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"Mesenchymal Stem Cell-Derived Extracellular Vesicles for Ocular Regeneration: From Bench to Scalable Clinical Translation"

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Abstract

Mesenchymal stromal cell-derived extracellular vesicles (MSC-EVs) have been of recent interest in academia and industry due to the potential for being an effective cell-free therapy for various ocular conditions. The nanoscale vesicles contain bioactive molecules, including proteins, lipids, and nucleic acids, that enable them to modulate immune responses, reduce inflammation, and contribute to tissue restoration, all the while evading many roadblocks found in the application of direct cell transplantation. Preclinical studies have shown the therapeutic potential of MSC-EVs for age-related macular degeneration, diabetic retinopathy, dry eye syndrome, and corneal injury by improving the functions of the retina, promoting tear secretion, inhibiting the activities of inflammatory cells, and enhancing the healing of the corneal tissue. Various techniques for isolation and modification have been explored to enhance the therapeutic potency of MSC-EVs, including standardizing production, scaling up production, and enhancing delivery. Future research needs to focus on optimizing culture conditions, identifying appropriate MSC donors, and creating targeted delivery systems to advance clinical translation. The present study aims to review MSCs and their application in clinical medicine, EVs and their isolation approaches, as well as the potential of MSC-EVs in the treatment of ocular diseases and existed challenges.

Keywords: Mesenchymal stem cells; Extracellular vesicles; Ocular diseases; Retinal regeneration; Cell-free therapy; EV engineering; Immunomodulation

Introduction

Ocular diseases, such as glaucoma, diabetic retinopathy, corneal injury, and age-related degeneration, continue to be the leading causes of vision loss globally, with available therapy frequently having limited ability to restore vision completely or prevent disease progression. The search for innovative and effective treatment modalities has been heightened, with special focus given to regenerative and acellular therapy strategies (Delplace et al. 2015; Hassan et al. 2023).

Multipotent stromal cells, known as mesenchymal stem cells (MSCs), are found in a variety of tissues, including bone marrow, adipose tissue, and umbilical cord (Kobolak et al. 2016; Park and Jung 2021). The hallmark features of MSCs are their inherent immunomodulatory and regenerative capabilities, largely due to paracrine secretion mechanisms (Zhou et al. 2019; Alvites et al. 2022). Of the many secreted elements, extracellular vesicles (EVs)—membrane-bound nanoparticles that measure between 30 and 5000 nm in size—have been identified as key players in mediating intercellular communication (Lo Cicero et al. 2015). There are multiple bioactive molecules contained in these vesicles, including messenger RNAs (mRNAs), microRNAs (miRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), and other forms of RNA and DNA, all of which are involved in tissue repair and immune regulation (Abels and Breakefield 2016; Eirin et al. 2017).

MSC-derived extracellular vesicles (MSC-EVs) harness various therapeutic advantages of parent cells but with improved safety and reduced immunogenicity (Zhao et al. 2020; Liu et al. 2022). Their nanometer size enables them to cross biological barriers, which is especially attractive for ophthalmic use, where direct cell transplantation is jeopardized by immune rejection and poor engraftment (Liu et al. 2022; Wu et al. 2023).

This review critically evaluates recent progress in the biology and therapeutic potentials of MSC-EVs in the field of ophthalmology. Emphasis is placed on their mechanisms of action, preclinical and clinical evidence to date, EV isolation and modification challenges, and perspectives for future clinical application. By consolidating information from molecular to clinical settings, this article seeks to give a state-of-the-art overview of MSC-EV-based therapies with promise as a useful approach to ocular disease treatment.

An overview of MSCs and their application in cell therapy approaches

Unlike other stromal cells, MSCs self-renew and differentiate into multiple lineages. In addition to the umbilical cord and endometrial polyps, menstrual blood, bone marrow, and adipose tissue, they are found in multiple connective tissues. A variety of substances are secreted by MSCs that are anti-inflammatory and immunomodulating. Based on these characteristics and the effects observed in cultured MSCs in vitro, they may prove to be an attractive therapeutic option (Pittenger et al. 2019).

MSCs play a crucial role in cell therapy, especially in the areas of tissue regeneration and the treatment of immune disorders. These cells exhibit exceptional properties and are presently utilized in a range of therapeutic applications to improve treatment effectiveness (Wei et al. 2013). An important example is allogeneic cell transplantation, which takes advantage of the low immunogenicity of MSCs, thus removing the need for immunosuppressive therapy, a significant benefit (Mastri et al. 2014). The initial clinical study involving MSCs was published in 1995 by Lazarus et al, focusing on patients with blood cancers. They showed that expanding human bone marrow-derived stromal progenitor cells (BMMSCs) outside the body and then infusing them into patients was not followed by significant adverse effects (Lazarus et al. 1995).

Furthermore, MSCs have been employed in the management of various conditions, such as myocardial infarction (22.9%), diabetes (10.3%), spinal cord injury (9.2%), Crohn's disease (3.8%), and aplastic anemia (1.5%) (Wei et al. 2013). It is primarily their ability to release paracrine factors that gives MSCs their therapeutic benefits, rather than their capability to differentiate into different lineages, which contributes to their therapeutic effectiveness. Cytokines and growth factors released by MSCs contribute to cytoprotection, angiogenesis, tissue repair, normalization of the extracellular matrix, and inflammation reduction. The immune system is also supported by them, assisting with immune disorders (Mastri et al. 2014).

