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Review

# Extracellular vesicles as a potential therapeutic for age-related macular degeneration

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### *From the Contents*



## Abstract

Age-related macular degeneration is a major global cause of central visual impairment and severe vision loss. With an aging population, the already immense economic burden of costly anti-vascular endothelial growth factor treatment is likely to increase. In addition, current conventional treatment is only available for the late neovascular stage of age-related macular degeneration, and injections can come with potentially devastating complications, introducing the need for more economical and risk-free treatment. In recent years, exosomes, which are nano-sized extracellular vesicles of an endocytic origin, have shown immense potential as diagnostic biomarkers and in the therapeutic application, as they are bestowed with characteristics including an expansive cargo that closely resembles their parent cell and exceptional ability of intercellular communication and targeting neighboring cells. Exosomes are currently undergoing clinical trials for various conditions such as type 1 diabetes and autoimmune diseases; however, exosomes as a potential therapy for several retinal diseases have just begun to undergo scrutinizing investigation with little literature on age-related macular degeneration specifically. This article will focus on the limited literature available on exosome transplantation treatment in age-related macular degeneration animal models and *in vitro* cell cultures, as well as briefly identify future research directions. Current literature on exosome therapy using age-related macular degeneration rodent models includes laser retinal injury, N-methyl-N-nitrosourea, and royal college of surgeon models, which mimic inflammatory and degenerative aspects of age-related macular degeneration. These have shown promising results in preserving retinal function and morphology, as well as protecting photoreceptors from apoptosis. Exosomes from their respective cellular origins may also act by regulating the expression of various inflammatory cytokines, mRNAs, and proteins involved in photoreceptor degeneration pathways to exert a therapeutic effect. Various findings have also opened exciting prospects for the involvement of cargo components in remedial effects on the damaged macula or retina.

**Key Words:** age-related macular degeneration; exosomes; extracellular vesicles; miRNA; neuroprotection; photoreceptors; retina; retinal pigment epithelium

## Introduction

### Retina, photoreceptors, and the retinal pigment epithelium

The retina belongs to the central nervous system and is a thin complex layer of tissues lining the inner wall of the eye. In the retina, light triggers electrochemical responses in photoreceptors, which send signals to the brain to produce a visual image through a process called phototransduction. Photoreceptors play an important role in the detection and absorption of light and have a high turnover of waste products such as photoreceptor discs or tips (Boulton and Dayhaw-Barker, 2001). The retinal pigment epithelium (RPE) regulates nutrients and metabolic waste transport, and functions as an outer blood-retinal barrier that partially filters particle movement between the choroid and subretinal space. These two are just some of the many mechanisms by which RPE function to maintain photoreceptors (Nowak, 2006; Campbell and Humphries, 2012; Nowak, 2014). Photoreceptors have been proven to be more susceptible to damage than other retinal neurons and their loss is a primary characteristic of a series of diseases known as age-related macular degeneration (AMD) (Stone et al., 1999).

### Age-related macular degeneration

AMD is one of the leading causes of irreversible vision loss in the world, accounting for 9% of blindness (Wong et al., 2014). In addition, studies have shown that incidences are rising at an alarming rate, especially within the elderly population, with an expected increase to 288 million by 2040 (Wong et al., 2014; Li et al., 2020). AMD consists of both an early (drusen and pigmentary changes) and late stage (geographic atrophy and neovascular AMD). Various risk factors have been identified with major ones being smoking, previous cataract surgery, age, family history, disputable correlations with a dietary habit, sunlight exposure, and lifestyle (Chakravarthy et al., 2010; Armstrong and Mousavi, 2015). Despite substantial research dedicated to AMD, its pathogenesis remains poorly understood due to its complex and multifactorial nature. However, research throughout the years has attributed the main cellular disease contributions to drusogenesis (drusen formation causing Bruch's membrane degeneration), lipofuscinogenesis (photoreceptor degeneration under continuous oxidative stress), local inflammation (immune system activated by drusen), and lastly neovascularization (disbalance between angiogenic and anti-angiogenic factors) seen in the late choroidal neovascularization (CNV) AMD, resulting in Bruch's membrane degeneration, RPE damage and subsequent photoreceptor cell damage and death (Nowak, 2006, 2014). AMD is a substantial global burden to health services, hence the rising need for further allocation of research and healthcare resources towards novel treatments.

