Proceedings of ICBioMed 2025 Symposium: AI for Healthcare: Advanced Medical Data Analytics and Smart Rehabilitation
DOI: 10.54254/2753-8818/2025.AU27718

Exosomes as a Double-Edged Sword in Neurodegenerative Diseases: Roles and Engineering Strategies

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Abstract. Central nervous system (CNS) disorders, particularly neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD), represent major challenges in contemporary medical research. The blood–brain barrier (BBB), an essential protective interface of the CNS, helps maintain brain homeostasis but also restricts the entry of most drugs and therapeutic molecules into the brain, severely limiting clinical efficacy. As nanoscale extracellular vesicles, exosomes possess an intrinsic capacity to traverse biological barriers—including the BBB—and can transport a wide range of bioactive cargos to modulate various physiological and pathological processes. Research indicates that exosomes play pivotal roles in the pathological progression of neurodegenerative diseases: they can mediate the spread of pathogenic proteins while also contributing protective effects such as the clearance of pathological aggregates and the attenuation of neuroinflammation. Moreover, engineering strategies (e.g., drug loading and targeted surface modification) expand the potential of exosomes as drug-delivery vehicles. Despite promising diagnostic and therapeutic prospects, challenges including exosomal heterogeneity, limited loading efficiency, imperfect targeting specificity, and stability in clinical settings remain significant bottlenecks. With further advances in exosome engineering, standardized characterization, and scalable production, exosomes hold promise as important tools for the early diagnosis and precision treatment of neurodegenerative diseases.

Keywords: Exosomes, Neurodegenerative Diseases, Exosome Engineering, Targeted Drug Delivery, Blood–Brain Barrier (BBB)

1. Introduction

The central nervous system (CNS) has a very limited capacity for self-repair following injury. In adult mammals, neurons regenerate poorly, and the physical and biochemical barriers of the blood–brain barrier (BBB) further complicate treatment of CNS disorders [1]. The BBB, formed by brain microvascular endothelial cells, pericytes, astrocytes, and tight junctions, maintains a highly selective permeability that effectively blocks harmful exogenous substances from entering the brain. However, this same property also makes it a formidable obstacle to drug delivery. More than 98% of small-molecule drugs and nearly all macromolecular drugs fail to cross the intact BBB, posing a major challenge to pharmacological intervention [2]. The BBB also prevents pathological molecules

DOI: 10.54254/2753-8818/2025.AU27718

in the brain from reaching the periphery, further hindering early diagnosis. As a result, recovery and treatment of CNS injuries and neurodegenerative disorders remain highly difficult.

Among CNS disorders, Alzheimer's disease (AD) and Parkinson's disease (PD) are the most representative neurodegenerative conditions. AD is the leading cause of dementia, affecting nearly 50 million people worldwide, while PD is the second most prevalent, with more than 8.5 million cases reported globally in 2019. Both diseases involve highly complex pathogenic mechanisms that remain incompletely understood. Clinically, available treatments focus primarily on symptom management and show limited efficacy. Early diagnosis is especially challenging due to the absence of reliable biomarkers and standardized detection methods [3]. The heavy burden of disease and the persistent diagnostic and therapeutic barriers underscore the urgent need for innovative strategies to address AD, PD, and other neurodegenerative CNS disorders.

In recent years, exosomes have emerged as a novel approach for crossing the BBB and intervening in CNS diseases. Exosomes are nanoscale extracellular vesicles secreted by cells, typically 30–150 nm in diameter. They are capable of efficiently traversing the BBB [3] and act as mediators of communication between the CNS and the periphery. Increasing evidence shows that exosomes play important roles in the pathology of AD and PD: they transport and disseminate misfolded proteins—such as Aβ and tau in AD, and α-synuclein in PD—between neurons, thereby facilitating the spread of disease pathology [4]. At the same time, exosomes can be isolated from body fluids including blood and cerebrospinal fluid, where they reflect pathological changes in the brain, making them promising biomarker candidates. Furthermore, exosomes can serve as natural drug carriers, delivering therapeutic molecules across the BBB directly to brain lesions. In sum, due to their unique ability to penetrate the BBB and mediate intercellular communication, exosomes exhibit dual potential as both diagnostic and therapeutic tools in AD and PD, representing an emerging strategy for combating neurodegenerative CNS diseases.

2. Exosomes

2.1. Definition and classification: extracellular vesicles and exosomes

Extracellular vesicles (EVs) are lipid bilayer-enclosed particles secreted by cells, incapable of autonomous replication, and found in nearly all body fluids and tissues [5]. The International Society for Extracellular Vesicles (ISEV), in its Minimal Information for Studies of Extracellular Vesicles guidelines, defines EVs as an umbrella term covering various subtypes.

EVs can be categorized according to multiple criteria. Based on particle size, they are divided into small EVs (<200 nm in diameter) and large EVs (>200 nm). Based on density, they may be classified as low-, medium-, or high-density vesicles. From a subcellular origin perspective, ectosomes bud directly from the plasma membrane, whereas exosomes are generated within multivesicular bodies (MVBs) and subsequently released into the extracellular environment [5].

Exosomes, typically ranging from 30 to 150 nm in diameter, are the most intensively studied class of EVs and fall within the category of small EVs. They are secreted by nearly all cell types and carry a diverse array of bioactive molecules derived from their parent cells, including lipids, proteins, nucleic acids, amino acids, and metabolites. The molecular composition of exosomes reflects not only the identity of their originating cells but also their physiological or pathological states (e.g., oxidative stress, oncogenic transformation). As such, exosomes are widely regarded as critical mediators linking cellular functions with disease phenotypes. Growing evidence highlights their essential roles in both the maintenance of physiological homeostasis and the progression of multiple pathological processes.

DOI: 10.54254/2753-8818/2025.AU27718

2.2. Historical trajectory and conceptual evolution of exosome research

2.2.1. Early research on exosomes: from discovery to nomenclature and research expansion

The origins of exosome research can be traced back to the mid-20th century in the field of hematology. In 1945, Chargaff discovered that "the addition of high-speed sediment to plasma supernatant significantly shortens clotting time," which is considered the starting point of exosome research. Subsequently, Peter Wolf first described a substance derived from platelets but separable from them through ultracentrifugation, which he termed platelet dust [6]. Later, Crawford visualized these vesicles for the first time and referred to them as microparticles [7].

It was not until the 1980s that the concept of exosomes gradually took shape. Independently, Johnstone and Stahl discovered a novel mechanism of intracellular sorting and transport that laid the foundation for understanding exosome biogenesis [8,9]. In 1987, R. M. Johnstone and colleagues were the first to use the term exosome to describe these vesicles [10]. These early studies provided the groundwork for subsequent exploration. With advances in molecular biology and flow cytometry, the structural and functional aspects of exosomes were studied in greater depth. Researchers identified a series of exosomal marker molecules such as Rab and ARF proteins [11], as well as tetraspanins [12]. Links between exosomes and various diseases were then uncovered, including vascular occlusion [13] and angina pectoris [14]. In 1996, Raposo and colleagues demonstrated that exosomes play a role in antigen presentation. Specifically, B lymphocytes were found to secrete exosomes carrying MHC class II molecules, which could induce T-cell immune responses, thus bringing exosome research into the field of immunology. With continued investigations, the physiological functions of exosomes in other systems also began to attract increasing attention.

2.2.2. Standardization of research: the establishment and evolution of ISEV and MISEV guidelines

In the 21st century, exosome-related research expanded exponentially. However, accompanying this growth was confusion in terminology, which became a major obstacle to progress. A survey of more than 4,000 papers published between 2000 and 2010 revealed that the most frequently used term was microparticles. Yet, this term was ambiguous, as it could refer not only to platelet-derived microparticles but also to iron oxide particles commonly used as imaging agents, or even to synthetic drug carriers. To address this, the International Society for Extracellular Vesicles (ISEV) was established in 2011. In 2014, it released the first "Minimal Information for Studies of Extracellular Vesicles" (MISEV) guidelines. The founding of ISEV marked a milestone, providing an effective platform for researchers worldwide to collaborate and share their work on extracellular vesicles (EVs) [5,15]. With the deepening of research, ISEV issued updated versions of MISEV in 2018 and 2023. These successive editions reflect the state of exosome research. Notably, participation expanded dramatically—from dozens in 2014, to hundreds in 2018, and ultimately over a thousand researchers contributing to MISEV2023—underscoring the growing interest in EVs. The key developments can be summarized in three aspects: terminology, characterization standards, and functional assays.