EVs Composition and Classification

EVs are nanoscale structures derived from cellular constituents, marked by significant heterogeneity and variability in size as well as in molecular composition, which affect intercellular communication (Shao et al. 2018; Doyle and Wang 2019). Vesicles contain a wide range of biomolecules, including lipids, nucleic acids, proteins, glycolipids, glycoproteins, and membrane receptors. These elements play a critical role in the transport of cytokines, particularly interleukin 1, which is the most important (Yáñez-Mó et al. 2015; Zaborowski et al. 2015; Sedgwick and

D'Souza-Schorey 2018). Based on their origin, molecular content, biological functions, and specific protein markers, EVs are divided into different types. The major subtypes of EVs are microvesicles (MVs), exosomes, and apoptotic bodies. The present study will be confined to MVs and exosomes only. Based on their significant impact on intercellular processes and inherent properties, EVs have extensive therapeutic potential.

Exosomes

Exosomes form a unique class of EVs produced by cells in response to particular requirements within the endosomal pathway. The process includes inward folding of the membrane of the early endosome, which further develops into multivesicular bodies (MVBs) that are distinguished by endocytic and trafficking characteristics, eventually leading to the formation of exosomal vesicles. The destiny of MVBs is regulated by cholesterol content, which defines whether they move towards lysosomal decay or fuse with the plasma membrane, thereby releasing their biomaterials into the extracellular space. Exosomes are crucial for intercellular communication, cellular health maintenance, tumor growth, and the initiation of immune responses attributable to their role as antigens. They are also crucial in the mechanisms of tissue repair and regeneration in the nervous system. Recent research has shown that exosomes, which contain detectable biomarkers in body fluids, are associated with many pathological conditions such as acute kidney injury, Parkinson's disease, pancreatic cancer, and lung cancer. Due to their occurrence in disease situations and their unique features—such as extended circulation half-life, biocompatibility, targeted cell interaction, and the ability to cross cellular membranes—exosomes have been found to be a promising option for therapeutic interventions in these diseases (Doyle and Wang 2019).

Microvesicles

Microvesicles, commonly known as ectosomes or microparticles, are a specific subtype of EVs distinguished by their size, which varies from 100 nm to 1 μ m. The formation of MVs is linked to a range of biomaterials, including lipid cholesterol derived from the plasma membrane, actin, and microtubules, along with molecular motors such as kinesins and myosins, as well as elements of the fusion machinery like SNAREs and tethering factors. Membrane vesicles are produced via the outward blebbing of the plasma membrane, which is subsequently followed by the fission of these

membrane blebs (Yáñez-Mó et al. 2015; Zaborowski et al. 2015; Sedgwick and D'Souza-Schorey 2018; Shao et al. 2018; Doyle and Wang 2019). MVs primarily consist of proteins, which include cytosolic proteins, plasma membrane-associated proteins, cytoskeletal proteins, heat shock proteins, integrins, and proteins that experience post-translational modifications like glycosylation and phosphorylation (Doyle and Wang 2019). MVs fulfill multiple roles, such as aiding in cell invasion, enhancing cell growth, circumventing immune responses, promoting angiogenesis, and playing a part in drug resistance. Furthermore, cancer cells within the body can produce these factors, impacting their invasive capabilities by facilitating the horizontal transfer of signaling proteins among cancer cells (Sedgwick and D'Souza-Schorey 2018). The general principles of EV formation, content, and therapeutic implications are illustrated in **Figure 1**.

