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REVIEW

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Exosome-powered neuropharmaceutics: unlocking the blood-brain barrier for next-gen therapies

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Abstract

Background The blood-brain barrier (BBB) presents a formidable challenge in neuropharmacology, limiting the delivery of therapeutic agents to the brain. Exosomes, nature's nanocarriers, have emerged as a promising solution due to their biocompatibility, low immunogenicity, and innate ability to traverse the BBB. A thorough examination of BBB anatomy and physiology reveals the complexities of neurological drug delivery and underscores the limitations of conventional methods.

Main body This review explores the potential of exosome-powered neuropharmaceutics, highlighting their structural and functional properties, biogenesis, and mechanisms of release. Their intrinsic advantages in drug delivery, including enhanced stability and efficient cellular uptake, are discussed in detail. Exosomes naturally overcome BBB barriers through specific translocation mechanisms, making them a compelling vehicle for targeted brain therapies. Advances in engineering strategies, such as genetic and biochemical modifications, drug loading techniques, and specificity enhancement, further bolster their therapeutic potential. Exosome-based approaches hold immense promise for treating a spectrum of neurological disorders, including Alzheimer's, Parkinson's, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), brain tumors, stroke, and psychiatric conditions.

Conclusion By leveraging their innate properties and engineering innovations, exosomes offer a versatile platform for precision neurotherapeutics. Despite their promise, challenges remain in clinical translation, including large-scale production, standardization, and regulatory considerations. Future research directions in exosome nanobiotechnology aim to refine these therapeutic strategies, unlocking new avenues for treating neurological diseases. This review underscores the transformative impact of exosome-based drug delivery, paving the way for next-generation therapies that can effectively penetrate the BBB and revolutionize neuropharmacology.

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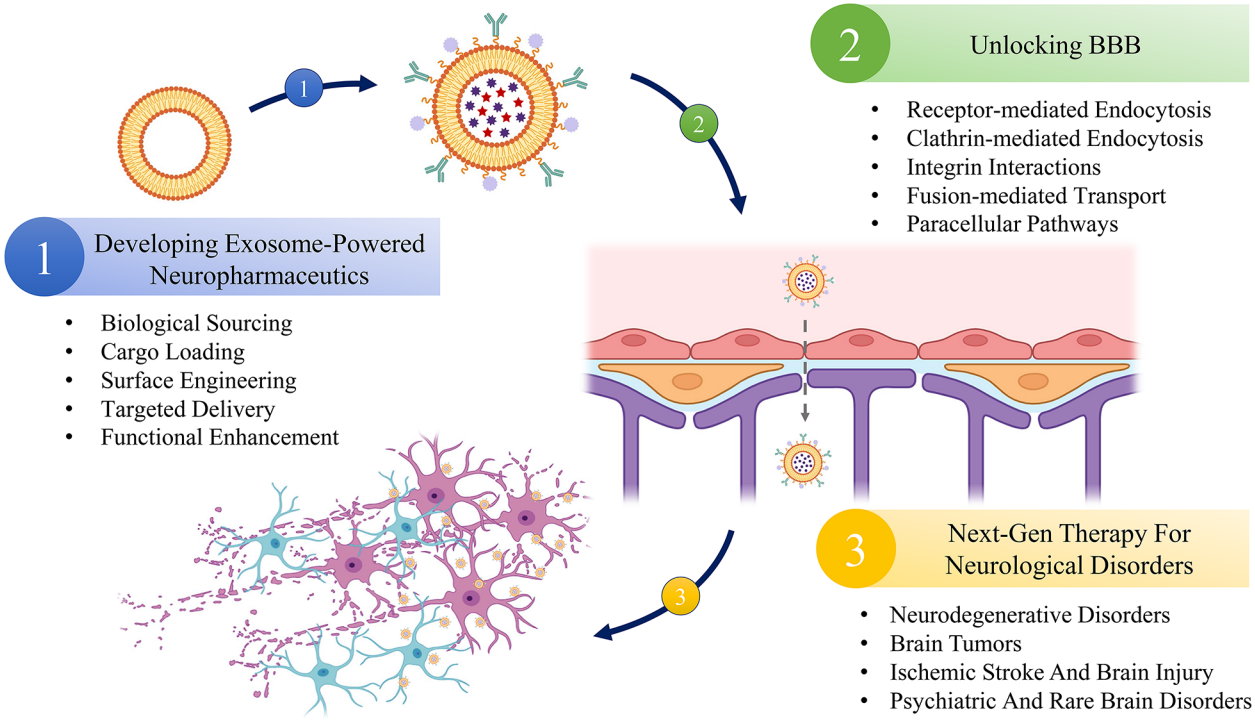
Graphical abstract



Keywords Exosome-mediated drug delivery, Targeted drug delivery, Blood-brain barrier, Neurodegenerative diseases, Brain cancer, Stroke, Psychiatric disorders

Introduction

Nanobiotechnology has revolutionized medicine, particularly in drug delivery systems. Among the latest advancements, exosome-mediated drug delivery has emerged as a promising strategy for treating neurological disorders. Exosomes are nanometer-scale extracellular vesicles secreted by virtually all cell types, playing key roles in intercellular communication by transporting biomolecules such as proteins, lipids, and nucleic acids. Given their natural ability to traverse biological barriers, exosomes are now being explored as vehicles for delivering therapeutics across the formidable Blood-Brain Barrier (BBB), a challenge that has long hindered effective neuropharmaceutical interventions [1].

Neuropharmaceuticals refer to therapeutic agents specifically designed to diagnose, prevent, or treat neurological disorders, including neurodegenerative diseases, psychiatric conditions, and brain tumors. These compounds encompass a broad range of drugs, from small molecules and peptides to biologics such as monoclonal antibodies, gene therapies, and RNA-based treatments. Despite their therapeutic potential, the successful delivery of neuropharmaceuticals to the central nervous system (CNS) remains a significant obstacle due to the BBB's highly selective nature [2]. Exosome-based drug delivery represents a transformative approach to overcoming this limitation, enabling precise, non-invasive, and effective transport of neuropharmaceutical agents into the brain [3].

The rationale for using exosomes in neuropharmaceutical applications lies in their unparalleled biocompatibility, stability, and immune-evasive properties. Unlike synthetic nanoparticles, exosomes are inherently non-immunogenic, allowing for prolonged circulation without triggering adverse immune responses. This is particularly crucial for CNS-targeted therapies, as traditional drug delivery strategies often fail due to rapid clearance, limited penetration, or off-target effects. The endogenous nature of exosomes makes them uniquely suited for crossing the BBB while preserving its structural integrity, thereby minimizing potential toxicity and inflammatory responses [4].

The BBB represents the most significant impediment to effective neurological treatment. This protective structure consists of endothelial cells joined by tight junctions, preventing most therapeutic agents from accessing the brain parenchyma [5, 6]. While various strategies have been developed to enhance BBB permeability—including

invasive techniques, chemical modifications, and synthetic nanocarriers—these methods often present limitations such as inefficacy, cytotoxicity, or disruption of normal brain function [7]. In contrast, exosomes, particularly those derived from neural or immune cells, exhibit intrinsic brain-targeting capabilities, leveraging receptor-mediated interactions to facilitate transcytosis across the BBB [8].

Recent research has highlighted the potential of exosomes as versatile carriers for neuropharmaceuticals, delivering drugs, RNA-based therapies, and CRISPR-Cas systems directly to the CNS [9]. Their lipid bilayer structure, enriched with specific surface proteins, enables dynamic interaction with cellular receptors, promoting endocytosis and transcytosis across the BBB. Preclinical studies have demonstrated the therapeutic efficacy of exosome-mediated drug delivery in neurodegenerative diseases such as Alzheimer's and Parkinson's, where conventional treatments struggle to achieve sufficient brain penetration. Exosomes not only enable targeted drug delivery but also protect encapsulated therapeutics from enzymatic degradation, enhancing bioavailability and therapeutic impact [10].

Beyond neurodegeneration, exosome-based strategies hold promise for treating brain tumors, psychiatric disorders, and traumatic brain injuries [11]. Functionalized exosomes can be engineered with surface ligands, antibodies, or peptides to enhance specificity and efficacy, thereby reducing off-target effects. For example, in glioblastoma models, engineered exosomes have successfully delivered chemotherapeutic agents with improved tumor targeting while sparing healthy tissues [12]. Similarly, exosome-mediated delivery of small molecules offers new avenues for modulating neural circuits in psychiatric conditions such as depression and schizophrenia, where conventional drugs often fall short due to poor BBB penetration and systemic side effects [13, 14].

The objective of this review is to provide a comprehensive and analytical exploration of exosome-powered neuropharmaceuticals, emphasizing their potential to revolutionize CNS drug delivery. By synthesizing the latest advancements in exosome research, nanobiotechnology, and neuropharmaceutical engineering, this paper aims to elucidate the mechanisms, applications, and challenges of leveraging exosomes to address the BBB challenge. Additionally, emerging trends, including artificial intelligence (AI)-driven optimization of exosome engineering and multimodal therapeutic approaches, will be discussed. Ultimately, this review aspires to establish a foundational framework for advancing exosome-based neuropharmaceuticals from experimental research to clinical application, redefining drug delivery paradigms for neurological health.

Exosomes: Nature's nanocarriers

Exosomes are thereby the key players in intercellular communication and, at the same time, also bear fundamental knowledge about increasing application in nanobiotechnology and therapeutic treatment. Thereby, they uniquely position due to structural and functional properties, in addition to breakthroughs achieved recently during engineering and applications, laying a platform for them among biomedical sciences today, mostly regarding the unmet healthcare challenges like neurological disorders, hard to be treated to date.

Cellular messengers: structure and function of exosomes

The size of exosomes, another name for extracellular vesicles (EVs), usually ranges between 30 and 150 nm in diameter; they are bound by a lipid bilayer. Their secretion occurs in almost every type of cell, transporting biomolecules such as proteins, lipids, and even nucleic acids like mRNA, miRNA, and long non-coding RNAs (Fig. 1). It is with the help of these cargo molecules that intercellular targeting is achieved in communication between two or more different types of cells [15, 16].

Their protective lipid bilayer protects the cargo from enzymatic degradation and confers stability to exosomes in biological fluids such as blood, cerebrospinal fluid, and saliva. The embedded membrane tetraspanins, for example, CD9, CD63, and CD81, integrins, and heat shock proteins play a critical role in determining exosome-cell interactions and selective uptake via receptor-mediated endocytosis [17]. Table 1 summarize the various proteins involved in exosome biology and functions. Several new exosomal biomarkers associated with disease states have been also identified by emerging research, thus enabling their use in diagnostics and prognostics [18].

Exosomes are enriched with a diverse array of molecular components that play vital roles in intercellular communication and physiological processes. Their membranes contain key proteins, including tetraspanins (CD9, CD63, CD81), glycoproteins, adhesion molecules, antigen-presenting molecules, and cell-specific receptors. Additionally, exosomal membranes incorporate transmembrane proteins, internal membrane transport proteins, fusion proteins, and heat shock proteins (HSPs). Within their lumen, exosomes house cytoskeletal proteins, ESCRT complexes, growth factors, and cytokines. Their lipid composition, essential for structural integrity and stability, includes cholesterol, ceramides, sphingomyelin, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), and gangliosides (GM). Furthermore, exosomes carry various nucleic acids, including DNA, mRNA, microRNA (miRNA), non-coding RNA (ncRNA), and other RNA species, contributing to their critical roles in gene regulation and cellular signaling.

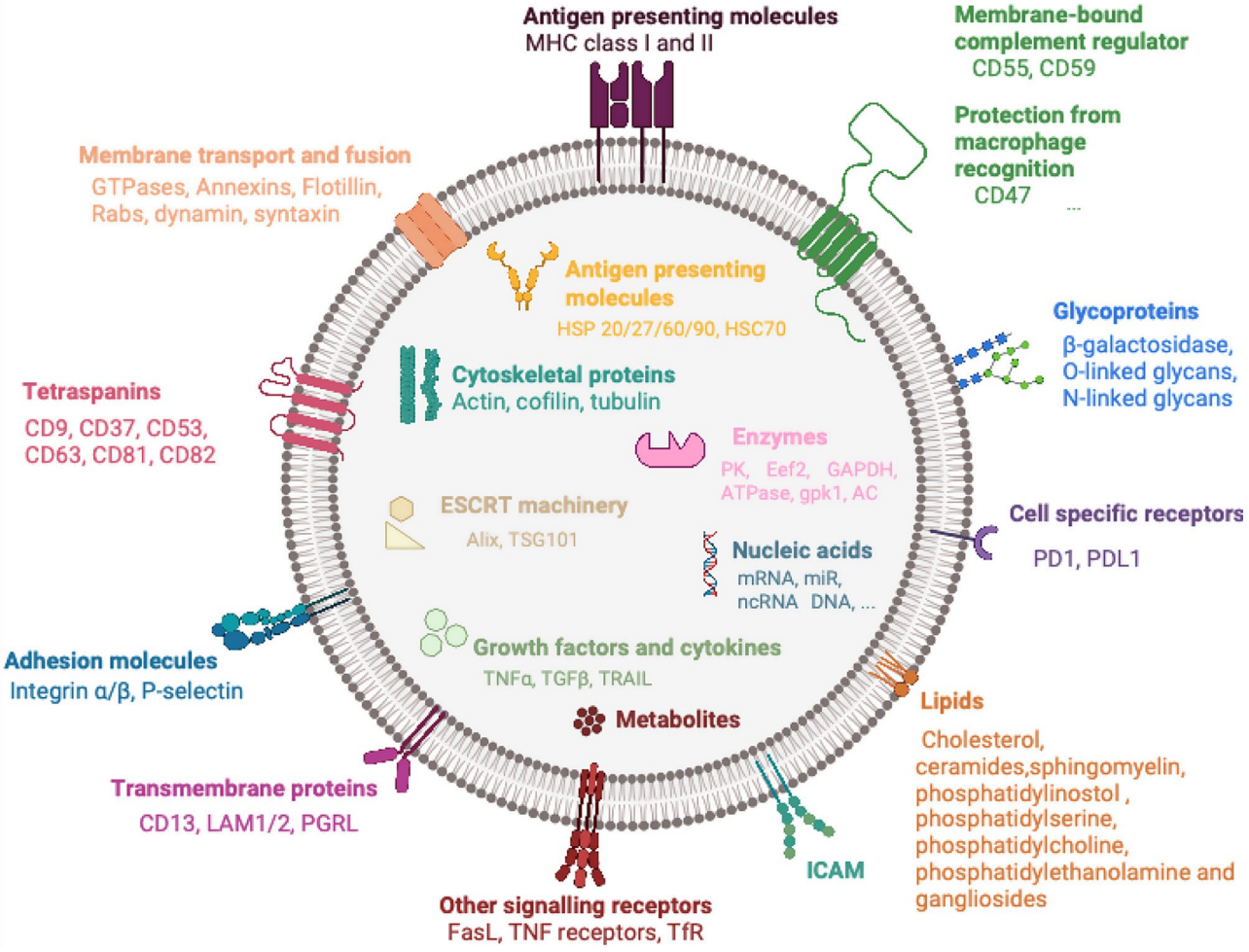


Fig. 1 Comprehensive Composition of Exosomes

Biogenesis and release mechanisms

The process of exosome formation is tightly regulated and occurs in the endosomal compartment. Multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs) are generated by inward budding of the endosomal membrane. When MVBs fuse with the plasma membrane, the ILVs are released as exosomes into the extracellular space [19].

Recent studies have established a refined view of the molecular mechanisms regulating exosome biogenesis. The coordinated action of the Endosomal Sorting Complex Required for Transport (ESCRT) complex, together with ESCRT-independent pathways, including ceramide and tetraspanins, ensures cargo sorting and vesicle formation. Rab GTPases-Rab27a and Rab35, in particular-tune MVB docking and fusion at the plasma membrane, allowing spatiotemporal regulation of exosome release [20]. Figure 2 represent the exosome biogenesis and release in detials. Exosome release is highly influenced by environmental cues, such as hypoxia and oxidative stress. For example, hypoxic conditions enhance the secretion of proangiogenic and metastatic exosomes, thus linking their biogenesis to tumor progression and tissue repair [21].