First, regarding terminology: In 2014, the overuse of the term exosome was a critical issue, as many studies in fact investigated mixed EV populations. MISEV therefore recommended the adoption of the general term extracellular vesicles (EVs). By 2018, significant progress had been made: the guidelines explicitly discouraged the use of exosome and microvesicle unless their biogenesis was strictly demonstrated, and instead recommended operational categories such as small

DOI: 10.54254/2753-8818/2025.AU27718

EVs and large EVs, with defined size ranges. The 2023 version further refined the nomenclature by distinguishing non-vesicular extracellular particles (NVEPs), such as lipoproteins and protein aggregates, and introduced the broader umbrella term extracellular particles (EPs). New concepts such as EV mimetics and EV corona were also added, reflecting a deeper understanding of EV surface complexity.

Second, for characterization standards, the 2014 guidelines required only minimal criteria: reporting three classes of protein markers (transmembrane/lipid-anchored, cytosolic, and non-EV-associated proteins) along with electron microscopy or atomic force microscopy imaging. In 2018, emphasis was placed on orthogonal validation using multiple methods (e.g., EM + nanoparticle tracking analysis + Western blotting), single-vesicle characterization (TEM, flow cytometry), and topological analyses distinguishing surface from luminal components (via protease/nuclease digestion). The 2023 update was even more targeted, specifying preprocessing requirements for different biofluids (blood, urine, cerebrospinal fluid, etc.), and integrating advanced techniques such as high-resolution flow cytometry, cryo-EM, and Raman spectroscopy. These advances enabled multiparametric analysis at the single-vesicle level, including size, composition, and surface markers.

Finally, regarding functional assays: In 2014, the minimum requirement was to include negative controls and dose–response curves to confirm whether a function was EV-dependent. By 2018, stricter measures were proposed, requiring exclusion of non-EV components through density gradient centrifugation or size-exclusion chromatography, and recommending comparisons across EV subtypes (e.g., small vs. large EVs) to avoid attributing functions of mixed populations to a single subtype. The 2023 guidelines incorporated more systemic considerations, emphasizing the potential interference of EV coronas, and requiring verification of whether functional molecules are intrinsic EV components or adsorbed NVEPs. Furthermore, they added standardized protocols for in vivo tracking and uptake mechanism studies, highlighting the field's increasing shift toward clinical translation.

2.3. Biogenesis mechanisms: ESCRT-dependent and ESCRT-independent pathways

The biogenesis of exosomes mainly involves four steps: the formation of early endosomes (EEs), the formation of multivesicular bodies (MVBs), the release of intraluminal vesicles (ILVs), and the degradation of ILVs [16]. Exosomes originate from invaginations of the plasma membrane. Caveolin-1, a hallmark protein, can induce membrane invagination, while asymmetric lipid distribution also drives membrane curvature. Subsequently, clathrin mediates inward budding by forming an icosahedral cage-like structure that encapsulates cargo [17]. Rab proteins play a crucial role in the formation of early endosomes by regulating vesicle fusion rates through continuous binding and hydrolysis of guanosine triphosphate (GTP) [17]. In the second stage, early endosomes undergo inward budding and maturation to form late endosomes, eventually producing MVBs containing ILVs. This process can occur through either endosomal sorting complex required for transport (ESCRT)-dependent or ESCRT-independent mechanisms. In the ESCRT-dependent pathway, budding is thought to be a passive process. ESCRT-0 first binds to the endosomal membrane via its domains that recognize ubiquitinated cargo and clathrin-binding motifs [18]. Subsequently, ESCRT-I and ESCRT-II are recruited to initiate the budding process, which is completed by ESCRT-III, whose terminal helical structures drive scission of the vesicle neck [19,20].

Exosomes can also be generated through ESCRT-independent pathways, in which ceramide plays a key role. Owing to its unique cone-shaped molecular structure, ceramide alters membrane

DOI: 10.54254/2753-8818/2025.AU27718

curvature when inserted into the endosomal membrane, thereby promoting ILV formation [21]. In this process, ceramide transfer protein (CERT) and neutral sphingomyelinase 2 (nSMase2) are two critical enzymes. nSMase2 converts sphingomyelin (SM) into ceramide, which is subsequently transferred to MVBs by CERT, further facilitating membrane invagination and ILV formation, ultimately leading to exosome release [22]. In addition, flotillin proteins and tetraspanins (e.g., CD9, CD81, Tspan7, Tspan9) have also been implicated in ESCRT-independent ILV generation [23].

After formation, ILVs face two potential fates: secretion as exosomes or degradation in lysosomes. Recent studies have identified a mechanism determining ILV destiny. ILV formation is not irreversible; a retrograde process known as back-fusion or retrofusion exists. This process requires continuous acidification of MVBs, while interferon-induced transmembrane protein 3 (IFITM3) inhibits back-fusion, thereby increasing the proportion of ILVs secreted as exosomes. Notably, approximately two-thirds of secreted exosomes originate from ILVs whose back-fusion was suppressed, suggesting that retrofused vesicles may also re-enter the secretory pathway. For ILVs destined for secretion, docking of MVBs to plasma membrane proteins followed by membrane–membrane fusion is required to release them as exosomes.

Beyond the classical endosomal pathway, studies have revealed that exosomes can also be generated via non-classical routes. Vesicles derived from other intracellular membranes may enter MVBs and be released together with ILVs. These include vesicles budding from mitochondria [24,25], the nuclear envelope [26,27], recycling endosomes [28], secretory autophagy [28], and autolysosomes [29]. These findings highlight the diversity of exosome biogenesis. Although “non-classical exosomes” represent only a minor fraction of the total secretome, their formation is often associated with pathological conditions such as tissue infection, viral invasion, and cancer [21]. Thus, the existence of different exosome subtypes often reflects the specific physiological or pathological state of the cell.

2.4. Molecular basis and cargo characteristics: membrane components and intravesicular contents

Exosomes are characterized by their typical lipid bilayer structure, which provides strong protection for their molecular cargo. Both membrane-associated molecules and intravesicular contents exhibit tissue specificity. These molecular features not only reflect the cellular origin of exosomes but also mirror the physiological state of the parent cell (e.g., oncogenic transformation or oxidative stress). Therefore, elucidating the molecular composition of exosomes is of great importance for disease detection. Exosomes are formed through a double invagination of the plasma membrane, processed via the endosomal pathway, and ultimately released upon fusion of MVBs with the plasma membrane. Consequently, exosomes retain a lipid bilayer orientation consistent with that of the cell membrane. Their components can thus be broadly categorized into membrane constituents (outer and inner leaflet) and luminal cargo.

2.4.1. Membrane components: proteins and lipids

The exosomal membrane is enriched with proteins, among which tetraspanins (CD9, CD63, CD81, CD82) are the most representative and are widely used as canonical markers. Additional membrane proteins include lysosome-associated membrane glycoproteins (LAMP-1 and LAMP-2B), adhesion molecules (CD45 and CD11b), major histocompatibility complex molecules (MHC-I and II), Rab GTPases, and annexins [30]. Phospholipids form the structural backbone of the exosomal membrane, playing a central role in maintaining vesicle integrity and function. The membrane

DOI: 10.54254/2753-8818/2025.AU27718

primarily consists of sphingomyelin, phosphatidylserine, phosphatidylcholine, phosphatidylethanolamine, cholesterol, and ceramide [31]. These lipids are asymmetrically distributed between the two leaflets: sphingomyelin is enriched in the outer membrane, phosphatidylserine is localized mainly to the inner leaflet, while phosphatidylethanolamine is more randomly distributed.

Beyond structural stabilization, lipids regulate vesicle fusion, signal transduction, and receptor recognition. In particular, phosphatidylinositol and its phosphorylated derivatives (PIPs) have gained increasing attention. PIPs act as precursors for secondary messengers in signal transduction and regulate membrane dynamics and vesicular trafficking [31]. Cholesterol has also been shown to be essential for exosome biogenesis, release, and subsequent uptake by recipient cells [32].