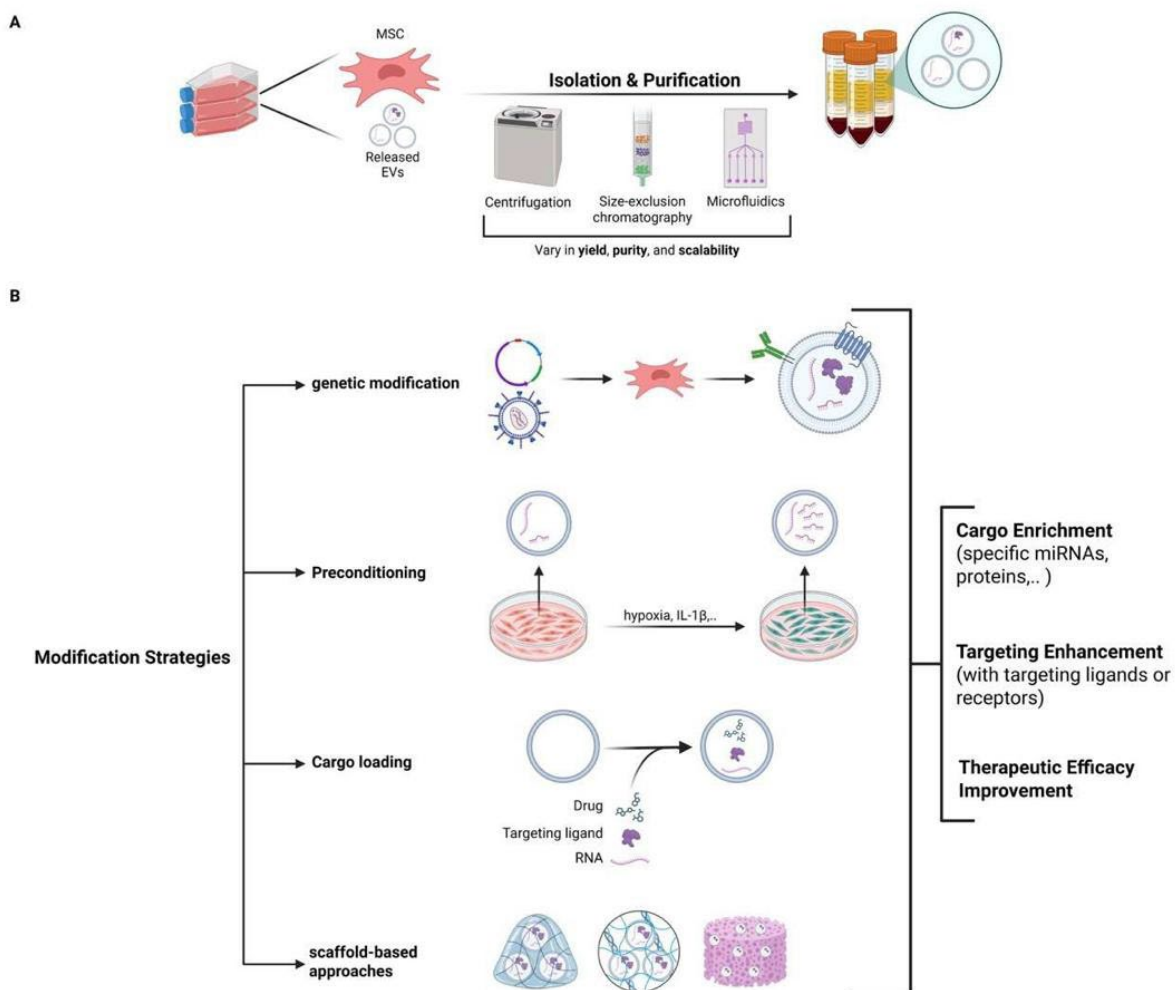


Figure 1. Schematic illustration of the biogenesis, molecular cargo, and functions of extracellular vesicles (EVs). Exosomes and microvesicles arise via distinct pathways—endosomal maturation and plasma membrane budding, respectively. Both EV types carry biomolecules such as proteins, RNAs, and lipids, and are involved in intercellular communication, tissue regeneration, immune modulation, and drug delivery. Their size range and key molecular features are summarized.

Isolation and Engineering of EVs

To facilitate clinical use of EVs, effective preparation and, if necessary, targeted modification are required to improve their therapeutic efficacy. The isolation of EVs has been achieved by using several methods, including density gradient centrifugation, ultracentrifugation, novel microfluidics, and size-exclusion chromatography (Wu et al. 2023). The methods vary in yield, purity, and scalability. After isolation, there are opportunities to modify EVs for maximum optimization of cargo and targeting capabilities. For example, loading functional therapeutic agents such as drugs, RNAs, or targeting ligands has been used to optimize the functional efficacy of EVs and to facilitate delivery to diseased tissues while limiting off-target effects (Wu et al. 2023). It is also possible to modify EVs in order to increase their therapeutic potential. Genetic engineering, for instance, can enhance the bioactivity and regenerative capacity of MSC-EVs through the enrichment of specific miRNAs or proteins. At the same time, scaffold-based approaches have been studied to improve EV survival and retention (Duan et al. 2024; Hindle et al. 2024).

A schematic summarizing the various isolation, purification, and engineering strategies employed to enhance the therapeutic potential of MSC-EVs is presented in **Figure 2**.

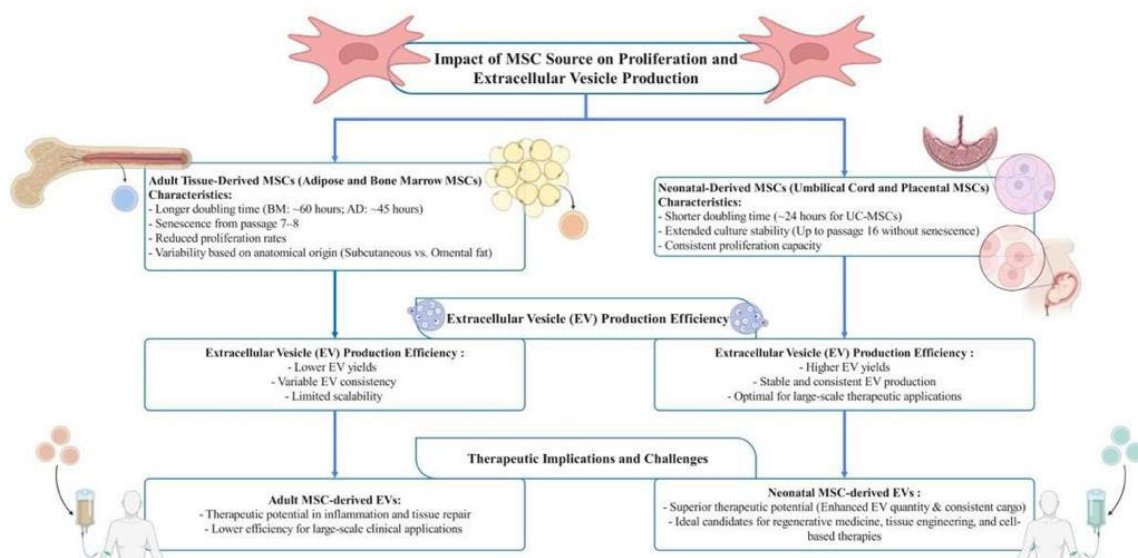


Figure 2. Overview of mesenchymal stromal cell-derived extracellular vesicle (MSC-EV) isolation, purification, and engineering strategies. (A) EV isolation and purification methods include ultracentrifugation, size-exclusion chromatography, and microfluidic techniques, each offering varying purity, scalability, and yield. (B) Engineering approaches enhance EV therapeutic performance and include genetic modification (e.g., miRNA/protein enrichment), cargo loading (drugs, RNAs, targeting ligands), preconditioning (e.g., hypoxia, IL-1 β), and scaffold incorporation for prolonged tissue retention.

Immune Modulation and Regeneration by MSC-EVs

Several studies have demonstrated the therapeutic potential of MSC-EVs in immune modulation and tissue repair contexts. Due to the interaction between MSCs and various target cells, such as macrophages, microglia, chondrocytes, articular chondrocytes, endothelial cells, fibroblasts, pericytes, neural stem cells (NSCs), neurons, hepatic stellate cells, and podocytes, this may be the case (Bruno et al. 2015).

