Trends in Neurosciences

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Review

Mesenchymal stromal cell extracellular vesicles as immune modulators and drug carriers in neurodegenerative disorders

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Mesenchymal stromal cells (MSCs) hold significant therapeutic potential, but their clinical application is often hindered by limitations such as donor variability. MSC-derived extracellular vesicles (EVs) present a promising alternative, offering comparable or superior therapeutic effects while overcoming some of these challenges. MSC-EVs exhibit strong anti-inflammatory and immunomodulatory properties, which could be leveraged in neurodegenerative diseases given the central role of neuroinflammation in these conditions. Additionally, MSC-EVs can be engineered for targeted drug delivery, enhancing their clinical utility. In this review we highlight the dual role of MSC-EVs as immunomodulators and drug carriers in neurodegenerative disorders. We discuss the current challenges, and outline strategies for clinical translation. Future advances in understanding MSC-EVs and their mechanisms of action could support their development into effective therapies for neurodegenerative diseases.

Highlights

Extracellular vesicles (EVs) derived from mesenchymal stromal cells (MSCs) represent a promising therapeutic tool to treat the complex pathology of neurodegenerative diseases.

MSC-EVs can deliver molecules to the brain, opening new possibilities for targeted therapeutic delivery.

MSC-EVs modulate neuroinflammation by shifting immune cells toward anti-inflammatory states, a key mechanism in slowing disease progression.

Clinical translation of MSC-EV-based treatment strategies is challenged by variability in MSC sources and limited scalability, raising the need for more standardized production methods.

Advances in EV engineering may enhance brain targeting and drug loading, increasing the therapeutic potential of MSC-EVs and moving the field closer to clinical application.

Transitioning from MSCs to their EV counterpart

MSCs are multipotent cells with diverse physiological effects, playing key roles in tissue homeostasis, regeneration, and immune modulation [1–4]. These characteristics make them promising therapeutic candidates. Despite more than 1700 clinical trials, including several Phase 3 studies, only a handful of MSC-based products have received regulatory approval for commercialization: mostly in Asia, one in the USA, and formerly one in Europe [5–7]. For example, Mesoblast's Remestemcel-L, used in pediatric acute graft-versus-host disease (aGVHD), was rejected twice before eventual approval by the US Food and Drug Administration (FDA) in 2024. By contrast, the European Medicines Agency (EMA) withdrew authorization for Alofisel, citing that its risks outweighed its benefits. These challenges in gaining clinical approval reflect, among other reasons, donor-dependent variability of human tissue-derived MSCs.

MSC therapeutic effects are believed to be driven largely by paracrine mechanisms, including via the release of extracellular vesicles (EVs) [8,9]. These nanosized membrane vesicles ferry bioactive molecules such as proteins, lipids, and nucleic acids, influencing recipient cells [10]. MSC-EVs retain the potential of their parent cells but also display low immunogenicity, high biocompatibility, and the ability to cross biological barriers.

This review explores the current and potential use of MSC- EVs in neurodegenerative diseases, particularly Alzheimer's disease (AD), where inflammation and immune dysregulation are central. MSC-EVs can act as 'dual agents', offering on the one hand anti-inflammatory, immunomodulatory, and pro-regenerative effects, and on the other hand serving as drug delivery vehicles that can be engineered for targeted delivery, potentially overcoming the challenge of crossing CNS barriers

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such as the blood–brain barrier (BBB). Despite the promise of MSC-EVs, challenges remain in their clinical translation, and we discuss obstacles and strategies to advancing MSC-EV therapies.

The therapeutic potential of MSC-derived EVs in the neurodegenerative landscape

Among the early demonstrations of the therapeutic effects of MSC-derived EVs was their application in a patient with treatment-refractory aGVHD [11]. Research on therapeutic effects of MSC-EVs in multiple preclinical models ensued. Box 1 outlines the proposed mechanisms of action of MSC-EVs in various neurological conditions.

Preclinical studies in AD mouse models

MSC-EVs show therapeutic promise in AD by targeting amyloid-β (Aβ) pathology through various mechanisms. *In vitro,* adipose-derived MSC-EVs reduce Aβ42 and Aβ40 in neuronal stem cells from AD mice and reduce both secreted and intracellular Aβ in human cells expressing amyloid precursor protein (APP), likely by delivering neprilysin, an Aβ-degrading enzyme [12,13]. *In vivo,* bone-marrow MSC-EVs reduced Aβ plaques and dystrophic neurites in APP/PS1 mice, an AD model, through a mechanism involving neprilysin [14]. Additionally, microRNA (miRNA) cargoes of MSC-EVs have been shown to modulate inflammation, synaptic density, and cognitive function. In AD mouse models, miR-146a suppresses nuclear factor κB (NF-κB) in astrocytes, thereby

Box 1. Mechanisms of action of MSC-EVs in neurodegenerative disorders

MSC-EVs offer a potential therapeutic approach for neurodegenerative diseases by promoting neuroprotection, modulating inflammation, and supporting tissue repair through their diverse molecular cargo, including proteins, lipids, and regulatory RNAs.

Neuroprotective mechanisms and regulation of cell survival pathways

MSC-EVs can support neuronal survival by mitigating oxidative stress, restoring mitochondrial function, and modulating key apoptotic and inflammatory signaling pathways. In cellular models relevant to Parkinson's disease (PD), they have been shown to protect dopaminergic neurons from damage induced by neurotoxins such as 6-hydroxydopamine, thereby enhancing cell viability [141,142]. In *in vitro* models of Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS), MSC-EVs reduce mitochondrial dysfunction, decrease aggregation of toxic proteins such as mutant huntingtin (mHtt) and mutant SOD1, and restore cellular energy metabolism [143,144]. In *in vitro* and mouse models of Alzheimer's disease (AD), they preserve hippocampal neurons by attenuating Aβ-induced oxidative stress and synaptic loss [145,146], with some studies implicating activation of the Nrf2 pathway, a key regulator of cellular antioxidant responses [147]. These neuroprotective effects are mediated largely by EV-contained miRNAs – such as miR-106b, miR-181a-2-3p, and miR-188-3p – which suppress oxidative stress and apoptosis by targeting NOX4/p38 mitogen-activated protein kinase (MAPK) and NLRP3 pathways [16,17,144,148,149]. Additionally, exosome delivery of miR-29b to the hippocampus of Aβ-injected rat models has been shown to improve spatial learning and memory deficits and reduce Aβ pathology [150].

