Review

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Exploring the multiple therapeutic mechanisms and challenges of mesenchymal stem cell-derived exosomes in Alzheimer's disease

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SUMMARY Alzheimer's disease (AD) is a severe neurodegenerative disorder, and the current treatment options are limited. Mesenchymal stem cell-derived exosomes (MSC-Exos) have garnered significant attention due to their unique biological properties, showcasing tremendous potential as an acellular alternative therapy for AD. MSC-Exos exhibit excellent biocompatibility and low immunogenicity, enabling them to effectively cross the blood-brain barrier (BBB) and deliver therapeutic molecules directly to target cells. They are highly efficacious in delivering nucleic acid-based drugs. Moreover, the production process of MSC-Exos benefits from a high proliferation capacity and multilineage differentiation potential, allowing for production while maintaining a stable composition. Despite the significant theoretical advantages of MSC-Exos, their clinical use still faces multiple challenges, including cross-contamination during isolation and purification processes, the complexity of their components, and the presence of potential adverse paracrine factors. Future research needs to focus on optimizing separation and purification techniques, enhancing delivery methods to improve therapeutic efficacy, and performing detailed analyses of the components of MSC-Exos. In summary, MSC-Exos hold promise as an effective option for the treatment of AD and other neurodegenerative diseases, driving their clinical research and use in related fields.

Keywords mesenchymal stem cell-derived exosomes, Alzheimer's disease, drug delivery, blood-brain barrier, immunogenicity, cell therapy

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline, primarily affecting the elderly population. According to the World Health Organization, approximately 55 million people worldwide suffer from dementia, with AD being the most common form, accounting for 60% to 70% of cases (*1*). As global aging accelerates, the incidence of AD continues to rise, making it a serious public health issue that poses a heavy burden on patients, their families, and society.

The exact pathogenesis of AD remains unclear; however, growing evidence suggests that the progression of the disease is closely linked to multiple pathological changes. Among these, the deposition of β-amyloid (Aβ) is considered a hallmark of AD. Aβ accumulates between neurons, forming plaques that disrupt neuronal function and ultimately lead to neuronal death. In addition to Aβ accumulation, the abnormal phosphorylation of tau protein is regarded as a critical pathological mechanism in AD, leading to the formation of neurofibrillary tangles that further exacerbate neurodegeneration. Moreover, neuroinflammation plays a crucial role in the progression of AD. Excessive inflammatory responses not only damage neurons but also contribute to the aggregation of Aβ and tau, creating a vicious cycle that accelerates disease progression.

Current treatment options for AD primarily focus on managing symptoms. Commonly used drugs include cholinesterase inhibitors and NMDA receptor antagonists (*2,3*). However, these treatments have been largely ineffective in halting the progression of the disease, underscoring the urgent need for new therapeutic strategies. Targeting the multiple pathological mechanisms of AD and integrating modern biomedical technologies to develop novel therapies has thus become a major area of research (*4*).

Over the past few years, mesenchymal stem cells (MSCs) and the exosomes secreted by them have gained considerable attention in the field of regenerative medicine. MSCs are adult stem cells with the capacity

for self-renewal and multipotent differentiation and are found in various tissues such as bone marrow, adipose tissue, and the umbilical cord (*5-7*). Mesenchymal stem cell-derived exosomes (MSC-Exos) are small vesicles secreted by MSCs that contain numerous bioactive molecules, including proteins, lipids, and RNA. These exosomes mediate intercellular communication and regulate various biological processes (*8*). Research has shown that MSC-Exos have promising therapeutic potential in a variety of diseases, demonstrating anti-inflammatory, immunomodulatory, neuroprotective, and regenerative properties.

MSC-Exos can protect neurons through various mechanisms, including reducing the toxicity of Aβ and tau proteins and preventing neuronal apoptosis. Studies suggest that the growth factors and antioxidant molecules contained within exosomes significantly enhance neuronal survival rates. Given that neuroinflammation plays a major role in AD pathology, MSC-Exos also exhibit the ability to modulate immune cell function, effectively suppressing excessive inflammatory responses and thereby reducing neuronal damage. In addition, MSC-Exos can promote the proliferation and differentiation of neural stem cells, aiding in the restoration of neural networks. This points to a novel therapeutic approach for AD treatment.

Although preliminary studies have indicated that MSC-Exos may offer benefits to patients with AD, their use in AD treatment remains in the exploratory phase, with several challenges yet to be addressed. These challenges include the processes of preparation, purification, and storage, as well as ensuring consistency and efficacy in clinical use. At present, there are various methods for exosome preparation and purification, but many are costly, operationally complex, and have limited efficiency. Therefore, there is an urgent need to develop more efficient and cost-effective technologies for isolating and purifying exosomes to increase their yield and purity. The bioactivity of MSC-Exos largely depends on their specific components. However, a comprehensive analysis of the precise proteins, RNA, and lipids present within MSC-Exos is still lacking. In this regard, evaluating the efficacy and safety of MSC-Exos, while ensuring consistency in quality and therapeutic outcomes for clinical use, is a key focus for future research.

In conclusion, MSC-Exos represent a promising and innovative therapeutic strategy for AD treatment. By further exploring their multiple therapeutic mechanisms, we can gain a deeper understanding of their potential in combating AD. As research advances, the clinical translation of MSC-Exos could significantly improve the quality of life for patients with AD and reduce the social burden of the disease. The aim of this study was to provide new insights and directions for future research, fostering the development and use of MSC-Exos in the field of neurodegenerative diseases.

2. Pathological mechanisms of AD

AD is a complex neurodegenerative disorder primarily characterized by memory impairment, cognitive decline, and loss of daily living abilities. While the exact mechanisms underlying AD remain unclear, mounting research indicates that its pathology involves multiple interrelated factors, including the deposition of Aβ, abnormal tau protein phosphorylation, neuroinflammation, and neurodegeneration.

2.1. Aβ deposition

Aβ deposition is a hallmark pathological feature of AD. Aβ is generated through the cleavage of amyloid precursor protein (APP) by β- and γ-secretases (*9*). Under normal physiological conditions, APP metabolism is tightly regulated. In patients with AD, however, this balance is disrupted, resulting in the overproduction and aggregation of Aβ, which subsequently forms amyloid plaques (Figure 1). Research has demonstrated that Aβ deposition impairs neuronal function and induces cell death by triggering oxidative stress, releasing pro-inflammatory factors, and ultimately causing neuronal dysfunction.

Aβ aggregation occurs in three stages: monomers, oligomers, and fibrils, with oligomers being the most toxic form. Oligomeric Aβ binds to neuronal cell membranes, disrupting intracellular signaling pathways and leading to calcium homeostasis imbalances, ultimately resulting in apoptosis. Moreover, amyloid plaque deposition is closely associated with microglial activation. Microglia, the immune cells of the central nervous system, respond to Aβ deposition by attempting to clear the harmful substance. However, excessive microglial activation can trigger neuroinflammation, creating a vicious cycle that exacerbates neuronal damage.

2.2. Tau protein phosphorylation

Tau protein, a microtubule-associated protein primarily found in neurons, plays a key role in stabilizing microtubules and facilitating intracellular transport. In AD, tau undergoes abnormal hyperphosphorylation, causing it to detach from microtubules and aggregate into neurofibrillary tangles (NFTs), another major pathological hallmark of AD closely linked to cognitive impairment (*10*).

Research has shown that tau hyperphosphorylation is regulated by several kinases, including glycogen synthase kinase 3β (GSK-3β) and cyclin-dependent kinase 5 (Cdk5) (*11,12*). These kinases are influenced by Aβ deposition, which further promotes tau phosphorylation. Tau aggregation disrupts intracellular signaling and metabolic pathways, leading to neurodegeneration (Figure 1). Studies indicate that abnormal tau phosphorylation not only directly contributes to neuronal death but also plays a

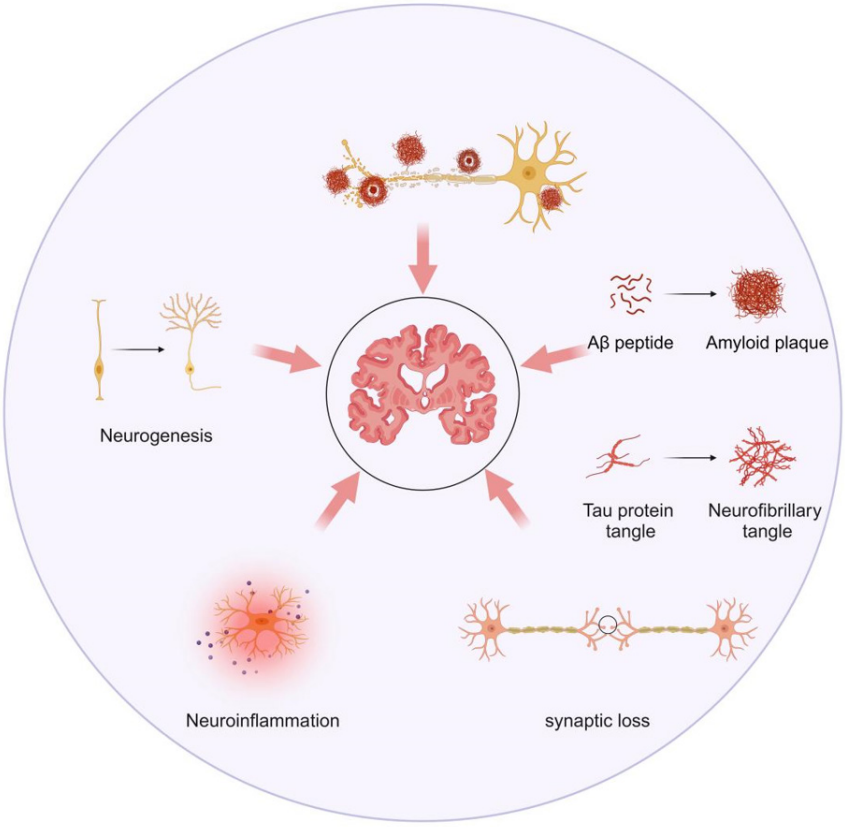

Figure 1. Pathogenesis of Alzheimer's disease. The progression of Alzheimer's disease is driven by various interconnected factors, such as Aβ deposition, abnormal tau protein phosphorylation, neuroinflammation, and neurodegeneration.

significant role in synaptic dysfunction and neural network disruption.

2.3. Neuroinflammation

Neuroinflammation plays a dual role in AD progression. Initially, the inflammatory response may be protective, aimed at clearing cellular damage or pathogens. As the disease advances, however, chronic inflammation persists, leading to further neuronal damage and death. Microglia and astrocytes, the primary immune cells of the central nervous system, are central to the inflammatory response in AD.

Activated microglia release various pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), that can induce neuronal apoptosis and synaptic damage (*13*). In addition, chronic inflammation can accelerate AD pathology by influencing Aβ metabolism and tau phosphorylation (Figure 1). Studies have shown that inhibiting neuroinflammation can alleviate cognitive impairment in mouse models of AD, suggesting that targeting neuroinflammation may be a therapeutic approach to AD treatment.

2.4. Neurodegeneration

Neurodegeneration is the final consequence of AD and is marked by the loss of neurons, synaptic dysfunction, and the breakdown of neural networks. This process is driven by a combination of Aβ deposition, abnormal tau protein aggregation, and chronic neuroinflammation. As neurons are lost, patients experience progressive cognitive decline, manifesting in memory loss, diminished learning capacity, and impaired executive functions (*14*).

Neurodegeneration in AD is often accompanied by a reduced capacity for neuronal regeneration, which is associated with impaired neural stem cell function and a lack of growth factors. Research has indicated that decreased neural stem cell activity and reduced neurogenesis may contribute to the neurodegenerative processes observed in the brain of patients with AD (*15*). Consequently, restoring neural stem cell function and promoting neurogenesis represent promising directions for future AD therapies.