In summary, factors released by MSCs, including interleukin-6 (IL-6), interleukin-10 (IL-10), prostaglandin E2 (PGE2), transforming growth factor beta (TGF- β), hepatocyte growth factor, and indoleamine 2,3-dioxygenase (IDO), have been shown to impact the target cells within both the innate and adaptive immune systems (Allan et al. 2020). Signaling derived from MSCs can suppress the proliferation and activity of natural killer cells, diminish the activation of B lymphocytes, and encourage T cells to differentiate into effector T cells that produce IL-17. Concurrently, MSC-related signaling has been linked to promoting T regulatory cells that express CD25 and are positive for FoxP3 (Kou et al. 2022).

EVs generated from samples of bone marrow by MSCs derived from individuals with type 1 diabetes were able to contain both mRNA and protein for TGF β . Treatment with these MSC-EVs enhanced TGF and PGE2 secretion from T cells. Further, MSC-EVs suppressed Th1 responses, increased regulatory T cell (Treg) numbers, and decreased Th17 differentiation (Favaro et al. 2014).

Several studies have shown that human bone marrow-derived MSCs-EVs (BMSCs) play a crucial role in the treatment of osteoarthritis by suppressing pro-inflammatory pathways and promoting chondrocyte proliferation and migration. Research conducted by Vonk et al. revealed that MSC-EVs inhibit NF- κ B signaling through the prevention of I κ B phosphorylation. This leads to a reduction in TNF- α -induced COX2 expression, as well as interleukin and collagenase activity. Moreover, MSC-EVs enhanced SOX9 and WNT7A expression, facilitating proteoglycan and type II collagen synthesis in vitro (Vonk et al. 2018).

In various animal studies, MSC-derived EVs have been associated with vascular repair. The mechanism underlying vascular repair is still being explored, but it has been shown that the activation of vascular endothelial growth factor receptors and subsequent angiogenesis pathways contributes to the repair of hindlimb ischemia in mice. Also, MSC-EVs improve recovery from myocardial infarction, reduce infarct size, and maintain cardiac function in rats. Moreover, small EVs from MSCs that express hypoxia-inducible factor-1 $^{+}$ overexpress Jagged-1, which activates Notch signaling pathways in endothelial cells under ischemic conditions (Allan et al. 2020).

Treatment of ocular diseases with MSC-EVs

EVs derived from MSCs are nanosized vesicles that are secreted by the MSCs and are recognized to be an important mode of cell-to-cell communication and tissue regeneration. EVs include exosomes, microvesicles, and apoptotic bodies, differing in size and method of biogenesis (Habibi et al. 2023; Sun et al. 2023). MSCs contain large numbers of bioactive molecules that include proteins, lipids, mRNAs, and microRNAs that are responsible for their regenerative and immunomodulatory potential (Habibi et al. 2023; Yu et al. 2023). The potential of MSC-EVs in ophthalmology has been appreciated in recent years, especially for the treatment of dry eye disease, corneal injuries, and retinal degeneration (Habibi et al. 2023; Massoumi et al. 2023). The regenerative potential of MSCs is thought to be mainly attributed to the EVs' ability to modulate immune responses, enhance cellular repair, and promote angiogenesis (Li et al. 2023).

Bone marrow-derived EVs offer an exciting cell-free option for stem cell therapy, especially to treat diseases of the eye. Preclinical and clinical studies confirm their potential to limit inflammation, promote healing, and protect against oxidative stress damage in the eye (Yu et al. 2024). Biological barriers can be crossed by these molecules, which makes them useful for treating diseases of the eye, such as macular degeneration and glaucoma (Li et al. 2023). Clinical trials are investigating the safety and efficacy of the application of marrow-derived EVs for ocular function. A recent study represented the promise of engineered mEVs to promote corneal regeneration, establishing their role in vision loss regenerative medicine strategies (Deng et al. 2024). With improved EV engineering and delivery, marrow-derived EVs will have an impact on ocular therapeutics.

In addition to physical injuries and chemical burns, infections, age-related degeneration, and corneal malnutrition, corneal disease is one of the most common causes of vision loss (Klingeborn et al. 2017). Despite corneal transplantation's promise, there are still limitations, such as a limited number of donors, immune rejection, and long-term immunosuppression (Liu et al. 2022).

Various studies have demonstrated that MSC-based therapy facilitates the healing of corneal stroma. The secretion of small extracellular vesicles (sEVs) appears to be the main mechanism by which MSCs exert their therapeutic effects. Cell viability and proliferation were significantly enhanced, while their plasticity was enhanced when corneal stromal cells (CSCs) were co-cultured with MSCs, according to Shen et al. Additionally, MSC-derived sEVs influenced matrix metalloproteinase and collagen levels in CSCs, increasing extracellular matrix production and proliferation (Yu et al. 2020). Also, Long et al showed the value of MSCs and their derivatives in improving surgical efficacy through their importance in the regeneration of corneal endothelium and retinal pigment epithelium (Long et al. 2024). Tao et al conducted a study to explore whether human placenta-MSCs-derived EVs could repair alkali injury of the cornea in the mouse model. Their findings indicated that these EVs promoted proliferation, reduced inflammation, and prevented apoptosis in corneal epithelial cells, improving corneal wound healing in mice by decreasing angiogenesis and inflammatory responses. So, their study results supported the potential of human placenta-MSC-derived EVs in corneal wound healing (Tao et al. 2019). Furthermore, Tati et al evaluated the effects of MSCs-derived EVs isolated from bone marrow on corneal epithelial repair. They reported that these vesicles stimulated corneal epithelial repair by

promoting proliferation and inhibiting apoptosis, mediated by the regulation of the expression of P-53, BAD, BCL-2, and CASPASE-3. Thereby, they supported the potential of BMSC-EVs in the treatment of injury-induced corneal epithelial defects (Tati et al. 2024).

