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Review article

Polymer-based nanocarriers to transport therapeutic biomacromolecules across the blood-brain barrier

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ABSTRACT

Therapeutic biomacromolecules such as genetic material, antibodies, growth factors and enzymes represent a novel therapeutic alternative for neurological diseases and disorders. In comparison to traditional therapeutics, which are mainly based on small molecular weight drugs that address the symptoms of these disorders, therapeutic biomacromolecules can reduce undesired side effects and target specific pathological pathways, thus paving the way towards personalized medicine. However, these biomacromolecules undergo degradation/denaturation processes in the physiological environment and show poor capacity to cross the blood-brain barrier (BBB). Consequently, they rarely reach the central nervous system (CNS) in their active form. Herein, we critically overview several polymeric nanocarriers that can protect and deliver therapeutic biomacromolecules across the BBB. Polymeric nanocarriers are first categorized based on their architecture (biodegradable solid nanoparticles, nanogels, dendrimers, self-assembled nanoparticles) that ultimately determines their physicochemical properties and function. The available polymeric formulations are then thoroughly analyzed, placing particular attention on those strategies that ensure the stability of the biomacromolecules during their encapsulation process and promote their passage across the BBB by controlling their physical (e.g., mechanical properties, size, surface charge) and chemical (e.g., surface functional groups, targeting motifs) properties. Accordingly, this review gives a unique perspective on polymeric nanocarriers for the delivery of therapeutic biomacromolecules across the BBB, representing a concise, complete and easy-to-follow guide, which will be of high interest for chemists, material scientists, pharmacologists, and biologists. Besides, it also provides a critical perspective about the limited clinical translation of these systems.

Statement of significance: The increasing incidence of central nervous system disorders is a major health concern. The use of therapeutic biomacromolecules has been placed in the spotlight of many investigations. However, reaching therapeutic concentration levels of biomacromolecules in the central nervous system is restricted by the blood-brain barrier and, thus, this represents the main clinical challenge when developing efficient therapies. Herein, we provide a critical discussion about the use of polymeric nanocarriers to deliver therapeutic biomacromolecules into the central nervous system, highlighting potential future directions to overcome the current challenges.

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1. Introduction

In the last thirty years, there has been a substantial rise in the global occurrence and death rates associated to neurological disorders, currently representing a major cause of morbidity and mortality worldwide. This phenomenon can be ascribed to the expansion and maturation of the world population, along with increasing vulnerability to environmental, metabolic, and lifestyle hazards. According to the "Global Burden of Disease, Injuries, and Risk Factors Study (GBD) 2021", around 3.4 billion people worldwide experienced a nervous system disorder in 2021 [1]. These include, among others: (i) neurodegenerative diseases, namely Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD); (ii) autoimmune diseases like multiple sclerosis (MS); (iii) brain cancer, particularly glioblastoma multiforme (GBM); (iv) cerebrovascular accidents (e.g., ischemic stroke); (v) traumatic brain injury (TBI); and (vi) other diseases (e.g., epilepsy, transient ischemic attack, and cerebral palsy) affecting the central nervous system (CNS). In the future, it is anticipated that brain disorders will emerge as a significant global health concern, posing substantial mortality rates and financial burdens [2].

To face this challenge, great efforts are being currently invested in developing treatments that not only alleviate the symptoms of these diseases but also treat the underlying pathology. Several factors contribute to the failure of current medication treatments (mainly based on small molecular weight (MW) drugs) for brain diseases, including the requirement for large therapeutic doses and prolonged and highly invasive intraventricular injections [3]. These variables strongly impact the mediocre results revealed by standard treatment options in the clinic [4,5]. The use of biomacromolecules such as oligopeptides, monoclonal antibodies, growth factors, antioxidant enzymes or nucleic acids, represents a novel therapeutic alternative and has garnered considerable attention from researchers, prompting extensive endeavors to facilitate their translation into clinical applications. This new class of therapeutics offers higher specificity and can greatly reduce off-target effects (Table 1).

Although significant advances in molecular biology permits now the large-scale synthesis of such delicate biomacromolecules, their use as a therapy still faces unmet challenges. The cell membrane has evolved over billions of years to accurately control the movement of molecules into and out of the cytosol, protecting the intracellular environment from extracellular disturbance. Hence, the high MW and the hydrophilic nature of most of the biomacromolecules results in a limited permeability through biological barriers like cell membranes [29]. Besides, macromolecular structures can undergo conformational changes due to the disruption of native non-covalent interactions and the cleavage of peptide bonds, resulting in impaired therapeutic agents. Furthermore, delivering biomacromolecules to the brain systemically faces diverse challenges such as: insufficient drug administration, degradation/denaturation in the bloodstream, first-pass clearance, immune response, toxicity to normal tissues, and restrictions imposed by the blood-brain barrier (BBB); most of the biomacromolecules are unable to noninvasively cross the BBB and enter the brain parenchyma [30].

The seek for strategies for the efficient trespassing of biomacromolecules through the BBB has opened new research opportunities in which state-of-the-art technologies are merged with engineered delivery systems. Thus, new approaches encompass the disruption of the BBB via the utilization of magnetic resonance-guided focused ultrasound [31] or the lipidization of water-soluble drugs [32]. In this regard, nanotechnology could provide sophisticated strategies to design and formulate nanomaterials loaded with biomolecules, significantly enhancing patient prospects and achieving positive pharmaco-economic results [33,34]. A clear example is the application of antioxidants [35] in the management of brain injuries and neurodegeneration, wherein antioxidant enzymes such as superoxide dismutase (SOD1) might

Table 1
Current medications and biomacromolecules under research for the treatment of CNS disorders or brain cancer [3].

Neurological disorder	Characteristic	Current medications	Common side effects	Studies with biomacromolecules
Alzheimer's disease (AD)	Gradually advancing dementia accompanied by memory impairment.	Cholinesterase inhibitors, NMDA antagonists.	Nausea, vomiting, diarrhea, decreased appetite, dizziness, headache, confusion.	Monoclonal antibody against Aβ aggregates [6] (Aducanumab [7], Lecanemab [8] and Donanemab [9]).
Parkinsonś disease (PD)	Pathological degeneration of nigrostriatal dopamine neurons characterized by motor rigidity, resting tremor, and bradykinesia.	Levodopa, dopamine agonist, Monoamine oxidase-B inhibitors, catechol o-methyltransferase (COMT) inhibitors, surgical therapies.	Dyskinesias, nausea, vomiting, orthostatic hypertension, hallucinations, liver damage, infections.	Neurotrophic factors (glial cell-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF)) [10,11], monoclonal antibodies against α-synuclein (Snca) (Prasinezumab) [12,13], shRNA targeting Snca [14].
Huntington's disease (HD)	Autosomal neurodegenerative disorder. Patients exhibit a range of symptoms, including chorea, mental disturbances, and cognitive deterioration.	Chorea and antipsychotic medication, antidepressants (Selective serotonin reuptake inhibitors), mood stabilizers.	Drowsiness, parkinsonism, depression, insomnia, anxiety, akathisia, excess weight gain and dyslipidemia.	Divalent small interfering RNA (siRNA) silencing the huntingtin gene [15]. Chaperone proteins to combat the neurodegenerative effect [16].
Multiple sclerosis (MS)	Demyelinating disease characterized by the immune system's attack on the myelin sheath surrounding nerve fibers, resulting in communication impairments between the brain and the body.	gamma-aminobutyric acid analogs, activation of α2-adrenergic receptors, mitochondrial dihydro-orotate dehydrogenase (DHODH) inhibitors, immunomodulators (Glatiramer acetate).	Diarrhea, nausea, liver problems, weakness, hallucinations, hypotension, increased risk of infections.	Neurotrophic factors (BDNF) [17]. Remyelination-promoting proteins (Insulin-like growth factor 1 (IGF-1)) [18]. Monoclonal antibodies (Natalizumab) [19].
Stroke	Neurological deficiency attributed to a severe focal lesion of the central nervous system (CNS) due to a vascular disease, including cerebral infarction, subarachnoid hemorrhage (SAH), and intracerebral hemorrhage (ICH).	Serine protease tissue-type plasminogen activator.	Nausea, vomiting, hypotension, dizziness, allergic reactions.	Neurotrophic factors (NGF) [20]. RNAi targeting tumor necrosis factor (TNF)-α or interleukin (IL)-1β to reduce post-ischemic inflammation [21]. miR-124, which promotes human neurogenesis [22]. siRNAs targeting caspase [23].
Glioblastoma	Most common and aggressive tumor among glial neoplasms.	Radiation therapy, temozolomide administration (chemotherapy DNA base alkylation), and surgery.	Cerebral edema, convulsions, fatigue, vomiting, skin damage, alopecia, bone marrow suppression.	Monoclonal antibodies: Bevacizumab that selectively binds to vascular endothelial growth factor (VEGF) [24], Cetuximab and Panitumumab that target the mutated epidermal growth factor receptor (EGFR)vIII variant, common in glioblastoma [25,26], siRNA against Bcl-2 [27] or miR-124 [28].

assume a pivotal role. Nonetheless, due to their limited stability and administration, the literature lacks relevant studies. The potential for clinical transfer arises solely when utilized in conjunction with nanocarriers [36–38].

Over the years, significant advancements have been made in the development of tailored nanocarriers, with special attention to lipid nanoparticles as delivery systems to enhance the effectiveness of these therapeutics [39–41]. However, the body recognizes these nanoparticles as foreign materials and activates the innate immunity, thereby influencing adaptive immunity [42]. In such situations, polymer nanoparticles may offer superior characteristics such as reduced immunogenicity, increased stability, and enhanced reproducibility [43–45]. Interestingly, these promising nanocarriers, when combined with cell-penetrating techniques, can address the challenges associated with the delivery of biomacromolecules for the treatment of CNS disorders [46,47].

In this review, we emphasize the sophisticated architectures available in the recent literature (i.e., ~60% of the references used in this review belong to studies performed in the last 5 years) for the fabrication of polymeric nanocarriers capable of crossing the BBB from the bloodstream, together with the current approaches for encapsulating therapeutic biomacromolecules (Fig. 1). This review does not aim solely to provide examples of polymeric nanocarriers that deliver biomacromolecules across the BBB, which are summarized in Table 2. Instead, our goal is to offer a critical perspective on the features that make these polymeric nanocarriers ideal for this purpose, as well as to

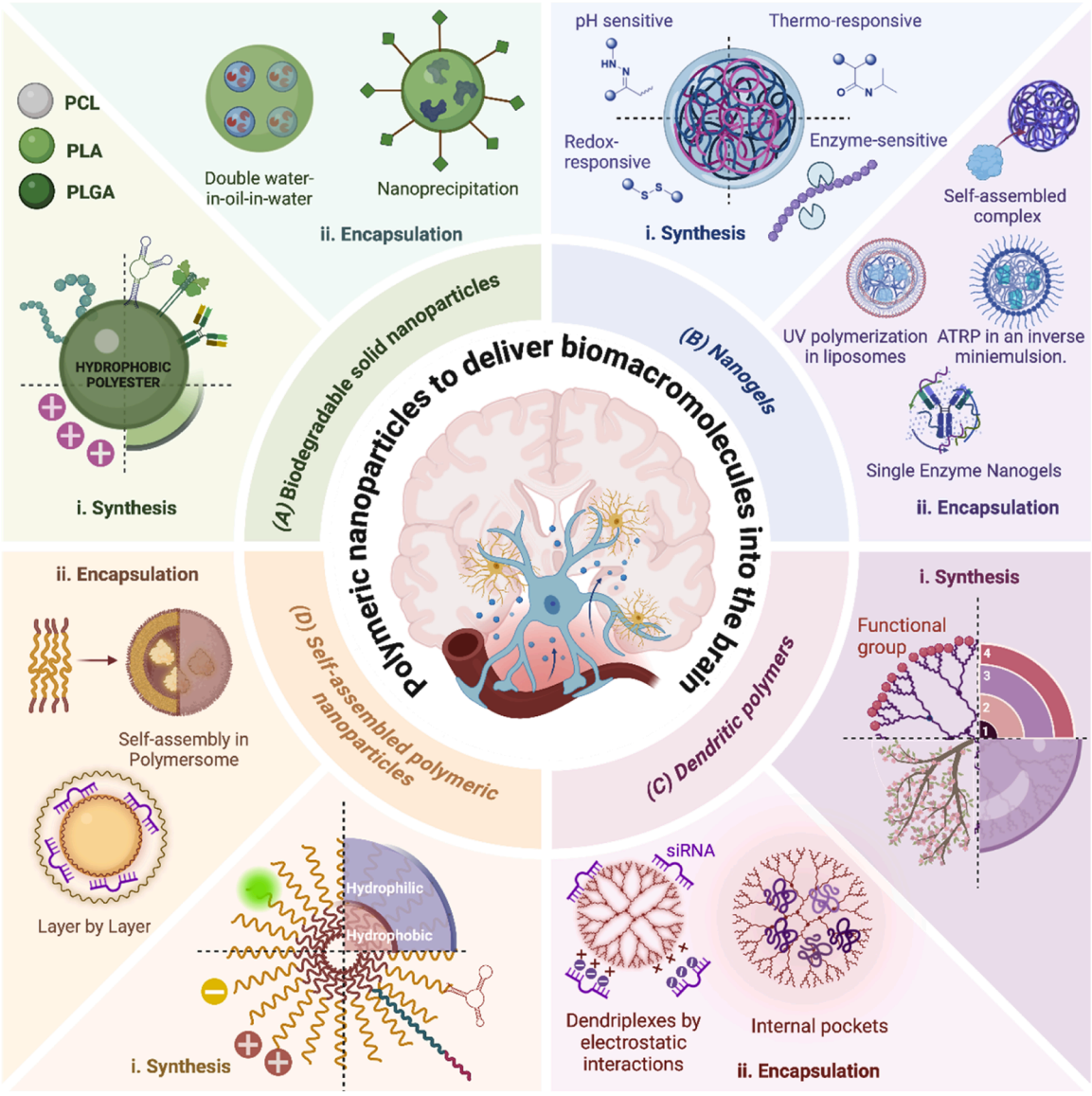

Fig. 1. General illustration that summarizes the main polymeric nanocarriers described in this review to transport therapeutic biomacromolecules into the brain. (A) Biodegradable solid nanoparticles encompass nanoparticles that present (i) a broad of synthetic alternatives to decorate their surface with specific ligands to enhance an active target delivery, and (ii) allow two main encapsulation approaches, such as double water in-oil-water or nanoprecipitation techniques to encapsulate biomacromolecules. (B) Nanogels are soft polymeric nanoparticles, that (i) permit the use of a broad multi-responsive crosslinkers to lead different stimuli-responsive nanomaterials and also (ii) allow the encapsulation of biomacromolecules mediated by different techniques such as the formation of self-assembled complex, UV polymerization in liposomes, atom transfer radical polymerizations (ATRP) in inverse micelles and in situ radical polymerization leading single enzyme nanogels. (C) Dendritic polymers have a controlled synthesis in multi-branched architectures, that opens the possibility to expose multivalently specific groups on their surface to enhance ligand-receptors interactions, and, at the same time, modulate the surface charge to encapsulate biomacromolecules as nucleic acids by electrostatic interaction inside their internal pores. (D) Self-assembled polymeric nanoparticles can be synthetized through spontaneous interaction of molecules (physic forces) to form organized structures that facilitate the implementation of encapsulation approaches. Biorender has been used for creating the descriptive image.

Table 2
Polymer-based nanocarriers that transport therapeutic biomacromolecules across the BBB to treat CNS disorders or brain cancer.

Classification	Drug delivery system	Cargo	Disease	BBB Penetration	Reference
Biodegradable solid nanoparticles (NPs)	Poly(lactic-co-glycolic acid) PLGA NPs	Enzyme galactosylceramidase	Krabbe disease	Angiopep-2 (Ang2), g7 and transferrin (Tf).	[48]
	PLGA NPs	Small interfering RNA (siRNA)	Traumatic brain injury	Polysorbate 80, poloxamer 188, DSPE- PEG-glutathione, and DSPE-PEG-Tf.	[49]
	PLGA NPs	Vitamin D-binding protein	Alzheimer's Disease (AD)	-	[50]
	PLGA NPs	Epidermal growth factor receptor siRNA	Gliomas	Ang2.	[51]
Nanogels	PLA-PEG-PLA NGs	Rituximab (RTX)	Non-Hodgkin lymphoma	2-methacryloyloxyethyl phosphorylcholine (MPC).	[52]
	PLA-PEG-PLA NGs	Nerve growth factor and RTX	Central nervous system (CNS) diseases	MPC.	[53]
	Poly(N-(3-aminopropyl) methacrylamide-co-methacryloyloxy ethyl phosphorylcholine) and MMP sensitive peptide sequence as crosslinker	Nimotuzumab and trastuzumab	Brain tumors	MPC.	[54,55]
	Poly(ethylene glycol)-b-poly(methacrylic acid) (PEG-b-PMAA) diblock copolymer	Cisplatin	Treatment of glioma	Monoclonal antibodies against Cx43 and BSAT1.	[56]
Dendritic polymers	Polyamidoamine dendrimer	Heme oxygenase-1 (HO-1) plasmid	Inflammatory diseases including ischemic stroke	-	[57]
	Dendritic polyglycerol sulfate. Dendritic polyglycerolamine.	Peptidomimetic of the anti-angiogenic protein thrombospondin-1 (TSP-1 PM) microRNA-34a (miR-34a)	Glioblastoma Glioma	Tf.	[58,59]
	Carbosilane dendrimer. Lactoferrin bearing polypropylenimine dendriplex	siRNA. 2G-(SNMe3I)11-FITC.	Neurological disorders	Lactoferrin.	[60,61]
Self-assembled particle	Cationic micelles assembled from vitamin E succinate grafted ε-polylysine polymers	TRAIL plasmids (pDNA)	Gliomas	Apolipoprotein E (ApoE).	[62]
	Glucosylated-polyion complex polymeric micelle	Antisense oligonucleotide (ASO)	Central nervous system (CNS) disorders	Glucose coating (GLUT-1-mediated transport strategy).	[63]
	Chimeric polymersomes	siRNA	Glioblastoma	Ang2.	[64]
	Chimeric polymersomes	Saporin	Glioblastoma	(ApoE) peptide.	[65]
Other polymeric carriers	Chitosan-PMMA-PAA NPs	Topoisomerase I inhibitor SN-38	Brain tumors	Retroenantio peptide shuttle H-pwvpswmpprht-NH2.	[66]
	Chitosan NPs	Green Fluorescent Protein (GFP)-tagged plasmid in HEK293–293	Brain cancer	-	[67]
	Hyaluronate Nanoparticles	Neuroglobin	Stroke	-	[68]

highlight the advantages of using certain types of polymeric systems over others. Additionally, it highlights clinical translation, explores current challenges, and offers insights into future prospects in this field. This approach allows the reader to draw their own conclusions, considering that each nanosystem possesses important features for different potential applications.