3. General characteristics of exosomes

3.1. Biogenesis of exosomes

Exosomes are small membrane-bound vesicles secreted by cells, ranging in diameter from 30 to 150 nm. The process of exosome biogenesis primarily involves the inward budding of the plasma membrane, leading to the formation of multivesicular bodies (MVBs) within the cell. This process begins with the invagination of

the plasma membrane, which captures surface proteins and soluble proteins from the extracellular environment, thus marking the start of exosome biogenesis. In addition, the Golgi apparatus and the endoplasmic reticulum contribute to the formation and fusion of early endosomes (ESE) (*16-18*). As these early endosomes mature into late endosomes, a secondary invagination occurs, resulting in the formation of intraluminal vesicles (ILVs), which are the precursors of exosomes. The size and number of ILVs vary depending on the extent of invagination. Some MVBs fuse with lysosomes or autophagosomes, leading to the degradation of their contents, while others fuse with the plasma membrane, releasing ILVs into the extracellular space, where they become exosomes (*18,19*). Once MVBs fuse with the plasma membrane, the ILVs are released into the extracellular space as exosomes that possess a lipid bilayer structure similar to that of the plasma membrane (*16,20*). Figure 2 provides an illustration of this process. Proteins involved in exosome biogenesis include Ras-related proteins (Rab GTPases), ESCRT proteins, exosome marker proteins (*e.g.*, TSG101, flotillin, ceramide, and Alix), and exosome surface proteins (*e.g.*, integrins, immunomodulatory proteins, and tetraspanins) (*17,21,22*). Alterations in the function of Rab and ESCRT proteins can indirectly affect the autophagic-lysosomal pathway and vesicular transport from the Golgi apparatus, thereby impacting exosome biogenesis. Moreover, factors such as cell type, culture conditions, and the genomic health of cells can influence key regulatory factors of exosome biogenesis both *in vivo* and *in vitro* (*17*).

3.2. Composition of exosomes

Exosomes consist of lipids, proteins, nucleic acids, and metabolites, reflecting the characteristics and physiological state of their donor cells. Their membrane structure consists of a lipid bilayer rich in cholesterol, sphingolipids, and ceramides (lipid rafts), which not only maintain structural integrity but also play a pivotal role in exosome formation and signal transduction (*23 24*).

Exosomes contain a variety of proteins, including ESCRT proteins and Rab GTPases, which participate in their biogenesis. In addition, exosomes carry marker proteins such as TSG101, flotillin, ceramide, and Alix and surface proteins like integrins, immunomodulatory proteins, and tetraspanins (*17,21,22*). Cell-specific proteins, such as MHC class I and II molecules, are also present in exosomes and reflect the unique characteristics of their donor cells (*25*).

Moreover, exosomes contain various nucleic acids, including DNA, mRNA, and non-coding RNA, with microRNA (miRNA) being the most abundant. These nucleic acids can be transferred to recipient cells *via* exosomes, influencing gene expression and cellular functions (*26,27*). In addition, exosomes carry small metabolites, such as growth factors and cytokines, that play critical roles in cellular metabolism and signal transduction (*26,28,29*). The composition of exosomes can vary depending on the cell type, cellular state, and environmental factors.

3.3. Exosome isolation and purification

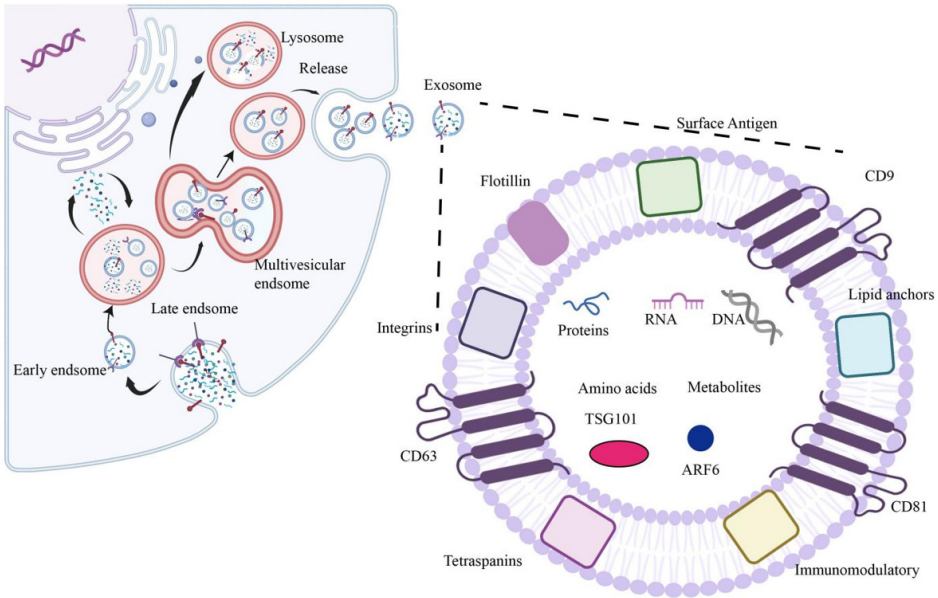

Figure 2. The process of exosome biogenesis. Exosome biogenesis begins with the invagination of the plasma membrane, capturing surface and extracellular proteins to form MVBs. The Golgi apparatus and endoplasmic reticulum aid in the formation of early endosomes, which mature into late endosomes. During this process, ILVs are formed as exosome precursors. Some MVBs fuse with lysosomes or autophagosomes for degradation, while others fuse with the plasma membrane, releasing ILVs as exosomes into the extracellular space with a lipid bilayer similar to the plasma membrane. Abbreviations: ILVs: intraluminal vesicles; MVBs: multivesicular bodies.

Isolating exosomes is a critical step in studying their functions and potential uses. Currently, there are several effective methods of isolation, including differential ultracentrifugation, size- and molecular weight-based ultrafiltration, polymer precipitation, washing separation, immunomagnetic bead separation, microfluidic separation, and mass spectrometry. Each method has its own set of advantages and disadvantages.

Differential ultracentrifugation is the most commonly used technique, separating particles by altering or gradually increasing centrifugation speeds. This method has been widely used for exosome isolation and purification (*30*). While it requires specialized equipment and can be time-consuming, it yields relatively pure exosomes (*31*). Another variation is density gradient centrifugation, which uses sucrose or cesium chloride to establish a density gradient that helps separate exosomes from other components. Although this method enhances exosome purity, it is labor-intensive and time-consuming, and repeated centrifugation or exposure to high centrifugal forces may irreversibly damage the vesicles (*32,33*). Ultrafiltration, based on size and molecular weight, uses membranes with varying pore sizes to filter cell culture supernatants. Although this method is simple and fast, it may cause damage to or loss of exosome structures. It is efficient, economical, and easy to perform, making it suitable for large-scale processing, but the ultrafiltration membranes can become clogged, and excessive pressure during the process can lead to exosome denaturation (*34*). Polymer precipitation involves adding solvents to a solution to alter the polarity and solubility of components, causing exosomes to precipitate. This method, which utilizes commercially available kits, enhances exosome separation from biological fluids (*35,36*). Immunomagnetic bead separation uses the specific binding between antibodies and exosome surface markers for isolation. It is a fast and highly specific method with a high yield and purity (*37,38*). However, the process is expensive and can affect the functionality of exosomes. Microfluidic separation allows precise control and manipulation of micro-scale fluids for exosome isolation in micron/nanometer-sized spaces. This technology is also widely used in cancer diagnosis and detection (*39-41*). Size exclusion chromatography (SEC) separates exosomes based on particle size using a gel filtration column. This method preserves exosome bioactivity, is time-efficient, cost-effective, and highly reproducible. However, it has relatively low recovery rates and purity (*42*). Given the advantages and limitations of each method, combining multiple approaches may help maximize exosome enrichment. One of the main challenges to clinical use of exosomes is the reproducibility of the isolation techniques, thus requiring further optimization and standardization.

Exosome yield is influenced by various factors, with cell type being one of the most critical. Different cells, such as tumor cells, immune cells, and stem cells, differ significantly in exosome yield and content (*43,44*). For example, MSCs secrete large amounts of exosomes, whereas immature dendritic cells produce fewer (*45,46*). In addition, the growth stage and physiological state of the cells affect exosome secretion. Under conditions of starvation, stress, or hypoxia, certain cell types may increase exosome production (*47,48*).

Culture conditions, including medium composition, pH, and temperature, also influence exosome secretion (*49*). Cell seeding density and growth density further affect yield (*50*). To optimize exosome yield, several strategies can be used. Biochemical strategies (*e.g.*, LPS, BMP-2, HIF-1α, interferon-γ (IFN-γ), and TNF-α), physical strategies (*e.g.*, hypoxia, heat stress, and starvation), mechanical strategies (*e.g.*, shear stress and 3D culture), and instrumental strategies (*e.g.*, hollow fiber bioreactors and stirred-tank bioreactors) can significantly enhance exosome production. Optimizing these techniques can not only increase exosome yield but also modulate their properties (*51*).

3.4. Characterization of exosomes

The characterization of exosomes is a crucial step in understanding their functions and mechanisms of action. Current methods primarily focus on analyzing the size, morphology, surface charges, and contents of exosomes. Common techniques for determining exosome size include nanoparticle tracking analysis (NTA), dynamic light scattering (DLS), and tunable resistive pulse sensing (TRPS) (*40*).

NTA measures the size and concentration of exosomes by tracking the Brownian motion of particles in a liquid (*52*). One key advantage of NTA is that it can detect a variety of extracellular vesicles (EVs), including exosomes, with relatively simple sample preparation. In addition, the sample can be recovered after measurement. Fluorescently labeled antibodies may also be used to detect EV antigens. However, a significant limitation is the difficulty in distinguishing exosomes from contaminants, such as proteins (*52-54*).

DLS, in contrast, analyzes the scattered light of particles in suspension to determine their size and is often used to measure the average particle size and distribution of exosomes (*54*). Its benefit lies in its ability to measure particles ranging from 1 nm to 6 μm, which makes it ideal for measuring particles in suspension (*55*).

TRPS measures electrical resistance changes when particles pass through a membrane with tunable pore sizes, allowing for single-particle characterization and concentration measurement of exosomes. However, TRPS is susceptible to issues like system instability, pore clogging, and sensitivity (*56*).

To analyze exosome morphology, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are frequently used. TEM is a high-

resolution imaging technique that uses an electron beam to penetrate the sample, making it ideal for visualizing EV morphology and measuring vesicle diameter. Despite its utility, TEM requires complex sample preparation and is not suitable for rapid analysis of large sample quantities, as it may cause morphological changes in EVs (*57*). In contrast, SEM scans the sample's surface with an electron beam, quickly generating high-resolution images (*53*). SEM offers the advantage of fast imaging and direct observation of solid samples without the need for complex sectioning or staining. However, it cannot be used directly on liquid samples, and sample vacuum stability must also be considered.

Exosome contents are typically analyzed in terms of proteomics, lipidomics, and genomics. Common methods include Western blotting, ELISA, flow cytometry, mass spectrometry, and PCR (*53,58*). These techniques can be used together to obtain comprehensive information about exosomes, including their morphology, size, concentration, surface markers, and molecular composition. In practice, researchers often use a combination of methods such as NTA, TEM, and Western blotting to ensure accurate exosome characterization.

3.5. Exosome heterogeneity

Exosome heterogeneity manifests in their origin, size, composition, and effects on recipient cell functions. Different cells secrete exosomes with diverse biological characteristics (*42,59,60*). Nearly all human cell types, including adipocytes, macrophages, olfactory mucosa cells, dendritic cells, stem cells, and tumor cells, are capable of releasing exosomes.

