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Cell Free Regenerative Extracellular Vesicle Therapy for Ocular Diseases

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Abstract

Purpose: To assess the therapeutic potential of extracellular vesicles (EVs) derived from stem cells and ocular tissues as a cell-free alternative to traditional stem cell therapies for a broad spectrum of ocular diseases.

Methods: A comprehensive literature review was performed, focusing on preclinical studies involving EVs derived from mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs), neural progenitor cells, immune cells, and ocular-resident cells. Data were extracted on EV cellular origin, isolation methods, routes of administration, preclinical disease models, therapeutic outcomes, and proposed mechanisms of action. Registered clinical trials were also evaluated.

Results: EVs exhibited regenerative and immunomodulatory effects across a range of ocular conditions, including dry eye, uveitis, glaucoma, retinal degenerations, and optic neuropathies. Various cell sources have been explored for EV production, including MSCs, iPSCs, hESCs, retinal organoids, and other ocular tissue-resident cells. In addition, bioengineered EVs have been developed to modify surface properties or enhance therapeutic cargo. Reported mechanisms of action include miRNA-mediated gene regulation, immune modulation, and oxidative stress reduction. Several early-phase clinical trials are currently underway to translate these findings into human therapies.

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Conclusion: Stem cell-derived EVs represent a promising next-generation, cell-free regenerative therapy for ocular diseases. While preclinical data are promising, successful clinical translation will require optimal EV source selection, scalable and GMP-compliant production, identification of disease-relevant mechanisms of action, rigorous cargo characterization, and alignment with regulatory standards.

Keywords

Extracellular vesicles; ocular therapy; regenerative medicine

Introduction

Over the past few decades, cell-based therapies have been explored as a treatment for a wide range of diseases, including degenerative conditions, immune disorders, and injuries.[1] The foundation of cell therapy lies in the transplantation or administration of live cells, such as pluripotent stem cells or fully differentiated cells derived from them, to repair or regenerate damaged tissues.[2] In ophthalmology, stem cell-based therapies have been actively studied as potential treatments for retinal degenerations, glaucoma, and corneal diseases.[3–9] Vision research has been at the forefront of cell-based therapies by leveraging the eye's small size, immune-privileged environment, robust blood-ocular barriers, and the accessibility for local delivery.[10] While cell therapies in vision disorders have demonstrated potential in preclinical and ongoing clinical trials, they have also presented significant challenges. Key lessons from these studies by far include challenges such as limited functional integration (e.g. lack of synaptic formation) of the implanted cells, immune rejection, tumorigenicity, and complications related to the delivery and engraftment of live cells, all of which underscore the need for safer and more controllable alternatives.[3,4,11–17] Recent insights have revealed that cell secreted molecules along with their paracrine and autocrine actions play a significant role in the mechanism of action in cell therapy, sparking interest in the potential applications of extracellular vesicles (EVs) in regenerative medicine.

EVs represent heterogenous nano-sized vesicles, which include exosomes, microvesicles, and other vesicular structures, are released by virtually all cell types and carry a diverse array of bioactive molecules, including proteins, lipids, and nucleic acids.[18]

EVs play key roles in cellular processes such as signaling, immune modulation, and tissue repair, positioning them as promising next-generation diagnostics, therapeutics, and mediators of both physiological and pathological processes. Accordingly, there has been a surge in EV-related studies and publications.[19] Reflecting this growing interest, the National Eye Institute (NEI) recently hosted a dedicated workshop to identify current gaps and outline future directions for EV research in ocular diseases.[20]

Stem cell-derived EV therapies, in particular, offer several advantages over conventional cell therapies, including a lower risk of immune rejection, easier storage and handling, and the ability to cross biological barriers such as the blood-retinal barrier.[20] These therapies hold significant promise for advancing regenerative medicine by introducing cell-free EV-based approaches to treat various ocular diseases. Leveraging their bioactive molecular cargo, EVs can target ocular cells and tissues while addressing challenges

associated with direct cell therapy.[20] A key advantage of stem cell-derived EVs lies in their native cargo, which comprises a diverse array of molecules that interact effectively with the complex microenvironments of ocular tissues. Moreover, compared to cell-based therapies, which often requires invasive surgical procedures, EV therapies offer the benefit of minimally invasive local treatments. This makes them particularly suitable for early-stage ocular diseases, providing consistent therapeutic potency over time without the risk of aging or degradation commonly observed in implanted stem cells within hostile disease microenvironments. Additionally, stem cell-derived EVs can be engineered to further enhance their therapeutic potential, enabling precise targeting of specific tissues or the delivery of tailored therapeutic cargos, such as proteins or microRNAs.

Nonetheless, important unmet needs must be addressed to successfully translate cell-free EV-based therapeutics into clinical applications. Careful attention must be paid to the cellular source of EVs, such as pluripotent stem cells, induced pluripotent stem cells, differentiated stem cells or immune cells, and the conditions under which they are cultured, as these factors significantly impact the composition and therapeutic potential of the vesicles.[21] Furthermore, elucidating their mechanisms of action and developing new strategies to evaluate their preclinical efficacy are essential for developing EV-based therapies.

The primary goal of this review is to highlight the current preclinical studies in various ocular disease models including those affecting the conjunctiva, cornea, glaucoma, uveitis, optic nerve, and retina, with a particular focus on EV cell source, efficacy measures and proposed mechanisms action (Figure 1). Additionally, the review examines registered EV clinical trials that merit attention.

Cell sources and preclinical applications of EV-based therapies in ocular diseases

EVs are posited to exert their actions *via* three crucial steps - (i) establishment of the intercellular communication by binding to the surface receptor of the target cells (ii) transfer of cargo to target cells through endocytosis, membrane fusion or receptor-ligand interactions and (iii) regulating signaling pathways that physiological functions such as immune modulation, stem cell maintenance, tissue repair and regeneration.

The successful therapeutic application of EVs depends on selecting an appropriate cellular source, employing an effective isolation strategy and optimizing EV delivery to target cells and tissues, including determining the optimal route of administration and ensuring vesicle stability. Here we focus on reviewing cell secreted EVs treatment for various ocular diseases, in which EVs were isolated from cell culture conditioned medium (CCM).

EVs derived from mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), and embryogenic stem cells (ESCs) have been the most extensively studied while a smaller body of literature has explored EVs derived from specialized cell types such as retinal ganglion cells (RGCs), neural progenitor cells (NPCs), retinal pigment epithelial (RPE) cells, and other ocular tissue resident cells. Tables 1 and 2 provide an overview of the current literature on both *in vitro* and *in vivo* applications of EVs in ocular disease respectively.

Below, we review the key findings from these studies, organized by the cellular source of the EVs. It is well-established that EV isolation methodologies influence their composition and purity.[61] While we do not discuss the pros and cons of each strategy, we summarize the isolation methods used in each study, along with the route of EV administration, measures of efficacy, and proposed mechanisms of action as these are critical components in evaluating prior research. Finally, we examine these same factors in the context of developing bioengineered EV therapeutics.

