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ADVANCED REVIEW

Envisioning the future of polymer therapeutics for brain disorders

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The growing incidence of brain-related pathologies and the problems that undermine the development of efficient and effective treatments have prompted both researchers and the pharmaceutical industry to search for novel therapeutic alternatives. Polymer therapeutics (PT) display properties well suited to the treatment of neuro-related disorders, which help to overcome the many hidden obstacles on the journey to the central nervous system (CNS). The inherent features of PT, derived from drug(s) conjugation, in parallel with the progress in synthesis and analytical methods, the increasing knowledge in molecular basis of diseases, and collected clinical data through the last four decades, have driven the translation from "bench to bedside" for various biomedical applications. However, since the approval of Gliadel® wafers, little progress has been made in the CNS field, even though brain targeting represents an ever-growing challenge. A thorough assessment of the steps required for successful brain delivery via different administration routes and the consideration of the disease-specific hallmarks are essential to progress in the field. Within this review, we hope to summarize the latest developments, successes, and failures and discuss considerations on designs and strategies for PT in the treatment of CNS disorders.

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1 | INTRODUCTION

Central nervous system (CNS) diseases represent a major health problem, constituting 16.8% of deaths worldwide (Group, 2017) and an enormous economical and societal burden. Moreover, CNS disease prevalence is expected to increase, mainly due to an increase in the proportion of elderly patients in the population and the lack of disease-modifying treatments (Oller-Salvia, Sánchez-Navarro, Giralt, & Teixidó, 2016). The major limiting factors for effective CNS treatments are the anatomical and metabolic barriers that hinder access of therapeutics to the CNS. The blood–brain barrier (BBB), the most relevant example, is impenetrable to 98% of small drugs and almost 100% of macromolecular biologicals in blood circulation (Cerna,

Stiborova, Adam, Kizek, & Eckschlager, 2016; Theodorakis, Müller, Craster, & Matar, 2017). Overall, most of CNS therapies under development are ineffective due to poor pharmacokinetics (PK) and low BBB penetration, as well as their inability to diffuse within the brain parenchyma to target the disease site in effective concentrations.

Many efforts to overcome this hurdle have been made over the past decades and the challenge of brain drug delivery has been partly accomplished. Food and Drug Administration (FDA) approval of Gliadel® wafer (Arbor Pharmaceuticals LLC., Atlanta, GA, USA) implants for glioma treatment made real the possibility of using polymeric systems to reach CNS (de Boer & Gaillard, 2007; Regina et al., 2008). Nonetheless, this treatment is based on brain implants, a highly aggressive strategy, and so current research is focused on noninvasive methods with many examples of CNS-specific drug nanocarriers recently reported (Gendelman et al., 2015). Results are encouraging, but these systems are in early stages of development and we require further investigation to unravel their real potential. Nonetheless, the use of nanotechnology is one of the most appealing approaches, especially when chronic treatments are required.

In this respect, polymer therapeutics (PT) represent promising candidates, as their key characteristics can help to overcome the main limitations associated with low-molecular weight (MW) tracers/drugs. This family of new chemical entities (NCEs) includes polymeric drugs, polymer–drug conjugates, polymer–protein conjugates, polymeric micelles with drugs covalently bound, and polyplexes designed as nonviral vectors for gene delivery (Duncan, 2003; Figure 1). Other than providing water solubility to hydrophobic drugs, the nanoscale size of PT combined with the rational design of the covalent drug linkage offer unique advantages: (a) *passive targeting* due to the enhanced permeability and retention (EPR) effect, (b) *the ability to cross biological barriers* and *overcome chemoresistance* (Markman, Rekechenetskiy, Holler, & Ljubimova, 2013), (c) *controlled PK* (Kobayashi, Turkbey, Watanabe, & Choyke, 2014) and sustained and stimuli-responsive release driven by rational linking chemistry (Duro-Castano, Conejos-Sanchez, & Vicent, 2014), and (d) the capacity to accommodate several active agents, probes, and/or targeting moieties, *allowing for combination therapy, theranostics, and active targeting*, respectively (Theek, Rizzo, Ehling, Kiessling, & Lammers, 2014; Vicent et al., 2005).

PT emerged to address unmet medical needs in cancer (Hare et al., 2017) but have been recently extended to applications in other diseases, with around 25 nanosized products for healthcare in clinical use and numerous under clinical development (Duncan, 2017). Indeed, two PT were among the U.S. top 10 selling drugs in 2013, thereby highlight the real potential of PT. Of note, one of these PT, Copaxone® (Teva Pharmaceutical Industries Ltd., Petah Tikva, Israel), is a polymeric drug used in the treatment of patients with relapsing–remitting multiple sclerosis (Duncan, 2014), a CNS disorder. The most appealing strategies pursued for CNS delivery in the nanomedicine field involve two main routes of administration: intravenous (IV) and intranasal (IN) administration. Systemic IV administrations employ active targeting provided by conjugation of ligands of specific receptors present in BBB (so-called Trojan horses, TH; Pardridge, 2006, 2007, 2012), while IN approaches take advantage of the direct nose-to-brain route (Chapman et al., 2013; A. R. Khan, Liu, Khan, & Zhai, 2017). Both approaches are still at very early stages of development and while promising, PT as a platform has been scarcely explored.

Herein, we hope to provide a review of the major findings during the development of PT for the treatment of CNS disorders. We note that invasive techniques which employ temporal BBB disruption or surgical approaches are out of the scope of this review (Oller-Salvia et al., 2016). Noninvasive techniques have focused on the chemical modification of drugs or the use of nanotechnology (Lu et al., 2014). Those nanotechnological approaches based on PTs using the patient-friendly IV or IN administration routes will be covered herein. We discuss the main challenges encountered for the development of successful therapies, with particular attention to the implications of the biological barriers in specific CNS pathological conditions. Finally, we also highlight the main limitations of the most frequently applied in vitro/in vivo models for brain delivery screening and how the lack of consensus in the biological data produced hinders clinical progress and success in many cases.

1.1 | IV delivery

The IV route is the most widely applied in the clinic and most of PTs developed to this date are designed to follow this route. For successful IV treatment, PTs must be engineered to display sufficient blood stability and compatibility (low interaction with plasma proteins and cells) and long circulation times to maximize the possibility of reaching their target, while avoiding hepatic clearance and renal filtration (England, Conejos-Sánchez, & Vicent, 2012). Polymer conjugates generally have exhibited a clinical blood clearance, stability in the bloodstream, and elimination rate/route that correlates well with preclinical studies (Duncan, 2017). In the case of brain disorders, targeting relevancy keeps crucial to increase brain accumulation after IV and avoid that most of the administered dose goes to the clearance organs. Regarding CNS delivery, two additional hurdles must be taken into consideration: crossing the BBB and the diffusion across the brain parenchyma to reach their target once in the brain.
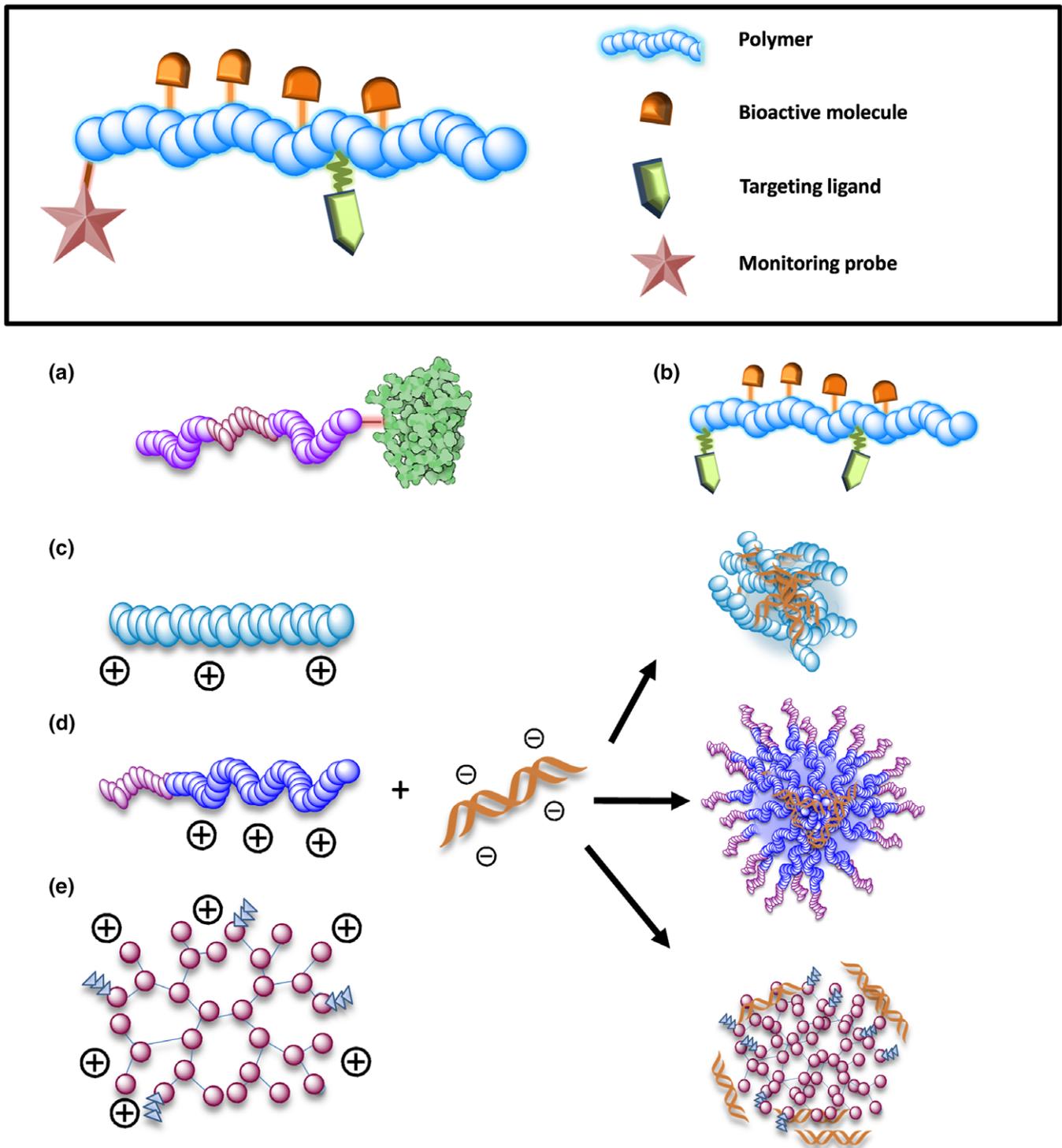
FIGURE 1 Schematic representation of polymer therapeutics examined for central nervous system (CNS) drug delivery systems. (a) Polymer-protein conjugate (e.g., Pluronic85®-leptin), (b) polymer-drug conjugate bearing a targeting ligand (e.g., Lf-HA-Dox) (c–e) Polyplexes: (b) with a linear polymer, (c) with a block copolymer forming micelles, (d) with dendrimers (e.g., arginine-modified PAMAM G4 dendrimer + siRNA)

