (ZO), afadin (such as AF-6), cingulin, calcium/calmodulin-dependent serine protein kinase (CASK), monoclonal antibody 7H6, and many more. JAMs, members of the immunoglobin subfamily, are usually expressed by platelets, leukocytes as well as endothelial cells. They are highly localized on the tight junctions of BBB [55,56] and regulate endothelium permeability, cell polarity, and leukocytes migration [57]. The extracellular domain of JAMs regulates the interaction between the endothelial cell and leukocytes by combining synergies of β_1 and β_2 integrins; whereas the cytoplasmic domain of JAMs interacts with various tight junction-associated proteins such as ZO-1 and AF-6 [56,58]. Moreover, JAMs located on the surface of endothelial cells can also contribute to adhesive interactions with circulating platelets [55]. To date, four distinct types of JAMs are identified in the BBB: JAM-A, JAM-B, JAM-C, and JAM-D. Although JAMs have specific roles in BBB, the most critical transmembrane proteins for constructing tight junctions are claudins and occludins [5,59]. Claudins are small (27 kDa) transmembrane proteins and so far, four diverse types (claudin −1, −3, −5 and −12) are identified at the BBB. The extracellular domains of claudins form the tight junctions between two neighboring endothelial cells and seal the para-cellular cleft, while the intracellular parts of claudins connect actin filaments through cytoplasmic scaffolding proteins. Occludins are a type II transmembrane protein and have similar functions of claudins [60]. Occludin is also expressed in brain microvascular endothelial cells and exclusively localized at the tight junctions. Besides these above-mentioned transmembrane proteins, several other cytoplasmic proteins also contribute to constituting the intact tight junction structures. For instance, the monoclonal antibody 7H6 creates a link between scaffolding proteins and the actin cytoskeleton. The intracellular scaffolding proteins, which connect claudins and occludins to actin filaments include zonula occludens-1 (ZO-1), zonula occludens-2 (ZO-2), and zonula occludens-3 (ZO-3) [61–64]. In order to connect tight junction proteins with the actin filament, zonula occludens distribute its C-terminal over the surface of the plasma membrane and other actin-rich structures, while N-terminal part link with the tight junctions proteins, such as claudins and occludins [61].

2.2.2 Adherens junction—Adherens junction is particularly important for the BBB structural integrity and proper assembly of tight junction proteins. It is formed by transmembrane glycoproteins cadherins, which present at the basal side of cell-cell junctions in BBB endothelial cells. Vascular endothelial cadherin (VE-cadherin or cadherin-5) is a homo-dimeric transmembrane protein that spans the para-cellular cleft. In the para-cellular cleft, the extracellular domain of VE-cadherin of one endothelial cell forms a homo-dimer by connecting to VE-cadherin molecules of neighboring endothelial cells. This cleft holds the cells together giving the structural support to tissue. The cytoplasmic domain of VE-cadherin interacts with the actin filament through scaffolding proteins, such as p120, α-

catenin, β-catenin, and γ-catenin [5,65,66]. The distal region of VE-cadherin binds to the β-catenin which interacts with the α-catenin to link the VE-cadherin with actin filament [67]. The β-catenin is located in cell-cell junctions areas of normal human brain cells. Their stabilization can enhance the expression of claudin-3, the formation of BBB tight junction, and maintenance of BBB characteristics *in-vivo* and *in-vitro* [68]. Moreover, α-catenin can mediate the interaction of β-catenin with the actin cytoskeleton and γ-catenin (also known as plakoglobin) which can bind to the cytoplasmic domain of cadherin-5 to links cadherin complex to the cytoskeleton [65]. Other transmembrane proteins associated with the adherens junction are PECAM-1, CD99, and nectin. Unfortunately, the specific roles of these associated proteins are still unclear. Overall, the adherens junction is essential for the structural integrity of interendothelial cell connections, and any alteration of the adherens junction leads to the BBB disruption [69].

2.2.3 Gap junction—Gap junction is located between the tight and the adherens junction. In chordates, gap junctions are intercellular channels that are formed by hexamers of medium-sized families of integral proteins: connexins and pannexins [70]. Three connexins, Cx37, Cx40 and Cx43, have been identified in BBB, among which Cx43 is the most ubiquitously expressed connexins in brain endothelial cells Each connexin can form gap junctions following oligomerization in the endoplasmic reticulum and homo- or hetero-hexamerization at the plasma membrane. Connexins typically have four transmembrane-spanning domains with unstructured C- and N-terminal cytoplasmic tails. While the C-terminal cytoplasmic tail regulates gap junctions and hemichannels function, the N-terminal regulates their oligomerization in the endoplasmic reticulum. In BBB, gap junctions permit the exchange of ions and small metabolites between adjacent endothelial cells. Since BBB is an extremely hermetic system, this junctional exchange of small molecules is crucial for maintaining tissue homeostasis. In addition, in the blood-brain barrier, gap junctions are responsible to transduce metabolic signals [71]. Furthermore, gap junctions regulate BBB permeability by interacting with scaffolding proteins ZO-1 through the linkages of afadin-6 protein.

2.3 Transport pathways across the blood-brain barrier

Although BBB works as a barrier for transport of molecules between the circulating blood and the brain parenchyma, several transport routes exist for transporting proteins and peptides to maintain brain homeostasis. These transport routes include diffusional transport in the form of paracellular and transcellular transcytosis, transporter proteins mediated transcytosis, receptor-mediated transcytosis, adsorptive mediated transcytosis, and cell-mediated transcytosis (Fig. 2C) [11].

Paracellular diffusion is the transport of solute molecules through a space between two neighboring endothelial cells (Fig. 2C, inset a). The driving force for this non-specific transport mechanism is the negative concentration gradient from blood to the brain. Only small water-soluble molecules (molecular weight < 500 Da) can transport through the paracellular space [72]. It has been found that tight junction modulations can increase paracellular diffusion [73]. However, the tight junction modulation may also increase the permeability of BBB for other unwanted substances.

The diffusion of solute particles through the endothelial cell is called transcellular diffusion (Fig. 2C, inset b). Only selective small size substances with desirable lipid solubility, high hydrophilicity, and non-ionized compounds can transport BBB through this route [74]. Like paracellular diffusion, the driving mechanism of the transcellular diffusion is simply the negative concentration gradient. Nevertheless, lipid solubility and hydrophilicity help solutes to cross the endothelial cells. For example, alcohol and steroid hormones can penetrate BBB through transcellular diffusion by dissolving themselves into the cell plasma membrane [11]. Similar to the paracellular diffusion, transcellular diffusion is also a non-specific approach.

Transporter proteins, such as glucose transporter isoform GLUT-1 and large amino-acid transporter (LAT), can transport molecules across the BBB through an active transport mechanism (Fig. 2C, inset c) [75]. In this process, glucose or amino acids first bind with the transporter proteins at the blood side of the BBB. Then, the conformational change of transporter proteins is responsible for the transfer of glucose or amino acids into the brain side [11]. Antibody conjugation on the drug surfaces is not needed for this process, but drugs must be modified to satisfy the structural binding requirements of the transporter proteins. Moreover, these transporter proteins carry only specific substances (such as GLUT-1 transport only glucose) across BBB, which limits the applicability of this mechanism for drug delivery.