2.4.2. Luminal cargo: proteins, metabolites, and nucleic acids

Exosomal cargo is highly diverse, comprising proteins, nucleic acids, and metabolites. Heat shock proteins (HSP70 and HSP90) are among the most characteristic exosomal proteins, indicating cellular stress and alterations in proteostasis. In addition to structural and stress-related molecules, exosomes contain metabolic enzymes, such as glycolytic enzymes (GAPDH, ENO1, PKM2, LDHA), pentose phosphate pathway (PPP) enzymes (G6PD, PGD), and tricarboxylic acid (TCA) cycle or shuttle-related enzymes (MDH2). The presence of these enzymes suggests metabolic reprogramming in parent cells under different pathological conditions [33]. Exosomes also selectively package RNA-binding proteins, including YBX1, hnRNPA2B1, and NPM1. These proteins not only facilitate the loading of non-coding RNAs (miRNAs, lncRNAs) into exosomes but also stabilize them. For example, hnRNPA2B1 recognizes EXOmotif sequences on miRNAs, thereby promoting their selective incorporation into exosomes [34].

As crucial mediators of intercellular communication, exosomes carry a wide variety of RNA species, including small RNAs, long non-coding RNAs (lncRNAs), messenger RNAs (mRNAs), and circular RNAs (circRNAs). These RNA molecules regulate gene expression in recipient cells and participate in diverse physiological and pathological processes. Small RNAs (<200 nucleotides), including microRNAs (miRNAs), small interfering RNAs (siRNAs), and PIWI-interacting RNAs (piRNAs), modulate gene expression, RNA splicing, and post-transcriptional modifications [35]. LncRNAs (>200 nucleotides), though non-coding, play essential roles in chromatin remodeling, transcriptional regulation, and RNA processing [36]. Although less abundant, exosomal mRNAs can be transferred to recipient cells, where they are translated into proteins, thereby modulating cellular function and extending exosome-mediated signaling [37]. CircRNAs, characterized by covalently closed loop structures, exhibit high stability. They function in recipient cells largely through miRNA sponging. For instance, exosomal circRNA-100338 promotes proliferation, migration, and invasion of highly metastatic hepatocellular carcinoma cells via the miR-29a-3p/GUCD1 axis [38]. Beyond these, exosomes also contain small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), and transfer RNAs (tRNAs), which play auxiliary roles in RNA splicing, translation, and modification. These RNA species can also be transferred to recipient cells to influence RNA processing and gene regulation [35].

2.4.3. Selective loading mechanisms

These cargos are selectively enriched rather than passively encapsulated, and both protein and RNA profiles are tightly regulated by the state of the parent cells. The loading of cytoplasmic proteins is mainly mediated by the ESCRT complex. ESCRT has been widely recognized as the central

DOI: 10.54254/2753-8818/2025.AU27718

machinery for exosomal cargo sorting. Specifically, ESCRT-0 initiates the multivesicular body (MVB)-dependent cargo-sorting process by recognizing ubiquitinated signals and recruiting ESCRT-I. Notably, the ESCRT complex itself can undergo ubiquitination, thereby further regulating sorting efficiency. In addition to ubiquitination, other post-translational modifications (PTMs) such as SUMOylation, ISGylation, phosphorylation, oxidation, citrullination, glycosylation, and myristoylation can also influence protein loading into exosomes, and interactions among different PTMs have been observed.

Besides ESCRT-dependent pathways, multiple ESCRT-independent sorting mechanisms also exist. For example, Alix facilitates exosomal loading by binding to specific cargos, a process through which Argonaute 2 (Ago2) and transferrin receptor (TfR) are incorporated into exosomes [16]. Moreover, proteins containing the KFERQ pentapeptide motif can be selectively loaded into exosomal subpopulations through a process dependent on the membrane protein LAMP2A [39]. In addition to actively sorted proteins, certain ESCRT components required for vesicular trafficking, such as TSG101 and CHMP4, are also present in exosomes [40].

The selective loading of RNA into exosomes is a complex and highly regulated process that involves specific sequence signals, RNA-binding proteins, and RNA chemical modifications. For instance, modifications at the 3′ end of microRNAs (miRNAs), such as 2′-O-methylation, have been identified as key determinants of their selective loading [41]. Furthermore, EXOmotif sequences can interact with the RNA-binding protein hnRNPA2B1 to promote the packaging of miRNAs into exosomes [42]. Studies have shown that the 3′ untranslated region (UTR) of long noncoding RNAs (lncRNAs) may contain specific signal sequences that mediate their selective incorporation into exosomes [43]. Similarly, the 3′UTR of messenger RNAs (mRNAs) contains specific regulatory sequences involved in exosomal loading, and RNA-binding proteins such as YBX1 can bind to mRNAs to promote their incorporation into exosomes [43,44].

2.5. Mechanisms and regulation of exosome crossing the blood–brain barrier

The blood–brain barrier (BBB) is the most critical protective interface of the central nervous system. It is composed of brain microvascular endothelial cells, pericytes, astrocytes, as well as tight junctions and adherens junctions that form a highly specialized structure. The tight junctions between endothelial cells are strictly regulated by proteins such as occludin and junctional adhesion molecules (JAMs), thereby preventing most molecules from traversing the barrier. Under physiological conditions, the BBB maintains cerebral homeostasis through its highly selective permeability, effectively blocking toxins, pathogens, and blood-borne harmful substances from entering the brain, thus ensuring the stability of the neuronal microenvironment and the maintenance of normal neural function. However, this protective mechanism also presents major challenges for drug delivery. Studies have shown that an intact BBB not only restricts the passage of most hydrophilic and polar molecules but also prevents more than 98% of small-molecule drugs and nearly all macromolecular therapeutics from entering the brain [45]. Consequently, although small-molecule inhibitors, monoclonal antibodies, and gene therapies have shown great potential in treating neurological disorders, their clinical efficacy is often significantly reduced due to the difficulty of penetrating the BBB [46]. In addition to functioning as a physical barrier, the BBB also exhibits active efflux mechanisms, the most prominent of which is the ATP-dependent efflux transporter P-glycoprotein (P-gp). P-gp is located on the membranes of brain microvascular endothelial cells and actively pumps a wide variety of drug molecules back into the systemic circulation, thereby reducing their accumulation and bioavailability within the central nervous system. Research has shown that P-gp can efficiently expel multiple substrates, including anti-HIV

DOI: 10.54254/2753-8818/2025.AU27718

inhibitors and chemotherapeutic agents, resulting in markedly reduced drug concentrations in the brain and severely limiting therapeutic efficacy [46,47].

Crossing the blood–brain barrier (BBB) efficiently remains a critical challenge in the diagnosis and treatment of neurological disorders. Traditional approaches include invasive methods (e.g., intraventricular injection), pharmacological modifications (e.g., increasing drug lipophilicity), and physical or chemical strategies to enhance permeability. However, these methods are often accompanied by tissue damage, inflammatory responses, or limited therapeutic efficacy. By contrast, exosomes have attracted considerable attention due to their wide availability, excellent biocompatibility, and innate ability to traverse the BBB. Existing studies have demonstrated that exosomes can bidirectionally cross the BBB through multiple mechanisms, including receptor-mediated transcytosis, lipid raft–dependent pathways, and macropinocytosis; under pathological conditions such as inflammation or tumors, this capacity becomes even more pronounced. This suggests that exosomes can serve not only as disease biomarkers detectable in peripheral blood but also as novel drug delivery vehicles, capable of transporting therapeutic molecules effectively into the central nervous system, thereby offering new strategies for the diagnosis and treatment of neurological diseases.

Although exosomes are generally believed to possess intrinsic BBB-penetrating abilities, their precise transport mechanisms and regulatory conditions remain unclear. Nevertheless, their bidirectional crossing ability provides a bridge for inter-compartmental communication. Exosomes carrying disease biomarkers can enter the bloodstream, facilitating convenient disease detection, while also acting as carriers for therapeutic agents into the brain. Current research has identified three major endocytic pathways for exosomal entry into cells: receptor-mediated transcytosis, lipid raft–mediated uptake, and macropinocytosis. Yet, the ultimate fate of exosomes after BBB translocation remains largely unknown [48]. Importantly, several studies have shown that exosomal transport across the BBB is selective; for example, reduced CD46 expression was reported to decrease exosomal BBB translocation by twofold [48].