Intrinsic advantages of exosomes in drug delivery

Compared with synthetic nanoparticles, exosomes have unparalleled advantages in many ways: (1) since exosomes originate from natural cellular processes, they show high biocompatibility with reduced immunogenicity. This minimizes the risk of adverse immune reactions and prolongs systemic circulation [22, 23]. (2) Exosomes are capable of crossing complex biological barriers, including the BBB. Their surface molecules, like LAMP2B and integrins, allow receptor-mediated transcytosis across endothelial cells, making them good vehicles for CNS drug delivery [24]. (3) Targeted delivery with exosomes can be achieved either by inheriting parent cell markers or through engineered surface modifications. For instance, rabies virus glycoprotein (RVG)-peptide-modified exosomes can deliver therapeutic siRNA at a higher efficiency into neuron cells than

Table 1 Proteins involved in exosome biology and their functions

Category	Examples	Role
Tetraspanins	CD9, CD63, CD81, CD82, CD53	Play crucial roles in exosome biogenesis, cargo selection, targeting, and uptake.
ESCRT Machinery/ MVB Biogenesis	Alix, TSG-101, Gag	Essential in the formation of multivesicular bodies (MVBs) and exosome biogenesis through the endosomal sorting complex required for transport (ESCRT).
Heat Shock Proteins	Hsp70, Hsp90, Hsc70, Hsp60, Hsp20, Hsp27	Involved in exosome release and signal transduction, stabilizing proteins during exosome biogenesis and secretion.
Membrane Transport and Fusion	Rab GTPases, Annexins (I, II, IV, V, VI), Dynamin, Syntaxin, AP-1, Arp2/3, SNAP	Crucial for exosome formation, trafficking, and fusion of membranes during secretion and uptake, with Rab5 and Rab7 being particularly important in sorting and release.
Antigen Presentation	MHC Class I, MHC Class II, CD86	Involved in antigen presentation for immune responses, facilitating T-cell activation.
Cytoskeletal Proteins	Actin, Vimentin, Talin, Ezrin, Tubulin, Cofilin, Moesin	Involved in maintaining vesicle integrity and facilitating exosome biogenesis and secretion.
Adhesion	P-selectin, CD146, CD166, ICAM-1, ALCAM, MAC-1, Integrin α chain, Integrins α4 ß1, LFA-3, CD53, CD326, CD11a, CD11b, CD11c, MFG-E8/lactadherin	Facilitates exosome interaction with target cells for uptake and immune modulation.
Glycoproteins	β-galactosidase, O-linked glycans, N-linked glycans	Found on the surface of exosomes, contributing to targeting and uptake by recipient cells.
Growth Factors and Cytokines	TNF-α, TGF-β, TRAIL	Involved in exosome-mediated signaling and immune modulation, impacting inflammation and tumor progression.
Signal Transduction	Erk2, Fyn, RhoA, Catenin, Syntenin, LCK	Essential for exosome-mediated signal transduction, influencing cellular processes and intracellular signaling pathways.
Enzymes	Peroxiredoxin 1, Fatty acid synthase, Pyruvate kinase, ATP Citrate lyase	Involved in exosome cargo selection and intracellular signaling processes, participating in metabolism and cell growth.
Other Signaling Receptors	FasL, TNF receptor, Transferrin receptor	Found in exosomes and contribute to immune responses and apoptotic signaling.
Anti-apoptosis	Alix, Thioredoxine peroxidase	Plays a critical role in preventing apoptosis and regulating intracellular signaling during exosome trafficking.
Lipid Rafts	Flotillin-1, Cholesterol, LBPA, Stomatin	Integral in exosome membrane composition, contributing to biogenesis, cargo sorting, and vesicle stability.
Miscellaneous	Histone 1, 2, 3, Clathrin, Ferritin light chain 1 and 2, CD18, CD147, Complement factor 3, CD55, CD59	Includes diverse proteins involved in cargo selection, exosome trafficking, immune modulation, and intracellular signaling.

nontargeting particles [25]. (4) Exosomes are functioning as carriers for a whole range of therapeutic agents-carried small molecules, proteins, and nucleic acid. The recent development indeed extends its application to their use as transporters with genome-editing tools at the CRISPR-Cas9 component level of resolution [26]. (5) Encapsulation within the lipid bilayer secures exosomal cargo from metabolic degradation and confers, in turn, increased robustness that can enhance its salutary effect [27]. (6) Exosomes inherit the tropism of their parental cells, which means they target active delivery to specific tissues or organs. This attribute, in particular, is valuable in cancer therapy and regenerative medicine [28]. (7) Large-scale production of exosomes was made possible with advances in biomanufacturing, featuring high purity and yield. Ultracentrifugation, tangential flow filtration, and size-exclusion chromatography are some of the techniques that have helped in making these processes more clinical for scalability [29].

Navigating the BBB

Anatomy and physiology of the BBB

The BBB is a dynamic and specialized vascular interface that plays a pivotal role in maintaining the homeostasis of the CNS. Acting as a gatekeeper between the systemic circulation and the neuronal milieu, the BBB meticulously regulates the exchange of nutrients, ions, and metabolites while preventing the entry of harmful substances and pathogens. Advances in molecular biology and neuroimaging have provided transformative insights into the structural and functional dynamics of the BBB, underscoring its significance in health and disease.

The BBB is a complex structure formed by specialized endothelial cells, pericytes, and astrocytic end-feet, collectively known as the neurovascular unit (NVU) (Fig. 3A). The endothelial cells of the cerebral microvasculature form the primary structural component of the BBB. These cells are characterized by the presence of tight junctions (TJs), which are composed of proteins such as claudins, occludin, and zonula occludens-1

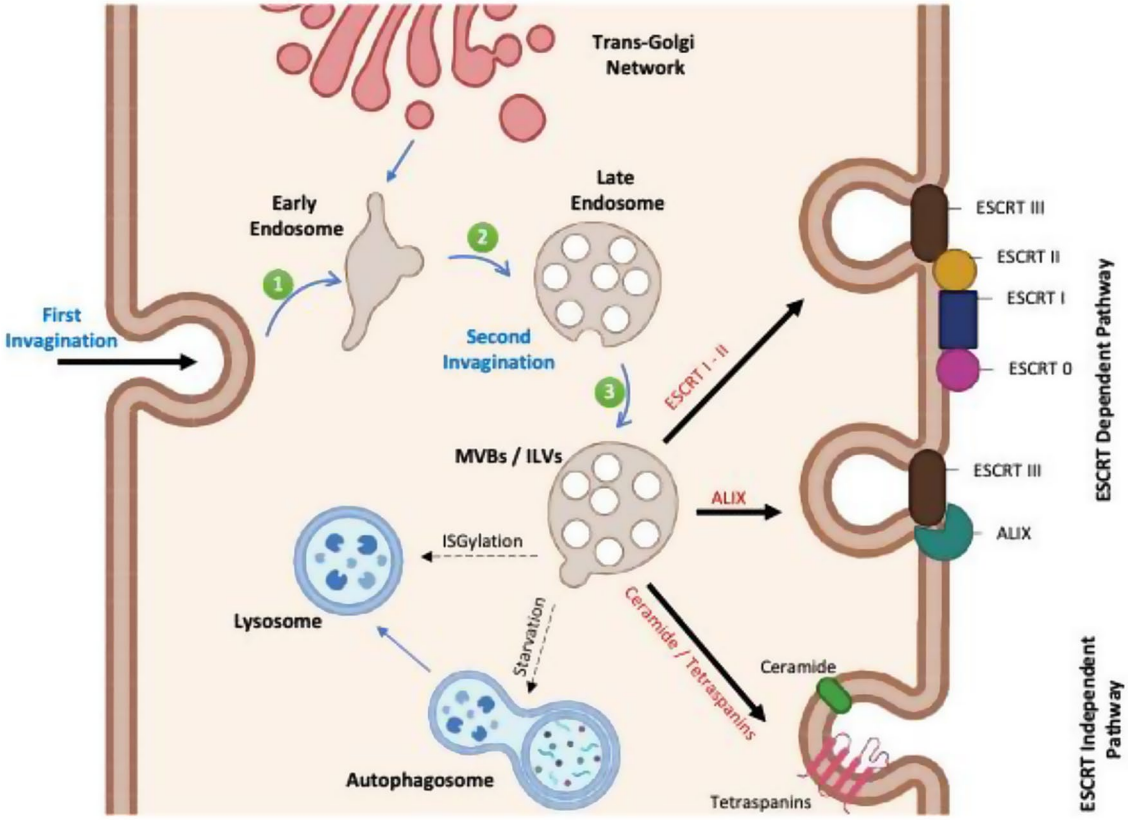


Fig. 2 Diagrammatic representation of exosome biogenesis and release. The four primary phases in exosome biogenesis—cargo sorting, maturation, and production of MVBs, MVB transport, and MVB fusion with the plasma membrane—are depicted in the image. The figure also labels the various molecules and mechanisms involved in each step. There are three possible ways that exosomes can be formed from endosomes, depending on the molecules and mechanisms involved. These are: ceramide-tetraspanin way, ALIX way, and ESCRT1-2 way. The figure also shows that besides these pathways, exosomes can become lysosomes or autolysosomes. Numerous exosomal surface proteins interact with cellular receptors to enable exosomes to adhere to recipient cells. Exosomes can cause a variety of effects after this adherence: (1) By initiating the transduction of signals through intracellular signaling pathways and subsequent release (referred to as juxtacrine signaling). (2) By merging with the cellular membrane, enabling the transfer of both proteins and genetic contents into the recipient cell's cytoplasm (fusion). (3) By being internalized through mechanisms such as receptor-mediated endocytosis, macropinocytosis or phagocytosis

(ZO-1) (Fig. 3B). These proteins work together to restrict paracellular permeability, ensuring the selective transport of substances. Recent studies have highlighted the role of Wnt/β-catenin signaling in maintaining endothelial TJ integrity, as well as the regulatory influence of shear stress on endothelial cell function and tight junction assembly [30]. Additionally, endothelial cells possess specialized transport mechanisms, including solute carriers and efflux pumps like P-glycoprotein, which prevent the accumulation of xenobiotics and maintain brain homeostasis [31]. Pericytes are embedded within the basement membrane and make extensive physical contact with endothelial cells. These cells play a critical role in regulating BBB permeability, angiogenesis, and the immune response. Pericytes communicate with endothelial cells through gap junctions and signaling molecules, such as transforming growth factor-beta (TGF-β) and platelet-derived growth factor (PDGF) [32]. In pathological conditions, such as stroke and neurodegenerative diseases, the loss of pericytes disrupts BBB integrity, leading to increased vascular permeability and inflammation [33]. Astrocytic end-feet ensheath the endothelial cells, playing a vital role in the induction and maintenance of the BBB. Astrocytes secrete a variety of factors, including glial-derived neurotrophic factor (GDNF) and sonic hedgehog (SHH), which promote endothelial barrier properties. They also regulate water and ion homeostasis via the expression of aquaporin-4 (AQP4) channels [30]. Recent advances in three-dimensional (3D) modeling have demonstrated the synergistic interactions between astrocytes, pericytes, and endothelial cells in maintaining BBB function, revealing their coordinated response to injury or inflammation [34]. The basement membrane is a specialized Extracellular matrix (ECM) that provides structural and biochemical support to the BBB. It is composed of laminins, collagen IV, and heparan sulfate proteoglycans, which contribute to cellular adhesion and signaling. Studies indicate that pericytes and astrocytes

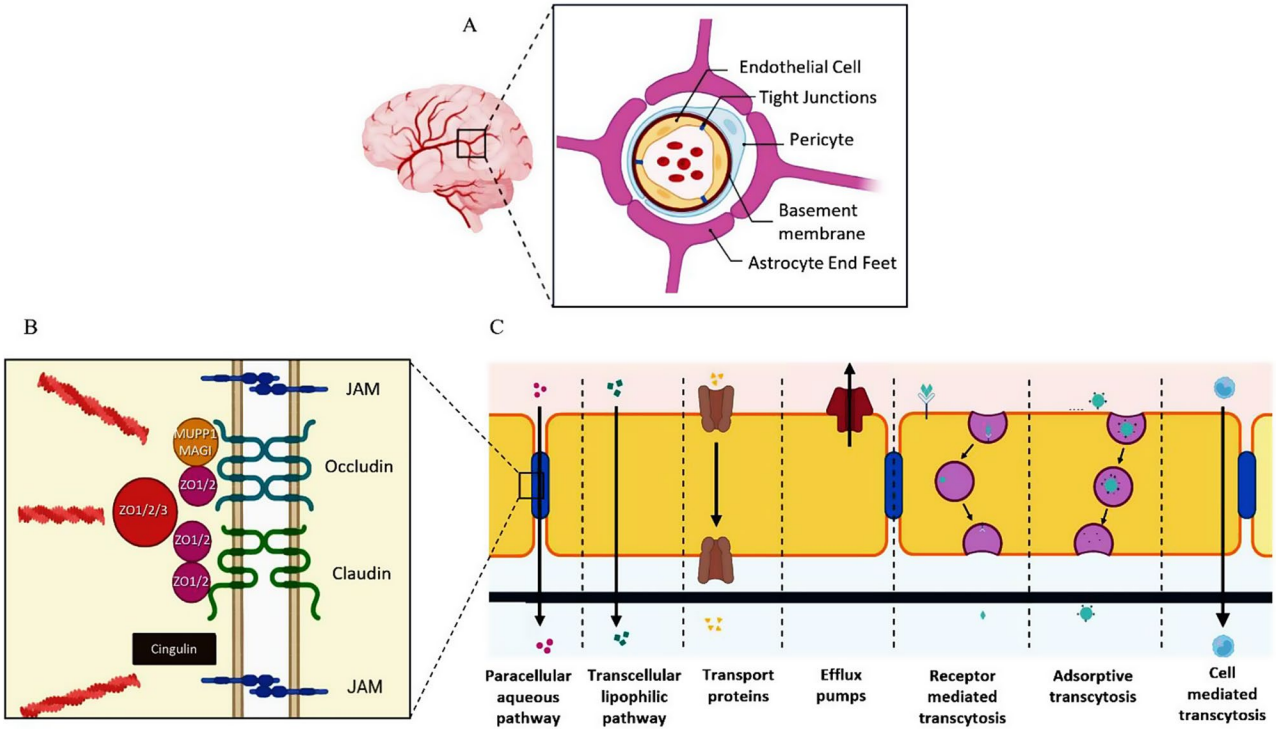


Fig. 3 BBB structure and mechanisms for BBB crossing. (**A**) Schematic diagram of BBB structure. (**B**) Brain microvascular endothelial cells of the BBB are characterized by the presence of tight junctions (TJs) composed of proteins such as claudin, occludin, and zonula occludens-1 (ZO-1). (**C**) Different mechanisms for BBB crossing

regulate the composition and morphology of the ECM, ensuring the stability of the neurovascular unit [35]. Damage to the basement membrane has been linked to age-related BBB dysfunction, emphasizing its critical role in barrier integrity [36].

The BBB plays a critical role in maintaining the homeostasis of the CNS by selectively regulating the passage of molecules and cells. Its selective permeability facilitates the transport of essential nutrients, such as glucose via glucose transporters (GLUT1) and amino acids, while effectively excluding potentially neurotoxic substances. This ensures an optimal microenvironment for neuronal function and activity. As a neuroprotective barrier, the BBB prevents the entry of pathogens, toxins, and peripheral immune cells into the CNS, thus safeguarding against infections and inflammation. Additionally, the metabolic barrier function of the BBB involves enzymatic systems within brain microvascular endothelial cells (BMECs) that degrade neuroactive compounds and xenobiotics, preventing their accumulation in the brain. The BBB also acts as a signaling interface, dynamically interacting with systemic and neuronal signals to regulate its permeability in response to various physiological and pathological stimuli. This adaptability is critical for responding to changes in the body's metabolic state and protecting the CNS under stress conditions. Recent studies continue to enhance our understanding of these functions, highlighting the complexity and significance of the BBB in both health and disease.

Mechanisms for BBB crossing

The BBB is a highly selective semipermeable border of endothelial cells that protects the brain from potentially harmful substances in the bloodstream while regulating the transport of essential nutrients and molecules. Despite its critical role in maintaining CNS homeostasis, the BBB poses a significant challenge for delivering therapeutic agents to the brain. Recent advancements in nanobiotechnology have illuminated various mechanisms through which substances can traverse the BBB, facilitating innovative approaches to drug delivery (Fig. 3C).

Among these mechanisms, paracellular transport, which occurs between adjacent endothelial cells, is tightly regulated by proteins such as claudins, occludins, and junctional adhesion molecules (JAMs). Normally, this route is impermeable due to tight junctions, but hyperosmotic solutions like mannitol can temporarily shrink endothelial cells and create transient openings. This strategy enables drug delivery but carries risks of nonspecific permeability and neurotoxicity [37]. This underscores the need for precise and localized application.

Another approach, transcellular lipophilic diffusion, allows small, lipophilic molecules to diffuse across endothelial cell membranes. The efficiency of this mechanism

depends on a compound's lipophilicity and molecular weight. For instance, the design of lipophilic prodrugs and molecular docking simulations has been instrumental in optimizing drug permeability [38]. However, challenges such as off-target effects necessitate careful design.

Carrier-mediated transport (CMT) involves endogenous transport proteins like GLUT1 and amino acid transporters. These systems are hijacked by therapeutic agents conjugated with molecules recognized by these carriers. For example, GLUT1-targeted nanoparticles have been effective in delivering antineoplastic agents to glioblastomas, demonstrating the potential of this strategy [39]. Advances in synthetic biology continue to enhance carrier specificity and affinity.

Similarly, receptor-mediated transcytosis (RMT) uses specific receptors on BBB endothelial cells to facilitate transport. Ligands such as transferrin, insulin, and low-density lipoprotein are commonly exploited in this context. Nanocarrier systems, functionalized with ligands or antibodies, have achieved effective BBB crossing, as seen in transferrin-conjugated nanoparticles [40]. Innovations like bispecific antibodies further improve targeting efficiency, exemplifying the synergy between molecular engineering and targeted delivery.