### Existing AMD treatments

The treatment option for AMD depends on the stage of the disease. Currently, apart from controversial evidence on antioxidant diet and supplements such as vitamins C & E, beta-carotene, and zinc in prevention and reduction of AMD progression from an age-related eye diseases study (ARED) study (AREDS-Research-Group, 2001), there is no direct proven treatment for dry AMD.

There are a few approved treatments available for patients with wet AMD, including photodynamic therapy, laser therapy, and the recent vicennial discovery of anti-VEGF injection or a combination of the above treatments. However, despite anti-VEGF injections being the current gold standard and conventional treatment for wet AMD, significant risks can still come with it despite careful handling. Anti-VEGF injections require intensive and frequent administration for lengthy periods with no guarantee for improved vision (Stahl, 2020). The injections also come with high risks of severe systemic and ocular complications per injection, such as endophthalmitis, ocular hemorrhages, inflammation, and retinal detachment (Fletcher and Chong, 2008; Falavarjani and Nguyen, 2013), and specifically in AMD: incidences of RPE tear in anti-VEGF injections (Ahn et al., 2022). Current wet AMD treatments only work through the blocking of CNV growth and vessel leakage reduction; therefore, there is an absence of a true cure and treatments catered to AMD's multifactorial nature or initial stages of the disease (Rattner and Nathans, 2006).

### Exosomes

In the recent decade, exosomes have garnered growing attention due to their promising potential and prospects in pathological research and uses. Exosomes are a nanosized subset of extracellular vesicles (EVs; along

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with apoptotic bodies and microvesicles), secreted by all cell types within the human body (Doyle and Wang, 2019). Exosomes are formed in the endosomal system, where inward budding of the plasma membrane and partial invagination of endocytic membrane forms intraluminal vesicles within multivesicular bodies (Colombo et al., 2014; Théry and Witwer, 2018; Kalluri and LeBleu, 2020). These multivesicular bodies then fuse with the plasma membrane cell surface to be secreted as exosomes and be released extracellularly through exocytosis. Substantial research has shown that exosomes facilitate various essential functions, such as mediating intercellular communication, influencing target cells (such as stimulating and reprogramming neighboring cells), regulating immune responses and wound healing, proving to be an important mediator in the retina (Klingeborn et al., 2017; Zhang et al., 2019b; Mead and Tomarev, 2020). The extensive functions of exosomes may be due to their expansive cargo, consisting of various bioactive molecules such as proteins, DNA, and RNA, which closely resemble their parental cellular origins, both in their characteristics and behavior, and can be successfully delivered to recipient cells (Valadi et al., 2007; Muthu et al., 2021).

**Exosomal therapy**

Exosomes are being actively studied as a potential therapeutic candidate and a potential alternative to cell therapy. Exosomes show supremacy over their parent cell due to various advantages such as quicker diffusive rate to target cells (Kastelowitz and Yin, 2014) and versatility of movement through the blood-brain/retinal barrier (Zagrean et al., 2018; Banks et al., 2020). Furthermore, exosomes solve previously raised issues that arose with cell-based therapy such as the possibility of immune rejection (Hmadcha et al., 2009), excessive cell replication which has blinded several AMD patients receiving stem cell therapy (Kuriyan et al., 2017; Hinkle et al., 2021) and damage to retinal tissues as exosomes have low immunogenicity, toxicity and good biocompatibility and stability (Samanta et al., 2018).