Fusion-mediated exosomal transport represents a mechanism ensuring intact delivery of exosomal cargo across the BBB by circumventing endosomal degradation. This process involves lipid-driven fusion between exosomal and cellular membranes. Lipid rafts are thought to underlie the high fusion efficiency of exosomes. Additionally, lipids such as cholesterol, phosphatidylserine, and sphingomyelin contribute to promoting fusion [46]. Clathrin-mediated endocytosis (CME) is another major pathway that facilitates exosomal membrane translocation. In fibroblasts, approximately 95% of plasma membrane receptors undergo internalization via endocytosis [49]. This process depends on the formation of clathrin-coated pits. Endothelial cells employ this mechanism to selectively transport exosomes into the brain, requiring transferrin receptor (TfR) and low-density lipoprotein receptor-related protein-1 (LRP1) as “molecular switches” for this pathway.

Receptor-mediated transcytosis (RMT) plays a critical role in exosomal BBB transport, relying on receptors expressed on BBB endothelial cell membranes, such as transferrin, insulin, and low-density lipoprotein receptors. Among them, the transferrin receptor (TfR) has been most extensively studied [40,50]. Evidence shows that surface modification of exosomes with TfR-binding peptides enhances their ability to cross the BBB [40].

Adsorptive-mediated transcytosis (AMT) is a receptor-independent pathway for BBB transport. Its core mechanism is based on electrostatic interactions between carriers and the endothelial cell membrane. Positively charged carriers bind to negatively charged regions on brain microvascular endothelial cells via electrostatic adsorption, initiating endocytosis and subsequent intracellular transport for release into the brain parenchyma. Unlike RMT, AMT does not rely on specific receptor expression, thereby offering higher binding efficiency and greater transport flexibility. It is

DOI: 10.54254/2753-8818/2025.AU27718

considered a promising strategy for drug delivery across the BBB [51]. Notably, studies have reported that red blood cell–derived exosomes carrying α-synuclein successfully cross the BBB via the AMT pathway, providing novel insights into the mechanisms of Parkinson's disease progression and potential therapeutic delivery [51].

Importantly, BBB permeability is not static. Under pathological conditions such as stroke, neuroinflammation, and tumors, BBB integrity is disrupted. Inflammatory states, in particular, enhance BBB permeability, allowing increased exosomal passage. Abnormal exosomal leakage across the BBB is closely associated with central nervous system disorders. Supporting this notion, exosomes containing inflammatory signals such as α-synuclein and prion proteins have been implicated in the progression of neurodegenerative diseases. Morad et al. demonstrated that tumor-derived exosomes could disrupt the BBB via transcytosis. Their study investigated exosomes from triple-negative breast cancer cells, highlighting their role in promoting brain metastasis and exploring their translocation mechanisms. Results revealed that certain exosomes may compromise BBB integrity by altering endothelial tight junctions. However, the study did not further clarify whether all implicated subpopulations were indeed exosomes [40].

3. The "double-edged sword" role of exosomes in neurodegenerative diseases

3.1. Exosome-mediated pathological progression of neurodegenerative diseases

3.1.1. α-Synuclein propagation and pathological spreading in Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide, after Alzheimer's disease. Its pathological hallmarks include the selective loss of dopaminergic neurons and the accumulation of misfolded presynaptic protein α-synuclein (α-syn) in Lewy bodies [52]. Neuropathological observations reveal that the spatial and temporal distribution of Lewy bodies correlates closely with disease progression, suggesting a prion-like spreading mechanism. Similar to prion disorders and other neurodegenerative diseases, PD may be triggered by toxic α-syn species that convert endogenous normal α-syn into its pathological form, thereby propagating progressively between cells. Mounting evidence indicates that exosomes represent a key pathway for α-syn dissemination. Various forms of α-syn—including monomers, oligomers, and fibrils—have been detected within exosomes [53]. Studies using conditioned media from neuroblastoma cells have shown that α-syn secretion follows a calcium-dependent mechanism, implying that it is an active and regulated process rather than a mere passive leak. When lysosomal function is impaired, exosomal α-syn levels increase, accelerating the spread of pathogenic α-syn. In the cerebrospinal fluid of PD patients, exosomes carrying pathogenic α-syn have been identified, and these vesicles can induce oligomerization of soluble α-syn in recipient cells. Although only a small fraction of secreted α-syn is exosome-associated, exosomal α-syn is more readily internalized and retained by neighboring cells compared to freely suspended extracellular proteins [54]. Furthermore, vesicles can actively promote α-syn oligomerization, and these oligomeric forms—more so than monomers or large aggregates—exert greater cytotoxic effects [55].

At the cellular level, multiple types of glial cells are involved in exosome-mediated α-syn propagation. Microglia have been shown to actively participate in intercellular α-syn transfer by releasing exosomes [56]. Astrocyte-derived exosomes can also induce protein aggregation in the mouse brain, suggesting their potential contribution to α-syn dissemination [57]. In addition to proteins, non-coding RNAs carried by exosomes exhibit characteristic alterations in PD. For example, exosomal cerebrospinal fluid from PD patients shows marked upregulation of let-7g-3p

DOI: 10.54254/2753-8818/2025.AU27718

and miR-409-3p, along with significant downregulation of miR-1. Among these, let-7 can activate the TLR7 signaling pathway upon entering neurons, triggering neurodegenerative injury. Further transcriptomic analysis revealed 15 upregulated and 24 downregulated lncRNAs in exosomes from PD patients, with expression levels of lnc-POU3F3 and lnc-MKRN2-42:1 positively correlating with the severity of motor dysfunction [38].

3.1.2. Aberrant deposition of Aβ and Tau in Alzheimer's disease

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder and is rapidly emerging as one of the most costly, lethal, and burdensome diseases of this century. In China alone, approximately 10–11 million individuals over the age of 60 suffer from dementia, with more than 60% diagnosed with AD [58]. Globally, the number of dementia cases reached around 50 million in 2018 and is projected to more than double by 2050. The major pathological hallmarks of AD are extracellular deposition of amyloid-β (Aβ) plaques and intracellular accumulation of hyperphosphorylated tau protein [59]. Hyperphosphorylation of tau reduces microtubule stability, thereby impairing synaptic plasticity and axonal transport, ultimately leading to cognitive deficits [60]. Aβ-induced tau hyperphosphorylation and aberrant sorting are considered key events driving the formation of dysfunctional neurons [61].

Exosomes play a dual role in AD pathogenesis. On the one hand, they contribute to the propagation of pathological proteins. Evidence has shown that exosomes can carry and release Aβ, and exosome-associated proteins such as Alix and Flotillin-1 are highly enriched in plaque regions of AD brains [62]. In addition, neurofibrillary tangles composed of misfolded and hyperphosphorylated tau have been identified in the cerebrospinal fluid of AD patients [63].

Genomic studies further implicate exosomes in AD pathology. Several susceptibility genes are associated with Aβ clearance, among which at least two (PICALM and CD2AP) are expressed in brain endothelial cells (BECs) and participate in endocytosis. PICALM, a ubiquitously expressed clathrin adaptor protein, has been extensively studied for its role in Aβ trafficking. PICALM interacts closely with LRP1, a critical receptor mediating endocytosis and Aβ efflux from the brain [49]. Other studies have demonstrated that neurotoxic tau and Aβ exhibit prion-like behavior, with intercellular transfer occurring even before overt cell degeneration. Exosomes are thought to play a pivotal role in this process [63,64]. For example, full-length amyloid precursor protein (APP) undergoes clathrin-mediated endocytosis into early endosomes, where it is cleaved by β-secretase to generate Aβ, which is subsequently released via multivesicular bodies (MVBs) in association with exosomes [52].