1.2 | IV route

1.2.1 | The blood brain barrier

Due to the high relevance of CNS functions, the brain is one of the most (if not the most) protected organs in the human body, being guarded by various barriers: the BBB, the blood-cerebrospinal fluid barrier (BCSFB), and the arachnoid epithelial membrane barrier or meningeal barrier (Abbott, Patabendige, Dolman, Yusof, & Begley, 2010). Such barriers (a) control the highly selective and specific uptake/efflux mechanisms, as well as the metabolism of endogenous and exogenous molecules, (b) contribute to ion homeostasis, (c) preserve neural connectivity in the CNS, (d) separate central and peripheral
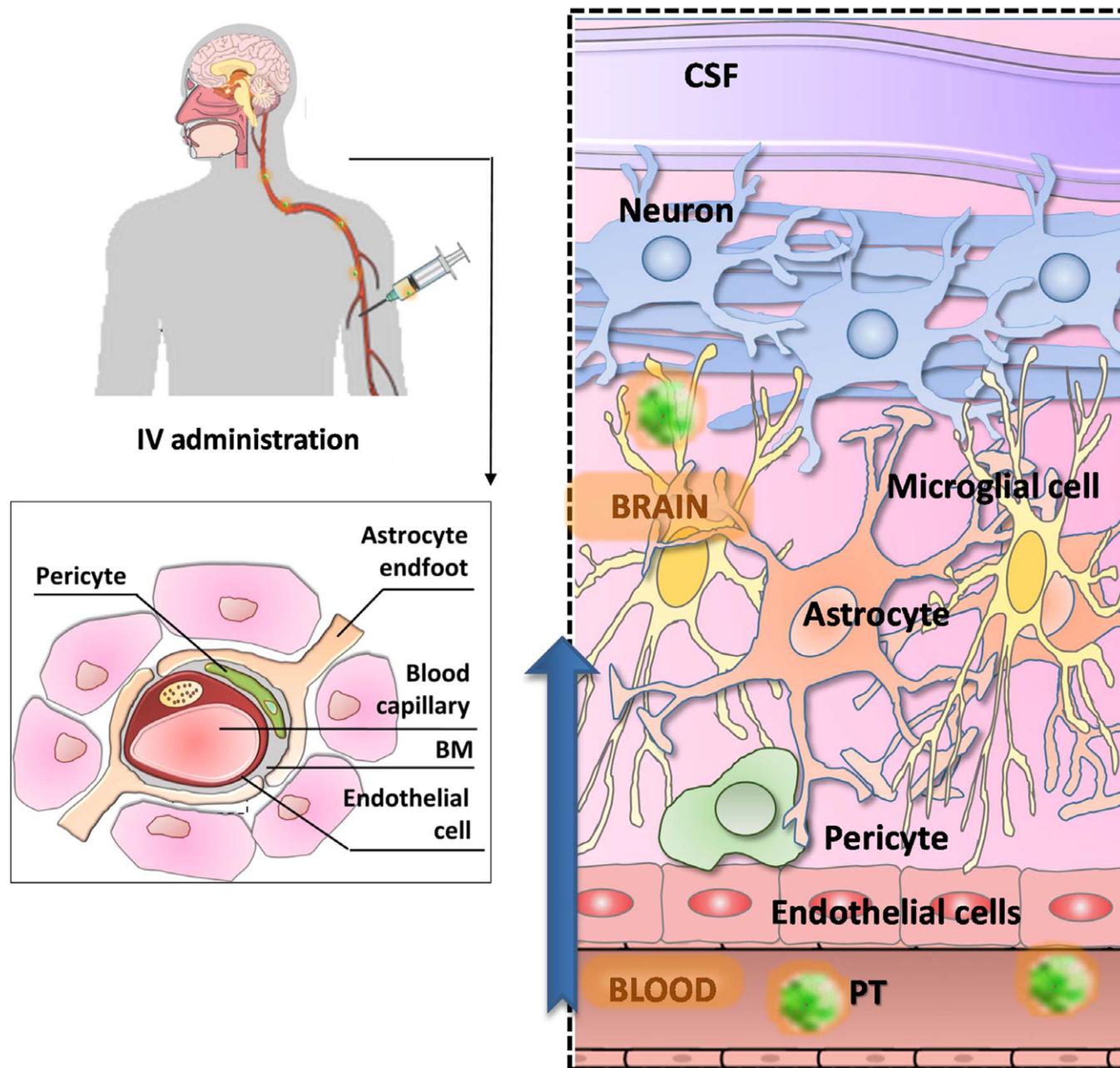
FIGURE 2 Schematic representation of PT administration through intravenous route. The neurovascular unit from blood–brain barrier (BBB) and its structure. PT = polymer therapeutics; BM = basement membrane

neurotransmitters pools, reducing cross talk and allowing nonsynaptic signaling in the CNS, and (e) allow immune surveillance and response with minimal inflammation and cell damage (Abbott, 2013).

The BBB is the most relevant obstacle to target, as it represents the widest exchange area between blood and brain. The BBB is a physical and metabolic barrier that comprises the brain vasculature whose endothelial cells (BECs) are closely connected by the presence of intercellular tight junctions (TJ) forming a continuous endothelium. This endothelium is surrounded on its abluminal side by a basement membrane into which the pericytes are embedded, while the astrocytes embrace the whole structure with their end feet. To complete the so-called neurovascular unit (NVU), neurons, and microglial cells and, optionally, peripheral immune cells also give functional and structural support to the BBB (Oller-Salvia et al., 2016; Figure 2). BECs show exceptional features due to their specific location: (a) lower number of endocytic vesicles compared to endothelial cells (ECs) in other endothelia, thus, limiting transcellular transport, (b) lack of fenestrations, (c) high electrical resistance provided by the presence of the TJ, which restricts paracellular flux, (d) specialized transport systems, (e) higher volume of mitochondria suggesting higher metabolism, and (f) a lack of lymphatic drainage (Theodorakis et al., 2017).

1.2.2 | Transport mechanisms across the BBB

Both paracellular and transcellular pathways are limited in the BBB. Paracellular diffusion is a passive process, where molecules travel through TJ of the ECs following electrochemical, hydrostatic, osmotic, or concentration gradients. Only water, and very few small water-soluble molecules and lipophilic solutes cross the BBB by paracellular diffusion, thus, it plays a minor role in brain drug delivery (Theodorakis et al., 2017). In the transcellular pathway, molecules travel across ECs using passive or active mechanisms. Small lipophilic molecules (<500 Da) and gases (e.g., O_2 and CO_2) can cross the BBB by passive transcellular diffusion through the EC cell membrane (Obermeier, Daneman, & Ransohoff, 2013). Indeed, most CNS drugs follow this mechanism (Alam et al., 2010). Some small hydrophilic molecules are transported by carrier-mediated transport (CMT) using specific membrane transporters in the BBB that can be energy dependent or independent (Lajoie & Shusta, 2015). Receptor-mediated transcytosis (RMT), an active transcellular pathway followed by hormones, growth factors, and lipoproteins among other macromolecules, is one of the most investigated routes in brain delivery and in PT development, due to the possibility to promote transcytosis (Tian et al., 2015). This approach is based on TH strategies, which employ conjugation of high-affinity and low-avidity ligands of specific BBB receptors to the nanocarrier, aiming for intact BEC crossing. Differential expression of BBB receptors with respect to other tissues and in different pathological conditions makes this strategy highly selective. However, this is a two-way route meaning that the targeted PT can be extruded from the brain if the concentration gradient changes. Although this pathway is neither size limited nor lipophilicity-dependent, some studies have shown that transcytosis is more efficient when the size of the particle is between 50 and 60 nm (Fullstone, Nyberg, Tian, & Battaglia, 2016). Among the most employed receptors for drug delivery approaches are: insulin receptor (IR), transferrin receptor (TfR), and low-density lipoprotein receptor (LDLR)-related proteins 1 and 2 (LRP-1 and 2) (discussed further in the *polymer therapeutics to bypass the BBB* section). Finally, positively charged compounds, such as plasma macromolecules (albumin), can be transported by adsorptive-mediated transcytosis (AMT). Although AMT can be exploited by positively charged PTs, it is not a preferred route, due to its lack of specificity (Yin et al., 2016).

The presence of efflux transport systems hinders the accumulation of many lipophilic compounds in the CNS (Lajoie & Shusta, 2015). Present in the BBB and in the BCSFB, this mechanism is the reason why most of drugs that cross the BBB, do not reach therapeutically relevant concentrations in the brain. One of the best-known efflux transporters that forms part of the multidrug resistance receptors (MDRs), is P-glycoprotein (P-gp), a member of the ATP-binding cassette (ABC) transporters. Of note, tumor cells also overexpress these efflux transporters as a mechanism of chemoresistance. Therefore, these transporters imply a double hurdle to surpass when treating brain tumors. In this concern, pluronic block copolymers should be highlighted, as members of this family (e.g., Pluronic®85) have been identified as efflux transporters inhibitors, thus acting also as a polymeric drug (England et al., 2012; Yi et al., 2014).

1.2.3 | BBB models and limitations

Interaction of PTs and their permeation across the BBB can be studied in vivo or using in vitro BBB models. In vivo, PTs can be administered in the periphery and allowed to reach the brain after traveling through the bloodstream (which will represent the actual scenario) or administered following surgically restrict of circulation to the brain to avoid metabolism by other organs and to facilitate the analysis (Bickel, 2005). In situ brain perfusion is the gold standard for the measurement of BBB permeability (Abbott, 2013) and the method of choice for studying RMT as the time of exposure to the BBB is longer than with other techniques. In in situ brain perfusion, the circulation of the compound is restricted to the brain by retrograde perfusion from the external carotid into the internal carotid (Stanimirovic, Bani-Yaghoub, Perkins, & Haqqani, 2015). Additionally, composition of the intravascular fluid (perfusate) can be modified depending on the variables of the study (e.g., pH, ligands for competition assays, etc.; Bickel, 2005).

However, such techniques require complex surgical interventions and/or the use of a large number of animals for screening purposes. Thus, a broad variety of in vitro BBB models have been developed, with different degrees of complexity and using different sources of biological material. These models are based on the culture of primary culture or immortalized BECs (Lippmann, al-Ahmad, Palecek, & Shusta, 2013) in static or dynamic conditions (microfluidics) involving a fluid flow on the endothelial luminal surface. Microfluidics may mimic better the in vivo scenario but static models offer better resolution and quantification of permeation using minimal amounts of compound (Abbott, 2013). The most frequently used BBB model is static and consists of a monolayer of BECs. However, monoculture models lack BBB in vivo characteristics derived from interactions between the various NVU-forming cells. Thus, bi and triple coculture models have been developed to closely reproduce the in vivo scenario (Gomes, Mendes, Martins, & Sarmento, 2016). Primary BECs cocultured with primary astrocytes better reflect the expression of receptors and transcytosis mechanisms found in vivo and promotes TJ formation between BECs, thereby reducing paracellular permeability and increasing transendothelial electrical resistance (TEER; Abbott, 2013; Lippmann et al., 2013). Triculture with primary pericytes appears to further improve barrier function mimicking (Lippmann et al., 2013). However, the wide variety of in vitro models available has impeded the general evaluation of their predictability.