Mesenchymal stem cells (MSC) derived EVs: Mesenchymal stem cells (MSCs), found in tissues such as bone marrow, adipose tissue, and the umbilical cord, have gained significant attention in regenerative medicine due to their ability to integrate into host tissues, their paracrine and immunomodulatory properties, and their abundant production of EVs. MSC-derived EVs have shown therapeutic potential in treating various ocular conditions. Each MSC source presents distinct advantages and challenges that affect their suitability for EV-based therapies. Bone marrow-derived MSCs (BM-MSCs) are among the most well-researched and established, with established immunomodulatory properties that make them effective for autoimmune diseases.[62–66] However, harvesting BM-MSCs is relatively invasive, painful, and yields are often low, requiring multiple aspirations. Additionally, the quality and functionality of BM-MSCs can decline with age, limiting their therapeutic potential. Umbilical cord-derived MSCs (UC-MSCs) are harvested non-invasively, pose no risk to donors, and have lower immunogenicity, reducing rejection risks in allogeneic applications. They also have strong proliferative capacity, enabling significant *in vitro* expansion. However, UC-MSCs are dependent on cord banks, which may be limited by ethical concerns. Variability in processing can affect their quality and functionality, and there is relatively limited clinical data compared to other MSC sources. Adipose-derived MSCs (AD-MSCs) can be obtained through minimally invasive liposuction, offering higher yields and easier expansion. These cells are versatile, with the ability to differentiate into multiple cell types and have also been shown to possess significant immunomodulatory properties rivaling or even exceeding those of BM-MSCs.[66] However, the quality and quantity of AD-MSCs are influenced by the anatomical source of adipose tissue, can vary between individuals, and the isolation process may be complicated by the presence of other cell types.[67–70] Moreover, clinical data for AD-MSCs remain limited compared to BM-MSCs.[66]

BM-MSC derived EVs: In *Mathew* et al.'s 2019 report, human BM-MSC derived EVs (BM-MSC-EVs) were isolated using System Biosciences' proprietary Exo Quick-TC EV Precipitation Solution.[31] When cocultured with R28 retinal cells in their *in vitro* model of retinal ischemia, EVs reduced cellular death and increased proliferation. When intravitreally administered in their rat retinal-ischemia reperfusion injury model, EVs significantly improved the recovery of the a and b-wave amplitudes of the ERG in comparison to both PBS vehicle and EV-depleted conditioned medium from MSCs. The authors also observed reduced apoptosis in retinal ganglion cells and outer retinal layers as well as decreased neuroinflammatory markers, TNF-α and IL-6, in retinal homogenate, leading the authors to conclude that the functional improvement was achieved *via* reduction of apoptosis and neuroinflammation.

Lending credence to the immunomodulatory properties of BM-MSC-EVs is *Shigemota-Kuroda* et al. *'s* 2017 study in a murine experimental autoimmune uveoretinitis (EAU) model.[53] Here, human BM-MSC-EVs were isolated using an anion exchange resin column. Systemic administration of these EVs prevented disruption of the retinal architecture and reduced inflammatory cell infiltration into the retina at 21-days post EAU induction. Accordingly, transcript levels of pro-inflammatory cytokines, interferon gamma (IFN-γ), interleukin (IL)-17A, IL-2, IL-1β, IL-6, and IL-12A were reduced in mouse eyes following systemic administration. The same group also evaluated the effects of BM-MSC-EVs in a mouse model of ocular Sjögren's syndrome. One week after injection into the lacrimal glands, EV administration improved corneal epithelial integrity and reduced T cell infiltration into the lacrimal glands.[46] There was also a significant decrease in the expression of inflammatory cytokines, including TNF-α, IL-1β, and IFN-γ, in both the ocular surface and lacrimal glands, supporting the anti-inflammatory properties of BM-MSC-EVs. Topical administration of BM-MSC derived exosomes (BM-MSC-exo) was also effective in treating two murine models of ocular surface disease: Benzalkonium chloride (BAK) induced keratopathy and the ocular graft-versus-host disease (oGVHD).[48] Topical EV administration preserved corneal epithelial integrity, reduced apoptosis, and reduced corneal infiltration of CD11b + macrophages with down-regulation of proinflammatory genes including *Il-6*, *Il-1*β, *Il-17a*, and *Cd86* in the cornea and conjunctiva. BM-MSC-exo also reprogrammed pro-inflammatory M1 macrophages to the anti-inflammatory M2 phenotype. Knockdown of microRNA (miR) −204 in the parent BM-MSC abrogated the anti-inflammatory and antixerophthalmic effects of the exosomes indicating that microRNAs play a significant role in their therapeutic action, which is supported by other studies mentioned below. Complementary work by *Tati* et al. demonstrated that BM-MSC-EVs enhanced epithelial cell proliferation, promoted wound closure, and reduced apoptosis in a corneal scratch assay, further supporting their potential in ocular surface repair.[25]

Outside of inflammatory and ischemic ocular disease, BM-MSC-EVs have shown promise in the DBA/2J mouse model of glaucoma.[50] Intravitreal injection of human BM-MSC-EVs isolated *via* a polyethylene glycol-based enrichment method improved retinal ganglion cell (RGC) survival compared to sham or fibroblast derived EV treated mice. Functionally, scotopic ERG amplitudes were also improved by BM-MSC-EV treatment. This same group found similar results in two separate rat models of glaucoma.[28] Interestingly, knockdown of Argonaute2 (AGO2), a protein essential for miRNA maturation in BM-MSCs, reduced the neuroprotective effects of BM-MSC-EVs. These findings were mirrored in a rat optic nerve crush (ONC) injury model, suggesting a critical role for miRNA in EVs therapeutic actions.[30] Intravitreal injection of BM-MSC-EVs, isolated *via* ultracentrifugation, promoted RGC survival following ONC injury compared to sham or fibroblast derived EV treated rats. They also promoted RGC survival as well as neurogenesis in *in vitro* cultures in a dose-dependent manner.

UC-MSC derived EVs: UC-MSC derived EVs (UC-MSC-EVs) have demonstrated therapeutic potential in preclinical studies. Two independent studies by *Guo* et al. and *Wang* et al. demonstrated that topical administration of UC-MSC-EVs improved tear production and ocular surface integrity in a murine model of dry eye disease induced

by scopolamine and a desiccating environments.[42,43] Both studies also highlighted the anti-inflammatory effects of UC-MSC-EVs. In a separate dry eye disease model involving rabbit autoimmune-induced dacryoadenitis, subconjunctival injection of UC-MSC-EVs alleviated disease severity. Consistent with the earlier findings, both *in vitro* and *in vivo* data from this study further confirmed the anti-inflammatory properties of UC-MSC-EVs.[47]

Li et al. *'s* 2019 report showed that UC-MSC-EVs isolated *via* ultracentrifugation inhibited RPE cell migration by targeting the epithelial mesenchymal transition (EMT).[33] Furthermore, the authors found that intravitreal injection of UM-MSC-EVs ameliorated subretinal fibrosis in an *in vivo* murine laser-induced choroidal neovascularization (CNV) model. Based on the *in vitro* data, the authors suggested that these effects were due to miR-27b modulating HOXC6 expression.