The size heterogeneity of exosomes may result from uneven invagination of the plasma membrane during biogenesis or contamination with other types of extracellular vesicles during isolation (*61*). These size differences can influence exosome density. In addition, exosomes contain various biomolecules such as proteins, lipids, RNA, and DNA, although the abundance of these molecules varies between exosomes (*62-65*). Notably, there are significant differences in protein and RNA composition between exosomes derived from different sources. Exosome impacts on recipient cells are closely related to surface receptor expression. For example, in cancer therapy, exosomes may either inhibit or promote tumor progression depending on their origin (*66*). The cellular microenvironment and biological characteristics of the donor cells also influence exosome cargo and markers, making exosome heterogeneity a key focus of research. Exosomes are found in various bodily fluids, such as blood, tears, saliva, amniotic fluid, and even breast milk, where they act as stable disease biomarkers that are useful for clinical liquid biopsies (*67-69*).

3.6. Exosome uptake and biodistribution

Exosomes reach their target cells through various mechanisms, including membrane fusion, receptor-mediated interactions, and endocytosis. Membrane fusion involves the direct release of exosomal contents into the cytoplasm of recipient cells when the exosome membrane merges with the recipient cell membrane. Proteins like SNAREs, Rab family proteins, exosomal lipid domains, integrins, and adhesion molecules are crucial to this fusion process (*70,71*). Receptor-mediated interactions occur when transmembrane ligands on the exosome surface bind to recipient cell receptors, triggering downstream signaling cascades in pathways that are often involved in immune regulation and apoptosis (*72,73*).

Endocytosis is another major uptake mechanism, allowing exosomes to be internalized by recipient cells. This can occur through clathrin-mediated endocytosis, phagocytosis, or lipid raft-mediated endocytosis. These processes may occur simultaneously and are not mutually exclusive (*74,75*). After internalization, exosomal cargo is released into the cytoplasm, depending on factors like the exosome's origin, cargo, and the metabolic state of the recipient cell (*76*). For instance, research has demonstrated that oncogenic signaling induced by mutant KRAS expression promotes the uptake of exosomes by human pancreatic cancer cells *via* macrophages (*77,78*). In contrast, human melanoma cells have been found to internalize exosome contents through plasma membrane fusion (*79*), while neurosecretory PC12 cells (derived from rat adrenal medullary tumors) exhibit a higher dependency on lectin-mediated endocytosis for exosomal cargo uptake (*80*).

The biodistribution of exogenous exosomes is influenced by factors such as the route of administration, exosome size, and surface composition. Different routes result in distinct distribution patterns. For instance, intravenously administered exosomes primarily accumulate in the liver, spleen, lungs, and gastrointestinal tract but are quickly cleared from circulation (*81,82*). Oral administration allows exosomes to reach multiple organs, including the brain (*83*), while intranasal administration efficiently delivers exosomes to the brain (*84,85*). Intratumoral injection ensures exosomes remain within tumors longer (*86*).

Exosome size also plays a role in biodistribution, with larger vesicles accumulating in bones, lymph nodes, and the liver (*61*). In addition, surface molecular composition influences *in vivo* distribution. For example, rabies virus glycoprotein (RVG) can direct exosomes to the brain (*87*), while cancer-derived exosomes rich in mannose and sialic acid target specific cell types (*88*). Another notable example involves RVG, which has been found to direct exosomal delivery specifically to the brain (*87*). Moreover, glycoproteins enriched with mannose and sialic acid on the surface of cancer cells have been identified as key contributors to the targeting of specific cell types (*88*). Glioblastoma-derived

exosomes, which are rich in phosphatidylethanolamine, show a greater propensity to target glioblastoma cells, while sphingomyelin-rich melanoma-derived exosomes demonstrate enhanced targeting capabilities within the tumor microenvironment (*79*).

3.7. Storage of exosomes

The storage of exosomes is essential to maintaining their stability and biological functionality. To prevent degradation, exosomes are typically stored at low temperatures. Common methods of preservation include cryopreservation, freeze-drying, and spray-drying. The optimal storage temperature is -80°C, which ensures long-term stability, whereas storage at 4°C may result in compromised biological activity and reduced protein content (*89,90*). For short-term storage, -20°C may suffice.

Non-permeable disaccharide cryoprotectants such as sucrose and trehalose are often used to enhance exosome protection during low-temperature storage (*91*). Freeze-drying, which converts exosomes into a stable powder form, is another effective method of long-term preservation that also aids in transportation. Freeze-drying (using lyophilization) removes moisture, preventing degradation, and studies suggest that exosomes treated with cryoprotectants can retain their protein and RNA activity for up to four weeks at room temperature (*92*).

Spray-drying offers another way to convert exosomes into dry powder, enhancing their stability and facilitating their transport (*93*). Prior to use, exosome quality needs to be assessed, including particle size and concentration, to ensure their integrity and activity.

3.8. The role and therapeutic potential of exosomes in neurodegenerative disorders

Exosomes play a crucial role in the pathogenesis of neurodegenerative diseases, largely by promoting or mitigating the aggregation of misfolded or unfolded proteins in the brain (*94-96*). Research has indicated that exosomes facilitate disease progression by spreading and aggregating misfolded proteins. For example, exosomes have been implicated in the transmission of the infectious prion protein PrPSc, which is associated with prion diseases (*97*). In AD, Tau and Aβ amyloid proteins have been detected in exosomes derived from cerebrospinal fluid (CSF) of patients (for Tau) and from the culture supernatants of mouse and human cells (for Aβ). Exosomes are also involved in propagating Tau aggregation pathology (*98,99*). In HeLa and Neuro-2a cells, APP cleavage occurs in early endosomes, leading to the release of Aβ into the extracellular space *via* exosomes (*98*). However, whether exosomes promote the oligomerization of neurotoxic Aβ *in vivo* remains unclear, and this warrants further investigation.

In addition to their role in the spread of misfolded proteins, exosomes have also been found to engage in the clearance of such proteins, acting as detoxifying agents and having a neuroprotective effect. By inhibiting the formation of neurotoxic oligomers or facilitating their expulsion from the cell, exosomes contribute to cellular protection (*100,101*). For instance, in APP transgenic mice, dysfunction of neuron-secreted exosomes, specifically in relation to endothelin-converting enzyme 1/2 (ECE1/2), leads to an accumulation of oligomeric Aβ within exosomes, accelerating the spread of Aβ amyloid (*102*). A similar phenomenon has been observed in Parkinson's disease (PD) and other proteinopathies, where exosomes from the CSF of patients with PD contain aggregates of α-synuclein (*103*). Moreover, α-synuclein inhibits the endosomal sorting complex required for transport (ESCRT) system, thereby limiting its intracellular degradation and promoting intercellular propagation (*104*). In amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration, cytoplasmic aggregation of TDP-43 is a hallmark pathology. In TDP-43 A325T transgenic mice, the secretion of exosomes facilitates the clearance of TDP-43 from neuronal cell bodies (*105*).

While the primary role of exosomes in neurodegenerative diseases is related to regulating misfolded proteins, other components, such as nucleic acids, also contribute to disease progression or amelioration (*106*). Importantly, exosomes can cross the BBB, making them a promising therapeutic approach for neurodegenerative disorders (*107*).

3.9. Comparison of MSC therapy and MSC-Exos therapy

Research has shown that MSCs derived from bone marrow, adipose tissue, and dental pulp can differentiate into neurons (*7,108,109*). In addition, MSCs play a crucial role in tissue repair and regeneration through paracrine signaling. By secreting a variety of bioactive factors, MSCs regulate the behavior of surrounding cells, promoting tissue repair, anti-inflammatory responses, immune modulation, and angiogenesis. Specifically, MSCs secrete growth factors (such as TGF-β, VEGF, and epidermal growth factor (EGF)), anti-inflammatory and immune-modulating factors (such as IL-10, TGF-β, Prostaglandin E2 (PGE2), and Indoleamine 2,3-dioxygenase (IDO)), angiogenic factors (such as VEGF, FGF, and PDGF), and anti-apoptotic and antioxidant factors (such as HGF and IGF-1), all of which provide vital support to damaged neurons and which enhance neural tissue repair and regeneration (*110*). Due to their ability to differentiate into neurons and their potent immunosuppressive and pro-angiogenic properties, MSCs have emerged as a promising treatment strategy for neurological disorders (*111*).

However, several safety concerns must be addressed when using MSCs in clinical settings. Factors such as

donor age, genetic characteristics, and medical history significantly influence the therapeutic potential of MSCs (*112*). This is particularly important for autologous MSC transplantation in elderly patients, where age-related changes, such as diminished proliferation and differentiation potential, may reduce therapeutic efficacy (*112*). Although MSCs typically exhibit low immunogenicity, they can still trigger immune responses (*113*). In addition, MSCs from different sources may exhibit varying immunogenic profiles, requiring close monitoring of potential immune reactions, especially in allogeneic transplants. MSCs are generally considered to have low tumorigenic potential, but there is still a risk of abnormal proliferation or transformation in certain *in vivo* microenvironments, particularly in long-term cultures or with genetically modified MSCs (*112,114*). The behavior of MSCs may also vary depending on the tissue and disease context; in some cancer environments, MSCs have been found to promote tumor growth and metastasis. Therefore, a thorough understanding and monitoring of MSC behavior in specific pathological settings is critical.

Moreover, when MSCs are used in combination with immunosuppressive drugs, respiratory and gastrointestinal infections have occurred in some patients, indicating that MSCs should not be used concurrently with other immunosuppressive therapies (*112,114*). In contrast, MSC-Exos contain bioactive factors that regulate immune responses, vascular function, and neuronal repair. Due to their nanoscale size and lipid-encapsulated structure, MSC-Exos can easily penetrate neural tissues and reach target cells (*115*). The exosomal membrane, which is rich in cholesterol, sphingolipids, ceramides, and lipid raft proteins, protects the exosomal contents from degradation. In addition, adhesion molecules such as CD29, CD44, and CD73 expressed on MSC-Exos promote their migration to inflamed or damaged tissues. Once they reach their destination, MSC-Exos directly fuse with cell membranes, delivering their contents into the cytoplasm of target cells and modulating their phenotype and function (*116*). MSC-Exos contain nucleic acids, proteins (such as cytokines and chemokines), and lipids that can alter the phenotype, function, and viability of neuronal and immune cells (*116*). Thus, MSC-Exos play an important role in treating neuroinflammatory diseases.

Importantly, no adverse effects have been observed in animal models or patients treated with MSC-Exos, suggesting that these exosomes could serve as a safer alternative to MSC therapy for treating inflammatory and degenerative neurological diseases (*115*). MSC-Exos inherit the therapeutic properties of MSCs, including anti-inflammatory, immune-modulatory, and regenerative effects, and they represent a viable alternative to MSC therapy while mitigating the risks associated with MSC use (Table 1).

4. Use of MSC-Exos in AD

MSC-Exos have a neuroprotective effect against AD *via* a variety of mechanisms, including the clearance of abnormal protein accumulations, alleviation of inflammation, and reduction of oxidative stress. Collectively, these effects significantly alleviate the pathological changes associated with AD and improve cognitive ability (Table 2).

4.1. Clearance of abnormal protein accumulation

Table 1. Comparison of MSC therapy and MSC-Exos therapy

Characteristics	MSC therapy	MSC-Exos therapy
Mechanism	Regeneration and repair *via* living cells	Therapy through exosomes released by MSCs
Multilineage differentiation potential	Present	Absent
Applicability	Suitable for treating various diseases	MSC-Exos have efficacy comparable to that of stem cells but are smaller in size
Isolation	MSCs are easily isolated and are scalable for large-scale production	Lower yield in large-scale production
Safety	May induce immune responses and carry a risk of tumor formation	Minimal risk of tumor formation and immune response
Ethical concerns	Ethical issues exist	No ethical concerns
Transportation and storage	Requires strict storage and transportation conditions	Stable, suitable for long-term storage and transport
Regulatory approval	Regulatory guidelines have been established	Lacks standardized quality control and faces regulatory challenges

Abbreviations: MSC: mesenchymal stem cell; MSC-Exos: mesenchymal stem cell-derived exosomes.