As stated earlier, due to the stringent characteristics of BBB offered by the tight junction, the transport of drugs through the brain capillary endothelial cells is very difficult. The challenge of drug delivery is further increased because of the presence of efflux pumps as shown in Fig. 2C (inset d) at the luminal side of BBB endothelial cells. These efflux pumps include P-glycoprotein, members of the multidrug resistance proteins and breast cancer resistance proteins [76]. These proteins collectively limit the accumulation of various hydrophobic molecules and potentially toxic substances in the brain. These proteins also prevent the therapeutics accretion in the brain through two phases. In the first phase, they collectively prevent the uptake of drug molecules by endothelial cells, while in the later stage they actively expel out the anticancer therapeutics, such as doxorubicine, daunorubicine, and vinblastine etc., from the brain. It is believed that ATP provides the necessary energy for the transportation of drugs against a negative concentration gradient [11]. In BBB, these efflux pumps have both positive and negative contributions. For instance, they are responsible for reducing neurotoxic harmful effects of drugs. On the other hand, they severely restrict the therapeutics distribution in the CNS that are beneficial to treat neuro-diseases. Therefore, the alteration of efflux pumps at the BBB might be a potential approach to boost the access of therapeutics into the brain and may offer new therapeutic options for many neurodegenerative diseases.

Another important mechanism for transporting drugs through BBB is to use the receptors on the cell surface, which is usually termed as receptor-mediated transcytosis (RMT). Nowadays, this specific transport mechanism is widely used for NP-based drug delivery because it could easily take advantage of receptors expressed on the apical surface of the BBB endothelial cells [77]. As shown in Fig. 2C (inset e), the transport of substances under this mechanism relies on endocytosis, a process by which materials enter into the cells from the outside world. In this process, the ligand binds with receptor specifically and then they

form an intracellular vesicle through membrane invagination. The most commonly targeted receptors for RMT are transferrin receptors, lactoferrin receptors, insulin receptors, diphtheria toxin receptors, and low-density lipoprotein receptors. In RMT, the membrane invagination is occurred either through clathrin or caveolae-mediated mechanism. In clathrin-mediated RMT, during the binding of ligands to surface receptors, clathrin triskelions assemble into a basket-like convex structure which helps to form the clathrin-coated pit on the cytoplasmic side of the endothelial cells [78]. On the other hand, caveolae, a caveolin enriched invaginations of the plasma membrane, forms a particular type of lipid draft, which leads to the formation of endocytic vesicles in caveolae-mediated RMT [79]. After vesicle formation, these vesicles are detached from the membrane and trafficked to three different destinations. Some vesicles are recycled to the apical side and a significant portion is directed to the basolateral membrane where they fuse and release their contents. The remaining go through the endosome-lysosome maturation process for degradation of their contents [80].

The adsorptive mediated transcytosis (AMT), a technique for transporting charged nanoparticles or macromolecules across BBB, is illustrated in inset f of Fig. 2C. The AMT technique takes advantage of the induced electrostatic interactions between positively charged drug carriers and negatively charged microdomains on the cytoplasm membrane [81]. Since this process does not involve any specific surface receptors, a large number of particles can bind on the cell surface with a lower binding affinity. Thus, AMT can potentially allow concentrated form of therapeutics delivery. However, cationic modifications of therapeutics or its carrier are needed during this process, which may affect the function of the therapeutics. Moreover, the AMT drug delivery method remains a non-specific process that may cause the accumulation of drugs in other organs.

Besides the aforementioned transport routes, cell-mediated transcytosis can also be used for drug delivery across BBB. The cell-mediated transport route (Fig. 2C, inset g) relies on immune cells (such as neutrophils, monocytes, and macrophages) which has the capability to cross the BBB in both healthy and disease conditions [82]. In cell-mediated transcytosis (aka “Trojan horse”). drugs are encapsulated in a liposome so that they can be quickly absorbed by immune cells of the circulating blood. These immune cells (along with the absorbed drug-loaded liposome) then cross the BBB and migrate toward the inflammation sites in the brain by using their unique properties called diapedesis and chemotaxis.

3. Types of nanoparticles for drug delivery across BBB

To treat the growing number of patients with neurodegenerative diseases, there is an urgent need for the development of non-invasive drug delivery methods that can mitigate the high cost and risk factors of traditional surgery, radiotherapy, and chemotherapy [83]. As shown in Fig. 1A, various nanoparticle-based delivery systems are widely used to transport drugs or other molecules (such as nucleic acids, proteins, or imaging agents) across the BBB without disrupting the normal function of the brain [84]. Here, we classify them into three common types including polymer-based, biomimetic-based and inorganic-based nanocarriers. In addition, some recently developed representative nanoplatforms are highlighted in Table 1.

3.1 Polymer-based NPs

Polymeric NPs provide several advantages for delivering drugs across the BBB. For example, they can improve the bioavailability of drugs by reducing enzymatic and hydrolytic degradation [11]. Most importantly, enhanced brain permeation and higher concentrations of drugs in the tumor can be achieved by using drug-loaded polymeric nanocarriers [25]. Poly(lactide-co-glycolic-acid) (PLGA), polyethylene glycol (PEG), and poly(lactic acid) (PLA) are three common polymer-based transport carriers [85,86]. Among them, PLGA NPs show the advantages of low toxicity, high biocompatibility, and highly controlled drug release [87]. Besides, the problems of drug solubility and the passive selectivity across the BBB can be avoided by PLGA NPs. For example, Ghosh and co-workers have demonstrated the transportation of PLGA NPs across the BBB for the therapy of glioma, in which they achieved high drug solubility and passage selectivity [88]. In their experiment, a novel synthetic peptide against somatostatin receptor 2 was grafted on PLGA NPs, which further enhanced NP transport efficiency. Moreover, results showed that this system could internalize drugs inside of glioma and induce apoptosis successfully. Because of the excellent biocompatibility of PEG and PLA NPs, this type of drug carrier can decrease the cytotoxicity of the drugs [89]. Moreover, biodegradable PEG and PLA were usually coated on the surface of NPs as a gatekeeping layer, which could enable controlled drug release. For example, Shen and co-workers utilized PLA as a ROS-responsive gatekeeper to coat mesoporous silica NPs, which could improve the drug release under high oxidative stress [86]. A dense PEG coating can benefit NPs to diffuse passively in the brain because PEG has a lower reticuloendothelial system uptake which can slow down the clearance of PEG-modified NPs [90]. Thus, PEGylation method is used to modify polymeric vectors to enhance their circulation time in the system and achieve efficient penetration and higher accumulation in the brain. As shown in Fig. 3A, researchers utilized these properties of PEGs and coated PEG on the surface of Au NPs. Their biostability and biocompatibility allow them to shuttle back and forth across the BBB for a long time under normal conditions. Moreover, they can dissolve in brain tumor cells quickly and aggregate drugs in the cancer region because of the acid-labile character of cancer cells [91].