**Isolation and characterization criteria for exosomes**

Exosomes can be isolated through different methods which each have their strengths and weaknesses (Thery et al., 2006). These include ultra-centrifugation, sucrose gradients, and polyethylene glycol-based centrifugation. Historically, the terms "exosome" and "extracellular vesicles" have been used interchangeably in the literature base (Mead and Tomarev, 2020). However, since exosomes refer to EVs formed by a specific biogenesis pathway, the International Society for Extracellular Vesicles (ISEV) recommends that unless this biogenesis pathway is confirmed, they should instead be referred to as "small EVs" (Théry and Witwer, 2018). The papers reviewed in this manuscript do not follow these guidelines and typically just call them "exosomes", and there is still much contention in the field over preferences for different nomenclature (Witwer and Théry, 2019). Thus, while we fully support the ISEV guidelines, we will continue to refer to them as exosomes in this manuscript to avoid confusion and maintain consistency with the studies being reviewed. We will however highlight any differences or shortcomings in the isolation techniques of the reviewed papers. Typically, exosomes/EVs will have undergone ultracentrifugation and another isolation method of either ultrafiltration (such as 0.22 μm filtering membrane or tangential flow filtration) or density gradient centrifugation (such as sucrose), which gives highly purified exosome solutions (Chen et al., 2021a). In addition, filtered exosomes should be sized 30-150 nm, have cup-shaped morphological characteristics, and have common markers such as CD63, CD81, and CD9.

**Focus of this review article**

Numerous studies have investigated the role of exosomes either as passive biomarkers in AMD (Biasutto et al., 2013; Kang et al., 2014), or potential mediators of the disease (Wang et al., 2009; Atienzar-Aroca et al., 2016; Kannan et al., 2016; Atienzar-Aroca et al., 2018; Elbay et al., 2019; Mukai et al., 2021) and this has been reviewed alongside their role in other eye diseases (Klingeborn et al., 2017). There is continued research on the use of exosomes as a drug delivery vehicle into the eye, utilizing exosome ability to deliver compounds while loading them with known therapeutic agents (Wassmer et al., 2017; Zhang et al., 2019a; Dong et al., 2021). These studies are too few however to provide a compelling review, as this area of research is still incredibly novel.

This review will focus on the therapeutic potential of exosomes, which, while also a very niche area of research, has now had several exciting studies published. Exosomal therapy may act as a potential cell-free therapy that aids rehabilitation and improvement of vision in AMD.

## Search Strategy

The studies cited in this review were identified through PubMed and Google Scholar, alongside any in-text citations found within this primary body of literature. Keywords used are the same as the keywords associated with this manuscript.

## Exosomal Therapy in Age-Related Macular Degeneration Models

Below we will discuss the therapeutic effects of exosomes in different models of AMD, with a brief introduction to the model used. These studies have had their findings summarized in **Additional Table 1**.

**Laser-induced retinal injury mouse/rat model**

The laser-induced-retinal injury rat model is an *in vivo* model of CNV in neovascular AMD. The model utilizes a technique known as laser photocoagulation, where a laser is directed at the RPE of the rat's eye to induce the rupturing of Bruch's membrane, subsequently triggering an inflammatory reaction in the eye and resultant growth of new blood vessels (Shah et al., 2015).