UC-MSC-EVs also possess significant immunomodulatory properties. Mirroring the findings of *Shigemota-Kuroda* et al.,[67] Bai et al. found that periocular injection of UC-MSC-EVs isolated *via* ultracentrifugation significantly reduced intraocular inflammation in a rat EAU model compared to fibroblast derived EVs injection.[54] Histological analysis showed reduced retinal infiltration of macrophages and $CD4^+IFN\text{-}\gamma^+$ and $CD4^+IL\text{-}17^+$ T-cells. Functionally, periocular administration of UC-MSC-EVs rescued ERG a and b-wave amplitudes following EAU induction. Outside of EAU, another study by *Zhang* et al. found that intravitreal injection of UC-MSC-EVs in the *rd10* murine retinal degeneration model enhanced photoreceptor survival and visual function while attenuating intraocular inflammation, with concordant *in vitro* data.[35]

AD-MSC derived EVs: A smaller body of literature exists on AD-MSC derived EVs (AD-MSC-EVs). As with UC-MSC-EVs, topical administration of AD-MSC-EVs resulted in improvement in two separate models of mouse dry eye disease. *In vitro* and *in vivo* data from both studies indicated that this effect was in part mediated by inhibition of the NLPR3 inflammasome.[22,44] AD-MSCs-EVs have also demonstrated promise in several preclinical models of diabetic retinopathy.[39,60] Subconjunctival and intravitreal administration of AD-MSC-EVs, but not intravenous, preserved retinal architecture following STZ-induced diabetic retinopathy in rabbits.[60] Similarly, *Gu* et al. *(2020)* found that intravitreal administration of AD-MSC-EVs, isolated *via* ultracentrifugation, reduced expression of intraocular inflammatory cytokines in a dose-dependent manner in STZ-induced diabetic retinopathy model in rats.[39] AD-MSC-EVs also inhibited retinal angiogenesis, macrophage infiltration, and inflammatory Müller cell activation while preventing RGC loss following STZ induction through a mir-192 dependent mechanisms, and this was also supported by their *in vitro* results in Müller cell and human retinal microvascular endothelial cell (HRMECs) cultures.

AD-MSC-EVs are also capable of mitigating damage from oxidative stress. *Hwang* et al. (2024) isolated AD-MSC-EVs using a tangential flow filtration system.[36] Pretreatment of a human RPE cell line with these EVs prevented apoptosis and reduced intracellular reactive oxygen species (ROS) following H_2O_2 treatment. Intravitreal injection of AD-MSC-EVs also preserved outer retinal architecture and functionally preserved ERG a and b-wave amplitudes in the Royal College of Surgeons (RCS) rats.

EVs from pluripotent stem cell derived MSC: MSCs differentiated from pluripotent stem cells could potentially overcome the limitations associated with somatically derived MSCs, such as the need for low passage numbers, which restrict scalability, and the variability in donor and cell source characteristics. Embryonic-stem cell derived MSCs EVs (ESC-MSC-EVs), isolated *via* ultracentrifugation, have been shown to preserve visual function and RGC number following optic nerve crush in mice.[51]

Induced pluripotent stem cells (iPSCs) derived EVs: iPSCs, first characterized in 2006 by *Yamanaka* et al. were classically generated by transfecting and overexpressing four transcription factors in somatic cells, Octamer-binding transcription factor 4 (Oct4), SRY-Box Transcription Factor 2 (Sox2), Kruppel-like factor-4 (Klf4), and cellular Myelocytomatosis oncogene (c-Myc).[71] However, more recent work has found that pluripotency can be induced by expression of other transcription factors, genes, or even small molecules. Because of their pluripotency, self-renewing capacity, and their freedom from the ethical constraints of sourcing embryonic stem-cells (ESCs), iPSCs have revolutionized the field of regenerative medicine. In ophthalmology, iPSCs offer an attractive solution in treating retinal disease characterized by significant cellular loss.[72]iPSC derived EVs have shown promise in models of ocular degenerative diseases. Interestingly, most studies have characterized the effects of EVs derived from iPSCs differentiated into the more restricted cellular lineages. *Han* et al. (2023) found that intravitreal administration of EVs secreted from human iPSC derived retinal organoids (hiPSC-RO-EVs), isolated utilizing Qiagen's proprietary miRCURY exosome isolation kit, reduced photoreceptor apoptosis, prevented outer nuclear layer thinning, and preserved visual function in RCS rats.[58] Scotopic electroretinogram (ERG) and optomotor response (OMR) testing revealed that intravitreal injection of hiPSC-RO-EVs preserved retinal function in RCS rats. From RNA sequencing analysis, the authors postulated that the hiPSC-RO-EVs may have achieved these effects by targeting the MAPK pathway.

Another study utilizing hiPSC-RO-EVs from *Lee* et al. *(2023)*, found that topical administration of these EVs in a murine model of corneal epithelial wounds led to significantly enhanced rates of wound closure 24h and 36h post-injury compared to control.[49] In contrast to *Han* et al. *'s* study, these EVs were isolated by ultracentrifugation. Furthermore, transcriptome analysis demonstrated that hiPSC-RO-EVs treated corneas had significantly decreased expression of TNF-α, CCL2, and CCL5 suggesting anti-inflammatory property of hiPSC-RO-EVs. Genes related to retinoic acid and eicosanoid metabolism were also upregulated, which have been implicated in wound healing.

Several studies have characterized the effects of EVs derived from iPSCs differentiated into tissue specific stem cells, including MSCs and neural progenitor cells (NPCs).[52,73] *Li* et al. (2025) isolated EVs from hiPSCs (hiPSC-EVs) and hiPSCs-differentiated neural progenitor cells (hiPSC-NPC-EVs) using ultracentrifugation.[52] Intravitreal injection of hiPSC-NPC-EVs, but not undifferentiated hiPSC-EVs, in their murine *in vivo* ONC injury model significantly mitigated retinal ganglion cell degeneration at days 7 and 14 following ONC and reduced inflammatory microglial cell migration at day 7 post-ONC. Functionally, intravitreal administration of hiPSC-NPC-EVs preserved flash visual-evoked potential N1-P1 wave amplitudes day 7 post-crush.

Embryonic stem cells (ESC) derived EVs: Few studies have explored the use of ESC-derived EVs in the treatment of ocular diseases. *Gao* et al. (2023) examined EVs from embryonic stem cell-derived retinal organoids (ESC-RO-EVs) as a potential treatment for retinal degeneration.[37] ESC-RO-EVs inhibited lipotoxicity and oxidative stress, and enhanced phagocytosis and survival of an oleic acid-treated RPE cell line (ARPE-19). Another study by *Liu* et al. found that ESC-derived EVs (ESC-EVs) reversed RPE cell senescence in an *in vitro* model.[74] Specifically, ESC-EVs enhanced proliferation of RPE cells, reduced the senescence-associated galactosidase (SA-β-gal) staining rate, and the levels of mitochondrial membrane potential (MMP) and ROS by inhibiting the p38MAPK pathway.

Neural progenitor cells derived EVs: Intraocular NPC transplantation has been shown to protect against retinal degeneration in preclinical and clinical models. We previously mentioned that EVs derived from iPSCs-differentiated NPCs ameliorated RGC loss in an optic nerve crush injury model.[52] Subretinal injection of primary NPC-derived EVs (NPC-EVs) were also found to prevent photoreceptor degeneration, preserved visual function, decreased thinning of the outer nuclear layer (ONL), and decreased apoptosis of photoreceptors in RCS rats.[38] EV treatment also inhibited microglial migration into the outer nuclear layer and subretinal space while inhibiting microglial cell activation and reducing photoreceptor cell death in a co-culture model, likely due to their anti-inflammatory properties.