2. The blood-brain barrier issue for therapy design

2.1. Physiology of the blood-brain barrier

The BBB serves as a protective, semi-permeable membrane that intricately regulates the exchange of essential nutrients, oxygen, and ions from the bloodstream into the CNS, while it prevents the infraction by exogenous substances, toxins, and pathogens [69,70]. Anatomically, the BBB is a complex and integrated network of endothelial, neural, and immune cells, known as the neurovascular unit [71–73]. The endothelial cells, representing the main component of the BBB, form the inner line of brain capillary walls with largest surface area. They are polarized with negative surface charge and no fenestrations, preventing the rapid diffusion and exchange of negatively-charged molecules between the blood and the brain [69,70]. Primarily, endothelial cells are allied with one another by tight junctions (TJs), which is distinctive for endothelial cells of CNS along with adherens junction, which together form a robust physical barrier [72]. Under the endothelial cells lies the basement membrane. It consists of extracellular matrix proteins like collagen, heparin, laminin, nidogen, and perlecan [69,70,72,73]. Within the basement membrane, pericytes (a type of mural cells) grow abluminal to endothelial cells, and cover 90% of capillary beds [73]. Pericytes are responsible for maintaining the diameter of the vessels and regulate the specific gene expression of endothelial cells from BBB, essential for maintaining the TJs [71,74]. Due to their close association with endothelial cells, they play a pivotal role in regulating BBB integrity, homeostasis, and in controlling cerebral blood flow in capillaries along with exchange of ions and metabolites between them [69,75,76]. Additionally, they are also important in vascular development, regulation of inflammatory response by controlling leucocyte infiltration, secretion of inflammatory mediators, and neurotoxin removal [75,76]. At the interface between neurons and endothelial cells are the astrocytes, a type of glial cells connected with the end walls of endothelium. They cover most of the CNS and give integrity by strengthening the TJs, while providing biological support to the cells [69,71]. They play a major role in regulation of vascular function, expression of transporters such as P-glycoprotein (P-gp) and glucose transporter-1 (GLUT-1), water and ion homeostasis, pH regulation, neurovascular coupling (as astrocytes are the central link between microvasculature and neural network), and provide energy-rich substrates and insulation to the neurons [70,71,76]. Accordingly, close association of astrocytes with endothelial cells and pericytes holds control over permeability across the BBB [69,77]. Microglia acts as the macrophages of the CNS, migrating to pathologically affected regions to phagocytose nervous tissue [77]. Microglia are vigilant, and ensure proper response to any

injury, infection, and inflammation for maintaining CNS homeostasis [76]. These highly complex, dynamic, and selective features of the BBB are of utmost importance for the correct homeostasis of the CNS. Understanding the role of BBB transporters for maintaining homeostasis is very crucial, since they govern the translocation of various molecular entities across the BBB (Fig. 2a).

2.2. Blood-brain barrier disruption under pathological conditions

The BBB undergoes distinctive changes in various neurological disorders and diseases [70,71,77,78]. The pathology of BBB involves structural, molecular, and functional modifications of BBB constituents, that impact the overall physiological integrity and function of the barrier. Even though the prime cause of different diseases impacting BBB differs, the primary focus of BBB pathology centers on its permeability. In normal conditions, BBB is selectively permeable (Fig. 2b), but most of these disorders result in increased permeability due to alterations of endothelial cells (shrinkage), loss of TJs proteins, augmented transport of molecules (transcytosis), and alteration in transport (paracellular and transcellular) systems [69,78]. Additionally, BBB breakdown is evident by the loss of basement membrane, enhanced leucocyte activity, deterioration of pericytes resulting in impairment of vascular network, and detached astrocytes, as described in Fig. 2c [71,76]. The other hallmark of BBB pathogenesis is the release of inflammatory mediators such as interleukin (IL)-1β, interferon (IFN)-γ, tumour necrosis factor (TNF)-α, oxidative compounds (e.g., NO, H_2O_2), lipid mediators (prostaglandin E2 and F2a), vasogenic agents (e.g., histamine), and enzymes (e.g., matrix metalloproteinases) by both local and infiltrating immune cells [77,78].

2.3. Strategies for blood-brain barrier crossing

2.3.1. Physical and chemical disruption of the blood-brain barrier

External stimuli-mediated methods that can regulate BBB permeability for delivery of therapeutic agents have been widely explored, and they showed high effectiveness. They involve physical and biochemical approaches (Fig. 3).

Physical disruption: External physical approaches, like focused ultrasound, magnetic resonance imaging, high pulse electric fields, lasers, and electro acupuncture, have been used to promote the permeability of the BBB and the following delivery of therapeutic agents and (bio) macromolecules in the brain tissue [69,79–82]. Focused ultrasound, along with microbubbles (gas-filled), involves high-frequency sound waves that are precisely targeted to a specific area within the brain to trigger microbubbles in the blood for cavitation [77]. This mechanical interaction results in temporary and reversible disruption of the BBB, allowing the delivery of therapeutic agents in CNS. There have been recent advancements in using this approach, some of which are undergoing clinical trials [83]. Additionally, focused ultrasound has been coupled with magnetic resonance imaging and microbubbles for precise targeting of drugs [78,80,83]. Low- and high-pulsed electrical fields have also been used to transport small molecules by disrupting the TJs of the BBB [81]. Recently, laser stimulation has been explored for modulating BBB permeability. For this, plasmonic gold nanoparticles (AuNPs) were conjugated with antibody BV11 (AuNPBV11) for targeting junctional adhesion molecule A (JAM-A) and were injected to mice

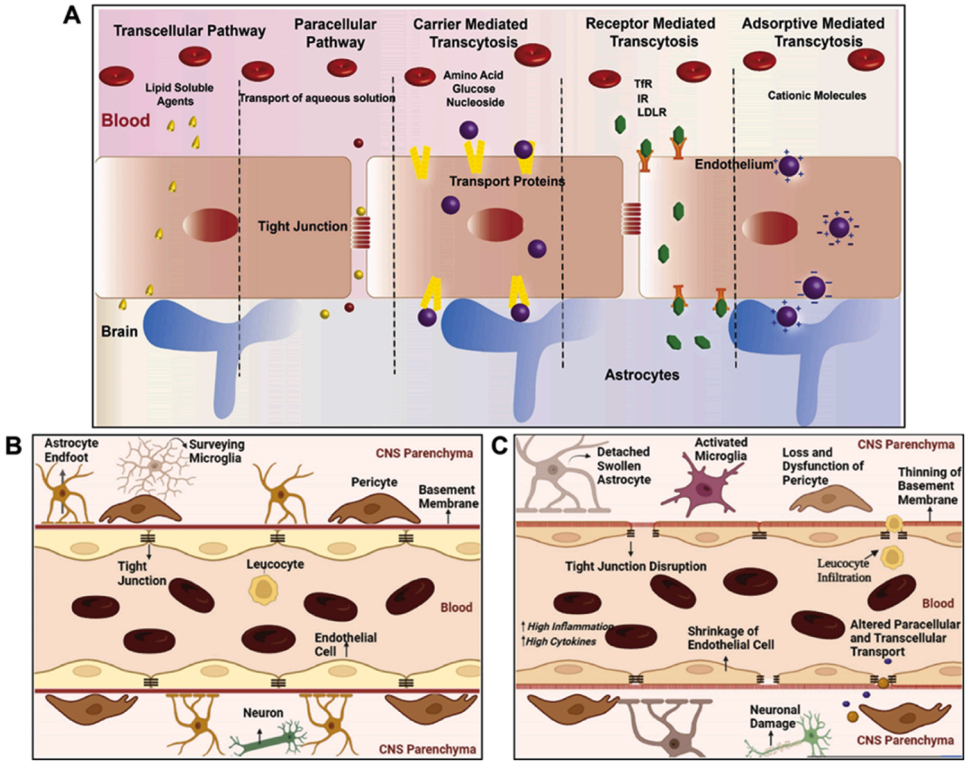

Fig. 2. (A) Schematic representation of various transcytosis pathways for blood-brain barrier (BBB) that regulates the transportation of different molecules, biomacromolecules and ions. Small lipophilic molecules passively diffuse through BBB via transcellular lipophilic pathway whereas hydrophilic molecules pass via a paracellular pathway. Essential nutrients and ions along with glucose, vitamins, electrolytes, amino acids, and nucleosides cross the BBB through carrier mediated transcytosis (CMT) via glucose transporter isoform 1 (GLUT-1) and large neutral amino acid transporter 1 (LAT1). Larger biomacromolecules like proteins, lipoproteins, or peptides, cross the BBB through transcytosis mechanisms via interaction with specific receptors and is known as receptor mediated transcytosis (RMT). This involves transferrin receptor (TfR), insulin receptor (IR), lipoprotein receptors, and lactoferrin (Lf) receptor. Adsorptive mediated transcytosis (AMT) allows transcytosis of larger biomacromolecules (e.g., peptides) and is based on electrostatic interactions between the positively-charged substrates and the negatively-charged plasma membrane. (B) Schematic representation of transverse section of BBB with its components and function during healthy state, and (C) after BBB breakdown. Biorender has been used for creating the descriptive image.

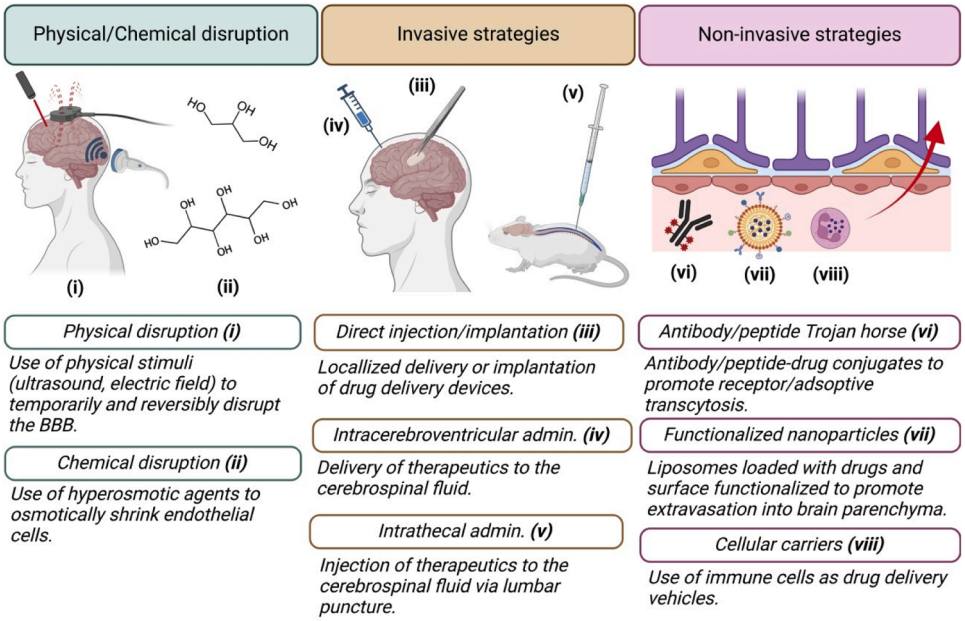

Fig. 3. Schematic representation of the most common strategies for blood-brain barrier (BBB) crossing. Physical/chemical disruption includes transient opening of the BBB to facilitate the delivery of therapeutics. Invasive strategies involve direct implantation/injection of therapeutics either directly in the brain or through the cerebrospinal fluid (CSF). Non-invasive strategies require the use of carriers capable of delivering therapeutics across the BBB. Biorender has been used for creating the descriptive image.

intravenously. One-hour post-administration, 532 nm picosecond laser was applied transcranially, leading to activation of AuNPBV11. This resulted in the enhancement of BBB permeability, facilitating the delivery of immunoglobulins, adeno-associated viral vectors, and liposomes [82]. The effect of irradiation on the brain is intricately associated with power and irradiation time, along with the distance between the source and the targeted area. Notably, near infrared light wavelength in the range from 700 to 1600 nm has garnered profound interest due to its ability to penetrate deep tissues and has been used to regulate BBB permeability. Recent studies have applied near infrared irradiation to improve the ability of light-sensitive drug delivery systems with photothermal effects to penetrate BBB, for effective treatment of depression [69]. The use of electroacupuncture for improving the permeability of BBB has also gained interest. One of the studies highlights the augmentation of BBB permeability through the application of electroacupuncture targeting the GV20 and GV26 acupoints in rat models [69].

Chemical disruption: The most commonly used chemical reagents to disrupt the integrity of the BBB, together with their working conditions, applications, advantages and disadvantages are summarized in Table 3. [69,77,78,80,84–92] Some other pharmaceutical biological compounds like zonula occludens toxins, Cereport (a synthetic peptide analog of bradykinin), LipoBridge (a nonimmunogenic formulation containing short-chain oligoglycerolipids), vascular endothelial growth factor (VEGF), and chemical compounds such as oleic acid, lysophosphatidic acid, cyclodextrins, and sodium dodecyl sulfate are also involved in transient opening of the BBB [77,78,86].

The viruses also serve as stimulating biological agents that help in the opening of TJs by upregulating chemokines. As example, studies on human brain microvascular endothelial cells highlighted the involvement of the human immunodeficiency virus (HIV) type 1 gp 120 virus in enhancing BBB permeability by degrading TJs [78]. Additionally, adeno-associated virus and West Nile virus also exhibited promising ability to target CNS, without disrupting the BBB [78,95].

2.3.2. Invasive strategies

Direct injection/implantation: Direct injections involve localized delivery of therapeutics or their sustained release via implants into the brain. This is used to treat conditions such as cancers, stroke, neurological and mental disorders [96]. Surgically placed implants can provide sustained release of drugs by opening the skull. The carmustine-loaded biodegradable implants (Gliadel® wafer), approved by the Food and Drug Administration (FDA), placed during tumour resection, have shown improved survival rates in patients facing GBM. Also, nicardipine-releasing solid implants have been successfully used to prevent neurological disorders following strokes [96]. At the same time, long-term (5 months) delivery systems have been explored for schizophrenia to address treatment adherence issues associated with mental disorders [96].

Intracerebroventricular administration: In this approach, the therapeutics are delivered into the cerebrospinal fluid, specifically in the lateral ventricle system of the brain, via an outlet catheter of intracerebroventricular port implanted under the scalp or by pump [77,88]. Out of this, the use of pumps is more prevalent, as it maintains continuous and elevated drug concentrations in cerebrospinal fluid [77]. Intracerebroventricular implants have been used to deliver chemotherapeutics for brain cancer, opioids for pain management, and drugs for the treatment of neurological disorders like lysosomal storage disorders (LSD), mucopolysaccharidosis, and for disorders associated with cerebral palsy (e.g., baclofen) [90]. Additionally, clinical trials are going on for amyotrophic lateral sclerosis (ALS) and PD by administering VEGF and platelet-derived growth factors, respectively [88,90].

Intrathecal administration: Here, the therapeutics are directly injected into the space surrounding the spinal cord by lumbar puncture and are delivered to CNS parenchyma via cerebrospinal fluid. This approach has been approved for the delivery of antisense oligonucleotide for the treatment of spinal muscular atrophy. Clinical trials are going on for ALS and HD [77,90].

2.3.3. Non-invasive strategies

Non-invasive strategies for the delivery of therapeutics rely on pharmacological approaches that exploit the endogenous transport mechanisms for enhanced BBB permeability. These strategies, that include chemical modification of the therapeutic drugs, transport carriers, chimeric peptides, nanocarriers, Trojan horse, etc., have

Table 3
Summary of various chemical reagents used for BBB disruption.

Agents	Examples	Mechanism	Working Condition	Application	Advantages/Disadvantages
Hyperosmotic Agents	Mannitol [77,78,80,84,87–89]	Osmotic pressure gradient created, which leads to shrinkage of endothelial cells and disruption of tight junction, and resulting in temporary opening of the blood-brain barrier (BBB)	Arterial Injection/infusion (25%)	Drug Delivery, (chemotherapeutics, imaging agents)	**Advantages**: Clinically approved, enhanced permeability, non-invasive, rapid activity, broad range of agents, cost effective **Disadvantages**: Non selective, inconsistent barrier disruption, neurological toxicity
	Glycerol [80,85]			Neurological Study, drug delivery	**Advantages**: Availability, low cost **Disadvantages**: Lower efficacy, Immunogenic risks
	Arabinose [77,78,80,87,89,93]		Intravenous administration	Drug Delivery	**Advantages**: Biodegradable, effective at low concentration, used for hydrophilic and lipophilic drugs **Disadvantages**: Limited information, risk of metabolic imbalances
	Saline [89,90,93]		Intravenous administration (23.4%)	Used in preclinical models for drug delivery and contrast agents to CNS	**Advantages**: Cost effective, availability, easy administration **Disadvantage**: Edema, tissue damage, hypernatremia (high sodium levels)
	Urea [85,89,90,93]			Used in preclinical studies for drug delivery	**Advantages**: Cost effective, disrupts BBB at controlled doses **Disadvantages**: Short lived disruption, electrolyte imbalance
	Fructose [85]		Intravenous administration	Used in preclinical studies of drug delivery and contrast agents	**Advantages**: Less toxic, biocompatible **Disadvantages**: Limited Information, metabolic imbalances
Vasoactive Compounds	Histamine [77,80,89,90]	Binds with B2 receptors of endothelial cells leading to disruption of tight junction and enhanced drug permeability	Intravenous (10 to 100 μM)	Used in preclinical models for drug delivery	**Advantages**: Minimal toxicity **Disadvantages**: short duration of action, non-specific
	Bradykinine [77,80,87,88,88,90]		Intravenous or intracerebral administration	Drug delivery (enhanced delivery of chemotherapeutics, small molecules)	**Advantages**: Selective, minimal toxicity **Disadvantage**: Short duration of action, limited efficacy for large molecules, cost
	RMP-7 (analogues of brandykinin [88,89]				**Advantages**: More potent and specific to B2 receptors than bradykinin, higher half-life as resistant to degradation
	Alkylglycerols (e.g., 1-O-pentylglycerol [80,88,89]		Intracarotid injection (200 mM)	Drug Delivery (Chemotherapeutics)	**Advantages**: Reversible, increase in BBB permeability **Disadvantages**: Limited effectiveness, side effects
	Leucotrienes (e.g., cysteinyl leukotrienes) [88,89]	Binds to the G-protein coupled receptors CysLTR1 and CysLTR2, triggering the contraction of endothelial cells and disrupting tight junction resulting in enhanced permeability	Intracerebrove–ntricular injection (6pmol)	Drug Delivery	**Advantages**: Immune response facilitation **Disadvantages**: Tissue damage, long term toxicity
	Tumor necrosis factor A (TNF-A)/interferon c (INF-c) [94]	Tight junction proteins are altered via inflammatory cytokines	Systemic or local administration	Drug Delivery, neuroinflammation and CNS research	**Advantages**: Target specific, mechanistic insights **Disadvantages**: Systemic side effects, risk of neuroinflammation
Chemical compounds	Oleic Acid [77,78]	It changes protein kinase C-induced protein phosphorylation, resulting in reversible opening of BBB	Intracarotid infusion	Used in preclinical studies of drug delivery	**Advantages**: Mild and reversible effect **Disadvantages**: Low specificity, limited information
	Lysophosphatidic acid [77,78]		Intravenous Injection		**Advantages**: Reversible effect **Disadvantages**: Limited information, risk of neuroinflammation
	Sodium lauryl sulphate [77,78]	Disrupts endothelial cell membrane by altering lipid bilayer	Direct Infusion on BBB	Drug Delivery and BBB studies	**Advantages**: Cost, Dose dependent

(*continued on next page*)

Table 3 (*continued*)

Agents	Examples	Mechanism	Working Condition	Application	Advantages/Disadvantages
					Disadvantages: Nonspecific, Side effects
	Sodium dodecyl sulfate [77,78]	Interacts with lipid and protein of cell membrane			**Advantages**: Dose dependent, reversible
	Sodium caprate (C10) [89,91]	Integrates into the lipid bilayer of endothelial cells which increases membrane fluidity and loosening the tight junction favoring paracellular transports	Intracarotid administration (5–25 mM)	Drug Delivery	**Advantages**: Reversible, effective for both small and large molecules **Disadvantages**: Limited information, non-selective, inflammation
	Cyclodextrins [78]	BBB disruption by extracting cholesterols from endothelial cells	Intravenous (1mM-10 mM)	Drug Delivery (hydrophobic drugs)	**Advantages**: Biodegradable, selective action on cell membrane **Disadvantages:** Limited efficacy for hydrophilic drugs, side effects
Aromatic substances	Borneol [84]	Reversibly disassembles the tight junction proteins (claudins and occludins), transiently disrupting the BBB integrity		Drug Delivery, neuroprotection	**Advantages:** Reversible effect, natural origin, broad applicability **Disadvantages**: Concentration dependent toxicity, nonspecific, limited activity

demonstrated promising results with minimal adverse effects. This review focusses on the Trojan horse approach, and highlights their mode of action and the potential application in the delivery of biomacromolecules across the BBB.