Beyond pathogenic proteins, exosomal RNAs also play important roles in AD progression. Neurotoxic Aβ42 is produced by sequential β- and γ-secretase cleavage of APP. In AD brain tissue, miR-34a is markedly upregulated; exosomes overexpressing miR-34a can be taken up by neighboring cells, thereby promoting APP processing and Aβ deposition. Conversely, levels of miR-193b are significantly reduced in blood-derived exosomes, and this miRNA normally targets the 3′ untranslated region (UTR) of APP mRNA to suppress APP expression [3,65,66]. Oxidative stress, a well-recognized pathogenic mechanism in neurodegeneration, is also modulated by exosomes. Notably, downregulation of miR-125b-5p and miR-141-3p in exosomes from AD patients is strongly associated with enhanced oxidative stress. Mechanistically, miR-125b-5p activates CDK5 signaling, promoting tau phosphorylation and elevating ROS levels, while miR-141-3p—present in exosomes released from inflammation-stimulated astrocytes—suppresses PTEN, thereby impairing antioxidant defenses and exacerbating neuronal damage [38,66,67].

DOI: 10.54254/2753-8818/2025.AU27718

Importantly, exosomes also exhibit potential protective effects. Neuron-derived exosomes can induce conformational changes in Aβ, facilitating the formation of non-toxic amyloid fibrils and enhancing microglial uptake of Aβ [68]. Moreover, the cellular prion protein (PrPC) present on exosomal membranes promotes the conversion of Aβ oligomers into fibrillar forms, thereby reducing their neurotoxicity.

3.2. The significance of exosomes in the diagnosis of neurological diseases

3.2.1. Exosomal biomarkers in Alzheimer's disease

In the field of biomarker research for Alzheimer's disease (AD), dynamic changes at the protein level have demonstrated remarkable potential for early diagnosis. Fiandaca et al. found that phosphorylated tau protein and Aβ levels were significantly elevated as early as 10 years prior to an AD diagnosis, with Aβ concentration continuing to rise as the disease progressed, thus providing a molecular basis for ultra-early warning of AD [69]. Moreover, Winston et al. reported that the expression levels of neurogranin (NRGN) and repressor element 1-silencing transcription factor (REST) were significantly reduced in patients during the non-cognitive dysfunction (NED) stage and in those transitioning from mild cognitive impairment (MCI) to AD, suggesting that these two proteins may be closely associated with the progression of cognitive dysfunction in AD [70].

At the RNA level, exosomal miRNAs have emerged as promising molecular biomarkers. Kumar et al. identified a characteristic panel of seven miRNAs in plasma (hsa-let-7d-5p, hsa-let-7g-5p, hsa-miR-15b-5p, hsa-miR-142-3p, hsa-miR-191-5p, hsa-miR-301a-3p, hsa-miR-545-3p), which distinguished AD patients from healthy controls with an accuracy of over 95% [70]. Additionally, Lugli et al. discovered another panel of seven miRNAs (miR-342-3p, miR-342-5p, miR-141-3p, miR-23b-3p, miR-185-5p, miR-338-3p, miR-3613-3p) that achieved diagnostic accuracy ranging from 83% to 89%, further supporting the reliability of miRNAs as biomarkers for AD [71].

3.2.2. Exosomal biomarkers in Parkinson's disease

In biomarker research for Parkinson's disease (PD), the pathogenic protein α-synuclein (α-syn) has become a central focus and is considered a highly promising diagnostic biomarker. Multiple clinical studies have confirmed that compared with healthy controls, the levels of α-syn in blood exosomes of PD patients are significantly elevated, and its concentration continues to increase with disease progression. This characteristic endows α-syn with particular value for the early diagnosis of PD [72]. In addition to α-syn, other protein biomarkers have also been identified. Recent studies have reported that transferrin receptor (TfR) in plasma neuron-derived exosomes may serve as a novel potential biomarker for PD [73].

Evidence at the nucleic acid level is equally noteworthy. He et al. revealed differential expression patterns of miRNAs in PD: miR-331-5p was significantly upregulated in plasma exosomes of PD patients, whereas miR-505 was significantly downregulated, thereby providing new candidate indicators for the molecular diagnosis of PD [74]. Furthermore, non-coding RNAs, such as long non-coding RNAs (lncRNAs, e.g., lnc-MKRN2-42:1) and circular RNAs (circRNAs), have exhibited specific expression patterns in PD patients. These molecules are regarded as potential diagnostic biomarkers, though further studies are needed to clarify their mechanisms and clinical utility [75].

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4. Therapeutic functions and neuroprotective roles of exosomes

4.1. Exosomes derived from natural cells

4.1.1. Mesenchymal stem cell-derived exosomes

Mesenchymal stem cells (MSCs), owing to their relatively broad sources, immunomodulatory capacity, and roles in tissue regeneration and protection, have become one of the most widely used stem cells in research and clinical applications. Similar to exosomes derived from other cells, MSC-derived exosomes (MSC-Exos) contain proteins, mRNAs, and microRNAs (miRNAs). Inheriting part of the functional characteristics of MSCs, MSC-Exos exhibit remarkable therapeutic potential in tissue repair by maintaining and recruiting endogenous stem cells, inhibiting apoptosis, modulating immunity, and stimulating angiogenesis. Notably, exosomes derived from different MSC sources display functional differences: adipose-derived MSC-Exos demonstrate stronger pro-angiogenic effects, while bone marrow-derived MSC-Exos (BM-MSC-Exos) are more advantageous in immune regulation and anti-inflammatory responses [76,77].

Importantly, BM-MSCs possess not only strong anti-inflammatory capacity but also the ability to degrade Aβ. Their exosomes contain enkephalinase, a proteolytic enzyme capable of directly degrading Aβ plaques. Consequently, BM-MSCs can reduce the Aβ plaque burden and dystrophic neurites in the cortex and hippocampus of mice, indicating their potential for early intervention in AD [78].

4.1.2. Neural stem cell-derived exosomes

Neural stem cells (NSCs) are undifferentiated cells in the central nervous system with self-renewal ability and multipotent differentiation potential. Exosomes derived from NSCs (NSC-Exos) carry features reflecting their parental cell composition. Retaining specific membrane proteins and certain intracellular contents, NSC-Exos can selectively target cells with similar phenotypes and deliver signals that regulate neural repair [79]. Among their protein cargo, pentraxin 3 (PTX3) is the most abundant protein in NSC-Exos; PTX3-containing exosomes enhance the anti-inflammatory activity of microglia [80]. In addition, their highly expressed Netrin-1 protein can upregulate neuron differentiation-related transcription factors Hand2 and Phox2b, thereby promoting neuronal repair [79]. At the RNA level, NSC-Exos are characterized by selective loading of miRNAs. The five most enriched miRNAs in NSC-Exos are hsa-miR-1246, hsa-miR-4488, hsa-miR-4508, hsa-miR-4492, and hsa-miR-4516 [81]. Differences exist in the miRNA profiles of NSC-Exos depending on cell source and conditions. For example, studies have shown that miRNAs are expressed at higher levels in exosomes derived from murine hypothalamic NSCs compared to hippocampal NSCs, possibly reflecting the crucial role of hypothalamic NSCs in regulating the pace of aging [82]. Furthermore, exosomes secreted by hypoxia-preconditioned NSCs exhibit decreased expression of miR-98-3p and increased expression of miR-210-3p, thereby enhancing their therapeutic efficacy for stroke [82].

Exosomes derived from murine hypothalamic neural stem/progenitor cells (htNSCs) have been found to contain PIWI-interacting RNAs (piRNAs), a class of RNAs predominantly present in mammalian reproductive and neural tissues [83]. piRNAs are unique in that they rely on PIWI-like proteins to target other DNA or RNA molecules. Current studies suggest that piRNAs participate in the antiviral functions of NSC-Exos, though their additional roles remain to be elucidated [82,84].

In addition, exosomes derived from glial cells also exhibit neuroprotective properties. For instance, synapsin I carried by these exosomes can act as an oligomannose-binding lectin to protect

DOI: 10.54254/2753-8818/2025.AU27718

neurons under oxidative stress conditions [85].

4.1.3. Plant-derived extracellular vesicles

In addition to exosomes of animal origin, plant-derived extracellular vesicles (PEVs) can also be employed for the treatment of neurological disorders. These vesicles have been found to originate from early endosomes, although the precise molecular mechanisms underlying their biogenesis remain unclear (possibly similar to those of animal-derived exosomes). For this reason, they are referred to in this paper as extracellular vesicles (PEVs) [86]. PEVs are specialized secretory products synthesized and released by plant cells into the environment. They share many similarities with exosomes, such as possessing a phospholipid bilayer that facilitates blood–brain barrier penetration and cellular uptake. Moreover, they are enriched in plant-specific bioactive molecules, including proteins, miRNAs, and lipids. These unique components from plant cells provide new therapeutic strategies for central nervous system (CNS) diseases.