The loss of retinal ganglion cells (RGCs) signifies the conclusive and irreversible phase of optic neuropathy. Glaucoma includes various progressive optic neuropathies characterized by the gradual loss of retinal ganglion cells (RGCs). In a regulated in vitro setting, MSCs have the capability to differentiate into neuroectodermal cells, which encompass neuronal cells. In the context of glaucoma treatment, MSCs play a crucial role in enhancing neurotrophin production, promoting the differentiation into functional RGCs, and engaging with the trabecular meshwork (TM), which collectively contributes to a decrease in intraocular pressure linked to glaucoma. The neuroprotective and immunomodulatory properties of MSC-EVs in the context of retinal diseases are primarily linked to their miRNA-dependent mechanisms and the modulation of inflammatory responses (Harrell et al. 2019; Liu et al. 2022). Mead et al investigated the effectiveness of BMSC-EVs in the treatment of rat models of glaucoma. Their observations showed that these EVs significantly enhanced neuroprotection of retinal ganglion cell, preventing retinal nerve fiber layer degeneration. When administered via intravenous injection weekly or monthly, sEV demonstrated therapeutic effectiveness, whereas longer intervals between treatments proved ineffective. They also claimed that this protective effect is mediated by miRNAs and knocking down argonaute2 (AGO2), a protein essential for miRNA activity and incorporation into sEV before isolation, markedly diminished these protective effects. RNA sequencing revealed 43 miRNAs upregulated in BMSC-EVs compared to fibroblast EVs, which did not exhibit neuroprotective effects (Mead et al. 2018).

In retinal degenerative conditions, alongside induced pluripotent stem cell (iPSC)-based therapies, MSC-EVs have emerged as a valuable and potentially limitless therapeutic option. iPSC-derived cells, including retinal pigment epithelial (RPE) cells, have demonstrated the ability to replace damaged cells and support tissue regeneration within the eye. Preclinical studies indicate that iPSC-RPE cells can survive in the injured ocular microenvironment and secrete neuroprotective factors, such as pigment epithelium-derived factor (PEDF), thereby contributing to the preservation of photoreceptors (Liu et al. 2022).

Furthermore, exosomes (a type of extracellular vesicle) have shown the capacity to protect retinal neurons and RPE cells from damage. These vesicles enhance cellular survival and improve retinal function. Notably, electroretinography assessments have revealed increased a-wave and b-wave amplitudes following MSC-EV treatment, suggesting a neuroprotective effect of MSC-EVs in various animal models of retinal degeneration (Zhang et al. 2022).

Retinal ischemia is an important cause of vision loss. Mathew et al, exploring the role of MSC-EVs in ameliorating retinal ischemia, reported that injection of MSC-EVs into the vitreous humor 24 hours following retinal ischemia in a rat model markedly improved functional recovery, while reducing neuroinflammation and apoptosis. The EVs were internalized by retinal neurons, ganglion cells, and microglia. They persisted in the vitreous humor for up to four weeks post-intravitreal delivery, demonstrating saturable binding to vitreous components. Thereby, their findings underscore the significant value of MSC-EVs as biomaterials for neuroprotection and regeneration in retinal diseases (Mathew et al. 2019).

Idiopathic macular holes are common fundus conditions that can result in blindness. It is difficult to achieve an optimal prognosis using the existing treatment options, particularly when the hole is large or refractory. A study was conducted on seven patients who received intravitreal injections of either MSCs or MSC-derived EVs. Five of the seven patients had significant improvements in visual acuity as well as successful closure of macular holes. The extraction of an epiretinal fibrotic membrane was required in one case following MSC injection. Additionally, the administration of EVs provided a greater safety profile and ease of use compared to MSC therapy (Zhang et al. 2018; Yu et al. 2020).

Blindness and vision impairment are the most common outcomes of diabetic retinopathy. Symptoms usually do not appear until the disease has advanced considerably and has caused considerable vision loss. Despite being preventable, diabetic retinopathy causes irreversible blindness (Yu et al. 2020). A study conducted by Zhang et al. demonstrated that the intravitreal delivery of MSC-derived sEVs into the vitreous body of rats with streptozotocin-induced diabetes significantly reduced the levels of inflammatory markers and adhesion molecules (Zhang et al. 2019).

The administration of MSC-derived sEVs through multiple routes, such as intravenous, subconjunctival, and intraocular injections in rabbits with streptozotocin-induced diabetes, was the focus of a study performed by Safwat et al. MSC-derived sEVs were successfully administered

subconjunctivally as well as intraocularly to maintain retinal structural integrity, while intravenous injection did not improve diabetic retinopathy progression (Safwat et al. 2018).

Challenges in MSC-EV-Based Therapy

For the treatment of various diseases, MSCs have been utilized in both animal and human models (Tan et al. 2022). Recent advances have highlighted the importance of MSC-EVs, which are secreted by MSCs. In comparison with intact MSCs, these vesicles have a smaller size (nano-scale), which makes them safer and easier to produce. Furthermore, they improve viability, streamline storage and administration, and enhance pharmacological predictability, establishing MSC-EVs as promising candidates for therapeutic applications, whether as alternatives to whole cells or as targeted drug delivery systems (Maumus et al. 2020; Tan et al. 2022; Giebel and Lim 2025). Significant therapeutic applications of MSC-EVs encompass treatment for myocardial infarction, prevention of ischemic injury in chronic renal failure, acute renal failure, respiratory failure, liver regeneration, infectious diseases, and ophthalmic disorders (Maumus et al. 2020). While MSC-EVs possess beneficial properties and significant potential, their application encounters various challenges. Critical considerations encompass the choice of cell sources, culture conditions, enrichment methodologies, intrinsic heterogeneity, and the tailored design of MSCs. It is essential to thoroughly evaluate these factors in connection with the underlying pathogenesis and pathological processes, along with the genetic or non-genetic engineering techniques utilized to generate appropriate EVs for therapeutic applications (Liu et al. 2022; Giebel and Lim 2025).