Molecular Trojan horses are designed to deliver therapeutic molecules (e.g., small-molecule drugs, recombinant proteins, and genes) across the BBB by mimicking natural biological processes and structures. This strategy involves non-invasive approaches, using antibodies, peptides, cells, and nanoparticles as Trojan horses, targeting endogenous BBB receptors such as receptor-mediated transcytosis (RMT) and adsorptive-mediated transcytosis (AMT) for transcytosis of therapeutic biomacromolecules [97–99]. Examples of some of the Trojan horse approaches for BBB penetration are described below (Fig. 3).

Antibody Trojan horses: This technique uses genetic engineering of therapeutics (neurotrophins, therapeutic antibodies, enzymes and decoy receptors) with monoclonal antibodies (mAb) (IgG domain) having specificity towards transferrin receptor (TfR) and human insulin receptor (HIR) to give TfRmAb and HIRmAb. These act as a Trojan horse for delivery of attached drugs via RMT [100,101]. For example, Trojan horse Pabinafusp alfa, engineered by fusion of iduronate 2-sulfatase (lysosomal enzyme) and heavy chain of TfRmAb, has been used for the Hunter syndrome and received market approval for the treatment of brain disorders in Japan [100,102].

Peptide Trojan horses: Angiopep-2 peptide (Ang2) is a low-density lipoprotein receptor (LPR1)-targeting peptide, that enhances the BBB penetration of anti-human epidermal growth factor-2 mAb [103]. Similarly, a novel derivative of paclitaxel with Ang2 (ANG1005) revealed improved drug uptake in gliomas and is under clinical trial [99, 104]. TAT is a positively-charged cell penetrating peptide (CPP) derived from the transactivator of transcription protein of the HIV. It has been found that many cationic peptides are taken up by the cells through AMT in vitro. However, poor brain permeability was observed in vivo [98, 103]. Contrarily, another CPP, SynB peptides, derived from a natural mammalian antimicrobial peptide, have shown better delivery of polar biomolecules such as morphine-6-glucuronide to the brain in a clinical trial, both in vitro and in vivo [78]. Additionally, SynB3 conjugated with various low brain-penetrating chemotherapeutic drugs like doxorubicin, benzylpenicillin, paclitaxel, and dalargin has demonstrated significant brain penetration and can be a potential strategy for brain cancer treatment [78].

Functionalization of nanoparticles: Therapies based on approved nanocarriers (e.g., liposomes and polymeric nanoparticles) and associated technologies (invasive and non-invasive) are currently being investigated to treat CNS diseases [99]. In this context, liposomes with high biocompatibility, biodegradability, and intrinsic competence for BBB have been extensively explored as Trojan horses. Liposomes functionalised with polyethylene glycol (PEG) and biomolecules (Tf and insulin) have been used for the delivery of DNA encoding lysosomal enzyme to the brain for the treatment of mucopolysaccharidosis [105]. PEGylated liposomes further functionalised with glutathione (GSH) for targeting GSH transporters for delivery of doxorubicin, methylprednisolone, and ribavirin are in preclinical studies [99]. For example, PEGylated doxorubicin-loaded liposomes targeting GSH transporters have shown positive outcomes in terms of antitumour activity, and have reached phase I/II clinical trials. The treatment was found to be safe and well-tolerated [105]. Similarly, treatment with GSH PEGylated liposomal methylprednisolone for phase I trials on 42 patients, was deemed safe and well tolerated at therapeutic doses for MS [105]. In the case of intravenous administration of ribavirin encapsulated GSH PEGylated liposome, a fourfold increment of the drug in brain microdialysates was observed [99]. Two other liposome-based Trojan horse systems, SGT-53 (with Tf Ab and plasmid p53), and doxorubicin-loaded PEGylated liposomes with cetuximab are in phase I and phase II clinical trials for GBM [105].

Cellular carriers: Innovative paradigms, wherein the immune cells (monocytes, neutrophils, and macrophages) are harnessed for BBB therapeutic interventions, have gained interest in recent times. In this approach, cells are used as Trojan horses. Cells are either genetically modified to produce therapeutically active peptides and RNA molecules/proteins or can carry therapeutics targeting BBB [99]. Immune cells have inherent properties to transmigrate across BBB by changing their shape (diapedesis) and have been used for brain delivery in diseases involving inflammation. Engineered macrophages expressing glial cell line-derived neurotrophic factor (GDNF) in a PD model demonstrated long-term neuroprotective effects at the onset of disease progression and reduced brain inflammation [99]. Further, neural precursor stem cells and hematopoietic stem cells can also interact with BBB cells under inflammation. In recent studies, neural precursor stem cells and hematopoietic stem cells were engineered to express the antitumour biomolecule tumour-selective proapoptotic death receptor ligand (TRAIL), resulting in treating metastatic brain tumour [99]. In another set of cell-mediated drug delivery examples, magnetic liposomes loaded with the drug diclofenac and functionalised with the peptide motif RGD (arginylglycylaspartic acid) were able to target monocytes and neutrophils in an IL-1β induced brain inflammation rat model. In that study, RGD acted as a targeting ligand for integrin

receptors expressed on monocytes and neutrophils, which enhanced the uptake of diclofenac-loaded liposomes by the cells. As a result, it was observed 9.1 fold increment of drug reaching the brain. Table 4 summarizes the different strategies of BBB crossing described in this section, highlighting the advantages and disadvantages of each approach [69,77,84,87,88,90,93,94,106–109].

Among the strategies explained above, we will consider herein the use of polymeric nanocarriers to deliver therapeutic biomacromolecules (e.g., enzymes, growth factors, genes) across the BBB from the bloodstream. The molecular structure of polymers can be finely adjusted during the synthetic approach or via post-polymerization approaches, resulting in polymeric nanocarriers with tuneable mechanical, physicochemical, and surface properties. The wide variety of available polymers (e.g., synthetic vs. natural, biodegradable vs. non-biodegradable, stimulus-responsive) allows the fabrication of a plethora of nanoformulations that have shown promising results regarding the protection of sensitive biomacromolecules from external conditions (e.g., presence of proteinases, changes in pH and ionic strength) and BBB crossing.

3. Polymer-based nanocarriers to cross the blood-brain barrier

In the present review, the various polymeric nanocarriers are organized according to their architecture, which ultimately dictates their physicochemical properties and functionality. In this context, biodegradable solid nanoparticles are notable for their compactness, hydrophobicity, and tunable biodegradability; nanogels are distinguished by their soft, three-dimensional structure and environmental adaptability; dendrimers are recognized for their well-defined, monodisperse, highly branched, and symmetrical architecture; and self-assembled nanoparticles/nanocapsules are characterized by their versatility in terms of synthetic approaches, enabling the encapsulation of sensitive biomacromolecules.

3.1. Biodegradable solid nanoparticles

Biodegradable solid nanoparticles are mainly formulated by using aliphatic polyesters. In this context, biocompatible and biodegradable nanoparticles based on FDA-approved poly(ε-caprolactone) (PCL), poly (lactide) (PLA), and its copolymers with glycolide (PLGA) have received particular attention as materials for the fabrication of brain-targeted drug delivery systems. A wide variety of cargos, mainly based on small MW drugs, have been successfully encapsulated into these nanoparticles to address neurodegenerative diseases such as AD, PD, and other diseases and disorders of the CNS [110]. Moreover, these nanoparticles have been proved to be promising nanocarriers to deliver (bio) macromolecules across the BBB. Hence, they are postulated as potential entities for enzyme replacement therapies (ERT) [48], small interfering RNA (siRNA) therapies [49,51], brain-derived neurotropic factor therapies (BDNF) [111] and vitamin D-binding protein (DBP) [50] for LSDs, GBM, or TBI, among others.

As (bio)macromolecule nanocarriers, PCL, PLA, and PLGA nanoparticles present certain advantages over other delivery systems. They are commercially available, easy to manufacture, and offer versatile nanoparticle formulations with adjusted physical, chemical, and mechanical properties. The MW, as well as the co-monomer constituents and ratio, directly affect the viscosity, solubility, glass transition temperature (T_g), crystallinity degree, and phase behavior of the polymer [112,113]. Amorphous matrices facilitate a more uniform distribution of the active/therapeutic compounds and endow the polymer with higher degradation rates. In this sense, the PLA component (i.e., lactide) plays an important role. The crystalline phase can be suppressed by modifying the stereochemistry of lactide: while stereoregular PLA (pure D- or L-lactide) is crystallizable, the stereo-irregular PLA (D,L-lactide) is amorphous [114]. Therefore, (co)polymers based on D,L-lactide, such as poly(D,L-lactide) (PDLLA), poly(D,L-lactide-co-ε-caprolactone) (PDLCL), and poly(D,L-lactide-co-glycolide) PDLLGA (usually reported in bibliography and commercialized as PLGA) are the most suitable candidates for this drug delivery applications. Among these polymers, PLGAs are the most reported and used as standard nanoparticles. In general, the biodegradation kinetics of these nanoparticles, which is essential for cargo release, is easily predicted by adjusting the MW and the composition of the copolymers. The increase in MW and D,L-lactide units generally reduces the biodegradation rate in PLGA copolymers. This feature allows the design of nanoparticles with controlled and tailorable release profiles of active agents ranging from months to years of degradation times. Moreover, MW and the co-monomer ratio play an important role in tuning nanoparticle elasticity. This parameter has been reported to be an important condition to control the biological interactions of nanoparticles with the brain endothelium, thereby having a direct impact on their transport across the BBB. In this sense, high MW and lactide-rich polymers will exhibit higher stiffness, which according to reported studies, might promote higher association with endothelial cells and transport through them [115,116].

The fabrication technique is essential to design specific nanoparticles with the ability to carry hydrophilic (bio)macromolecules to the brain

Table 4
Comparative analysis of different strategies of BBB crossing, highlighting the advantages and disadvantages of each strategy.

Strategies	Examples	Advantages	Disadvantages
Physical Disruption	Focused Ultrasound (FUS) [77,84,87,88,93]	Precise targeting, non-invasive, temporary opening, controlled and adjustable parameters.	Tissue damage, requirement of high-end equipment, operational expertise, cost.
	High Pulse Electric Field [94]	Permeability through electroporation, reversible.	Cellular damage, precise delivery, limited research.
	Lasers and Electropuncture [69,94]	Non-invasive, adjustable parameters.	Cellular damage, side effects, limited clinical data, technical challenges.
Chemical Disruption	Hyperosmotic Agents [84,90]	Clinically approved, enhanced permeability, non-invasive, rapid activity, broad range of agents, cost effective.	Non-targeting, cellular damage, transient and inconsistent therapeutic effect, systemic toxicity, regulatory and safety concern.
Invasive Strategies	Direct Injection/Implantation [84,88,90,106]	Localized delivery, circumventing BBB, higher drug concentration, reduced systemic toxicity.	Invasive, limited drug distribution, adverse local reactions, technical complexity, cost.
	Intracerebroventricular and Intrathecal Administration [77,88,90,93,106,109]	Direct CNS delivery, circumventing BBB, uniform drug distribution with enhanced therapeutic efficiency.	Invasive, procedure related complications, technical complexity, side effects.
Non-Invasive Strategies	Antibody and Peptide Trojan Horse [103,108,109]	Targeted delivery, high selectivity and specificity, reduced systemic toxicity, high transcytosis, versatility in payload.	Complex engineering and optimization, immune response, variable BBB penetration, production and scalability, stability issue, cost.
	Functionalization of Nanoparticles [69,77,88,90,107,109]	Targeted delivery, specificity, improved penetration, control-led release, versatility, multifunctional.	Batch to batch variability, toxicity and clearance, stability issues.
	Cellular Carriers [77,88,90,107,109]	Targeted delivery, reduced immunogenicity, biodegradable, multifunctional, versatility, efficient gene delivery.	Pathogenicity, stability, culturing and maintenance, scalability, limited payload, regulatory and ethical issues.

prior to the BBB permeation. Although in situ polymerization from monomers is reported as a feasible fabrication approach (i.e., bottom-up), the use of pre-synthesized polymers (i.e., top-down) is preferred due to a better control over the formulation characteristics. The choice of the fabrication technique enables the control of the structure, physicochemical properties (e.g., shape, size, and surface charge), and the encapsulation efficiency (EE) in the nanoparticles. Even though there are several different methods for manufacturing biodegradable polyester nanoparticles, such as salting-out, rapid expansion of supercritical solution process, spray drying, or microfluidics, in this review we highlight the double-emulsion method (e.g., water-in-oil-in-water, W/O/W) and the nanoprecipitation method (Fig. 4). Both techniques allow the encapsulation of hydrophilic agents with acceptable EE and enable obtaining nanoparticles with sizes similar and below 100 nm, which is beneficial to promote BBB crossing. In the double emulsion method, an aqueous solution containing the active (bio)macromolecule (W_1) is initially mixed with a polyester solution (O) to obtain a primary emulsion (W_1/O). In a second step, the primary emulsion is poured into a surfactant-containing second aqueous solution (W_2), to obtain the secondary emulsion ($W_1/O/W_2$). Finally, the organic solvent is removed by evaporation and the nanoparticles are collected by centrifugation (Fig. 4a). The final size of the nanoparticles is influenced by the energy applied in the homogenization process, volume of the internal aqueous phase, temperature and solvent evaporation rate. In general, double-emulsion method generates larger nanoparticles than the nanoprecipitation method. Nanoprecipitation is a simple and reproducible technique, well suited to encapsulate hydrophobic substances. However, good results are also obtained with hydrophilic molecules [48,49]. In this method, all the components (i.e., polymer, targeting ligands, and (bio)macromolecules) are dissolved together in a water-miscible solvent, which is added to an aqueous solution (Fig. 4b). Subsequently, the organic solvent is displaced and the nanoparticles precipitate. In this case, the size of the nanoparticles is tuned by controlling the stirring rate, volume of aqueous phase, polymer and surfactant concentration, and MW of the polymer.

The hydrophobic character of polyester-based nanoparticles makes them vulnerable to opsonization and consequent phagocytosis (Fig. 5a (i)). As pre-transcytosis strategy, hydrophilic surfaces have been proved to endow the nanoparticles with stealth properties, hence prolonging their circulation in the bloodstream (Fig. 5a(ii)). In this sense, PEGylation (covalently grafted or adsorbed low MW PEG) and the surfactant-based coatings are the most reported strategies. To improve the stability of nanoparticles *via* electrostatic or steric repulsion, sodium dodecyl sulfate, poly(vinyl alcohol), polysorbate 80 (Tween® 80) or poloxamer 188 (Pluronic® F68; a poly(ethylene oxide)-poly(propylene oxide) block copolymer) are commonly used. In addition, the surfactant used for the fabrication of the nanoparticles also plays a crucial role in their capacity to cross the BBB; Tween® 80 outperformed as coating material of polyester-based nanoparticles in the design of BBB-targeted carriers [49]. Tween® 80 facilitates the adsorption of apolipoproteins, which enables the interaction with lipoprotein receptors present on BBB, thereby allowing the transcytosis across the BBB. Another transcytosis strategy involves the AMT by exposing cationic compounds, such as cationic bovine serum albumin (BSA), on the surface of the nanoparticles. The most common transcytosis strategy is based on the amenability of nanoparticles to chemically modify their surfaces with ligands for targeting specific receptors at the BBB. Thanks to their carboxylic acid or ester endings, different chemistry routes can be followed

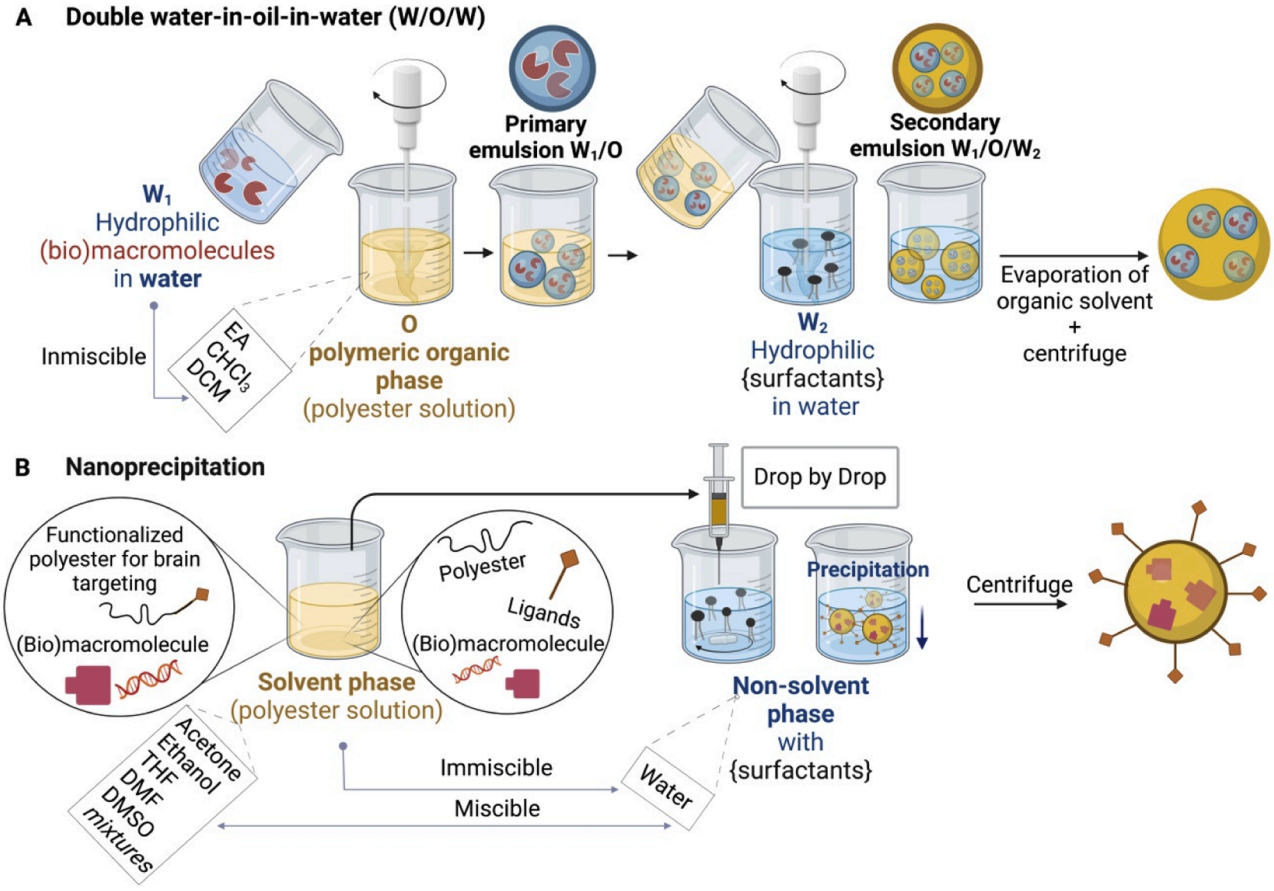

Fig. 4. Nanoparticles fabrication techniques to efficiently encapsulate hydrophilic (bio)macromolecules. (A) Double emulsion method, relying on the formation of a primary emulsion (W_1/O), followed by a secondary emulsion ($W_1/O/W_2$) carrying the (bio)macromolecule of interest. (B) Nanoprecipitation, which is based on the precipitation of a polymeric solution carrying the (bio)macromolecule of interest in a non-solvent phase. Biorender has been used for creating the descriptive image.