A hallmark of AD pathology is the presence of Aβ plaques and hyperphosphorylated tau proteins, which contribute to synaptic disruption and neuronal degeneration. Therefore, clearing these abnormal proteins is crucial to improving synaptic function and neuronal survival, representing a significant therapeutic target for AD (*130*). Notably, MSC-Exos demonstrate the ability to modulate the levels of key proteins involved in the progression of AD.

For instance, sphingosine-1-phosphate (S1P) receptors, which are widely expressed throughout the body, play vital roles in various physiological processes, including angiogenesis, neurogenesis, immune cell trafficking, endothelial barrier function, and vascular tone regulation (*131*). Bone-marrow mesenchymal stem cell-derived exosomes (BMSC-Exos) can reduce Aβ deposition and promote cognitive recovery in mice with AD by enhancing the expression of S1P (*117*). In addition, neprilysin (NEP), a membrane-bound metallopeptidase capable of degrading neuropeptides and amyloid proteins, represents a potential therapeutic target for AD (*132,133*). Research by Elia *et al.* demonstrated that injecting BMSC-Exos into the cortex of APP/PS1 mice significantly increased the expression and activity of NEP, leading to reduced Aβ protein deposition in both the hippocampus and cortex (*118*). Similarly, Wang *et al.* administered human umbilical cord mesenchymal stem cell-derived exosomes (hUC-MSC-Exos) to 9-month-old APP/PS1 mice *via* the tail vein. This intervention resulted in a reduction in Aβ accumulation and neuronal loss in the hippocampus, along with improvements in cognitive function, likely mediated by its effects on the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway, which is a critical mediator of neuronal damage in AD (*119*).

Moreover, MSC-Exos have been found to possess the capability to clear pathological proteins through non-coding RNAs. The pathology of AD begins with the cleavage of APP by β-secretase (BACE), making BACE1 inhibitors potential therapeutic agents for AD (*134,135*). Research by Sha *et al.* revealed that microRNA-29c-3p, delivered through BMSC-Exos, suppressed BACE1 expression (*120*) and activated the Wnt/β-catenin pathway, thereby lowering BACE1 levels and demonstrating efficacy against AD (*136*).

4.2. Anti-inflammatory effects

Neuroinflammation is a significant feature in the pathological process of AD and is primarily characterized by the abnormal activation of immune cells, such as microglia and astrocytes, within the brain, along with excessive production of inflammatory factors. In the early stages of AD, activated microglia typically exhibit an M2 phenotype, which is associated with neuroprotective and anti-inflammatory properties. As the disease progresses, however, this M2 phenotype gradually shifts towards a pro-inflammatory M1 phenotype, exacerbating neuroinflammation and neuronal damage.

The immunomodulatory effects of MSC-Exos in the treatment of AD primarily involve the inhibition of neuroinflammation and the regulation of microglial polarization. Specifically, MSC-Exos can reduce the production of pro-inflammatory cytokines and inhibit the activation of both microglia and astrocytes. In addition, MSC-Exos promote the polarization of microglia from the M1 phenotype to the M2 phenotype, thereby mitigating neuroinflammation and facilitating neural repair, which ultimately alleviates AD-related neurodegeneration. For instance, research by Ding *et al.* demonstrated that injecting hUC-MSC-Exos in a mouse model of AD modulated the phenotype and function of microglia in APP/PS1 mice, thereby alleviating neuroinflammation (*121*).

Moreover, the findings of Nakano *et al.* corroborate these results, showing that miR-146a present in BMSC-Exos can inhibit TRAF6 and IRAK1 in microglia, subsequently reducing NF-κB activity. This reduction leads to decreased gene expression of inducible nitric oxide synthase (iNOS), TNF-α, IL-1β, and IL-6, resulting in diminished production of the pro-inflammatory M1 phenotype in microglia treated with MSC-Exos (*122*). In addition to miR-146a, MSC-derived miR-21 also plays a role in modulating immunity and protecting neurons. Research has indicated that hypoxia-preconditioned MSC-Exos inhibit microglial activation through miR-21, decreasing inflammatory factors (such as TNF-α and IL-1β) while increasing the anti-inflammatory factor IL-10 (*123*). Thus, MSC-Exos induce anti-inflammatory effects by inhibiting activated microglia, reactive astrocytes, and the release of cytokines (*137*).

Importantly, overexpression or dysregulation of pro-inflammatory cytokines (such as iNOS) can promote AD pathology (*138*). Studies have shown that MSC-derived EVs have beneficial effects in mouse models of AD by inhibiting Aβ-induced iNOS expression in APP/PS1 mice (*139*). Moreover, research by Liu *et al.* demonstrated that direct injection of exosomes into the lateral ventricles of mice with AD significantly reduced the activation of glial cells in the hippocampus and lowered levels of pro-inflammatory markers such as IL-1β and TNF-α. This indicates that exosomes have an inhibitory effect on inflammatory responses, potentially mitigating the damage caused by neuroinflammation (*129*).

In addition, Godoy *et al.* found that MSC-Exos naturally contain and carry endogenous catalase. This enzyme can be delivered to neurons *via* exosomes, providing antioxidant protection and preventing synaptic loss in neurons exposed to Aβ. This finding suggests that MSC-Exos may offer a novel therapeutic possibility for AD by reducing oxidative stress and its associated neuronal damage (*124*).

Table 2. Use of MSC-Exos in AD

Source of Exosomes	Method of extraction	Administration	Mechanism	Effect	Mouse model	Reference
BMSCs	Total exosome isolation reagent	Intravenous injection *via* the tail vein	Reduces Aβ deposition in mice with AD by activating the SphK/S1P signaling pathway	Promotes the recovery of cognitive function	Female double-transgenic APP/PS1 mice	(*117*)
BMSCs	Ultracentrifugation	Intracortical injection	BMSC-EVs enhance the expression and activity of neprilysin	Reduces Aβ plaque load and dystrophic neurites in the cortex and hippocampus	Male APP/PS1 mice	(*118*)
hUC-MSCs	Ultracentrifugation	Intravenous injection *via* the tail vein	Modulates nuclear factor E2-related factor 2 (Nrf2)	Reduces Aβ accumulation and neuron loss in the hippocampus and improves cognitive function	Male APP/PS1 mice	(*119*)
BMSCs	Ultracentrifugation	Intracerebroventricular injection	BMSC-EVs deliver miR-29c-3p to target BACE1 and activate the Wnt/β-catenin pathway	Lowers BACE1 levels and reduces Aβ aggregation	SD rats injected with oligomeric Aβ1-42 (5 μg/μL)	(*120*)
hUC-MSCs	ExoQuick-TC (polymer precipitation)	Intravenous injection *via* the tail vein	Reduces acute inflammation and selectively activates microglia, increasing "M2 microglia" and decreasing "M1 microglia"	Relieves neuroinflammation, clears Aβ deposition, and restores cognitive function	APPswe/PS1-dE9 mice	(*121*)
BMSCs	MagCapture exosome isolation kit (magnetic bead-based isolation)	Intracerebroventricular injection	miR-146a reduces NF-κB activity by inhibiting TRAF6 and IRAK1 in microglia	Promotes synaptogenesis and alleviates cognitive impairment	APP-695swe/PS1-dE9 mice	(*122*)
BMSCs	ExoQuick exosome precipitation solution (polymer precipitation)	Intravenous injection *via* the tail vein	miRNA-21 inhibits microglial activation, decreases inflammatory factors (TNF-α and IL-1β), increases anti-inflammatory IL-10, enhances synaptophysin expression, and restores synaptic function	Improves learning and memory and alleviates Aβ pathology	Male APP/PS1 mice	(*123*)
BMSCs	Ultracentrifugation	NA	Catalase antioxidant protection	Reduces AβO-induced oxidative stress and synaptic damage *in vitro*	Hippocampal neurons induced by AβO	(*124*)
MSCs	Ultracentrifugation	Bilateral dentate gyrus injection	Stimulates neurogenesis in the ventricles	Promotes neurogenesis and recovery of cognitive function	C57BL/6 mice injected with Aβ aggregates in the bilateral dentate gyrus	(*125*)
ADMSCs	Ultracentrifugation	Intranasal administration	Upregulates genes related to neuronal neurogenesis and neurite outgrowth	Promotes neurogenesis and alleviates memory deficits	APP/PS1 mice	(*126*)
hWJ-MSCs	Ultracentrifugation	NA	Catalase-mediated protection	Attenuates AβO-induced oxidative stress and synaptic damage	Primary hippocampal cultures exposed to AβOs	(*127*)
WJ-MSCs	Ultracentrifugation	Intravenous injection	Increases expression of NR2B, GluR1, GluR2, NR2A, Syp, and BDNF, all related to synaptic plasticity and memory	Significantly improves brain glucose metabolism and cognitive function	AD (JAX-006293) mice	(*128*)
BMSCs	Exosome isolation kit (ultrafiltration tube kit)	Intravenous/ intracerebroventricular injection	Upregulates the expression of BDNF and synapse-associated proteins in the hippocampus	Improves cognitive deficits in AD mice	Male C57BL/6 mice, sporadic AD model established by streptozotocin	(*129*)

Abbreviations: AβOs: amyloid β oligomers; AD: Alzheimer's disease; ADMSCs: adipose-derived mesenchymal stem cells; BDNF: brain-derived neurotrophic factor; BMSCs: bone-marrow mesenchymal stem cells; hUC-MSCs: human umbilical cord mesenchymal stem cells; hWJ-MSCs: human Wharton's Jelly MSCs; MSCs: mesenchymal stem cells; WJ-MSCs: Wharton's jelly MSCs.

4.3. Promotion of neurogenesis

MSC-Exos have displayed the ability to alleviate the neuropathology associated with AD and improve cognitive function by promoting the proliferation and differentiation of neural stem cells, as well as by enhancing synaptic plasticity. A study has indicated that MSC-Exos can stimulate the generation of new neurons within the brain ventricles, thereby alleviating cognitive impairments caused by β1-42 amyloid protein (*125*). A notable study by Ma *et al.* found that EVs secreted by adipose-derived mesenchymal stem cells (ADMSCs) and delivered intranasally rapidly entered the brain, significantly repairing neural damage and improving spatial learning and memory abilities (*140*).

Moreover, MSC-Exos promote the expression of nerve growth factor (NGF), which enhances the survival and functionality of neural cells. Research by Li *et al.* demonstrated that MSC-Exos upregulate NGF expression and activity, stimulating the proliferation and differentiation of adult neural stem cells. This process promotes neurogenesis and synaptic plasticity, ultimately alleviating cognitive deficits associated with AD (*141*).

As research on the pathological mechanisms of AD progresses, the potential use of MSCs and their derived exosomes in treating neurodegenerative diseases has garnered increasing attention. Notably, MSC-Exos demonstrate significant potential for the treatment of AD through various mechanisms, including the clearance of abnormal protein accumulation, inhibition of neuroinflammation, and promotion of neurogenesis. However, an important point to note is that most of the current research involves animal models, underscoring the need for more clinical trials to verify the efficacy and safety of MSC-Exos.

5. Advanced techniques for optimizing exosome function

With advances in research on MSC-Exos, these nanoscale vesicles have shown significant promise in regenerative medicine and disease treatment. Exosomes are essential carriers of intercellular signaling, and their bioactivity and functionality are influenced by several factors, including the physiological state of the source cells, culture conditions, and changes in the extracellular environment (*142*). Therefore, exploring cutting-edge technologies to optimize exosome function is crucial to enhancing their therapeutic efficacy in neurodegenerative diseases such as AD.