***Exosome/EV therapy in laser-induced retinal injury mouse/rat models***

The first paper to show prospects of exosomal therapy for AMD in a laser-induced injury mouse model was by Hajrasouliha and coauthors (Hajrasouliha et al., 2013). Periocular injection of exosomes derived from B6 mouse retinal astrocytes significantly suppressed blood vessel leakage and inhibited CNV in the model. No signs of retinal vessel leakage were found in comparison to the 42% leakage found in phosphate-buffered saline (PBS) injected control group. Administrations every two days had the same efficacy as every day, and late treatment after 7–14 days of injury also had similar efficacy to immediate treatment, demonstrating the potential of exosomal therapy for early and later stages of neovascular AMD. Astrocyte exosomes also significantly reduced the migration of macrophages in an *in vitro* chemoattractant migration assay and *in vivo* (with a reduction of 55% per laser spot). Astrocyte exosomes also inhibited mouse retinal microvascular endothelial cell migration and suppressed the formation and branch length of VEGF-induced tubule network formation, which plays a role in CNV formation. The mechanism of action may be partially explained by the internalization of astrocyte exosomes by macrophages and microvascular endothelial cells. Anti-angiogenic factors and proteins such as endostatin and pigment epithelial-derived factor were exclusively found in astrocyte exosomes and a greater expression of tissue inhibitor of metalloproteinase-1 was also found in astrocyte exosomes compared to exosomes derived from B6 mouse RPE (RPE-exos). A lack of expression in endostatin within astrocyte exosomes completely reversed the suppression of leakage and partially suppressed CNV, showing endostatin is responsible for retinal vessel leakage suppression but not exclusively for CNV. The paper suggests a strong potential of endostatin in therapeutics and demonstrates the need for further research on the involvement of exosome's expansive cargo in its therapeutic action.

Interestingly, exosomes isolated from RPE-exos had no therapeutic effect but instead, induced a higher amount of blood vessel leakage than PBS control. However, a significance test was not performed and the potential influence on macrophages was not investigated as RPE-exos were not the focus of the paper. In addition, RPE-exos were found to contain placenta growth factor-2, a growth factor within the VEGF family that can amplify VEGF2 secretion and hence may partially explain the increase in blood vessel leakage. Further investigation is required to validate RPE-exos results to identify the significance of findings and potential protein involvements.

In addition, He and coauthors (He et al., 2018) tested exosomes in an AMD model in which the early stages of AMD (oxidative stress) are mimicked by inducing blue light injury on RPE cells. Exosomes were isolated from human umbilical cord mesenchymal stem cells (hucMSCs-exos) but were not filtered through a 0.22 μm filter. After administration intravitreally, they were found to downregulate VEGF production, a key component of the CNV process. Exosomes were injected in 1, 2, and 3 μL of 50 μg/mL dosages. hucMSCs-exos reduced VEGF protein levels and mRNA expression with a greater reduction found with greater concentration and over longer time, as determined by PCR and western blot assay. By day 21, exosomal injections alleviated the laser injuries, demonstrating a reduced amount of large vascular channels, fibroblasts, and collagen fibers. Moreover, the retinal structure which should appear disorganized under laser injury resembled a normal retina. hucMSCs-exos also reduced VEGFA protein expression, especially in the group with the highest dosage (3 μL). Similar to findings from the previous paper, hucMSCs-exos also reduced retinal vessel leakage, which was again most prominent at 3 μL. This study showed exosome involvement in reducing VEGF expression and supports the previous findings (Hajrasouliha et al., 2013).

In another study, hucMSCs-exos as well as exosomes derived from mouse adipose tissue suppressed inflammatory tissue damage and exhibited protective effects on photoreceptor degeneration in a mouse laser injury model (Yu et al., 2016). Exosomes (also unfiltered) were intravitreally injected and attenuated outer nuclear layer (ONL) defect areas with a greater reduction over time, fewer $TUNEL^+$ cells, and best efficacy in the 0.5–2.0 mg/mL concentrations. Light-adapted and dark-adapted electroretinogram (ERG) responses, including alpha and beta wave amplitude, were significantly greater when treated with both types of exosomes, compared to PBS control. The results were only seen exclusively in exosomes derived from MSC and not in fibroblast-derived exosomes, further demonstrating that exosome action varies depending on their cellular origin. Similar efficacy was also found between MSC treatment and MSC-exosomes, which further supports that exosomes have comparable properties to their parental cells.

In addition, protective effects on tissue wound healing were also observed in eyes treated with MSC-exosomes. The inflammatory cytokine tumor necrosis factor-α, intercellular adhesion molecule-1 (which regulates adhesion and migration of leukocytes of the retina under stress), and most significantly monocyte chemoattractant protein-1 (MCP-1; key role in the escalation of inflammatory reaction, including monocyte recruitment and photoreceptor apoptosis) all aggregate at laser injury sites and their mRNA expression was downregulated by MSC-exosomes. MCP-1 downregulation was also confirmed via an *in vitro* thermal injury model. Immunohistochemical staining

also showed fewer macrophages in the MSC-exosome-treated group over time. The authors explained that the mechanism of action was through the inhibition of MCP-1 mRNA expression, as evidenced by the reversal of the alleviated effects when injected with exogenous MCP-1.