Modulation of inflammation

Anti-inflammatory effects of MSC-EVs are observed across various disease models. MSC-EVs suppress microglial activation in a mouse model for PD, restoring immune balance and cognitive function [151], and they reduce neuroinflammation in murine and rat models for multiple sclerosis (MS) by inhibiting the NLRP3 inflammasome and modulating TLR4 signaling [152–154]. Also, they downregulate proinflammatory signaling *in vitro* in astrocytes and microglia, creating a more neuroprotective environment [155]. MSC-EVs were also shown to deliver miR-146a, which downregulates NF-κB signaling in astrocytes, leading to reduced cytokine production and a less inflammatory microglial phenotype *in vitro* [143,144].

Neural repair and functional recovery

MSC-EVs support structural repair and functional recovery by enhancing cell survival and regenerative processes. In murine models of MS and AD, they promote remyelination through improved oligodendrocyte survival and reduced DNA damage [156,157], and they improve synaptic density and cognitive performance, largely through anti-inflammatory effects and delivery of reparative miRNAs [16,17], respectively. *In vitro*, MSC-EVs have been shown to stimulate neurite outgrowth and synaptic repair in dopaminergic neurons [141], and to preserve motor neurons and neuromuscular junctions [158].

reducing proinflammatory microglia [15], while miR-21-5p improves synaptic function and decreases Aβ plaques [16,17].

MSC-EVs are also relevant in Niemann–Pick type C1 (NPC1), a pediatric neurodegenerative disorder sometimes referred to as 'childhood Alzheimer's'. In NPC1-deficient mice, MSC-EVs reduced systemic and brain inflammation and glial activation, highlighting potential to target central and peripheral disease features [18]. These studies highlight the multifaceted mechanisms by which MSC-EVs exert neuroprotective effects in AD, including Aβ degradation, modulating inflammation, and altering glial activity.

Ongoing clinical studies in neurological diseases

Several clinical trials are evaluating MSC-EVs for neurodegenerative and neurodevelopmental disorders, differing in EV source, formulation, administration route, dosing, and patient population (Table 1). In NCT04388982[i], adipose-derived EVs are delivered intranasally to AD patients twice weekly for 12 weeks (5–20 μg protein). By contrast, NCT03384433[ii] uses bone-marrow EVs engineered with miR-124, injected stereotactically into post-stroke patients at a single ~200 μg dose. Two umbilical-cord-derived EV trials (NCT06607900[iii] and NCT06598202[iv]) test the same product intranasally across AD, Parkinson's disease, multiple system atrophy, Lewy body dementia, frontotemporal dementia, and amyotrophic lateral sclerosis (ALS). They differ in design (randomized versus open-label) and donor sourcing (pooled versus single-donor). Another study, NCT05490173[v], targets extremely low birth weight infants at risk of neurodevelopmental impairment. In this study, EVs from conditioned media of 120 million MSCs are given intranasally once daily for 5 days soon after birth, contrasting with longer-term dosing in adults. Collectively, these trials show wide variability in MSC source, EV engineering, dose, and schedule. Intranasal delivery is used in many of the trials, whereas in some trials of acute injury stereotactic injection is used. Engineered EVs, such as miR-124-enriched vesicles, illustrate emerging strategies to boost potency. These studies underscore the experimental stage of MSC-EV therapy and the need for systematic comparisons to guide clinical translation.

Table 1. Ongoing clinical trials as of September 2025 using MSC-derived EVs for neurological disorders

NCT Number	Title	Phase	Disease	Source of EV	Administration route
NCT04388982[i]	The safety and the efficacy evaluation of allogenic adipose MSC-exos in patients with Alzheimer's disease	1, 2	Alzheimer's disease	Allogenic adipose tissue	Nasal drip
NCT03384433[ii]	Allogenic MSC-derived exosome in patients with acute ischemic stroke	1,2	Acute ischemic stroke Cerebrovascular disorders	Allogenic MSC-derived EVs enriched by miR-124	Stereotaxis Intraparenchymal
NCT06607900[iii]	HUC-MSC-sEV-001 nasal drops for neurodegenerative diseases	1	Alzheimer's disease, Parkinson's disease, multiple system atrophy, Lewy body dementia, frontotemporal dementia	Human umbilical cord MSC-derived small extracellular vesicles	Nasal drops
NCT06598202[iv]	Exploring nasal drop therapy with small extracellular vesicles for ALS (de-ALS)	Recruiting	Amyotrophic lateral sclerosis	Human umbilical cord blood MSC-derived exosomes	Nasal drops
NCT05490173[v]	The pilot experimental study of the neuroprotective effects of exosomes in extremely low birth weight infants	Not yet recruiting	Long-term neurodevelopmental outcomes in extremely low birth weight infants	A daily conditioned culture medium of 120 million MSCs	Nasal drops

The immunomodulatory capacity of MSC-derived EVs in neurodegenerative disorders

The brain hosts resident (parenchymal) immune cells, namely microglia; in addition, immune cells patrol barriers such as the meninges, choroid plexus (ChP), and perivascular spaces [19]. Dysregulated immunity is now recognized as a key factor in neurological disorders, including AD [20]. Since MSC-EVs display immunomodulatory properties, they are promising candidates for neurodegenerative therapy. Here we describe the direct and indirect effects of MSC-EVs on immune cells, including those already reported in preclinical studies as well as their potential effects (Figure 1). Emphasis is placed on AD as one of the primary neurodegenerative disorders investigated in MSC-EV research.