5.1. The impact of preconditioning techniques on exosomes

Preconditioning techniques involve manipulating the parent cells under specific culture conditions, such as hypoxia, three-dimensional (3D) culture, and serum deprivation. In addition, biochemical stimuli like lipopolysaccharides, nitric oxide, pro-inflammatory cytokines, or exogenous genes (*e.g.*, plasmid DNA and miRNAs) can be introduced to alter the culture environment, thereby modulating the function of the cells (*143-145*). Recent research has indicated that hypoxia or pro-inflammatory cytokine preconditioning can be effectively used to produce MSC-Exos for the treatment of AD. For example, a study by Cui *et al.* demonstrated that exosomes derived from hypoxia-preconditioned mesenchymal stem cells (PC-MSCs) significantly increased the levels of miR-21 in the brains of mice with AD. This increase not only reduced Aβ deposition but also decreased pro-inflammatory cytokines such as TNF-α and IL-1β, thereby enhancing therapeutic efficacy in transgenic mice with AD (*123*). Similarly, Liu *et al.* found that exosomes derived from hypoxia-preconditioned ADMSCs improved cognitive function in mice with AD by upregulating circRNA-Epc1. This modulation of microglial M1/M2 polarization reduced neuronal damage and enhanced cognitive function (*146*). Hypoxic preconditioning of MSCs enhances their neuroprotective effects primarily by inducing the secretion of HIF-1α, reactive oxygen species (ROS), and anti-inflammatory cytokines (*147*). Moreover, preconditioning MSCs with pro-inflammatory cytokines enhances the immunomodulatory properties of MSC-Exos. For instance, Losurdo *et al.* found that MSC-Exos preconditioned with IFNγ and TNF-α suppressed microglial activation and increased dendritic spine density, thereby displaying both immunomodulatory and neuroprotective effects in AD (*148*).

In addition, Chen *et al.* demonstrated that preconditioning MSCs with an prostaglandin E_2 receptor 4 (EP4) antagonist inhibited the proliferation of reactive astrocytes, reduced widespread inflammation, enhanced BBB integrity, and alleviated learning and memory deficits (*149*). Moreover, altering MSC culture conditions, such as use of 3D culture methods, can significantly modify the miRNA and protein profiles of MSC-Exos (3D-MSC-Exos) compared to traditional two-dimensional culture systems. Studies have confirmed that 3D culture upregulates α-secretase expression and inhibits β-secretase activity, thereby reducing Aβ production in pathological AD cells and transgenic mice (*142*).

These findings suggest that preconditioning strategies can enhance MSC functionality by modifying the culture environment, thus optimizing their performance before transplantation. However, an important point is to carefully consider the effects of preconditioning on the parent cells, as changes in physiological conditions may impact therapeutic outcomes in particular. Therefore, assessing whether the components generated within exosomes can negatively affect these outcomes is essential.

5.2. Drug-loaded MSC-Exos

Loading drugs into MSC-Exos capitalized on the natural delivery capabilities of exosomes to transport therapeutic agents more effectively to targeted disease sites. Drug-loading strategies can be broadly categorized into two main approaches: the first involves loading drugs into the parent cells, which subsequently transfer these drugs to exosomes. This can be achieved by co-culturing the parent cells with the drug or by using chemical methods (*e.g.*, liposomal transfection) to introduce the drug into the parent cells, after which the drugs are encapsulated within the exosomes (*44,84,150*). The second approach is direct exosome loading, where exogenous drugs are directly introduced into isolated exosomes. This method includes co-incubating the drug with isolated exosomes or using physical techniques (*e.g.*, electroporation, low-permeability dialysis, and ultrasound treatment) and chemical methods (*e.g.*, liposomes) to transfer the drug to exosomes (*151,152*).

Both approaches aim to optimize drug delivery efficiency and enhance therapeutic efficacy. Current research has shown that nucleic acids, proteins, and drugs can be effectively loaded into MSC-Exos for the treatment of AD. For instance, Zhai *et al.* successfully loaded miRNA-22 into ADMSCs, producing miRNA-22-loaded exosomes that suppressed neuroinflammation in mice with AD, resulting in improved behavioral and memory function (*153*). Similarly, Jahangard *et al.* loaded miR-29b into BMSCs, generating miR-29b-enriched exosomes that helped reduce Aβ peptide pathology in a rat model of AD (*154*). Moreover, Xu *et al.* used genetic engineering techniques to transfect MSCs with the gene encoding tyrosine phosphatase-2 (SHP2), resulting in exosomes enriched with SHP2. These SHP2-expressing MSC-Exos significantly induced mitophagy in neuronal cells, alleviating mitochondrial damage-induced apoptosis and NLRP3 inflammasome activation in mice with AD (*155*). Another study demonstrated that exosomes derived from enkephalinase gene-modified human umbilical cord mesenchymal stem cells (hUC-MSCs) significantly enhanced the effects of hUC-MSCs on memory and cognitive improvement in mice with AD (*132*).

The advantages of using MSC-Exos for drug loading are twofold. First, MSC-Exos possess inherent neuroprotective properties, including the ability to reduce Aβ accumulation, inhibit inflammatory responses, and improve neuronal function. Second, MSC-Exos have strong targeting capabilities, efficiently delivering drugs to neurons and diseased regions. In addition, they have excellent biocompatibility, lower immunogenicity, and the ability to cross the BBB, delivering therapeutic agents directly to brain tissue and influencing AD pathology. A strategy that combines the therapeutic benefits of both drugs and MSC-Exos offers new possibilities and directions for AD treatment.

5.3. Surface modification and artificial exosomes

Surface modification techniques allow for the endowment of natural exosomes with unique functions, such as targeted delivery, through genetic engineering or chemical modification of specific peptides or ligands (*156*). Surface functionalization can be divided into two categories: endogenous and exogenous modifications. Endogenous functionalization involves modifying the exosome surface by transfecting vectors into the parent cells. This approach preserves the essential functions and integrity of the exosomes; however, it may introduce heterogeneity and requires complex purification processes to isolate the functionalized exosomes (*157*). In contrast, exogenous functionalization uses physical or chemical methods to directly modify the exosome membrane. Physical methods include extrusion, sonication, and freeze-thaw cycles, while chemical methods utilize various click chemistry techniques (*158*). However, these methods may affect the internal cargo of the exosomes, and further research needs to be conducted to fully understand their impact on functionality, integrity, and therapeutic potential.

In addition to natural exosomes, artificial exosomes, which act as substitutes, offer greater scalability and flexibility. They can be produced through techniques such as extrusion and microfluidics (*159*). Artificial exosomes can retain multiple intracellular components from the parent cells, and their RNA and protein content may be double that of natural exosomes (*160*). During the production process, drugs and therapeutic molecules can also be loaded into artificial exosomes. However, several challenges remain for the clinical use of artificial exosomes. These include potential contamination with other organelles during purification, low encapsulation efficiency due to quality control issues, and alterations in the ratio of cellular membrane components (*161*). Therefore, the establishment of standard operating procedures and further exploration into their use in AD treatment are essential.

6. Therapeutic advantages and challenges

The use of MSC-Exos in the treatment of AD has gained increasing attention. As a cell-free alternative to stem cell therapy, these exosomes exhibit immense potential for the treatment of neurodegenerative diseases because of their unique biological properties and therapeutic benefits. Compared to traditional stem cells and nanocarriers, MSC-Exos offer superior biocompatibility and lower immunogenicity. Since exosomes are non-replicating entities, they eliminate the tumorigenic risks associated with cell proliferation. In addition, unlike artificial nanoparticles, they do not trigger strong immune responses, which reduces toxicity and contributes to their excellent *in vivo* stability. This safety profile makes MSC-Exos ideal for allogeneic applications, minimizing

the risk of immune rejection.

Another key advantage is their nanoscale size, which enables MSC-Exos to effectively cross the BBB—an essential feature for AD treatment. Their natural properties allow exosomes to remain stable within the body and efficiently reach their target cells. As a drug delivery system, MSC-Exos can transport therapeutic molecules directly to specific cells within brain tissue, and they display a particular affinity for packaging and delivering nucleic acid-based drugs. Moreover, engineered MSC-Exos with surface modifications can enhance targeting capabilities, further improving the efficiency of drug delivery. The high proliferative capacity and multi-differentiation potential of MSCs also make the large-scale production of consistent exosomes feasible, a critical factor for clinical use. In addition to these advantages, MSC-Exos have multiple biological effects—such as anti-inflammatory, antioxidative, immunomodulatory, and neuroprotective properties—enabling them to mitigate AD pathology through various mechanisms.

Despite these significant theoretical advantages, several challenges still impede the clinical use of MSC-Exos. First, the isolation, purification, and storage processes for MSC-Exos require further optimization and standardization. Current methods of isolation, which mainly rely on density and size, may result in cross-contamination with other biological molecules like lipoproteins and viruses, potentially compromising purification quality and therapeutic efficacy. Thus, there is an urgent need for more efficient and cost-effective isolation techniques, as well as standardized production processes, to ensure the consistency and reproducibility of MSC-Exos. In addition, the complex composition of MSC-Exos may include harmful paracrine factors that could interfere with therapeutic outcomes or cause adverse effects. To address these concerns, detailed analyses of the components within MSC-Exos need to be performed to identify and remove potential harmful factors while maintaining rigorous quality control before clinical use.

Moving forward, future research and clinical efforts should focus on addressing these challenges and further exploring the therapeutic potential of MSC-Exos in AD treatment. Developing efficient production and purification methods will help improve the yield and consistency of MSC-Exos. Moreover, investigating novel delivery methods—such as local delivery and the incorporation of biomaterials to protect and enhance exosome delivery—could significantly improve therapeutic efficacy. Grappling with these aspects collectively would position MSC-Exos to play a crucial role in treating AD and other neurodegenerative diseases, paving the way for their emergence as a viable therapeutic option.

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References

1. Hu X, Ma YN, Karako K, Song P, Tang W, Xia Y. Guardians of memory: The urgency of early dementia screening in an aging society. Intractable Rare Dis Res. 2024; 13:133-137.
2. Booker SA, Wyllie DJA. NMDA receptor function in inhibitory neurons. Neuropharmacology. 2021; 196:108609.
3. Marucci G, Buccioni M, Ben DD, Lambertucci C, Volpini R, Amenta F. Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease. Neuropharmacology. 2021; 190:108352.
4. Hu X, Ma YN, Xia Y. Association between abnormal lipid metabolism and Alzheimer's disease: New research has revealed significant findings on the APOE4 genotype in microglia. Biosci Trends. 2024; 18:195-197.
5. Liu ZZ, Huang Y, Hong CG, Wang X, Duan R, Liu JY, He JL, Duan D, Xie H, Lu M. Autologous olfactory mucosa mesenchymal stem cells treatment improves the neural network in chronic refractory epilepsy. Stem Cell Res Ther. 2023; 14:237.
6. Kuang Y, Zheng X, Zhang L, Ai X, Venkataramani V, Kilic E, Hermann DM, Majid A, Bahr M, Doeppner TR. Adipose-derived mesenchymal stem cells reduce autophagy in stroke mice by extracellular vesicle transfer of miR-25. J Extracell Vesicles. 2020; 10:e12024.
7. Lin H, Sohn J, Shen H, Langhans MT, Tuan RS. Bone marrow mesenchymal stem cells: Aging and tissue engineering applications to enhance bone healing. Biomaterials. 2019; 203:96-110.
8. Harrell CR, Volarevic A, Djonov V, Volarevic V. Mesenchymal stem cell-derived exosomes as new remedy for the treatment of neurocognitive disorders. Int J Mol Sci. 2021; 22:1433.
9. Orobets KS, Karamyshev AL. Amyloid precursor protein and Alzheimer's disease. Int J Mol Sci. 2023; 24:14794.
10. Trejo-Lopez JA, Yachnis AT, Prokop S. Neuropathology of Alzheimer's disease. Neurotherapeutics. 2022; 19:173-185.
11. Ao C, Li C, Chen J, Tan J, Zeng L. The role of Cdk5 in neurological disorders. Front Cell Neurosci. 2022; 16:951202.
12. Lai S, Wang P, Gong J, Zhang S. New insights into the role of GSK-3beta in the brain: From neurodegenerative disease to tumorigenesis. PeerJ. 2023; 11:e16635.
13. Leng F, Edison P. Neuroinflammation and microglial activation in Alzheimer disease: Where do we go from here? Nat Rev Neurol. 2021; 17:157-172.
14. Marogianni C, Sokratous M, Dardiotis E, Hadjigeorgiou GM, Bogdanos D, Xiromerisiou G. Neurodegeneration and inflammation-An interesting interplay in Parkinson's disease. Int J Mol Sci. 2020; 21:8421.