**MNU mouse/rat model**

Another model of AMD is the N-methyl-N-nitrosourea (MNU) model, which is suggested to imitate the retina undergoing oxidative stress during lipofuscinogenesis. In the model, MNU, a DNA alkylating agent which is toxic to the retina, is injected intraperitoneally into rats, inducing retinal photoreceptor degeneration through photoreceptor outer segment loss, evidenced by the thinning of ONL (Chen et al., 2014).

***Exosome/EV therapy in MNU mouse/rat model***

Using the MNU model, exosomes derived from human adult retinal pigment epithelial cell line 19 (ARPE-exos) (Wang et al., 2021), as well as exosomes derived from mouse bone marrow (BMSC-exos) (Deng et al., 2021) demonstrated common findings with Yu et al. (2016). Both papers found that exosome treatment significantly preserved ONL thickness and scotopic ERG amplitudes of alpha and beta waves, retained normal retinal architecture and protected cone photoreceptors as shown in immunohistochemical staining (Wang et al., 2021) and TUNEL analysis (Deng et al., 2021).

Furthermore, Wang et al. (2021) also showed copious punctate and opsin staining in the retina which indicated cone photoreceptors were undamaged, with M & S cones best protected against MNU-induced injury, whereas no staining was found in the untreated MNU control group. Visual acuity, contrast sensitivity, and optokinetic behaviors tests displayed all-round protection of visual functions from ARPE-exo treatment which was not previously shown. Significant improvement of local field potential amplitudes, which indicate nerve response to electrical signal transmissions from the retina, was also seen in ARPE-exos treated group. Comparison analysis showed a difference in local field potential amplitudes were 18.3%, 42.4%, and 69.2% for central, mid-peripheral, and peripheral zone, respectively, showing that there was greater protection of peripherally regionalized photoreceptors.

The MNU model exhibits a significant increase in the expression of inflammatory cytokine factors, such as MCP-1, tumor necrosis factor-α, interleukin-1β, and interleukin-6 and these were reduced when treated with exosomes. The mechanism of ARPE-exos may be that it inhibits the caspase-independent apoptotic pathway that encourages photoreceptor degeneration, as exosomes downregulated the mRNA levels of the pro-apoptotic BAX, Calpain-2, and Caspase-3 and increased mRNA levels of anti-apoptotic BCL-2. In addition, retinal malonyldialdehyde concentration analysis which quantifies the level of oxidative stress showed a significant reduction, showing promising results that exosome therapy may be able to target multiple aspects of AMD, through regulating expressions, neural signals and reducing pro-inflammatory cytokine factors.

Deng et al. (2021) found that GW4869, an inhibitor of the exosome biogenesis pathway, completely reversed the therapeutic efficacy (photoreceptor protection) when added to BMSC-only injections, which suggests that BMSC-exos mediate the therapeutic effects of the BMSC. Therapeutic effects of BMSC-exos were also demonstrated in an *in vitro* cellular apoptosis assay which showed a reduction in MNU damaged 661W photoreceptor cell line. In addition, a single injection of BMSC-exos was still efficacious even at 8 weeks, showing long-term therapeutic effects, which may be an advantageous alternative to conventional anti-VEGF injections administered every month.

It was speculated that exosomes target photoreceptors directly to exert their therapeutic effects, as BMSC-exos labeled with PKH26 dye aggregated linearly posterior to the edge of ONL upon injection, and the photoreceptor 661W cell line was capable of BMSC-exos uptake *in vitro*. miR-21, part of the exosomes cargo, is suggested to play a role in mediating the protective effect of BMSC-exos on photoreceptors, as BMSC-exos isolated from rats deficient in miR-21 had their therapeutic efficacy reduced. The pro-apoptotic protein, Pdcd4, which is a downstream target of miR-21, is reduced following BMSC-exos treatment. miR-21 may be one among many miRNAs involved in therapeutic action of exosomes.