Electrostatic interactions also play a role in adsorptive-mediated transcytosis (AMT), where cationic molecules bind to the negatively charged surface of endothelial cells. Nanoparticles coated with polyethyleneimine (PEI) leverage this mechanism but require careful optimization to mitigate cytotoxicity and non-specific uptake [41]. These strategies illustrate the balance between efficacy and safety in AMT-based designs.

Beyond molecular interactions, cellular-based transport strategies employ macrophages, stem cells, and leukocytes as "Trojan horses" to deliver therapeutic agents. These cells naturally traverse the BBB, providing a platform for nanoparticle or gene-editing tool delivery. Such approaches have shown promise in neurodegenerative disease treatment [42]. Cellular vehicles thus offer immunomodulation advantages alongside targeted drug delivery.

Nanoparticles and exosomes represent versatile tools for BBB penetration due to their customizable properties. Exosomes, which are naturally occurring extracellular vesicles, exploit endogenous communication pathways to cross the BBB. Functionalizing exosomes with targeting peptides or antibodies enhance their delivery efficiency. Similarly, nanoparticles such as dendrimers and lipid-based carriers have been tailored to exploit multiple BBB-crossing mechanisms [43]. These innovations highlight the adaptability of nanotechnology.

In addition to these strategies, focused ultrasound (FUS) combined with microbubbles offers a non-invasive means to transiently disrupt the BBB. The cavitation effect of microbubbles under ultrasound stimulation creates temporary openings in tight junctions, enabling drug delivery without significant endothelial damage. Clinical trials using FUS for glioblastoma treatment have shown promising results [44]. Combining FUS with nanocarriers further enhances the precision and efficacy of this technique.

Emerging research has also highlighted endogenous mechanisms such as extracellular vesicles and peptide shuttles. Vesicles secreted by brain endothelial cells can encapsulate therapeutic agents, while peptides derived from natural ligands like apolipoproteins mimic receptor interactions to facilitate transport [45]. These approaches offer a biomimetic solution, minimizing immune responses while enhancing delivery efficiency.

Finally, pathological conditions like inflammation, tumors, and infections can be exploited to enhance drug delivery. For example, inflammatory mediators increase BBB permeability, allowing the delivery of larger molecules. Glioblastomas, with their leaky vasculature, permit selective targeting through the enhanced permeability and retention (EPR) effect [46]. Such strategies underscore the potential of leveraging pathological states to overcome BBB challenges.

Challenges in neurological drug delivery

The highly selective nature of the BBB poses formidable challenges for the delivery of therapeutic agents to the CNS. Although essential for neuroprotection, the barrier restricts the entry of many therapeutic molecules, including hydrophilic drugs and large biomolecules, thereby limiting treatment options for neurological disorders [47]. Drug physicochemical properties such as lipophilicity and molecular size play critical roles in determining BBB permeability, yet many advanced therapies, including proteins and gene-editing tools, fail to achieve therapeutic concentrations in the brain [48]. Efflux transporters such as P-gp further complicate drug delivery by actively expelling many molecules back into circulation, significantly reducing their bioavailability. While protective against toxins, these mechanisms represent a substantial bottleneck for CNS drug development [49].

Neurological diseases themselves can alter the BBB, either exacerbating or complicating these challenges. Conditions like Alzheimer's disease (AD), Parkinson's disease (PD), and brain tumors are associated with structural and functional changes in the BBB. These alterations are often stage-dependent; for example, increased permeability in early-stage AD may allow the entry of therapeutic agents, but at the cost of homeostatic disruption, while advanced stages may see tighter barrier regulation, complicating drug delivery further [50].

Traditional drug delivery routes, such as intravenous administration, are inherently limited in their ability

to effectively target the CNS. Systemic delivery results in widespread distribution of therapeutic agents, often diluting their concentration at the brain while increasing the likelihood of off-target effects. This inefficiency significantly hampers therapeutic efficacy within the CNS. Intranasal drug delivery, which exploits direct anatomical pathways through the olfactory and trigeminal nerves to bypass the BBB, has emerged as a promising alternative. However, it faces persistent challenges, including limited efficiency, restricted payload capacity, and variability in patient response, highlighting the need for continued optimization [51].

Emerging technologies such as nanoparticles and liposomal systems offer innovative approaches to overcome the restrictive nature of the BBB. Nanoparticles, capable of encapsulating drugs, shield therapeutic agents from systemic degradation and enhance permeability across the BBB. Similarly, liposomes, which mimic cell membranes, have shown considerable promise in transporting hydrophilic drugs into the brain. Despite these advantages, both systems face significant challenges, including instability, scalability issues for clinical use, and achieving specificity to target diseased brain regions without off-target effects [52].

Biocompatibility and immunogenicity are critical obstacles for advanced drug delivery systems. Engineered carriers, such as nanoparticles and liposomes, are frequently identified by the immune system as foreign entities, leading to rapid clearance and inflammatory responses that reduce their efficacy. Efforts to mitigate immune recognition include surface modifications, such as polyethylene glycol (PEG) coating, which can create a steric shield to evade immune detection. However, such modifications may also compromise the ability of these carriers to penetrate the BBB effectively, presenting a trade-off that necessitates careful optimization [53].

Despite promising preclinical results, translating these innovative drug delivery methods into clinical practice remains a formidable challenge. Regulatory frameworks for nanomedicine are still evolving and often require comprehensive safety and efficacy evaluations, delaying clinical application. Manufacturing these advanced drug carriers at a scale suitable for widespread clinical use introduces additional hurdles, including maintaining stability and ensuring quality control. Furthermore, transitioning from laboratory research to clinical application requires the demonstration of both therapeutic efficacy and long-term safety, along with cost-effectiveness, posing further complexities in the development pipeline [54].

Why exosomes?? A natural key to the BBB

The BBB is a highly selective and tightly regulated structure that effectively limits the passage of most therapeutic agents into the CNS. While this impermeability is essential for protecting the CNS, it presents significant challenges in the treatment of neurological diseases. Exosomes have emerged as a promising solution for overcoming the BBB due to their unique biological properties and inherent compatibility with physiological systems. Exosomes possess several intrinsic advantages that make them effective in traversing the BBB. These nanosized vesicles are naturally secreted by various cell types, including neurons, glial cells, and endothelial cells of the BBB. Their endogenous origin allows exosomes to interact seamlessly with brain endothelial cells and other CNS components, eliciting minimal immune responses [55]. Additionally, the exosomal membrane is enriched with specialized surface proteins and lipids, such as tetraspanins and integrins, which facilitate interactions with receptors on BBB endothelial cells. These receptor-mediated processes, including endocytosis and transcytosis, enable efficient transport of exosomes across the BBB [56]. Surface modification of exosomes further enhances their ability to penetrate the BBB. For instance, Alvarez-Erviti et al. demonstrated that exosomes loaded with siRNA could effectively cross the BBB and deliver therapeutic cargo to neurons, validating their potential as targeted drug delivery systems for CNS disorders [57].

The biocompatibility of exosomes, derived from their endogenous origin, represents a critical advantage over synthetic nanoparticles. Unlike synthetic carriers, exosomes exhibit minimal immunogenicity, making them suitable for repeated administration. This characteristic is particularly valuable in the management of chronic neurological diseases such as AD and PD, which require sustained therapeutic intervention [58]. Moreover, exosomes are capable of evading phagocytosis and clearance by the reticuloendothelial system, thereby prolonging their circulation time and enhancing their efficacy as drug carriers [59].

Recent advancements in bioengineering have enabled the customization of exosomes to optimize their BBB penetration and therapeutic potential. Functionalization of the exosomal surface, such as the incorporation of brain-targeting ligands like transferrin or lactoferrin, significantly enhances their specificity and efficiency in BBB transport [60]. Additionally, genetic or biochemical modifications of donor cells can yield exosomes with tailored surface markers, improving their interaction with BBB endothelial cells and reducing off-target effects.

Exosomes' ability to carry diverse therapeutic payloads, including small molecules, proteins, RNA, and CRISPR/Cas9 components, sets them apart from traditional delivery systems. Their structure protects therapeutic cargo from enzymatic degradation in the bloodstream, ensuring that the payload reaches its CNS target intact [61]. In brain cancer therapy, for instance, exosomes have been

employed to deliver chemotherapeutic agents across the BBB, achieving precise targeting of glioblastoma cells while sparing healthy tissue. This precision minimizes systemic toxicity and maximizes therapeutic efficacy [56].

Mechanisms of exosome translocation across the BBB

The transport of exosomes across the BBB leverages their unique biological properties and the specialized structure of the endothelial interface. Several distinct mechanisms facilitate this process, including receptor-mediated endocytosis, clathrin-mediated endocytosis, integrin interactions, fusion-mediated transport, and paracellular pathways (Fig. 4). Understanding these pathways in detail is critical for optimizing exosome-based drug delivery systems for neurological disorders [62].

Receptor-mediated endocytosis plays a crucial role in exosome uptake at the BBB. Exosomal ligands such as integrins, tetraspanins, and heat shock proteins (HSPs) bind to specific receptors on endothelial cells, triggering vesicular internalization. The transferrin receptor (TfR) is one of the most widely studied receptors in this context, facilitating targeted exosome transport into the CNS [63]. Studies have demonstrated that surface modification of exosomes with TfR-binding peptides enhances their BBB permeability, significantly improving brain delivery of therapeutic cargo [60]. In addition to TfR, other receptors such as low-density lipoprotein receptor (LDLR) and insulin receptor (INSR) have been identified as potential mediators of exosome internalization. Studies suggest that exosome surface engineering with LDLR-binding ligands can further enhance BBB penetration, offering alternative pathways for brain-targeted therapies [64]. Furthermore, nanoparticle-functionalized exosomes have been developed to improve receptor-targeting efficiency, reducing off-target effects and enhancing CNS delivery [65]. The efficiency of receptor-mediated transcytosis also depends on ligand-receptor affinity and receptor recycling mechanisms, making the selection of optimal targeting moieties essential for successful therapeutic applications [66].

Clathrin-mediated endocytosis (CME) is a major pathway facilitating exosome transcytosis across the BBB. This process relies on the formation of clathrin-coated pits that selectively engulf exosomes, allowing their intracellular trafficking and transport into the brain [67]. The TfR and low-density lipoprotein receptor-related protein-1 (LRP1) play crucial roles in mediating exosome uptake via CME, ensuring efficient transport of exosome-bound molecules [65]. Upon ligand binding, clathrin-coated vesicles undergo dynamin-dependent scission from the plasma membrane, leading to the internalization of exosomes into early endosomes. These vesicles are subsequently trafficked to late endosomes or lysosomes for degradation or undergo sorting for transcytosis across endothelial cells [68]. Exosome surface markers, such as tetraspanins CD63 and CD81, have been shown to play an essential role in modulating interactions with clathrin-associated receptors, thereby enhancing selective uptake and transport across the BBB [69]. Beyond receptor-mediated interactions, studies suggest that HSP70 on exosome surfaces further influences their recognition by endothelial cells, enhancing clathrin-dependent transcytosis [70]. Additionally, exosomal interactions with LDLRs have been implicated in

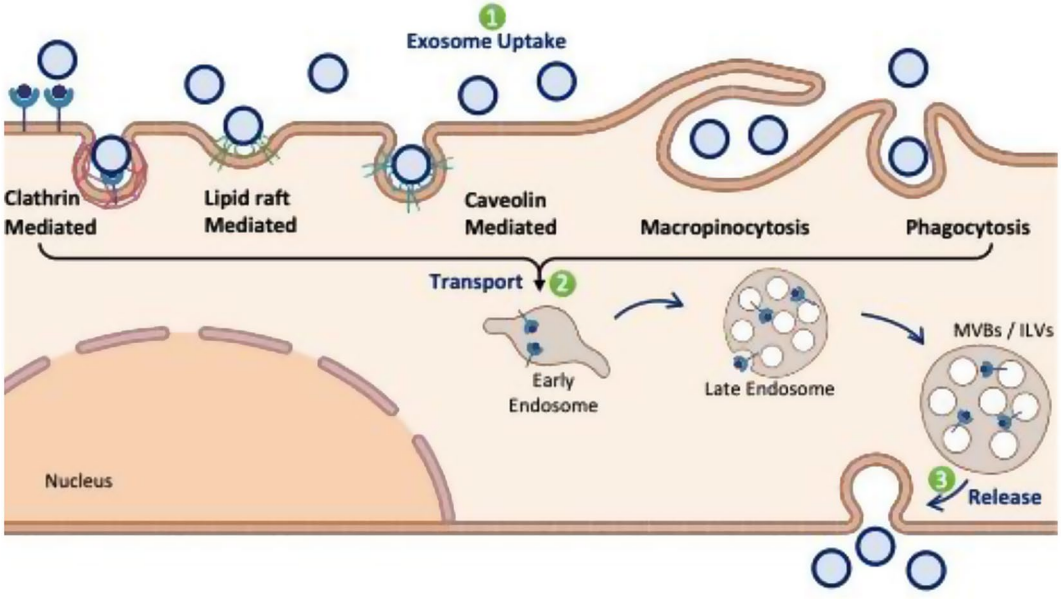


Fig. 4 Mechanisms of Exosome Translocation Across the BBB

facilitating LDLR-clathrin interactions that promote vesicle trafficking across the endothelial layer [71].

Studies in in vitro BBB models indicate that up to 10% of exosomes can successfully cross from the luminal to the abluminal chamber via clathrin-mediated transcytosis [72]. This highlights the efficiency of CME as a key pathway for extracellular vesicle transport across the BBB.

Integrins play a pivotal role in exosome-mediated transport across the BBB, facilitating both receptor-mediated uptake and clathrin-independent pathways. Exosomes expressing integrin αvβ3 interact with vascular endothelial adhesion molecules, thereby enhancing their ability to traverse the endothelial layer by leveraging existing cellular adhesion pathways [73]. Recent studies have highlighted that integrin-expressing exosomes actively engage ECM components, facilitating BBB penetration without compromising its structural integrity. For instance, macrophage-derived exosomes equipped with integrins have been observed to efficiently cross the BBB and deliver therapeutic cargoes to neural tissues [74]. This process is further influenced by integrin signaling, which modulates endothelial permeability and enhances transcytosis efficiency [75]. Exosome transport across the BBB also involves molecular regulators such as milk fat globule-EGF factor 8 (MFG-E8), which enhances integrin αvβ3-mediated exosomal trafficking through endothelial layers [76]. These mechanisms highlight the specificity of integrin-exosome interactions, underscoring their potential as therapeutic delivery vehicles and biomarkers for disease progression.

Fusion-mediated transport is a critical mechanism that allows exosomes to bypass endosomal degradation, ensuring the intact delivery of their cargo across the BBB. This process involves direct membrane fusion between exosomes and endothelial cells, facilitated by lipid components such as phosphatidylserine, cholesterol, and sphingomyelin. The high fusogenic potential of exosomes is attributed to the presence of lipid rafts, which promote membrane curvature and fusion with target cells [77]. A key factor influencing fusion efficiency is the lipid composition of both the exosomal and endothelial membranes. Phosphatidylserine, for instance, plays a pivotal role in mediating the interaction between exosomes and recipient cells by engaging phosphatidylserine-recognizing receptors, such as TIM-4 and lactadherin, which facilitate docking and subsequent fusion [78]. Cholesterol-rich lipid rafts further enhance membrane fluidity, promoting efficient fusion events that drive cargo delivery into the cytoplasm of target cells [79]. Additionally, sphingomyelin contributes to membrane rigidity and helps stabilize the formation of fusion-competent domains, ensuring that exosomal contents are delivered without degradation [80]. Among the various pathways EVs utilize for BBB crossing, fusion-mediated transport is particularly advantageous as it circumvents lysosomal processing, which often degrades cargo before it reaches the cytoplasm [81]. Other pathways, such as receptor-mediated endocytosis and macropinocytosis, involve internalization into endosomes, where EVs risk degradation unless they successfully escape into the cytoplasm. In contrast, direct fusion allows for the immediate cytoplasmic release of bioactive molecules such as proteins, RNA, and lipids, increasing the efficiency of therapeutic cargo delivery [82].

Comparative studies indicate that different EV subtypes exhibit varying propensities for fusion-mediated transport, depending on their lipid composition and surface protein expression. For instance, neural-derived exosomes exhibit enhanced fusion with endothelial cells due to the presence of neural adhesion molecules that facilitate targeted binding [83]. Additionally, artificially engineered exosomes with modified lipid compositions, such as increased phosphatidylserine content, have been shown to exhibit improved fusogenicity, highlighting the potential for optimizing fusion-mediated transport for therapeutic applications [84]. In addition to lipid composition, specialized proteins such as SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) and Rab GTPases facilitate membrane docking and fusion, ensuring the directed transport of exosomal cargo into the cytoplasm of target cells [85]. Recent studies indicate that modulating lipid raft dynamics and incorporating engineered fusogenic peptides can further enhance exosomal fusion efficiency [86]. This process not only optimizes therapeutic delivery but also enables exosome-mediated intercellular communication within the CNS, paving the way for novel neurotherapeutic strategies.