MSC-EV-mediated modulation of microglia

Microglia make up approximately 5–15% of cells in the CNS. They are involved in neuronal support, synaptic remodeling, myelin homeostasis, and immune surveillance [21]. In AD, microglia are thought to play a dual role: early on, they clear oligomeric Aβ and limit plaque growth [22,23], while with persistent activation they release proinflammatory cytokines – tumor necrosis factor (TNF), interleukin 1β (IL-1β) – causing chronic neuroinflammation and neurotoxicity [24]. Chronically activated microglia adopt an ameboid morphology with shorter dendrites, fewer branches, and reduced phagocytic capacity [25].

MSC-EVs have been shown to modulate microglia, shifting them toward an anti-inflammatory phenotype. EVs from TNF/interferon γ (IFNγ)-preconditioned MSCs reduce secretion of

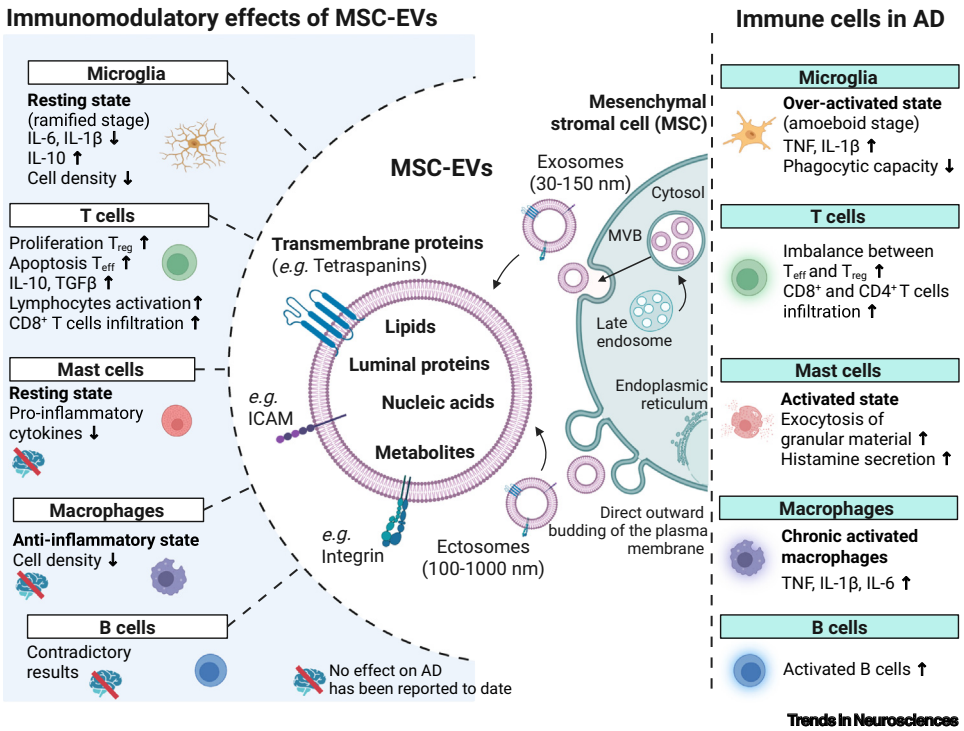

Figure 1. Immunomodulation of immune cells by mesenchymal stromal cell (MSC)-derived extracellular vesicles (EVs). Schematic overview of current knowledge about different immune cells in neurodegenerative disorders and how MSC-EVs may modulate them. Abbreviations: AD, Alzheimer's disease; CD, cluster of differentiation; ICAM, intercellular adhesion molecule; IL, interleukin; MVB, multivesicular body; T_{eff}, effector T cells; TGF, transforming growth factor; TNF, tumor necrosis factor; T_{reg}, regulatory T cells. Figure created with BioRender.

proinflammatory cytokines such as IL-6 and IL-1β, while promoting the release of the anti-inflammatory cytokine IL-10 in murine primary microglia exposed to proinflammatory stimuli [26]. MSC-EVs also induce macrophages to shift from pro- to anti-inflammatory states under normoxic and hypoxic conditions [27]. These findings suggest that MSC-EVs can effectively drive the functional polarization of microglia, and may counteract the inflammatory response associated with neurodegeneration. *In vivo*, intranasal administration of MSC-EVs reduced the number of $IBA1^+$ microglia in 3xTg AD mice [26], and – in a lipopolysaccharide (LPS)-induced neuroinflammation mouse model – lower IBA-1 expression, intracellular ROS levels, and Icam-1 mRNA, indicative of a less activated microglial state [28].

MSC-EV-mediated modulation of infiltrating innate immune cells

Peripheral innate immune cells originating from the blood have been observed in the brains of AD animal models and patients with AD [29,30]. Like microglia, their role can be both harmful and beneficial, depending on disease stage and model.

Mast cells respond in phases, rapidly releasing granular material and later synthesizing mediators, thereby amplifying vasoactive and immune responses [31]. In AD, mast cells containing tryptase infiltrate brains and colocalize with Aβ plaques [32]. Fibrillar Aβ peptides can trigger histamine secretion [33], contributing to neuroinflammation. A clinical study showed that masitinib mesylate, a tyrosine kinase inhibitor regulating mast cell survival and degranulation, slowed cognitive decline in AD patients [34], suggesting that mast cell modulation may influence disease progression, albeit with important limitations and possible side effects that warrant careful consideration. Given the dual roles of mast cells in both amplifying neuroinflammation and contributing to tissue homeostasis, MSC-EVs have been proposed as a potential means of modulating mast cell activity, though their efficacy and safety in this context remain uncertain and require critical evaluation. While to the best of our knowledge no studies have directly examined MSC-EV effects on mast cells in neurodegenerative diseases so far, research in other contexts demonstrates the immunomodulatory potential of MSC-EVs. Notably, MSC-derived EVs have been shown to reduce mast cell degranulation and activation in a mouse model of intracranial aneurysm by increasing prostaglandin E2 (PGE2) and prostaglandin E2 receptor 4 (EP4) expression via a PGE2-mediated mechanism [35]. Regulation of mast cell activation has been shown to reduce the excessive release of inflammatory mediators in non-neurological disease models, which may suggest – but does not yet confirm – a mechanism by which MSC-EVs could modulate mast-cell-driven neuroinflammation in AD. Additionally, MSC-EVs can decrease TNF, tryptase, and chymase – enzymes that contribute to vascular damage and neuroinflammation [36] – indicating a possible but as yet unproven role in stabilizing mast cell responses**.** In neurodegenerative diseases, mast cells are further activated by chemokines secreted by microglia or astrocytes, perpetuating a feedback loop that exacerbates neuroinflammation and neuronal damage [37]. By reducing mast cell activation, MSC-EV-based therapies have the potential to disrupt this cycle and prevent excessive neuroinflammatory responses in AD. However, further research is necessary to determine the precise mechanisms through which MSC-EVs influence mast cells in neurodegenerative conditions and their potential therapeutic benefits.