Regardless of the complexity of the system selected, several aspects are of critical importance: (a) (over)expression of the receptor of interest (for RMT), (b) adequate quantification of BBB entry versus binding to BECs, as well as specific (RMT) versus unspecific (i.e., AMT) transport, (c) establishment of adequate protocols for data acquisition and analysis to normalize procedures, thus enabling the direct comparison of results between different studies, both in vitro and in vivo.

1.3 | Polymer therapeutics strategies to cross the BBB

Among strategies based on nanosystems for IV administration, two main approaches are followed to cross the BBB: the use of delivery systems relying on unspecific BEC uptake and AMT or the use of targeted delivery systems targeting BBB receptors for RMT. The first approach is followed by liposomal formulations (neutral or cationic), colloidal nanoparticles (neutral or cationic), and solid lipid nanoparticles (Tajes et al., 2014; Vlieghe & Khrestchatisky, 2013). Regarding PTs, few examples with intrinsic ability to cross the BBB can be found, being pluronics, the most relevant example. Pluronic® block copolymers, consisting of hydrophilic poly(ethylene oxide) (PEO) and hydrophobic poly(propylene oxide) (PPO) blocks (PEO-b-PPO-b-PEO), are drug delivery systems (DDSs) that can act as polymeric drugs themselves or be used as polymeric micelles for imaging probes and/or drug delivery (Batrakova & Kabanov, 2008). Pluronics have already reached the clinic, with Pluronic® 407 gel (LeGoo®, Pluromed Inc., Woburn, MA, USA) employed for vessel occlusion and many Pluronic®-containing formulations currently in clinical trials (Pitto-Barry & Barry, 2014). This technology has also been extensively explored for CNS delivery due to their inherent ability to interact with hydrophobic surfaces and therefore, promote the crossing of biological barriers. Moreover, pluronics have been shown to inhibit drug efflux transporters (Kabanov, Batrakova, & Alakhov, 2002), specially P-gp, which is widely expressed on the BBB (Alakhova & Kabanov, 2014; Batrakova et al., 2001). Pluronics have been applied for peptide/protein delivery to the CNS, with examples including opioid peptides (Witt, Huber, Egleton, & Davis, 2002), horseradish peroxidase (Batrakova et al., 2005), leptin (Banks et al., 2011; Price et al., 2010; Yi et al., 2014), or superoxide dismutase 1 (Yi et al., 2010). Also for small drugs such as digoxin (Batrakova et al., 2001), lamotrigine (Liu et al., 2014), and other P-gp efflux transporter substrates such as Rho123 (Meng et al., 2017).

Nevertheless, the second approach, relying on RMT by means of TH, is the most applied strategy for brain delivery driven by PTs (England et al., 2012). This approach involves the conjugation of the receptor-targeting moieties to the therapeutic agent of interest, that is, monoclonal antibodies (mAb), recombinant proteins, RNA, DNA, or nanomedicines (Pardridge, 2008). The most well-studied BBB RMT targets include TfR (Moos & Morgan, 2000), IR (Banks, Owen, & Erickson, 2012; Duffy & Pardridge, 1987), and LDLRs (Candela et al., 2015).

TfR mediates blood-to-brain iron delivery by binding and intracellular trafficking of the iron-binding protein transferrin (Tf; T. Moos & Morgan, 2000) and is highly expressed on the BBB (Y. Uchida et al., 2011). Initially, Tf found use as a vector for BBB delivery; however, the high concentration of endogenous Tf in the bloodstream competes with injected Tf. Thus, antibodies targeting epitopes distal to the Tf-binding site have been developed to overcome this limitation (Lajoie & Shusta, 2015). The most used antibodies are OX26 (Johnsen et al., 2017; Loureiro et al., 2017) and 8D3 (Cabezon et al., 2015). Furthermore, advances in genetic engineering have allowed the development of fusion proteins and chimeric antibodies to optimize antibody–receptor affinity and improve BBB transport (Chang et al., 2017; Sehlin, Fang, Meier, Jansson, & Syvänen, 2017; Syvanen et al., 2017). For example, a recombinant fusion protein between the single chain variable fragments (scFv) of 8D3 and an antibody against Aβ fibrils achieved a 2% of the injected dose per gram (ID/g) of brain tissue 2 hr after IV administration in a transgenic mouse model of Alzheimer's disease (AD), an 80-fold increase over the nontargeted antibody (Hultqvist, Syvänen, Fang, Lannfelt, & Sehlin, 2017). Another TfR-targeting moiety is the iron-mimicking cyclic CRT peptide (CRTIGPSVC), which has demonstrated promising results despite binding to the Tf-binding site (Huang et al., 2017; Nathanson & Mischel, 2011).

However, TfR does display certain limitations as a target for RMT. First, TfR expression in vascular beds and parenchyma of other organs can lead to undesired widespread distributions. Also, full transcytosis of TfR to the brain may be limited and depends on the affinity of the ligand for the receptor (Lajoie & Shusta, 2015). Despite that, TfR has been used as an RMT target for PTs. For example, a Tf-tagged generation 3-diaminobutyric polypropylenimine dendrimer (DAB) developed for gene delivery to the brain increased gene expression of β-galactosidase in mouse brain more than twofold over the nontargeted complex (Somani, Blatchford, Millington, Stevenson, & Dufès, 2014). Also, polyamidoamine (PAMAM) dendrimers modified with polyethylene glycol (PEG) and bearing Tf were developed for gene delivery to the brain, increasing by 2.25-fold the percentage of ID/g of tissue compared to naked dendrimer (Huang et al., 2007).

Another target for RMT is the IR, which is expressed in the BBB and is responsible for the transport of insulin to the brain (Uchida et al., 2011). Insulin itself cannot be used as vector due to its short half-life in serum and the possibility of causing hypoglycemia (Bickel, Yoshikawa, Landaw, Faull, & Pardridge, 1993). As an alternative, a human mAb against IR (HIRMAb) has been used for brain delivery (Pardridge, 2017). The company ArmaGen Technologies Inc. has developed a myriad of fusion proteins with HIRMAb (armagen.com), with two currently in clinical trials: AGT-181, an HIRMAb-α-L-

iduronidase fusion protein for the treatment of mucopolysaccharidosis type I (Hurler's syndrome; Boado & Pardridge, 2017), and AGT-182, an HIRMAb-Iduronate 2-sulfatase fusion protein for the treatment of mucopolysaccharidosis II (Hunter's Syndrome; Boado, Ka-Wai Hui, Zhiqiang Lu, & Pardridge, 2014). However, this approach is considered risky due to the involvement of the IR in glucose homeostasis and no PT examples have been described to our knowledge.

Other exploited receptors include the LDLRs and LRP 1 and 2, which are expressed by BECs (Daneman et al., 2010; Uchida et al., 2011) and mediate the transport of lipoproteins and other ligands (e.g., ApoE, HIV-1, TAT protein, etc.) across the BBB via RMT (Candela et al., 2015). Antibodies against these receptors have not been reported for this family, although a large number of studies have explored the use of LDLR and LRP ligands and peptide-ligand mimics as vectors for brain delivery. The most relevant example is angiopep-2 (ANG-2), a 19-amino acid peptide that was reported as a ligand targeting LRP receptor (Demeule et al., 2008) and has shown promising results as delivery vector. ANG-2 has been used to deliver genes, peptides, proteins, antibodies, and enzymes (Demeule et al., 2014; Ke et al., 2009; Lachowicz et al., 2013; Regina et al., 2015). The company Angiochem has developed several ANG-2-drug conjugates for the treatment of brain tumors (Ché et al., 2010; Régina et al., 2008) with ANG1005 reaching phase II clinical trials for metastatic breast cancer with brain metastases (alone or in combination with Trastuzumab), recurrent high-grade gliomas, and nonsmall cell lung cancer with brain metastasis. ANG-2 has also been used to transport nanomedicines across the BBB (Endo-Takahashi et al., 2016; Hao et al., 2015; Joseph et al., 2017b; Luo et al., 2017; Ruan et al., 2015; Wang et al., 2015). For instance, pH-sensitive poly[oligo(ethylene glycol) methyl methacrylate]-co-poly(2-(diisopropylamino)ethyl methacrylate) (POEGMA-PDPA) polymersomes modified with ANG-2 have been developed for the transport of antibodies, leading to increased polymer accumulation in the brain compared to free antibody. The antibody was detected inside glia and neurons in midbrain and hippocampus suggesting good penetration throughout brain parenchyma (Tian et al., 2015). ANG-2-tagged poly-(L-lysine)(PLL)-grafted polyethylenimine (PEI) was developed as a nonviral vector for the treatment of glioblastoma multiforme (GBM) in nude mice, displaying higher accumulation in the brain compared with all the controls at any time point studied, achieving its maximum at 48 hr, in nude mice (Gao et al., 2016; Figure 3). Other nonviral gene delivery systems, such as PAMAM dendrimers, have also used ANG-2 as targeting moiety. ANG-2-conjugated PEGylated PAMAM dendrimers display increasing brain penetration and accumulation with ANG-2 loading (Ke et al., 2009). Examples of Apolipoprotein E (ApoE) as a vector can also be found in the literature (S. Khan, Chen, Nadimidla, & Kanapathipillai, 2017; Neves, Queiroz, Lima, & Reis, 2017; Song et al., 2016).
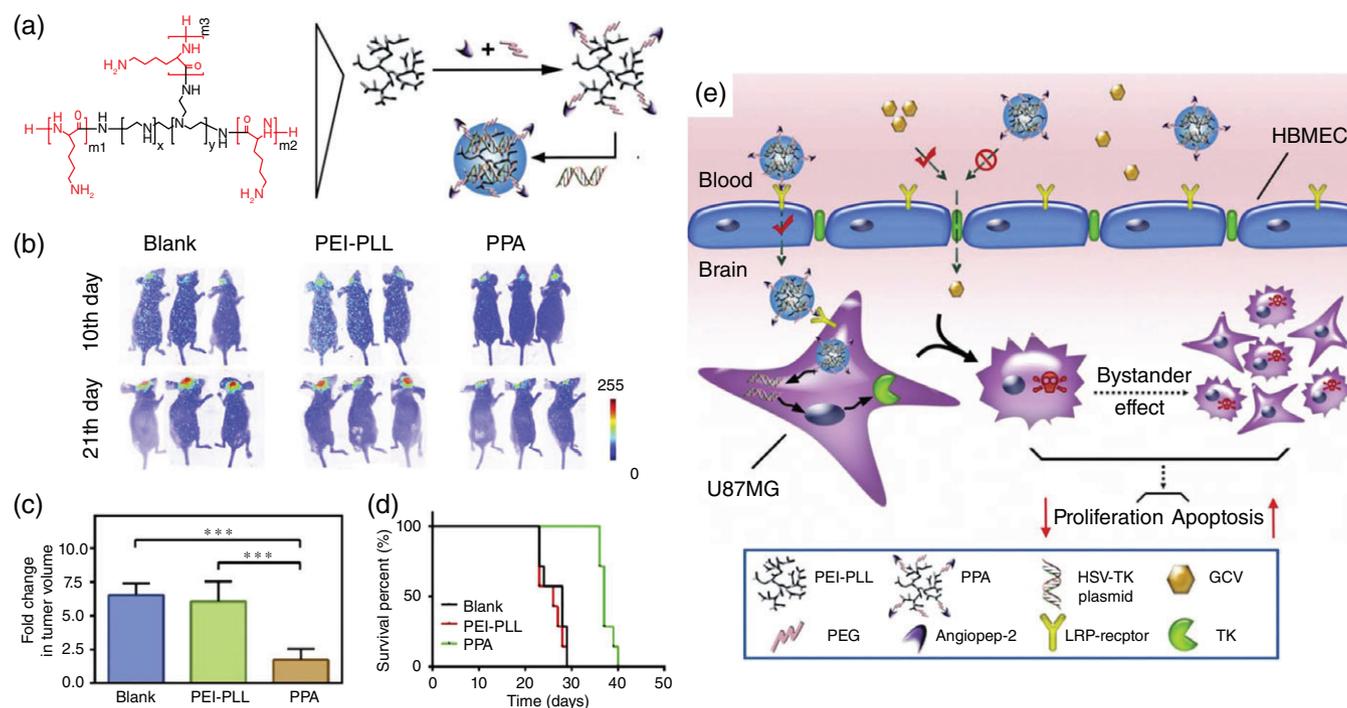