Ocular tissue derived EVs: More recently, investigators have also explored the therapeutic potential of EVs derived from ocular tissue–resident cells, aiming to harness their intrinsic paracrine functions in a tissue-specific context. *Sameekia* et al. successfully isolated MSCs from human cadaveric corneas, and demonstrated that EVs from these cells significantly enhanced wound healing in both an *in vitro* corneal epithelial scratch assay and an *in vivo* murine model of corneal epithelial debridement.[26] Interestingly, *Tati* et al. found that EVs from corneal epithelial cells were even more effective than BM-MSC-EVs in enhancing corneal epithelial repair and exhibited superior anti-apoptotic and anti-inflammatory activity *in vitro*.[27]

Beyond the treatment of ocular surface disease, Iswarya et al. showed that EVs from trabecular meshwork (TM) stem cells accelerated TM cell wound healing in an *in vitro* scratch assay and conferred protection against oxidative stress, highlighting their potential for therapeutic use in glaucoma.[29] Other studies have investigated the role of EVs derived from retinal astroglial cells (RAC) and retinal pigment epithelial (RPE) cell in ocular disease. *Hajrasouliha* et al. (2013) isolated EVs from murine RAC and RPE cell cultures using ultracentrifugation.[34] Periocular administration of RAC-derived EVs (RAC-EVs) inhibited choroidal neovascularization (CNV) in mouse laser-induced CNV model and reduced macrophage migration whereas RPE-derived EVs (RPE-EVs) did not. Accordingly, *in vitro* chemotaxis assays also showed RAC-EVs' ability to inhibit macrophage migration and prevent tubule forming in mouse retinal microvascular endothelial cells. While this study did not find therapeutic benefit of RPE-EVs in treating CNV, another study found that EVs from ARPE-19 cells inhibited cell growth, migration, and tube formation in

human umbilical vein endothelial cells (HUVECs) under high-glucose conditions.[40] The authors further postulated that EV miR-202–5p targeted TGFβR2, inhibiting endothelial-mesenchymal transition in HUVECs which has been implicated in proliferative diabetic retinopathy associated pathologic fibrosis. Finally, a recent study by *Pollalis* et al. (2024) investigated intravitreal injection of EVs from human ESC-derived RPE cells (hESC-RPE-EVs) in the RCS rats. hESC-RPE EVs restored photoreceptor function, as demonstrated by the preservation of scotopic ERG a- and b-wave amplitudes, preserved outer retinal structure on OCT imaging, and increased RPE engulfment of photoreceptor outer segments, suggesting a direct effect on RPE cells.[59]

Immune cells EVs: Other studies have sought to harness the immunomodulatory potential of immune cell-derived EVs. *Kang* et al. (2020) isolated EVs from *ex-vivo*-generated IL-35-producing regulatory B-cells (i35-B-regs-EVs). Retro-orbital injection of these EVs mitigated disease severity in a mouse model of EAU, showing a significant reduction of Th17 cells in eyes of mice treated with i35-B-regs-EVs.[55] This same group also found retro-orbital administration of EVs from IL-27-producing regulatory B-cells (i27-B-reg-EVs) also ameliorated uveitis in EAU mice.[56] Topical administration of EVs from M2-macrophages reduced disease severity in a murine BAK-induced dry eye disease model and decreased ocular surface pro-inflammatory cytokine levels.[45]

Bioengineered EVs: EV bioengineering strategies include surface modification, intraluminal or extraluminal cargo loading, and various forms of cell engineering to modify and enhance EVs. Efforts to utilize EVs as delivery systems for drugs, genes, or vaccines are also actively underway. While a comprehensive review of these strategies is beyond the scope of this review, we will highlight selected bioengineering approaches applied to stem cell-derived EVs to potentially enhance therapeutic efficacy for ocular diseases.

Topical administration of murine BM-MSC-derived modified with cerium oxide nanocrystals (MSC-EVs-Ce) enhanced tear production and reduced fluorescein staining in a mouse model of dry eye disease. These MSCExo-Ce also exhibited antioxidant properties both *in vitro* and *in vivo*, and significantly promoted corneal epithelial wound healing in an *in vitro* scratch assay.[23] Similarly, murine BM-MSC EVs conjugated with ascorbic acid improved clinical features of dry eye diseases in mice and demonstrated antioxidant properties and accelerated corneal epithelial wound healing *in vitro.*[24] *Jiao* et al. overexpressed splicing factor proline/glutamine-rich (SFPQ) in human iPSCs and isolated the EVs (hiPSC-SFPQ-EVs) by ultracentrifugation.[32] When hiPSC-SFPQ-EVs were co-cultured with human Müller cells in an *in vitro* model of hypoxic injury, they reduced Müller cell apoptosis and promoted cell proliferation. In their *in vivo* rat retinal ischemia/reperfusion injury model, intravitreal administration of hiPSC-SFPQ-EVs significantly decreased retinal cell apoptosis and rescued scotopic a-wave and b-wave attenuation on ERG.

Bioengineered UC-MSCs derived EVs have shown promise in several inflammatory retinal diseases. A single intravenous injection of IL-10-overexpressing UC-MSC-EVs in EAU mice ameliorated disease by suppressing T-cell proliferation and promoting their differentiation into Treg cells.[57] Another study demonstrated that intravitreal injection of

miR-126-overexpressing UC-MSC derived EVs (USC-MSC-EVs) reduced inflammatory factor expression and retinal blood vessel leakage in a streptozotocin (STZ)-induced diabetic rat model, outperforming fibroblast-derived EVs or native UC-MSC-EVs.[41] *In vitro* tests on human retinal vascular endothelial cells showed that EVs overexpressed miR-126 inhibited HMGB1 signaling pathway. In both studies, EVs were isolated by ultracentrifugation.

EVs can also be bioengineered to target specific tissues or cells. EVs modified with Arginylglycylaspartic acid (RGD) on their surface have demonstrated active targeting properties toward CNV.[75] While detailed strategies for EV cargo loading or other modifications are beyond the scope of this review, it is worth noting that stem cell-derived EVs can also be modified by a wide range of bioengineering approaches. Efforts are also underway to use immortalized cell lines as EV sources, which offer the advantage of scalable production.

Clinical trials for ocular diseases using EVs

Clinical trials involving EVs for vision disorders are still in their nascent stages. Since 2017, several Phase I/II clinical trials focusing on EV-based therapies for ophthalmic diseases have been registered on ClinicalTrials.gov (Table 3). These trials include three trials for patients with Dry Eye Disease (ID # NCT04213248, NCT065543667, NCT05738629), one for patients with Retinitis Pigmentosa (ID # NCT05413148), and one for patient with refractory macular holes (ID # NCT03437759). A number of phase I/II trials are evaluating the efficacy of cell therapies using transplanted MSC-, iPSC-, and ESC-derived retinal pigment epithelium (RPE) and photoreceptors for diseases such as age-related macular degeneration (AMD), Stargardt's disease, and retinitis pigmentosa (RP),[8,70,76–82] suggesting that EVs from these cells may hold therapeutic potential encouraging the investigation of extracellular vesicles (EVs) derived from these cells as potential therapeutic agents..