**Royal College of Surgeon rat model**

The Royal College of Surgeon (RCS) rat strains are born with a genetic defect, which causes the deletion of the MertK protein-coding gene and stimulates apoptotic cell phagocytosis (Ryals et al., 2017). The absence of the MertK gene induces thinning and disorganization of retinal layers as well as the accumulation of photoreceptor outer segment debris due to RPE dysfunction, causing subsequent loss of photoreceptors and retinal ganglion cells. RCS rats gradually lose visual function including ERG responsiveness, contrast sensitivity and visual acuity, mimicking progression in AMD (Lund et al., 1998).

***Exosome/EV therapy in Royal College of Surgeon rat model***

Two papers from the same/overlapping group tested the efficacy of exosomes derived from human retinal progenitor cells (hRPC-exos) (Chen et al., 2021b), as well as the efficacy of exosomes derived from mouse neural progenitor cells (mNPC-exos) (Bian et al., 2020). Both published papers found findings consistent with previously published papers that their exosomes reduced the percentage of apoptotic cells and preserved visual acuity, as well as maintained retinal layer thickness, and downregulated inflammatory cytokines in the RCS model. In addition, both papers found their respective exosomes exerting long-term therapeutic action with Chen et al. (2021b) finding exosomes exerting therapeutic effects four weeks after administration.

Interestingly, Chen et al. (2021b) found that hRPC-exos had better efficacy in preventing temporal ONL thinning compared to nasal. hRPC-exos also induced downregulation of BAX and Caspase 3 but increased BCL-2, which is consistent with results from Wang et al on ARPE-exos (Wang et al., 2021). A finding unique to this article was that an *in vitro* co-culture showed hRPC-exos co-localized with human microglial cell line HMC3 and significantly reduced protein and gene levels of inflammatory proteins, which may be yet another mechanism of exosomes.

Bian et al. (2020) found reduced alpha and beta waves (as recorded by ERG), and ONL thickness thinning on days 2–4 before therapeutic effect increased and exceeded PBS injected control, which may suggest that mNPC-exos exert an initial toxic effect prior to their therapeutic one. Bian's paper also focused heavily on the mechanism of mNPC-exo therapy in reducing local inflammation through microglia inhibition in the RCS model. mNPC-exos that migrated to the subretinal space were internalized by $IBa1^{+}$ microglial cells. Furthermore, by day 7, there was a significant reduction in the number of internalized exosomes in the microglia and microglia formed a ramified morphology indicating a switch to a resting inactivated state.

Co-culture of BV2 microglial cells and 661W photoreceptor cell lines alongside treatment with lipopolysaccharide was used as an additional *in vitro* model of AMD (Bian et al., 2020). mNPC-exos inhibited a series of inflammatory actions including activation of BV2 microglial cells, inflammatory cytokines, and increase of apoptotic 661 W photoreceptor cells. mNPC-exos treatment also downregulated CCL3 and COX-2 inflammatory-related genes, as well as targeted genes that activated a substantial number of pathways including well-known anti-inflammatory pathways, such as interleukin-17, MAPK, and tumor necrosis factor pathways, which can alter RPE or choroidal tissues during AMD.

While most findings from research articles focusing on exosome treatment of AMD demonstrate congruence, findings from Park et al. (2021) tell a different story. BMSC-exos demonstrated no significant protective effect in RCS rats, whether injected subretinal or intravitreal. Instead, they found the cells themselves to be an effective therapy, suggesting that exosomes are not mediating this effect. However, it is to be noted that this paper did not perform statistical significance tests on BMSC-exos injection alone compared to PBS injected control. Park and coauthors also acknowledge the lack of investigation allocated to potentially subtle therapeutic effects that BMSC-exos alone may have on RCS mice.