FIGURE 3 (a) Schematic illustration for the synthesis of PPA and the formation of PPA/DNA nanoparticles. Tumor volumes imaging (b), fold change in tumor volume by calculating the mean fluorescence of the luciferase signal (c) and survival rates (d) of U87MG orthotopic glioma bearing mice after treatment. The weight ratio of polymer/DNA was 5/1. ***$p < .001$. Data represent mean ±standard deviation ($n = 3$). (e) Schematic elucidation of in vivo circulation, blood–brain barrier (BBB) crossing, tumor targeting, cellular uptake, and biological effects of the PPA/HSV-TK NPs. U87MG (human primary glioblastoma cell), HBMEC (human brain microvascular endothelial cells), PPA = PEI-PLL-PEG-ANG2.(Reprinted with permission from Gao et al. (2016). Copyright 2018 Elsevier)

Glutathione (GSH) has recently been investigated as targeting vector, as cells of the BBB express transporters for this molecule. For instance, GSH-tagged PEGylated liposomal doxorubicin (2B3-101) has reached phase I/II clinical trials for the treatment of brain tumors (Aftimos et al., 2015; Kerklaan et al., 2014). The iron binding glycoprotein lactoferrin (Lf) whose receptor (LfR) is highly expressed at the BBB has also been used as a vector for brain delivery (M. M. Song et al., 2017; Xu et al., 2017). The rabies virus glycoprotein (RVG) peptide targeting acetylcholine receptors (AChR) has gained popularity (Gooding et al., 2015; Wei et al., 2015; Zou et al., 2017). For example, PEGylated trimethylated chitosan modified with RVG peptide allowed the delivery and accumulation of Cy5-labeled small interfering RNA (siRNA) to the brain of mice 12 hr after injection, with a notable increase in signal when compared to the nontargeted counterpart (Gao et al., 2014).

The main drawback in crossing the BBB using a TH strategy is the presence of the targeted receptor in other tissues, leading to undesired biodistribution of the nanosystem. One possible solution is the design of "superselective" nanosystems that only bind to surfaces with a receptor density above a given threshold (Martinez-Veracoechea & Frenkel, 2011). This way, the binding of the nanosystem displays an *on–off* behavior allowing selective binding. This can only be achieved using multivalent materials, which can display a variable number of ligand molecules. By tuning their properties (i.e., affinity and density of the ligand, linker used to conjugate the ligand, etc.) multivalent polymers can be directed toward a specific surface density of receptors (Dubacheva, Curk, Auzély-Velty, Frenkel, & Richter, 2015). Due to their multivalency and tunability, PTs are ideal candidates for the development of superselective nanosystems for biomedical applications.

1.4 | Polymer therapeutics for CNS disorders via IV route

In both primary and secondary brain tumors, the integrity of the BBB is compromised at late stages; a feature that can be exploited by PTs to reach the CNS without additional targeting (Adkins et al., 2015; Ofek et al., 2016). However, the BBB maintains its integrity at early stages and so, inclusion of targeting moieties has been widely used to improve permeation to the brain. For example, Yin et al. (2016) developed an Lf-tagged hyaluronic acid-doxorubicin (Lf-HA-DOX) conjugate for the treatment of glioma. Importantly, both LfR and the CD44 receptor, which is bound by HA, are overexpressed in glioma cells. Encouragingly, Lf-HA-DOX displayed progressive and sustained accumulation in the tumor tissue (up to 24 hr after IV injection) in a mouse model of glioma.

Delivery of nucleic acids has also been explored for the treatment of brain tumors. ANG-2-tagged T-shaped succinoyl tetraethylenepentamine (Stp)-based redox-responsive polyplexes for the delivery of siRNA targeting the BAG3 cochaperone gene in glioma cells demonstrated greater accumulation in tumor tissue and improved downregulation of the target gene when compared to the nontargeted polyplexes (An, He, Wagner, & Jiang, 2015). PEGylated PAMAM dendrimers modified with ANG-2 used for the delivery of a plasmid encoding tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) increased permeation to the brain and significantly prolonged survival in a glioma mouse model (Huang et al., 2011). Poly-β-L-malic acid-based PTs have been developed for the treatment of glioma combining antibodies against TfR to target the BBB and the blood–brain tumor barrier, trileucine units as endosomal disruptors, and an antisense oligonucleotide against laminin-411 for tumor inhibition, so permitting enhanced delivery to tumor tissue (Ding et al., 2010; Fujita et al., 2007). Liu et al. combined gene therapy and drug therapy for the treatment of glioma by conjugating a chemotherapeutic agent (doxorubicin) through an acid-sensitive linkage to a T7-PEG-modified dendrigraft PLL (DGL) used to complex a TRAIL expression plasmid (Liu, Guo, et al., 2012). The T7 peptide is a novel targeting ligand for TfR and its conjugation enhanced BBB crossing and brain accumulation of polymer/DNA complexes, although the study did note nonspecific uptake through other endocytic pathways (Kuang et al., 2013; Liu, Guo, et al., 2012). Of note, in vivo antitumor activity studies demonstrated a prolonged survival of animals treated with the targeted systems (Liu, Guo, et al., 2012).

The treatment of neurodegenerative disorders such as Parkinson's (PD) or AD has recently focused on neuroprotective strategies as a potential complement to other approaches, such as cell replacement. Liu, Guo, et al. (2013) developed nonviral gene vectors based on RVG-tagged PEGylated DGLs complexed with a caspase-3 short hairpin RNA (shRNA)-encoding plasmid to treat PD. Encouragingly, this approach achieved a sustained reduction of caspase-3 mRNA levels (below 50% of that on nontreated controls) in a rat model of the disease, leading to a reduction in TNF-α and nitric oxide (NO) levels in the midbrain (indicating an anti-inflammatory effect), an increase in tyrosine hydroxylase (TH)-positive (dopaminergic) neurons in *substantia nigra*, and an improvement in the parameters of behavioral analysis (Liu, Guo, et al., 2013). The same group used ANG-2 to deliver a PEGylated DGL complexed with a human glial cell line-derived neurotrophic factor (*hGDNF*)-encoding plasmid to dopaminergic neurons in a rat model of PD, achieving percentages of TH-positive neurons similar to that of the control group (Huang et al., 2013). The enzyme beta-secretase 1 (BACE1) is a therapeutic target for AD due to its involvement in Aβ production, the main component of the characteristic plaques found in the gray matter of patients. Thus, inhibition of BACE1 activity has been extensively pursued. For example, a PEGylated poly(mannitol-co-PEI) (PEG-PMT) gene transporter tagged with RVG peptide was developed for the delivery of BACE1 siRNA, obtaining a 5.8-fold increased accumulation in the brain when compared with nontargeted polyplexes. Moreover, only targeted complexes significantly reduced the

expression of BACE1 mRNA in the hippocampus (≈50% of control), which was accompanied by a reduction of Aβ levels in mice (Park et al., 2015).

Additionally, PTs can include moieties that bind to protein deposits to target treatment toward an affected area and trigger protein disaggregation at the same time. For example, Zheng et al. (2017) obtained multifunctionalized delivery systems for the treatment of AD by coassembling different PEGylated poly(2-(N,N dimethylamino) ethyl methacrylate) (PEG-PDMAEMA) polymers; the naked polymer, the polymer bearing the CGN peptide for brain targeting, and the polymer bearing an Aβ-targeting peptide. The CGN peptide is the retroinverse isomer of the phage-displayed TGN peptide (TGNYKALHPHNG) that serves as a vector for BBB crossing, although the exact target remains unknown (Oller-Salvia et al., 2016; Zheng et al., 2017). In an AD mouse model, BACE1 siRNA dual-targeted complexes reduced the expression of BACE1 mRNA to 63%, which was translated into a reduction of soluble amyloid precursor protein (sAPPβ), Aβ, and neural loss in the hippocampus (Zheng et al., 2017).

1.5 | IN delivery

IN administration, with numerous advantages over oral or parenteral administration, has emerged as an alternative route to directly target therapeutics to the CNS, avoiding the BBB completely. Other than its noninvasiveness nature, IN administration offers: (a) facile self-administration, (b) rapid drug absorption via the highly vascularized mucosa, (c) improved drug bioavailability and avoidance of the gastrointestinal (GI) tract and first-pass metabolism, and (d) rapid onset of action with lower side effects, among other advantages. In general, IN administration permits elevated brain targeting (about 10-fold increase) as compared with IV administration and, so, involves lower drug doses to achieve successful clinical outcomes and reduces off-target effects. IN delivery has been already used to target a wide variety of therapeutics to the CNS as a simple solution or coadministered with permeation enhancers (Ozcan, Ozpolat, Coleman, Sood, & Lopez-Berestein, 2015). However, not all the drugs/biologicals can bypass the nasal barriers and only 1% of the total dose of those who do are able to reach the brain, revealing the need of DDSs/novel technologies to enhance this percentage (Illum, 2004; Mistry, Stolnik, & Illum, 2015).

1.6 | IN route

1.6.1 | The nasal pathway: Barriers

As mentioned, PTs aiming to reach brain delivery through the IN route, must overcome diverse conditions and barriers. The size of the nasal cavity itself limits the volume of concentrated drugs (25–200 μL in humans) (Van Woensel et al., 2016). while the nasal microenvironment is the major barrier for nasal permeation due to (a) low membrane permeability, (b) rapid mucociliary clearance mechanisms, (c) possible enzymatic degradation in the nasal cavity, and (d) the slightly acidic pH in adults (5.5–6.5) (Misra & Kher, 2012).