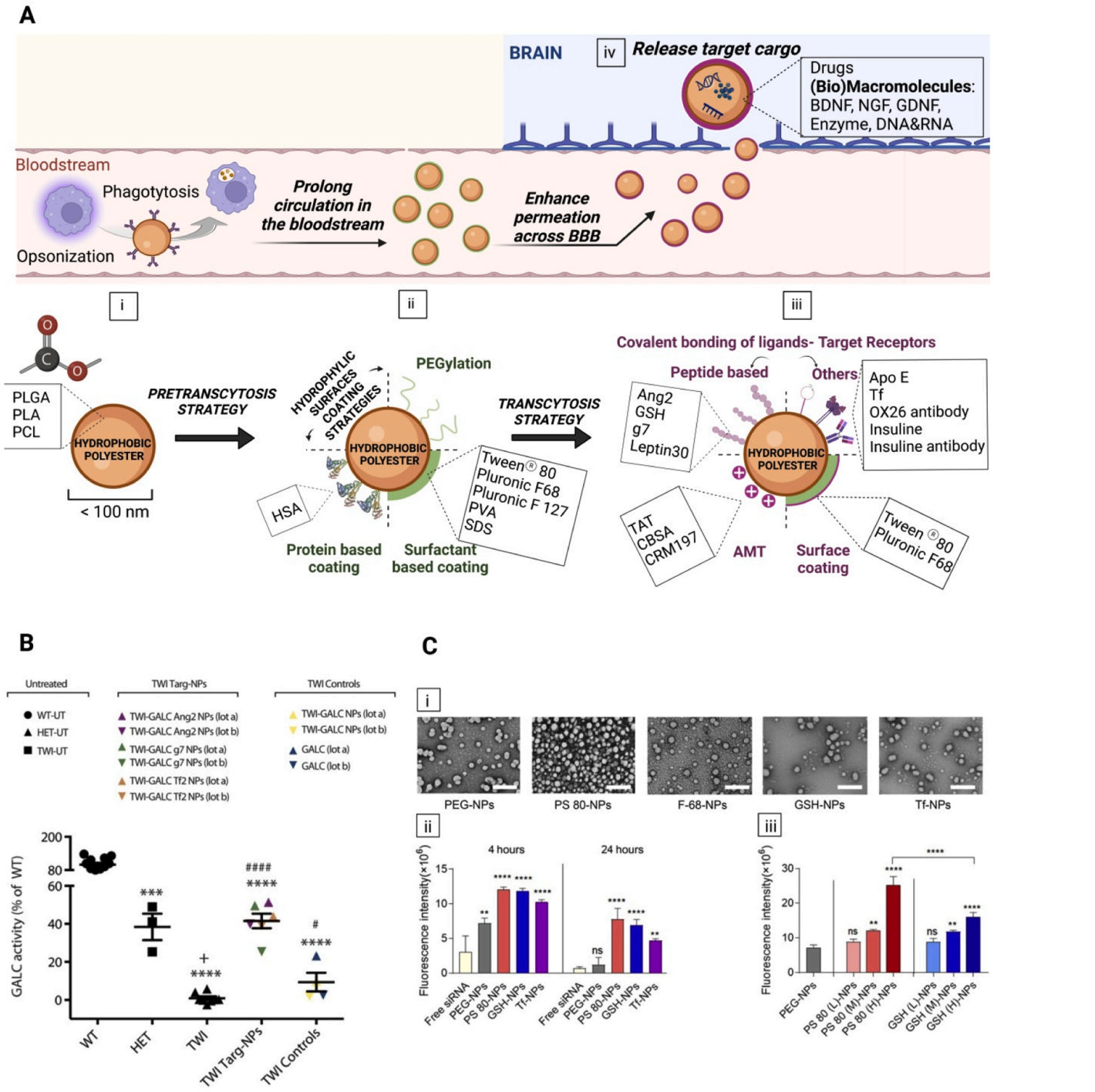

Fig. 5. (A) (i) Biodegradable polyesters highly used in the nanoparticle fabrication: polycaprolactone (PCL), polylactide (PLA), and its copolymer with glycolide (PLGA); (ii) Pretranscytosis strategies for prolonging circulation in the bloodstream; (iii) Transcytosis strategies for enhancing permeation across the blood-brain barrier (BBB); (iv) surface-tailored nanoparticles with brain-target (bio)macromolecules as delivery system. Biorender has been used for creating the descriptive image. (B) Enzyme-loaded (GALC) PLGA nanoparticles with different ligands (Ang2-, g7-, and Tf-NPs) for enzyme replacement therapy (ERT) in Krabbe disease. In vivo brain GALC activity. Adapted with permission from [48]. Copyright © 2019 AAAS. (C) siRNA loaded PLGA nanoparticles with different surface coating chemistries (PEG-, PS80-, F68-, GSH- or Tf-nanoparticles) and densities for traumatic brain injury (TBI): (i) Transmission electron microscopy images of nanoparticles. Scale bars, 200 nm. (ii) Penetration of nanoparticles across intact BBB depending on surface coating type. (iii) In vitro penetration of nanoparticles across intact BBB depending on surface coating density (Low, Medium, High). Adapted with permission from [49]. Copyright © 2021 AAAS.

to bind apolipoprotein E (ApoE), Tf, and anti-Tf antibodies (OX26), insulin and insulin antibodies, as well as peptide-based ligands through carbodiimide, amide, thioether, or disulfide bonds (Fig. 5a(iii)). Among the peptides, leptin 30, Ang2, GSH and glycopeptide g7 show efficacy in triggering BBB crossing of the nanoparticles [48].

Selecting the appropriate surface chemistry and coating density allows to maximize the active penetration of nanoparticles loaded with (bio)macromolecules across the BBB. For example, crosslinked galactosylaceramidase enzyme aggregates (GALC CLEA)-loaded PLGA nanoparticles have been proved to reach the brain and promote

enzymatic activity recovery when functionalized with targeting peptides (Ang2, g7, Tf). For instance, Del Grosso *et al.* followed the nanoprecipitation approach to encapsulate GALC CLEAs within PLGA nanoparticles modified with peptides for ERT in Krabbe disease [48]. All the obtained nanoparticles showed a hydrodynamic diameter below 200 nm and negative surface charge, with EEs ranging from 40% to 75%. In vivo results showed that Twitcher mice (i.e., a preclinical model of the Krabbe disease) treated with targeted nanoparticles displayed a GALC activity in the brain similar to the one reported for the heathy control (Fig. 5b), whereas mice treated with non-targeted nanoparticles or free enzyme did not show any significant increase in GALC activity. Additionally, Li *et al.*, reported the fabrication of siRNA-loaded PLGA nanoparticles (<100 nm, negative surface charge and EE = 55–65%) and their surface were easily modified with PEG, Tween® 80, GSH or Tf, using nanoprecipitation process for TBI treatment (Fig. 5c(i)) [49]. In vitro and in vivo assays clearly proved the outstanding performance of Tween® 80 and GSH coatings in facilitating the penetration across undamaged BBB, while also promoting the cellular uptake of nanoparticles by neural cells, leading to significant gene silencing. In vivo imaging of brains from healthy mice showed the strongest fluorescent signals for those nanoparticles coated with Tween® 80 and GSH (Fig. 5c(ii)). Moreover, the authors demonstrated that combined modulation of mentioned surface chemistry along with high coating density could further enhance the active penetration of nanoparticles within and outside the window of physically breached BBB after TBI (Fig. 5c(iii)). In this sense, mice injected with nanoparticles with high Tween® 80 modification showed the strongest fluorescence signal in the brain, as well as significant in vivo brain accumulation when administered during early or late injury periods; direct evidence of their ability to cross BBB.

It is unquestionable that biodegradable solid nanoparticles postulate as viable carriers for delivering (bio)macromolecules through the BBB, though they present some limitations. The production of acidic byproducts (e.g., lactic and glycolic acid) might locally decrease the pH, causing detrimental consequences in the brain and also compromising the integrity of the encapsulated (bio)macromolecules. Besides, most of the polyesters tested are limited to commercially available lactide and glycolide copolymers, which lack functionalities in the polymeric backbone; hence, they present limited anchor sites for further functionalization or binding ligands restricting the library of formulations. Another limitation is the fabrication process, namely, the use of organic solvents and the utilization of high shear-stress in the nanoprecipitation and double emulsion methods, respectively. Organic solvents might deteriorate the activity of the (bio)macromolecules; and the shear-stress might cause the unfolding and consequent denaturation of the entrapped (bio)macromolecule in the W_1/O interface. Optimized formulations and fabrication conditions are required to ensure inter-batch reproducibility and further scale-up. From the clinical translation perspective, it must be considered that most of the polyester-based nanocarriers for brain disorders are still in the lab-scale. There is extensive scientific literature reporting differences in engineered nanoparticles, types of (bio)macromolecules, and in vitro and in vivo disease and animal models. Thus, a direct comparison and evaluation of the results observed in the literature for further translation towards precision therapeutics for brain targeting is complex.

3.2. Nanogels

Nanogels are soft materials that accommodate therapeutic small molecules and biomacromolecules into their three-dimensional crosslinked structures [117], providing several significant advantages compared to alternative systems described in this review. These include their modular size (15 to 300 nm), diverse chemical composition, tuneable mechanical and physicochemical properties, high water absorption capacity, and high biocompatibility. Importantly, in addition to their reticular nature, the significant ratio of surface area to volume allows for the physical entrapment of pharmacological (bio)molecules, maintaining the chemical integrity of the drugs [33]. Overall, combining these exceptional features and the possibility to design smart systems with programmable responses to external stimuli, promotes nanogels as the material of choice in many drug delivery applications [118,119].

The responsiveness of the nanogels can be tailored to the target application and the local environment conditions, making use of a wide chemical palette of labile chemical bonds. To gain in-depth knowledge about the synthesis, composition, and application opportunities endowed by responsive nanogels, we recommend consulting specialized reviews [119–121]. Shortly, pH-sensitive nanogels are utilized when the environment experiences significant pH changes, such as in the vicinity of tumorous cells. In this case, crosslinkers bearing acidic-pH labile chemical bonds such as hydrazone, cis-aconityl, or acetal groups, among others, are utilized [122–124]. As an example, Morimoto *et al.* created an acid labile nanogel based on the hydrolysis of a vinyl ether group in the acidic condition capable of encapsulating and releasing BSA [125]. When a temperature gradient is identified (e.g., in the outer layers of the skin), the use of thermosensitive nanogels is an attractive option to deliver and release drugs. These nanogels are typically composed of poly (N-isopropylacrylamide) (PNIPAAm) because its phase transition occurs approximately at body temperature [126,127]. Calderon and co-workers carried out a series of exhaustive studies for promoting the controlled delivery of biomacromolecules such as BSA, insulin, and anti-TNF-α fusion protein, using hydrophilic thermo-responsive nanogels based on multifunctional-dendritic polyglycerol (dPG) as macromolecular crosslinker [128–130]. The reducing environment in the cytosol and cell nucleus compared to the extracellular microenvironment is also used as a target stimulus to release drugs inside the cells [131,132]. GSH, the most prevalent reducing agent in most cells, has a typical intracellular concentration of 10 mM and an external concentration of 0.002 mM. Redox-responsive nanogels with disulfide bonds, Se-Se, or Te-Te bonds are the most typical for this purpose [133–136]. For instance, Chen *et al.* prepared reduction-sensitive degradable nanogels in the presence of cystamine (via ring-opening reaction with cyclic carbonate groups), for the encapsulation and efficient in vitro release of fluorescently-labeled cytochrome C [137]. Additionally, the presence of specific enzymes in targeted environments, such as hyaluronidases in many cancer types or proteolytic enzymes (e.g., matrix metalloproteinases) in several severe conditions, provides the opportunity to design enzyme-sensitive nanogels. These nanogels are disassembled in the presence of those enzymes, releasing the cargo specifically in the targeted environment [138–142].

For the development of biopharmaceutical products, it is crucial to address the important issue of preserving (bio)macromolecule activity, structural integrity, and stability following encapsulation [117]. Nanogels can act as artificial chaperones, providing stability to the three-dimensional structure of large biomolecules, and making them excellent candidates for delivering proteins. In this regard, self-assembled polysaccharide nanogels have been widely utilized as a delivery system for cancer therapy. Nanogels of this nature have the ability to create a complex with proteins by means of hydrophobic and electrostatic forces, thereby maintaining their functionality [143,144]. Akiyoshi *et al.* provided one of the initial examples of cholesterol-modified pullulan-based self-assembled (CHP) nanogels capable of embedding protein (α-chymotrypsin and insulin) through hydrophobic interactions [145,146]. Further extension of the platform was achieved by cationizing the CHP nanogels to harness both hydrophobic and electrostatic interactions, and thereby improve the protein trapping (BSA and β-galactosidase) and enhance cellular internalization of the protein-loaded carriers [147]. Cholesterol-bearing hyaluronic acid (CHHA) was also reported as an alternative candidate of physically crosslinked nanogels that exhibited spontaneous protein binding without causing denaturation and improved protein binding capacity compared to typical non-ionic nanogels [148]. Recently, Antonia-Nancy *et al.* reported on the use of CHHA to protect antibodies from heat denaturation as a promising field of study [149].

One of the main challenges of the implementation of nanogels as platforms to deliver large cargoes lies in the failure to achieve high loading yields. The three-dimensional structure and the pore size can hinder the embedment of the (bio)macromolecules within the nanogels. To address this issue, several protocols have been developed for the in situ formation of nanogels in the presence of therapeutic proteins. For example, Van Thienen *et al.* developed biodegradable lipid-coated dextran nanogels using the UV polymerization of dextran modified with 2-hydroxyethyl methacrylate groups. The resulting nanogels demonstrated a 50% EE for BSA and lysozyme [150]. Using a different synthetic approach, Matyjaszewski and coworkers demonstrated that atom transfer radical polymerization (ATRP) in an inverse mini-emulsion facilitated the development of functionalized nanogels exhibiting a uniform network capable of efficiently encapsulating green fluorescent protein (GFP) in situ, with around 50% of incorporation and maintaining its tertiary structure [151,152]. In this context, the advancement of the in situ approach led to the development of single enzyme nanogels (SENs) [153–157]. The synthesis of SENs involves a free radical polymerization that occurs on the surface of the protein, resulting in small nanogels (<20 nm) loaded with single proteins. The shell that covers the protein is robust enough to confer stability to the protein against denaturation and is reasonably thin to allow the diffusion of small compounds to the core of the nanogel. This is crucial when delivering therapeutic enzymes, as the reactants must cross the polymeric shell, and the reaction (by)product needs to be released into the environment. This methodology has been applied to encapsulate antigens as vaccine nanocarriers to enhance the anti-tumoral immune response [158]. One illustrative instance involves the development of a weakly cell-interacted, nanosized, environment-responsive carrier, which represents a specific class of mAb (nimotuzumab) nanocarriers utilizing enzyme-responsive peptides [55]. This study emphasises the importance of developing strategies to not only produce high yields of encapsulation, but also to have precise control over the release process. Under this view, a specific peptide sequence was used as a crosslinker in the nanogel polymerization, making it biodegradable towards matrix metalloproteinases.

Thanks to their tuneable chemical composition, certain nanogel formulations show promise for programming drug delivery to the brain [33,159,160]. The introduction of zwitterionic groups has shown some promise to increase brain uptake through AMT [52]. It is also possible to tailor nanogels to actively cross the BBB by binding to cell membrane receptors [54]. For example, by coating the nanogels with polysorbate 80 [161] or using the oligopeptide Ang2, the RMT can be exploited [162]. As an illustration, Ye *et al.* reported Ang2-modified chitosan nanogels loaded with oxytocin to combat neuroinflammation for early intervention in AD [163]. A potentially more ground-breaking strategy is the utilization of nanogels coated with erythrocyte membranes or platelet membranes that are decorated with ApoE residues. This approach aims to prolong blood circulation and facilitate the penetration across the BBB [164,165]. Further, decorating nanogels with small signalling proteins such as insulin/Tf, or small molecules, like acetyl choline or choline that interact with specific receptors in endothelial cells, have also shown efficient delivery of (bio)macromolecules, as oligonucleotides, via transcellular pathways [166]. She *et al.* synthesized an azobenzene-based crosslinker to construct hypoxia-degradable zwitterionic phosphorylcholine nanogels. These nanogels were able to penetrate the BBB due to their ability to mimic the structure of cell membranes composed of phosphorylcholine polymers [167]. In this context, certain authors have encapsulated individual antibodies within nanogels that incorporate acetylcholine and choline analogues to cross the BBB and promote their subsequent delivery to the CNS [53–55]. Alternatively, Chekhonin and Kovanov produced nanogels conjugated with mAb to brain-specific anion transporter 1 that regulates transendothelial transport of thyroxine and some other amphiphilic anions through BBB [56,168]. Another factor that should be considered when developing nanogels for CNS delivery is the relationship between the density of crosslinking in nanogels and their transcytotic potential or uptake by polarized brain endothelial cells. It has been observed that nanogels with lower crosslinking density exhibit higher transcytotic potential, while nanogels with higher crosslinking density exhibit greater uptake by polarized brain endothelial cells [169].

Fig. 6. presents an overview of the fundamental principles involved in the development of nanogels for delivering biomacromolecules into the CNS. This includes a look at the many encapsulation methods described in the literature, as well as the often-employed strategies for effectively crossing the BBB using nanogels.

In summary, nanogels are highly modifiable nanoparticles with a significant ratio of surface area to volume that offer infinite possibilities as delivery systems, but certain critical factors must be considered. Their unique feature lies in their ability to induce release in response to stimulus such as temperature, oxidative stress, pH, or enzymes (physiological alterations that can arise from factors other than the disease itself). From this perspective, nanogels must overcome the significant limitation in their biodegradability, as they evade the kidney's glomerular filtration, implying a prolonged circulation within the body. This factor, in combination with their ability for gradual drug release, makes necessary to quickly reach nanogels to their intended therapeutic site. This urgency arises from the possibility that other physiological factors within the body also trigger a response to the stimulus, potentially exacerbating any off-target effects and causing adverse reactions. However, when treating nervous system diseases, the disruption of the BBB often leads to a faster accumulation of these nanocarriers at the target site by the enhanced permeability and retention effect (EPR). Furthermore, the use of (bio)macromolecules as therapeutic agents reduce the possibility of side effects if they are released outside the target tissue. In conclusion, although there is still room for improvement in terms of higher clearance from the body, reduced stimulus-responsive window, and active-target delivery, nanogels are promising polymeric nanoparticles for transporting large biomolecules and for the treatment of CNS disorders [170].