Discussion and future perspectives

While stem cell derived EVs hold tremendous potential for the next generation of cell free regenerative therapies, this emerging paradigm toward multimolecular biologics also highlights several gaps in our current knowledge. Identification of cellular source and optimization of culture conditions for ideal EV production is one of the most essential steps in achieving consistent and effective cell-free therapies. Without this foundation, therapeutic efficacy remains variable and difficult to optimize. Equally important is elucidating the mechanisms by which EVs exert their effects whether through immunomodulation, tissue repair, or other biological processes. Given that EV therapy may act through multiple pathways, understanding its primary or target mechanisms is essential not only for matching EVs with specific diseases, but also for guiding the selection of cell sources, optimizing culture conditions, and refining upstream EV production to enhance their therapeutic efficacy for various ocular diseases. Beyond the mechanisms of action, other factors, such as accessibility of cell source, scalability of cell expansion and EV production, batch-to-batch consistency, and long-term safety data play critical roles in determining the suitability of EV-based therapies.

For example, MSCs have the longest track record in pre-clinical and clinical cell therapies, and MSC-derived EVs benefit from an established safety profile, particularly for anterior segment and anti-inflammatory applications.[83–86] One of the key challenges associated with MSCs is their considerable batch-to-batch variability, which can potentially lead to inconsistent EV cargo and therapeutic outcomes.[87–89] Additionally, their long-term safety and efficacy in treating posterior segment ocular diseases remains to be determined.

RPE cells derived from hESCs or iPSCs, have shown greater consistency and demonstrated safety and early efficacy in clinical trials for macular degenerations such as AMD and Stargardt disease.[5,7–9] Although no head-to-head clinical comparisons have been made between hESC- and iPSC-derived RPE cells, several studies have explored their biological similarities *in vitro* and *in vivo*.[90–92] Compared to iPSC-RPE, hESC-RPE cells offer the advantage of consistent quality and scalability from well-characterized, standardized cell lines, making it suitable for off-the-shelf allogeneic therapies. A potential disadvantage is a higher risk of immune rejection requiring immunosuppression, along with ethical concern associated with embryonic tissue origin. Both cell sources are currently being evaluated in ongoing clinical trials, and lessons from these studies will be instrumental in shaping future EV-based therapeutic strategies. Meanwhile, comparative studies of EVs derived from hESC-RPE and iPSC-RPE remain scarce. Comprehensive analyses of EV cargo from these two may provide valuable insights into therapeutic potential and inform the development of optimized, cell-free therapies for treating a range of retinal diseases associated with RPE dysfunction. These studies may address a critical question: can EVs derived from functional RPE cells retain and deliver therapeutic effects?

Retinal organoid–derived EVs are intriguing due to their origin from the multicellular retinal environment, potentially enabling the delivery of more physiologically relevant and functionally diverse cargo. However, as the therapeutic application of retinal organoids remains in its early stages, EVs from these sources require further preclinical validation to determine the optimal maturation stage of the organoid and ensure reproducibility of RO-EVs.

Ultimately, whether derived from pluripotent stem cells or fully differentiated ocular tissues, the production of therapeutic EVs hinges on the establishment of a stable, scalable, and quality-controlled cell bank. Lessons from cell therapy also emphasize the importance of standardized manufacturing protocols, batch-to-batch consistency, and long-term genetic and phenotypic stability to ensure reproducible and clinically viable EV production.

Given the inherent heterogeneity of EVs, it is essential to establish rigorous, high-purity, and reproducible strategies for EV isolation and characterization that can later be adapted to Good Manufacturing Practice (GMP)-compliant process. Each isolation method used in laboratory settings offers distinct advantages and limitations, including variability in specificity, sensitivity, and yield.[20,61,93] While a comprehensive evaluation of each strategy is beyond the scope of this review, we detailed the methods employed in each study, as they are critical for interpreting therapeutic efficacy. Advances in EV isolation techniques, combined with the emergence of high-resolution and single-particle characterization tools, have made substantial progress over the past decade toward standardized, scalable, and

clinically applicable workflows. Notably, most EV-based therapeutics currently in clinical trials utilize tangential flow filtration (TFF) as the primary isolation method due to its scalability, reproducibility, and compatibility with regulatory requirements.[94]

Dosing and route of administration also require optimization, as EV pharmacokinetics (PK) differ significantly from cell-based therapies. Intraocular EV pharmacokinetics remain poorly characterized. A reasonable first step is to define the half-life of EVs within various compartments of the eye. Given their complex and multimolecular cargo, including proteins, lipids, and genetic material such as mRNA and microRNAs, the functional half-life of EVs may extend beyond their physical clearance. RNA cargos, in particular, have the potential to induce sustained biological effects through gene regulation and signaling cascades, leading to a prolonged therapeutic window beyond the physical clearance of EVs. Therefore, both physical clearance and downstream molecular activity must be considered in determining optimal dosing regimens.

For both naïve and bioengineered EVs, the functionality and potential off-target effects of native cargo must be considered. This concern is particularly relevant when EVs are derived from non-ocular tissue sources, such as MSC-EVs as their native cargo may reflect the physiological or immunomodulatory profile of their tissue of origin, potentially leading to unintended consequences in the eye. Therefore, characterizing the native cargo composition is critical to ensuring both the safety and specificity of EV-based ocular therapies. To mitigate or predict the off-target effect and enhance therapeutic precision, it would be prudent to establish a comprehensive multi-omic cargo atlas, including transcriptomic, proteomic, lipidomic, and metabolomic profiles, of EVs from key candidate cell lines. Given recent technological advances in high-throughput omics platforms, data integration, and bioinformatic tools, such efforts appear to be increasingly feasible. These data will serve as valuable references for evaluating batch-to-batch consistency, identifying functional cargo signatures, and ensuring the safety and reproducibility of EV-based ocular therapies. While controlling cargo content may offer a promising strategy to mitigate off-target effects, current methods for precise cargo engineering remain limited and are not yet fully developed or widely accessible.

Finally, the regulatory landscape for EV-based therapies presents unique challenges, as they occupy a novel space within the broader category of biologics. This underscores the urgent need for consensus-driven frameworks to standardize EV production, characterization, and dosing, thereby ensuring reproducibility and facilitating clinical use. While the mechanism of action (MOA) is likely to be multifactorial, as seen in cell therapies, identifying the primary or dominant MOA is essential for developing relevant potency assays, an essential step closely tied to therapeutic efficacy, dose optimization, and successful clinical translation. Encouragingly, several early-phase clinical trials involving EV therapeutics, both within and beyond the field of vision disorders, have successfully received regulatory approval to proceed with the clinical trials. This progress is, in part, a reflection of the growing efforts by the scientific community to advance regulatory science and establish foundational standards in the field. The International Society for Extracellular Vesicles (ISEV), founded in 2011, has released a set of quality control standard recommendations to enhance the utilization of EVs among researchers and clinicians. The first field consensus,

'Minimum Information for Studies of Extracellular Vesicles' (MISEV), was issued by ISEV members in 2014, embodying best practices and guidelines aimed at unifying the nomenclature and methodologies of EV research.[95] With the rapidly evolving knowledge in the EV field, the initial 2014 MISEV document has evolved into two additional iterations (2018 and 2023), refining the assessment of EV traits such as biophysical properties (size, density, isolation, purity, and immune characteristics), surface markers, and functional proteins.[61,96] The Regulatory Affairs and Clinical Use of EV-based Therapeutics Task Force under ISEV emphasizes the urgent need to cultivate the regulatory landscape and provides the platform to promote the clinical use of EV-based therapeutics. Close collaboration and ongoing discussions among scientists, clinicians, industry, and regulatory bodies are essential to accelerate the progress of this exciting field.