Once absorbed in the nasal mucosa, molecules can follow different pathways (Figure 4). Despite the large mucosal surface area of the nasal cavity (160 cm^2, or 96 m^2 if the microvilli are included), only 3% constitutes the olfactory region with a direct connexion to the CNS (Illum, 2015). Biologicals/drugs absorbed to this less-ciliated region can use axonal transport through the olfactory nerve (the first cranial nerve) to reach the olfactory bulb, which gives access to rostral brain areas including the piriform cortex, amygdala, and hypothalamus. Alternatively, active agents can also use axonal transport through the trigeminal nerves, that innervate the respiratory region, the largest region of the nasal cavity, promoting access to caudal and rostral parts of the brain through the pons (S. Khan, Chen, et al., 2017). Furthermore, axonal transport coexist with fast transcellular or paracellular mechanisms across both the olfactory and respiratory epithelium (nasal blood and lymphatic vessels) reaching then the perineural space, and subsequently the subarachnoid space which contacts with the cerebrospinal fluid (CSF; Figure 5). Once in the CSF, any active agent can diffuse into the brain tissue or be cleared back to systemic circulation. This transport occurs faster than axonal route (<30 min vs. few hours or days in the case of the axonal route; Badhan, Kaur, Lungare, & Obuobi, 2014). For a deeper analysis of the nasal anatomy and an exploration of nose-to-brain delivery, we direct the reader to recent excellent reviews (Almeida & Florindo, 2012; Pardeshi & Belgamwar, 2013).

1.6.2 | Nasal models and limitations

Nasal environment complexity gives rises to a general problem in CNS delivery that has impeded or limited clinical translation: the lack of realistic in vivo/in vitro models that resemble human scenario.

In vitro models have aided investigations into the molecular mechanisms behind drug/polymer absorption, including trans- and paracellular pathways, metabolism, cytotoxicity and irritation, as well as the mucoadhesive and mucodiffusive properties of the systems. Cellular models rely on a donor chamber and a receptor medium separated by a membrane and/or a cell monolayer, with a large array of procedures covering sampling procedures, cell preparation, and culture techniques (Sosnik, 2015). RPMI 2650 cell line and primary culture of human nasal epithelia (HNE) have been widely used models for permeability
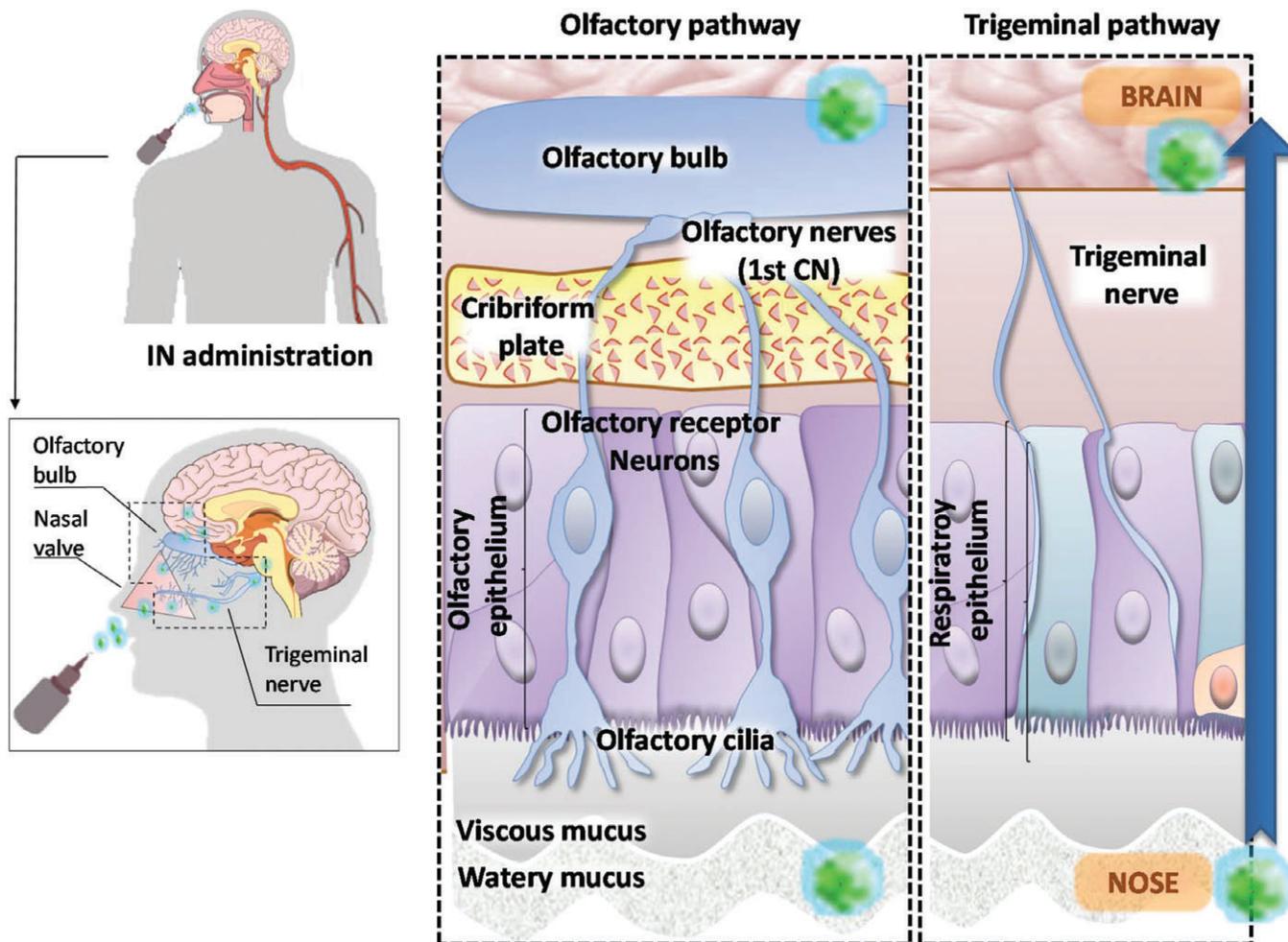
FIGURE 4 Schematic representation of polymer therapeutics (PT) administration through intranasal administration. The olfactory and the trigeminal routes
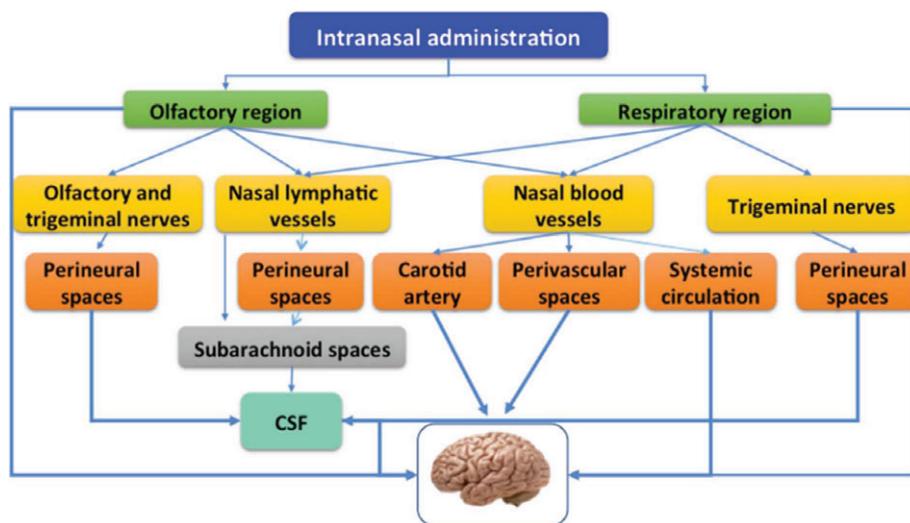
FIGURE 5 Detailed pathways for reaching the brain after intranasal (IN) delivery. CSF = cerebrospinal fluid

studies, with direct relevance to human patients (Kim, 2008). The human Calu-3 lung carcinoma cell line has also been employed, owing to their ability to produce mucus and form TJ in a Transwell® configuration in an air-liquid interface (ALI; Lechanteur, das Neves, & Sarmento, 2017). Alternatively, some models consider the incorporation of artificial mucus from animal or human sources or commercial mucus-like fluids to cell membrane models. Nevertheless, mucus sources differ in composition and three-dimensional (3D) architectures, conferring high heterogeneity and, often fail to reproduce the biophysical properties of human mucosa. Increased complexity is found in 3D models that combine cocultures and 3D mucus-secreting spheroids of primary bronchial epithelial cells (Kalashnikova, Albekairi, Ali, Al Enazy, & Rytting, 2016). Although poorly

explored yet, commercial 3D models of human airway epithelium such as EpiAirway™ (MatTek Corporation, Ashland, MA) and MucilAir™ (Epithelix, Plan-les-Ouates, Switzerland) are available and may represent a more biologically relevant model (Zavala et al., 2016). Variability derived from donor to donor also must be considered (Huang, Wiszniewski, & Constant, 2011). Animal and human excised nasal mucosa are useful ex vivo models to study the permeation of fluorescently labeled PTs across the epithelium when mounted in chambers (i.e., Franz cells), allowing the precise control of physiological conditions such as pH, temperature, and oxygenation. Among the animal models, ovine and porcine nasal mucosa exhibit similar cellular composition and morphology to human nasal mucosa (Mistry et al., 2015).

Even though these models represent an affordable and helpful predictive and screening tool, in vitro and ex vivo models fail to fully recreate the total complexity of the nasal mucosa and do not reproduce the entire nose-to-brain pathway, including axonal transports or brain localization (brain diffusion and bioavailability). In this case, in vivo models are strictly necessary. However, in vivo models must carefully consider the anatomical and physiological differences between species (see Table 1), with the percentage of olfactory epithelium surface the most relevant parameter (i.e., this value reaches 50% in rats, while only 3% in humans; Illum, 2015). Eve given the disparity, rodent models are the most popularly applied for these studies due to similarities in the number of olfactory receptors and transport rates.

However, the processes employed to validate IN CNS delivery strategies vary across the numerous studies. Apart from the use of different animal models, the broad range of reported PK/PD parameters or the indirect/semiquantitative biodistribution data collected hamper the extraction of conclusions concerning the evaluated systems. The application of homogenous analysis criteria to all in vitro and in vivo studies will facilitate clinical translation, including (a) the use of imaging techniques evidencing ability of nanocarriers to travel across neuronal transport (distinguishing between olfactory or trigeminal pathway), brain delivery, and subsequent diffusion, comparing techniques of the sliced brain tissues to prove drug presence at the target site (radioactive or fluorescent labeling, high-performance liquid chromatography [HPLC] detection, radioimmunoassays, staining techniques; Sekerdag, 2017), (b) the attainment of quantitative data through PK assessment (C_{max}, t_{max}, AUC, brain: plasma, nose-to-brain direct transport percentage [DTP], and drug targeting efficiency [DTE]; Westin et al., 2005), (c) the implementation of well-designed controls, including the demonstration of higher CSF/plasma ratio in comparison with parenteral administration, and (d) the application of behavioral and metabolic studies to prove drug efficacy and histopathology to prove safety.