Variability in MSC-EV Quality

MSC-EVs derived from different sources and batches have variable quality, which hampers the development of EV-based therapies. Different MSC-EVs derived from bone marrow, umbilical cord, adipose tissue, and embryonic stem cells possess a variety of therapeutic attributes, indicating that they are composed and function differently. As an example, EVs from umbilical cord MSCs were more effective at treating glioblastoma than those from adipose tissue, which, on the contrary, induced tumor cell proliferation (Del Fattore et al. 2015). Similarly, in neurodegenerative disorders, EVs from menstrual MSCs were more potent compared to EVs from bone marrow and umbilical cord in stimulating neuritic outgrowth, again pointing to source-dependent functional diversity (Lopez-Verrilli et al. 2016). Proteomic profiles showed that while there is a certain

overlap in protein content—approximately 60% similarity of bone marrow and umbilical cord MSC-EVs—their other composition is quite different, which suggests that these disparities likely explain their diverse quality and therapeutic potency (Angulski et al. 2017; van Balkom et al. 2019).

An analysis of MSC-derived EVs of different tissue sources using SWATH-MS identified 451 proteins in total. Among the three MSC sources, bone marrow stem cells, adipose-derived stem cells, and human umbilical cord perivascular cells, only 134 proteins were shared, and only 121 were quantifiable (Pires et al. 2016). There was a unique profile of proteins in all MSC sources that are involved in essential neuroprotective functions such as antioxidative stress response, anti-apoptosis, and excitotoxicity modulation. For example, proteins CYPB, CYPA, and DJ-1 were significantly upregulated in BMSCs compared to HUCPVCs and ASCs, suggesting the existence of greater antioxidative potential in BMSCs. DJ-1, as recognized for its antioxidative role, has already been established in Parkinson's disease and cerebral ischemia models (Yanagisawa et al. 2008; Kahle et al. 2009); in line with this, TRX and CYPA have demonstrated neuroprotection via their antioxidant properties (Hattori et al. 2004; Ge et al. 2009; Ma et al. 2012).

Additionally, even EVs from the same MSC origin can be different from batch to batch due to variations in culture conditions or pretreatment regimens. Pretreatment of MSCs with inflammatory cytokines IL-1 β or environmental stresses such as hypoxia was shown to modify the miRNA content of the EVs, enhancing some therapeutic activities such as anti-inflammation or tolerance to oxidative stress (Song et al. 2017; Zhu et al. 2018). This was illustrated in experiments in which pretreatment with IL-1 β loaded huc-MSC-derived EVs with miR-146a, enhancing their anti-inflammatory performance in sepsis models (Song et al. 2017), and hypoxia-induced miR-210 expression augmented myocardial repair through exosomal delivery (Zhu et al. 2018). These observations hint at the responsiveness of EV composition to extrinsic stimuli, resulting in batch-to-batch differences in efficacy. It must be mentioned that the absence of standardized manufacturing procedures accounts significantly for these variations, emerging as an impediment to the clinical translation and regulatory approval of MSC-EV treatments (Nassar et al. 2016). A comparative overview of EV characteristics from different MSC sources is provided in **Table 1**.

Table 2. Comparative features of extracellular vesicles (EVs) derived from different mesenchymal stromal cell (MSC) sources in ocular disease models.

Disease Model	MSC-EV Source	Therapeutic Approach	Key Findings	Year	Study Reference
Alkali-burn corneal injury	BMSCs	Topical (embedded in methylcellulose)	Reduced inflammation, modulated angiogenesis and apoptosis, accelerated wound healing	2022	(Saccu et al. 2022)
Glaucoma (OHT model)	ADSCs	Intravitreal injection	Promoted RGC survival and function; reduced microglial activation and neuroinflammation via TLR4/MAPK/NF-κB inhibition	2024	(Ji et al. 2024)
Autoimmune dacryoadenitis (Sjögren's syndrome model, rabbit)	UC-MSCs	Subconjunctival injection	Promoted M2 macrophage polarization and Treg generation via miR-100-5p; reduced inflammation and gland damage	2022	(Li et al. 2022a)
EAU	UC-MSCs engineered to overexpress IL-10	Intravenous injection (tail vein)	Reduced Th1/Th17 and enhanced Treg responses; suppressed inflammation via IL-10-loaded EVs	2022	(Li et al. 2022b)

MSC-EVs, mesenchymal stem/stromal cell-derived extracellular vesicles; BMSCs, bone marrow-derived mesenchymal stem cells; ADSCs, adipose tissue-derived mesenchymal stem cells; UC-MSCs, umbilical cord-derived mesenchymal stem cells; RGC, retinal ganglion cell; OHT, ocular hypertension; TLR4, Toll-like receptor 4; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; Treg, regulatory T cell; Th1/Th17, T helper cell subsets; miR-100-5p, microRNA-100-5p; EAU, experimental autoimmune uveitis.