Macrophages are central players in neuroprotection, contributing to Aβ plaque clearance, debris removal, and secretion of anti-inflammatory cytokines such as IL-10 and transforming growth factor β (TGF-β) [38]. Their functional states, however, are highly plastic and context-dependent. Under homeostatic or early disease conditions, macrophages often adopt an anti-inflammatory, pro-resolving phenotype characterized by IL-10 and TGF-β secretion and efferocytosis [39,40]. Yet, with persistent or dysregulated stimulation, they can transition toward a proinflammatory state marked by elevated TNF, IL-1β, and IL-6 production, enhanced antigen presentation, and oxidative activity [39,41]. This shift promotes chronic neuroinflammation and

contributes to AD pathology. The duality of macrophage responses – shaped by local cues, stimulus type, and disease stage – underscores the complexity of macrophage-mediated immunomodulation in AD [20]. However, direct evidence of MSC-EV interactions with brain-infiltrating macrophages in AD remains limited. For instance, MSC-EVs promote macrophage polarization toward an anti-inflammatory phenotype in mice on a high-fat diet, preventing excessive adipose tissue inflammation [42]. In a murine model for myocardial ischemia/reperfusion injury, MSC-EVs containing miR-182 suppressed NF-κB signaling via Toll-like receptor 4 (TLR4) inhibition, attenuating macrophage-driven inflammation [43]. Similarly, MSC-EVs enriched in miR-223 reduced LPS-induced responses in macrophages in a murine model of sepsis [44], while miR-17-rich vesicles blocked NOD-like receptor pyrin domain-containing 3 (NLRP3) inflammasome activation through downregulation of thioredoxin-interacting protein [45]. Given that NF-κB, TLR4, and NLRP3 pathways are also implicated in AD, these findings raise the possibility that MSC-EVs could modulate macrophage responses within the AD brain. Further work is required to examine these effects in neurodegenerative contexts.

Other innate immune populations also contribute to neuroinflammation and may be regulated by MSC-EVs. Monocytes, as precursors to macrophages and dendritic cells, aid in Aβ clearance but also release proinflammatory cytokines in AD [46,47]. In mice, MSC-EVs were shown to modulate monocyte polarization toward anti-inflammatory states and enhance IL-10 and TGF-β secretion, and to suppress differentiation into proinflammatory dendritic cells [48–50], largely through delivery of regulatory miRNAs and proteins [49,50]. Neutrophils, similarly, were influenced by MSC-EVs in infection and lung-injury murine models, where vesicles enhanced phagocytic activity while limiting tissue damage [51–53]. Mechanistic studies in murine lung models identified miR-145-mediated MRP1 suppression, boosting leukotriene B4/BLT1 signaling [54], and the transfer of miR-21-5p and α1-antitrypsin as drivers of anti-inflammatory effects [52,53]. Together, these data support a protective role for MSC-EVs in limiting neutrophil-mediated collateral injury, though the relevance to CNS processes remains to be examined.

MSC-EV-mediated modulation of infiltrating adaptive immune cells

Adaptive immune cells play a crucial role in neurodegeneration, with mounting evidence highlighting their detrimental impact in AD and underscoring the need for effective immunomodulatory strategies [55].

Among these, T cells have been shown to contribute to AD pathology, where an imbalance between proinflammatory effector T cells (Teff) and anti-inflammatory regulatory T cells (Treg) exacerbates neuroinflammation [55]. Misfolded aggregated self-proteins can disrupt immune tolerance, leading to a reduction in Tregs and an increase in autoreactive Teffs, which in turn activate microglia and promote neuroinflammation [56]. Notably, MSC-EVs have been shown to have the potential to influence this imbalance by inducing Teff apoptosis and enhancing Treg proliferation [57]. *In vitro* studies demonstrate that MSC-EVs stimulate IL-10 and TGF-β secretion, further reinforcing their anti-inflammatory and immunoregulatory potential [58,59].

B cells are known for their role in antibody production against Aβ plaques [60], but their broader impact on AD remains less well understood. Studies in mouse models indicate that B cell depletion can reduce Aβ plaque burden, suggesting their potential contribution to AD pathology [61]. The immunomodulatory effects of MSCs on B cells appear to be largely independent of secreted EVs, as MSC-EVs have not been consistently shown to modulate activated B cells [62].

Natural killer (NK) cells, recognized for their ability to release inflammatory molecules, also contribute to neurodegeneration by promoting microglial polarization toward a proinflammatory state

and hindering Treg infiltration, as observed in ALS mouse models [63]. Depleting NK cells has been shown to delay disease progression and improve survival in an ALS mouse model, though conflicting reports suggest a potential neuroprotective role in Parkinson's disease (PD) [64]. While in mice MSCs can suppress NK cell proliferation by reducing IL-2 and IL-15 production and downregulating activating receptors such as NKp30, NKp44, and NKG2D [65], the specific role of MSC-EVs in modulating NK cell activity remains to be explored.