1.7 | Polymer therapeutics strategies for the IN route

Envisioning the whole nasal route, there are key physicochemical parameters that PT formulation must display for tailored nasal absorption: a balance between bioadhesion and mucodiffusion, permeation, and further distribution using a specific transport route, and the ability to reach a specific brain area without causing inflammatory or immune responses (Karavasili & Fatouros, 2016; Misra & Kher, 2012). Those parameters are their stability, solubility, lipophilicity, Mw and size, pH, osmolarity, viscosity, and density.

Each single stage of the route has been deeply studied in the nanomedicine field to confer the DDS determined characteristics to overcome the problematic, for example, drug protection, mucoadhesive polymers (Sosnik, das Neves, & Sarmento, 2014), targeting moieties to nasal mucosa to improve permeability (Samaridou & Alonso, 2017), enzyme inhibitors (Thwala, Préat, & Csaba, 2017), PEGylation to facilitate diffusion (Huckaby & Lai, 2017), and/or the use of peptides as cell-targeting (F. Zhang, Lin, Kannan, & Kannan, 2016).

TABLE 1 Interspecies comparison of nasal cavity characteristics according their complexity (scroll = nasal turbinate). (Reprinted with permission from Sosnik (2015). Copyright 2016 Elsevier)

	Estimated weight (kg)	Nasal volume (mL)	Surface area (cm^2)	Volume to be administered (μL)	Clearence half-life (min)
Single scroll					
Man	70	20	160	150	15
Monkey	7	8	62	58	10
Double scroll					
Guinea pig	0.6	0.9	27	25	7
Mouse	0.03	0.03	2.8	3	1
Rat	0.25	0.4	14	13	5
Sheep	60	114	327	307	42
Branching					
Dog	10	2	221	207	20
Rabbit	3	6	61	58	10

The ideal nanocarrier should be retained at the mucosa and must penetrate the mucus faster than its turnover rate, so protecting the cargo from premature degradation and promoting cell internalization. Devoted efforts in this direction have exploited mucoadhesive polymers or PEGylation (see Section 1.9). Cationic polymers such as chitosan has been extensively explored either as a coating or as vehicle for IN drug delivery owing to their electrostatic interaction with anionic mucin (Samaridou & Alonso, 2017). Polymers like PEG, polymethacrylates, and poly(acrylic acid)-derivatives may propagate mucoadhesion via hydrogen bonding, hydrophobic interactions, polymer-mucin entanglements, or their combination (Huckaby & Lai, 2017). In addition, PEG can reduce particle aggregation and adhesion to mucin fibers, thereby allowing diffusion through the low viscosity interstitial fluids in between (X. Gao et al., 2006; Huckaby & Lai, 2017). Mucoadhesive properties seem to be intimately related to the intrinsic physicochemical nature of the nanosized carriers not only in terms of structural properties, such as size, shape, zeta-potential, deformability and elasticity, but also to the chemical composition based on the ability to interact through specific noncovalent interactions forces (e.g., lectins) or covalent bond formation (thiolation groups for in situ crosslinking with mucin components) (Bernkop-Schnürch, 2005b). For instance, thiolated chitosan affords up to 140-fold greater mucoadhesion (Bernkop-Schnürch, 2005a). Other established strategies to promote mucoadhesion include the use of absorption enhancers, for example, inclusion of targeting moieties such as lectins (detailed below).

In this regard, structure–activity relationships (SARs) based on physicochemical descriptors of mucoadhesion and/or mucodiffusion properties in mucosa mimicking environment are vital for the prediction of PT in vivo performance. Other than the previously mentioned in vitro/in vivo models, rapid and noninvasive techniques such as nuclear magnetic resonance (NMR), nanoparticle tracking analysis (NTA), small-angle neutron scattering (SANS) have been extensively applied. Specific and advanced NMR experiments (e.g., Diffusion Ordered SpectroscopY (DOSY), Nuclear Overhauser Effect (NOE) NMR, or relaxation times $T1$–$T2$ measurements) have been used to quantify diffusion coefficients, relaxation times and possible noncovalent interactions for the designed nanoconstructs (Occhipinti & Griffiths, 2008). Descriptors such as size, conformation, deformability and 3D shape can be extrapolated from SANS data. Studies combining DOSY NMR and SANS for the evaluation of PT and mucin interactions demonstrated that neutral polymers such linear or branched PEG (10 and 100 KDa) and dextrin exhibited moderate mucin interaction. However, positively charged PAMAM dendrimers and hyperbranched PEI exhibited strong interactions, leading to reduced diffusion rates (Griffiths et al., 2010). Finally, NTA experiments in reconstituted mucin gels or respiratory mucus have been used to test nanosystem motion and stability in mucus at high spatial and temporal resolutions. Several parameters can influence these studies for example, mucin concentration can affect diffusion, while pH affects interactions between mucin and charged particles (Schuster, Ensign, Allan, Suk, & Hanes, 2015). Using he NTA technique, cationic-surfactant poly(lactic-co-glycolic acid (PLGA) polyplexes showed 10-fold higher diffusion than polystyrene NPs in reconstituted pig gastric mucin. Additionally, PEGylation has demonstrate to provide diffusive properties (Yang et al., 2012).

Biorecognitive ligands targeting the olfactory region (i.e., lectins, specific proteins or glycoproteins) that bind to saccharides present in the mucus layer (e.g., N-acetylglucosamine and L-fucose) have been applied to enhance transepithelial transport. An example is the smallest known lectin, odorranalectin (OL), a 17-amino acid sequence with lectin-like activity that binds specifically to the monomeric sugar L-fucose. L-fucose is found on the olfactory epithelium of nasal mucosa and presents less immunogenicity than other larger natural lectins (Li et al., 2008) such as wheat germ agglutinin (WGA), *Solanum tuberosum* lectin (STL) and ulex europeus agglutinin I (UEA-1; Gao et al., 2007). Even though immunogenicity represents an important obstacle to clinical translation, several studies have studies the application of such ligands. WGA functionalization enhances epithelium crossing transit through the extraneuronal pathway and promotes localization in deep brain areas 30 minutes after administration (Gao et al., 2007; Liu et al., 2011; Liu, Shen, et al., 2012), while STL conjugation provided 2.5-fold more brain targeting efficiency (AUCBrain/AUCBlood) than nontargeted systems (Chen et al., 2012). Additionally, OL-targeted nanoparticles achieved 2 times higher accumulation than nontarget nanoparticles via IN, and almost 150-fold more than IV administration of the same nanoparticle (Wen et al., 2011). Lactoferrin (Lf), a natural binding iron protein, has been also exploited due to high expression of LfR on the apical surface of respiratory epithelial cells, brain endothelial cells, and neurons (Elfinger, Maucksch, & Rudolph, 2007) and the specific overexpression of LfR in the CNS of patients suffering from age-related neurodegenerative disorders (Gao, 2016).

Upon conjugation, cell-penetrating peptides also enhance brain delivery with reduced clearance and improved absorption. Cell-penetrating peptides (CPPs) are short cationic amino acid sequences that interact with the plasma membrane and promote internalization, often via endocytosis (Skotland, Iversen, Torgersen, & Sandvig, 2015). Low-MW protamine (LMWP) and the human immunodeficiency virus transactivator of transcription peptide (TAT) have been explore for IN administration (Kanazawa, Taki, Tanaka, Takashima, & Okada, 2011; A. R. Khan, Liu, et al., 2017), although both present serious limitations due to their nonspecific interaction with biological membranes, which increases accumulation and unwanted side effects, thereby limiting their application (Koren & Torchilin, 2012; D. Zhang, Wang, & Xu, 2016). Furthermore, TAT can induce cargo-dependent cytotoxicity (El-Andaloussi, Järver, Johansson, & Langel, 2007; Kilk, Mahlapuu, Soomets, & Langel, 2009)

and does not always resulted in enhanced IN administration (McGowan, Shao, Vig, & Bidwell, 2016), while one study found significant levels of LMWP-conjugated PEG–PLA in liver, kidneys, and other organs after IN administration (Xia et al., 2011). However, covalent linkage of LMWP to proteins such as bovine serum albumin (BSA), horseradish peroxidase (HRP) and β-galactosidase (β-gal) enhanced brain delivery via IN administration and maintained protein activity (Lin et al., 2016). These results highlight the requirement for physicochemical descriptors to establish SAR. A recently reported arginine-based CPP (CHHRRRRHHC)-conjugated PEG-poly(caprolactone) (PEG-PCL) demonstrated enhanced brain delivery of Alexa-dextran (as model drug) after IN administration when compared to free Alexa-dextran. More importantly, the polymeric version spread throughout brain tissue, a fact that the authors attribute to PEG's ability to diffuse together with a combination of the IN pathways used (olfactory/trigeminal pathways and passage to the CSF) (Kanazawa et al., 2017). Finally, omega-3 fatty acids such as docohexaenoic acid (DHA) have been shown to also facilitate mucosal transport through natural diffusion across the BBB through their natural receptors CD36 and FABP (Picq et al., 2010). However, a recent study of human brain tissue samples discovered increased levels of DHA during the progression of AD (Snowden et al., 2017). Therefore, alternative unsaturated fatty acids, such as eicosapentaenoic acid (EPA), which presents with reduced levels in AD patients, may represent an interesting molecule to evaluate in nasal transport.

Although all the described approaches have contributed to ameliorate brain delivery, there still remain problems to overcome. Efficient developed vectors may permit the specific selection of nasal route (e.g., olfactory, trigeminal, passage to CSF) to promote delivery to a specific brain area and improve the bioavailability of the drug. Moreover, once in brain, DDS diffusion to reach the target still represents a great challenge. Methods to improve brain diffusion (see section 1.9) as well as other approaches mentioned for IV, can be exploited for IN although there are not yet evidences on literature for PT, for example, conjugation of specific cell-targeting ligands to unique receptors in a brain cell type (dual targeting), use of smart linkers for example, susceptible to cathepsin B release, reductive environment, or oxidative stress, etc.

1.8 | Polymer therapeutics for CNS disorders via IN route

Although the IN administration route has been extensively applied in the past, this route represents a "rediscovered" strategy for the delivery of active agents using polymeric nanocarriers and only few examples, most of them related with nucleic acid delivery, can be found in the literature. The IN route has been effectively targeted for the delivery of siRNAs for pulmonary diseases, with ALN-RSV01, a siRNA for respiratory syncytial virus (RSV)-infected lung transplant patients, the most advanced example (in phase IIb clinical trials; Gottlieb et al., 2016). Nevertheless, most CNS treatments via the IN route are in early preclinical stages and PTs targeting the IN route are almost unexplored in the field. Several examples will be discussed in this section.

Linear PEI polyplexes carrying siRNA to silence Beclin-1 have been designed to treat HIV-associated dementia pathology, by attenuating HIV replication in mouse after IN administration, reducing its protein expression up to a third without inducing additional neuroinflammation of neurotoxicity (Rodriguez et al., 2017). Nevertheless, the application of PEI does display toxicity-related problems related to its cationic charge density, a fact that may hamper clinical translation.