Limitations of Adult MSCs for EV Production

Proliferation of MSCs is a valuable characteristic for regenerative medicine and cellular therapy. Adult tissue-derived MSCs can be derived from adipose tissue (AD-MSCs) and BMSCs. AD-MSCs have a doubling time of 45.2 hours, while BMSCs have a doubling time of 61.2 hours, according to Peng et al. (Peng et al. 2008). Similarly, Kern et al. noted that AD-MSCs could be cultured to passage 8 without signs of senescence, whereas BMSCs underwent senescence from passage 7 (Kern et al. 2006). Furthermore, the anatomical origin affects AD-MSC proliferation: those derived from subcutaneous fat grew faster (doubling time 4 ± 1 days) compared with those derived from omental fat (5 ± 1 days) (Van Harmelen et al. 2004). On the contrary, neonatal or birth-tissue-derived MSCs—i.e., UC-MSCs and PL-MSCs—are consistently reported to have higher proliferative potential compared to adults. UC-MSCs exhibit shorter and more consistent population doubling times with average doubling times of 24 hours up to passage 10, while BM-MSCs were at a mean of approximately 40-60 hours and had elevated doubling times beyond passage 6 (Lu et al. 2006; Peng et al. 2008). Moreover, UC-MSCs kept on proliferating through multilayering, unlike BM-MSCs, which showed contact inhibition (Baksh et al. 2007). MSCs derived from the placenta also showed greater expansion and engraftment potential when compared with BM-MSCs (In 't Anker et al. 2004; Barlow et al. 2008; Brooke et al. 2008). These neonatal MSCs show very little indication of senescence even after extended culture; for example, UC-MSCs showed morphology and proliferative capacity for as many as 16 passages without any senescence (Conconi et al. 2006).

MSCs derived from adult tissues such as bone marrow and adipose tissue produce EVs, but with lower yield and batch consistency compared to neonatal sources (Liu et al. 2024). Although BM-MSC-derived EVs have shown therapeutic effects in osteoarthritis and renal injury models, they have not yet shown the same results in humans (Bruno et al. 2017; Vonk et al. 2018; Matsukura et al. 2019), but production is less effective than from perinatal sources. Human umbilical cord MSCs (hUC-MSCs) produced a higher proportion of EVs compared to BM-MSCs, according to analysis of EV production (Ragni et al. 2017). AD-MSCs are typically isolated from adipose tissue of healthy adult donors (Pascucci et al. 2014), and, even though the source is less limiting than bone marrow, yield and consistency of EV secretion are still variable. Studies showed that although AD-MSC-derived EVs possess beneficial properties such as anti-inflammatory and tissue repair-

promoting activity (Tofiño-Vian et al. 2017; Tofiño-Vian et al. 2018), cellular uptake efficiency and therapeutic efficacy could be variable compared to EVs from BM-MSCs due to not fully understood reasons (Gebraad et al. 2018). These inconsistencies, together with lower EV productivity, point toward the limited capabilities of adult MSCs as efficient EV producers for therapeutic purposes at large scales. In contrast, MSCs isolated from the human umbilical cord offer higher yields and more reproducible EV production, indicating a relative lack of adult MSC-derived EV production (Ragni et al. 2017). A visual comparison of EV production efficiency across different MSC sources is shown in **Figure 3**.

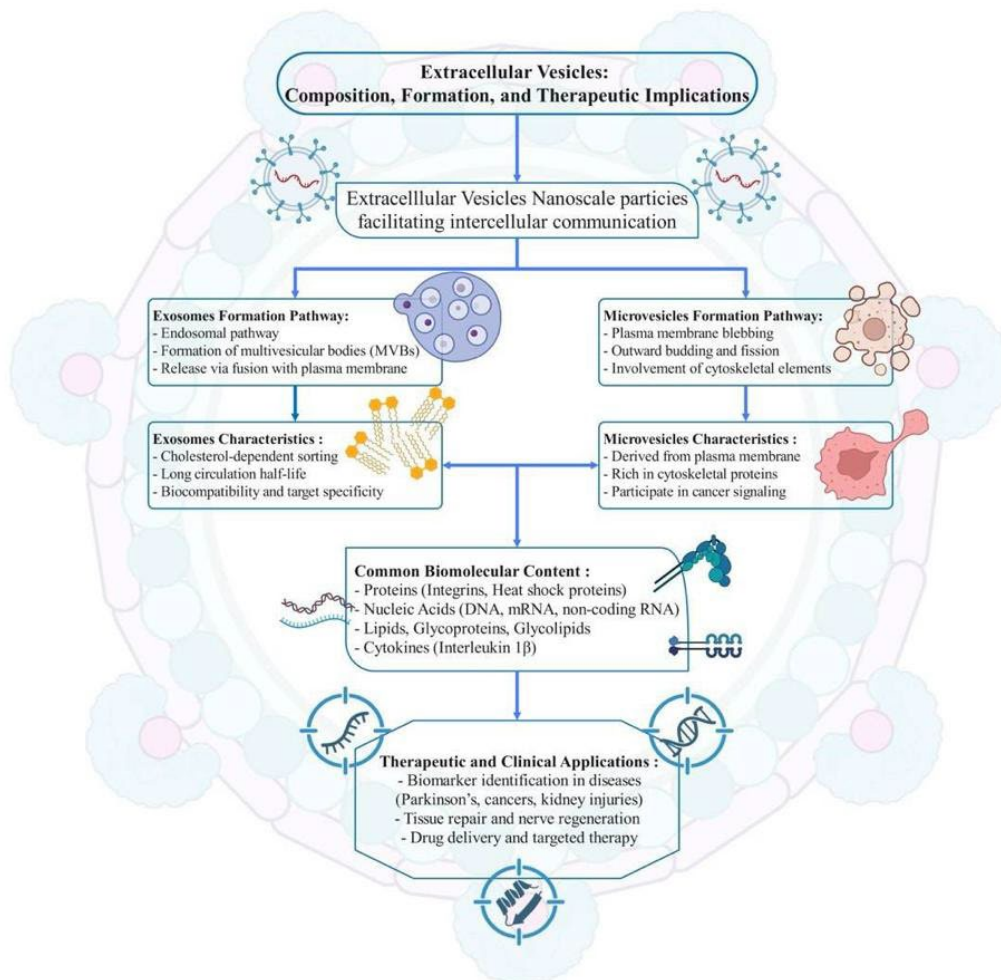


Figure 3. Schematic illustration of the biogenesis, molecular cargo, and functions of extracellular vesicles (EVs). Exosomes and microvesicles arise via distinct pathways—endosomal maturation and plasma membrane budding,