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Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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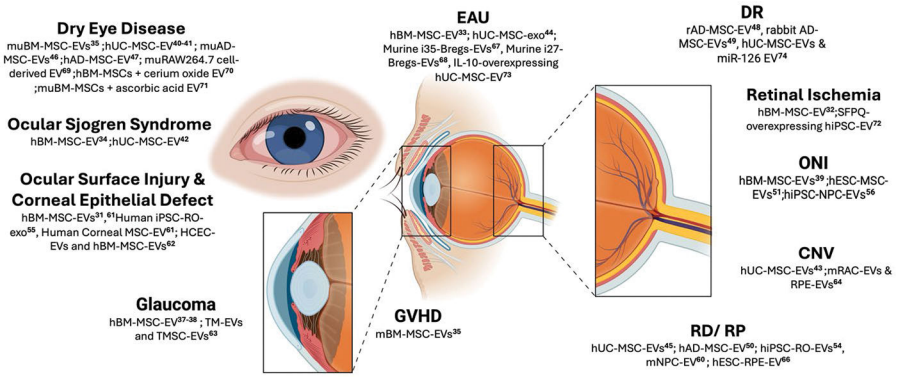

Figure 1.
Extracellular vesicles derived from various cell sources tested in ocular diseases.

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Table 1

In vitro evaluation of EV therapies for ocular diseases.

EV origin	Ocular disease	Isolation method	Model	Findings
Ocular Surface Disease				
Human AD-MSC-EVs[22]	Dry Eye Disease	Ultracentrifugation	HCECs	Reduced hyperosmotic stressed induced HCEC apoptosis (TUNEL+ cells) and decreased expression of NLRP3, caspase-1, and IL-1β.
Human BM-MSCs functionalized with cerium oxide nanocrystals[23]	Dry Eye Disease	Ultracentrifugation	HCECs	Accelerated scratch wound closure rate and reduced intracellular ROS.
Murine BM-MSCs functionalized with ascorbic acid[24]	Dry Eye Disease	Ultracentrifugation	HCECs	Accelerated scratch wound closure rate and reduced intracellular ROS.
Human BM-MSC EVs[25]	Ocular Surface Injury	Total Exosome Isolation Kit	HCECs	Accelerated scratch closure rate, increased HCEC proliferation, and decreased apoptosis.
Human Corneal MSC-EVs[26]	Ocular Surface Injury	Ultracentrifugation	HCECs	Increased proliferation of HCECs and accelerated scratch wound closure rate.
HCEC-EVs and Human BM-MSC-EVs[27]	Ocular Surface Injury	Total Exosome Isolation Kit	HCECs	hBM-MSC-EVs treatment accelerated scratch wound locusts rate, reduced H_2O_2 induced apoptosis, and decreased IL-1β stimulated inflammation.
Glaucoma and Optic Nerve Pathology				
Human BM-MSC EVs[28]	Glaucoma	ExoQuick-TC	RGC Culture	Reduced death of RGCs.
TM-EVs and TMSC-EVs[29]	Glaucoma	Ultracentrifugation	TM Cells	TMSC-EVs increased TM cell proliferation, accelerated scratch wound wound closure rate, reduced ROS.
Human BM-MSC-exo[30]	ONI	Ultracentrifugation	RGC Culture	Reduced RGC death and increased neuritogenesis.
Retina Disease				
Human BM-MSC EVs[31]	Retinal Ischemia	Exo Quick-TC EV Precipitation Solution	OGD-R28 Cells (Adherent Retinal Precursor Cell Line)	Reduced cytotoxicity of cells. Improvement in the number of proliferating cells (Edu + cells).
SFPQ overexpressing Human iPSC-EVs[32]	Retinal Ischemia	Ultracentrifugation	Human Müller Cells	Decreased Müller cell apoptosis and increased proliferation in face of hypoxic stress. SFPQ recruited HDAC1 to downregulate HIF-2α by regulating its acetylation.
Human UC-MSC-exo[33]	Subretinal Fibrosis	Ultracentrifugation	TGF-β2 Induced EMT-ARPE19 Cells	Inhibited EMT migratory ability of ARPE19 cells, Improvement of intercellular tight junctions (ZO-1), Upregulated occluding marker, decreased MSC markers (Vimentin, N-cadherin, α-SMA).
Murine RAC-exo and RPE-exo[34]	CNV	Ultracentrifugation	B6 Murine Retinal Microvascular Endothelial Cells (mRMVECs)	Inhibited mRMVEC tubule formation.
Human UC-MSC-exo[35]	RD	Ultracentrifugation	661 W Cells Co-cultured with LPS-Stimulated BV2 Cells	Reduced BV2 cell apoptosis (TUNEL+ cells) and decreased inflammatory cytokines.
Human AD-MSC EV[36]	RD	Tangential Flow Filtration System	ARPE-19 Human RPE Cell Line	Reduced H_2O_2 induced cytotoxicity, apoptosis, and activated the intracellular Nrf2 signaling pathway.

EV origin	Ocular disease	Isolation method	Model	Findings
Human ESC-RO[37]	RD	Ultracentrifugation	ARPE-19 Human RPE Cell Line	Reduced lipotoxicity and enhanced survival of oleic acid treated cells with upregulation of fatty acid β-oxidation related proteins.
Murine ESC-EVs[74]	RD	Ultracentrifugation	Human RPE Cells	Enhanced proliferation and cell cycle transition of senescent RPE cells. Reduced SA-β-gal staining rate, levels of MMP and ROS, downregulated cellular senescence markers p2 p16.
Murine NPC-EV[38]	RD	Ultracentrifugation	Co-culture of LPS-Activated BV2, a Murine Microglia Cell Line, and 661 W, a Murine Photoreceptor Cell Line	Suppression of inflammatory microglial activation and inhibition of photoreceptor apoptosis in a coculture model.
Rat AD-MSC-EV[39]	DR	Ultracentrifugation	HG Treated-HRMEC, RPE-J Cells and rMC1	Apoptosis inhibition of RPE-J cells (reduced caspase-3 activity and TUNEL+ cells), Weakened effect of Müller cell activation (GFAP) & decreased levels of inflammatory cytokines (IL-1β, IL-6, TNF-α, VEGF) in rMC1 cells, Inhibition of HRMEC proliferation (EdU staining and CCK-8 assays), decreased expression of angiogenesis-related factors (CD31, VEGF) and the number of tubes in HRMEC cells.
ARPE-19 human RPE cell line[40]	DR	Ultracentrifugation	HG Treated Human Umbilical Vein Endothelial Cells (HUVECs)	Suppression of HUVEC cell growth, migration, and tube formation.
Human UC-MSC-exo and UC-MSC-exo-miR-126[41]	DR	Ultracentrifugation	HG Treated Human Retinal Endothelial Cells (HREC)	HMGB1, NLRP3 inflammasome, and NF-κB/P65 decreased following hUC-MSC-exo-miR-126 treatment as did secretion of lL-1β, IL-18, and caspase-1.