For Alzheimer's disease therapy, PEVs derived from Lycium ruthenicum Murray (black goji berry) have been shown to inhibit oxidative stress and apoptosis, thereby protecting neurons from Aβ-induced damage [87]. In transgenic Caenorhabditis elegans models of Alzheimer's disease, black goji berry-derived PEVs suppress Aβ aggregation via the DAF-16 pathway, while also exerting anti-inflammatory and antioxidant effects [88]. In addition, surface-modified ginger-derived PEVs not only activate M1 macrophages but also induce phenotypic switching of microglia through the PI3K–AKT signaling pathway [89]. Kudzu root-derived PEVs have demonstrated the ability to protect dopaminergic neurons and alleviate Parkinson's disease symptoms [90]. Furthermore, PEVs derived from Salvia hairy roots contain triterpenoids that help maintain metabolic homeostasis and inhibit apoptosis [88,91].

4.2. Engineered exosome strategies

Engineering of exosomes refers to the use of genetic engineering, chemical modification, or physical methods to enhance the functions of natural exosomes or to design them for specific therapeutic purposes. This technological platform offers several advantages. First, engineered exosomes inherit membrane proteins and molecular characteristics from their parent cells, conferring high biocompatibility and immune evasion capacity. This substantially reduces the risk of clearance and extends their circulation time in vivo. Second, the transmembrane proteins enriched on exosomal membranes, such as LAMP2B and integrins, mediate receptor-dependent endocytosis–exocytosis processes, enabling them to traverse complex biological barriers, including the blood–brain barrier. Third, exosomes retain adhesion molecules and receptor repertoires from their parent cells, which endow them with natural tissue tropism, allowing accumulation in specific microenvironments such as tumors or sites of inflammation. Meanwhile, engineering strategies can further enhance their functionality by improving targeting specificity, extending stability, or conferring novel therapeutic properties. Exosomes possess versatile cargo-loading capacities, allowing encapsulation of small-molecule drugs, proteins, RNAs (including miRNAs, mRNAs, and siRNAs), and even CRISPR-Cas9 gene-editing systems. With advances in technologies such as continuous-flow centrifugation, tangential flow filtration, and size-exclusion chromatography, the large-scale production of exosomes with high purity, high yield, and batch-to-batch consistency has gradually become feasible, laying a foundation for clinical translation [46].

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4.2.1. Endogenous engineering

Endogenous loading refers to manipulating donor cells so that therapeutic molecules are actively or passively incorporated into exosomes during their biogenesis. Incubation and transfection are commonly used methods [92]. Incubation is the most basic biological loading strategy, in which a concentration gradient drives small molecules to cross the cell membrane, diffuse into the cells, and subsequently become incorporated into exosomes during vesicle formation. Compared with approaches such as sonication, incubation better preserves the bioactivity of both the drug and the exosomes. However, its major drawback lies in the relatively low encapsulation efficiency. For example, researchers incubated coenzyme Q10 (CoQ10) with human adipose-derived stem cells (hADSCs) and obtained adipose-derived stem cell exosomes (ADSC-Exos). In an Alzheimer's disease mouse model, these ADSC-Exos exhibited superior therapeutic effects compared with free CoQ10, significantly upregulating BDNF and SOX2 expression, achieving the highest hippocampal neuron density, and demonstrating improved outcomes in both the passive avoidance test and the Morris water maze [93].

Another strategy is virus-based transduction. Adenoviruses and lentiviruses have been widely applied in gene therapy, owing to their stable and well-characterized transfection capability. Viral transduction can also be employed to load exosomes, since transduced cells express specific genes whose products are subsequently sorted into exosomes. Transfection is considered an effective approach for nucleic acid loading and has been applied to various RNAs, including mRNA, miRNA, and siRNA. For instance, Monfared et al. established a stable HEK293T cell line expressing miR-21 and successfully isolated EVs loaded with the target RNA [94]. DNA modification represents a primary approach in gene therapy, aiming to achieve defect repair or functional enhancement at the genomic level to correct disease-associated molecular mechanisms. With ongoing improvements in delivery vectors, both efficiency and specificity of gene transfer have been significantly enhanced, while safety concerns have been alleviated. Multiple studies have validated the feasibility of this method. Kanada et al. transfected cells with plasmid DNA encoding Cre and successfully isolated exosomes containing plasmid DNA [95]. Tran and Boedicker not only demonstrated effective conditions for plasmid DNA loading into E. coli but also found that DNA loading capacity was related to plasmid replication ability [96]. More recently, this method has been extended to loading the CRISPR/Cas9 system. This system consists of the Cas9 protein and a single-guide RNA (sgRNA). Cas9 is an endonuclease capable of cleaving double-stranded DNA, while the sgRNA binds to a specific DNA sequence by complementarity, directing Cas9 to the target site. Once the Cas9–sgRNA complex enters the cell, it induces DNA breaks, which are subsequently repaired by the cell's endogenous repair mechanisms, resulting in deletions, insertions, or modifications for gene editing [97,98]. Genetic engineering can also be applied to modify exosomal membrane proteins. Bai et al. transfected HEK293T cells with tLyp-1-lamp2b, generating exosomes containing tLyp-1 that exhibited enhanced targeting efficiency toward lung cancer stem cells [99]. Similarly, Curley et al. truncated the transmembrane helices of CD63 and observed that the truncated variants not only retained strong membrane anchoring but also improved exosomal targeting activity [100].

4.2.2. Exogenous engineering

Exogenous engineering refers to the modification of natural exosomes outside the cell, which mainly involves three strategies: cargo loading, surface modification, and construction of hybrid vesicles.

DOI: 10.54254/2753-8818/2025.AU27718

4.2.2.1. Drug loading

Drug loading is usually achieved through physical or chemical methods, with the key principle being the temporary enhancement of exosomal membrane permeability to allow drug molecules to enter the vesicular lumen. Physical approaches include electroporation, sonication, freeze–thaw cycles, and extrusion. Electroporation applies short electrical pulses to create transient pores on the exosomal membrane, enabling drug entry. This method is widely used due to its simplicity and high controllability [101,102]. Freeze–thaw cycles induce membrane reorganization through repeated freezing and thawing, providing a relatively mild method for loading nucleic acids and proteins. Extrusion involves repeatedly forcing a mixture of exosomes and cargo through polycarbonate membranes with pore sizes of 100–400 nm using an extruder equipped with a heating block. This method achieves high cargo encapsulation efficiency and produces exosomes with uniform size distribution, although the strong mechanical forces may alter the exosomal membrane [102].

Chemical loading approaches include surfactant-assisted permeabilization and transfection reagent-based encapsulation. Saponin-assisted permeabilization is one of the most commonly used chemical methods. Saponin, a natural surfactant, interacts with cholesterol in the exosomal membrane to create pores, thereby facilitating drug entry into exosomes [102]. Among transfection reagent-based methods, calcium phosphate is frequently used. Traditionally employed for cell transfection, calcium phosphate forms co-precipitates with nucleic acids, promoting their encapsulation into exosomes. It is commonly applied for loading siRNA and miRNA.

4.2.2.2. Surface engineering

Surface engineering involves directly modifying the exosomal membrane to improve stability, targeting ability, and delivery efficiency. Common strategies are broadly divided into non-covalent and covalent modifications. Non-covalent strategies typically rely on hydrophobic interactions or molecular ligands to functionalize the exosomal surface. For example, Pi and colleagues functionalized exosomes with cholesterol-conjugated RNA aptamers or folic acid [103]. By contrast, covalent modifications provide more stable and durable effects, among which “click chemistry” has emerged as a research hotspot. Click chemistry comprises efficient and highly selective reactions applicable to proteins, lipids, and nucleic acids. A typical example is the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reaction [103]. Jia et al. employed a sulfonyl azide-based cycloaddition reaction to conjugate RGE peptides (a neuropilin-1–targeting ligand) onto the exosomal surface, thereby constructing an exosome delivery system with glioma-targeting capability [104].