15. Culig L, Chu X, Bohr VA. Neurogenesis in aging and age-related neurodegenerative diseases. Ageing Res Rev. 2022; 78:101636.
16. Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. Cell Mol Life Sci. 2018; 75:193-208.
17. Mathieu M, Martin-Jaular L, Lavieu G, Thery C. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. Nat Cell Biol. 2019; 21:9-17.
18. van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol. 2018; 19:213-228.
19. Kahlert C, Kalluri R. Exosomes in tumor microenvironment influence cancer progression and metastasis. J Mol Med (Berl). 2013; 91:431-437.
20. Pegtel DM, Gould SJ. Exosomes. Annu Rev Biochem. 2019; 88:487-514.
21. Ciardiello C, Cavallini L, Spinelli C, Yang J, Reis-Sobreiro M, de Candia P, Minciacchi VR, Di Vizio D. Focus on extracellular vesicles: New frontiers of cell-to-cell communication in cancer. Int J Mol Sci. 2016; 17:175.
22. Bebelman MP, Smit MJ, Pegtel DM, Baglio SR. Biogenesis and function of extracellular vesicles in cancer. Pharmacol Ther. 2018; 188:1-11.
23. Skryabin GO, Komelkov AV, Savelyeva EE, Tchevkina EM. Lipid rafts in exosome biogenesis. Biochemistry (Mosc). 2020; 85:177-191.
24. Skotland T, Hessvik NP, Sandvig K, Llorente A. Exosomal lipid composition and the role of ether lipids and phosphoinositides in exosome biology. J Lipid Res. 2019; 60:9-18.
25. Mashouri L, Yousefi H, Aref AR, Ahadi AM, Molaei F, Alahari SK. Exosomes: Composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. Mol Cancer. 2019; 18:75.
26. Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: Biogenesis, biologic function and clinical potential. Cell Biosci. 2019; 9:19.
27. Ge L, Zhang N, Li D, Wu Y, Wang H, Wang J. Circulating exosomal small RNAs are promising non-invasive diagnostic biomarkers for gastric cancer. J Cell Mol Med. 2020; 24:14502-14513.
28. Shelke GV, Yin Y, Jang SC, Lasser C, Wennmalm S, Hoffmann HJ, Li L, Gho YS, Nilsson JA, Lotvall J. Endosomal signalling *via* exosome surface TGFbeta-1. J Extracell Vesicles. 2019; 8:1650458.
29. Munich S, Sobo-Vujanovic A, Buchser WJ, Beer-Stolz D, Vujanovic NL. Dendritic cell exosomes directly kill tumor cells and activate natural killer cells *via* TNF superfamily ligands. Oncoimmunology. 2012; 1:1074-1083.
30. Yang D, Zhang W, Zhang H, Zhang F, Chen L, Ma L, Larcher LM, Chen S, Liu N, Zhao Q, Tran PHL, Chen C, Veedu RN, Wang T. Progress, opportunity, and perspective on exosome isolation - Efforts for efficient exosome-based theranostics. Theranostics. 2020; 10:3684-3707.
31. Shao H, Im H, Castro CM, Breakefield X, Weissleder R, Lee H. New technologies for analysis of extracellular vesicles. Chem Rev. 2018; 118:1917-1950.
32. Xu K, Jin Y, Li Y, Huang Y, Zhao R. Recent progress of exosome isolation and peptide recognition-guided strategies for exosome research. Front Chem. 2022; 10:844124.
33. Melo SA, Luecke LB, Kahlert C, *et al.* Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. Nature. 2015; 523:177-182.
34. Jalaludin I, Lubman DM, Kim J. A guide to mass spectrometric analysis of extracellular vesicle proteins for biomarker discovery. Mass Spectrom Rev. 2023; 42:844-872.
35. Soares Martins T, Catita J, Martins Rosa I, O ABdCES, Henriques AG. Exosome isolation from distinct biofluids using precipitation and column-based approaches. PLoS One. 2018; 13:e0198820.
36. Menon R, Dixon CL, Sheller-Miller S, Fortunato SJ, Saade GR, Palma C, Lai A, Guanzon D, Salomon C. Quantitative proteomics by SWATH-MS of maternal plasma exosomes determine pathways associated with term and preterm birth. Endocrinology. 2019; 160:639-650.
37. Doyle LM, Wang MZ. Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. Cells. 2019; 8:727.
38. Que RS, Lin C, Ding GP, Wu ZR, Cao LP. Increasing the immune activity of exosomes: The effect of miRNA-depleted exosome proteins on activating dendritic cell/cytokine-induced killer cells against pancreatic cancer. J Zhejiang Univ Sci B. 2016; 17:352-360.
39. Iliescu FS, Vrtacnik D, Neuzil P, Iliescu C. Microfluidic technology for clinical applications of exosomes. Micromachines (Basel). 2019; 10:392.
40. Gurunathan S, Kang MH, Jeyaraj M, Qasim M, Kim JH. Review of the isolation, characterization, biological function, and multifarious therapeutic approaches of exosomes. Cells. 2019; 8:307.
41. Popovic M, Mazzega E, Toffoletto B, de Marco A. Isolation of anti-extra-cellular vesicle single-domain antibodies by direct panning on vesicle-enriched fractions. Microb Cell Fact. 2018; 17:6.
42. Kalluri R, LeBleu *VS.* The biology, function, and biomedical applications of exosomes. Science. 2020; 367:eaau6977.
43. Tian Y, Li S, Song J, Ji T, Zhu M, Anderson GJ, Wei J, Nie G. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. Biomaterials. 2014; 35:2383-2390.
44. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol. 2011; 29:341-345.
45. Chen TS, Arslan F, Yin Y, Tan SS, Lai RC, Choo AB, Padmanabhan J, Lee CN, de Kleijn DP, Lim SK. Enabling a robust scalable manufacturing process for therapeutic exosomes through oncogenic immortalization of human ESC-derived MSCs. J Transl Med. 2011; 9:47.
46. Yeo RW, Lai RC, Zhang B, Tan SS, Yin Y, Teh BJ, Lim SK. Mesenchymal stem cell: An efficient mass producer of exosomes for drug delivery. Adv Drug Deliv Rev. 2013; 65:336-341.
47. Jiang H, Zhao H, Zhang M, He Y, Li X, Xu Y, Liu X. Hypoxia induced changes of exosome cargo and subsequent biological effects. Front Immunol. 2022; 13:824188.
48. Kucharzewska P, Christianson HC, Welch JE, Svensson KJ, Fredlund E, Ringner M, Morgelin M, Bourseau-Guilmain E, Bengzon J, Belting M. Exosomes reflect the hypoxic status of glioma cells and mediate hypoxia-dependent activation of vascular cells during tumor development. Proc Natl Acad Sci U S A. 2013; 110:7312-7317.

49. Li J, Lee Y, Johansson HJ, Mager I, Vader P, Nordin JZ, Wiklander OP, Lehtio J, Wood MJ, Andaloussi SE. Serum-free culture alters the quantity and protein composition of neuroblastoma-derived extracellular vesicles. J Extracell Vesicles. 2015; 4:26883.
50. Yuan X, Sun L, Jeske R, Nkosi D, York SB, Liu Y, Grant SC, Meckes DG, Jr., Li Y. Engineering extracellular vesicles by three-dimensional dynamic culture of human mesenchymal stem cells. J Extracell Vesicles. 2022; 11:e12235.
51. Bei HP, Hung PM, Yeung HL, Wang S, Zhao X. Bone-a-Petite: Engineering exosomes towards bone, osteochondral, and cartilage repair. Small. 2021; 17:e2101741.
52. Dragovic RA, Gardiner C, Brooks AS, Tannetta DS, Ferguson DJ, Hole P, Carr B, Redman CW, Harris AL, Dobson PJ, Harrison P, Sargent IL. Sizing and phenotyping of cellular vesicles using nanoparticle tracking analysis. Nanomedicine. 2011; 7:780-788.
53. Tan F, Li X, Wang Z, Li J, Shahzad K, Zheng J. Clinical applications of stem cell-derived exosomes. Signal Transduct Target Ther. 2024; 9:17.
54. Szatanek R, Baj-Krzyworzeka M, Zimoch J, Lekka M, Siedlar M, Baran J. The methods of choice for extracellular vesicles (EVs) characterization. Int J Mol Sci. 2017; 18:1153.
55. Hoo CM, Starostin N, West P, Mecartney ML. A comparison of atomic force microscopy (AFM) and dynamic light scattering (DLS) methods to characterize nanoparticle size distributions. Journal of Nanoparticle Research. 2008; 10:89-96.
56. Anderson W, Lane R, Korbie D, Trau M. Observations of tunable resistive pulse sensing for exosome analysis: Improving system sensitivity and stability. Langmuir. 2015; 31:6577-6587.
57. Colombo M, Raposo G, Thery C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol. 2014; 30:255-289.
58. Pospichalova V, Svoboda J, Dave Z, Kotrbova A, Kaiser K, Klemova D, Ilkovics L, Hampl A, Crha I, Jandakova E, Minar L, Weinberger V, Bryja V. Simplified protocol for flow cytometry analysis of fluorescently labeled exosomes and microvesicles using dedicated flow cytometer. J Extracell Vesicles. 2015; 4:25530.
59. Shan X, Zhang C, Mai C, Hu X, Cheng N, Chen W, Peng D, Wang L, Ji Z, Xie Y. The biogenesis, biological functions, and applications of macrophage-derived exosomes. Front Mol Biosci. 2021; 8:715461.
60. Li M, Li S, Du C, Zhang Y, Li Y, Chu L, Han X, Galons H, Zhang Y, Sun H, Yu P. Exosomes from different cells: Characteristics, modifications, and therapeutic applications. Eur J Med Chem. 2020; 207:112784.
61. Zhang H, Freitas D, Kim HS, *et al.* Identification of distinct nanoparticles and subsets of extracellular vesicles by asymmetric flow field-flow fractionation. Nat Cell Biol. 2018; 20:332-343.
62. Chevillet JR, Kang Q, Ruf IK, *et al.* Quantitative and stoichiometric analysis of the microRNA content of exosomes. Proc Natl Acad Sci U S A. 2014; 111:14888-14893.
63. Lasda E, Parker R. Circular RNAs co-precipitate with extracellular vesicles: A possible mechanism for circRNA clearance. PLoS One. 2016; 11:e0148407.
64. Pathan M, Fonseka P, Chitti SV, Kang T, Sanwlani R, Van Deun J, Hendrix A, Mathivanan S. Vesiclepedia 2019: A compendium of RNA, proteins, lipids and metabolites in extracellular vesicles. Nucleic Acids Res. 2019; 47:D516-D519.
65. Keerthikumar S, Chisanga D, Ariyaratne D, Al Saffar H, Anand S, Zhao K, Samuel M, Pathan M, Jois M, Chilamkurti N, Gangoda L, Mathivanan S. ExoCarta: A web-based compendium of exosomal cargo. J Mol Biol. 2016; 428:688-692.
66. Liu J, Wu F, Zhou H. Macrophage-derived exosomes in cancers: Biogenesis, functions and therapeutic applications. Immunol Lett. 2020; 227:102-108.
67. Boukouris S, Mathivanan S. Exosomes in bodily fluids are a highly stable resource of disease biomarkers. Proteomics Clin Appl. 2015; 9:358-367.
68. Zhou B, Xu K, Zheng X, Chen T, Wang J, Song Y, Shao Y, Zheng S. Application of exosomes as liquid biopsy in clinical diagnosis. Signal Transduct Target Ther. 2020; 5:144.
69. Kluszczynska K, Czernek L, Cypryk W, Peczek L, Duchler M. Methods for the determination of the purity of exosomes. Curr Pharm Des. 2019; 25:4464-4485.
70. Mulcahy LA, Pink RC, Carter DR. Routes and mechanisms of extracellular vesicle uptake. J Extracell Vesicles. 2014; 3.
71. Prada I, Meldolesi J. Binding and fusion of extracellular vesicles to the plasma membrane of their cell targets. Int J Mol Sci. 2016; 17:1296.
72. Tkach M, Kowal J, Zucchetti AE, Enserink L, Jouve M, Lankar D, Saitakis M, Martin-Jaular L, Thery C. Qualitative differences in T-cell activation by dendritic cell-derived extracellular vesicle subtypes. EMBO J. 2017; 36:3012-3028.
73. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007; 9:654-659.
74. Tian T, Zhu YL, Hu FH, Wang YY, Huang NP, Xiao ZD. Dynamics of exosome internalization and trafficking. J Cell Physiol. 2013; 228:1487-1495.
75. Joshi BS, de Beer MA, Giepmans BNG, Zuhorn IS. Endocytosis of extracellular vesicles and release of their cargo from endosomes. ACS Nano. 2020; 14:4444-4455.
76. Kwok ZH, Wang C, Jin Y. Extracellular vesicle transportation and uptake by recipient cells: A critical process to regulate human diseases. Processes (Basel). 2021; 9:273.
77. Kamerkar S, LeBleu VS, Sugimoto H, Yang S, Ruivo CF, Melo SA, Lee JJ, Kalluri R. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. Nature. 2017; 546:498-503.
78. Commisso C, Davidson SM, Soydaner-Azeloglu RG, Parker SJ, Kamphorst JJ, Hackett S, Grabocka E, Nofal M, Drebin JA, Thompson CB, Rabinowitz JD, Metallo CM, Vander Heiden MG, Bar-Sagi D. Macropinocytosis of protein is an amino acid supply route in Ras-transformed cells. Nature. 2013; 497:633-637.
79. Parolini I, Federici C, Raggi C, Lugini L, Palleschi S, De Milito A, Coscia C, Iessi E, Logozzi M, Molinari A, Colone M, Tatti M, Sargiacomo M, Fais S. Microenvironmental pH is a key factor for exosome traffic in tumor cells. J Biol Chem. 2009; 284:34211-34222.
80. Tian T, Zhu YL, Zhou YY, Liang GF, Wang YY, Hu FH, Xiao ZD. Exosome uptake through clathrin-mediated endocytosis and macropinocytosis and mediating miR-21