AD-MSC-Adipose tissue derived mesenchymal stem cell; BM-MSC-Human bone marrow derived mesenchymal stem cells; CNV-Choroidal neovascularization; DR-Diabetic retinopathy; EMT-Epithelial to mesenchymal transition; EVs-Extracellular vesicles; exo-exosomes; ESC-Embryonic stem cells; HG-High glucose; HCEC-Human corneal epithelial cells, HRMEC-Human retinal microvascular endothelial cells; iPSC-Induced pluripotent stem cell; MC1-Müller cell line; OGD-Oxygen glucose deprived; ONI-Optic nerve injury; NPC-Neural progenitor cells; RAC-Retinal astroglial cells; RD-Retinal degeneration; RGC-Retinal ganglion cell; ROS-Reactive oxygen species; RPE-Retinal pigment epithelium; TM-Trabecular Meshwork; TMSC-Trabecular meshwork stem cells; UC-MSC-Human umbilical cord derived mesenchymal stem cells.

Table 2.

In vivo evaluation of EV therapies for ocular diseases.

EV origin	Ocular disease	Isolation method	*In vivo* models		
			Animal model	EV-administrative route	Findings
Ocular Surface Disease					
Human UC-MSC-EV[42]	Dry Eye Disease	Ultracentrifugation	Scopolamine and desiccating environment murine model	Eye drops	Increased tear production, decreased fluorescein staining, increased goblet cell density, reduction of CD4 T cell infiltration and inflammatory cytokines.
Human UC-MSC-EV[43]	Dry Eye Disease	Ultracentrifugation	Scopolamine and desiccating environment murine model	Eye drops	Increased tear production, decreased fluorescein staining, increased goblet cell density, reduction of inflammatory cytokines, reduction of corneal dendritic cells and inhibition of maturation.
Mouse AD-MSC-exo[44]	Dry Eye Disease	Ultracentrifugation	BAC-induced mice	Eye drops	Increased tear production, decreased corneal fluorescein staining, decreased corneal epithelial cell apoptosis and decreased inflammatory cytokine expression.
Human AD-MSC-EV[22]	Dry Eye Disease	Ultracentrifugation	Scopolamine and desiccating environment murine model	Eye drops	Increased tear production, decreased corneal fluorescein staining, decreased corneal epithelial cell apoptosis and inhibited NLRP3 inflammasome activation.
Murine RAW264.7 macrophage cell line[45]	Dry Eye Disease	Ultracentrifugation	BAC-induced mice	Eye drops	Increased tear production, improved tear film stability and reduced corneal surface damage. Reduction in ocular surface proinflammatory cytokine level.
Human BM-MSCs functionalized with cerium oxide nanocrystals[23]	Dry Eye Disease	Ultracentrifugation	BAC-induced mice	Eye drops	Increased tear production, decreased corneal fluorescein staining, increased ROS scavenging, decreased tear film inflammatory cytokine levels.
Murine BM-MSCs functionalized with ascorbic acid[24]	Dry Eye Disease	Ultracentrifugation	BAC-induced mice	Eye drops	Increased tear production, decreased corneal fluorescein staining, increased ROS scavenging, decreased tear film inflammatory cytokine levels.
Human BM-MSC-EV[46]	Ocular Sjogren Syndrome	Size Exclusion Chromatography	NOD.B10.H2b murine model of Sjogren Syndrome	Intraorbital Lacrimal Gland injection	Improved corneal epithelial integrity, increased tear production and reduced T cell infiltration into the lacrimal glands. Decrease in the expression of inflammatory cytokines, including TNF-α, IL-1β, and IFN-γ, in both the ocular surface and lacrimal glands.
Human UC-MSC-EV[47]	Ocular Sjogren Syndrome	Ultracentrifugation	Rabbit autoimmune dacryoadenitis model	Subconjunctival injection	Increased tear production, decreased fluorescein staining, decreased conjunctival and lymphatic gland inflammatory cell infiltration, promote M2 macrophage polarization.
Murine BM-MSC-exo[48]	GVHD-Associated Dry Eye Disease	Ultracentrifugation	BAC-induced mice & NCG-GVHD mice	Eye drops	Transparent cornea with less fluorescein staining. Elevated tear secretion. Prevented corneal epithelium degeneration. Increased thickness of central cornea and epithelium layer. Restoration of well-organized corneal structure. Decreased $TUNEL^+$ cells and CD11b + macrophages, increased proliferative $Ki67^+$ corneal epithelial cells, downregulation of proinflammatory (IL6, IL1β, IL-17A, CD86) and upregulation of classical M2 markers (CD206, Arg1).

EV origin	Ocular disease	Isolation method	*In vivo* models		
			Animal model	EV-administrative route	Findings
Human iPSC-RO-exo[49]	Ocular Surface Injury	Ultracentrifugation	C57BL/6J mice with 2 mm central epithelial defect	Eye drops	Higher wound-healing ratio at 24 and 36h after exo-treatment. Clear stroma at 36h after treatment. Promoted wound healing seen by cell proliferation. Suppression of inflammatory cytokines (TNF-α, CCL2, CCL5). Upregulation of genes related to retinoic acid and eicosanoid metabolism.
Human Corneal MSC-EVs[26]	Ocular Surface Injury	Ultracentrifugation	Murine corneal epithelial debridement	Eye Drops	Accelerated corneal epithelial wound healing.
Glaucoma and Optic Nerve Injury					
Human BM-MSC-EV[50]	Glaucoma	Polyethylene Glycol-Based Enrichment	DBA/2J murine model	Intravitreal injection	Neuroprotection with 3.7-fold greater RBPMS+ RGC cells. Reduction in the number of degenerating axons seen in the optic nerve. Prevented the early decline of RGC function.
Human BM-MSC-EV[28]	Glaucoma	ExoQuick-TC	Two separate rat ocular hypertension models	Intravitreal injection	Preserving RGC density and preventing RNFL degeneration thinning and loss of positive scotopic threshold response on ERG. Knockdown of Ago 2 attenuated therapeutic effects of hBM-MSC-EVs.
Human BM-MSC-exo[30]	ONI	Ultracentrifugation	ONC Rat Model (Sprague Dawley)	Intravitreal injection	Preserved RGC axonal density, reduced RGC loss, and prevented decline in RGC function. Knockdown of Ago2 attenuated therapeutic effects of MS-MSC-exo.
Human ESC-MSC-EV[51]	ONI	Ultracentrifugation	ONC Murine model (C57BL/J6)	Tail vein injection	Increased Brn3a + RGCs, Tuj1+ cells and GAP43+ axons in optic nerve and downregulation of cis p-tau. Retro and anterograde tracing of RGC projections to the brain. Preservation of RNFL thickness. Improved cognitive visual behavior.
Human iPSC-NPC-EV[52]	ONI	Ultracentrifugation	ONC Murine model (C57BL/J6)	Intravitreal Injection	Prevented RGC loss, reduced number of retinal microglial cells, and preserved N1-P1 wave amplitude in flash visual evoked potentials.
Uveitis					
Human BM-MSC-EV[53]	EAU	Anion Exchange Resin	Murine model of EAU	Tail-vein injection	Suppresses Th1 and Th17 cell development. Inhibited the activation of APC and T cells. Restoration of retinal photoreceptor layer structural damage. Reduced retinal inflammatory cells (CD3+ T cells). Decreased expression of pro-inflammatory cytokines (IFN-γ, IL-17A, IL-2, IL-1β, IL-6, IL-12A).
Human UC-MSC-exo[54]	EAU	Ultracentrifugation	Lewis rat model of EAU	Periocular injections	Reduced infiltration of T cell subsets, other inflammatory cells (Gr-1, CD161, CD68, CD4, IFN-γ, IL-17, CD161, Foxp3). Improved retinal function (Increased α and β-wave amplitudes).
Murine i35-Bregs-exo[55]	EAU	Exoquick TC Reagent	Murine model of EAU	Retro-orbital injection	Reduction in EAU disease severity on fundoscopic OCT, histologic analysis. Rescue of a-wave and b-wave ERG amplitude. Expansion of intraocular Treg cells.
Murine i27-Breg-exo[56]	EAU	Ultracentrifugation	Murine model of EAU	Retro-orbital injection	Reduction in EAU disease severity on fundoscopic OCT, histologic analysis.
IL-10 overexpressing hUC-MSC-EV[57]	EAU	Ultracentrifugation	Murine model of EAU	Tail vein injection	Reduction in clinical and histological EAU disease severity. Inhibited proliferation of T-cells and differentiation of Th1 and Th17 cells while promoting differentiation of Treg cells.