Collectively, these findings position MSC-EVs as promising immunomodulatory agents capable of reshaping adaptive immune responses in AD by reducing Teff-driven neuroinflammation, enhancing Treg activity, and modulating lymphocyte infiltration into the CNS. However, further research is necessary to fully elucidate their effects and therapeutic potential.

MSC-EVs as a drug delivery vehicle to treat neurodegenerative disorders

The CNS barriers play a crucial role in maintaining brain homeostasis, but they also pose a significant challenge for the delivery of macromolecular drugs from the bloodstream into the brain. In fact, more than 98% of small molecules and nearly all larger molecules, such as peptides, proteins, and antibodies, face difficulty in crossing these barriers [66]. The limited capacity of numerous therapeutic compounds to penetrate the brain's barriers and target specific regions highlights the need for the development of efficient drug delivery platforms [67]. EVs have gained substantial attention as potential drug-delivery vehicles due to their biocompatibility, low immunogenicity, and capacity to cross biological barriers (Table 2). In therapeutic applications, EVs are first distributed via biological fluids, after which they engage target cells through uptake/interaction mechanisms (e.g., receptor-mediated endocytosis or membrane fusion) to deliver cargo either locally or at a distance. Engineering of EVs presents an opportunity to enhance their targeting capabilities toward specific recipient cells, including those designed to target brain barriers [68]. Furthermore, EVs can protect their cargo from degradation and enhance the stability and bioavailability of potential encapsulated drugs.

Given that MSC-EVs possess all the aforementioned favorable EV characteristics alongside their intrinsic immunomodulatory properties, their capacity to transport bioactive molecules can be further leveraged to better modulate neurodegenerative processes in the brain. MSC-EVs, like other EVs, can be loaded using two approaches: post- and pre-loading [69]. In post-loading, both hydrophobic and hydrophilic drugs can be incorporated into EVs via passive methods like co-incubation [70] or active methods such as sonication [68,71], electroporation [72], extrusion, dialysis, freeze–thaw cycles, and saponin treatment [73–75] (Figure 2). Another innovative approach to post-loading involves fusing MSC-EVs with nanoparticles containing a cargo of interest. This fusion results in a hybrid structure that retains the ideal characteristics of MSC-EVs while incorporating the

Table 2. Advantages and disadvantages of synthetic nanoparticles and EVs as a drug delivery system

Carrier	Advantages	Disadvantages
Synthetic nanoparticle	• Scalable and cost-effective • Controlled and targeted drug release possible • Easy to modify the synthetic nanoparticle to custom specific requirements: e.g., loading of exogenous drug or increasing stability of the nanoparticle	• Low barrier-crossing properties • Toxicity and immunogenicity issues
Extracellular vesicles (EVs)	• Good bioavailability and biocompatibility • Sometimes have inherent barrier-crossing properties • Sometimes have inherent targeting capacity	• Lack of controlled release mechanism • Low efficiency of exogenous drug loading • Non-specific effects of natural EV cargo