3.3. Dendritic polymers

Dendritic polymers (DPs) have been deeply studied in brain targeting, motivated by their ability of crossing the BBB, high EE, and easy surface decoration. Benefiting from their versatile properties, numerous (bio)macromolecules like pDNA [61], proteins [58,171], microRNA [59], and siRNA [60] have been targeted to the brain using DPs, showing high cerebral uptake and accumulation [172], fast brain tumour regression, and high gene expression [173]. DPs are tree-like architectures with different branching levels and a variety of morphologies including dendrons, hyperbranched polymers, dendrigraft polymers, and perfect dendrimers [174]. Among them, dendrimers are considered unique in their nature by their remarkable characteristics such as compact globular topology, highly symmetric structure, well-defined MW, controllable size, and high density of functional groups on the surface. From the synthetic point of view, they can be obtained using convergent or divergent approaches. Traditional strategies involve a series of deprotection and activation, resulting in the gradual formation of dendrimer generations at each iteration cycle [174]. However, due to inherent limitations found in synthesis and purification procedures, these polymeric structures are usually obtained at the laboratory scale with relatively low MW, typically below 10–20 kg/mol. For higher dendrimer generations, some degree of MW polydispersity is often observed [174]. Moreover, a few dendrimers available in the market have high prices ascribed to elevated costs of manufacture. On the other hand, the synergy of their nanoscale size, surface chemistry, and multivalent features makes them promising candidates for transporting therapeutic (bio)macromolecules into the brain.

Delivery of (bio)macromolecules to the brain in an efficient and noninvasive way is still challenging due to the existence of the BBB. As previously described, this barrier is highly selective, and diverse factors

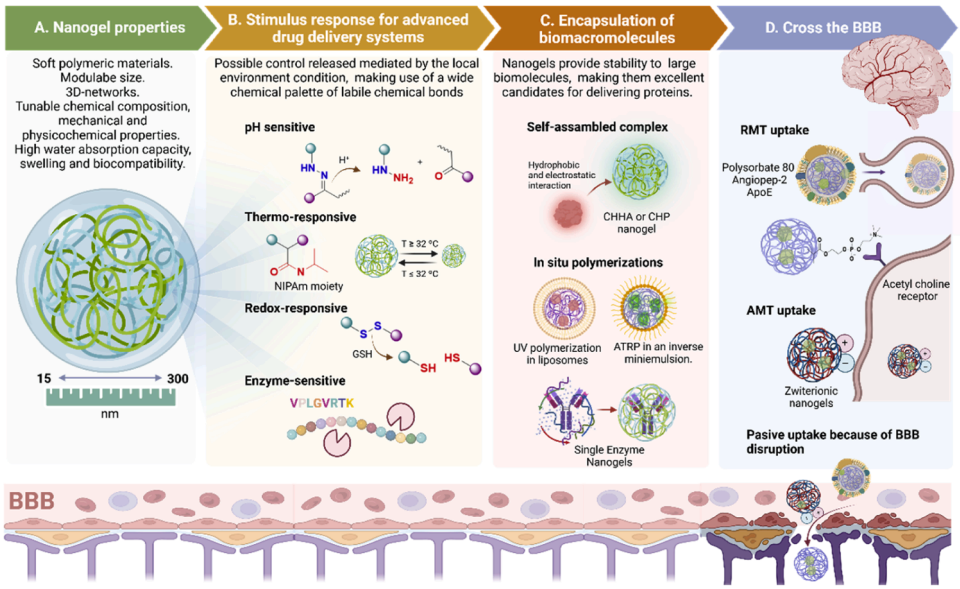

Fig. 6. The potential to integrate multiple strategies by exploiting the unique characteristics of nanogels presents an opportunity to create optimal nanocarriers for the treatment of central nervous system (CNS) disorders. (A) The first possibility is to modify the physicochemical and mechanical properties of nanogels during their synthesis process. (B) Experimenting with different labile chemical bonds can create a stimulus-responsive behaviour influenced by local environmental conditions. (C) Nanogels offer a substantial surface area for encapsulating (bio)macromolecules using different approaches; (i) complexation, a process that explores hydrophobic and electrostatic interactions: cholesterol-modified pullulan-based self-assembled (CHP) and cholesterol-bearing hyaluronic acid (CHHA) nanogels; or (ii) in situ polymerization with mild reaction conditions: UV polymerization in liposomes, atom transfer radical polymerization (ATRP) in an inverse miniemulsion and single enzyme nanogels (SEN). (D) Finally, it is feasible to decorate the surface of the nanogels with ligands to exploit receptor mediated transcytosis (RMT), modify their surface charge to enhance the adsorptive mediated transcytosis (AMT), or take advantage of the compromised integrity of the blood-brain barrier in CNS disorders (enhanced permeability and retention effect). Biorender has been used for creating the descriptive image.

impact the penetration across it. In fact, Brown *et al.* demonstrated that the composition is a key aspect that predominantly affects the uptake process and transport across the BBB [175]. Consequently, dendrimers are usually modified by including electrostatically charged functional groups, targeting ligands, fluorescent dyes, and solubilizing agents. A typical case is the dendrimer known as poly(amidoamine) (PAMAM), which is a cationic dendrimer with functional amine groups on the surface. In fact, these positive residues have been exploited in the generation of complexes with genes and for transporting negatively-charged cargos in the internal pockets (Fig. 7a,c). These two mechanisms help to increase the solubility of the (bio)macromolecule and its permeability across the BBB [57]. Also, when a therapeutic agent is carried using the internal voids of these branched structures, it is inherently protected from degradation, and more intact cargo reaches the target organ. Besides, positive charges can promote the interaction with the endothelial cells and induce cellular uptake, promoting the internalization process across the BBB. However, it is well-documented that polymeric architectures with a high density of positive charges induce cytotoxicity by the strong interaction with cells [57]. Therefore, in many cases, PAMAM has been partially or fully decorated for modulating its biocompatibility [176]. Typically, hydroxyl or carboxyl molecules are used to functionalize PAMAM because they show higher safety profiles than amino moieties. In other cases, scientists prefer to apply dendrimers based on PEG [177], polyglycerol (PG) [58], poly-L-lysine [178], or carbosilane [60], among others. In addition, targeting ligands have also been incorporated on the surface of dendrimers to induce a certain affinity towards antibodies, proteins, and factors overexpressed by the endothelial cells of the BBB. Also, the specificity of the system can be tailored to target certain proteins that tend to fold, producing a degenerative disease [179]. For instance, amyloid fibrils are produced as a result of proteins that fold, forming this final structure that is highly associated with AD. In Fig. 7b, it is showcased the most popular targeting ligands used to decorate systems for inducing selectivity towards specific (bio) macromolecules. As discussed above, RMT and AMT are among the most common pathways used to penetrate the BBB. Consequently, multiple strategies have been developed for decorating the dendrimer surface with multiple targeting ligands and stimulating the BBB permeation through these pathways. For example, Gao *et al.* developed PAMAM dendrimers that were modified with Tf, using PEG linkages [173]. This ligand was selected to facilitate brain targeting, considering that TfRs are overexpressed by the endothelial cells of the BBB and tumor cells. It is also known that TRAIL can induce apoptosis of glioma cells by binding to TRAIL-R1 and TRAIL-R2/Killer receptors, which are expressed by these kinds of cells. Thus, a human TRAIL-encoding plasmid was the therapeutic gene carried by the dendrimer, exploiting the internal cavities and the opposite charges between this polymeric architecture and the cargo. In vitro studies revealed that Tf-functionalized dendrimers had higher cellular uptake and gene expression in a glial cell line (i.e., C6 cells). According to in vivo results, Tf-decorated dendrimers showed preferred targeting towards glioma and were able to induce a greater tumor apoptosis [173]. The difference observed between naked and modified dendrimers is attributed to Tf, which can mediate both the BBB penetration using RMT pathway and induce the accumulation of the dendrimers in glioma cells *via* endocytosis [173].

At the same time, dendrimers can emulate a mechanism that usually occurs in nature. The presence of multivalent ligands on the surface allows cooperative binding events, generating stronger interactions. This property is a powerful tool that has been shown to facilitate cellular entry, promote BBB permeation, and induce accumulation in target organs, as it is illustrated in Fig. 7d. For instance, Gao *et al.* exploited the multivalent effect and the receptor binding affinity by preparing different batches of PAMAM dendrimers and modifying them with varying quantities of Ang2 peptides, using PEG as linker [172]. Ang2 was used to increase the system affinity towards the low-density lipoprotein receptor-related protein, which can be found on brain endothelial cells and also expressed in different types of brain cancer cells. Flow cytometry studies demonstrated that the receptor binding affinities increased with the substitution degree of the peptide, being 7.5 times

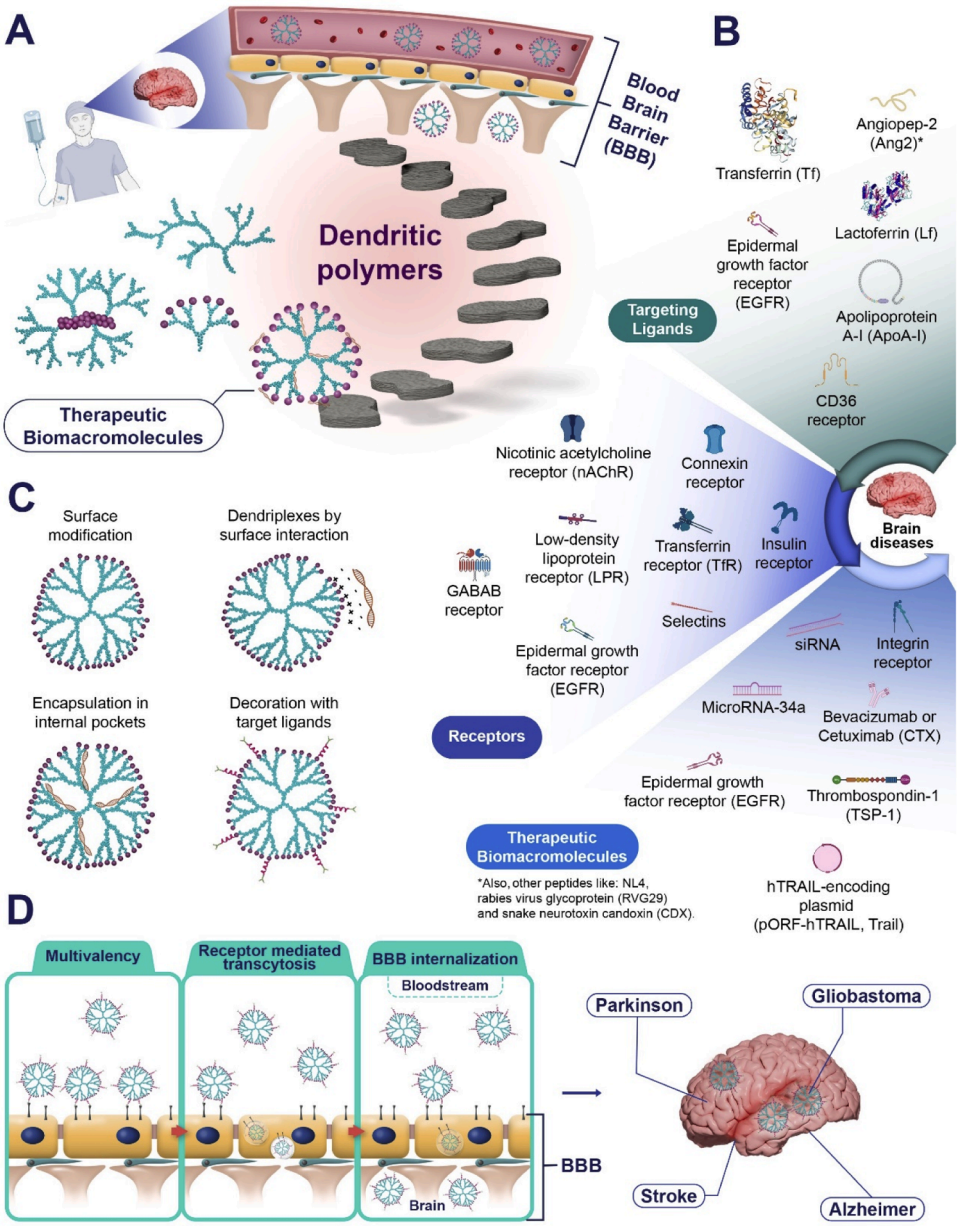

Fig. 7. Therapeutic biomacromolecules transported across the BBB using dendritic nanocarriers. (A) Dendritic polymer with different morphologies that have been used for transporting a variety of cargo through the BBB. (B) Important biomacromolecules involved in different brain diseases including therapeutic biomacromolecules, targeting ligands that have been exploited to decorate dendrimers and the receptors that these ligands usually bind with. (C) Attributes exploited and strategies applied with dendrimers for enhancing efficiency of the transportation process like surface modification with functional moieties, formation of dendriplexes, encapsulation of cargo in internal pockets and decoration with target ligands. (D) Dendrimer penetration of the BBB by receptor mediated transcytosis pathway. Biorender and Blender have been used for creating the descriptive images.

higher in the dendrimer decorated with eight peptides than in the dendrimer without peptides [172]. The biodistribution results indicated that the dendrimer that showed the highest concentration in the brain was the one functionalized with four peptides, overcoming the BBB *via* RMT [172]. Besides, another innovative idea for promoting cargo penetration through the BBB was described by Gao and collaborators [180]. In this recent research, a temporary opening of inter-endothelial TJs was performed by specific activating adenosine receptors on murine brain endothelial cells [180]. In this case, nano-agonists of PAMAM were decorated varying the quantities of the A2A AR agonist regadenoson on their surface. In vitro and in vivo studies revealed that nano-agonists with higher ligand density had the longer BBB opening time-window, resulting in the highest model drug crossing efficiency [180]. The authors explained that nano-agonists activate ARs on brain endothelial cells, which drives intracellular signal transduction, causing the TJ opening [180]. The efficiency of the activation is justified by the prolonged circulation time and multivalent association between nano-agonists and receptors on brain endothelial cells [180].

Briefly, dendrimers have attracted a growing interest in brain targeting based on their nanometric size, surface functionality, and the strong interaction that can be established with cargo or receptors by the multivalent effect. These are advantageous attributes that showed a high impact on the penetration of the BBB or internalization into target cells. Even though the synthesis process is tedious, with multiple steps and difficulties to obtain high MW structures, dendrimers are considered efficient vehicles for transporting and being applied in controlled-release applications of biomacromolecules in brain for the treatment of GBM, or neurodegenerative diseases.

3.4. Self-assembled polymeric nanoparticles

Inspired by biology and combining supramolecular chemistry with nanotechnology, self-assembled nanocarriers can be produced through spontaneous interaction of molecules to form organized structures [181–183]. There are various self-assembled nanocarriers that have been studied to cross the BBB, such as liposomes [62,184], niosomes (using solute carrier transporters) [185], lipid nanocarriers [186], polymeric micelles [187], polymersomes (polymer vesicles) [188], and layer-by-layer (LbL) capsules, among others. As the focus of the present review is on polymer-based nanocarriers, herein we elaborate upon three self-assembled polymeric nanocarriers: polymeric nanoparticles based on amphiphilic block and graft copolymers, polymersomes, and LbL capsules.

Amphiphilic block and graft copolymers represent a notable category of polymeric nanoparticles, featuring distinct hydrophilic and hydrophobic polymer blocks chemically linked to create structures with exceptional self-assembly capabilities. These blocks can either be neutral polymers (hydrophilic or hydrophobic) or polyelectrolytes (anionic, cationic, or zwitterionic) [189–191]. In an aqueous environment, these amphiphilic block copolymers self-assemble to form polymeric micelles above their critical micelle concentration (CMC). Above this concentration, the hydrophobic inner core of the copolymer comes closer to aggregate and distances itself from water molecules [192]. These are generally made of biocompatible, non-immunogenic, biodegradable blocks such as polyesters, poly(propylene oxide), or poly (amino acids) linked to biologically compatible corona-forming blocks like PEG [193,194] and display a core-shell nanostructure. In addition, graft copolymers produced by the hydrophobization of hydrophilic polymeric backbones (e.g., polysaccharides, polyols) can give place to more complex nanostructures such as multimicellar systems that enable greater EE [195]. Their ease of functionalization makes them an efficient nanosystem for brain targeting by both intravenous and intranasal administration [196,197]. However, depending on the administration strategy, nanocarrier features such as size and surface must be properly engineered. For example, the group of Sosnik developed chitosan-based self-assembled nanocarriers that were surface-modified with shuttle peptides and increased the delivery of small-molecule anticancer drugs to the CNS [66,198]. Their investigation in the CNS delivery of (bio) macromolecules is currently being investigated. Various approaches used for the functionalization of polymer micelles for brain targeting are shown in (Fig. 8) [199]. Pluronic® block copolymers, also known as poloxamers, exemplify this phenomenon and have garnered significant interest due to their ability to impede drug efflux transporters, such as the inhibition of P-gp expressed on the BBB, thus enhancing drug delivery to the CNS [200,201]. Pluronic® are poly(ethylene oxide)-poly (propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) triblocks, forming an amphiphilic copolymer. The manipulation of the number of hydrophilic EO and hydrophobic PO units provides a versatile means of tailoring these copolymers for specific applications [200,202]. With over 50 Pluronic® molecules commercially available from BASF Corp. (Parsippany, NJ, USA), each characterized by different hydrophilic-lipophilic balance (HLB) and CMC, these copolymers offer a spectrum of possibilities for tailoring particle size, drug loading, stability, and drug release profiles in the pursuit of optimized therapeutic outcomes.

The classification of Pluronic® copolymers into four categories based on differences in HLB and hydrophobic PPO chain length further elucidates their diversity [203]. The first class includes hydrophilic Pluronic® with an HLB of 20–29, such as F68, F108, and F127, where F means that the product is supplied in the form of flakes [190,204,205]. These not only exhibit good hydrophilicity but also boast excellent biocompatibility [206], contributing to drug accumulation and prolonged blood circulation time [207]. The second class comprises

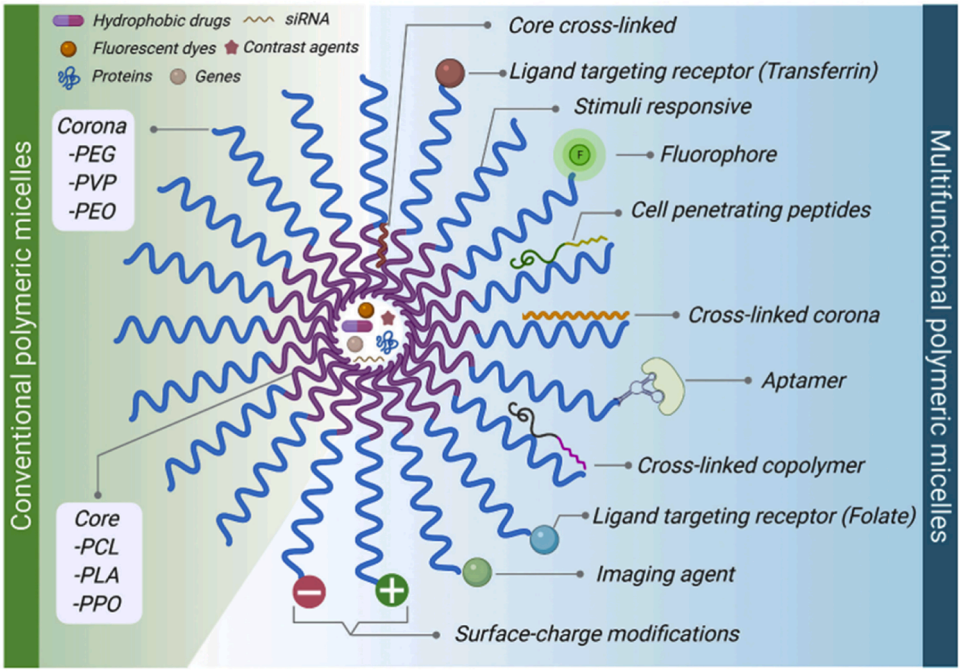

Fig. 8. Schematic representation of polymeric micelles for drug delivery. The image illustrates multifunctional polymeric micelles with various modifications for enhanced drug delivery. The core is hydrophobic, consisting of polymers like PCL (polycaprolactone), PLA (polylactic acid), and PPO (polypropylene oxide), while the corona is hydrophilic, formed by PEG (polyethylene glycol), PVP (polyvinylpyrrolidone), or PEO (polyethylene oxide). Modifications include core cross-linking for stability, ligand targeting receptors (e.g., transferrin, folate) for receptor-mediated targeting, and stimuli-responsive elements for controlled drug release. Additional features such as fluorophores and imaging agents allow in vivo tracking, cell-penetrating peptides enhance cellular uptake, and cross-linked coronas improve structural integrity. Aptamers are used for specific targeting, and surface charge modifications optimize interactions with biological membranes. These micelles encapsulate drugs, siRNA, proteins, and other agents for targeted and efficient therapeutic delivery. Adapted with permission from [199].