EV origin	Ocular disease	Isolation method	*In vivo* models		
			Animal model	EV-administrative route	Findings
Retinal Disease					
Human BM-MSC-EV[31]	Retinal Ischemia	Exo Quick-TC EV Precipitation Solution	Rat ocular ischemia/ reperfusion injury	Intravitreal injection	Improved recovery of a-and b-wave amplitudes (ERG). Decreased apoptosis (TUNEL+ cells and cleaved caspase-3 levels) in ONL, INL and GCL layers. Declined levels of neuroinflammatory cytokines (TNF-α, IL-6).
SFPQ overexpressing hiPSC-EV[32]	Retinal Ischemia	Ultracentrifugation	Rat ocular ischemia/ reperfusion injury	Intravitreal injection	Reduced retinal cell apoptosis and preserved a-wave and b-wave ERG amplitudes.
Human UC-MSC-exo[33]	Subretinal Fibrosis	Ultracentrifugation	Laser-induced CNV subretinal fibrosis and murine model	Intravitreal injection	Decreased sub retinal fibrotic areas (Type 1 collagen) and vascular channels (Isolectin B4).
Murine RAC-exo and RPE-exo[34]	CNV	Ultracentrifugation	Laser-induced CNV mice and Laser-induced subretinal fibrosis model	Periocular injection	RAC-exo inhibited retinal vascular leakage and CNV whereas RPE-exo did not.
Human UC-MSC-EV[35]	RD	Ultracentrifugation	Rd10 mice	Intravitreal injection	Improved photoreceptor survival, decreased reactive gliosis, decreased retinal macrophage infiltration, and decreased inflammatory cytokine expression. Improved ERG a-wave and b-wave amplitude as well as optomotor response.
Human AD-MSC EV[36]	RD	Tangential Flow Filtration System	RCS Rats	Intravitreal injections	Preservation of outer nuclear layer and photoreceptor segments and increased inner nuclear Nrf2 levels. Early preservation of ERG and a-wave and b-wave amplitude.
Human iPSC-RO-exo[58]	RD	miRCURY Exosome Isolation Kit	RCS Rats	Intravitreal injection	Reduced photoreceptor apoptosis. Prevented ONL thinning. Preserved visual function by inhibiting MAPK pathway.
Murine NPC-EV[38]	RD	Ultracentrifugation	RCS rats	SRS	Improved scotopic activity. Reduced TUNEL+ apoptotic cells in ONL. Preservation of ONL thickness. Inhibiting microglia activation: decreased number of migratory Iba1+ cells in ONL and SRS.
Human ESC-RPE-EV[59]	RD	Tangential Flow Filtration System	RCS Rats	Intravitreal Injection	Preserved scotopic ERG a-wave and b-wave amplitude, reduced photoreceptor death and increased photoreceptor outer segment engulfment.
Rat AD-MSC-EV[39]	DR	Ultracentrifugation	STZ-induced SD rats	Intravitreal injection	Significant decrease of ITGA1 expression in retinal tissues. Decreased levels of inflammatory cytokines in vitreous (TNF-α, IL-6, IL-1β, VEGF, MCP-1). Inhibition of angiogenesis (VEGFA), macrophage infiltration (CD31), absence of proliferation factor (Ki67) and decreased glial hyperplasia (GFAP). Restoration of RGC number.
Rabbit AD-MSC-exo[60]	DR	Ultracentrifugation	STZ-induced diabetes rabbits	IV, SC and IO injections	Regenerative changes in retinal tissue: well organized retinal layers with well-observed vacuoles in ONL, GCL and IPL in SC and IO-exo groups. Significant increase in micRNA-222 levels seen for SC, IO & IV-exo groups.
Human UC-MSC-exo and hUC-MSC-exo-miR-126[41]	DR	Ultracentrifugation	STZ-induced SD rats	Intravitreal injections	Alleviated inflammation. Decreased apoptosis (caspase-1), inflammatory factors (IL-1β, IL-18), HMGB1, adhesion molecules (ICAM-1, VCAM-1), NF-κB/P65 and NLRP3 inflammasome.

AD-MSC-Adipose tissue derived mesenchymal stem cells; APC-Antigen presenting cells; BAC-Benzalkonium chloride; BM-MSC-Bone marrow derived mesenchymal stem cell; CNV-Choroidal neovascularization; DR-Diabetic retinopathy; EAU -Experimental autoimmune uveoretinitis; EMT-Epithelial to mesenchymal transition; ESC-MSC-Embryonic stem cell derived mesenchymal stem cells; ESC-RPE-Embryonic stem cell derived retinal pigment epithelium, EV-Extracellular vesicles; exo-Exosomes; GCL-Ganglion cell layer; GVHD-Graft versus host disease; iPSC-RO-Induced pluripotent stem cells derived retinal organoids; INL-Inner nuclear layer; IO-Intraocular; IPL-Inner plexiform layer; ITGA1-Integrin subunit α 1; IV-Intravenous; MAPK-Mitogen-activated protein kinase; mNPC-Murine neural progenitor cells; MSC-Mesenchymal stem cells; N/A-Not applicable; NPC-neural progenitor cell; ONC-Optic nerve crush; ONI-Optic nerve injury; ONL-Outer nuclear layer; RAC: retinal astroglial cell; RBPMS-RNA binding protein with multiple splicing; RCS-Royal college of surgeons' rats; RGC-Retinal ganglion cells; RNFL-Retinal nerve fiber layer; RD-Retinal degenerations; RP-Retinitis Pigmentosa; RPE-retinal pigment epithelium; UC-MSC-Umbilical cord derived mesenchymal stem cells; SC-Subconjunctival; sEV-Small extracellular vesicles; SD-Sprague-Dawley rat; SRS-sub retinal space; STZ-Streptozotocin

Table 3.

Summary of clinical trials for ocular diseases using EV-based therapies.

Clinical trial ID	Disease	EV source	Delivery method	Phase
NCT04213248	Dry Eye Disease	UC-MSC	Topical (Eye drops)	Phase I
NCT06543667	Dry Eye Syndrome	Limbal stem cell	Topical (Eye Drops)	Phase I/II
NCT05738629	Dry Eye Syndrome	iPSC derived MSC	Topical (Eye Drops)	Phase I/II
NCT05413148	Retinitis Pigmentosa	UC-MSC	Subtenon Injection	Phase I/II
NCT03437759	Refractory Macular Hole	UC-MSC	Intravitreal injection	Phase I/II