The IN route is an appealing strategy to treat Parkinson's disease (PD), whose affected areas are reachable via the trigeminal pathway (substantia nigra and corpus striatum; Aly & Waszczak, 2015). Copernicus Inc. has developed a gene therapy strategy based on a PEG–PLL copolymer complexed with a DNA plasmid encoding hGDNF. IN administration of the complex permitted brain delivery, wide distribution, and successfully induced sufficient GDNF expression to protect dopamine neurons in the rat 6-hydroxydopamine (6-OHDA) model of PD (Aly et al., 2015). This exciting system was previously evaluated for eGFP expression (Harmon et al., 2014) and encouraging results were also noted in an initial clinical trial in cystic fibrosis subjects (Konstan et al., 2004). This trial reported no adverse events and positive functional assays; however, no further applications of this complex have been reported in the literature.

IN administration of chemotherapeutics for recurrent GBM patients is currently under assessment in a phase I/II trial with monoterpene perillyl alcohol (POH) (REF CT NCT02704858), proving the feasibility of the technique to treat brain tumors. The mPEG-PCL-TAT micelles complexed with siRNA against Raf-1 codelivered with the anticancer-drug camptothecin (encapsulated, not conjugated) have been evaluated as an IN treatment for glioma (Kanazawa, Morisaki, Suzuki, & Takashima, 2014). Four percent of the total administered dose of these nanomicelles was able to reach the brain through olfactory and trigeminal nerves, while only 1.5% was achieved via IV administration (Kanazawa, Akiyama, Kakizaki, Takashima, & Seta, 2013). Furthermore, the authors observed a synergistic effect of this combination therapy: a reduction in tumor size and an increase in mean survival in treated glioma-bearing rats from 18 to 28 days.

Use of the IN route also represents an attractive noninvasive approach to reach infarcted area following brain ischemia (Kim et al., 2006). For instance, Kim et al. (2012) developed IN polyplexes based on an arginine-modified PAMAM G4 dendrimer and siRNA targeting high-mobility group box 1 (HMGB1), a protein known to aggravate inflammation, within the infarcted area. The study detected the polyplex in multiple brain regions for up to 12 hr, with presence detected after only 1-

hr postadministration. Moreover, IN administration in a middle cerebral artery occlusion (MCAO) rat model produced a significant depletion of the target gene, accompanied by a reduction in the infarct volume by 43% and enhance neuroprotection, as shown by neurological and behavioral deficit recovery. In a different study, Perez et al. developed a radioactive labeled siRNA PAMAM dendriplex formulated within in situ forming mucoadhesive gels, based on blended Pluronic®F127 for gelling properties and polyacrylic acid (Carbopol) for its mucoadhesiviness (Perez, Mundiña-Weilenmann, Romero, & Morilla, 2012). The polymeric blend displayed several advantages over buffer dilution, showing higher brain/blood ratio. Nevertheless, the study did not see comparable IV and IN dosages, but did observe drainage to the lungs and the gastrointestinal system, which may lead to off-target effects, especially from the cationic PAMAMs (Catalan-Figueroa et al., 2016).

PTs have also been applied following IN route to treat sensory nerve disorders, owing to their ability to provide stability and enhanced bioavailability of the commonly applied growth factors or peptides used to treat these diseases. For instance, Baba, Itaka, Kondo, Yamasoba, and Kataoka (2015) administered polyplex nanomicelles based on the PEG-polyaminoacid block copolymer poly[N′-[N-(2-aminoethyl)-2-aminoethyl] aspartamide] [PAsp(DET)] complexing mRNA encoding the brain-derived neurotrophic factor (BDNF) protein in a mouse model of experimentally induced olfactory dysfunction (Figure 6). Another studies found the mRNA-nanomicelles to be safe and stable in the CNS (Uchida et al., 2013) and this approach provided an efficient and sustained protein expression for 48 hr in nasal tissues and particularly in the lamina propia, which contains olfactory nerve fibers. Moreover, the authors found a recovery in both olfactory epithelium architecture and function after IN, providing proof the success for this strategy.

Finally, Kabanovet al. (2002) developed conjugates of the antiobesity hormone leptin with Pluronic 85® (LepNP85; Yi et al., 2014; Yuan et al., 2017) to improve brain delivery in obese patients displaying leptin deficiency in the brain. This systematic study evaluated (a) hormone conjugation specificity, by comparing conjugation via random lysines or the N-terminal group, (b) hydrophobicity, comparing PEG versus pluronic, and (c) differences between administration routes (IV, intracerebroventricular [ICV], and IN). The authors concluded that N-terminal linkage was optimal, due to the avoidance of steric hindrance in the protein–receptor interaction, and that noncleavable linkage was required for protein activity. By IV administration, brain entry of LepNP85 was inhibited by coinjection of cold leptin, suggesting that peripheral resistance was not overcome IV. However, LepNP85 improved leptin absorption across the nasal epithelia (NE) following IN administration, due to increased hydrophobicity in comparison with the PEG conjugated form, leading to higher accumulation and effective activity in the hypothalamus and hippocampus (in disease and healthy mice models) when compared with unmodified leptin.
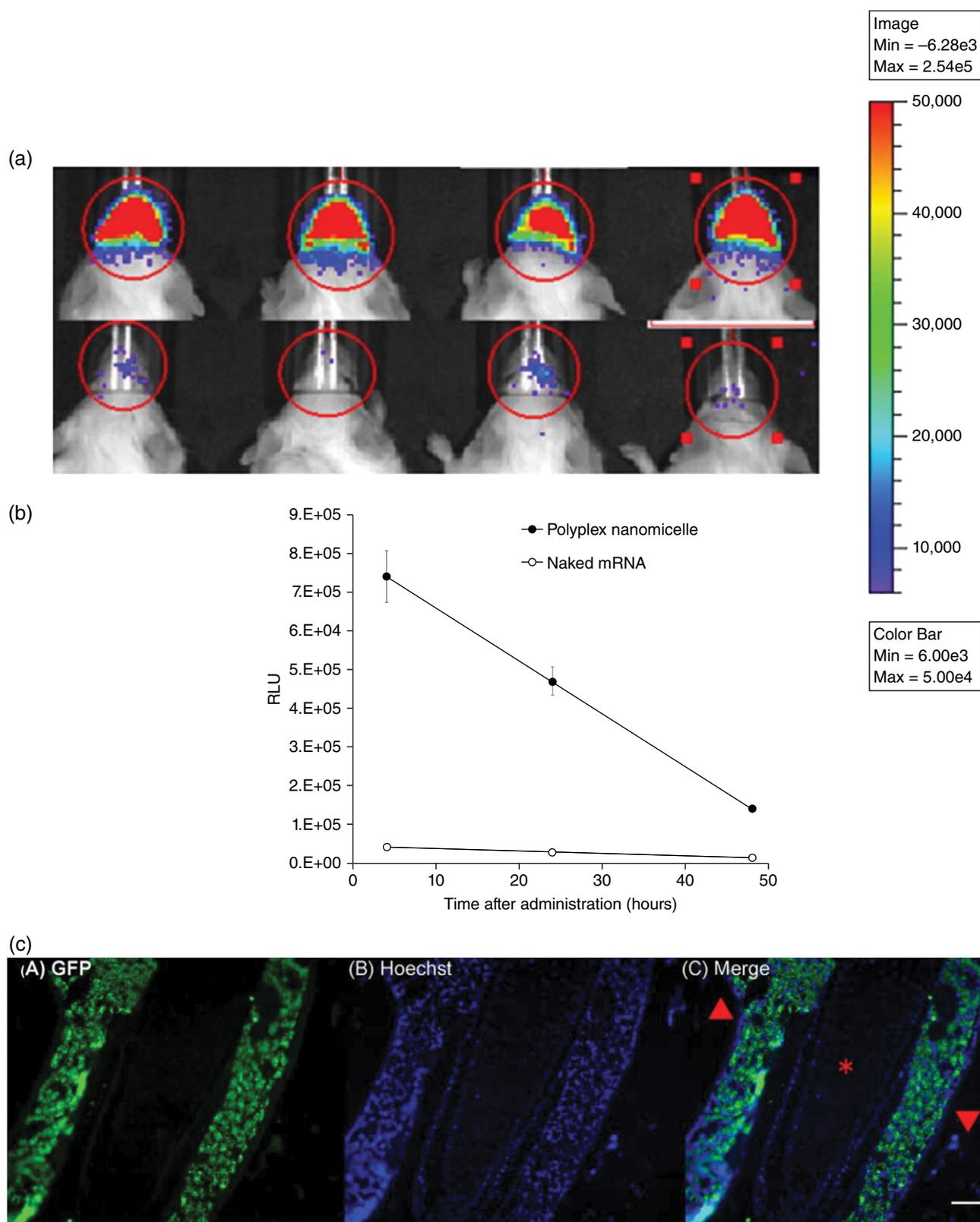
Many other CNS diseases would also benefit from PT treatment through this route, where other types of nanosystems are already being assayed (Liu, Jiang, et al., 2013). For example, IN insulin is in phase II/III of clinical trials for AD (starts in the entorhinal cortex, itself closely connected to the olfactory nerves (Liu, Guo, et al., 2012)) and phase II trial for mild cognitive impairment, PD, and multiple system atrophy. This approach achieved memory and mood improvement in AD patients, as well as modulated plasma levels of amyloid beta (Claxton et al., 2015; Reger et al., 2008) and phase II trial for mild cognitive impairment, PD, and multiple system atrophy. This approach achieved memory and mood improvement in AD patients, as well as modulated plasma levels of amyloid beta (Quintana, Guastella, Westlye, & Andreassen, 2016).

1.9 | Additional design considerations and strategies

The paramount rationale in PT design for brain delivery relies on the need to cross biological barriers to reach the site of action, with the BBB representing the main hurdle in this pathway. Overcoming the BBB through noninvasive PT-based techniques is a plausible alternative, either bypassing it with TH-based strategies after IV administration or by employing the IN route. From the literature covering PT-based studies, several key design considerations can be drawn.

The advantages of PT are well established, for example, protecting and stabilizing the active agent during passage through the bloodstream and promoting controlled release of said active agent under specific biological conditions at the required target (Duncan & Vicent, 2013). In addition, PT can exploit polymeric multivalency to conjugate multiple drugs and establish synergistic combination therapies (Greco & Vicent, 2009) or the construction of theranostic platforms (Duncan, 2011). But specifically for drug brain delivery, either through IN or IV, *active targeting of BBB or NE* is underscored as an essential asset as addressed in this review. The compiled results on the named targeting moieties highlight the need of physicochemical descriptors to establish SAR are fundamental to develop efficient PT.