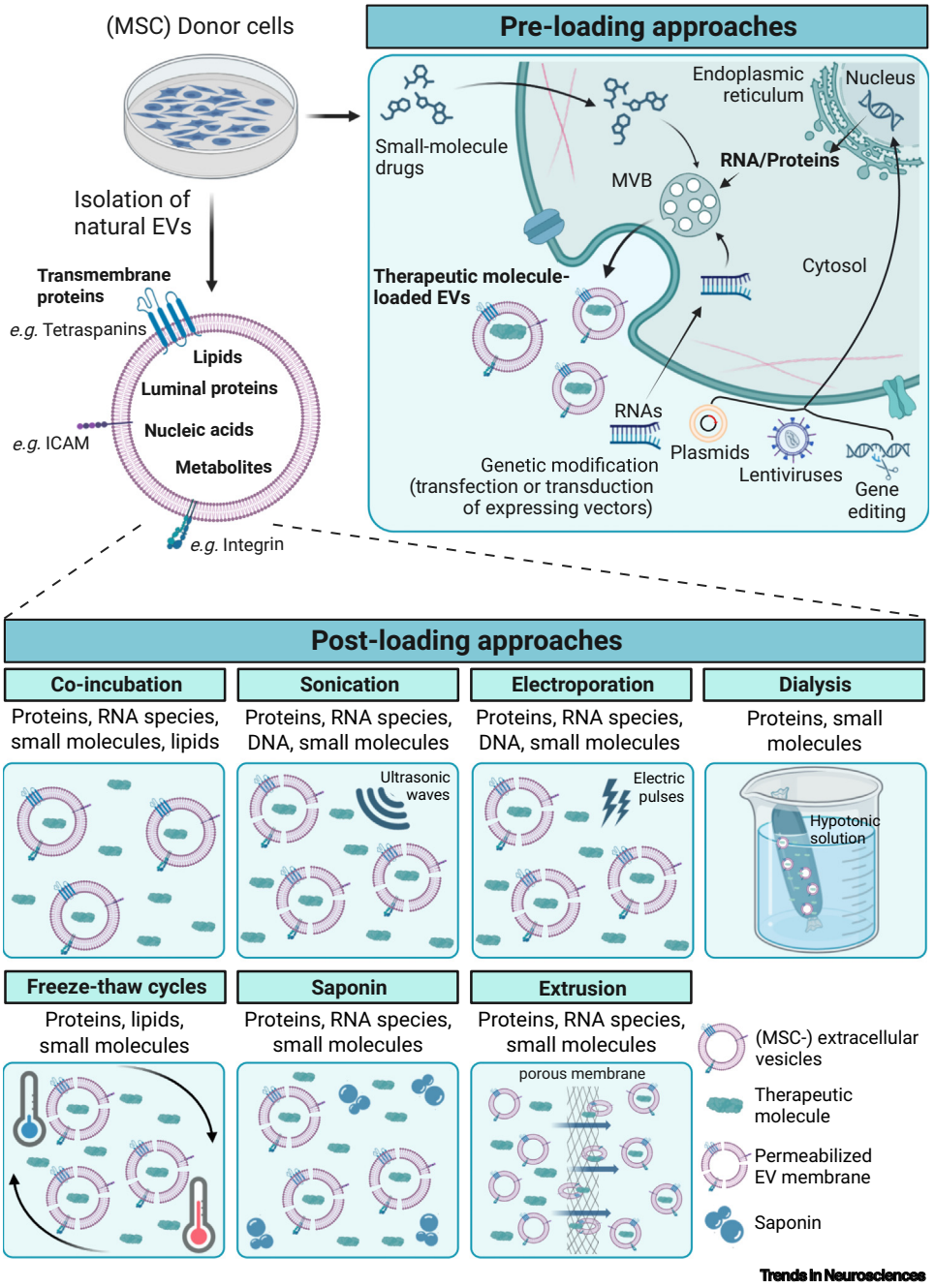

Figure 2. Schematic representation of the most common extracellular vesicle (EV) pre- and post-loading approaches. Pre-loading strategies incorporate therapeutic molecules (e.g., RNA, DNA, proteins, or small-molecule drugs) during EV biogenesis by engineering parental cells: for example, by overexpressing proteins or genetic constructs that are packaged into multivesicular bodies (MVBs) prior to EV release. Post-loading approaches, by contrast, rely on exogenous insertion of therapeutic molecules into EVs after their isolation. Several methods transiently increase EV membrane permeability to facilitate cargo entry: co-incubation promotes passive cargo association; sonication loads molecules into EVs through mechanical disruption, electroporation applies electrical pulses, dialysis in hypotonic solutions encourages cargo diffusion; freeze–thaw cycles repeatedly disrupt and reseal membranes; saponin solubilizes the EV membrane; and extrusion forces EVs through porous membranes. Abbreviations: ICAM, intercellular adhesion molecule; MSC, mesenchymal stromal cell. Figure created with BioRender.

cargo. Notably, there is growing interest in fusing MSC-EVs with lipid nanoparticles, one of the most clinically advanced nanoparticles [76]. However, pre-loading approaches involve encapsulating drugs into the EVs during their formation, which can be advantageous for incorporating drugs unsuitable for post-loading [68,71]. The molecule of interest that needs to be loaded is often attached to proteins associated with EVs, such as tetraspanins CD63, CD9, and CD81, but also other EV proteins [68,77]. While a full benchmarking of loading techniques is outside the scope of this review, we note several method-dependent trade-offs. Post-loading strategies are cargo-dependent: hydrophobic small molecules often load efficiently by incubation, while nucleic acids and proteins typically require active methods that boost loading but risk aggregation, yield loss, or membrane/proteome changes. Pre-loading via producer-cell engineering enhances protein/RNA packaging and delivery but may affect EV biogenesis and complicate scale-up and release testing. Reported efficiencies vary widely by quantification metrics, with single-particle assays showing divergent outcomes. For detailed comparisons and standards see [78–80].

Various studies have explored the potential of loading EVs with therapeutics for treating brain disorders. For instance, EVs carrying the anti-inflammatory compound curcumin were delivered to microglial cells, which resulted in an inhibitory effect on brain tumor growth in GL26 tumor model mice [81]. Another study in mice successfully loaded dopamine into EVs using the co-incubation method, achieving a 15-fold increase in brain delivery compared with injection of free dopamine, with both formulations administered via intravenous (IV) injection [82]. Moreover, this approach exhibited low toxicity and higher therapeutic efficacy [82]. More recently, a study employed MSC-EVs packed with the anticancer drug doxorubicin and compared three loading methods: passive loading, active loading via electroporation, and sonication [83]. The findings demonstrated the feasibility of loading MSC-derived EVs with doxorubicin, with electroporation proving to be the most effective method. By contrast, passive loading yielded suboptimal results, and sonication was shown to be detrimental to EV integrity.

Taken together, various methods exist for effectively loading MSC-EVs with drugs, proteins, or miRNAs. However, translating these approaches into clinical settings requires further validation in advanced disease models.

Current challenges of MSC-EVs for clinical use in treating neurodegenerative disorders and potential strategies to overcome them

Despite their promises, several obstacles still hinder the full transition of MSC-EVs from bench to bedside. In the following sections we look into the current primary challenges, also recently reviewed in [84], and outline possible strategies for overcoming them.

Restricted expandability and heterogeneity of primary MSCs

A significant obstacle to the translation of MSC-EV products into clinical practice lies in the limited expandability and heterogeneity of primary MSCs. It is likely that this heterogeneity contributes to the inconsistent results observed in numerous clinical trials [85–88]. These challenges with cellular heterogeneity also extend to MSC-EV products. Recent preclinical investigations have shown that, due to inter- and intra-donor MSC heterogeneity, only a portion of MSC-EV products exhibit therapeutic effects [18,89,90]. Despite being manufactured using standardized procedures from the same MSC stocks, significant batch-to-batch variations in EV products are observed [90–92]. EVs obtained from MSCs that have undergone fewer passages tend to have greater immunomodulatory potency than EVs isolated from late-passage MSCs [93]. In addition, factors such as proliferation rate and differentiation potential of MSCs can influence the potency of their derived EVs [94–96]. For example, late-passage MSCs may undergo senescence-related changes, including telomere shortening, DNA damage, and altered cytokine secretion, which can negatively

impact their therapeutic efficacy [95]. The combination of MSC heterogeneity and the limited expandability of primary cells is likely to hinder the large-scale production of clinically applicable MSC-EV products.