Although polymeric NPs have taken key roles in this field, some issues still limit their further expansion and encourage us to seek alternatives. One important issue is that traditional linear polymers have few interaction sites and drug-loading areas. Currently, some exquisitely designed polymeric NPs with large specific surface areas are introduced for drug delivery through the BBB. For instance, dendrimers are a type of special stretched polymers which possess much more accurate controlled structures. A large number of controllable ‘peripheral’ functional groups can be attached to dendrimer NPs compared to traditional linear polymers [92]. As shown in Fig. 3B, polyamidoamine dendrimers (G5) were attached with PEG, CGS, Cy5.5, and cyclic[RGDyK] peptide, which furnishes biocompatibility, BBB penetration ability, signal responsiveness, and tumor targeting properties, respectively to these polymeric NPs [93]. For instance, the utilization of CGS can activate the A2A adenosine receptor and temporarily increase the intercellular space between brain capillary endothelial cells, and hence, more NPs pass through the BBB and spread into the brain side. Moreover, studies have shown that enhancing the generations of dendrimers may have potential in extending blood circulation times and increasing

accumulation in the injured brain [94]. However, one potential shortcoming of these carriers is that most of the polymeric NPs cannot track them in cells without attaching them with at least one fluorescent dye. Therefore, polymeric NPs require a complex synthesis process to attach fluorescent dye tracing molecules. Recently, we developed a novel fluorescent polymeric NPs based on poly [Triphenylamine-4-vinyl-(Pmethoxy-benzene)] (TEB), where we avoided the complex dye tracing method. In addition, this nanoparticle shows an improved transcytosis across BBB when functionalized with different ligands such as transferrin, lactoferrin, and lipoprotein [95]. Moreover, we have also developed a new mathematical model to predict the transport efficiency of TEB NPs across BBB [96].

3.2 Biomimetic-based NPs

Exogenous NPs used for drug delivery can easily be recognized by the immune system and cleared by the liver and kidney. Thus, the design of biomimetic NPs is getting tractions because these NPs can recognize and target ligand easily, remain in the blood circulation for a long term, and escape the immune system [97]. Chitosan (CS), derived from chitin by partial deacetylation, is considered to be a common biomimetic drug carrier due to its biocompatibility, minimal immunogenicity, biodegradability, and its ability to open cellular tight junctions [98]. Moreover, some natural vesicles (formed with membranes) such as liposomes, exosomes, red cell membranes, or “Leukolike” coated NPs have been studied widely in the field of brain drug delivery as important biomimetic NPs [99,100]. It is not surprising that phospholipid bilayer is responsible for its high biocompatibility. Fig. 3C shows the morphologies of one liposome-based drug delivery platform. Here liposome NPs were conjugated with six peptides and used to penetrate the BBB for chemotherapeutics of glioma [101]. Based on IVIS spectrum results (Fig. 3D and E), peptide modified liposomes could traverse the BBB and enhance the internalization of liposomes in the tumor site. Moreover, multifunctional or self-assembled proteins, like commonly used ferritin, are also utilized to form biomimetic nanovesicles for drug delivery [102–104]. Protein-based nanomaterials, as one of the colloidal systems, could enhance the cellular uptake and also possess many virtues such as non-toxic, biodegradable, non-antigenic and easy surface modification [105]. Based on these properties, protein-based nanoparticles have the potential to carry drugs that normally cannot cross the BBB after intravenous injection [106]. Fig. 3F and 3G show a work, in which researchers evaluated the stability of protein corona Au NPs transporting across the BBB [104]. In addition to protein-based materials, virus-like NPs (VLPs), a kind of noninfectious capsid protein-based NPs derived from several types of virus self-assembly, have been considered as vaccine and drug delivery candidates [107]. Herein, the capsid protein offers a Trojan horse strategy for encapsulated drugs or agents. An interesting work on engineered VLPs (as a nanocarrier) was reported by Anand et al (Fig. 3H) to transport across BBB, where they selected *Salmonella typhimurium* bacteriophage P22 capsid as a precursor and transported the analgesic marine snail peptide ziconotide into an in vitro BBB model successfully via an endocytic strategy [108].

3.3 Inorganic-based NPs

Due to high stability and distinct material- and size-dependent physicochemical properties, inorganic NPs have advantages over polymeric and biomimetic NPs in brain drug delivery [109]. Nowadays, versatile inorganic-based NPs with different structures have been widely

investigated [110,111]. It is easy to modify inorganic-based NPs with polymer or specific ligands to facilitate the delivery of therapeutics and macromolecules across the BBB. Silica nanoparticles (Si NPs), as an approved food additive by U.S Food and Drug Administration (FDA) [112], is one of the promising candidates for brain drug delivery due to its relatively low cost, good biocompatibility and manufacturing controllability [113–115]. In our group, the lactoferrin (Lf) modified Si-NPs have been prepared for investigating the size-dependent transport effciency of Si-NPs across the BBB model (Fig. 4A) [116]. Polyethylene glycol was conjugated on the surface of Si NPs to reduce protein adsorption. This Lf attached Si-NPs enhanced transport effciency across the BBB compared to bare Si-NPs. Lf modified Si-NPs with different sizes were also studied to evaluate transport efficiency. Experimental results showed that particles with the sice of 25 nm diameter have the highest transport efficiency, which is almost 4 times (21.3%) higher than that of bare Si-NPs. Moreover, we also compared the Si-NP transport efficiencies in one-cell (monolayer of endothelial cells) and three-cell (coculture of endothelial cells, pericytes, and astrocytes) BBB models [117]. Mesoporous silica nanoparticles (MSNs), as porous Si-based material, are also popularly used in the drug delivery system. They not only inherit excellent biocompatibility but also own substantial specific surface area for loading drugs or ligands [118]. As shown in Fig. 4B, Kuang et al. investigated a typical MSNs-based drug delivery system for treating glioma [119]. Au nanomaterials are another inorganic material that offers high potential in drug delivery. As an ideal photothermal therapy (PTT) candidate, some special Au nanomaterials could transfer photo energy into thermal energy under near-infrared (NIR) laser irradiation conditions. Owing to their excellent NIR absorption property, Yin et al. used Au-based NPs to dissociate the fibrous Aβ, which is a crucial factor in Alzheimer's disease [120]. The absence of fibrils network in the transmission electron microscope and atomic force microscope images indicated the effective ability of Au-based NPs for dissociating Aβ fibrils upon NIR irradiation (Fig. 4C). Silver and titanium dioxide NPs have also been used to cross the BBB [121,122]. Fig. 4D shows an example of Ag ion, Ag NPs and TiO_2 NPs crossing an in vitro BBB model [123].

Iron oxide NPs are actively being developed as drug carriers due to their magnetic properties which subsequently eliminates the off-target effects. Zhao's group developed a magnetic SiO_2@Fe_3O_4 nanoparticle-based carrier, attached to cell-penetrating peptide Tat, and studied its fates in accessing BBB [124]. Their experimental observations indicate that these particles could penetrate the brain endothelial cells effectively by virtue of cell-penetrating peptide Tat and magnetic properties of Fe_3O_4. Although inorganic NPs offer several advantages, they can bring several side effects on BBB properties and function. For example, a research team studied the adverse effects of SiO_2 NPs exposure on BBB and found that SiO_2 NPs could disturb BBB structure and induce BBB inflammation through ROS and ROCK-mediated pathways [125].

In summary, different NPs have different advantages and disadvantages. For example, the preparation of inorganic NPs still needs organic solvents or inorganic reagents which are very expensive. Moreover, the toxicity and in vivo clearance of inorganic NPs are still a major concern. On the contrary, polymeric and biomimetic-based NPs exhibit excellent biocompatibility, biodegradability and surface manipulation, but large NP size, poor

targeting efficacy, and production difficulty still limit their further application in the brain drug delivery.