respectively. Both EV types carry biomolecules such as proteins, RNAs, and lipids, and are involved in intercellular communication, tissue regeneration, immune modulation, and drug delivery. Their size range and key molecular features are summarized.

iPSC-Derived MSCs for Scalable, Standardized EV Production

Pluripotent stem cells and iPSCs are highly promising solutions to the enormous challenges of mass production and quality variations that generally plague traditional MSCs. Single-donor MSCs can only be harvested in limited numbers, and longer culture periods negatively impact proliferating and differentiating MSCs, causing inconsistent therapeutic effects (Nejati-Koshki et al. 2017; Pilehvar-Soltanahmadi et al. 2017; Saei Arezoumand et al. 2017). iPSCs, on the other hand, offer an unlimited source of cells that can be routinely differentiated into MSCs in controlled environments, thus enabling the large-scale and reproducible generation of EVs from MSCs. Importantly, EVs derived from iPSCs can be harvested repeatedly from immortalized cell lines, thus avoiding the limitations of low yield and inter-batch variation generally experienced with EVs derived from primary MSCs (Collino et al. 2020).

Aside from their scalability, MSCs derived from iPSCs address the quality heterogeneity issue frequently faced in the generation of MSC-EVs from primary tissues very effectively. The therapeutic activity and biochemical constitution of MSC-EVs are known to vary depending on various parameters such as tissue source, donor characteristics, and culture conditions (Yin et al. 2020; Zhang et al. 2020). In contrast, iPSCs can be directed by well-characterized differentiation methods to generate MSCs, which have more consistent phenotypic and functional characteristics, leading to EVs of standardized bioactive contents. For example, EVs obtained from iPSC-derived cardiac progenitor cells supplemented with miR-373 presented consistent anti-fibrotic and pro-regenerative effects in models of ischemia-induced heart disease (Wei et al. 2015). Similarly, iPSC-derived EVs presented enhanced mitochondrial protective effects and anti-inflammatory activities in models of acute kidney injury when compared with those from adipose tissue-derived MSCs, highlighting their improved and consistent therapeutic potential (Collino et al. 2020). Thus, the use of iPSC-derived MSCs represents an important advancement toward the generation of high-quality, standardized MSC-EVs for clinical use.

The use of iPSCs to produce EVs on a large scale offers numerous advantages, especially for clinical use and industrial manufacturing. The intrinsic ability of iPSCs to undergo indefinite propagation allows scalable EV production, which is an important factor since therapeutic use frequently requires large amounts of EVs; for instance, in vivo delivery might require 50 to 500 µg of EV protein/mouse (Riazifar et al. 2017). This scalability problem is overcome by the use of bioreactor systems, e.g., hollow fiber or 3D-printed scaffold perfusion reactors, which have shown considerable productivity improvements compared to other traditional culture methods (Watson et al. 2016; Watson et al. 2018; Patel et al. 2019). In addition, undifferentiated iPSCs have also been found to produce about 2200 EVs/cell/hour, a productivity rate about 16 times higher than that of different types of MSCs in a defined medium (Zhou et al. 2016; Liu et al. 2019), thus emphasizing their value for high-yield EV production.

Along with increasing yield, iPSC-derived MSCs enable the minimization of quality heterogeneity that is normally inherent with EVs produced from conventional MSC sources. EV cargo composition variability with adult MSC-derived EVs can be affected by donor age, passage number, and culture conditions and could in turn impact the predictability of therapeutic responses (Patel et al. 2017; Wang et al. 2018). In contrast, iMSCs, which are derived from a standardized iPSC line and differentiated in controlled and reproducible environments, demonstrate more homogeneous EV profiles. Comparative studies have shown that EVs from iMSCs show stronger enhancement of endothelial cell migration, angiogenesis, and extracellular matrix (ECM) deposition in comparison with EVs from synovial membrane MSCs, thereby reflecting a greater and more consistent therapeutic potential (Zhang et al. 2015; Zhu et al. 2017). In addition, proteomic characterizations demonstrate unique and targeted cargoes in iMSC-EVs, including proteins involved in stromal modulation and ECM organization, thus highlighting their regulated and specialized functional capabilities (La Greca et al. 2018).

Conclusion and Future Directions

In recent years, there has been a significant increase in research focused on the therapeutic effects of MSC-EVs for the management of various eye diseases. MSC-EVs also serve as excellent nanocarriers for drug delivery due to their remarkable biocompatibility and bilayer lipid membrane structure. As research continues to demonstrate their therapeutic potential, with advances in engineering, scalable production, and targeted delivery, MSC-EVs—particularly those from iPSC-

derived MSCs—are poised to become a cornerstone of future ophthalmic regenerative therapies (Yu et al. 2020; Liu et al. 2022).

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Conflicts of Interest

The authors declare no conflicts of interest related to this manuscript.

Ethics Approval

Not applicable.

Consent to Participate

Not applicable.

Consent for Publication

All authors consent to the publication of this manuscript.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

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