Moreover, once in the brain, targeting to the specific site of action (cell, deposits, etc.) should be considered provided that unselective distribution may cause severe neurotoxicity and low therapeutic response. The *dual targeting* constitutes an appealing strategy due to its ability to conquer different barriers sequentially. Some literature examples of dual-targeted nanosystems support this fact, for example, combining ANG-2 for BBB penetration and EGF1 for neuroglial cell targeting (Huile et al., 2011), the TGN peptide (TGNYKALHPHNG) for BBB and the AS1411 aptamer for brain tumor targeting (Gao et al., 2012) or the QSH peptide (QSHYRHISPAQV) against $A\beta_{1-42}$ (C. Zhang et al., 2014). Some ligands can meet both criteria by targeting receptors expressed at the BBB and diseased brain cells as in the case of brain tumors, such as LRP or Tf receptor

FIGURE 6 (a) Bioluminescence after intranasally administering luciferase-expressing mRNA. (a) Bioluminescence images obtained by an IVIS imaging system 4 hr after administering luciferase expressing mRNA-loaded polyplex nanomicelles (upper) and an equal quantity of naked mRNA (lower). (b) Time course of bioluminescence after intranasally administering mRNA using polyplex nanomicelles (closed circle) and naked mRNA (open circle). Statistical analyses were performed by two-tailed Student's t-test, ***$p < .001$, **$p < .01$. RLU = relative luminescence units. Results are means ±SEMs ($n = 4$). (c) Histological analysis after intranasally administering GFP-expressing mRNA. Mice were sacrificed and decapitated 24 hr after administering GFP-expressing mRNA. (A) GFP expression visualized by immunostaining using an anti-GFPmonoclonal antibody. (B) Cell nuclei stained by Hoechst. (C) Merged image. GFP-positive staining was widely observed in the lamina propria (arrowheads), but not in nasal septal cartilages and bones (asterisk). Scale bar: 50 μm. (Reprinted with permission from Baba et al. (2015). Copyright 2015 Elsevier)

(e.g., ANG-2 and lactoferrin). For IN administration, lactoferrin can target its receptor on the NE as well as brain endothelial cells and neurons later on in the pathway (Liu, Jiang, et al., 2013).

While the scientific community has always concerned about the limitations owing to the BBB, attention has been paid only recently to the need of *brain diffusion*. Once in the brain, PTs must avoid its clearance mechanisms (i.e., efflux transporters, enzymatic breakdown, and paravascular drainage) and diffuse through the extracellular space to reach their target (Wadajkar et al., 2017). This extracellular space consists of a negatively charged network of extracellular matrix (ECM) and interstitial fluid. The ECM network is formed by hyaluronic acid, heparan and chondroitin sulphate proteoglycans, and several glycoproteins, laminins, and collagens (Wolak & Thorne, 2013). PTs will face narrow extracellullar spaces (38–64 nm average width pore with 28% of pores less than 100 nm in width with a high degree of tortuosity and electrostatic interactions leading to repulsion, attraction, transient binding, and/or sequestration effects (Wadajkar et al., 2017). Thus, size, surface charge, composition, and architecture of PTs can directly influence brain diffusion. PTs with near-neutral surface charge might be favored (Curtis, Zhang, Liao, Wood, & Nance, 2017), being one of the reasons why brain widespread is favored by PEGylation (Joralemon, McRae, & Emrick, 2010; Nance et al., 2012) or hampered due to lipidization (Albertazzi et al., 2013). Polymer architecture can also directly influence PTs performance, by (a) increasing effectivity due to higher drug/bioactive loading capacity and exposure and (b) modifying in vivo distribution and cell trafficking (Duro-Castano et al., 2015; Liechty, Kryscio, Slaughter, & Peppas, 2010) owing to a better presentation of targeting ligands and the adopted solution conformation. For instance, an enhanced rate of transfer across biological barriers has been seen for branched architectures (Duro-Castano et al., 2015; van der Poll et al., 2010), a fact that may be suited for brain delivery. Additional considerations for clinical translation include the differences in interspecies brain volume and diffusion distances. Human brain volume is 2,800-fold larger than mouse and four-fold larger than nonhuman primates (Curtis et al., 2017). Moreover, diffusion distances may vary depending on the disease target site (brain area, intracellular/extracellular target, etc.) and on the pathway used to reach the brain. Chemotaxis represents an interesting concept in the enhancement of PT brain diffusion, that is, engineering PTs to navigate by endogenous chemical gradients (Vorotnikov, 2011). This mechanism allowed a fourfold increase in brain penetration with polymersomes encapsulating glucose oxidase alone or in combination with catalase using a gradient of glucose to promote movement (Joseph et al., 2017).

It is noteworthy to remark that well-recognized and sophisticated imaging techniques to monitor BBB crossing and diffusion across the brain parenchyma own a high risk of artifacts and do not ensure precise brain delivery. To prove this fact, experimental work often requires behavioral assessment or direct drug quantification after tissue digestion (ideally, after perfusion). The detection of intact drug carriers inside the brain as well as carrier-drug dissociation monitoring through this type of techniques is still challenging and it is rarely shown. The use of radiolabelled polymers and/or drugs incorporating ^{14}C, and ^{3}H can contribute to this goal (Wolf, 2016).

Due to its key function, brain is particularly sensitive to toxic agents and the proper assessment of nanomedicine-induced neurotoxicity represents a crucial checkpoint for clinical development Neurotoxicity can result from direct alteration to neuronal structure or activity or indirect effects derived from glial activation, leading to severe acute toxicity or even death. Neuronal loss is irreversible and leads to the loss of particular functions, with cell death caused by diverse mechanisms including induction of oxidative stress, Ca^{2+} overload, cytoskeleton disruption, or mitochondria damage (Li & Martin, 2017). Toxicity to nanomedicine is often associated with increased intracellular reactive oxygen species (ROS) levels and/or the levels of proinflammatory mediators that upregulating various proinflammatory genes, for example, TNF-α, and Interleukins (IL)-1,-6, and -8. Ideally, the polymeric vehicle employed as part of the PT, as well as its metabolites, must be able to exert its therapeutic action without nonspecific long-lasting extracellular brain accumulation, which would enhance the risk of toxicity. Only a few studies report neurotoxicity assessment, and most cases only employ simple cytotoxic test, so ignoring possible effects due to microglial activation, astrocyte reactivity, brain homeostasis maintenance, BBB disruption, inflammatory responses, ROS production, and so on. (Newland, Newland, Werner, Rosser, & Wang, 2015; Nunes, Al-Jamal, & Kostarelos, 2012). Several studies on the neurotoxicity of cationic polymers indicate that PEI could induce acute brain toxicity, PAMAM dendrimers could promote neuron death, ROS production, apoptosis and activation of CNS immune system microglia, and PLGA could generate oxidative stress, and so on (Li & Ju, 2017).

The process of *PT elimination from the CNS* is equally as relevant as PT activity in the brain. Four routes are involved in this mission: (a) enzymatic extracellular degradation, (b) intracellular degradation after glial or neuron internalization, (c) entering CSF circulation and then the bloodstream or the cervical lymphatics, and (d) brain-to-blood passage through the pump-efflux systems such as P-gp, multidrug resistance associated proteins (MRPs), and breast cancer resistance protein (BCRP; Gao, Gu, & Chen, 2017). In particular, cellular uptake of PT by endocytosis in neurons or glial cells leads to endolysosomal degradation, and at this point, the resulting metabolites should also be biocompatible. Shape and size also can impact endolysosomal degradation (Canton & Battaglia, 2012). In this respect, biopersistent carriers (Canal, Sanchis, & Vicent, 2011) present disadvantages in chronic administrations due to the potential to generate "lysosomal storage disease" syndrome.

Preclinical evidence of intracellular vacuolation (Webster et al., 2009) and clinically reported hypersensitivity reactions (Duncan, 2014) with certain PEG-protein conjugates has raised awareness of the potential advantages of biodegradable polymers (Duncan, 2014) with certain PEG-protein conjugates is raising awareness of the potential advantage of biodegradable polymers regarding safety benefit (Vicent & Duncan, 2006). The acid environment either in the extracellular space of inflamed areas or extracellular glioma can also trigger the degradation of hydrolysable polymers. For example, the marketed brain implant Gliadel® wafer is composed the combination of hydrophobic monomer units and highly water-reactive anhydride linkages between monomers.

Additional challenges include manufacturing (e.g., reproducibility, scaling up, sterilization, and storage requirements; van Woensel et al., 2013), and adaptation to personalized medicine (e.g., therapies based on expression levels of a specific target). With all their components, PT constitute complex systems and thus, rationality within this complexity must be correctly addressed, and the specific needs toward a disease application is highly necessary (Gaspar & Duncan, 2009).

2 | CONCLUSION

The increased prevalence of neurodegenerative diseases is undeniable and while vital strides have been made concerning CNS drug delivery, more efficient and safer systems are required for treating these complex natured diseases. PT embody great capacities to meet this goal through nondisruptive or invasive administration routes such as IV or IN pathways. Although still in their early development in this field, the reviewed preclinical studies of PT have demonstrated effective brain delivery via IV or IN of small drugs, proteins or nucleic acids and have revealed that active targeting reports greater brain accumulation enhancing the therapeutic outcome. For this purpose, the selection of the appropriate targeting ligand is crucial and subordinated to the expression of its receptor, which could variate under disease hallmarks/evolution and be present in other organs. The synthesis of superselective PTs could influence on their behavior allowing only a selective binding, thus improving their output.

Future directions to boost PT development must tackle several key points. PT diffusion ability within the brain parenchyma to target the disease site in effective concentrations must be considered. PEGylation and the construction of chemotactic PTs are promising strategies for this goal. Dual targeting to traverse BBB or the nasal epithelia in conjunction with brain cell-specific uptake will increase treatment efficiency. Finally, an exhaustive assessment of nanomedicine-induced neurotoxicity as well as the tracking of the elimination route is crucial for clinical development and their study is frequently obviated in preclinical studies. Both constitute key aspects not only for the therapy but also in the regulatory pathway for clinical translation. The use of biodegradable polymers for PT construction, for example, polypeptides, constitutes a potential advantage regarding safety benefits.

It is certain that for all these studies it is mandatory to have realistic disease models. As remarked in this review, the wide variety of in vitro models (for BBB and nasal pathway) and their lack in completely mimicking the real scenario constitutes a real handicap to predict the potential of each system. in vivo studies resemble essential to this objective. In this regard, there is also the need to stabilize common protocols and analysis to normalize procedures to report brain uptake and PK data, and thus enabling the direct comparison of results between different studies for both in vitro and in vivo studies. The establishment of SARs based on physicochemical descriptors will also further aid PT development.

The use of noninvasive and patient-compliant approaches is particularly noteworthy in chronic administrations, such as those required for CNS diseases such as AD or PD. Finally, there exists a marked research trend for the exploration of immunotherapies as adjuvant treatments in brain metastasis and neurodegenerative disorders. Synergism combinations of such therapies with nanomedicines, and specifically PT, may contribute greatly to the treatment of CNS disorders.

After all the named considerations, we do believe in the opportunities offered by PT became real treatments for CNS disorders. Precedents with polymer-based systems in clinical trials for brain disorders and marketed PT with applications in other diseases sponsor this affirmation.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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