4. Controlling parameters for drug delivery by NPs

As a newly emerging class of research, NPs have attracted significant interests in brain drug delivery due to their unique structures and multi-functionalities, such as mechanical properties (lightweight, good flexibility), high tunability and adaptability to determine the transport mechanism across the BBB [126–128]. It is widely acknowledged that the morphology and surface chemistry of nanomaterials have significant impacts on their physicochemical properties [129,130]. Tuning these properties (such as size, shape, surface charge, and coating ligands) of NPs could improve the therapeutic agent stability, avoid the RES, improve the controllability of the drug release mechanisms and enhance transport efficiency [131].

4.1 Size

Typically, several parameters affect the transport efficiency of NPs through BBB and drug delivery into the brain. The size of nanoparticles is one of the most important parameters for intracellular localization of NPs as well as NPs transport across the BBB [132]. For example, some research work suggests that the internalization of NPs of diameter around 50 nm is easier than other sizes for receptor-mediated endocytosis within endothelial cells [133,134]. Another group compared the permeability of silica NPs with different sizes (30, 100, 400 nm, and the micro-particles) through the BBB model [135]. They observed that the NPs with a diameter of 30 nm had the highest permeability coefficient among all the silica NPs, indicating a size-dependent property of BBB permeability. Similarly, 30 nm biocompatible NIR NPs were proved to have the superior capability for BBB damage evaluation than 10 nm and 60 nm NPs in the photothrombotic ischemia (PTI) model [136]. Although NPs with a smaller size can transport easily across the BBB, they are not suitable for drug delivery because of limited encapsulation efficiency, rapid drug release and excretion. Generally, NPs with a size of around 20 nm is large enough to avoid renal excretion and small enough to cross BBB which makes them an ideal candidate as nanocarriers for brain drug delivery.

4.2 Shape

The shape of nanomaterials also influences the cellular uptake of drugs [137–139]. In recent years, different shapes of NPs have been tested to identify the optimum shape for neuro disease treatment. These include spherical [140,141], cubic [142], rod-like [143], and ellipsoidal [144,145]. Towards this front, spherical NPs indeed hold significant advantages for drug delivery applications because of relatively easier preparation and surface modification [146]. However, nanorods coated with specific antibodies have been proved to have a higher adhesion capability than their spherical counterparts. For example, rod-shaped polystyrene NPs coated with transferrin showed a 7-fold increase in brain accumulation when compared to their spherical NP counterparts [147].

4.3 Surface charge

Nowadays, more and more attention has been placed on the effect of surface charge of NPs for drug delivery across BBB [148]. Zeta potential can directly affect the uptake of NPs due to the negatively charged nature of the cellular membranes. Thus, the internalization of neutral or negatively charged NPs is much more difficult compared to positive charged NPs. Apart from this, several other key factors such as particle biodistribution and blood circulation half-life are also associated with the surface charge of NPs. Alexis el al. presented the factors which can influence blood residence time and organ-specific accumulation of NPs [149]. They reached a conclusion that the neutral or negatively charged NPs could reduce the plasma protein adsorption and achieve a low rate of nonspecific cellular uptake resulting in a longer blood circulation half-life than positively charged NPs. Moreover, positively charged NPs have a toxic effect, which can disturb BBB integrity [148]. To avoid BBB destruction, NPs with negative zeta potential are favored for drug delivery in the brain. For example, Zhang et al. [150] conjugated the negatively charged peptide (peptide-22) to decrease the zeta potential of NPs, which has resulted in a significantly higher transport efficiency across the BBB. Another group reported high stability and excellent transport of poly(*n*-butyl cyanoacrylate) nanoparticles across BBB while coated with a negatively charged (− 35.2 ± 1.1 mV) polysorbate 80 [151].

4.4 Drug loading strategies

Efficient and convenient methods for drug loading is also crucial in designing an excellent drug delivery system since this will influence the amount and binding strength of loaded drugs. Thus, an optimal interaction between drugs and nanoparticles are also important. Too strong or too weak interactions will make it tough to release drugs or cause unnecessary early leakage, respectively. Similarly, too low drug loading would affect the treatment while too high may cause some side effects. Therefore, it is imperative to determine the appropriate binding between drugs and nanocarriers. Currently, three strategies including covalent bonding, non-covalent adsorption and direct embedding are mainly utilized to bind various CNS disease-related drugs with nanoparticles [6,152,153].

4.4.1 Covalent bonding—The covalent bond connection is a classic approach to bind drugs with nanoparticles. This method usually applies readily reversible condensation reactions consisting of ketals/acetal [154], boronate esters [155], and Schiff's base [156]. For example, anti-cancer drugs were immobilized on the surface of quantum dots via dehydration condensation between -NH_3 and -COOH [157]. However, due to the limited reversible condensation reactions, covalent bonding is considered as a less flexible strategy [158]. Moreover, time to reach the thermodynamic equilibrium is very long because of slow binding and dissociation caused by strong covalent interactions [159].

4.4.2 Non-covalent adsorption—Recently, non-covalent binding has become one of the most popular drug loading methods because of its simple operation and fast binding & release rate. Adsorption of drugs by the non-covalent methods can proceed through ion-ion electrostatic interactions, hydrogen bonding, halogen bonding, π–π stacking, coordination bonding, van der Waals interactions, or hydrophilic and hydrophobic properties [160,161]. Recently, the halogen bond is also used as a hit-to-lead-to-candidate to enhance drug-target

binding affinity for rational drug design [162,163]. Some works also focused on anchoring bio-medicines on nanocarriers by employing the synergetic effect of multiple non-covalent interactions, which could provide more interaction sites and stronger binding force [164,165].

4.4.3 Drug encapsulation—Another drug loading method is entrapping drugs inside a vesicle formed by a closed phospholipid bilayer membrane [166]. In comparison to covalent or non-covalent immobilization, drug entrapment could potentially reduce unwanted early drug-tissue interaction. In addition to lipid nanovesicles, molecular imprinting technology is also employed to directly entrap drugs inside the 3D nanomaterials cavities, which could provide specific molecularly controlled delivery systems [6,167,168]. Tang et al. entrapped the aminoglutethimide drug and built a drug delivery system by a molecularly imprinted polymer [169]. Experimental results show that this material achieved rapid drug release rate and high bioavailability.

4.5 Ligands

Some research groups are conjugating ligands on polymeric NPs to increase drug delivery efficiency to the brain through the receptor-mediated system. When polymeric NPs conjugated to specific targeting agents, therapeutics delivery to tumors enhanced significantly [170]. For example, Gint4.T aptamer is specifically used to recognize the platelet-derived growth factor receptor β [170]. Results show that Gint4.T-conjugated PNPs efficiently cross the BBB and highly aggregate into U87MG glioblastoma (GBM) cells.