## Discussion

Concluding based on the limited articles available on retinal exosomal therapy, there is congruent evidence that exosomes or EVs from respective origins specified in their papers can preserve the retinal structure and layers, protect photoreceptors from apoptosis as well as retain retinal function and ERG response (Yu et al., 2016; He et al., 2018; Bian et al., 2020; Deng et al., 2021; Chen et al., 2021b; Wang et al., 2021) in multiple models of AMD. In all studies except Park et al. (2021), exosomes were also observed to exert a therapeutic effect through influencing the expression of various proteins, cytokines, growth factors and mRNA levels. Few papers have also highlighted exosomes' role in blood vessel leakage suppression and CNV inhibition (Hajrasouliha et al., 2013; He et al., 2018; Wang et al., 2021); however, more research is required to support those findings. Individual papers have also demonstrated the involvement of exosomal cargo such as miR-12 and endostatin in its therapeutic action (Hajrasouliha et al., 2013; Deng et al., 2021). While there is valid evidence of exosome or EVs as a promising cell-free therapy, the exact mechanism of action has yet to be fully determined. Some papers have also found rapid diffusion of exosomes or EVs upon administration (Hajrasouliha et al., 2013; Yu et al., 2016) and long-lasting therapeutic effects up to 21 days (Bian et al., 2020; Deng et al., 2021), showing optimistic prospects of exosomes as a possible alternative or adjunctive treatment to anti-VEGF with less risk.

Differential findings were observed by Park et al. (2021) on BMSC-exos and Hajrasouliha et al. (2013) on ARPE-exos in comparison to other literature. A lack of therapeutic effect of BMSC-exos in RCS rodent model may be a methodology discrepancy, as BMSCs were cultured under 1% oxidative stress for 48 hours before exosome isolation and characterization, which could have caused stress-induced changes in the exosomal cargo. Conflicting results found between ARPE-exos and RPE-exos on exosome therapeutic effects may be due to a difference in expressed complement factors between mouse and humans RPE exosomes (Bennis et al., 2015), or a potential difference in protein composition between normal RPE and ARPE-19 cell line. Another reason for the lack of therapeutic effect from RPE-exos may be due to the function of RPE cells (and potentially their exosomes) in the pathogenesis of AMD, including drusen formation, involvement with macrophages and stimulation of inflammatory cytokines, angiogenesis, and vessel formation (Wang et al., 2009; Atienzar-Aroca et al., 2016, 2018; Mukai et al., 2021; Saada et al., 2022).

Therapeutic effects of progenitor cells and MSC-derived exosomes were also found to differ across the literature. After treatment with mNPC-exos, scotopic ERG response was higher at 2–4 weeks and $TUNEL^{+}$ cells were lower at 1–2 weeks compared to hRPC-exos (Bian et al., 2020; Chen et al., 2021b) despite a lower dosage of exosomes used. ERG response from BMSC-exos also appear higher (especially scotopic beta waves) and retinal layers were less

disrupted compared to mouse adipose- or hucMSC-exos in the MNU model, again, despite a lower dose used (Yu et al., 2016; Deng et al., 2021). These findings demonstrate a strong need for direct research into the relationship between dosage and exosomal therapeutic efficacy but simultaneously, also support that exosomal properties depend largely on which cell in the body it is released from and as a result can alter the function and efficacy of exosomes.

In the current literature, exosome therapy is tested on rodent models including MNU, laser retinal injury, RCS, and blue light-induced oxidative stress. While these models partially cover the oxidative stress, local inflammation, and neovascularization of AMD's pathophysiology, there are currently no *in vivo* rodent models available on drusen formation and subsequent buildup of photoreceptor waste product. In addition, current AMD models available are far from perfect, mimicking only limited parts of AMD's multifactorial nature and they do not reflect the exact pathway undergone and pathological features such as the chorioretinal environment in AMD (Shah et al., 2015). Furthermore, current models including MNU and laser retinal injury induce rapid and indiscriminate damage to the retina rather than a progressive development like in AMD, demonstrating a need for further model development or uses of other animal species.