4.2.2.3. Hybrid vesicle construction

Hybrid exosomes integrate the dual advantages of synthetic materials and natural exosomes: they combine the controllability, high drug loading capacity, and stability of artificial nanomaterials with the intrinsic tropism, barrier-penetrating ability, long circulation, low immunogenicity, and biocompatibility of exosomes. Consequently, strategies fusing exosomes with nanomaterials have become a research hotspot, primarily achieved through exosome–liposome hybridization. Freeze–thaw hybridization is a common approach, in which exosomes and liposomes are frozen in liquid nitrogen and then thawed at room temperature over several cycles to induce fusion. Multifunctional liposomes (e.g., pH-sensitive, photosensitive, or thermosensitive types) can be incorporated in this way, enabling hybrid vesicles with enhanced delivery properties [105].

DOI: 10.54254/2753-8818/2025.AU27718

Incubation is another mild and widely used strategy. By exploiting the shared lipid bilayer structure of liposomes and exosomes, natural fusion occurs during co-incubation. However, due to the limited internal volume of exosomes, this approach faces constraints in delivering large exogenous nucleic acids such as the CRISPR/Cas9 system. Lin et al. successfully developed hybrid vesicles by incubating cell-derived exosomes with liposomes carrying CRISPR/Cas9 components [106].

4.2.2.4. Co-extrusion

Co-extrusion employs mechanical force to drive recombination and fusion of exosomes and liposomes as they are passed through membranes with defined pore sizes. Compared with other methods, co-extrusion provides better control over the size of hybrid vesicles. Rayamajhi et al. fused macrophage-derived exosomes with liposomes through co-extrusion, producing hybrid vesicles with slightly larger sizes than the precursors but with a narrower size distribution [107]. Importantly, this approach also offers advantages for large-scale production. The inherently low yield of natural exosomes limits their applications, but recent studies suggest that co-extrusion can overcome this challenge. Jhan et al. mixed exosomes with synthetic lipid suspensions and extruded them sequentially through membranes with pore sizes of 400 nm, 200 nm, and 100 nm, successfully generating size-controlled layered vesicles. Compared with isolated EVs, this strategy increased vesicle yield by 6- to 43-fold [108,109].

4.2.3. Exosomal membrane engineering

Exosomal membrane engineering involves ligand conjugation or molecular modification of the exosomal membrane to enhance stability, targeting efficiency, and blood–brain barrier (BBB) penetration. Although natural exosomes possess intrinsic BBB-crossing abilities and partially retain donor cell characteristics, their therapeutic efficacy in vivo is often limited by the complex vascular network and phagocytosis by immune cells. Membrane modification strategies are therefore widely employed to improve the therapeutic performance of exosomes. Overall, these strategies focus on three main objectives: enhancing targeting specificity, improving BBB penetration, and prolonging circulatory stability.

4.2.3.1. Enhancing targeting specificity

Membrane engineering can significantly improve exosome targeting toward specific tissues or cell types. The rabies virus glycoprotein (RVG) peptide selectively binds to acetylcholine receptors, making it widely used for constructing neuron-specific exosomes for drug delivery to the central nervous system [110]. In glioblastoma therapy, researchers conjugated RGE peptides (RGERPPR) to exosomal surfaces via sulfonyl azide cycloaddition, enabling specific recognition of tumor cells. After intravenous injection, RGE-modified exosomes efficiently crossed the BBB and accumulated in tumor regions [104]. Moreover, RGE-modified exosomes loaded with curcumin demonstrated significant antitumor efficacy in tumor-bearing mice. When combined with other drugs or imaging agents, this approach enhanced both therapeutic and imaging outcomes [104].

4.2.3.2. Improving blood–brain barrier penetration

Several membrane modification strategies have been developed to enhance exosome transport across the BBB. Transferrin ligands are commonly used to mediate receptor-dependent endocytosis via

DOI: 10.54254/2753-8818/2025.AU27718

transferrin receptors, improving delivery of chemotherapeutic agents to the brain and enhancing treatment of central nervous system tumors [111].

Folate (FA), a small molecule whose receptor is overexpressed on the BBB, can facilitate exosome uptake via endocytosis and can be anchored to the exosomal surface through electrostatic adsorption.

Certain viruses naturally cross the BBB through ligand–receptor interactions, providing inspiration for exosome engineering. The rabies virus capsid glycoprotein (RVG) is a surface protein with strong neurotropism and BBB penetration capability [112]. RVG-modified mesenchymal stem cell (MSC) exosomes display faster accumulation in the brain compared with unmodified exosomes. Furthermore, RVG-modified MSC exosomes improve learning and memory in APP/PS1 transgenic mice, reduce Aβ accumulation and glial activation, and help balance inflammatory cytokines in the brains of Alzheimer's disease (AD) model mice [113,114].

4.2.3.3. Prolonging circulatory stability

Although exosomes are naturally stable, pharmacokinetic studies show that intravenous administration typically results in circulation times of only 2–30 minutes, with rapid clearance by the mononuclear phagocyte system (MPS) in the liver, spleen, and lungs. Membrane engineering can extend in vivo half-life through "immune evasion" mechanisms. Phagocytes represent a major barrier to systemic exosome therapy; thus, membrane modification to avoid macrophage clearance can prolong circulation time, reduce the time required to achieve therapeutic concentrations at target tissues, and minimize off-target effects [115]. CD47 is a cell surface protein belonging to the immunoglobulin superfamily, expressed in most cell types. CD47 interacts with the immune inhibitory receptor SIRPα, suppressing macrophage activation and functioning as a specific immune checkpoint. CD47 has been identified in exosomes derived from mesenchymal stem cells (MSCs) and platelets. Studies have shown that overexpression of CD47 in exosomes extends their half-life in circulation by threefold, without inducing significant immunogenicity or in vivo toxicity in mice [116]. CD31, also known as platelet endothelial cell adhesion molecule-1 (PECAM-1), is primarily associated with vascular biology. It serves as an endothelial marker and is expressed in endothelial cells, hematopoietic cells, and others. In 2002, CD31 was first described as transmitting a "don't eat me" signal that prevents viable cells from being phagocytosed by macrophages [117]. β2-microglobulin (β2M), a component of the MHC class I complex, is another protein exploited by cancer cells to protect themselves from immune attack. β2M can bind to leukocyte immunoglobulin-like receptor B1 (LILRB1), which is highly expressed on the surface of macrophages, thereby suppressing immune cell activity [118]. Programmed death-ligand 1 (PD-L1) is also a potential candidate. PD-L1 interacts with programmed death protein 1 (PD-1) to evade immune surveillance. PD-1, an immune checkpoint molecule, is highly expressed on various lymphocytes, including B cells, NK cells, dendritic cells, and macrophages [115]. Tumor cell–mediated suppression of T cells via the PD-1/PD-L1 axis has been extensively studied. Gordon et al. demonstrated that blocking PD-1/PD-L1 enhances macrophage phagocytic activity. Thus, PD-L1 represents a promising molecule for prolonging exosome half-life [115,116].

4.2.4. Exosomal cargo engineering

The lipid bilayer structure of exosomes provides a natural physical barrier for cargo, effectively protecting nucleic acids and proteins from enzymatic degradation while significantly enhancing the stability and bioactivity of therapeutics. Numerous studies have demonstrated that exosomes are

DOI: 10.54254/2753-8818/2025.AU27718

promising carriers for therapeutic small molecules. For instance, curcumin encapsulated in exosomes achieves substantially higher concentrations than free curcumin, and paclitaxel delivered via exosomes exhibits approximately a 50-fold increase in cytotoxicity against drug-resistant cancer cells. These findings indicate that exosomes not only protect small-molecule drugs from degradation but also exert a concentration effect, improving therapeutic efficacy while minimizing side effects. Beyond small molecules, exosomes are also well-suited for delivering macromolecules such as peptides and proteins, including enzymes and antigens. For the treatment of inflammatory and neurodegenerative diseases, exosomes have been employed as delivery platforms for catalase, a potent antioxidant enzyme. Exosomes loaded with catalase can successfully deliver the enzyme to the brains of Parkinson's disease model mice, where it is taken up by neurons.