Ligands are very popularly used to transport NPs across BBB by receptor-mediated transcytosis [171]. For receptor-mediated transcytosis, several ligands including transferrin (Tf), low-density lipoprotein receptors (LDL) and lactoferrin (Lf) have been used to target receptors expressed on the BBB membrane [172]. Ligands, such as peptides [173], proteins [174], or antibodies [175], are usually conjugated on the surface of NPs to provide a high targeting affinity with receptors. As a matter of fact, most of the reported NP-based drug delivery systems have used ligands to cross the BBB by receptor-mediated transcytosis. Different kinds of ligands show various abilities to facilitate BBB penetration and can be categorized into different types as discussed below [176].

4.5.1 Use of ligands to develop protein corona—When NPs enter a physiological environment, their surface rapidly adsorbs proteins from the bloodstream and forms a protein coating, “protein corona” [177]. Over 70 different serum proteins from the bloodstream have been reported to adsorb onto the surface of NPs. Shubar et al. have modified Tween-80 by using surfactant-assisted synthetic methods on the surface of NPs to absorb apolipoprotein E from the bloodstream to form the protein corona for effective transportation through BBB [178]. The Tween-80 modified nanocomposites exhibited excellent biocompatibility and a significant amount of uptake when compared with NPs without coating. The biocompatibility of these NPs ensures drug delivery to the brain with lower cytotoxicity. In addition to biocompatibility, with appropriate designs, the protein corona may alter the surface chemistry of NPs, which improves drug delivery performance by enhancing surface functionalization and avidity [179]. But protein corona may accelerate

the clearance of NPs through the reticuloendothelial system (RES) in blood, which decreases the dose of NPs for brain drug delivery and induces inflammation [180]. Grafting NPs with surfactant molecules can minimize the surface fouling, decrease the clearance, and increase biocompatibility [181]. For example, PEG modification shows antifouling properties, minimal surface charge, low ionic interactions, and hydrogen bonding, which contribute to lower NP opsonization and long circulation time [90]. Lipka et al. indicated that a longer PEG chain with a length of 10 kDa improved the NP blood circulation time. They have reported that over 15% of the applied PEG-modified NPs were found in the bloodstream of mice subjects after 24 hours [182]. Thus, PEG grafting on NPs can effectively decrease protein adsorption and slows down the clearance of NPs [183], which results in more PEGylated NPs accumulation in the brain [181,184].

4.5.2 Use of ligands to target receptors on BBB—The ligand modified NPs can respond to receptors and increase BBB permeability than NPs without modification. Ulbrich et al. reported that the attachment of transferrin peptide on NPs can achieve well surface distribution even with smaller particle size [185]. The tunable surface peptide can target the transferrin receptor of the endothelial cells on BBB to initiate the transcytosis process. Very recently, several other targeting ligands have been reported, which could effectively attach to a variety of receptors [186].

4.5.3 Use of ligands to enhance NPs properties—The amphiphilic peptides monomers play a pivotal role in facilitating the uptake of NPs across the BBB, thus improves transport efficiency [187]. Generally, amphiphilic peptide modified NPs are stable and exhibit high affinity toward the BBB. The higher stability can be attributed to the energetic penalty associated with peptide strands, which increases unfavorable intermolecular electrostatic interactions.

The content of ligands and their receptor affinity have an important impact on transporting NPs across the BBB (avidity) [188]. Choi and coworkers investigated whether human transferrin (Tf) affect the PEGylated gold NPs (on tumor targeting) in mice bearing s.c. Neuro2A tumors. They found that the amount of targeting ligands significantly influences the number of NPs localized in cancer cells [189]. Moos et al. reported the optimal ligand density which yields the highest affinity towards targeting brain capillary endothelial cells and subsequent transport across the BBB [190]. Moreover, the modification of NPs with multiple targeting ligands showed higher targeting efficiency and better distribution for brain disease. Zhang et al. used a dual-targeting ligand to treat AD, in which TGN and QSH were used as ligands on PEG-PLA NPs. TGN is a specific target ligand at the BBB membrane, while QSH has a good affinity with AD disease cells. The NPs modified with both TGN and QSH had an excellent hippocampus-targeting effect compared to the bare NP and only TGN-modified NP [191]. Another group created a Y shaped liposome-based carrier conjugated with RGD and pHA ligands, which can penetrate both BBB (blood-brain barrier) and BBTB (blood-brain tumor barrier). In vivo, fluorescence imaging indicated that the liposome conjugated with two ligands exhibit superior nanocarrier distribution in tumor than single or no conjugation [192].

5. Conclusions

BBB is a primary obstacle in drug delivery to treat brain tumors and other neurodegenerative ailments. This review provides a comprehensive summary of the current achievements in NP-based drug carrier development for efficient drug delivery strategies across BBB. We specifically discussed various properties of NPs to shed light on the influencing factors for improved penetration efficiency in the pursuit of optimum drug delivery techniques. We do wish to emphasize here that several parameters influence the transport of NPs through the BBB. Among them, size, shape, ligand density, surface charges, as well as drug loading method are most notable. Although NPs-based systems have been widely exploited to develop a synthetic platform for brain drug delivery due to their unique properties, some critical problems were not well studied yet. Moreover, some challenges and obstacles still need to be addressed before functional nanocarriers could be effectively used for further clinical applications.

1. Biodegradation and biocompatibility of NPs are essential factors for biomedical applications and can directly decide their progress toward clinical translation. Although many studies have reported highly biocompatible NPs for transport across BBB, including polymer, biomimetic and inorganic NPs, their interactions with the immune system are complicated, and the potential health impacts are also unclear. It is worth noting that some polymeric NPs show higher biocompatibility and biodegradability in comparison with other nanomaterials. Therefore, further research is needed in solving the biological stability of polymeric NPs and achieving controllable drug delivery to the brain through the BBB.

2. The surface charge of NPs plays a contradictory role in crossing BBB and needs to be balanced. General knowledge dictates that cationic NPs are more favorable for crossing the BBB due to the complementary negative charge on the endothelial cells. On the other hand, anionic or neutral NPs show lower toxicity and longer circulation time compared to cationic NPs. Moreover, the existence of charge can cause non-specific adsorption with protein or peptide in the circulation system, which disturbs the normal drug delivery operation. Until now, the most useful strategy is to coat NPs with PEG chains, which results in minimal NP opsonization, decreased macrophage uptake, and prolonged blood circulation.

3. Designing nanocarriers for superior drug loading and effective drug release is a major challenge. Biocompatible nanocarriers suitable for controlled drug loading and release are rare. Moreover, limited drugs could be delivered to the brain tumor owing to the leakage of drugs during transportation. An ideal nanoparticle-based drug carrier must possess a high specific surface area and strong interactions to loaded drugs. Responsive porous materials can achieve high drug loading, and they have the potential to release drugs in a controlled manner in the targeted pathological area.

Nanotechnologies are providing unique opportunities for nano-carrier development to transport drugs at the targeted sites. Nowadays, along with overcoming BBB-crossing with special nanocarriers, multifunctional theranostic nanoplatforms are also developed, such as NP-based magnetic resonance imaging, computed tomography, and photoacoustic imaging. Although they are still in early-stage, ligand conjugated NPs show the best performance in transporting drugs through BBB and have led to promising results preclinically. Therefore, as the research continues, we believe that the NP-based drug delivery into the brain will has a huge opportunity and a broader prospect to cure cerebral diseases in the near future.

Acknowledgment

This work was supported by the National Institute of General Medical Sciences of the US National Institutes of Health under Award Number R01GM122081. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. S. Ding (CSC201806290030) would like to acknowledge the scholarships from the China Scholarship Council.