delivery. J Biol Chem. 2014; 289:22258-22267.
81. Murphy DE, de Jong OG, Brouwer M, Wood MJ, Lavieu G, Schiffelers RM, Vader P. Extracellular vesicle-based therapeutics: Natural *versus* engineered targeting and trafficking. Exp Mol Med. 2019; 51:1-12.
82. Morishita M, Takahashi Y, Nishikawa M, Takakura Y. Pharmacokinetics of exosomes-An important factor for elucidating the biological roles of exosomes and for the development of exosome-based therapeutics. J Pharm Sci. 2017; 106:2265-2269.
83. Munagala R, Aqil F, Jeyabalan J, Gupta RC. Bovine milk-derived exosomes for drug delivery. Cancer Lett. 2016; 371:48-61.
84. Zhuang X, Xiang X, Grizzle W, Sun D, Zhang S, Axtell RC, Ju S, Mu J, Zhang L, Steinman L, Miller D, Zhang HG. Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. Mol Ther. 2011; 19:1769-1779.
85. Haney MJ, Klyachko NL, Zhao Y, Gupta R, Plotnikova EG, He Z, Patel T, Piroyan A, Sokolsky M, Kabanov AV, Batrakova EV. Exosomes as drug delivery vehicles for Parkinson's disease therapy. J Control Release. 2015; 207:18-30.
86. Smyth T, Kullberg M, Malik N, Smith-Jones P, Graner MW, Anchordoquy TJ. Biodistribution and delivery efficiency of unmodified tumor-derived exosomes. J Control Release. 2015; 199:145-155.
87. Lentz TL, Burrage TG, Smith AL, Crick J, Tignor GH. Is the acetylcholine receptor a rabies virus receptor? Science. 1982; 215:182-184.
88. Escrevente C, Keller S, Altevogt P, Costa J. Interaction and uptake of exosomes by ovarian cancer cells. BMC Cancer. 2011; 11:108.
89. Yamashita T, Takahashi Y, Takakura Y. Possibility of exosome-based therapeutics and challenges in production of exosomes eligible for therapeutic application. Biol Pharm Bull. 2018; 41:835-842.
90. Maroto R, Zhao Y, Jamaluddin M, Popov VL, Wang H, Kalubowilage M, Zhang Y, Luisi J, Sun H, Culbertson CT, Bossmann SH, Motamedi M, Brasier AR. Effects of storage temperature on airway exosome integrity for diagnostic and functional analyses. J Extracell Vesicles. 2017; 6:1359478.
91. Bosch S, de Beaurepaire L, Allard M, Mosser M, Heichette C, Chretien D, Jegou D, Bach JM. Trehalose prevents aggregation of exosomes and cryodamage. Sci Rep. 2016; 6:36162.
92. Charoenviriyakul C, Takahashi Y, Nishikawa M, Takakura Y. Preservation of exosomes at room temperature using lyophilization. Int J Pharm. 2018; 553:1-7.
93. Kusuma GD, Barabadi M, Tan JL, Morton DAV, Frith JE, Lim R. To protect and to preserve: Novel preservation strategies for extracellular vesicles. Front Pharmacol. 2018; 9:1199.
94. Yuan L, Li JY. Exosomes in Parkinson's disease: Current perspectives and future challenges. ACS Chem Neurosci. 2019; 10:964-972.
95. Howitt J, Hill AF. Exosomes in the pathology of neurodegenerative diseases. J Biol Chem. 2016; 291:26589-26597.
96. Budnik V, Ruiz-Canada C, Wendler F. Extracellular vesicles round off communication in the nervous system. Nat Rev Neurosci. 2016; 17:160-172.
97. Guo BB, Bellingham SA, Hill AF. Stimulating the release of exosomes increases the intercellular transfer of prions. J Biol Chem. 2016; 291:5128-5137.
98. Rajendran L, Honsho M, Zahn TR, Keller P, Geiger KD, Verkade P, Simons K. Alzheimer's disease beta-amyloid peptides are released in association with exosomes. Proc Natl Acad Sci U S A. 2006; 103:11172-11177.
99. Baker S, Polanco JC, Gotz J. Extracellular vesicles containing P301L mutant tau accelerate pathological tau phosphorylation and oligomer formation but do not seed mature neurofibrillary tangles in ALZ17 mice. J Alzheimers Dis. 2016; 54:1207-1217.
100. Yuyama K, Sun H, Usuki S, Sakai S, Hanamatsu H, Mioka T, Kimura N, Okada M, Tahara H, Furukawa J, Fujitani N, Shinohara Y, Igarashi Y. A potential function for neuronal exosomes: Sequestering intracerebral amyloid-beta peptide. FEBS Lett. 2015; 589:84-88.
101. Falker C, Hartmann A, Guett I, Dohler F, Altmeppen H, Betzel C, Schubert R, Thurm D, Wegwitz F, Joshi P, Verderio C, Krasemann S, Glatzel M. Exosomal cellular prion protein drives fibrillization of amyloid beta and counteracts amyloid beta-mediated neurotoxicity. J Neurochem. 2016; 137:88-100.
102. Pacheco-Quinto J, Clausen D, Perez-Gonzalez R, Peng H, Meszaros A, Eckman CB, Levy E, Eckman EA. Intracellular metalloprotease activity controls intraneuronal Abeta aggregation and limits secretion of Abeta *via* exosomes. FASEB J. 2019; 33:3758-3771.
103. Stuendl A, Kunadt M, Kruse N, Bartels C, Moebius W, Danzer KM, Mollenhauer B, Schneider A. Induction of alpha-synuclein aggregate formation by CSF exosomes from patients with Parkinson's disease and dementia with Lewy bodies. Brain. 2016; 139:481-494.
104. Spencer B, Kim C, Gonzalez T, Bisquertt A, Patrick C, Rockenstein E, Adame A, Lee SJ, Desplats P, Masliah E. alpha-Synuclein interferes with the ESCRT-III complex contributing to the pathogenesis of Lewy body disease. Hum Mol Genet. 2016; 25:1100-1115.
105. Iguchi Y, Eid L, Parent M, Soucy G, Bareil C, Riku Y, Kawai K, Takagi S, Yoshida M, Katsuno M, Sobue G, Julien JP. Exosome secretion is a key pathway for clearance of pathological TDP-43. Brain. 2016; 139:3187-3201.
106. Tsilioni I, Theoharides TC. Extracellular vesicles are increased in the serum of children with autism spectrum disorder, contain mitochondrial DNA, and stimulate human microglia to secrete IL-1beta. J Neuroinflammation. 2018; 15:239.
107. Cooper JM, Wiklander PB, Nordin JZ, Al-Shawi R, Wood MJ, Vithlani M, Schapira AH, Simons JP, El-Andaloussi S, Alvarez-Erviti L. Systemic exosomal siRNA delivery reduced alpha-synuclein aggregates in brains of transgenic mice. Mov Disord. 2014; 29:1476-1485.
108. Han C, Wang YJ, Wang YC, Guan X, Wang L, Shen LM, Zou W, Liu J. Caveolin-1 downregulation promotes the dopaminergic neuron-like differentiation of human adipose-derived mesenchymal stem cells. Neural Regen Res. 2021; 16:714-720.
109. Bonaventura G, Incontro S, Iemmolo R, La Cognata V, Barbagallo I, Costanzo E, Barcellona ML, Pellitteri R, Cavallaro S. Dental mesenchymal stem cells and neuro-regeneration: A focus on spinal cord injury. Cell Tissue Res. 2020; 379:421-428.
110. Volarevic V, Gazdic M, Simovic Markovic B, Jovicic N, Djonov V, Arsenijevic N. Mesenchymal stem cell-derived factors: Immuno-modulatory effects and therapeutic