Engineering exosomes for precision therapies

The engineering of exosomes as precision delivery vehicles has revolutionized their therapeutic potential across diverse medical fields. By leveraging their inherent biocompatibility and ability to traverse biological barriers such as the BBB, researchers have devised innovative strategies to enhance exosome functionality for targeted drug delivery. Recent advancements in exosomal engineering can be categorized into genetic and biochemical modification techniques, drug-loading strategies, and brain-specific targeting mechanisms.

Genetic and biochemical modification techniques

Exosomes, by virtue of their biological compatibility and ability to cross barriers like the BBB, represent promising platforms for drug delivery. Engineering these vesicles through genetic and biochemical modifications has expanded their therapeutic potential, enabling

precise cargo delivery, improved targeting specificity, and enhanced stability.

Genetic engineering approaches

Genetic engineering involves modifying exosome-producing cells to generate vesicles with desired properties. This approach leverages cellular machinery to express specific proteins, peptides, or ligands on the exosomal membrane or load therapeutic molecules into their lumen. Incorporating targeting ligands through genetic engineering has been a cornerstone of exosome-based innovations. A notable strategy involves expressing the rabies viral glycoprotein (RVG) peptide on exosomal membranes, enabling selective interaction with acetylcholine receptors on neuronal cells. For instance, Khongkow et al. demonstrated that RVG-engineered exosomes effectively crossed the BBB and delivered RNA therapeutics to neuronal targets, paving the way for neurodegenerative disease therapies [87]. Similarly, Jia et al. developed neuropilin-1-targeted exosomes by genetically modifying donor cells to express ligands that bind glioblastoma cells. These exosomes exhibited enhanced targeting of brain tumors in vivo, significantly improving therapeutic outcomes [88].

Loading therapeutic cargo into exosomes by engineering donor cells has also proven highly efficient. For example, Alvarez-Erviti et al. successfully modified dendritic cells to produce exosomes carrying siRNA targeting the BACE1 gene, a critical player in AD. These exosomes traversed the BBB and silenced the target gene in neurons [25]. Similarly, CRISPR/Cas9 systems have been encapsulated in exosomes for genome editing. Duan et al. demonstrated that donor cells engineered to produce exosomes loaded with CRISPR/Cas9 targeting the PCSK9 gene achieved precise genome editing and significant therapeutic benefits in preclinical models [89].

Genetically engineered exosomes have also been explored for cancer immunotherapy. Ke Si et al. developed exosomes that co-deliver paclitaxel (PTX) and programmed death-ligand 1(PD-L1)-blocking Single-chain variable fragment (scFv), enhancing T-cell activation and reducing PD-L1-mediated immune suppression. These engineered exosomes demonstrated improved drug absorption and inhibited tumor growth, offering a promising strategy for cancer treatment [90].

Biochemical modifications

Biochemical modification techniques enhance the surface properties or cargo content of exosomes post-isolation, complementing genetic engineering by adding functionalities that cannot be achieved through cellular modifications alone. Surface functionalization through covalent or non-covalent bonding has significantly expanded the targeting capabilities of exosomes. For instance, dopamine-functionalized exosomes, created by conjugating dopamine molecules to exosomal surfaces, efficiently delivered therapeutic agents to dopaminergic neurons, offering potential in PD therapy [91]. Nanoparticle decoration is another promising approach, as demonstrated by Li et al., who developed a magnetic nanoparticle-exosome platform that enhances BBB penetration and tumor targeting, facilitating synergistic ferroptosis therapy through Fe_3O_4-mediated Fe^{2+} release and ferroptosis defense axis disintegration [92]. Exosomes can also be modified through fusion with liposomes or synthetic vesicles, enabling the incorporation of additional functional molecules such as peptides, drugs, or contrast agents. Wang et al. developed biomimetic pHybrid nanovesicles by fusing blood exosomes with tLyp-1 peptide-modified liposomes, enhancing BBB penetration and facilitating synergistic glioma therapy through cytotoxic and anti-angiogenic effects [93]. Chemical conjugation techniques have also been employed to enhance exosome stability in circulation. For example, PEG conjugation prevents opsonization, prolonging circulation time. This modification is particularly advantageous for applications requiring repeated systemic administration, such as chronic neurodegenerative diseases [94].

Drug loading strategies

The therapeutic efficacy of exosomes as drug delivery platforms hinges on their capacity to effectively encapsulate therapeutic agents. This improves bioavailability, protects payloads from enzymatic degradation, and ensures targeted delivery to desired sites [95]. Two primary approaches—pre-loading and post-loading—determine the stage at which therapeutic molecules are introduced, while passive and active loading methods govern the mechanism of encapsulation. Table 2 represents a comparative overview of pre-loading and post-loading techniques for exosome drug loading, highlighting their principles, advantages, and limitations.

Pre-Loading vs. Post-Loading strategies

Pre-loading involves the introduction of therapeutic molecules into exosomes during their biogenesis, prior to their release from parent cells. This process capitalizes on the natural ability of cells to incorporate agents such as miRs, siRNA, and mRNA through transfection or genetic engineering. Pre-loading ensures efficient encapsulation as therapeutic agents integrate into exosomes at the point of formation, resulting in homogeneous payload distribution [106]. Post-loading, by contrast, occurs after exosome isolation. Drugs are introduced into harvested exosomes using external techniques that manipulate the vesicle membrane [107]. Although this approach enables flexibility in selecting exosome sources and therapeutic payloads, loading efficiency, zeta potential, and size

Table 2 Exosome drug loading methods

Method	Principle	Advantage	Disadvantage	Ref.
Pre-loading before exosome isolation				
Co-cultivation	Co-cultivating cells to exchange cargo and release via exosomes	Simple and effective	Low loading efficiency, risk of membrane instability and degradation	[96]
Gene editing	Editing genes to overexpress specific molecules	Precise editing, overexpression	Low efficacy, gene-editing related toxicity	[97]
Transfection	Using reagents for molecule overexpression in exosomes	High loading capacity, molecular stability	Low efficacy, toxicity concerns with transfection agents, not suitable for all molecules	[98]
Post-loading after exosome isolation				
Co-incubation	Incubation and diffusion of drugs into exosomes via a concentration gradient	Simple process, preserves exosome integrity	Limited efficacy for hydrophilic drugs, risk of toxicity	[99]
Passive incubation	Passive diffusion of drugs into exosomes	Simple, inexpensive, effective for hydrophobic drugs	Not suitable for hydrophilic drugs, low loading capacity	[100]
Incubation with membrane permeabilizers	Using agents to create porous structures on the exosome membrane	High loading capacity	Risk of hemolysis and damage to cells	[101]
Electroporation	Electric pulses increase membrane permeability	Effective for hydrophobic drugs and nucleic acids, simple to use	Risk of exosome aggregation, requires process optimization	[96]
Sonication	Mechanical shear stress applied to exosomes to load drugs	High loading efficacy, efficient drug release	Possible damage to membrane integrity	[98]
Extrusion	Mechanical pressure applied to exosomes to reduce membrane integrity and facilitate loading	Uniform drug distribution, high efficacy	Possible membrane damage, drug leakage	[102]
Freeze-thaw cycles	Repeated freezing and thawing to deform exosome membranes for drug entrapment	Simple, industrially applicable	Low efficacy, risk of aggregation and inactivation	[97]
Thermal shock	Rapid temperature changes to increase exosome permeability	Preserves exosome morphology, may enhance immunogenicity	Affects membrane fluidity, potentially impacting stability and delivery	[96]
Detergent treatment	Formation of pores by combining with cholesterol on the exosome membrane	High loading capacity, effective with saponin	Risk of hemolysis and cytotoxicity, requires purification	[99]
Lipofectamine	Lipofectamine reagent facilitates drug transfer into exosomes	Widely used for nucleic acids		[103]
Nanoporation	Nanosecond electrical pulses reduce exosome membrane integrity	Effective for small molecules	Risk of aggregation	[104]
Surface treatment	Surface modification of exosomes to enhance drug loading and targeting ability	High loading efficiency	Risk of cargo degradation, requires purification	[105]
Hypotonic dialysis	Hypotonic solution causes exosome swelling and pore creation	Greater loading efficiency than passive incubation	Risk of protein degradation, size distribution alteration	[100]
pH gradient	pH gradient increases membrane permeability	Simple, effective for nucleic acids	Risk of protein degradation, exosome aggregation	[101]
Ultrasound	Reduces micro-viscosity of exosomal membrane	No effect on lipids or membrane proteins	Risk of membrane disruption	[98]

distribution can vary, influencing the stability and functionality of the exosomes [108].

Passive vs. Active strategies

Passive loading relies on the natural ability of exosomes to incorporate therapeutic molecules during their biogenesis or through diffusion into their lipid bilayer. This method is particularly effective for small, hydrophobic molecules that can seamlessly integrate into the exosomal membrane.

The inherent affinity of hydrophobic drugs for the lipid bilayer of exosomes makes passive diffusion a viable strategy. However, passive loading often suffers from limitations such as suboptimal encapsulation efficiency, particularly for molecules with low lipophilicity or larger molecular sizes. Payload leakage during purification and circulation further reduces therapeutic efficacy. These challenges underscore the need for more sophisticated and reliable active loading strategies [109]. Active loading methods utilize external physical or chemical forces to enhance the encapsulation of therapeutic agents into

exosomes. These techniques address the limitations of passive loading by enabling the efficient incorporation of hydrophilic molecules, macromolecules, and genetic materials. Prominent active loading strategies include electroporation, sonication, extrusion, and emerging methods such as freeze-thaw cycles and chemical conjugation.

Electroporation, one of the most widely used active loading techniques, employs short electrical pulses to create transient pores in the exosomal membrane, allowing therapeutic agents to diffuse into the vesicles. This method is particularly effective for loading genetic materials, such as siRNA, mRNA, and CRISPR/Cas9 components [110]. However, electroporation can cause aggregation of nucleic acids, potentially reducing their functional efficacy. Optimizing parameters such as pulse strength, duration, and solution conductivity is crucial for minimizing these effects.

Sonication utilizes ultrasonic waves to temporarily disrupt the exosomal membrane, facilitating the encapsulation of therapeutic agents [111]. While sonication ensures uniform drug encapsulation and is scalable for clinical applications, prolonged exposure can damage exosome structure, affecting their biodistribution and targeting.

Extrusion involves forcing a mixture of exosomes and therapeutic agents through nanoporous membranes under high pressure. This mechanical process achieves efficient and reproducible drug loading. Extrusion is highly scalable and suitable for industrial production. It also enables the encapsulation of larger therapeutic molecules, such as peptides and proteins. In contrast, the high-pressure process may alter the structural integrity of exosomes, necessitating post-loading characterization to ensure functionality [9].

Novel active loading methods are emerging to address the limitations of traditional techniques. Freeze-thaw cycles create temporary membrane pores by subjecting exosomes to alternating freezing and thawing, allowing the encapsulation of therapeutic agents [112]. Chemical conjugation techniques, such as click chemistry, offer high specificity and stability for drug payloads [89]. Magnetic manipulation has also been explored, demonstrating that iron oxide nanoparticle-loaded exosomes could be guided to inflamed tissues using magnetic fields, enhancing targeted delivery [9].

Targeting the brain: enhancing exosome specificity

The BBB presents a formidable obstacle for therapeutic delivery to the CNS. Exosomes, with their innate biocompatibility and ability to traverse the BBB, have emerged as promising carriers for brain-targeted therapies. Strategies to enhance exosome specificity include surface functionalization, cell-derived targeting, and hybrid systems that integrate natural and synthetic components.

Surface functionalization involves modifying exosomal membranes with ligands or molecules that enhance their interaction with CNS-specific targets. Transferrin ligands exploit TfR-mediated transcytosis to cross the BBB. For example, exosomes functionalized with transferrin ligands efficiently delivered chemotherapeutic agents to neuronal cells, significantly improving therapeutic outcomes in CNS oncology [113]. Similarly, exosomes engineered with neuropilin-1 ligands demonstrated superior targeting capabilities, reducing tumor growth in glioblastoma models [88]. Tian et al. further reported enhanced anti-inflammatory and neuroprotective effects of curcumin (Cur)-loaded exosomes in ischemic stroke models following surface modification, with notable reductions in infarct size [114].

Exosomes derived from specific CNS cell types, such as astrocytes or brain endothelial cells, possess inherent brain-targeting properties. Cao et al. demonstrated that brain endothelial cell-derived extracellular vesicles efficiently transport mitochondria-targeting photosensitizers across the BBB, enhancing photodynamic therapy efficacy and selectively inducing apoptosis in glioblastoma cells [115]. Astrocyte-derived exosomes have also been used to deliver genetic materials, such as siRNA, to neurons, showing promise in treating neurodegenerative conditions like AD and PD [116–118].

Hybrid systems combine exosomes with synthetic nanoparticles or liposomes to enhance functionality and targeting precision. Khongkow et al. developed a hybrid system by conjugating exosomes with gold nanoparticles, achieving improved BBB penetration and brain targeting in neurodegenerative disease models [87]. Similarly, liposome-exosome hybrids have been shown to improve membrane stability and targeting accuracy. Liu et al. developed exosome-liposome hybrid-based vehicles labeled with near-infrared-II (NIR-II) fluorescence dyes, exhibiting strong light-harvesting capability, high photoconversion efficiency (62.28%), and effective tumor ablation in glioblastoma models [119].

Therapeutic potential of Exosome-Based drug delivery

Exosome-based drug delivery systems represent a transformative approach to tackling CNS diseases, including neurodegenerative disorders, brain tumors, and psychiatric conditions. Their ability to cross the BBB and inherent biocompatibility position them as promising tools for precision medicine in neurology.

Treating neurodegenerative diseases: AD, PD, ALS, and MS

Neurodegenerative diseases, such as AD and PD, amyotrophic lateral sclerosis (ALS), and multiple sclerosis

(MS) pose significant therapeutic challenges due to the progressive nature of neuronal damage and the impermeability of the BBB. Exosome-based therapies offer innovative solutions to overcome these barriers and address underlying pathologies.

AD

AD, the most prevalent neurodegenerative disorder globally, is characterized by the accumulation of extracellular amyloid-β (Aβ) plaques and intracellular neurofibrillary tangles. Traditional drug therapies, such as cholinesterase inhibitors and N-methyl D-aspartate (NMDA) receptor antagonists, often provide symptomatic relief rather than addressing the underlying pathology, and their efficacy is limited by their inability to cross the BBB efficiently [120]. In contrast, exosome-based therapies present a paradigm shift by offering targeted delivery of therapeutic agents and early intervention strategies.

RNA-based exosome therapies have been particularly effective in modulating amyloidogenesis pathways. Jahangard et al. developed exosomes transfected with miR-29b using bone marrow-derived mesenchymal stem cells (BM-MSCs). These exosomes successfully delivered miR-29b to target cells, significantly enhancing miR-29b expression and inhibiting amyloidogenic pathways in Aβ-induced mouse models. Bilateral administration improved hippocampal learning, underscoring their therapeutic potential [121]. Yang et al. highlighted the advantages of exosomes derived from human MSCs cultured under 3D conditions compared to two-dimensional (2D) cultures. Exosomes from 3D cultures exhibited elevated levels of neprilysin (NEP), HSP70, and insulin-degrading enzyme (IDE), which promote Aβ degradation. APP/PS1 mouse models treated with these exosomes demonstrated reduced amyloid plaque burden and improved memory and cognitive function [122].

Despite their ability to cross the BBB, exosome therapeutics face challenges related to off-target accumulation in non-brain organs, such as the liver and spleen. Recent advancements in engineering brain-specific exosome targeting address this limitation. RVG-conjugated exosomes have demonstrated strong affinity for acetylcholine receptors, facilitating their uptake into neuronal cells. In AD models, these exosomes reduced Aβ plaques, inhibited glial fibrillary acidic protein (GFAP) production, and improved hippocampal and cortical functions by modulating neuroinflammation and enhancing spatial memory [123].

Drug-loaded exosomes represent another promising approach. Wang et al. developed macrophage-derived exosomes loaded with Cur for AD treatment. These exosomes crossed the BBB via active transport mediated by lymphocyte function-associated antigen 1 (LFA-1) integrins binding to Intercellular adhesion molecule 1(ICAM-1)and localized with hippocampal neurons. They reduced neuronal apoptosis, inhibited tau hyperphosphorylation via the AKT/GSK-3β pathway, and enhanced cognitive function in AD models [124]. Similarly, plasma-derived exosomes loaded with quercetin (Que) demonstrated improved cognitive performance in okadaic acid (OA)-induced AD mice. These exosomes reduced tau phosphorylation through cyclin-dependent kinase 5 (CDK5) suppression, minimized neurofibrillary tangle formation, and decreased caspase-3/9 activity, emphasizing their neuroprotective potential [125]. Coenzyme Q10 (CoQ10)-loaded exosomes derived from human adipose-derived stem cells (hADSCs) have also demonstrated efficacy. In a streptozotocin (STZ)-induced AD rat model, CoQ10-loaded exosomes improved spatial memory, increased hippocampal neuron density, and elevated brain-derived neurotrophic factor (BDNF) and SRY-related HMG-box 2 (SOX2) expression, highlighting their role in neuroprotection and neurogenesis [126]. Exosome-like liposomes have emerged as complementary systems. Fernandes et al. investigated Cur-loaded liposomes in zebrafish embryos and human neuronal cells. These liposomes reduced oxidative stress by 50%, promoting neuroprotection while minimizing adverse effects. Although further validation in mature organisms is needed, these findings suggest potential synergy between liposome-based and exosome-mediated approaches [127].