Abbreviations

ANG	angiopep-2
CGS	4-[2-[[6 Amino-9-(N-ethyl-β-d-ribofuranuronamidosyl)-9H-purin-2-yl] amino]ethyl]benzenepropanoic acid hydrochloride
CMC	Carboxymethyl cellulose
Den-RGD	poly amidoamine dendrimers-cyclo (Arg-Gly-Asp-d-Tyr-Lys)
HIV	Human immunodeficiency virus
LRP1	lipoprotein-receptor-related protein-1
MR	magnetic resonance
SERRS	surface-enhanced resonance Raman spectroscopy
SSTR2	Somatostatin receptor 2
TGN	TGNYKALHPHNG
QSH	QSHYRHISPAQV
DS	Dextran-spermine
Pen	Penetratin

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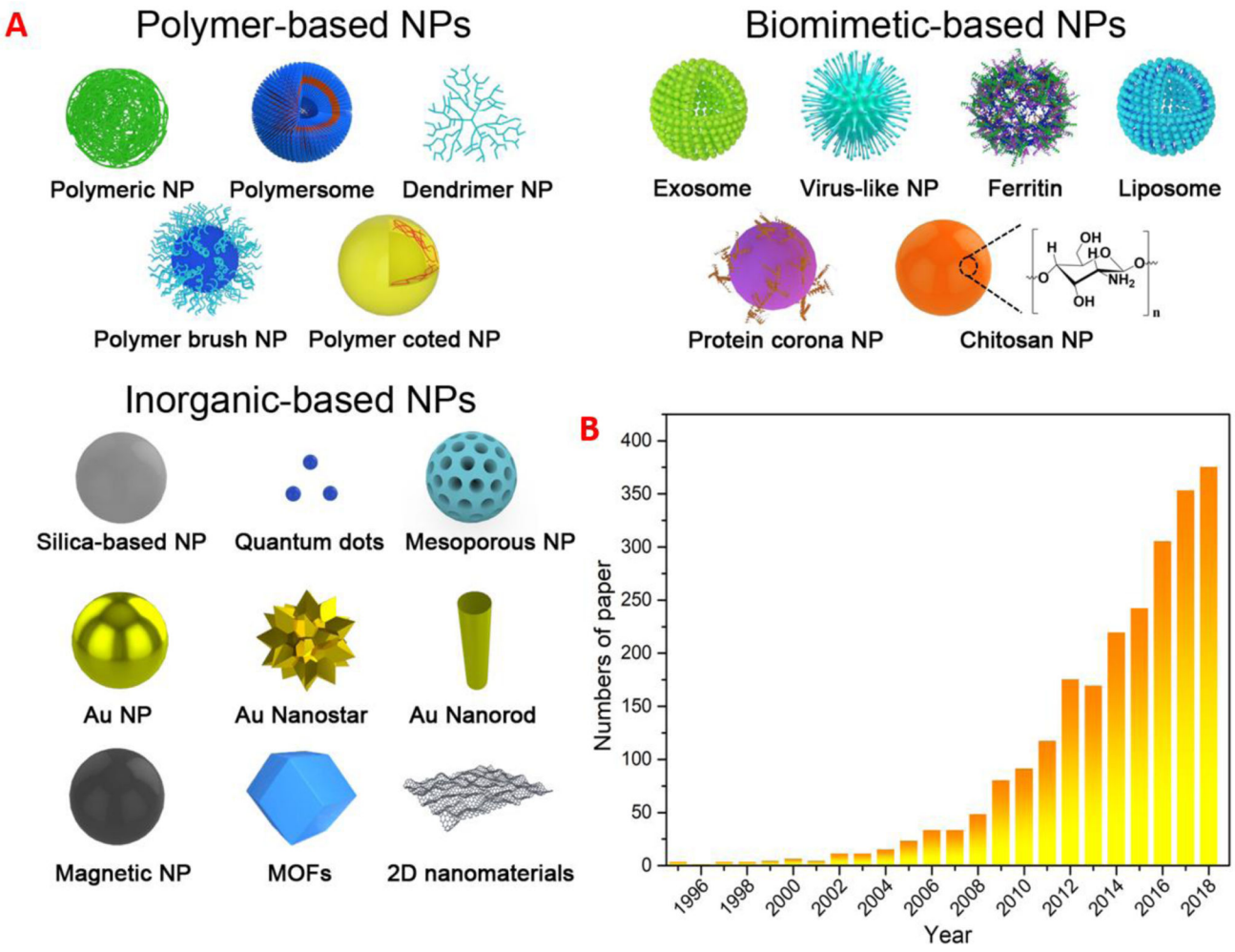

Figure 1.

(A) Representative BBB-crossing nanomaterials. (B) The number of published papers per year on nanoparticle-based drug delivery across the blood-brain barrier. Data are collected from the web of science on May 16, 2019 by advanced search with “Topics = (Blood-brain barrier AND Nanoparticles AND (Drugs OR Gene OR Therapeutics)) AND Language: (English)”.