**Exosome therapy in other retinal diseases**

Exosomes have also been researched substantially as a therapeutic tool in various other retinal diseases including diabetic retinopathy (Safwat et al., 2018; Li et al., 2021), glaucoma (Mead et al., 2018a, b) and retinopathy of prematurity (Xu et al., 2019; Cai et al., 2021). In addition, there are also various ongoing clinical trials including on dry eye and diabetes that show great promise in regenerative cell-free therapy and that exosome treatment is not far from reach.

## Future Research Directions

Exosomes are an incredibly novel area of research, particularly with regard to their use as a therapy. This means a variety of hurdles and unknowns still exist in the quest to translate these compounds into viable therapies for AMD patients.

**Definition, quantification, isolation, and characterization**

The exact definition of exosomes and the quantification criteria has yet to reach a consensus (Witwer and Théry, 2019) and while the guidelines established by ISEV go some way to rectify this, it is still contentious in the field and, as evidenced by the studies discussed here, largely ignored. In addition, various methods for the isolation and characterization of exosomes are available, all of which affect the purity and heterogeneity of the sample. Therefore, while highly purified exosomes have been isolated from many different bodily fluids, there is difficulty in comparing study outcomes with similar studies using different isolation/purification protocols. More research should be invested into methods of purifying exosomes to give a good yield of pure exosomes with a balance over cost as well as head-to-head comparisons between methods.

**Mass scale production**

In addition to overcoming isolation and characterization challenges through further research, to facilitate treatment across the world, large-scale production of exosomes is necessary. Isolation methods to produce a high yield to match the demand without compromising cell behavior are needed and demand further investigation (Whitford and Guterstam, 2019).

**Method of administration**

There is a huge variety of administration methods available. Further investigation on form of administration, including periorbital, subretinal, and intravitreal injections (Del Amo et al., 2017) may also prove useful to compare the efficacy and duration of effect as well as assess risks for injection. The delivery of exosomes into the systemic circulation to treat the eye is also a potential given their ability to cross the blood-retinal barrier, but again, needs further investigation regarding efficacy and toxicity.

**Exosome cargo**

As exosomes were first discovered 30 years ago, there are still complex aspects of exosomes within their cargo that have yet to be fully understood, especially due to their heterogeneity and different expression across different cells. Consistent concentrations, cargo contents, and stage of differentiation are essential to facilitate exosome therapy, hence further research should be allocated to identifying and comparing cargo contents and differentiation stage across the same cell type. Despite these low number of studies, however, there is a growing comprehensive database of exosomal cargo (http://www.exocarta.org/) that include many cargo analyses performed on exosomes.

**Toxicology**

If exosomal therapy is translational, exosomes will eventually be administered into humans. While there have been toxicology studies of exosomes on animals (Zhu et al., 2017) demonstrating their safety, some studies have highlighted the potential for toxicity (Cho et al., 2018) that correlates with their cargo (Rokad et al., 2019). The limited studies however highlight that toxicity of exosomes in specific tissues is limited, and with respect to AMD, these potential toxic effects in the eye should be investigated contemporaneously with any development of exosome-based therapies.

## Conclusion

Exosomes or EVs harbor immense potential as viable therapy both in retinal diseases and age-related macular degeneration. However, further research is required to solidify findings as well as investigate the mechanism of action.

**Author contributions:** *LLCC: Conception and design of the manuscript, collection and/or assembly of data, data analysis and interpretation, and manuscript writing; BM: Conception and design and manuscript writing. Both authors contributed equally to this article.*
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**Availability of data and materials:** *All data generated or analyzed during this study are included in this published article and its supplementary information files.*

**Open peer reviewer:** *Amany Tawfik, Oakland University, USA.*
**Additional files:**
***Additional file 1:*** *Open peer review report 1.*
***Additional Table 1:*** *Summary of exosomal or extracellular vesicle therapeutic effect on AMD model.*

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