Exosome-based therapeutics are rapidly evolving, with ongoing efforts to enhance brain-specific targeting and mitigate off-target effects. Advanced engineering techniques, such as peptide conjugation and CRISPR-Cas9 gene editing, are opening new frontiers for precision medicine. For instance, CRISPR-Cas9 delivered via exosomes has shown potential to correct genetic defects, such as ApoE4-associated mutations, offering novel approaches to treating AD [128]. Further, engineered exosomes with a photoinducible protein delivery system, termed MAPLEX, enable precise epigenome editing in AD models. MAPLEX utilizes mMaple3-mediated protein loading and blue-light-induced cargo release, allowing targeted delivery of gene-editing tools like dead Cas9 (dCas9) ribonucleoprotein complexes. In amyloid precursor protein transgenic (5xFAD) and triple-transgenic (3xTg-AD) mouse models of AD, MAPLEX-based delivery of β-site amyloid precursor protein cleaving enzyme 1 (Bace1)-targeting single guide RNA resulted in reduced amyloid plaque formation and improved cognitive function. This innovative platform highlights exosomes as promising therapeutic nanocarriers for neurodegenerative diseases [129].

Integrating exosomes with established therapeutics, like aducanumab, could amplify treatment efficacy by ensuring BBB penetration and sustained drug release.

Additionally, combining exosomes with neurotrophic factors and anti-inflammatory cytokines may restore synaptic density, reduce neuroinflammation, and improve cognitive functions, further expanding the therapeutic arsenal for AD and other neurodegenerative diseases.

PD

PD is characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta and the pathological aggregation of alpha-synuclein (α-Syn) proteins. This neurodegenerative disorder manifests with hallmark motor symptoms, including bradykinesia, rigidity, resting tremor, and postural instability. Traditional pharmacological therapies, such as levodopa and dopamine agonists, focus on symptom management rather than addressing disease progression. Furthermore, their efficacy diminishes over time due to side effects, including motor complications and systemic toxicity [130]. Recent advancements in nanobiotechnology, including exosome-mediated delivery systems, present innovative therapeutic strategies for overcoming these limitations and enabling early diagnosis and intervention.

The pathological aggregation of α-Syn plays a central role in PD pathogenesis. Exosome-based RNA therapies have shown significant promise in targeting α-Syn. Izco et al. demonstrated that exosomes loaded with small hairpin RNA (shRNA) effectively reduced α-Syn aggregation, protected dopaminergic neurons, and alleviated PD symptoms in preclinical models. These shRNA-loaded exosomes exhibited stable delivery across the BBB, ensuring controlled release and minimizing systemic degradation [131]. Similarly, Cooper et al. utilized RVG-modified exosomes to deliver siRNA targeting α-Syn transcripts. In PD mouse models, these siRNA-loaded exosomes reduced α-Syn accumulation in the substantia nigra, improved motor functions, and underscored the therapeutic potential of RNA-based exosome therapies [132].

Exosome-mediated delivery of CRISPR/Cas9 components represents an innovative approach to addressing genetic contributors to PD. Exosome-mediated delivery of CRISPR/Cas9 components represents an innovative approach to addressing genetic contributors to PD. Recent research by Weirong Kong et al. demonstrated an epigenetic regulation platform using exosomal CRISPR intervention. FUS facilitated the delivery of engineered exosomes (RVG-CRISPRi-Exo) into brain lesions, inducing specific methylation of SNCA (α-synuclein gene). This approach significantly improved motor performance, reduced α-synuclein levels, and rescued neuronal damage in PD mice. These findings highlight the potential of targeted brain nanodelivery in neurodegenerative diseases [133].

Dopamine (DA) replacement remains a cornerstone of PD treatment. Exosome-based delivery systems have demonstrated the ability to enhance the efficacy of this approach. Qu et al. engineered blood-derived exosomes to deliver DA across the BBB by incorporating transferrin ligands on their surface, enhancing targeting to dopaminergic neurons. In vivo studies reported a 15-fold increase in brain DA levels compared to free DA administration, restoring dopaminergic function and reducing oxidative stress in PD models. Neurobehavioral improvements and elevated striatal tyrosine hydroxylase levels further supported the therapeutic potential of DA-loaded exosomes [134].

Neuroinflammation is a critical factor in PD progression. Exosome-mediated delivery of anti-inflammatory agents offers a novel therapeutic avenue. Kojima et al. developed EXOtic constructs to engineer exosomes for delivering catalase mRNA to inflamed brain regions (Fig. 5). These catalase-loaded exosomes reduced oxidative stress and neuroinflammatory markers, such as GFAP and TNF-α, in PD models. Immunostaining revealed localized neuroprotection, highlighting the role of exosome-based mRNA therapies in mitigating reactive oxygen species (ROS)-induced neurotoxicity [135]. Ren et al. further developed RVG-exosomes loaded with aptamer F5R2 to selectively target α-Syn fibrils (Fig. 6). These exosomes reduced α-Syn aggregation in the substantia nigra and cortex, improving motor functions with high specificity while sparing endogenous α-Syn, providing a novel approach for treating synucleinopathies [136].

A recent study by Huang et al. demonstrated the therapeutic potential of intranasally administered umbilical cord mesenchymal stem cell (UC-MSCs)-derived exosomes in a PD mouse model. The study found that UC-MSCs-derived exosomes successfully crossed the BBB, were endocytosed by neuronal and glial cells, and significantly improved both motor and non-motor functions in PD models. Additionally, UC-MSCs-derived exosomes exhibited neuroprotective effects by mitigating dopaminergic neuronal loss in the substantia nigra pars compacta and enhancing olfactory bulb neuronal activity. Furthermore, the treatment reduced neuroinflammation by suppressing microglial and astrocyte activation, improving the local brain microenvironment. These findings provide compelling evidence for the potential clinical application of exosome-based nanotherapeutics in PD treatment [138].

These approaches, spanning RNA therapies, gene editing, DA replacement, and anti-inflammatory strategies, offer immense potential to mitigate PD progression and improve patient outcomes. However, further clinical studies are needed to optimize these therapies and translate them into viable treatments.

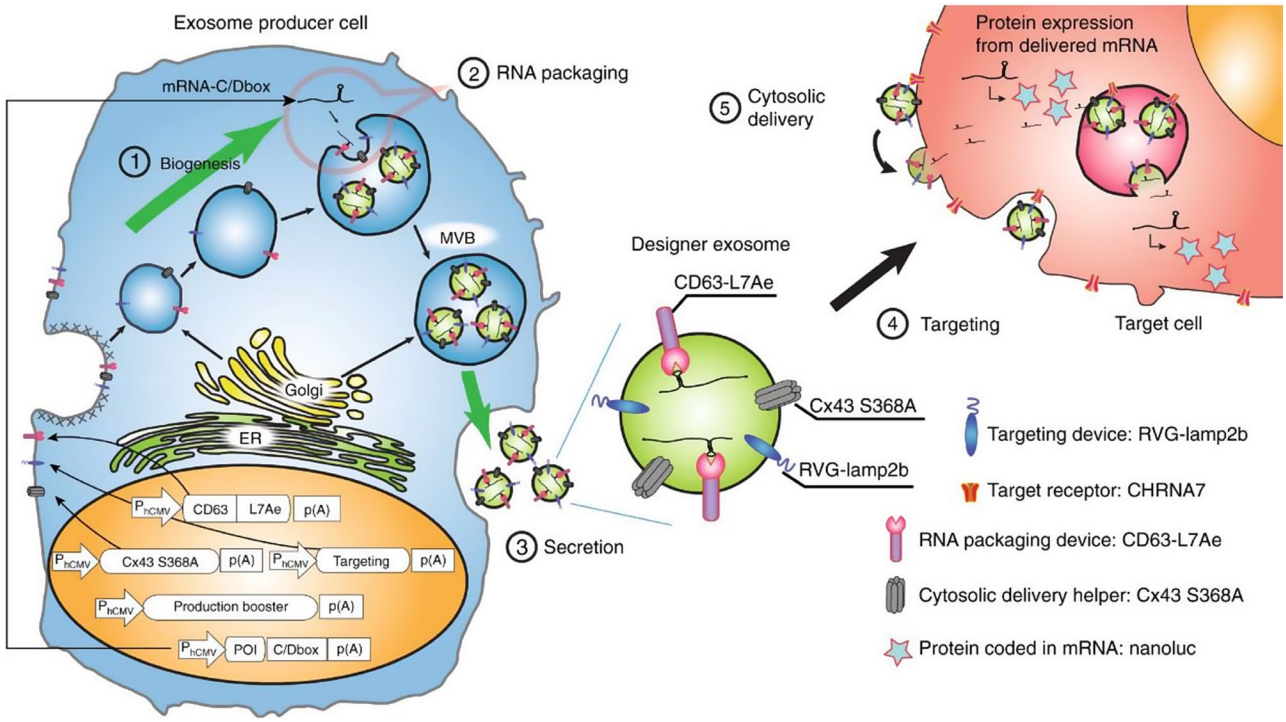


Fig. 5 EXOtic devices for mRNA delivery. In the schematic, exosomes containing various components—an RNA packaging device (CD63-L7Ae), a targeting module (RVG-Lamp2b to target CHRNA7), a cytosolic delivery helper (Cx43 S368A), and mRNA (e.g., nluc-C/Dbox)—are efficiently produced by exosome-producing cells with the aid of an exosome production booster. These engineered exosomes are then delivered to target cells (HEK-293T cells expressing CHRNA7), where the mRNA is released into the cytosol with the help of the cytosolic delivery helper. Finally, the encoded protein (e.g., nluc, depicted by stars) is expressed in the target cells igure adapted from Kojima et al. [135] under the terms of the Creative Commons Attribution 4.0 International License

Huntington's disease

Huntington's disease (HD) is a hereditary neurodegenerative condition caused by an expanded CAG repeat in the huntingtin (HTT) gene, leading to the production of toxic mutant huntingtin protein (mHTT). This protein forms aggregates that disrupt cellular processes, causing progressive motor dysfunction, cognitive decline, and psychiatric symptoms. Although the genetic mutation underlying HD was identified in 1993, disease-modifying treatments remain elusive. Current pharmacological therapies, such as tetrabenazine and deutetrabenazine, provide symptomatic relief but do not address the underlying pathology or alter disease progression. Moreover, these treatments are associated with side effects, including sedation, depression, and motor disturbances [139]. Exosome-mediated drug delivery offers an innovative solution for crossing the BBB and delivering targeted therapeutics to the brain, addressing the limitations of traditional approaches.

Exosomes have been extensively studied as carriers for siRNA and antisense oligonucleotides (ASOs) to target mHTT transcripts and reduce mutant protein production. Wu et al. investigated neuron-specific RVG-modified exosomes loaded with siRNA targeting human mutant huntingtin (HuHtt) mRNA in two HD mouse models, BACHD and N171-82Q. Intravenous administration reduced HTT expression by 46% and 54% in these models, respectively. Treated N171-82Q mice showed significant motor coordination improvements in rotarod tests, underscoring the potential of siRNA-loaded exosomes for silencing mHTT and improving disease outcomes [140]. Didiot et al. enhanced siRNA stability and uptake by using hydrophobically engineered siRNAs (hsiRNAs) encapsulated in exosomes. Cholesterol-conjugated hsiRNAs significantly reduced HTT mRNA levels in cortical and striatal regions by approximately 35%. Free hsiRNAs were far less effective, emphasizing the importance of exosomes in facilitating efficient BBB delivery and gene silencing [141].

ASOs provide another promising tool for targeting mHTT transcripts. These molecules bind complementary mRNA sequences, promoting degradation or blocking translation. Lee et al. demonstrated that miRNA-124-loaded exosomes reduced the expression of repressor element 1-silencing transcription factor (REST), implicated in HD pathology. Although a single injection did not significantly improve motor function, this study highlighted the potential of optimizing dosing strategies or exploring alternative miRNAs, such as miR-9 and miR-125b, for therapeutic efficacy [142].

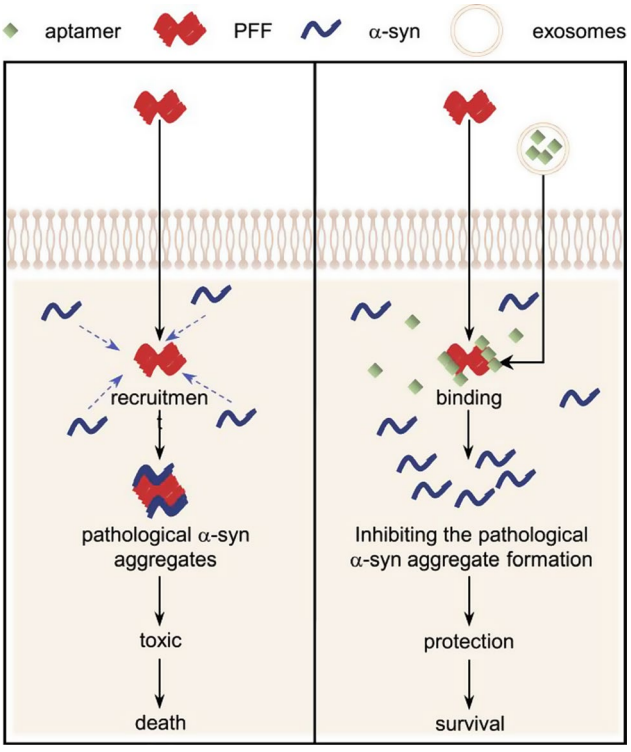


Fig. 6 The aptamers exert neuroprotective effects by inhibiting the recruitment of endogenous α-synuclein by preformed fibrils (pffs), preventing the formation of pathological aggregates. *Figure reprinted from Ren et al.* [137] *with permission from Elsevier.* © 2019 The Author(s). Published by Elsevier

Exosomes can be applied for delivering CRISPR-Cas9 systems to achieve allele-specific editing of mHTT. This approach might allow permanent genome modification, selectively targeting the mutant allele while sparing the wild-type HTT allele to minimize off-target effects. In addition to gene silencing, exosomes can be evaluated for delivering autophagy-enhancing molecules to facilitate the clearance of toxic mHTT aggregates. Combination therapies leveraging exosomes to co-deliver siRNAs and autophagy inducers might show synergistic benefits. This dual approach probably accelerate mHTT clearance, improves neuronal survival, and mitigates HD pathology.

ALS and MS

ALS and MS are devastating neurodegenerative diseases characterized by progressive motor dysfunction and immune-mediated demyelination, respectively. Despite significant advancements, current therapeutic strategies remain palliative, highlighting the urgent need for novel treatment modalities.

Traditional treatment strategies for ALS and MS primarily focus on symptom management rather than disease modification. For ALS, FDA-approved drugs such as riluzole and edaravone offer limited efficacy, only slightly prolonging survival and reducing oxidative stress, respectively. MS treatments include immunomodulatory drugs like interferon-beta and monoclonal antibodies such as natalizumab, which help mitigate relapses and slow disease progression. However, these approaches do not reverse neuronal damage and may come with severe side effects, including immune suppression and infection risk.

Exosome-based drug delivery has emerged as a promising alternative due to its ability to cross the BBB, carry neuroprotective cargo, and mediate immune modulation. Unlike traditional therapies, exosomes can deliver targeted treatments, reducing systemic toxicity while enhancing therapeutic efficacy. Their natural biocompatibility and ability to transfer bioactive molecules, including miRNAs and proteins, provide a novel mechanism to promote neuroregeneration and modulate inflammation directly at the site of injury.

Exosomes derived MSCs have demonstrated potent neuroprotective effects by transferring bioactive molecules such as miRNAs, proteins, and lipids to neural cells. For instance, a study by Gschwendtberger et al. showed that exosomal delivery of neurotrophic factors significantly reduced neurotoxicity in ALS motor neurons [143]. In MS, excessive neuroinflammation contributes to demyelination and neuronal damage. Exosome-based therapies can modulate the immune system by suppressing pro-inflammatory cytokines and promoting regulatory T-cell responses. Ojeda-Hernández et al. highlighted the role of engineered exosomes in reducing neuroinflammation and promoting remyelination [144].

Exosomes possess inherent capabilities to cross the BBB, making them ideal carriers for drug delivery. A study by Bonafede et al. demonstrated that hADSCs-exosomes successfully reached neuronal targets and improved motor function in ALS mice [145]. Recent studies have focused on exosomal therapy for ALS by delivering neurotrophic and anti-inflammatory molecules. Wang et al. demonstrated that exosomes derived hADSCs attenuated motor neuron degeneration by reducing oxidative stress and inflammation [146]. Another promising approach involves Schwann cell-derived exosomes. A case report by Goldschmidt-Clermont and Khan showed that administration of these exosomes through the FDA's Expanded Access Program resulted in notable symptomatic relief in an ALS patient [147]. More recently, Mazzini et al. reviewed the latest preclinical advancements using stem cells and their translation into clinical trials of ALS, emphasizing the role of exosome-derived therapies [148]. Furthermore, the FDA recently cleared Aruna Bio's exosome-based therapy AB126 for clinical trials in ALS, as reported by Ciccone, marking a significant step toward clinical application [149].