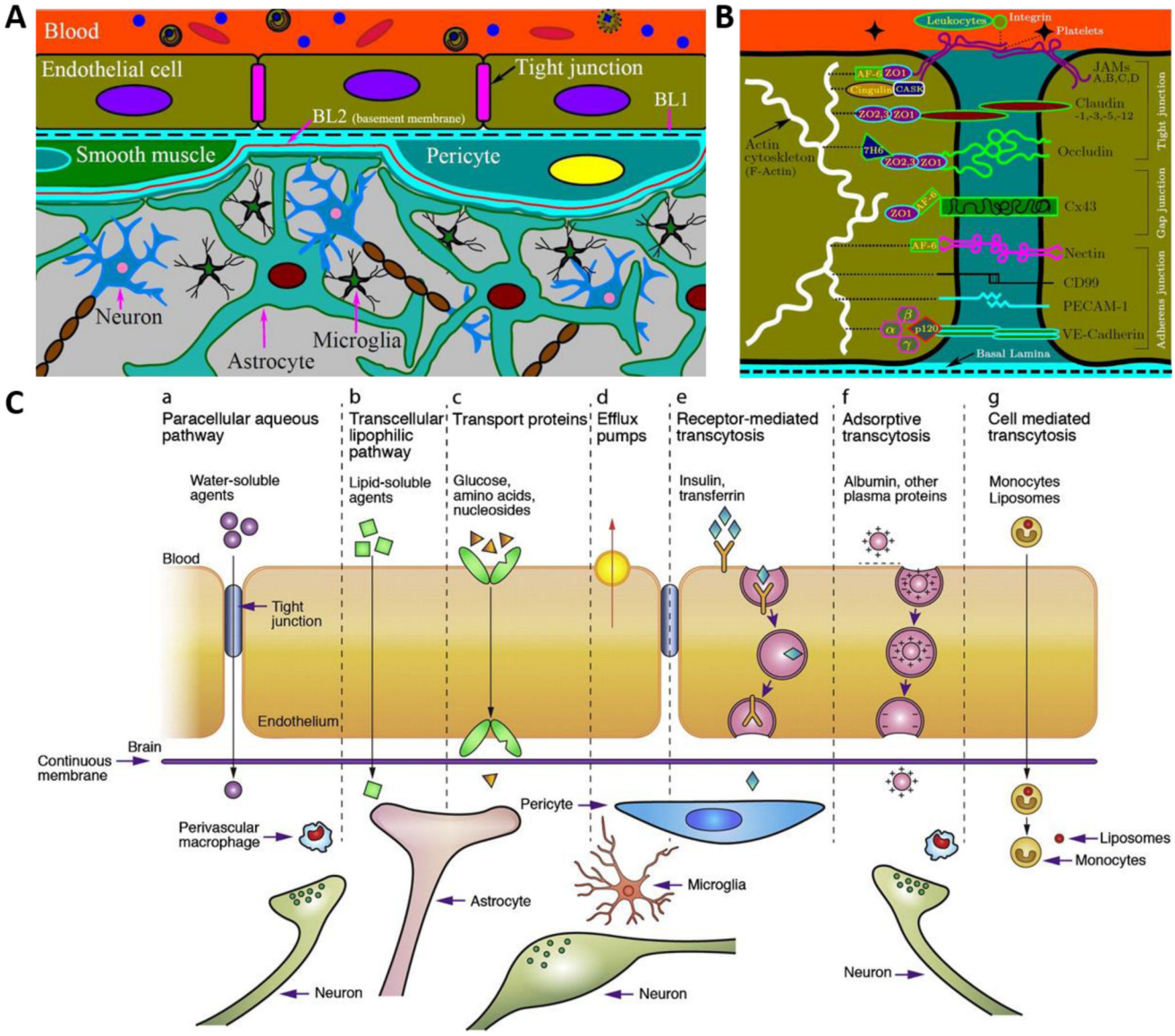

Figure 2.

(A) The cell associations at the BBB. Reproduced with permission Ref. [5]. (B) Structure of junctions at the BBB. (C) Transport routes across the BBB. Reproduced with permission Ref. [11].

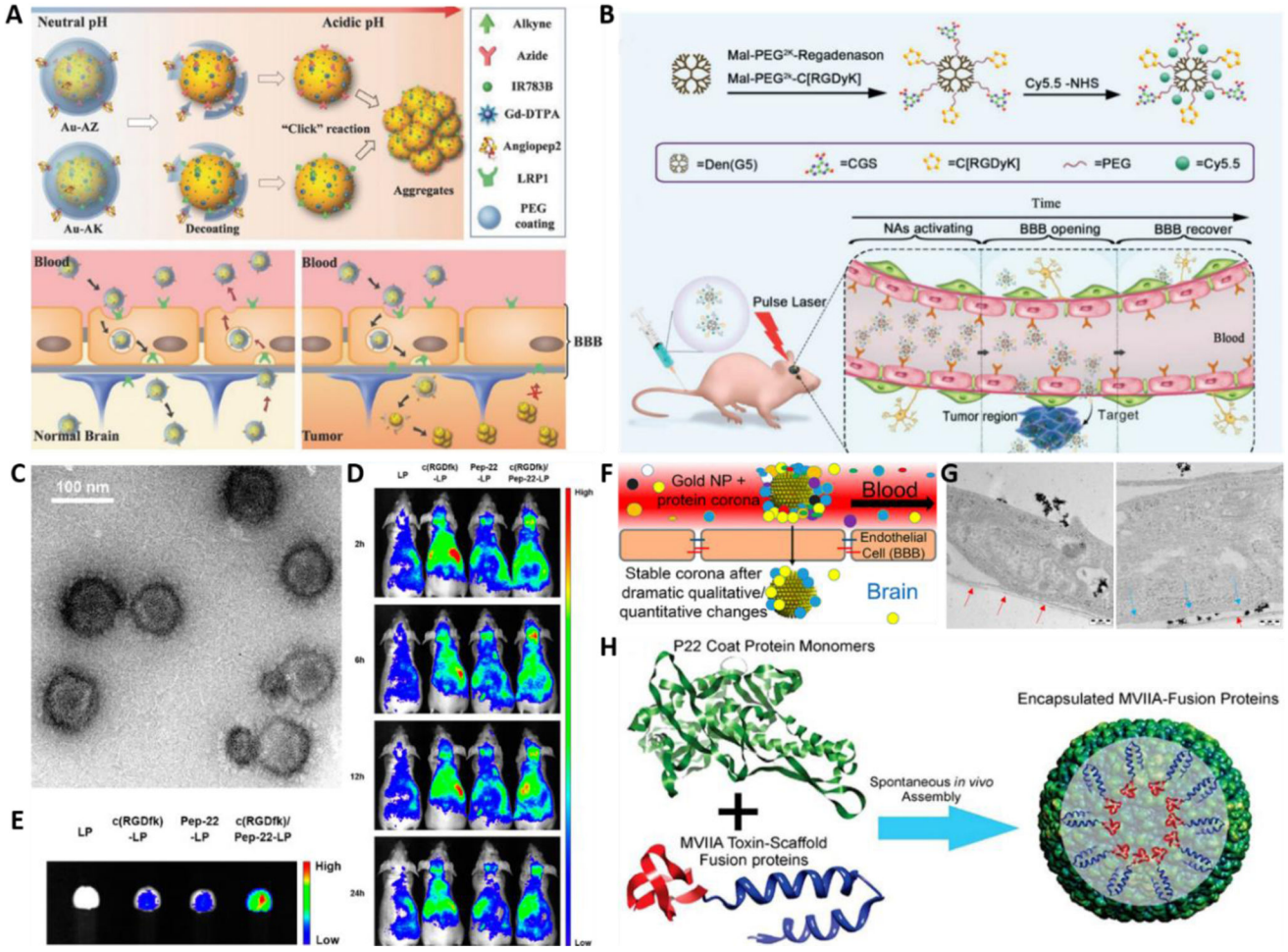

Figure 3.

(A) Transport of PGE-coated Au NPs through BBB and their different biostability in acidic or normal conditions. Reproduced with permission Ref. [91]. (B) The synthesis process of multifunctional dendrimers and crossing mechanisms based on activation of the A_2A adenosine receptor. Reproduced with permission Ref. [93]. (C-E) Structure of liposomes NPs and IVIS spectrum imaging of intracranial glioma-bearing mice and brains after injection of liposomes NPs. Reproduced with permission Ref. [101]. (F-G) Protein corona Au NPs across the BBB and the TEM images of their internalized process from the "blood" to the "brain" side. Reproduced with permission Ref. [104] (H) Synthesis schematic of one VLPs by the assembly of two proteins. Reproduced with permission Ref. [108].