Due to their ability to shield nucleic acids from degradation, exosomes have emerged as ideal carriers for gene therapy. This feature addresses one of the major challenges in nucleic acid-based treatments—susceptibility to degradation—thus providing a robust platform for effective therapeutic delivery. The CRISPR/Cas9 genome-editing system has attracted widespread attention for its unprecedented precision, efficiency, and flexibility in gene editing, representing a revolutionary tool for treating a wide range of diseases. The system consists of an RNA-guided endonuclease (Cas9), which cleaves double-stranded DNA, and a guide RNA that directs Cas9 to specific genomic loci [119]. Significant progress has been made using CRISPR/Cas9 for disease treatment. For example, Konstantinidis et al. demonstrated that CRISPR/Cas9 could selectively disrupt the PSEN1^M146L allele associated with Alzheimer's disease, partially reversing the abnormal Aβ42/40 ratio in patient-derived cells [120]. Another study targeting patient-derived fibroblasts showed that CRISPR/Cas9-mediated knockout of the Swedish APP (APP^swe) mutation reduced Aβ protein levels [121]. In Parkinson's disease research, CRISPR/Cas9 has been employed to investigate and intervene in familial PD-related mutations (e.g., SNCA, PRKN/PARK2, PINK1, LRRK2, PARK7/DJ-1, GBA, UCH-L1, MAPT), offering new avenues for targeted therapy [121]. However, the choice of delivery vehicle remains a critical challenge for CRISPR/Cas9 applications. Most current delivery systems are limited by immunogenicity, which can compromise both safety and therapeutic efficacy. As a natural carrier, exosomes exhibit significant potential for packaging and delivering CRISPR/Cas9 into cells, providing a promising solution to this limitation.

In addition to CRISPR/Cas9, exosomes are widely used to deliver other types of nucleic acids. For instance, Xu M. et al. successfully utilized exosomes loaded with siRNA to silence disease-causing genes in Huntington's disease, highlighting the potential of exosomes for treating neurodegenerative disorders. Collectively, these studies demonstrate that exosomes offer substantial advantages for delivering small-molecule drugs, macromolecular proteins, and nucleic acids, providing a versatile and promising platform for future therapeutic applications.

4.3. Exosome delivery routes and clinical translation

When employing exosome-based therapies, the choice of an appropriate administration route is critical for enhancing therapeutic efficacy. Common delivery methods include intravenous injection, intranasal administration, intraperitoneal injection, and local injection.

4.3.1. Intravenous injection

Intravenous (IV) injection is the most widely used delivery route due to its simplicity and relative safety. Exosomes administered intravenously distribute systemically via the bloodstream but have limited ability to cross the blood–brain barrier (BBB), often accumulating in organs such as the liver

DOI: 10.54254/2753-8818/2025.AU27718

and spleen. In Alzheimer's disease research, exosomes carrying β-secretase (BACE1) siRNA delivered intravenously successfully reduced Aβ production in the brains of AD model mice and improved pathological phenotypes [122]. Similarly, mesenchymal stem cell-derived exosomes (MSC-Exos) delivered via IV injection suppressed neuroinflammation and promoted Aβ clearance, reducing its accumulation in mouse brain tissue. In Parkinson's disease models, engineered exosomes loaded with antioxidant enzymes, such as catalase, have been shown to cross the BBB following intravenous injection and specifically target the substantia nigra pars compacta (SNpc), significantly alleviating neuroinflammation and oxidative stress while protecting dopaminergic neurons [123]. Currently, clinical studies on IV exosome administration are mainly focused on non-neurodegenerative conditions, including COVID-19 and various cancers. Applications for neurodegenerative diseases remain in the early exploratory stage and require further research and clinical validation [124].

4.3.2. Local injection

Local injection, such as intracerebroventricular administration, delivers exosomes directly to affected sites, significantly increasing local drug concentration while reducing peripheral clearance. However, the invasiveness and technical complexity of this method limit its widespread use for central nervous system (CNS) disorders. In a recent study, researchers employed a controllable 3D exosome–gel hybrid microneedle array patch for in situ repair of spinal cord injury [125]. In Alzheimer's disease models, intracerebral injection of exosomes modified with miR-29a/b into the CA1 region of rat hippocampus effectively alleviated memory and spatial learning deficits induced by Aβ, demonstrating notable neuroprotective effects [126]. For spinal muscular atrophy (SMA), neonatal mice receiving intracerebroventricular injections of ASC-EVs exhibited improved motor function and reduced neuronal apoptosis, indicating strong neuroprotection [126]. Clinically, local injection remains limited. A Phase I study in 2019 administered adipose-derived stromal vascular fraction (including exosomes) intracerebroventricularly to patients with neurodegenerative diseases such as Alzheimer's disease, ALS, and Parkinson's disease. A total of 113 injections were performed, with some patients receiving repeated injections over a period of up to three years. The procedure was generally safe, with no major adverse events observed; only one case of implant-related infection and four hospitalizations were reported [127].

4.3.3. Intranasal administration

Intranasal administration allows exosomes to reach the central nervous system (CNS) via two main routes: intracellular and extracellular. The intracellular route involves exosome uptake by epithelial neurons, followed by axonal transport and eventual exocytosis within the CNS. This internalization process can occur through both specific and nonspecific endocytosis, suggesting that surface modification of exosomes may enhance neuronal uptake. Studies indicate that the transport rate of exosomes within neurons is independent of cell size. Transport via the olfactory nerve occurs over approximately 0.74–2.67 hours, while transport via the trigeminal nerve takes 3.69–13.33 hours [128]. Olfactory nerve transport terminates in the olfactory bulb, whereas the trigeminal nerve ends in the pons. After exocytosis, exosomes can be reabsorbed by local cells or diffuse through the CNS interstitial fluid [129].

Extracellular transport does not rely on neuronal binding, endocytosis, or exocytosis. The primary challenge is traversing the nasal epithelium, which contains tight junctions that restrict molecular entry into the lamina propria. Interestingly, olfactory neurons are not permanent—they regenerate

DOI: 10.54254/2753-8818/2025.AU27718

every 30–60 days [130]. During the turnover of old cells, temporary gaps form in the epithelium, allowing molecules to penetrate the lamina propria. Certain agents, such as papaverine and poly-L-arginine, can transiently loosen tight junctions to facilitate transport. The second barrier is entry from the lamina propria into the brain. Studies have shown that when neurons exit the CNS into the periphery, they carry with them the arachnoid layer surrounding the nerve bundle [131]. Olfactory ensheathing fibroblasts (OEFs), which surround cerebrospinal fluid–filled nerves, connect the subarachnoid space to the lamina propria, providing a pathway for exosomes into the CNS [129,132].

Cone and colleagues reported that intranasally delivered human bone marrow–derived MSC exosomes reduced Aβ deposition in the hippocampus and attenuated glial activation in Alzheimer's disease model mice. Similarly, MSC-Exos preconditioned with cytokines demonstrated therapeutic efficacy following intranasal administration, requiring only two doses to inhibit microglial activation and reduce dendritic spine loss [132].

5. Conclusion

Exosomes are generated via the multivesicular body pathway and carry proteins, nucleic acids, lipids, and other cargoes. They can cross the blood–brain barrier and mediate intercellular communication within the CNS. In neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD), exosomes can facilitate the spread of pathogenic proteins such as Aβ and α-synuclein, accelerating disease progression, but they also possess neuroprotective potential. Engineered exosomes are increasingly being developed as drug delivery vehicles, capable of delivering therapeutic cargoes to the brain via intravenous, intranasal, or local administration. Current challenges include high heterogeneity, low cargo loading efficiency, poor stability, limited tissue targeting, difficulty in in vivo tracking, lack of standardized characterization, and challenges in large-scale production. Recent studies have made significant progress: engineered exosomes delivering siRNA or antioxidant enzymes have alleviated pathological damage in animal models of AD and PD. Moreover, the first clinical trial using MSC-derived exosomes in AD patients demonstrated safety and suggested trends of cognitive improvement. Future efforts should focus on genetic and membrane engineering of exosomes, artificial hybrid strategies, standardized characterization methods, and robust in vivo tracking techniques to improve targeting efficiency, cargo capacity, and functional validation. Exosomes hold promise as innovative tools for the diagnosis and treatment of CNS diseases such as AD and PD.

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