Table 3 The utility of exosomes in brain tumor treatment

EV source	Therapeutic cargo	Target cells	Result	Ref.
Brain endothelial bAND.3 cells	Vascular endothelial growth factor small interfering RNA	Neuronal glioblastoma, astrocytoma U-87, malignant glioma cells	Vascular endothelial growth factor RNA and protein level were inhibited by siRNA encapsulated in exosomes	[56]
Brain neuronal glioblastoma-astrocytoma U-87 MG cells, brain endothelial bAND.3 cells, neuroectodermal tumor PF SK-1 cells, and glioblastoma A-172 cells	Rhodamine 123, Paclitaxel, doxorubicin	Neuronal glioblastoma-astrocytoma U-87, malignant glioma cells	The effectiveness of this treatment technique is due to a higher level of CD63 expression.	[150]
HEK293T cells	miRNA-21	Brain tumor	Tumor growth inhibition; can pass across the BBB	[151]
Mouse fibroblast cell line L929	Methotrexate functionalized with therapeutic [Lys-Leu-Ala (KLA)] and targeted [low-density lipoprotein (LDL)] peptides	Human primary glioma cell line U87	LDL/KLA-LDL modification enhanced EV uptake, BBB permeation, and glioma targeting, leading to improved treatment outcomes and increased median survival in mice	[152]
Bone marrow stromal cells	miRNA-146b	Glioma	Intratumoral exosome injection into glioma rat xenograft reduced tumor volume considerably.	[153]
Mesenchymal Stem Cells	Anti-miR-9	Glioblastoma multiforme	MiR-9 reversal sensitized glioblastoma cells to Temozolomide, increasing cellular death and caspase activation.	[154]
HEK293T cells	Cytosine deaminase-uracil phosphoribosyl transferase (CD-UPRT) fusion protein	Schwannoma	Treatment of Schwannoma	[155]
Blood-Derived Exosomes	Cytosolic Phospholipase A2 (cPLA2) siRNA, Metformin	Glioblastoma multiforme	ncreased cellular uptake, strong antitumor effects, inhibition of tumor growth, and extended survival in mice	[156]
CpG-Loaded Exosomes (CpG-EXO/TGM)	CpG oligonucleotides, Temozolomide	Glioblastoma multiforme	Significantly extended median survival in glioma mouse models; synergistic effect with TMZ, preventing postoperative recurrence	[77]
Dendritic cell-derived exosomes (DEX)	Alpha-Galactosylceramide, Tumor-Derived Exosomes, iNKT cells	Glioma cells	Promoted immune response against glioma in mouse models, breaking immune tolerance	[157]
Neutrophil-derived exosomes (NEs-Exos)	Doxorubicin (DOX)	Glioma cells	Efficient BBB penetration, tumor targeting, and suppression of glioma growth	[158]
Neural stem cell-derived exosomes (NSCEXOs)	miR-124-3p	Glioma cells	Therapeutic effects on a mouse tumor xenograft model of glioma. Suppression of glioma growth via the EXOmiR-124-3p/*FLOT2/AKT1* pathway	[159]
Neural stem cells (NSCs)	CpG-STAT3 antisense oligonucleotide (CpG-STAT3ASO)	Glioma microenvironment	NSCs secreted exosomes loaded with CpG-STAT3ASO, which enhanced immune activation, induced NF-κB signaling and IL-12 production, improved oligonucleotide transfer, and resulted in enhanced antitumor effects in GL261 glioma models	[160]

Tackling brain tumors with Exosome-Based strategies

Brain tumors, especially, glioblastoma represent some of the most formidable challenges in oncology due to their aggressive progression, invasive behavior, and the significant obstacles posed by the BBB to therapeutic delivery. Exosomes, with their intrinsic capability to traverse the BBB and deliver bioactive molecules directly to target cells, have emerged as a groundbreaking approach in glioblastoma treatment. Table 3 reperesent the therapeutic applications of exosomes in treatment of brain tumors. These nanoscale EVs exhibit remarkable attributes, including biocompatibility, minimal immunogenicity, protection of their therapeutic cargo, and an inherent propensity for tumor targeting. This section delves into the diverse applications of exosomes in brain cancer therapy, focusing on chemotherapeutic delivery, RNA-based treatments, and CRISPR/Cas9 gene-editing advancements.

Exosomes have demonstrated exceptional promise as carriers for chemotherapeutic agents, enabling deeper tumor penetration while reducing systemic toxicity. Unlike traditional drug delivery methods, exosome-mediated transport permits therapeutic compounds to bypass the BBB and accumulate effectively at tumor sites. Their lipid bilayer not only shields therapeutic agents from enzymatic degradation but also minimizes clearance by the immune system, thereby enhancing both drug stability and bioavailability. Engineered exosomes

loaded with chemotherapeutic agents such as PTX and DOX have exhibited improved efficacy in preclinical glioblastoma models. For instance, research has shown that these exosomes significantly inhibit tumor growth while mitigating the systemic side effects typical of conventional chemotherapy [161]. Notably, exosomes derived from brain endothelial cells were employed to deliver PTX and DOX across the BBB in zebrafish glioblastoma models, achieving superior drug accumulation in brain tissues and greater cytotoxic effects against glioma cells compared to free-form drugs [56].

Recent innovations have centered on functionalizing exosomes to enhance the targeting and efficacy of chemotherapeutics. For example, exosomes engineered with neuropilin-1-targeting peptides have demonstrated increased tumor specificity and improved delivery efficiency [88]. Similarly, embryonic stem cell-derived exosomes modified with Cyclo (Arg-Gly-Asp-D-Tyr-Lys) peptide (c(RGDyK)) and loaded with PTX have shown enhanced targeting and antitumor effects in glioblastoma. These engineered exosomes exhibit superior drug delivery compared to free PTX, improving therapeutic outcomes in both in vitro and in vivo GBM models [162]. These findings underscore the transformative potential of the exosome-based delivery systems in redefining chemotherapy for glioblastoma. Exosomes are increasingly being integrated with complementary therapeutic modalities to optimize their efficacy. For instance, exosomes functionalized with ginsenoside Rg3 have been employed to co-deliver chemotherapeutics and amplify anti-tumor immune responses, effectively suppressing glioblastoma progression in preclinical settings [163]. These developments highlight the adaptability of exosomes as versatile drug delivery platforms capable of overcoming the inherent limitations of traditional chemotherapy.

In addition to chemotherapeutics, RNA-based therapies such as siRNAs, miRNAs, and circRNAs are emerging as promising tools to address the genetic and molecular underpinnings of glioblastoma. Exosomes offered an ideal vehicle for these fragile molecules, safeguarding them from enzymatic degradation and facilitating their efficient transport to tumor cells. Exosomes offered an ideal vehicle for these fragile molecules, safeguarding them from enzymatic degradation and facilitating their efficient transport to tumor cells. Shan et al. developed a macrophage-derived exosome-based nanodrug delivery system (cEM@DEP-siRNA) to enhance the therapeutic efficacy against diffuse intrinsic pontine glioma (DIPG). These exosomes were functionalized with a cyclic RGD (cRGD) peptide, which specifically binds to integrin αVβ3, a protein highly expressed on diffuse intrinsic pontine glioma (DIPG) cells, facilitating targeted drug delivery. The engineered exosomes successfully traversed the BBB, allowing for the efficient co-delivery of panobinostat and PPM1D (Protein Phosphatase, Mg2+/Mn2 + Dependent 1D)-targeting siRNA (Fig. 7). This dual-therapy approach inhibited tumor cell proliferation and prolonged survival in DIPG animal models. The biomimetic design of exosome membranes provided superior biocompatibility, prolonged circulation time, and reduced systemic toxicity. This study highlights exosomes as a powerful drug delivery platform, offering a promising avenue for improving DIPG treatment outcomes [164].

The therapeutic application of miRNAs within exosomes has been particularly noteworthy in glioblastoma research. For instance, Munoz et al. demonstrated that MSC-derived exosomes could effectively deliver functional anti-miR-9 to glioblastoma cells, conferring chemosensitivity. glioblastoma cells resistant to temozolomide (TMZ) exhibited increased miR-9 expression, which regulated P-glycoprotein, a drug efflux transporter. The transfer of anti-miR-9 via MSC-derived microvesicles reversed multidrug transporter expression, increasing caspase activity and promoting cell death. This study highlights exosome-mediated miRNA therapy as a promising strategy to overcome glioblastoma chemoresistance [154]. Similarly, exosomes loaded with miR-21 inhibitors have successfully reduced tumor proliferation and enhanced chemotherapy sensitivity [165]. By targeting molecular pathways implicated in angiogenesis, immune evasion, and drug resistance, these exosomes contribute to a less favorable microenvironment for tumor development. Emerging studies have also highlighted the potential of exosomal circRNAs in glioblastoma therapy. For example, exosomal circRNA 0001445 has been identified as a pivotal regulator of glioblastoma progression, suggesting new therapeutic directions in targeting circRNAs [166]. Furthermore, exosomes engineered to deliver siRNAs targeting VEGF pathways have demonstrated promise in suppressing angiogenesis and tumor growth, thereby advancing RNA-based glioblastoma treatment strategies [56].

In another strategy, an exosome-membrane (EM) and polymer-based hybrid complex has been developed for the systemic delivery of plasmid DNA (pDNA) encoding the Herpes simplex virus thymidine kinase (HSVtk) gene. This approach overcomes the challenge of pDNA penetration through the BBB by utilizing histidine/arginine-linked polyamidoamine (PHR) as a carrier, which binds to pDNA via electrostatic interactions. The hybrid complex, decorated with a T7 peptide for glioblastoma targeting, enhances transfection efficiency, leading to higher HSVtk expression and apoptosis levels in tumors, making it a promising gene therapy strategy [167].

The advent of gene-editing technologies such as CRISPR/Cas9 has further expanded the therapeutic possibilities for glioblastoma, with exosomes serving as a

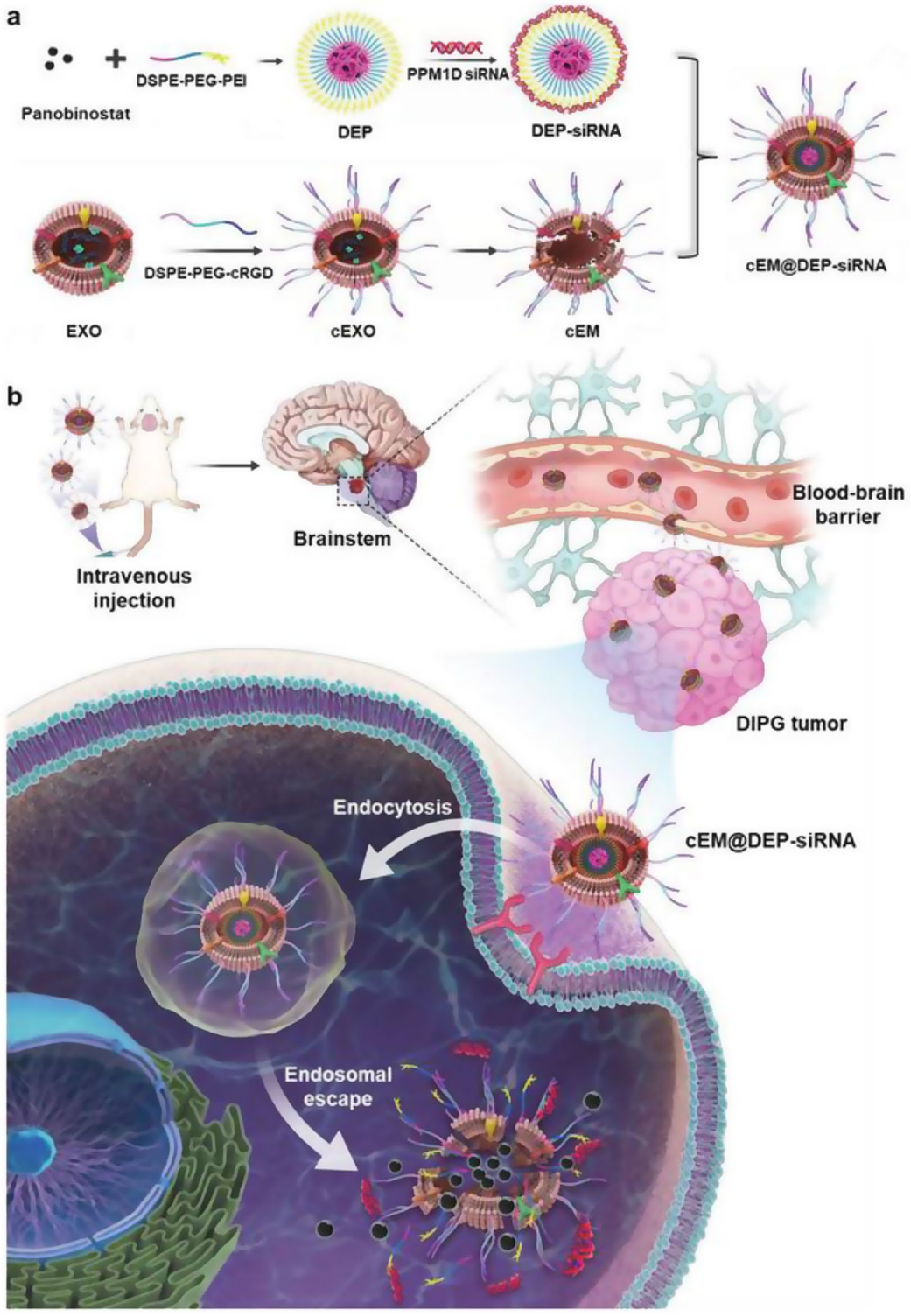


Fig. 7 A schematic representation of (**A**) the preparation process for the exosome-based drug delivery system (cEM@DEP-siRNA). First, exosomes (EXO) are extracted from macrophage cells and modified with a targeting peptide (cRGD) to enhance tumor selectivity. These modified exosomes (cEXO) are then used to form the final delivery system by encapsulating DEP-siRNA, which consists of a chemotherapeutic agent (panobinostat) and PPM1D-targeting siRNA. (**B**) The cEM@DEP-siRNA nanodrug is administered to animal models of diffuse intrinsic pontine glioma (DIPG) through intravenous injection via the tail vein. The drug delivery system successfully crosses the blood-brain barrier (BBB) and accumulates in the tumor due to its affinity for integrin αV, a protein highly expressed on the surface of DIPG cells. Once inside the tumor, the nanodrug is taken up by the cancer cells, allowing for targeted therapy. *Figure adapted/reprinted from Chen et al.* [164] *under a Creative Commons Attribution (CC BY) license.*

powerful delivery mechanism. Exosomes offer distinct advantages for gene-editing applications, including non-immunogenicity, precision targeting, and efficient BBB penetration. Preclinical studies have demonstrated the potential of exosome-mediated CRISPR/Cas9 delivery. For instance, radiotherapy remains a cornerstone of glioblastoma treatment. However, therapeutic resistance limits its efficacy, necessitating additional strategies. Recent studies employed in vivo loss-of-function genome-wide CRISPR screens in orthotopic tumor models under radiation treatment to identify synthetic lethal genes associated with radiotherapy. One such study highlighted glutathione synthetase (GSS) as a key regulator of radioresistance through ferroptosis suppression. High GSS levels correlated with poor prognosis and relapse in glioma patients. Mechanistically, GSS depletion disrupted glutathione synthesis, inactivated GPX4, and caused iron accumulation, amplifying radiotherapy-induced ferroptosis. To address delivery challenges, the study introduced an innovative genome editing system where CRISPR/Cas9 complexes were loaded into EVs modified with Angiopep-2 (Ang) and TAT peptides (Fig. 8). This dual modification allowed the EVs to cross the BBB and target glioblastoma tissue effectively. These EVs achieved remarkable GSS gene editing efficiency in glioblastoma (up to 67.2%) with minimal off-target effects, demonstrating the feasibility of combining unbiased genetic screens and CRISPR-Cas9-based gene therapy for identifying therapeutic targets and overcoming radioresistance [168]. This study underscores the promise of integrating CRISPR/Cas9 with exosome-mediated delivery systems to enhance glioblastoma treatment outcomes.

Exosomes for ischemic stroke (IS) and brain injury (TBI)

IS and TBI rank among the leading causes of long-term neurological disability, affecting millions globally. The complexity of these conditions, compounded by the challenges of delivering therapeutic agents across the BBB, has spurred significant interest in exosome-based interventions. Exosomes offer unparalleled advantages by enabling the targeted delivery of neuroprotective and regenerative biomolecules while bypassing the BBB and minimizing off-target effects.