potential. Biofactors. 2017; 43:633-644.
111. Shariati A, Nemati R, Sadeghipour Y, Yaghoubi Y, Baghbani R, Javidi K, Zamani M, Hassanzadeh A. Mesenchymal stromal cells (MSCs) for neurodegenerative disease: A promising frontier. Eur J Cell Biol. 2020; 99:151097.
112. Lukomska B, Stanaszek L, Zuba-Surma E, Legosz P, Sarzynska S, Drela K. Challenges and controversies in human mesenchymal stem cell therapy. Stem Cells Int. 2019; 2019:9628536.
113. Gazdic M, Volarevic V, Arsenijevic N, Stojkovic M. Mesenchymal stem cells: A friend or foe in immune-mediated diseases. Stem Cell Rev Rep. 2015; 11:280-287.
114. Volarevic V, Markovic BS, Gazdic M, Volarevic A, Jovicic N, Arsenijevic N, Armstrong L, Djonov V, Lako M, Stojkovic M. Ethical and safety issues of stem cell-based therapy. Int J Med Sci. 2018; 15:36-45.
115. Guy R, Offen D. Promising opportunities for treating neurodegenerative diseases with mesenchymal stem cell-derived exosomes. Biomolecules. 2020; 10:1320.
116. Harrell CR, Jovicic N, Djonov V, Arsenijevic N, Volarevic V. Mesenchymal stem cell-derived exosomes and other extracellular vesicles as new remedies in the therapy of inflammatory diseases. Cells. 2019; 8:1605.
117. Scheltens P, De Strooper B, Kivipelto M, Holstege H, Chetelat G, Teunissen CE, Cummings J, van der Flier WM. Alzheimer's disease. Lancet. 2021; 397:1577-1590.
118. Elia CA, Tamborini M, Rasile M, Desiato G, Marchetti S, Swuec P, Mazzitelli S, Clemente F, Anselmo A, Matteoli M, Malosio ML, Coco S. Intracerebral injection of extracellular vesicles from mesenchymal stem cells exerts reduced Abeta plaque burden in early stages of a preclinical model of Alzheimer's disease. Cells. 2019; 8.
119. Wang H, Liu Y, Li J, Wang T, Hei Y, Li H, Wang X, Wang L, Zhao R, Liu W, Long Q. Tail-vein injection of MSC-derived small extracellular vesicles facilitates the restoration of hippocampal neuronal morphology and function in APP / PS1 mice. Cell Death Discov. 2021; 7:230.
120. Sha S, Shen X, Cao Y, Qu L. Mesenchymal stem cells-derived extracellular vesicles ameliorate Alzheimer's disease in rat models *via* the microRNA-29c-3p/BACE1 axis and the Wnt/beta-catenin pathway. Aging (Albany NY). 2021; 13:15285-15306.
121. Ding M, Shen Y, Wang P, Xie Z, Xu S, Zhu Z, Wang Y, Lyu Y, Wang D, Xu L, Bi J, Yang H. Exosomes isolated from human umbilical cord mesenchymal stem cells alleviate neuroinflammation and reduce amyloid-beta deposition by modulating microglial activation in Alzheimer's disease. Neurochem Res. 2018; 43:2165-2177.
122. Nakano M, Kubota K, Kobayashi E, Chikenji TS, Saito Y, Konari N, Fujimiya M. Bone marrow-derived mesenchymal stem cells improve cognitive impairment in an Alzheimer's disease model by increasing the expression of microRNA-146a in hippocampus. Sci Rep. 2020; 10:10772.
123. Cui GH, Wu J, Mou FF, Xie WH, Wang FB, Wang QL, Fang J, Xu YW, Dong YR, Liu JR, Guo HD. Exosomes derived from hypoxia-preconditioned mesenchymal stromal cells ameliorate cognitive decline by rescuing synaptic dysfunction and regulating inflammatory responses in APP/PS1 mice. FASEB J. 2018; 32:654-668.
124. de Godoy MA, Saraiva LM, de Carvalho LRP, *et al.* Mesenchymal stem cells and cell-derived extracellular vesicles protect hippocampal neurons from oxidative stress and synapse damage induced by amyloid-beta oligomers. J Biol Chem. 2018; 293:1957-1975.
125. Reza-Zaldivar EE, Hernandez-Sapiens MA, Gutierrez-Mercado YK, Sandoval-Avila S, Gomez-Pinedo U, Marquez-Aguirre AL, Vazquez-Mendez E, Padilla-Camberos E, Canales-Aguirre AA. Mesenchymal stem cell-derived exosomes promote neurogenesis and cognitive function recovery in a mouse model of Alzheimer's disease. Neural Regen Res. 2019; 14:1626-1634.
126. Ma X, Huang M, Zheng M, *et al.* ADSCs-derived extracellular vesicles alleviate neuronal damage, promote neurogenesis and rescue memory loss in mice with Alzheimer's disease. J Control Release. 2020; 327:688-702.
127. Bodart-Santos V, de Carvalho LRP, de Godoy MA, Batista AF, Saraiva LM, Lima LG, Abreu CA, De Felice FG, Galina A, Mendez-Otero R, Ferreira ST. Extracellular vesicles derived from human Wharton's jelly mesenchymal stem cells protect hippocampal neurons from oxidative stress and synapse damage induced by amyloid-beta oligomers. Stem Cell Res Ther. 2019; 10:332.
128. Chen YA, Lu CH, Ke CC, Chiu SJ, Jeng FS, Chang CW, Yang BH, Liu RS. Mesenchymal stem cell-derived exosomes ameliorate Alzheimer's disease pathology and improve cognitive deficits. Biomedicines. 2021; 9:594.
129. Liu S, Fan M, Xu JX, Yang LJ, Qi CC, Xia QR, Ge JF. Exosomes derived from bone-marrow mesenchymal stem cells alleviate cognitive decline in AD-like mice by improving BDNF-related neuropathology. J Neuroinflammation. 2022; 19:35.
130. Scheltens P, De Strooper B, Kivipelto M, Holstege H, Chetelat G, Teunissen CE, Cummings J, van der Flier WM. Alzheimer's disease. Lancet. 2021; 397:1577-1590.
131. McGinley MP, Cohen JA. Sphingosine 1-phosphate receptor modulators in multiple sclerosis and other conditions. Lancet. 2021; 398:1184-1194.
132. Jeong H, Kim OJ, Oh SH, Lee S, Reum Lee HA, Lee KO, Lee BY, Kim NK. Extracellular vesicles released from neprilysin gene-modified human umbilical cord-derived mesenchymal stem cell enhance therapeutic effects in an Alzheimer's disease animal model. Stem Cells Int. 2021; 2021:5548630.
133. de Dios C, Bartolessis I, Roca-Agujetas V, Barbero-Camps E, Mari M, Morales A, Colell A. Oxidative inactivation of amyloid beta-degrading proteases by cholesterol-enhanced mitochondrial stress. Redox Biol. 2019; 26:101283.
134. Ferreira JPS, Albuquerque HMT, Cardoso SM, Silva AMS, Silva VLM. Dual-target compounds for Alzheimer's disease: Natural and synthetic AChE and BACE-1 dual-inhibitors and their structure-activity relationship (SAR). Eur J Med Chem. 2021; 221:113492.
135. Ghosh AK. BACE1 inhibitor drugs for the treatment of Alzheimer's disease: Lessons learned, challenges to overcome, and future prospects(dagger). Glob Health Med. 2024; 6:164-168.
136. Parr C, Mirzaei N, Christian M, Sastre M. Activation of the Wnt/beta-catenin pathway represses the transcription of the beta-amyloid precursor protein cleaving enzyme (BACE1) *via* binding of T-cell factor-4 to BACE1 promoter. FASEB J. 2015; 29:623-635.
137. Wu H, Fan H, Shou Z, Xu M, Chen Q, Ai C, Dong Y, Liu Y, Nan Z, Wang Y, Yu T, Liu X. Extracellular vesicles

containing miR-146a attenuate experimental colitis by targeting TRAF6 and IRAK1. Int Immunopharmacol. 2019; 68:204-212.
138. Cinelli MA, Do HT, Miley GP, Silverman RB. Inducible nitric oxide synthase: Regulation, structure, and inhibition. Med Res Rev. 2020; 40:158-189.
139. Wang SS, Jia J, Wang Z. Mesenchymal stem cell-derived extracellular vesicles suppresses iNOS expression and ameliorates neural impairment in Alzheimer's Disease mice. J Alzheimers Dis. 2018; 61:1005-1013.
140. Elelu N, Bankole AA, Daphne HP, Rabiu M, Ola-Fadunsin SD, Ambali HM, Cutler SJ. Molecular characterisation of Rhipicephalus sanguineus sensu lato ticks from domestic dogs in Nigeria. Vet Med Sci. 2022; 8:454-459.
141. Mukherjee M, Dutta S, Ghosh M, Basuchowdhuri P, Datta A. Performance of the nitrogen reduction reaction on metal bound g-C(6)N(6): A combined approach of machine learning and DFT. Phys Chem Chem Phys. 2022; 24:17050-17058.
142. Yang L, Zhai Y, Hao Y, Zhu Z, Cheng G. The regulatory functionality of exosomes derived from hUMSCs in 3D culture for Alzheimer's disease therapy. Small. 2020; 16:e1906273.
143. Domenis R, Cifu A, Quaglia S, Pistis C, Moretti M, Vicario A, Parodi PC, Fabris M, Niazi KR, Soon-Shiong P, Curcio F. Pro inflammatory stimuli enhance the immunosuppressive functions of adipose mesenchymal stem cells-derived exosomes. Sci Rep. 2018; 8:13325.
144. Liu X, Li X, Zhu W, Zhang Y, Hong Y, Liang X, Fan B, Zhao H, He H, Zhang F. Exosomes from mesenchymal stem cells overexpressing MIF enhance myocardial repair. J Cell Physiol. 2020; 235:8010-8022.
145. Gala D, Mohak S, Fabian Z. Extracellular vehicles of oxygen-depleted mesenchymal stromal cells: Route to off-shelf cellular therapeutics? Cells. 2021; 10:2199.
146. Liu H, Jin M, Ji M, Zhang W, Liu A, Wang T. Hypoxic pretreatment of adipose-derived stem cell exosomes improved cognition by delivery of circ-Epc1 and shifting microglial M1/M2 polarization in an Alzheimer's disease mice model. Aging (Albany NY). 2022; 14:3070-3083.
147. Yin T, Liu Y, Ji W, Zhuang J, Chen X, Gong B, Chu J, Liang W, Gao J, Yin Y. Engineered mesenchymal stem cell-derived extracellular vesicles: A state-of-the-art multifunctional weapon against Alzheimer's disease. Theranostics. 2023; 13:1264-1285.
148. Losurdo M, Pedrazzoli M, D'Agostino C, *et al.* Intranasal delivery of mesenchymal stem cell-derived extracellular vesicles exerts immunomodulatory and neuroprotective effects in a 3xTg model of Alzheimer's disease. Stem Cells Transl Med. 2020; 9:1068-1084.
149. Chen SY, Lin MC, Tsai JS, He PL, Luo WT, Herschman H, Li HJ. EP(4) antagonist-elicited extracellular vesicles from mesenchymal stem cells rescue cognition/learning deficiencies by restoring brain cellular functions. Stem Cells Transl Med. 2019; 8:707-723.
150. Xu M, Yang Q, Sun X, Wang Y. Recent advancements in the loading and modification of therapeutic exosomes. Front Bioeng Biotechnol. 2020; 8:586130.
151. Wang Z, Rich J, Hao N, Gu Y, Chen C, Yang S, Zhang P, Huang TJ. Acoustofluidics for simultaneous nanoparticle-based drug loading and exosome encapsulation. Microsyst Nanoeng. 2022; 8:45.
152. Wang J, Chen D, Ho EA. Challenges in the development and establishment of exosome-based drug delivery systems. J Control Release. 2021; 329:894-906.
153. Zhai L, Shen H, Sheng Y, Guan Q. ADMSC Exo-microRNA-22 improve neurological function and neuroinflammation in mice with Alzheimer's disease. J Cell Mol Med. 2021; 25:7513-7523.
154. Jahangard Y, Monfared H, Moradi A, Zare M, Mirnajafi-Zadeh J, Mowla SJ. Therapeutic effects of transplanted exosomes containing miR-29b to a rat model of Alzheimer's disease. Front Neurosci. 2020; 14:564.
155. Xu F, Wu Y, Yang Q, Cheng Y, Xu J, Zhang Y, Dai H, Wang B, Ma Q, Chen Y, Lin F, Wang C. Engineered extracellular vesicles with SHP2 high expression promote mitophagy for Alzheimer's disease treatment. Adv Mater. 2022; 34:e2207107.
156. Tian T, Zhang HX, He CP, Fan S, Zhu YL, Qi C, Huang NP, Xiao ZD, Lu ZH, Tannous BA, Gao J. Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. Biomaterials. 2018; 150:137-149.
157. Gaurav I, Thakur A, Iyaswamy A, Wang X, Chen X, Yang Z. Factors affecting extracellular vesicles based drug delivery systems. Molecules. 2021; 26:1544.
158. Rayamajhi S, Aryal S. Surface functionalization strategies of extracellular vesicles. J Mater Chem B. 2020; 8:4552-4569.
159. Li YJ, Wu JY, Liu J, Xu W, Qiu X, Huang S, Hu XB, Xiang DX. Artificial exosomes for translational nanomedicine. J Nanobiotechnology. 2021; 19:242.
160. Kim HY, Kwon S, Um W, Shin S, Kim CH, Park JH, Kim BS. Functional extracellular vesicles for regenerative medicine. Small. 2022; 18:e2106569.
161. Le QV, Lee J, Lee H, Shim G, Oh YK. Cell membrane-derived vesicles for delivery of therapeutic agents. Acta Pharm Sin B. 2021; 11:2096-2113.

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