Pluronic® with a lower HLB and shorter PPO chain, represented by liquid L64 [208], L44 [209], and L35 [210], which imparts nanostructures with the ability to self-assemble in aqueous media, offering a more suitable size (10–100 nm) and stable structure [211]. The third class features Pluronic® with a lower HLB and a PPO chain length ranging from 30 to 60 and in paste form at room temperature, including P85 [212], P105 [213], and L61 [214], which exhibit dissolution and self-assembly properties in water, making them suitable for loading hydrophobic drugs. Additionally, these copolymers consume adenosine triphosphate (ATP) in multidrug-resistant cancer cells, inhibiting P-gp and prolonging drug circulation time [215,216]. The fourth class of Pluronic® with a lower HLB and a PPO chain exceeding 60, featuring P123 and L121, with the longest PO chain, is widely used for loading drugs due to its greater hydrophobicity [217,218].

The encapsulation of (bio)macromolecules within self-assembled nanocarriers is a challenge that can be overcome by harnessing the advantages of self-assembled nanoparticles and strategically binding biomacromolecules through entrapment, adsorption, or covalent attachment, transforming these copolymers into Trojan horses, facilitating the enhanced transport and delivery of therapeutic payloads to the brain. Certain amphiphilic nanoparticles modified with proteins, peptides, RNA, and drugs have shown the ability to cross the BBB. For instance, Poly(β-L-malic acid-tri leucine)-copolymer has been used to transport miRNA across the BBB. This system utilizes the LRP-1 transcytosis pathway and amyloid beta (Aβ) pathway by conjugating a D-configured (D3)-peptide as a vector for specific targeting, achieving neuron-selective delivery of miRNA in an AD mouse model [219]. Another notable example involves the use of Leptin-Pluronic® 85 (P85) conjugates. This amphiphilic copolymer targets the leptin transporter and has shown promising results as an anti-obesity drug. Its benefits include enhanced peripheral bioavailability, increased brain uptake, and the ability to cross the BBB independently of the leptin transporter [220]. Furthermore, the TfR-T12-PEG-PLGA and TATH7-PEG-PLGA systems utilize TfR to transport the TfR-T12 peptide, TATH7 peptide, and paclitaxel across the BBB. The strategy involves conjugation with the TfR-T12 and TATH7 peptide shuttle, and in vivo pharmacodynamic evaluation demonstrated a potent anti-tumor effect in subcutaneous and normotopic glioma models, significantly extending the life cycle of tumor-bearing mice [221].

On the other hand, while amphiphilic copolymers can cross the BBB, specific targeting to desired brain regions remains challenging. Strategies like conjugation with brain-specific ligands or antibodies are crucial for maximizing efficacy and minimizing off-target effects. Efficient encapsulation and controlled release of (bio)macromolecules within amphiphilic copolymers remain key challenges. Optimizing polymer ratios, incorporating stimuli-responsive release mechanisms, and exploring co-assembly with other polymers are promising strategies. Extensive preclinical and clinical studies are necessary to establish safety and efficacy for regulatory approval. Despite these difficulties, amphiphilic copolymers hold immense potential for transporting biomacromolecules across the BBB. Continued research efforts focused on addressing these limitations and exploring innovative design strategies can help to move closer the technology for treating various neurological disorders.

Polymersomes are synthetic vesicles composed of amphiphilic block copolymers, with a hydrophobic bilayer membrane and greater degree of complexity in comparison to the aforementioned core-shell polymeric micelles (Fig. 9a). Polymersomes are usually fabricated using diblock, triblock or multiblock copolymers by following different methods to induce their self-assembly including solvent-switch (or microfluidic method), pH-tuning, polymer rehydration, polyion complex vesicles (PICsomes) fabrication approach, a combination of self-assembly/polymerization (i.e., polymerization-induced self-assembly), and centrifugation method. After their self-assembly, sonication, membrane extrusion, or alternative methods can be used to purify and/or adjust their size [222]. Some typical polymers to produce polymersomes for nanomedicine are PEG [223], diblock copolymers of dextran and PLGA (DEX–PLGA), poly(butadiene)(PBd)-PEG [224], and diblock copolymers of PEG and PCL [225,226]. Compared to liposomes, polymersomes have a thicker bilayer structure, which improves their physical stability and increase their blood circulation [224]. Contrarily to polymeric micelles, that are usually limited to the encapsulation of hydrophobic drugs, polymersomes can accommodate both hydrophilic and hydrophobic cargos in their structure. Polymersomes have gained attention in cancer nanomedicine and drug delivery systems due to their advantageous properties, such as great stability, versatility in design, and tunable capabilities for effective cargo encapsulation and controlled release of anticancer therapeutics [222].

Regarding the use of polymersomes to deliver therapeutic agents in the brain environment, several attempts have been described in literature. Yu *et al.* functionalized the surface of 100 nm PEG-PLGA polymersomes with lactoferrin to cross the BBB and improve the delivery of fluorescent 6-coumarin and S14G-humanin (a neuroprotective peptide) to the brain tissue. Pharmacokinetic studies following intravenous injection suggested that 101 lactoferrin ligands per polymersome was the optimized number to promote the permeability of the polymersomes across the BBB and ensure brain-targeted delivery [223]. Georgieva *et al.* presented a formulation of PBd-b-PEG polymersomes, which were decorated with peptide G23 to increase their capacity to target ganglioside GM1 for caveolae-mediated endothelial transcytosis. Preliminary in vitro studies of these polymersomes in an endothelial Transwell® model showed that the polymersomes were capable of penetrating the BBB through efficient endothelial cells transcytosis [224]. In addition, in vivo biodistribution studies after intravenous injection in mice showed that G23-functionalized polymersomes accumulate in the brain parenchyma [227]. This is an advantage of G23-functionalized polymersomes over some reported Tf-targeted systems, which mainly accumulate in the brain capillary and do not reach to the parenchyma region.

Active targeting strategies enable the nanocarriers to cross the BBB by means of an interaction through the specific ligands and they have been applied to both polymeric micelles and polymersomes, aiming to improve the uptake of the nanocarriers by endothelial cells. One active targeting strategy is to traverse the nanocarriers in the BBB using carrier proteins, such as GLUT-1, which is specifically and highly expressed on endothelial cells of the BBB. Su Min *et al.* developed glucosyl-poly (ethylene glycol)-b-poly(L-lysine) modified with 3-mercaptopropyl amidine and 2-thiolaneimine (Glu-PEG-PLL(MPA/IM)) polymeric micelles. As explained before, polymeric micelles are also obtained by self-assembly of amphiphilic polymers [228]. These glucosylated-polyion complex polymeric micelles were loaded with antisense oligonucleotide (ASO) that were functionalized with different quantities of glucose to study the effect of the ligand density and the accumulation of nanoparticles in brain. A group of nanocarriers that was coated with 52 glucose ligands per nanoparticle showed 17 times higher accumulation in the brain compared to the group without glucose coating, indicating the great potential of GLUT-1-mediated transport strategy [63]. Shi Yanan *et al.* discovered a new method to enhance the clinical use of RNAi in glioblastoma therapy. This method involves the development of an Ang2 peptide-decorated chimeric polymersome (ANG-CP), which effectively packs and protects anti-PLK1 siRNA (siPLK1). In vitro studies indicate that ANG-CP may efficiently traverse the immortalized mouse brain endothelial cell line (bEnd.3) monolayer, facilitate the transport of siRNA into the cytoplasm of U-87 MG glioma tumor cells through the LRP-1-mediated pathway, and markedly silence PLK1 mRNA and its associated oncoprotein in U-87 MG cells [64]. In this way, Jiang Yu *et al.* suggest using a chimeric polymersome (CP) decorated with ApoE instead of Ang2 as a targeted protein therapy for treating glioblastoma. This showed better penetration through the bEnd.3 monolayer in in vitro BBB models [65].

Among self-assembled polymeric nanocarriers, multilayer polymer capsules have attracted increasing attention in recent years. Multilayer polymer capsules are usually fabricated via the LbL approach, which

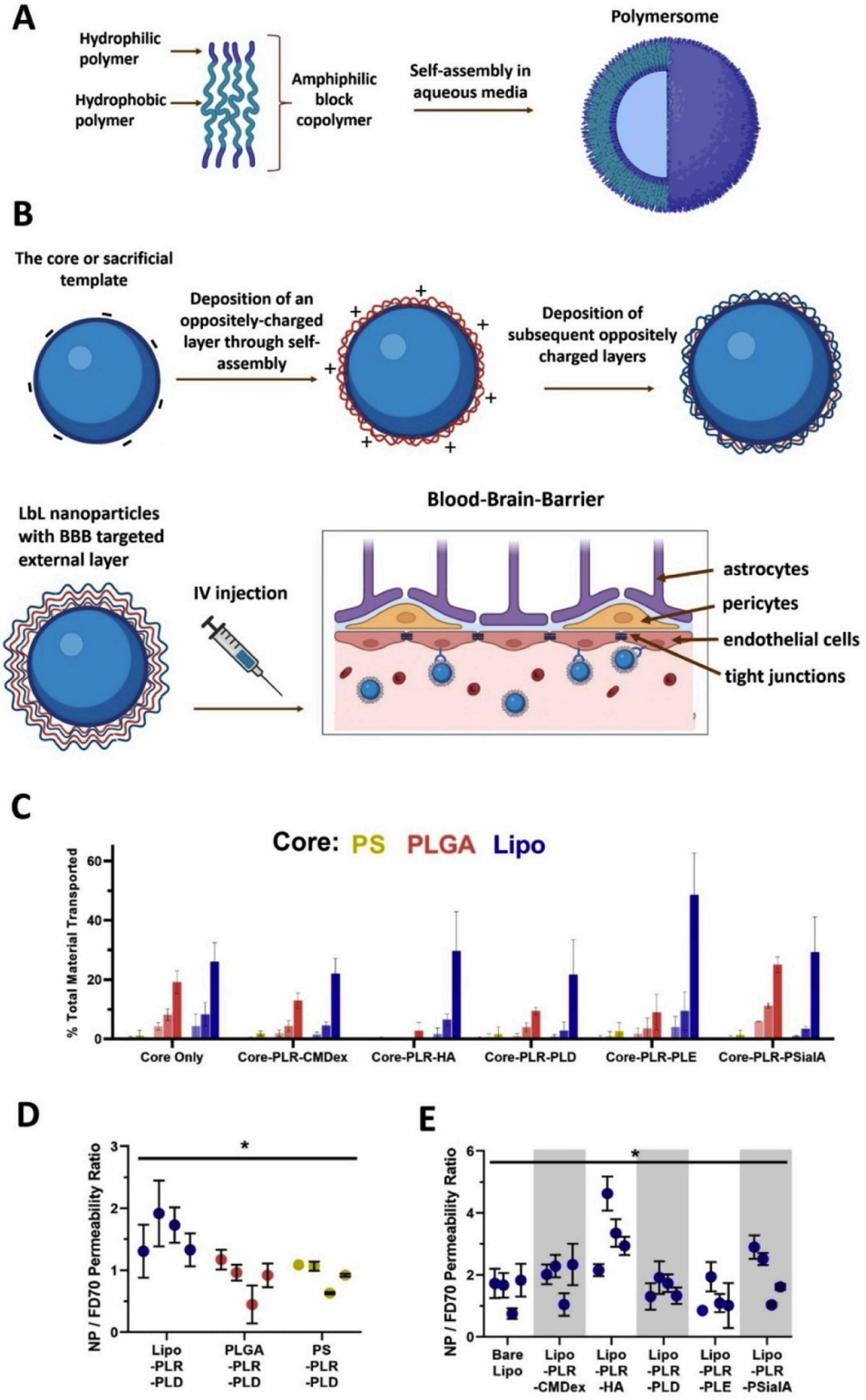

Fig. 9. (A) Schematic illustration of polymersome structures, which are obtained by self-assembly of amphiphilic block copolymers. (B) A schematic illustration of layer-by-layer (LbL) deposition of oppositely charged polyelectrolytes on a negative core or sacrificial template. Final LbL nanoparticle with blood-brain barrier (BBB)-targeted external layer to facilitate the passage of nanoparticles through the BBB and deliver its cargo into the brain. Biorender has been used for creating the descriptive image. (C) Uptake of LbL nanoparticles with three cores (lipo: liposomes, PLGA: poly (lactic-co-glycolic acid), PS: carboxylated polystyrene), which were deposited by one layer of poly-L-arginine (PLR) polycation and final layer of different polyanions (CMDex: carboxymethyldextran, HA: hyaluronic acid, PLD: polyL-aspartic acid, PLE: poly-L-glutamic acid, and PSialA: polysialic acid). The uptake by the monolayers that were grown in Transwell shows superiority of liposome core over other stiffer cores. (D) Permeability of the LbL nanoparticles with different cores but with the same last layer of PLD in the mice BBB demonstrates more permeability of LbL nanoparticles with liposomal core. (E) BBB permeability of bare liposomes and liposome-based LbL nanoparticles with different surface functionalization in mice showed that the permeability of LbL nanoparticles with hyaluronic acid (HA) external layer across mice BBB was the greatest. Adapted with permission from [243]. Copyright © 2023 John Wiley & Sons. Biorender has been used for creating the descriptive image.

relies on the alternate deposition of oppositely-charged polymers onto a template (Fig. 9b) [229]. The stepwise process in the LbL approach allows the precise control over the surface properties (i.e., charge and functionalities) and the size of the capsule [181,230]. LbL capsules have been accordingly considered as potential drug delivery and theranostic nanosystems for a wide variety of biomedical applications [229, 231–233]. Beyond drugs of small MW, LbL (nano)capsules have demonstrated their capacity to deliver therapeutic biomacromolecules, such as genes and enzymes [234–237]. As an illustration of this versatility and multi-functionality associated to LbL systems, Boehnke *et al.* introduced click chemistry into LbL nanoparticles, which enables them to combine a biosensing peptide and a targeting peptide within the same carrier. This multifunctional system demonstrated a sensitive detection to three different types of cancers and simultaneous gene silencing as a result of successful siRNA delivery [232]. This study highlights the potential of LbL nanoparticles to load high amounts of various large biomacromolecules, such as siRNA and peptides, in a single formulation.

One of the important aspects for targeted drug delivery to brain tissue is that nanoparticles are not cleared out from the blood stream via phagocytosis and the recognition of immune system. There are some strategies that have been implemented to avoid the uptake by macrophages, such as cell hitchhiking, surface modification of nanoparticles, and the modulation of the physiological environment [238]. Łukasiewicz *et al.* developed 100 nm LbL nanocapsules loaded with clozapine and functionalized with PEG to investigate their potential to cross the BBB [239]. Results demonstrated that the proposed surface modification strategy decreased their phagocytosis and uptake by macrophages, but did not decrease the endothelial cells uptake. Moreover, all the groups were able to cross the BBB in an in vitro BBB Transwell® model, and PEG functionalization improved the transcytosis and reduced the cytotoxicity of clozapine-encapsulated nanocapsules. In addition, their inhibition studies indicated that caveolae-dependent transcytosis played a role in the internalization of nanocapsules by endothelial cells [239].

Another strategy for BBB transport relies on facilitating transcytosis in BBB endothelial cells through a mechanism mediated by low-density lipoprotein receptor-related proteins, such as LRP1 [188,240]. Straehla *et al.* fabricated LbL nanocapsules using liposomes as a template and propargyl-modified poly(L-aspartic acid) as the external layer, which was further modified with Ang2 peptide [241]. They used these nanoparticles for cisplatin delivery in a microfluidic BBB-GBM. In comparison with bare liposomes and non-functionalized LbL nanoparticles, the LbL nanocapsules functionalized with Ang2 were preferentially accumulated in microvascular network near GBM spheroids. Thus, Ang2-functionalized LbL nanoparticles showed more effectiveness in GBM tumor cell killing with respect to bare liposomes and free cisplatin. These outcomes demonstrated the superiority of Ang2-functionalized nanoparticles, penetrating the BBB by LRP1-mediated transcytosis. This work also demonstrated the increased expression of LRP1 in the vascular network in presence of GBM spheroids.

Additionally, the intrinsic mechanical properties of the LbL particles/capsules can also determine their capacity to cross the BBB. It was shown that the stiffness and deformability of LbL nanoparticles is mainly dependent on the core rather than on surface functionalities. Kong *et al.* were able to tune the stiffness of LbL nanoparticles by altering the cholesterol content in liposome synthesis, which was used as a core. This study reports that more deformable, fluid, and compliant LbL nanoparticles were obtained by cholesterol addition to liposomal core. Compared to stiff LbL nanoparticles, compliant LbL ones can penetrate filter membranes (with 100 nm pore size) more easily, which could also have better BBB penetration [242]. In a similar study, Lamson *et al.* focused on the effect of core stiffness and surface functionalization of LbL nanoparticles on their transport through the BBB. To this end, they investigated three different negatively-charged cores, i.e., liposomes, PLGA, and carboxylated polystyrene, and modified the last layer of the nanoparticles by using different polyanions such as carboxymethyldextran, hyaluronic acid (HA), poly(L-aspartic acid), poly(L-glutamic acid,) and poly(sialic acid). Their results revealed that liposome-based LbL nanoparticles showed the highest or similar transport in Transwell® assay and the highest uptake in brain microvascular endothelial cell line (hCMEC/D3) monolayer association (Fig. 9c) compared to the nanoparticles with stiffer core. It was also revealed that the surface chemistry of the LbL nanoparticles determines the mechanism by which nanoparticles are transported intracellularly. After evaluating the transport in the BBB of mice, liposomal nanoparticles exhibited the highest permeability compared to other LbL nanoparticles with the same outer layer (Fig. 9d). Finally, liposome-based nanoparticles with the last layer of HA showed the highest permeability in mice BBB compared to other surface modifications (Fig. 9e) [243]. Although this work provides insightful research on the effect of core stiffness and surface chemistry on the capacity of LbL nanoparticles to cross the BBB, it is not exactly clear why HA functionalization improved permeability in vivo.