Current clinical therapeutic methods for IS commonly include drug therapy, such as thrombolytic and neuroprotective drugs, and intravascular stent therapy. While these treatments have proven beneficial, they have limited efficacy and potential side effects. Over the years, exosome-based therapies have emerged as a promising option in the treatment of IS, and their combination with existing therapies could reduce the side effects associated with drug and stent treatments. Research in this area is ongoing, and it appears that exosome-based therapies could open doors to innovative treatment options that could offer more effective and safer alternatives for IS patients. Exosome-based therapies could be a crucial step in advancing the clinical management of IS. Tissue plasminogen activator (tPA) is one of the most widely used thrombolytic drugs for the treatment of ischemic stroke. tPA is responsible for converting plasminogen to plasmin, which then degrades fibrin, facilitating the dissolution of blood clots. The therapeutic efficacy of tPA is limited, however, by its potential to cause intracerebral hemorrhage when it crosses the BBB and enters brain parenchyma [169]. Researchers have attempted to address this issue by using exosomes as carriers for tPA, ensuring that the drug is delivered directly to the thromboembolic site, thereby reducing the risk of hemorrhagic complications. Khalil and Kanapathipillai developed an exosome-coated tPA nanoformulation, Exo-tPA, which demonstrated superior stability compared to free tPA. The encapsulation of tPA within exosomes also reduced the likelihood of tPA penetration into the brain parenchyma, mitigating the risk of intracerebral hemorrhage and enhancing the overall therapeutic efficacy [170].

Neuroprotective drugs play a crucial role in managing stroke, particularly in cases where the thrombolysis window has passed, and the patient is no longer eligible for tPA therapy. Edaravone is a neuroprotective drug that has been widely used to mitigate the damage caused by oxidative stress following ischemia and hypoxia. However, edaravone has limitations, including its short half-life, poor BBB penetration, and low bioavailability [171]. To address these challenges, Li et al. used macrophage-derived exosomes to load edaravone (Exo + Edv). Their research demonstrated that edaravone-loaded exosomes exhibited improved brain targeting, enhanced bioavailability, and reduced neuronal damage [172]. Additionally, the use of plasma-derived exosomes has been shown to enhance edaravone's therapeutic effects by improving its ability to cross the BBB and targeting brain cells more effectively [173]. These findings indicate that exosome-based drug delivery systems offer significant potential in enhancing the efficacy of neuroprotective therapies for ischemic stroke. In addition to pharmacological therapies, mechanical therapies such as intravascular stenting are commonly used in the treatment of ischemic stroke, particularly in cases of large-vessel occlusion. While stenting is effective in restoring blood flow, the risk of in-stent restenosis, which occurs when the blood vessel narrows again after stent implantation, remains a major concern. Traditional drug-eluting stents have been developed to reduce restenosis, but the use of these stents can delay endothelial healing and increase the risk of late-stage restenosis [174]. Exosomes are now being investigated as potential candidates for coating stents due to their ability to promote endothelial growth, reduce inflammation, and enhance angiogenesis. Exosomes derived from MSCs

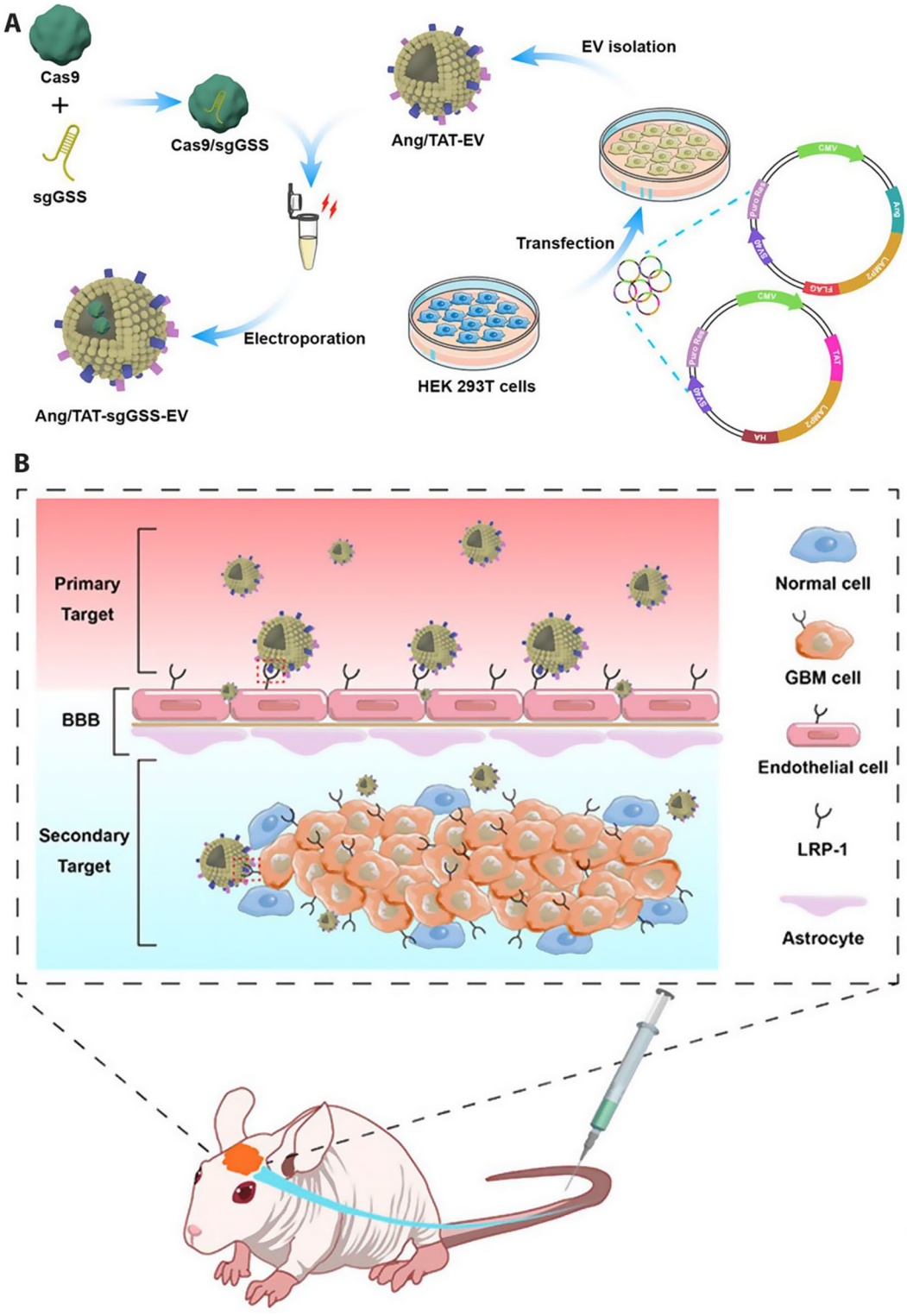


Fig. 8 Engineered Extracellular Vesicle-Delivered CRISPR/Cas9 for Radiotherapy Sensitization of Glioblastoma. (**A**) Ang and TAT peptide are modified to EVs membrane surface to obtain engineered EVs with glioma targeting and tumor-penetrating functions. (**B**) Schematic illustration of EVs for in vivo delivery of Cas9 protein and sgRNA for the treatment of brain tumor. *Figure reprinted from Liu et al.* [168] *with permission under the Creative Commons CC BY-NC-ND 4.0 license.* © 2023 The Authors. Published by American Chemical Society. (https://creativecommons.org/licenses/by-nc-nd/4.0/)

have shown great promise in preventing in-stent restenosis. Studies have shown that MSC-derived exosomes contain various bioactive molecules, including miRNAs and proteins, that can promote vascular repair and endothelial regeneration [175]. Moreover, exosomes are advantageous because they can be loaded with multiple drugs, including anti-inflammatory and angiogenic agents, which work together to enhance the therapeutic effects. Recently, Hu et al. developed an innovative bioresponsive exosome-eluting stent that releases exosomes in response to ROS generated during ischemia-reperfusion injury. This system improved endothelial cell regeneration and reduced the risk of restenosis compared to traditional drug-eluting stents. The potential of exosomes to serve as drug-eluting coatings for stents may lead to improved outcomes in the management of ischemic stroke [174]. One of the limitations of exosome-based therapies is their inability to specifically target ischemic lesion sites, which reduces their therapeutic efficacy. To overcome this, researchers are exploring various strategies to enhance the targeting capabilities of exosomes. Genetic engineering is one of the most widely used approaches, where targeting peptides or proteins are fused to the surface of exosomes to direct them to specific cells or tissues. For example, Alvarez-Erviti et al. demonstrated that fusing the RVG to exosomes allowed them to specifically target neurons in the brain, improving the efficacy of gene delivery in an animal model of ischemic stroke [25]. Similarly, other studies have used genetically engineered exosomes to deliver siRNAs and miRNAs to target ischemic lesions and promote neuroprotection [176, 177]. These approaches have shown promising results in preclinical studies. Chemical modification techniques are another strategy used to improve exosome targeting. One such method involves conjugating peptides, such as the RGD peptide, to the surface of exosomes. The RGD peptide binds to integrins on the surface of cerebral vascular endothelial cells in ischemic brain regions, enhancing the delivery of therapeutic exosomes to the target site. Tian et al. demonstrated that RGD-functionalized exosomes exhibited enhanced targeting of ischemic brain tissue and promoted neuroprotection in a mouse model of stroke [178]. Other targeting strategies, such as the use of mannose-conjugated exosomes, have been developed to specifically target microglia, which play an essential role in the immune response during stroke [179]. Chemical modification techniques are not only cost-effective but also more suitable for large-scale production, making them highly attractive for clinical application. Magnetic targeting is another innovative strategy to enhance exosome delivery to ischemic lesions. Iron oxide nanoparticles can be loaded into exosomes, and an external magnetic field can be applied to guide the exosomes to the target site. This method has been shown to improve the targeting and retention of therapeutic exosomes in ischemic brain tissue, further enhancing their therapeutic effects [180]. While magnetic targeting holds great promise, its application may be limited by the complexity of human anatomy, and further research is needed to optimize this technique for clinical use.

TBI represents a multifaceted therapeutic challenge due to its dual-phase pathology. Primary injuries involve direct mechanical trauma, while secondary injuries encompass delayed processes such as oxidative stress, neuroinflammation, and BBB disruption. Exosome-based therapies hold immense promise in mitigating these processes by delivering targeted molecular payloads, modulating neuroinflammation, and promoting neural regeneration [181]. Neuroinflammation serves as a double-edged sword in TBI recovery, offering initial protective mechanisms but potentially exacerbating damage through prolonged inflammation. Exosomes, particularly those derived from MSCs, effectively modulate this response by reducing the activity of pro-inflammatory cytokines (e.g., TNF-α and IL-6) and upregulating anti-inflammatory cytokines like IL-10. In a weight-drop-induced TBI rat model, intracerebroventricular microinjection of hADSC-derived exosomes promoted functional recovery by suppressing neuroinflammation, reducing neuronal apoptosis, and increasing neurogenesis. These exosomes were primarily taken up by microglia/macrophages, inhibiting their activation through NFκB and P38 mitogen-activated protein kinase pathways, ultimately facilitating brain repair and recovery [182]. Furthermore, neural stem cell-derived exosomes have shown unique benefits in reducing inflammation and oxidative stress. Zhong et al. highlighted their role in attenuating microglial activation, further emphasizing their neuroprotective potential (Fig. 9) [183].

The use of miRNAs within exosomes has gained significant attention for their ability to regulate gene expression in injured neural tissue. In the context of repetitive mild TBI, microglial exosomal miR-124-3p plays a crucial role in alleviating neurodegeneration and improving cognitive outcomes. exosomal miR-124-3p targets Rela, an inhibitory transcription factor of ApoE, thereby enhancing β-amyloid clearance and reducing neurodegeneration. Intravenously injected exosomal miR-124-3p is taken up by hippocampal neurons, leading to cognitive improvements, highlighting its potential as a therapeutic strategy for neurodegenerative disorders.

Besides, incorporating exosomes into biocompatible scaffolds has emerged as a novel approach to prolong their therapeutic presence at injury sites. Hajinejad et al. investigated the efficacy of a cell-free-based therapy strategy using exosomes derived from human neural stem cells and a novel nano-scaffold in rats subjected to TBI. Their findings demonstrated that exosomes in

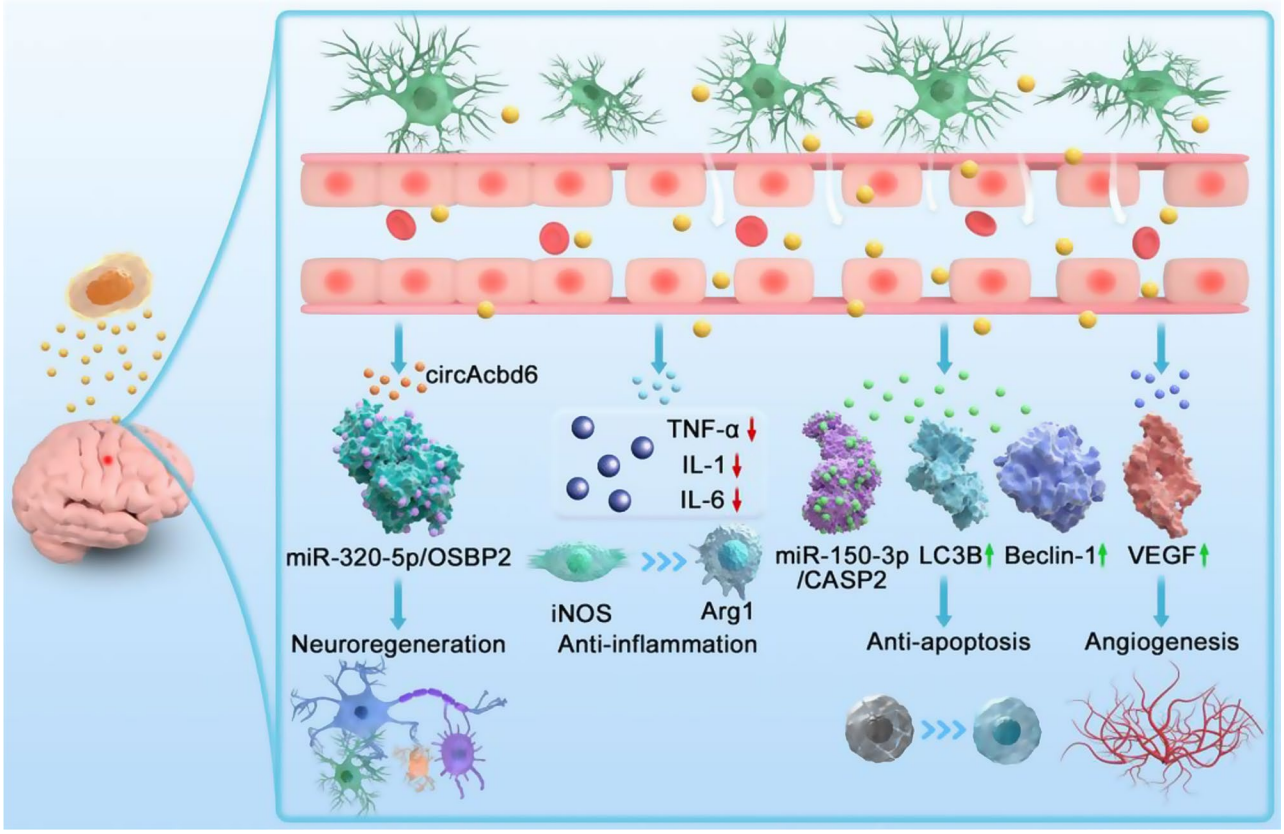


Fig. 9 A schematic representation illustrates the potential mechanisms through which exosomes secreted by neural stem cells (NSCs) contribute to the therapeutic effects of TBI. These exosomes, rich in bioactive molecules, can freely cross the BBB, suppress neuroinflammation and neuronal apoptosis, and enhance neuroregeneration and angiogenesis. In terms of neuroregeneration, NSCs release exosomes containing circAcbd6, which facilitates the differentiation of NSCs into cholinergic neurons via the miR-320-5p/oxysterol-binding protein–related protein 2 axis. Additionally, NSC-derived exosomes modulate immune responses by reducing inflammation, suppressing the expression of proinflammatory cytokines such as TNF-α, IL-1, and IL-6, and shifting microglial polarization from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype. Regarding anti-apoptotic effects, NSC-derived exosomes deliver miR-150-3p, a microRNA that effectively inhibits neuronal apoptosis. Furthermore, these exosomes enhance the expression of autophagy marker proteins LC3B and beclin-1, which contributes to reducing nerve cell apoptosis. Lastly, in promoting angiogenesis, NSC exosomes elevate VEGF expression, thereby accelerating the formation of new blood vessels. *Figure adapted/reprinted from Zhong et al.* [183] *under a Creative Commons Attribution (CC BY) 4.0 license.* © 2023 The Authors. Published by Stem Cell Research & Therapy. (https://creativecommons.org/licenses/by/4.0/)

combination with a 3D nano-scaffold containing a biomotif of (stromal cell-derived factor 1α) SDF1α (Nano-SDF) significantly decreased oxidative stress, reduced neuroinflammatory responses, and promoted neurogenesis in the sub-ventricular zone of the lateral ventricle. The study further revealed that exosomes with Nano-SDF suppressed the expression of Toll-like receptor 4 and its downstream signaling pathway, including NF-kβ and IL-1β, thereby mitigating reactive gliosis at the injury site. These findings suggest that a cell-free-based therapy strategy leveraging exosomes and Nano-SDF could serve as a promising treatment approach for TBI [184].