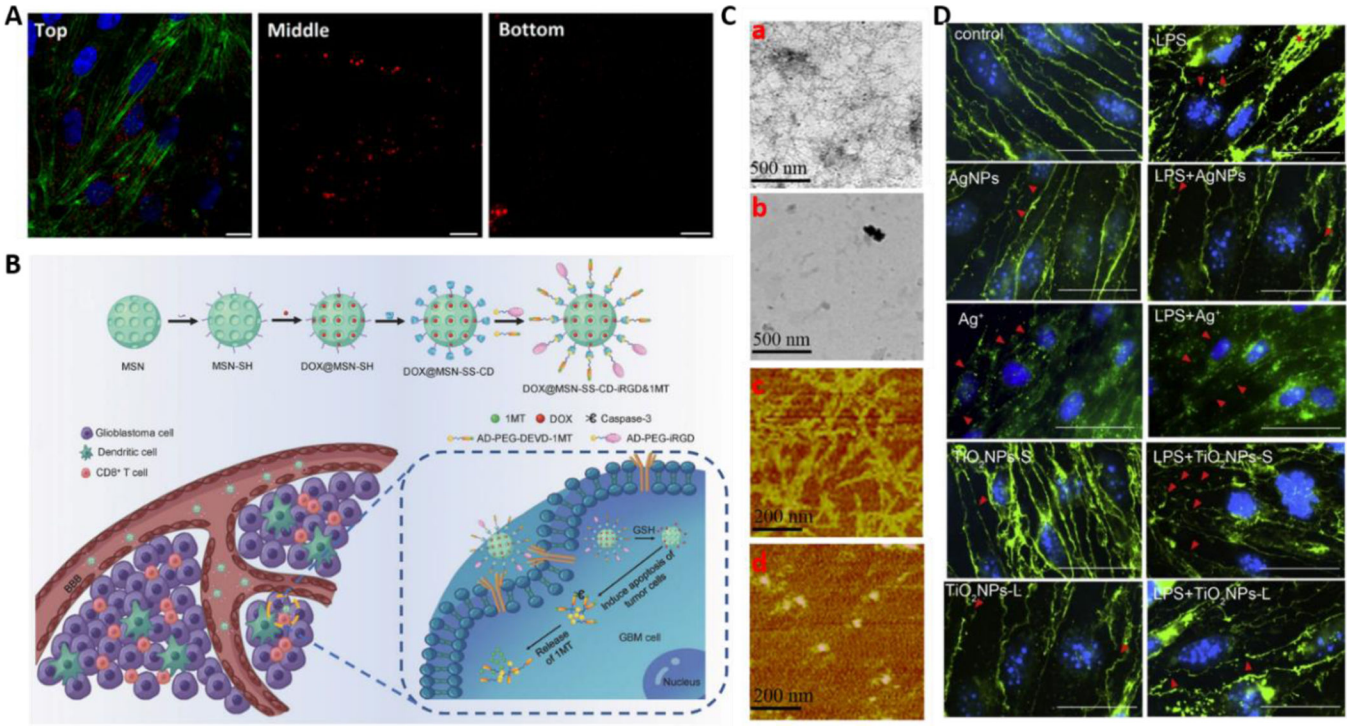

Figure 4.

(A) Confocal images of the in vitro BBB treated with PSi-Lf NPs. Reproduced with permission Ref. [116]. (B) Mechanism of combined chem-immunotherapeutic MSNs nanoparticles. Reproduced with permission Ref. [119]. (C) TEM and AFM images of Aβ fibrils under normal (a,c) condition and after coculturing with Au NPs under NIR condition (b,d). Reproduced with permission Ref. [120]. (D) Ag^{+}, Ag NPs and TiO_2 NPs in the BBB model. Reproduced with permission Ref. [123].

Table1

Representative Drug delivery across the blood-brain barrier by nanoparticles

Nanoparticles	Size (nm)	Drugs or agents	Drugs loading method	Methods of crossing BBB	Application	Ref
Polymersome	~76	Saporin	Covalent bond	Bonding ANG	Protein toxin delivery	[193]
Den-RGD	~10	Cy5.5	Covalent bond	Using CGS to activate the A_2A adenosine receptor	Photoacoustic shockwave therapy for glioblastoma	[93]
PLGA	~204	Curcumin	Encapsulation	Modified with glycopeptide	Inhibiting Abeta aggregation	[85]
PLGA	28–98	3,3'-diindolylmethane	Encapsulation	Synthesizing SSTR2 peptide	Preventing glioma progression	[88]
PEG-PLA NPs	111.30 ±15.64	Paclitaxel	Encapsulation	Decorating with penetrating peptide (tLyp-1 peptide)	Glioma therapy	[89]
PLA coated MSNs	200	Resveratrol	Covalent bond	functionalized by LDLR ligand peptide	Oxidative stress therapy in CNS	[86]
PEG-coated Au NPs	26	--	--	Using LRP1 receptor-mediated transcytosis	As nanoprobes by MR and SERRS signals	[91]
PEG–PLGA NPs	24.11 ± 1.36	doxorubicin	Encapsulation	Modified by I_6P_8 peptide	Glioma-targeted therapy	[173]
Liposomes	100 to 125	Doxorubicin	Ammonium sulfate gradient loading method	Decorating with six peptides (Angiopep-2, T7, Peptide-22, c(RGDfK), D-SP5 and Pep-1)	Chemotherapeutics for glioma therapy	[101]
Liposomes	96.24 ± 1.13	stroke homing peptide (SHp)	Encapsulation	Conjugated T7 peptide (T7) and	Treatment of brain ischemic stroke	[194]
Au NPs	3.5 ± 0.8	--	--	Coated with 11-mercapto-1-undecanesulfonate	Evolution of protein corona NPs across the BBB	[104]
VLPs	~54	venomous marine snail analgesic peptide ziconotide	Self-assembly	HIV-Tat peptide	Developing peptide therapeutics in the CNS	[108]
Si NPs	25, 50, 100	Ruby dye	--	Graft from Lactoferrin	Evolution of Si NPs across the BBB	[116,117]
CMC-coated Fe_3O_4 NPs	14.05±1.70	Dopamine hydrochloride	Covalent bond	--	An agent for MRI and targeted drug delivery	[195]
Solid lipid NPs	100–120	--	--	Binding human apolipoprotein E peptide	A feasible strategy for improving brain delivery of therapeutics	[196]
Lactoferrin NPs	70 ± 10	Temozolomide	Sol-oil method	Using lactoferrin as a matrix	Treatment of Gliomas cancer	[174]
CS NPs	235.7 ± 10.2	siRNA	Non-covalent bond	Transferrin and bradykinin B2 antibody	To prevent strategy for HIV infection	[197]
Amphiphilic polymer-lipid NPs	100.1 ± 2.6	Docetaxel	Hydrophobic interaction	Loading with PS 80	Treatment of brain metastasis of triple-negative breast cancer	[198]
DS NPs	~100	Capecitabine	Ionic gelation	Loading with transferrin	Administering for metastatic brain breast and colorectal cancer	[199]
TEB NPs	25	--	--	Loading with transferrin, lactoferrin and lipoprotein	Imaging probes to the brain	[95,96]

Nanoparticles	Size (nm)	Drugs or agents	Drugs loading method	Methods of crossing BBB	Application	Ref
Ag NPs, TiO_2 NPs, Ag^+	Ag NPs 8nm; TiO_2 NPs 6nm & 35nm	--	--	Ag NPs utilizing ROS-induced cell death; Ag^+ and TiO_2 NPs exposure disrupted BBB by cytokine secretion	In vitro testing BBB permeability	[123]
Lipid NPs (TLN)	<270	Paclitaxel	Encapsulation	Incorporating some lipophilic character by lipophilic surfactant	For treating glioblastoma	[200]
Au Nanostars	105	Ru (II) complex	Electrostatic bonding	Pen peptide to promote cellular internalization	Inhibiting the formation of Aβ fibrils	[120]