Despite previous attempts, there are few research works on self-assembled nanoparticles focusing on carrying biomacromolecules and crossing the BBB. Therefore, many more studies are needed to investigate different prospective formulations using various templates and surface chemistries, determine their effectiveness, and uncover the real potential of self-assembled polymeric nanocarriers. Core removal after layer deposition could result in nanoplatforms with more elastic and fluid membrane that can penetrate through the barrier [244]. All the mentioned examples show the versatility and potential of self-assembled nanocarriers, including polymeric micelles, polymersomes, and LbL nanoparticles/nanocapsules, to combine various functionalities in one system for effective delivery of therapeutic molecules across the biological barriers, such as the BBB.

3.5. Other polymeric carriers

Nanoparticles based on natural polymers like polysaccharides or proteins have been explored for their potential use in targeting BBB and deliver therapeutic biomacromolecules [95,245–247]. The presence of large surface areas containing functional groups (e.g., hydroxyl, carboxyl, amino) allows tailoring the physicochemical properties of polymeric nanocarriers for enhancing biomolecule conjugation and targeting receptors for crossing the BBB. Along with this, the size, aspect ratio (shape), surface charge and ligands of the particles can also be modulated, which also have an important role in BBB penetration [248–250]. In this section, we discuss examples of some of the nanocarriers derived from natural polymers for targeted delivery of drugs and strategies to improve their ability to cross the BBB.

Chitosan: Chitosan (CS) is a natural linear cationic copolymer derived from chitin and is a widely used material for nanocarrier fabrication having profound application in many CNS disorders [251,252]. CS consists of β-(1,4)-linked d-glucosamine and *N*-acetyl-d-glucosamine groups. Owing to its good biocompatibility, it is classified as 'generally recognized as safe' (GRAS) by the FDA. CS nanoparticles can be fabricated by various methods including ionic gelation, emulsification, and chemical crosslinking [253]. The ionic gelation method was followed to encapsulate methotrexate, dopamine, and sitagliptin drug for the treatment of GBM, PD, and AD, respectively [251,254,255]. Recently, proline-loaded CS nanoparticles, synthesized by ion crosslinking method, have been used to deliver proline, resulting in neuroprotection after ischemic injury [256]. Additionally, due to poor penetration of most of the CNS associated drugs, CS was used to decorate PLGA and PCL nanoparticles for targeting the drugs across BBB [251]. The positive surface-charge of the nanoparticles shows high affinity towards the endothelial cells for enhanced brain delivery and cellular adsorption, via AMT. For this, CS is modified with methyl iodide and glycidyl ethers to generate *N*-trimethyl CS (TMC) and alkylglyceryl-modified CS, respectively [249]. TMC improves mucoadhesion, solubility (in a pH-independent manner), enhances drug loading and absorption efficiency with respect to native CS. For example, nanoparticles formulated with TMC loaded with an anti-neuroexcitation peptide displayed

effective BBB penetration, and have been used for epilepsy treatment [251,257]. In case of alkylglyceryl CS, initially the primary hydroxyl functionalities of the low MW CS were modified through selective grafting with alkylglyceryl groups [249,257]. Following this, the nanoparticles were synthesized using ionic gelation method and the resulting nanoparticles displayed enhanced permeability of therapeutics across BBB [257]. Furthermore, the surface modification of CS nanoparticles with PEG has also emerged as a promising strategy to enhance the brain targeting efficiency of the therapeutics [257]. To further optimize this approach, the PEG-modified nanoparticles were functionalized with targeting antibodies like OX26 mAb or anti-TfR [257]. This targeted delivery strategy has been used to deliver Z-DEVD-FMK peptide and antisense oligonucleotides, showing improved therapeutic efficacy via RMT [252]. For a variety of therapeutic applications, CS nanoparticles have also shown promise to carry biomacromolecules across BBB. As an illustration, dual antibody targeting siRNA-loaded CS nanoparticles were engineered to block HIV replication in the CNS [95]. The two antibodies Tf and bradykinin B2 specifically bind with the TfR and bradykinin B2 receptor, respectively, facilitating siRNA delivery into astrocyte target cells. This resulted in inhibition of expression of proteins such as SART3 and hCycT1 that are associated with HIV replication [258]. In a similar work, CS nanoparticles were used for in vitro transfection of GFP-tagged plasmids on HEK293 (human embryonic kidney cells) and MG-U87 (brain cancer) cells on murine models [67]. The work highlights the effective BBB penetration of CS nanoparticles.

Alginate: Alginate is a linear unbranched anionic and hydrophilic natural polysaccharide obtained from brown seaweed. Structurally, it is composed of a copolymer of α-1-guluronic acid and β-D-mannuronic acid linked by 1,4-glycosidic moieties. FDA classified alginate as GRAS due to its very good biocompatibility and thus, it has widespread applications in the field of drug and gene delivery, wound healing, and tissue engineering [249]. Reports have shown that pH-responsive alginate nanoparticle formation involves methods like ionic gelation, emulsification-solvent evaporation, emulsification-gelation, water/oil emulsion method, inter-chain crosslinking, or by mixing with other polymers [259]. For example, alginate nanoparticles have been recently prepared along with CS to deliver small peptide SpBMP-9 derived from neural growth factor BMP-9. This promotes the differentiation of cholinergic neurons and inactivate GSK3beta which are promising outcomes in the treatment of brain degenerative disorders, such as AD [260]. The widely used ionic gelation method has been applied to prepare venlafaxine-loaded alginate nanoparticles, which have been utilized as anti-depressants [261]. Following this approach, an intranasal carrier system was developed, exploiting electrostatic interaction of alginate and doxorubicin to give alginate-doxorubicin nanocomplex, which was incorporated into CS nanoparticles. The work highlights the efficient delivery of doxorubicin to brain tissue with a targeting efficiency of 480%, signifying a promising strategy for intranasal targeted delivery to the brain [262]. In another example, sodium alginate along with doxorubicin and rhodamine were emulsified into dioctyl sodium sulfosuccinate [Aerosol OT (AOT)]–alginate nanoparticles and were able to overcome P-gp mediated drug resistance in tumour cells [263]. In recent progressions within regenerative medicine, the treatment of PD encompasses the delivery of therapeutic cells and neurotrophic factors capable of releasing dopamine to the brain. The approach of delivering cells aims to replace lost neurons and facilitate functional reinnervation by releasing dopamine that secretes neurotrophic factors. However, clinical trials revealed high rates of cell death post implantation. To overcome this, alginate has been used to make dual layer beads along with HA for slow and sustained release of therapeutic cells SH-SY5Y (neuroblastoma cell line) and immunosuppressant FK506 compound showing prolonged cell survival and functionality for PD treatment [264].

Hyaluronic Acid: HA is a water soluble, biocompatible linear polysaccharide composed of D-glucuronic acid and *N*-acetyl-D-glucosamine units found in the extracellular matrix of various tissues. The presence of hydroxyl groups makes it hydrophilic, whereas carboxyl, hydroxyl, and acetamido groups in its structure can be exploited for chemical modification [265]. HA has been extensively utilized in drug delivery systems due to its affinity towards CD44 (trans-membrane glycoprotein) receptors that are overexpressed in breast, lung and metastatic brain cancer [266,267]. The synthesis of nanoparticles involves electrostatic interactions between anionic HA and cationic CS, which were used to deliver curcumin and neuroglobin in the brain for GBM and stroke treatment [68,268]. Various HA-based modified nanoplatforms, like PEGylated HA, HA with lipoic acid- lysine, HA with vitamin E succinate copolymers, HA with glycyrrhetinic acid, doxorubicin, and sodium tripolyphosphate nanoparticles have been used for doxorubicin delivery for anticancer therapy and overcoming chemoresistance [269]. For enhanced targeted therapy for GBM, a hybrid HA nanocarrier system was functionalized with peptide HRK-19, containing RGD and NGR to bind $\alpha v\beta 3$ and aminopeptidase-N (CD13) receptors overexpressed in glioma cells and/or angiogenic vessels. With limited investigation on the potential use of alginate nanocarriers, there is plenty of room to improve these nanocarriers for biomacromolecule delivery across BBB. Both alginate and HA nanoparticles, being easily designable, and with long circulation time in the systemic bloodstream, can be easily modulated to achieve enhanced BBB permeation [249–270].

Besides, natural proteins are intended to serve as carriers or targeting moieties to enhance the transport of drugs and therapeutic agents to the CNS [271,272]. Proteins having high stability and activity with low enzymatic degradation, immunogenicity, phagocytosis, renal clearance, leading to increase in half-life of the drug, has shown promising outcomes [271].

Gelatin: Among all natural polymers, gelatin has been extensively used for the delivery of biomacromolecules to the brain. The synthesis of gelatin nanoparticles involves emulsification solvent evaporation, desolvation, nanoprecipitation, and microfluidic devices methods [245]. Ideally, gelatin needs crosslinking agents to overcome its limitations of low mechanical integrity and rapid decomposition. For CNS disorders, strategies like cationic cell penetration based on the peptide-mediated endocytosis are conducted to transverse the BBB. For example, a brain-penetrating peptide conjugated with PEG and TAT peptide has been used for modification of gelatin–siloxane nanoparticles for enhancing their efficiency in crossing BBB [245–252]. Further, gelatin nanoparticles have demonstrated their effectiveness as a reliable carrier for RNA delivery in the brain environment, along with transport of growth factors, microRNAs, and siRNA to differentiate stem cells and silence genes [245]. The intranasal delivery of inducible nitric oxide synthase siRNA and osteopontin peptide increased its efficacy for the treatment of ischemic stroke by inducing strong neuroprotective effect [245–252]. Recently, gelatin nanoparticles loaded with neuropeptide Substance P delivered in CNS enhanced the dopaminergic neuron recovery in hemiparkinsonian rats and can be an effective therapy for PD [273].

Human Serum Albumin: Human serum albumin (HSA) is a globular protein commonly used to improve the solubility and transport of hydrophobic drugs across the BBB. Drug-albumin complexes can increase drug stability and prolong circulation time, enhancing their potential to reach the CNS. The synthesis involves chemical (emulsion and complex coacervation), physical (electrospray and nano spray), and self-assembly (desolvation) methods. HSA has reactive amino, thiol and carboxylic groups on its surface, which favour covalent ligand and surface modifications whereas they also favour non-covalent interactions of various drugs and peptides. Albumin nanoparticles functionalised with the protein ApoE could target endothelial cells by receptor mediated endocytosis, whereas nanoparticles without ApoE were incapable of reaching brain tissues. Further, albumin particles can be modified with cell penetrating peptides like low MW protamine (LMWP) and brain penetration enhancers based on traditional Chinese medicines like borneol, muscone or menthol to improve BBB penetration [274,275]. As

an illustration, BSA nanoparticles and LMWP-modified BSA nanoparticles were fabricated based on self-assembly of LMWP and BSA with hydrophobic drugs paclitaxel and fenretinide (Fig. 10a). Both types of nanoparticles resulted in arresting tumour growth in both subcutaneous and orthotopic glioma models. In the orthotopic glioma model, the whole-body imaging data revealed uptake of both types of nanoparticles (Fig. 10b). Notably, LMWP-modified BSA nanoparticles demonstrated rapid and substantial accumulation in brain with higher fluorescence signals (Fig. 10c). In comparison, the uptake of BSA nanoparticles was notably lower than that of the LMWP (Figs. 10c and e). The results are further corroborated with *ex vivo* imaging of the dissected organs from mice (Fig. 10d). A major disadvantage of this model is the lack of BBB. Currently, for GBM, clinical trials are going on with FDA approved Abraxane (albumin bound paclitaxel) along with carboplatin and implanted ultrasound emitters device [246]. Further, albumin is used to modify the surface of polymeric nanoparticles for enhancing BBB translocation [246].

Further, modifications of nanoparticles with natural ligands/

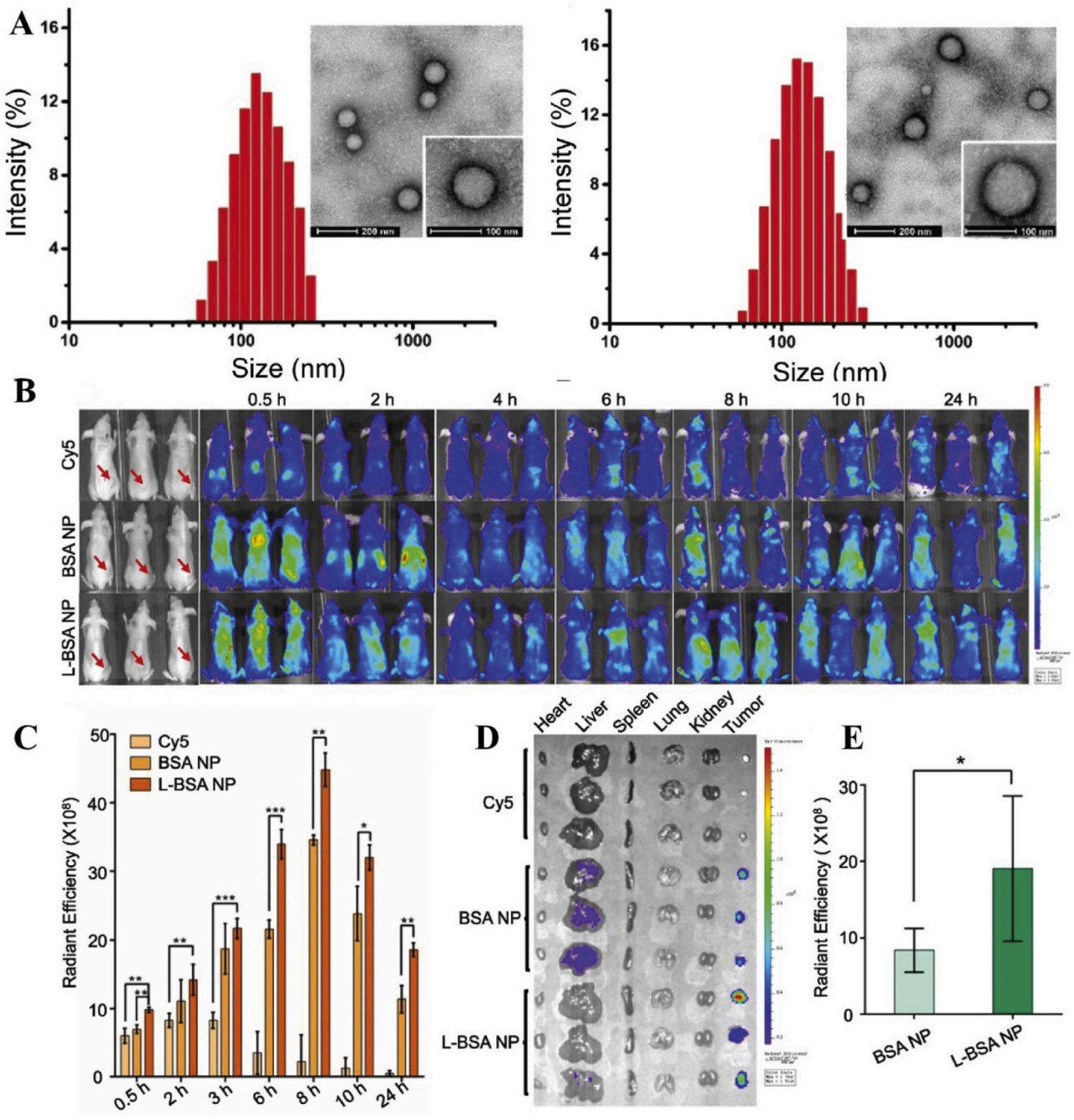

Fig. 10. Blood-brain barrier (BBB) penetrating low molecular weight protamine (LMWP) bovine serum albumin (BSA) nanoparticles (L-BSA-NPs) and BSA-NPs developed with self-assembly of LMWP and BSA along with hydrophobic drugs paclitaxel (PTX) and fenretinide(4-HPR). Characterization of the synthesized BSA-NPs and L-BSA-NPs. (A) Transmission electron micrography and the particle size distribution of the BSA-NPs and L-BSA-NPs. (B) In vivo imaging of mice bearing U87 xenograft tumours showing intertumoral penetration ability of BSA-NPs and L-BSA-NPs. Full body imaging showing biodistribution of BSA-NPs and L-BSA-NPs, with progressive rise in fluorescent intensity from 2 to 8 h, with L-BSA-NPs displaying enhanced tumor accumulation compared to BSA-NPs. (C) Quantification of the average fluorescence efficiency in vivo at the tumor site. (D) *Ex vivo* imaging of the major dissected organs, where tumor shows higher fluorescence intensity. (E) Quantification of fluorescence intensity of only tumours from D. Adapted with permission from [274]. Copyright © 2016 American Chemical Society.

proteins like Tf, lactoferrin, apolipoprotein, and melanoTf have been successfully used to target the expressed receptors on endothelial cells and improve BBB transcytosis [249,252]. Tf is a glycoprotein that interacts with TfR overexpressed on the surface of BBB through ligand-receptor recognition. Additionally, nanocarrier made from natural iron storage protein, ferritin, exhibits BBB-traversing and the glioma-targeting properties. Ferritin nanocarriers along with their modification with integrin α2β1 targeting ligand (DGEAGGDGEA) have been used to deliver doxorubicin for enhanced orthotopic glioma therapy [276].

Advances in material chemistry have suggestively augmented the polymeric nanocarriers formulations and its usage in CNS delivery. The naturally occurring polymers owing to its less toxicity, biocompatibility, targeted drug delivery, versatility, biodegradability, availability, and low cost offer great promise for improving drug delivery across the BBB. However, due to their low mechanical strength, polymeric nanocarriers have poor stability and structural integrity during circulation and drug leakage compared to synthetic polymers. Further, challenges like rapid clearance, batch to batch variability, lower drug encapsulation and potential immunogenicity should be carefully considered in the rational designed of strategies to facilitate drug transportation across the BBB for potential long-term and clinical application.

4. Clinical translation

Basic and applied neuroscience has brought to light new technologies and the discovery of new therapeutic interventions with potential to overcome some of the major CNS disorders. Furthermore, an increasing in-depth understanding and description of new molecular targets of CNS disorders is also enhancing the toolbox for the design of new therapies. Hence, both academia and industry efforts are focused on the worldwide burden of the neurological disorders in the last decades, which represent the leading cause of disability and the second leading cause of death [277]. Cases of brain cancer as well as the prevalence of CNS disorders are increasing due to the longer life-expectancy according to World Health Organization. However, some practical challenges remain in establishing novel drugs and biologicals when moving from the bench to in vivo studies or into the clinical use. Emerging approaches, such as intranasal delivery, are being implemented as a non-invasive option for the drug delivery to the CNS while reducing peripheral exposure, mostly at a basic research level. Thus, developing new strategies to reach the CNS, while limiting systemic effects is still one of the main clinical challenges due to the inherent high complexity of the BBB. Decades of neuroscience research have led to the development of biologic drugs that have given clinicians and patients renewed hope for improved treatment options for complex diseases like MS, AD, PD, or brain cancers, among others. The development and use of biomacromolecules are blossoming in the last years due to their ability to target specific pathways, which reduces undesired side effects, also allowing to personalized treatment that addresses the patient's particular condition. Although the availability of biologic treatments for CNS disorders is limited, there is no denying that the existing traditional drugs fundamentally change symptoms of these diseases but do not change the chronic onset of the disorder. Most of the available therapeutic biomacromolecules in the market are antibodies, such as LEMTRADA® (alemtuzumab), TYSABRI® (natalizumab), or OCREVUS® (ocrelizumab) for the treatment of MS, Aduhelm® (aducanumab) for AD, and Bevacizumab or Naxitamab-gqgk for brain cancer. Regardless of the pathology, clinical trials have failed for a multitude of reasons, such as severe adverse effects, minimal to no change in disease onset or poor bioavailability due to the inherent complexity of the disorders and the BBB crossing ability [278]. However, the increasing number of people affected by CNS disorders and brain tumors point out the need of new developments to tackle this global burden, especially in low and mid-income countries. Furthermore, about one third of the FDA approvals in 2022 were biologics, pushing them ahead of small molecules for the first time, with new approaches such as antibody–drug conjugates, bispecific proteins, and cell and gene therapies [279]. This trend is evidenced with more than 300 entries for "Alzheimer's Disease and enzymes", 34 entries for "multiple sclerosis and growth factors", 127 entries for "neuroblastoma and antibody", or 63 entries for "GBM and antibodies", in the website *clinicaltrials.gov* among others. When searching for trials involving "nanoparticles", the numbers decrease dramatically, being mostly focused on the use of metallic nanoparticles and liposomes (Table 5). The first liposomal based drug, Doxil, was approved by the FDA in 1995, while the first polymeric nanoparticle, Abraxane, got the approval in 2005 both using small molecules as their cargoes [280]. Two decades later, the FDA approved a nanometric carrier based on Lipid and Nucleic acids, called ONPATTRO®. In addition, PEGylation technology has been widely applied over the last 30 years to improve the pharmacokinetics and pharmacodynamics of different therapeutic modalities such as small molecules, peptides, or proteins, which leads to over 30 PEGylated drugs currently used in the clinic [281]. Furthermore, some clinical trials are focused on the use of Abraxane or Doxil on drug repurposing approaches nowadays (Table 5). Overall, it is expected that the tendency of using biomacromolecules follow up to the use of polymeric nanocarriers loading them to overcome their inherently poor physicochemical properties in the upcoming years.