Addressing psychiatric and rare brain disorders through Exosomal innovation

Exosomes are emerging as an innovative therapeutic platform for addressing psychiatric disorders and rare neurological diseases, with their ability to deliver neuroactive molecules and genetic therapies directly to the brain. Their natural capacity to traverse the BBB and selectively target specific cell populations has catalyzed significant interest in their potential to treat these complex, often neglected conditions.

Psychiatric disorders such as depression and schizophrenia frequently involve disruptions in neurochemical pathways, inflammation, and impaired neurogenesis—key therapeutic targets for exosome-based strategies. Liu et al. explored a novel approach for treating major depressive disorder (MDD) by engineering RVG-modified exosomes to overexpress BDNF (RVG-BDNF-Exos). These exosomes effectively crossed the BBB, delivering BDNF directly to neurons in the hippocampus and prefrontal cortex. In a mouse model of depression, RVG-BDNF-Exos significantly increased BDNF levels, modulated the BDNF/TrkB/AKT signaling pathway, and improved depressive-like

behaviors. The treatment also reduced neuroinflammation by decreasing microglial and astrocyte numbers while enhancing neurogenesis and synaptic plasticity, as indicated by elevated expression of neuronal markers MAP2 and DCX and synaptic proteins PSD95 and Syn-1. These findings highlight the potential of serum exosomal BDNF as both a biomarker and a therapeutic target for MDD, paving the way for personalized psychiatric treatments [185]. Additionally, recent research by Huang et al. has demonstrated that hippocampal exosomes derived from stroke models may exacerbate post-stroke depression (PSD) by altering key neurobiological mechanisms. In their study, injection of hippocampal exosomes from stroke significantly worsened depressive-like behaviors in mice, as evidenced by behavioral tests such as sugar water preference, open field, and forced swim tests. These exosomes increased the expression of depression-associated proteins, including proBDNF and p75NTR, while simultaneously decreasing synapse-associated proteins such as Synaptotagmin and PSD95. Furthermore, Golgi staining revealed a notable reduction in dendritic spine density following treatment with these exosomes. These findings suggest that hippocampal exosomes from stroke contribute to PSD progression by modulating neurogenesis and synaptic plasticity, highlighting their potential as both diagnostic markers and therapeutic targets for PSD [186]. Furthermore, inflammation has emerged as a significant contributor to psychiatric disorders. Exosomes enriched with anti-inflammatory miRNAs, including miR-124 and miR-146a, have demonstrated the ability to reduce neuroinflammation and alleviate behavioral deficits in rodent models of depression and schizophrenia [187, 188]. Rare neurological diseases, such as Niemann-Pick and Gaucher's diseases, lysosomal storage defects drive progressive neurodegeneration [189]. Exosomes offer an innovative solution for delivering therapeutic enzymes and genetic treatments across the BBB. Conventional enzyme replacement therapies are typically ineffective in treating CNS symptoms due to their inability to cross the BBB. However, exosome-based delivery systems have been shown to transport therapeutic enzymes directly to neuronal lysosomes, thereby correcting metabolic defects in preclinical models of Niemann-Pick and Gaucher's diseases [190, 191].

Exosomes are also being explored as carriers for CRISPR/Cas9 systems to address genetic mutations underlying orphan neurological conditions. In preclinical models of Rett syndrome, exosome-mediated delivery of CRISPR systems successfully restored MECP2 expression, resulting in symptomatic improvement [192].

Clinical translation and challenges

While exosome-mediated therapies hold immense potential, their transition from laboratory research to clinical practice faces significant challenges. These hurdles include preclinical validation, immune compatibility, and the complexities of regulatory approvals and manufacturing scalability. Below, we explore the progress and obstacles associated with the clinical translation of exosome-based therapies.

From lab to clinic: preclinical and clinical evidence

Although most exosome-based treatments remain in the preclinical phase, early clinical trials have yielded promising results, showcasing their safety, efficacy, and translational potential for neurological disorders. These advancements underline the growing feasibility of exosomes as therapeutic tools for CNS targeting.

One of the initial clinical applications of exosome therapy involved MSC-derived exosomes for IS. Among the notable studies, the NCT03384433 trial stands out. This randomized, single-blind, placebo-controlled, phase 1/2 trial evaluates the safety and efficacy of exosomes derived from allogenic MSCs in improving disability outcomes in patients with acute ischemic stroke. Conducted by Isfahan University of Medical Sciences, it is currently recruiting participants [193]. Similarly, the NCT06138210 trial is a randomized, double-blinded, placebo-controlled, dose-escalation study. It explores the safety and preliminary efficacy of intravenous exosomes derived from human induced pluripotent stem cells (GD-iExo-003) for treating acute ischemic stroke patients. This study is also actively recruiting participants [194]. These trials highlight the potential of exosome-based therapies in addressing neurological deficits caused by IS, offering hope for innovative treatments in stroke recovery.

Efforts to treat AD with exosome-based therapies have also reached clinical stages. A notable trial, NCT04388982, investigates the use of MSC-derived exosomes for treating AD. This study evaluates their safety, tolerability, and potential cognitive benefits in patients [195].

Across these trials, exosomes have consistently exhibited excellent safety profiles, with minimal immunogenicity and adverse reactions. This aligns with their endogenous origin, which minimizes the likelihood of triggering robust immune responses. However, challenges remain, particularly regarding the scalability of exosome production to meet clinical-grade standards.

Immunogenicity and biocompatibility of engineered exosomes

The immune profile of exosomes plays a pivotal role in their therapeutic application, directly influencing their efficacy and safety. Naturally derived exosomes,

especially those sourced from autologous cells, exhibit minimal immunogenicity due to their endogenous origin. This characteristic makes them particularly suitable for long-term or repeated administration in chronic neurological conditions, where sustained therapy is critical [196].

However, surface modifications designed to enhance the functionality of exosomes, such as ligand conjugation or the integration of nanoparticles, can influence their immunogenicity. For example, Tian et al. demonstrated that excessive chemical functionalization of exosomes could elicit an immune response, emphasizing the need to preserve the innate biocompatibility of these vesicles [114]. To counteract such risks, immune evasion strategies like PEGylation have been developed. These strategies not only improve circulation time by reducing immune recognition but also enhance the therapeutic viability of engineered exosomes [106].

Beyond mitigating immune responses, exosomes have also been utilized to actively modulate immune function. For instance, MSC-derived exosomes loaded with anti-inflammatory cytokines, such as IL-10, have shown promising results in reducing neuroinflammation in preclinical models of multiple sclerosis and depression. By suppressing inflammatory pathways, these exosomes contribute to restoring neural homeostasis and improving behavioral outcomes [197].

Regulatory and manufacturing hurdles in Exosome-Based therapies

The journey from laboratory research to clinical implementation of exosome-based therapies is fraught with challenges, particularly in the areas of manufacturing, regulation, and scalability. These obstacles present significant barriers to translating promising preclinical findings into viable therapeutic options.

One of the primary challenges lies in the methods used for exosome isolation. Techniques such as ultracentrifugation and size-exclusion chromatography are labor-intensive and often yield heterogeneous populations of exosomes. These heterogeneities can impact the consistency and efficacy of the final product. Advances in microfluidics-based isolation methods are emerging as a scalable and efficient alternative, offering higher purity and reproducibility [198]. Purity is especially critical for clinical-grade exosomes, as the co-isolation of non-vesicular contaminants such as lipoproteins could compromise safety and therapeutic efficacy.

Regulatory hurdles also complicate the clinical translation of exosome therapies. Agencies such as the FDA and EMA require comprehensive characterization of exosome preparations, including parameters like size, zeta potential, and molecular composition. Advanced analytical tools, such as nanoparticle tracking analysis (NTA) and proteomics, are increasingly being integrated into quality control workflows to meet these stringent requirements [199]. Furthermore, the classification of exosome-based treatments—whether as biologics, drugs, or devices—remains ambiguous. This lack of clear guidelines hampers regulatory approval processes and creates uncertainty for researchers and manufacturers alike. Establishing precise and consistent regulatory frameworks will be essential to streamline the approval and commercialization of exosome therapies [196].

Another critical issue is the scalability of exosome production. While bioreactor systems offer a promising solution for large-scale harvesting of exosomes, significant optimization is still required to reduce production costs while maintaining product quality. Without these advancements, scaling exosome-based therapies for widespread clinical use remains a formidable challenge [197].

Future directions in exosome nanobiotechnology

The field of exosome nanobiotechnology is advancing rapidly, offering transformative possibilities for treating neurological diseases. As researchers continue to uncover their potential, attention is shifting toward addressing existing limitations, refining exosome designs, and exploring cutting-edge applications.

Emerging trends in exosome research

Exosome research is transitioning from basic discoveries to translational and clinical applications. Several emerging trends are shaping the future of this field: First, advancements in exosome isolation techniques are enhancing efficiency and scalability. Traditional methods, such as ultracentrifugation, are being replaced by microfluidics and nanotechnology-based platforms. For example, Ortega-Sanchez et al. developed microfluidic systems capable of single-vesicle analysis, enabling unparalleled resolution in exosome characterization [42].

Second, innovative surface modifications are expanding the specificity and functionality of exosomes. Exosomes functionalized with peptide ligands targeting the transferrin receptor, for instance, have shown enhanced BBB penetration and improved targeting of neurological tissues [42].

Lastly, hybrid exosome-nanoparticle systems are emerging as next-generation platforms. These hybrids combine the biocompatibility of exosomes with the functional versatility of synthetic nanoparticles, enabling multi-modal therapies. Fatima et al. explored the use of exosomal shuttles for precision therapeutics, highlighting the role of hybrid exosome-nanoparticle formulations in enhancing targeted drug delivery to brain tumors. Their study discusses how engineered exosomes, functionalized with surface ligands, can improve nanoparticle

uptake and drug bioavailability, thereby optimizing therapeutic outcomes [200].

Multi-Modal approaches for neurological diseases

Multi-modal strategies, which integrate therapeutic and diagnostic functions, are increasingly being developed to tackle the complexity of neurological diseases. Theranostic exosomes represent a significant breakthrough in this area. These exosomes are engineered to deliver therapeutic agents while simultaneously enabling real-time imaging to monitor disease progression. In AD research, theranostic exosomes incorporating imaging agents and anti-inflammatory molecules have demonstrated the ability to track disease progression and evaluate treatment efficacy [201].

Combination therapies are another key focus of multimodal approaches. Exosomes are being engineered to deliver multiple agents targeting different pathways in neurodegenerative diseases. For instance, Yadav et al. investigated macromolecular neurotherapeutic approaches using exosome-based drug delivery systems. Their research focused on exosome-engineered therapies targeting neurodegenerative conditions such as AD and PD, where combination therapies involving siRNA and neurotrophic peptides showed promising results in reducing pathological hallmarks [202]. Similarly, Zhao et al. highlighted the use of extracellular vesicle-based theranostic platforms, where exosomes were engineered to not only deliver therapeutic agents but also serve as biomarkers for early disease detection. This dual function allows for real-time monitoring of treatment response, further optimizing combination therapy approaches [203].

Integrating AI for exosome engineering

AI is revolutionizing exosome research, providing powerful tools for optimizing their design and application. AI is being employed to identify optimal surface modifications for exosome targeting. Machine learning algorithms analyze molecular databases to predict the most effective ligands for crossing the BBB and engaging specific neuronal receptors. In addition, AI plays a crucial role in overcoming exosome heterogeneity by constructing novel targeting ligands through generative AI approaches. AI-driven techniques, such as Bayesian active learning and deep learning-based classifiers, enable the prediction of exosome uptake and tissue specificity. By leveraging support vector machines (SVM), random forests (RF), and convolutional neural networks (CNNs), AI enhances the characterization of exosome surface markers and their pharmacokinetic properties. Moreover, AI aids in exosome engineering by optimizing their cargo-loading efficiency, ensuring that therapeutic payloads such as proteins, RNA, or small molecules reach their intended targets with high precision. Recent developments include AI-designed tissue-targeting peptides that enhance exosome-based drug delivery for precision medicine. These AI-driven approaches, combined with multiplexed molecular profiling and high-throughput screening, have the potential to transform exosome therapeutics into hyper-personalized drug delivery systems [204]. This synergy between AI and exosome technology opens the door to more effective treatments for neurological disorders, cancer, and regenerative medicine applications.

Conclusion: the transformative impact of exosome nanotechnology

Exosome-based drug delivery systems herald a paradigm shift in the treatment of neurological disorders, offering unparalleled precision, efficacy, and biocompatibility. As naturally derived nanocarriers capable of crossing the BBB, exosomes have become a cornerstone of nanobiotechnology, redefining therapeutic strategies for some of the most challenging and debilitating conditions. This concluding synthesis highlights the transformative potential of exosome nanotechnology and its profound implications for the future of neurological health.

Neurological disorders such as AD, PD, glioblastoma, and major depressive disorder present formidable therapeutic challenges due to the highly selective and restrictive nature of the BBB. Traditional drug delivery systems have struggled to achieve adequate CNS bioavailability while minimizing systemic side effects. Exosome-mediated delivery offers a novel and effective solution by leveraging the natural ability of these vesicles to cross the BBB and deliver diverse therapeutic payloads, including small molecules, RNA, proteins, and CRISPR/Cas9 systems. By integrating endogenous compatibility with advanced engineering techniques, exosomes have elevated the concept of targeted therapy to unprecedented levels of sophistication. Surface modifications, such as the incorporation of transferrin ligands or neuropilin-1 peptides, have refined their targeting precision. Simultaneously, bioengineered hybrid systems have expanded their functional versatility, making it possible to selectively deliver drugs to specific neuronal populations. These advancements minimize off-target effects, reduce treatment toxicity, and maximize therapeutic efficacy. Exosome-based platforms transcend the limitations of traditional drug delivery systems by seamlessly integrating therapeutic and diagnostic functionalities. This theranostic capability enables real-time monitoring of disease progression, therapeutic efficacy, and patient response. For instance, exosome systems equipped with imaging probes and therapeutic agents have demonstrated remarkable potential in tracking neuroinflammation and tumor regression in both preclinical and clinical settings. Such multi-modal platforms are paving the way

for personalized medicine, wherein treatments can be tailored to the molecular profiles of individual patients, revolutionizing the approach to neurological care.

While exosomes hold immense promise, their integration into clinical practice is not without challenges. One of the foremost barriers lies in scaling up production to meet clinical-grade standards while maintaining the purity and functional integrity of exosomes. Current manufacturing protocols, such as ultracentrifugation, are labor-intensive and yield heterogeneous populations, posing bottlenecks for scalability. Advances in microfluidics-based isolation, AI-driven optimization of manufacturing pipelines, and standardized quality control protocols are actively addressing these challenges. Another significant hurdle is regulatory ambiguity regarding the classification of exosome-based therapies. Whether exosomes are categorized as biologics, drugs, or devices remains unresolved, complicating their pathway to regulatory approval. Clear and consistent regulatory frameworks are essential to streamline the clinical translation of exosome-based treatments. Long-term safety evaluations are equally critical to understanding the immunogenicity and biodistribution of engineered exosomes. While early clinical trials have demonstrated promising safety profiles, sustained research is necessary to confirm their viability for chronic and repeat administration in neurological disorders. Addressing these concerns is vital to ensuring the broader acceptance and adoption of exosome-based therapeutics.

The convergence of exosome nanotechnology, artificial intelligence, and advanced bioengineering heralds an era of unprecedented innovation in neurological health. By enabling targeted, efficient, and minimally invasive treatments, exosomes are not merely addressing unmet therapeutic needs—they are redefining what is possible in medicine. Looking to the future, the integration of AI-driven predictive models, patient-specific exosome platforms, and multi-modal approaches promises to accelerate the development of precision therapies. In the context of neurodegenerative diseases, exosomes have the potential to slow disease progression, enhance quality of life, and extend survival rates. For psychiatric disorders, they offer groundbreaking avenues to address neuroinflammation and synaptic dysfunction, laying the foundation for transformative treatment paradigms.

The future of exosome-based therapies lies in fostering collaboration among multidisciplinary researchers, clinicians, regulators, and industry stakeholders. Investment in translational research, robust clinical trials, and scalable manufacturing pipelines is essential to unlock the full potential of this technology. Furthermore, the establishment of regulatory clarity and a patient-centric approach will be critical to ensuring the successful clinical implementation of exosome therapies.

In conclusion, exosome nanotechnology represents a transformative force in the battle against neurological disorders. By redefining drug delivery paradigms, uniting therapeutic and diagnostic applications, and overcoming long-standing barriers to CNS treatment, exosomes have positioned themselves at the forefront of modern medicine. Their impact on neurological health is not just promising—it is revolutionary, offering a new era of hope for patients and clinicians alike.

Author contributions
SM conducted the literature review, performed data analysis, and contributed to writing the original draft. MM prepared figures and tables and assisted in drafting the manuscript. SHK validated the content and contributed to writing, reviewing, and editing. YP was responsible for conceptualization, supervision, and reviewing/editing the manuscript. YNE also contributed to conceptualization and manuscript review/editing.

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