To mitigate these challenges, careful selection of monoclonal MSC lines with stable genomic and functional characteristics is crucial. Consistency in passage numbers across production batches is also essential, as this influences EV composition and requires rigorous bioequivalence testing to confirm product identity and potency. Since primary MSCs have a limited lifespan, their heterogeneity cannot be fully resolved through good manufacturing practice protocols alone. In an effort to standardize MSC-EV production, a potential solution involves cloning and expanding MSCs to generate stable cell lines [97]. The development of immortalized MSC lines can provide an unlimited and more standardized source of EVs [98,99]. A proof-of-principle study demonstrated that EVs derived from clonally expanded MSC lines retained their therapeutic efficacy [99]. When administered intranasally in a mouse model of hypoxia–ischemia-induced brain injury, these EVs reduced microglial and astrocyte activation, decreased leukocyte infiltration, downregulated proinflammatory cytokines such as IL-1β, and increased levels of anti-inflammatory cytokines such as IL-4 and TGF-β.

However, despite their advantages, immortalized MSCs do not completely eliminate variability. Over time, factors such as genetic drift, epigenetic modifications, and asymmetric cell division may lead to re-emerging heterogeneity, even within clonal lines. Additionally, MSC lines derived from different subclones of primary cells may retain unique biological properties, resulting in variations in EV composition, including differences in proteomic and RNA profiles. While immortalized MSC lines hold promise for producing MSC-EVs with greater consistency, ongoing efforts are needed to ensure stability and minimize batch-to-batch variability.

Enhancing MSC-EV delivery and biodistribution to the brain

To effectively address neurodegenerative disorders, MSC-EVs must reach the brain. However, direct injections into the brain are highly invasive and not advisable, particularly for repetitive treatments [100]. IV administration offers a non-invasive alternative but presents another significant challenge, as only a small fraction of MSC-EVs successfully crosses the CNS barriers and reaches the brain in therapeutically relevant amounts [101]. Additionally, systemic clearance mechanisms and potential uptake by peripheral organs further limit the efficiency of IV delivery [102]. By contrast, intranasal administration allows MSC-EVs to bypass the BBB via the olfactory bulb or the trigeminal pathway from the nasal epithelium to the brain [103]. Numerous preclinical studies in rodent models have demonstrated the efficacy of intranasal EV delivery [26,104,105]. For instance, a comparison of intranasal and IV delivery showed that both could ameliorate symptoms of ALS [106]. While intranasal administration enhances patient compliance and facilitates repeated dosing regimens essential for neurodegenerative diseases [90,94,107], challenges remain. These include dosing accuracy, mucociliary clearance, and achieving targeted delivery to specific brain regions [90,94,107]. It is also important to note that most preclinical studies on EV delivery have been conducted in murine models, whose nasal epithelium morphology and olfactory bulbs differ significantly from those of humans, posing an additional translational challenge [108].

To enhance the brain targeting of MSC-EVs, one strategy is to fuse them with EVs known for their brain-targeting capacity. Several studies have underscored the efficacy of specific unmodified EVs in homing to the brain [68,109]. However, the precise mechanism by which naive EVs cross the brain barriers remains elusive [110,111]. Notably, the cellular origin of EVs significantly influences their biodistribution [112,113]. Emerging evidence suggests that EVs derived from various brain cell types, including brain endothelial cells [114], microglia [115,116], and astrocytes

[117,118], as well as those from brain metastasizing tumors [118,119], possess a remarkable brain targeting or 'homing' potential upon IV injection, possibly mediated by specific surface factors such as integrins. In a study from our group, we investigated the brain-targeting capabilities of peripherally administered ChP epithelial cell-derived EVs and observed an increase in accumulation of ChP-EVs in the brain compared with control EVs [120]. Various methods exist for fusing EVs with nanoparticle systems [121], and these techniques can also facilitate fusion between EVs sourced from different cell types. However, passive hybridization may encounter limitations due to the similar surface charge of diverse EVs. Therefore, approaches inducing transient disruption of EV lipid layers, such as sonication, freeze-thawing, and extrusion, should be explored to enhance EV fusion.

A second viable strategy involves modifying the membrane of MSC-EVs with molecules that recognize brain-specific receptors, a method already tested for nanoparticles [122]. Modification of EVs can occur through genetic engineering or chemical modification, offering versatile approaches for enhancement [77]. The efficacy of engineering EVs has been demonstrated by fusing rabies virus glycoprotein (RVG), a neuron-specific peptide, with an EV membrane protein (Lamp2b) [72]. This work illustrated the potential of engineered EVs to selectively target brain cells such as neurons, microglia, and oligodendrocytes upon IV administration. Similarly, another study in rats engineered EVs by attaching the T7 peptide-transferrin receptor-binding peptide to Lamp2b protein in glioblastoma, resulting in reduced tumor size compared with unmodified EVs, indicating improved targeted delivery [123]. Likewise, EVs linked with RVG peptide and Lamp2b protein successfully delivered miR-124 in a rat model of ischemic stroke, leading to observed neurogenesis upon IV administration, reiterating the success of EV modification in delivering therapeutics to the brain [124]. Also, MSC-EVs coupled with RVG have shown promising results in improving targeted delivery to the hippocampus and cortex in an AD mouse model [125]. This targeted delivery significantly reduced Aβ plaque deposition and enhanced memory and cognitive abilities compared with untagged MSC-EVs [125].

Despite these promising strategies, one of the major challenges in optimizing EV-based delivery is the accurate tracking and quantification of EV biodistribution [126]. Common techniques such as fluorescent labeling, bioluminescent reporters, or radiolabeling can suffer from significant limitations, including dye transfer, signal persistence unrelated to intact EVs, and limited spatial resolution in deep tissues [127]. These factors complicate the interpretation of where EVs accumulate and how long they remain functional. Moreover, some labeling approaches may alter EV surface properties, particle size, or biodistribution behavior [128,129]. Recent advances using genetically encoded reporters, and click-chemistry-based tracers show improved specificity and quantifiability, but these tools are largely restricted to preclinical settings [113,130,131]. A lack of standardized protocols across studies further hinders the comparability of biodistribution data. Therefore, careful methodological choices and validation strategies are critical to ensure accurate tracking and interpretation of EV fate *in vivo*, particularly in the context of brain-targeted therapies.