5. Pitfalls and future perspectives

As explained along this review, targeted nanoparticles have shown promise to overcome the BBB. However, they also accumulate and release the cargo in off-target body sites, which often results in systemic side effects. Nasal administration was initially utilized for local drug delivery in the treatment of allergic conditions and systemic administration in the so-called transnasal route [282]. For example, calcitonin salmon nasal spray is used to treat postmenopausal osteoporosis. The transnasal delivery of other biologicals such as the human growth factor and oxytocin has been also investigated [283,284]. The transnasal route bypasses hepatic first-pass metabolism and could lead to systemic bioavailability comparable to the intravenous and the intramuscular injections. An administration strategy that capitalizes on direct transport mechanisms between the olfactory nasal epithelium and the CNS, namely the intranasal or nose-to-brain route, was introduced by Prof. W. H. Frey II in the 1990s for the delivery of peptides and other therapeutic molecules [285–288]. Major advantages are the quick onset of action, no hepatic first-pass metabolism, and patient compliance. Dauntless efforts have been devoted to unravel the molecular and cellular pathways involved in the nose-to-brain transport, which are still under scientific debate [289]. The transport is an interplay among different intracellular and extracellular mechanisms with a level of contribution that depends on the properties of the drug (in solution or as a nanoparticle) and the formulation. Overall, there is broad agreement that the olfactory region in general and the olfactory neurons in particular play a key role [290,291]. Also branches of the trigeminal nerve are relevant players as neurons from these branches connect the nasal mucosa with the olfactory bulb. In addition, therapeutic compound can access the brain via the blood vasculature and the lymphatic system. The possible role of microglia in the nose-to-brain transport of different types of nanoparticles has been recently reported [292,293]. It is worth stressing that the region of the CNS in which the nanoparticles accumulate depends on features such as size and surface chemistry [197]. Thus, a more comprehensive investigation of this pathway has to be carried out for which the development of clinically relevant in vitro and *ex vivo* models that will enable the screening of the most promising prototypes is a crucial stage [197–294].

The development of in vitro models that faithfully recapitulate the complexity of the BBB and allow a rapid screening of therapeutics has been also placed in the spotlight of many investigations. These models are believed to circumvent, or at least minimize, the need of in vivo models in the near future, being in line with the 3R principles (replace,

Table 5
Ongoing clinical trials using nanoparticles to treat CNS disorders or brain cancer.

Study Title	Phase	System	Disease	Groups	Participants	Primary Outcome	Status	Identifier
High-Field MRI Iron-Based Contrast-Enhanced Characterization of Multiple Sclerosis and Demyelinating Diseases	NA	Feraheme (ferumoxytol)	RRMS	Interventions: Drug: Feraheme Drug: Gadolinium-based contrast	NA	Number and location of enhancing brain lesions seen on 7 tesla MRI following Feraheme administration.	Withdrawn	NCT01973517
In Vivo Characterization of Inflammation With Ferumoxytol, an Ultrasmall Superparamagnetic Iron Oxide Nanoparticle, on 7 Tesla Magnetic Resonance Imaging	1	Ferumoxytol, an Ultrasmall Superparamagnetic Iron Oxide Nanoparticle	MS	Experimental: Ferumoxytol A 510 mg dose (17 mL) of ferumoxytol diluted in 50 mL of 0.9% normal saline will be intravenous	14	To determine the change in gradient-echo T2*-weighted signal in an iron-rich brain structure, the globus pallidus [Time Frame: 6 months following ferumoxytol administration] determine if ferumoxytol induces long-lasting brain signal intensity changes in HV and MS	Completed	NCT02511028
Novel Imaging Markers in SPMS	1	Ferumoxytol	SPMS	Drug: Ferumoxytol infusion Drug: Gadoteridol Diagnostic Test: MRI Brain and Cervical Spine	10	To determine a signal change on T1-weighted and 3D UTE MRI brain (and upper cervical cord) before and 96 hours (±24 hours) after ferumoxytol administration [Time Frame: 96 hours ±24 hours]	Recruiting	NCT05357833
31P-MRS Imaging to Assess the Effects of CNM-Au8 on Impaired Neuronal Redox State in Multiple Sclerosis. (REPAIR-MS)	2	CNM-Au8 (Gold Nanocrystals)	MS PD	Interventions: Drug: gold nanocrystals Experimental: 60mg CNM-Au8 60mg suspension of clean-surfaced, faceted, gold nanocrystals in 120ml of sodium bicarbonate buffered water Interventions: Drug: gold nanocrystals	NA	The change from baseline to week 12 in CNS metabolic changes, based on 31P-MRS Redox Ratio. [Time Frame: At 12 Weeks]	Recruiting Completed	NCT03993171 NCT03815916
Therapeutic Nanocatalysis to Slow Disease Progression of Amyotrophic Lateral Sclerosis (ALS) (RESCUE-ALS)	2	CNM-Au8	ALS	Drug: CNM-Au8 Drug: Placebo	45	Electromyography measures of disease progression. [Time Frame: 36 weeks]	Completed	NCT04098406
Study of APH-1105 in Patients With Mild to Moderate Alzheimer's Disease	2	APH-1105 (Alpha Secretase Modulator)	AD	Drug: APH-1105 Other: Placebo	NA	Safety: Incidence of Treatment-emergent Adverse Events [Time Frame: Baseline through 30 days post final treatment dose up to day 60] Efficacy: Cognition Change [Time Frame: Baseline - day 60] Change in Alzheimer's Disease Assessment Scale-Cog (ADAS-COG) total score from baseline to post final treatment dose.	Not yet recruiting	NCT03806478
Radiosensitization of Multiple Brain Metastases Using AGuIX Gadolinium Based Nanoparticles (NANO-RAD)	1	AGuIX® Gadolinium chelated polysiloxane based nanoparticles with Magnetic Resonance	Brain Cancer	Interventions: Drug: AGuIX Radiation: whole brain radiation therapy	15	Maximum-tolerated dose (MTD) of polysiloxane gadolinium-chelates based nanoparticles (AGuIX) given concurrently to the whole brain radiation therapy for the	Completed	NCT02820454

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Table 5 (*continued*)

Study Title	Phase	System	Disease	Groups	Participants	Primary Outcome	Status	Identifier
						treatment of multiple brain metastases [Time Frame: 18 months]		
Evaluating AGuIX® Nanoparticles in Combination With Stereotactic Radiation for Brain Metastases (NANOSTEREO)	2	AGuIX®	Brain Cancer	Drug: AGuIX 2 IV injections (100 mg/Kg/injection) at day 4 and day 8 + Strereotactic Radiation from day 8 to day 15 as per standard practice.	1	Rate of local control [Time Frame: 1 year] The primary endpoint is the rate of local control defined as the proportion of patients with a complete response, a partial response or a stable disease.	Terminated	NCT04094077
Stereotactic Brain-directed Radiation With or Without Aguix Gadolinium-Based Nanoparticles in Brain Metastases	2	AGuIX®	Brain Cancer	Radiation: Stereotactic Radiation Drug: AGuIX gadolinium-based nanoparticles Radiation: Stereotactic Radiation Other: Placebo	134	Local Recurrence [Time Frame: From enrollment to 6 months] Assessed with Response Assessment in Neuro-Oncology (RANO) - Brain Metastasis Guidelines Time to local failure on a per metastasis basis will be performed using the log-rank test.	Recruiting	NCT04899908
Radiotherapy of Multiple Brain Metastases Using AGuIX® (NANORAD2)	2	AGuIX®	Brain Cancer	Interventions: Drug: AGuIX® Radiation: Whole Brain Radiation Therapy Interventions: Radiation: Whole Brain Radiation Therapy	NA	Evaluation of brain metastases response, according to RECIST v1.1 criteria (or modified RECIST) by MRI, with MRI centralized reading	Recruiting	NCT03818386
Pembro+Chemo in Brain Mets	2	Nab-Paclitaxel	Brain Cancer	Pembrolizumab with standard of care chemotherapy treatment: Patients will receive 200mg or 400mg of Pembrolizumab (standard of care dosing at the discretion of treating physician) every three or six weeks with standard of care chemotherapy treatment (carboplatin, pemetrexed, paclitaxel, nab-paclitaxel). Interventions: Drug: Pembrolizumab Drug: Nab paclitaxel Drug: Paclitaxel Drug: Pemetrexed Drug: Carboplatin	NA	Disease control rate [Time Frame: 6 months (baseline to 6 months)] Intracranial benefit defined as stable disease, partial response, and complete response	Recruiting	NCT04964960
Lapatinib and Paclitaxel in Treating Patients With Advanced Solid Tumors	1	Abraxane	Brain Cancer	Drug: lapatinib Drug: paclitaxel albumin-stabilized nanoparticle formulation Abraxane	28	Maximum tolerated dose (MTD) of lapatinib in course 1 [Time Frame: estimated to be 12 weeks]	Completed	NCT00313599
MTX110 by Convection-Enhanced Delivery in Treating Participants With Newly-Diagnosed Diffuse Intrinsic Pontine Glioma (PNOC015)	1 and 2	MTX110 (panobinostat nanoparticle formulation)	Brain Cancer	Interventions: Drug: Panobinostat Nanoparticle Formulation MTX110 Drug: Convection-Enhanced Delivery (CED)	7	Adverse events and clinically significant laboratory abnormalities which meet Grade 3, 4, or 5 criteria according to Common Terminology Criteria for Adverse Events (CTCAE)	Completed	NCT03566199
CED of MTX110 Newly Diagnosed Diffuse Midline Gliomas	1	MTX110	Brain Cancer	Interventions: Drug: Infusate with MTX110 and gadolinium Device: Convection-Enhanced Delivery (CED)	NA	Safety of repeated convection-enhanced delivery (CED) of MTX110 will be reported by summarizing the incidence rate of adverse events observed or reported.	Recruiting	NCT04264143

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Table 5 (*continued*)

Study Title	Phase	System	Disease	Groups	Participants	Primary Outcome	Status	Identifier
						Maximum Tolerated Dose (MTD) of MTX110 [Time Frame: 14 days]		
A Study to Evaluate the Safety, Tolerability and Immunogenicity of EGFR(V)-EDV-Dox in Subjects With Recurrent GBM (CerebralEDV)	1	EGFR(V)-EDV-Dox ((V)EDVDox contains doxorubicin (Dox) within the minicells and targets EGFR through Vectibix)	Brain Cancer GBM	Interventions: Drug: EGFR(V)-EDV-Dox	NA	Safety measures will be conducted from Study Day 1 as per study schedule to safety follow-up visit 30 (+5 days) post last dose.	Unknown	NCT02766699
AGuIX Nanoparticles With Radiotherapy Plus Concomitant Temozolomide in the Treatment of Newly Diagnosed GBM (NANO-GBM)	1 and 2	AGuIX®	GBM	Experimental: AGuIX + chemoradiotherapy (radiotherapy + temozolomide) Sham Comparator: chemoradiotherapy (radiotherapy + temozolomide)	NA	The recommended dose (phase I) of AGuIX in combination with TMZ and and radiotherapy during the radio-chemotherapy period [Time Frame: during 6 weeks after the first injection of AGuIX] 6-month Progression Free Survival (PFS) rate (phase II) [Time Frame: 6 months from the start of treatment]	Recruiting	NCT04881032
Phase II Study of Combined Temozolomide and SGT-53 for Treatment of Recurrent GBM	2	SGT-53 (a complex of cationic liposome encapsulating a normal human wild type p53 DNA sequence in a plasmid backbone)	GBM	Interventions: Genetic: SGT-53 Drug: Temozolomide	1	Tumor Response [Time Frame: 6 months] The 6-month progression-free survival (PFS) was evaluated using RANO Response Criteria.	Terminated	NCT02340156
NU-0129 in Treating Patients With Recurrent GBM or Gliosarcoma Undergoing Surgery	1	NU-0129 (gold base spherical nucleic acid (SNA) nanoconjugate targeting BCL2L12)	GBM	Experimental: Treatment (NU-0129)	8	To evaluate the safety of intravenous NU-0129 in patients with recurrent GBM or GS.	Completed	NCT03020017
A Phase I Trial of Nanoliposomal CPT-11 (NL CPT-11) in Patients With Recurrent High-Grade Gliomas	1	NL CPT-11	GBM	Interventions: Drug: Nanoliposomal CPT-11	34	To assess the safety and pharmacokinetics of NL CPT-11 in patients with recurrent malignant glioma stratified based on UGT1A1 genotyping. [Time Frame: 1–2 years]	Completed	NCT00734682

(Data from: https://clinicaltrials.gov). Trials with just expected number of participants were considered as not applicable (NA). Abbreviations: MS, Multiple Sclerosis; RRMS, Relapsing Remitting Multiple Sclerosis; SPMS, Secondary progressive Multiple Sclerosis; PD, Parkinson's Disease; ALS, Amyotrophic Lateral Sclerosis; AD, Alzheimer's Disease.

reduce, refine) and facilitating the pre-clinical selection of BBB-permeable therapeutics [295]. As explained along this review, the BBB is a heterocellular barrier, in which the crosstalk between the different cell types and the characteristics of the extracellular matrix play an important role. Thus, resembling this complex scenario in vitro is challenging. Most of the studies reported in literature rely on the use of two-dimensional models. The complexity of these two-dimensional models can be further expanded by including various cell types (e.g., pericytes, astrocytes, neurons) either primary, established cell lines or derived from induced pluripotent stem cells (iPSCs) [296,297]. These models have gained popularity thanks to their simplicity and possibility to obtain preliminary results in a relatively short period of time. However, they poorly represent the morphological features of brain capillaries in terms of geometry and structural organization. Besides, the semiporous membrane is made out of rigid polymers (e.g., polycarbonate or polyethylene terephthalate) with mechanical stiffness far above the ones observed in the extracellular matrix. Therefore, researchers are trying to combine the simplicity of these two-dimensional models with more biomimetic membranes to achieve permeability values closer to the in vivo scenario [298]. The use of microfluidics has emerged as a potent tool to develop more clinically relevant BBB models. Microfluidic devices usually contain interconnected compartments that allow the coculture of different cell types in individual chambers. Moreover, these compartments can be filled with cell-laden gels, thus creating a 3-dimensonal environment that better represent the physiological cell organization [299,300]. The use of microfluidic devices, together with the establishment of improved in vitro culture protocols, the use of human iPSCs and the possibility to mimic physiological conditions (e.g., fluid shear stress, blood-like viscosity) are allowing the development of robust in vitro platforms that represent a real alternative to in vivo models. As an illustration, Park *et al.* reported a microfluidic chip that maintained human BBB function (i.e., low barrier permeability, expression of efflux pumps, high levels of TJs) for up to one week in vitro, and showed *in vivo*-like selective transcytosis of peptides and antibodies [301]. Alternative models exploit the capacity of cells to self-assemble into three-dimensional vessel architectures. For example, the coculture of human endothelial cells, astrocytes and pericytes within fibrin gels resulted in the formation of a microvascular network that shows gene expression profiles and permeability values similar to those observed in vivo [302,303].

As explained before, this review examines various polymer carriers suitable for delivering biomacromolecules to the brain. These carriers have been developed using a trial-and-error-based approach, which is currently a significant bottleneck in the development of polymeric

nanoparticles for biomedical applications. Furthermore, their translation from the lab to clinical use is limited. Some of the proposed polymers are not yet FDA-approved, and a few biodegradable polymers deemed safe for administration still present off-target issues, while scalability of the therapeutics remains challenging. Consequently, the scientific community has embraced artificial intelligence (AI)-based tools to address design challenges and predict the composition and formulation of delivery systems, improving, thereby, the targeting efficiency. Nonetheless, the use of machine learning (ML) in pharmaceutical sciences is often constrained by the limited availability of open-source datasets for training models [304]. The progress of ML in this field relies on a feedback-loop scheme, which requires substantial sets of accurately annotated data to refine computational algorithms and effectively trace nano-bio data [305]. The convergence of lab automation, robotics, and advancements in ML has given rise to "self-driving labs" (SDL), where ML-assisted modular experimental platforms guide experimental procedures to achieve user-defined objectives [306]. SDL facilitates the collection of a comprehensive database covering all the chemical design spaces that need to be experimentally explored, thereby accelerating the discovery of materials or compounds [307–311]. Due to the multiple variables that impact the successful delivery of macromolecules to the brain, SDL applied to pharmaceutics appears to be a promising approach for understanding the fundamental aspects of efficient carrier design. Some progress has been made in the development of SDL for pharmaceutics, particularly in the design of therapeutic agents, such as drug delivery formulation, protein encapsulation, and polymer synthesis [312–314].

The use of ML and AI has been as well expanded to cover fields related to diagnosis of CNS diseases, re-purposing of drugs, and prognosis of efficacy of treatments. A field, in which ML and AI have already reached the clinical practice, is that of diagnosis, where accurate and rapid results are desired. This can be often challenging in CNS diseases, in which radiology and histology are not always conclusive and big amount of data should be analyzed to extract quantitative information [315]. ML and AI methods are currently being used to aid clinicians to analyze the data, to make informed decisions during surgery, and to predict the outcome and progression of the diseases and therapeutic interventions [316]. ML and AI also hold significant promise for predicting the biodistribution of nanomedicines. For instance, *in silico* methods have been used to correlate the capacity of a series of compounds to cross the BBB in spheroid cells [317]. These predictive tools emphasize the potential of identifying critical chemical parameters that should guide the design of new drugs. Moreover, modelling approaches traditionally applied to predict the pharmacokinetic of small-molecule drugs, such as computational fluid dynamics (CFD) [318] and physiologically based pharmacokinetic (PBPK) [319], could be exploited to envisage the therapeutic capacity of biomacromolecules making use of the tremendous potential of ML and AI.

Taken together, ML and AI are called to revolutionize the current standard of care of CNS diseases, improving the early diagnosis and efficacy of the personalized medicine. It is expected that in the future these tools will improve accuracy of the medical practice, reduce diagnosis and decision times, decrease the hospitals' workload, and improve the patient care [320].

CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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