Understanding the mechanisms of action

Achieving clinical approval for MSC-EV-based therapies relies in part on understanding their mechanism of action, a daunting challenge given their multimodal function. Upon entering the bloodstream, MSC-EVs' docking sites and the subsequent cascade of events are difficult to predict, despite attempts to track them via imaging techniques [132]. The multifaceted effects of EVs stem from the involvement of numerous proteins and RNAs, without a distinct predominant player among them [133]. From a regulatory standpoint, elucidating whether the effects arise primarily from receptor–ligand interactions or cargo delivery is a crucial first step. Physics-based analyses suggest that receptor–ligand interactions are more efficient than cargo delivery, given surface-to-volume ratio approximations.

While nanoparticles leverage small size for potent surface-cell interactions and homogeneous distribution, ensuring sufficient quantity/volume delivery requires large amounts. The role of lipids in MSC-EVs, particularly bioactive lipids like pro-resolving lipids, remains largely unexplored [134]. Moreover, *in vitro* experiments often involve exaggerated concentrations compared with *in vivo* EV concentrations and should be interpreted cautiously [135]. Although various experiments confirm the efficient entry of EVs into cells [136], their ability to deliver cargo effectively to the cytoplasm – that is, escaping the endosome – remains debatable. Initially hailed as major actors, miRNAs were found to be relatively sparse within EVs [137]. Furthermore, while fusion with the plasma membrane was initially proposed, internalization and passage through the endosome–lysosome pathway, associated with degradation, were observed instead. The quantification of these phenomena necessitates complex methodologies, highlighting the challenges in assessing efficient protein cytosolic delivery.

Safety and toxicity considerations

The toxicity and long-term safety profiles of MSC-EVs remain underexplored [138]. Most preclinical studies report no overt toxicity after IV or intranasal administration, even with repeated dosing, but these studies are typically short-term and limited in scope [139]. Many assessments emphasize acute readouts and lack comprehensive toxicology assessment (e.g., immune responses, off-target effects, organ-specific accumulation) [138,140]. For example, multiple animal studies report preferential EV accumulation in liver, spleen, and lungs after systemic delivery, which raises questions about potential long-term impacts on these organs. In addition, heterogeneity in EV isolation, purification, and storage can alter vesicle composition and thereby influence safety [140]. Regulatory agencies emphasize rigorous characterization and good manufacturing practice compliant production, yet harmonized toxicity testing frameworks for EVs are still emerging [138,140]. As MSC-EVs advance toward clinical use, particularly for chronic indications requiring repeated dosing, systematic studies of immunogenicity, biodistribution, and long-term toxicity will be essential.

Concluding remarks and future perspective

This review highlights the dual therapeutic potential of MSC-EVs in modulating immunity and in acting as a drug delivery system for neurodegenerative disorders, particularly AD. EVs, once considered primarily as carries of cellular waste, are now recognized as critical mediators with notable immunomodulatory properties. MSC-EVs can reduce neuroinflammation, enhance regulatory T cell activity, and suppress proinflammatory immune cells, including macrophages, mast cells, B cells, and NK cells. In parallel, engineered MSC-EVs offer a promising platform for targeted drug delivery to the CNS, overcoming barriers that limit conventional therapeutics. Their natural capacity to traverse biological membranes, shield cargo, and reach specific tissues provides clear potential advantages in brain-targeted therapies.

Despite these opportunities, the clinical application of MSC-EVs faces substantial challenges (see Outstanding questions). EV composition varies with parental cell source, route of administration, and cargo complexity, complicating reproducibility. Differences in MSC expansion methods, along with the need for optimized drug-loading techniques, add further variability. Standardized protocols for EV isolation, characterization, and potency testing are urgently needed to ensure consistency.

Future research should prioritize unraveling the mechanisms by which MSC-EVs regulate immune responses and interact with neural and glial cells. A deeper understanding of EV pharmacokinetics, biodistribution, and cargo delivery – especially for engineered EVs – will be critical. Parallel efforts should refine strategies for precise targeting, scalable production, and rigorous safety and efficacy testing.

Outstanding questions

How do MSC-EVs interact with different immune cell populations in neurodegenerative diseases, and what are the implications for long-term immune modulation?

What is the optimal MSC-EV dose for therapeutic impact in the brain, and how can this be achieved through non-invasive routes such as intranasal administration? What are the long-term effects of repeated MSC-EV administration for chronic neurodegenerative diseases, and how can they be monitored? How can risks like immune responses or tissue accumulation be minimized in MSC-EV drug delivery?

How can MSC-EVs be engineered to cross the BBB more efficiently, and which strategies work best for different neurodegenerative diseases? How can MSC-EVs be modified for precise targeting of specific brain regions, and what technologies could aid this? Could combining MSC-EVs with targeted delivery systems like brain-targeting peptides enhance their therapeutic efficacy?

How can challenges related to restricted MSC expandability and heterogeneity be addressed to ensure consistent MSC-EV production? What are the limitations of creating stable, standardized MSC-EV products for clinical use, considering MSC heterogeneity and expandability?

What are the key molecular mechanisms that determine the therapeutic effectiveness of MSC-EVs, especially regarding receptor–ligand interactions and cargo delivery? How do different types of cargo (e.g., miRNAs, proteins, lipids) within MSC-EVs contribute to their therapeutic effects, and can they be selectively targeted for specific brain regions?

Addressing these challenges will be essential to fully harness MSC-EVs as next-generation immunotherapies and delivery vehicles. If successful, these vesicles could represent a transformative approach for treating AD and other neurodegenerative diseases, uniting immunomodulation with advanced drug delivery in a single therapeutic platform.

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Declaration of interests

The authors declare no competing interests in relation to this work.

Resources

[i]https://clinicaltrials.gov/study/NCT04388982

[ii]https://clinicaltrials.gov/study/NCT03384433

[iii]https://clinicaltrials.gov/study/NCT06607900

[iv]https://clinicaltrials.gov/study/NCT06598202

[v]https://clinicaltrials.gov/study/NCT05490173

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