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Review Article

Advances in exosome therapies in ophthalmology–From bench to clinical trial

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ABSTRACT.

During the last decade, the fields of advanced and personalized therapeutics have been constantly evolving, utilizing novel techniques such as gene editing and RNA therapeutic approaches. However, the method of delivery and tissue specificity remain the main hurdles of these approaches. Exosomes are natural carriers of functional small RNAs and proteins, representing an area of increasing interest in the field of drug delivery. It has been demonstrated that the exosome cargo, especially miRNAs, is at least partially responsible for the therapeutic effects of exosomes. Exosomes deliver their luminal content to the recipient cells and can be used as vesicles for the therapeutic delivery of RNAs and proteins. Synthetic therapeutic drugs can also be encapsulated into exosomes as they have a hydrophilic core, which makes them suitable to carry water-soluble drugs. In addition, engineered exosomes can display a variety of surface molecules, such as peptides, to target specific cells in tissues. The exosome properties present an added advantage to the targeted delivery of therapeutics, leading to increased efficacy and minimizing the adverse side effects. Furthermore, exosomes are natural nanoparticles found in all cell types and as a result, they do not elicit an immune response when administered. Exosomes have also demonstrated decreased long-term accumulation in tissues and organs and thus carry a low risk of systemic toxicity. This review aims to discuss all the advances in exosome therapies in ophthalmology and to give insight into the challenges that would need to be overcome before exosome therapies can be translated into clinical practice.

Key words: clinical trial – exosome – extracellular vesicle – gene delivery – miRNA – nucleic acid – ophthalmology

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Introduction

Extracellular vesicles (EVs) is a broad term given to lipid particles secreted naturally by all cell types to facilitate cell-to-cell interaction. These particles are not self-replicated but instead are produced within the cell. EVs are lipid bilayer-encased extracellular structures with a diameter that can range from 30 to 2000 nm and can be divided into three main types: exosomes, microvesicles and apoptotic bodies (Borrelli et al. 2018) (Table 1). EVs are known for their natural cargo of RNAs, proteins and lipids (Kastelowitz & Yin 2004). Over the last decade, there has been increasing interest in using EVs, in particular exosomes, in therapeutic and regenerative nanomedicine.

Exosomes can be isolated from cultured cells and body fluids, including blood, saliva, urine, amniotic fluid and breast milk (Allenson et al. 2017) (Fig. 1). Their internal contents are tissue-specific and can thus be used to identify disease-specific biomarkers (Roberson et al. 2011; Fais et al. 2016). Another area of interest is the use of exosomes in regenerative medicine. Researchers are currently exploring the potential treatment of degenerative disorders, such as Parkinson's disease, by utilizing exosomes isolated from mesenchymal stem cells (MSC) (Vilaca-Faria et al. 2019; Zhao et al. 2019). Moreover, exosomes can act as an engineered drug vehicle to allow for site-specific drug delivery. Therefore, they can reduce the risk of adverse side effects and improve the therapeutic outcomes, as demonstrated by their recent use in cancer therapy (Frydrychowicz et al. 2014; Khalid et al. 2017).

Many studies have highlighted the variations in exosome cargo, particularly miRNAs, and have proposed that this might be responsible for the therapeutic effects associated with EVs (Sanz-Rubio et al. 2018). MiRNAs are non-coding RNAs, 17-21 nucleotides long, which are responsible for

Table 1. Classification of extracellular vesicles in terms of size, cargo and function.

	Exosomes	Microvesicles	Apoptotic bodies
Diameter	30–100 nm	100–1000 nm	500–2000 nm
Cargo	Proteins and nucleic acids (mRNA, miRNA)	Proteins and nucleic acids (mRNA, miRNA)	Nuclear fragments and organelles
Function	Intercellular communication	Intercellular communication	Phagocytosis and recycling

regulating gene expression at the post-transcriptional level (Frydrychowicz et al. 2014). There has been growing interest in using exosomes and miRNAs in medicine, in particular ocular diseases. Early efforts were made to characterize some of the miRNAs present in the aqueous humour as a potential source of biomarkers for

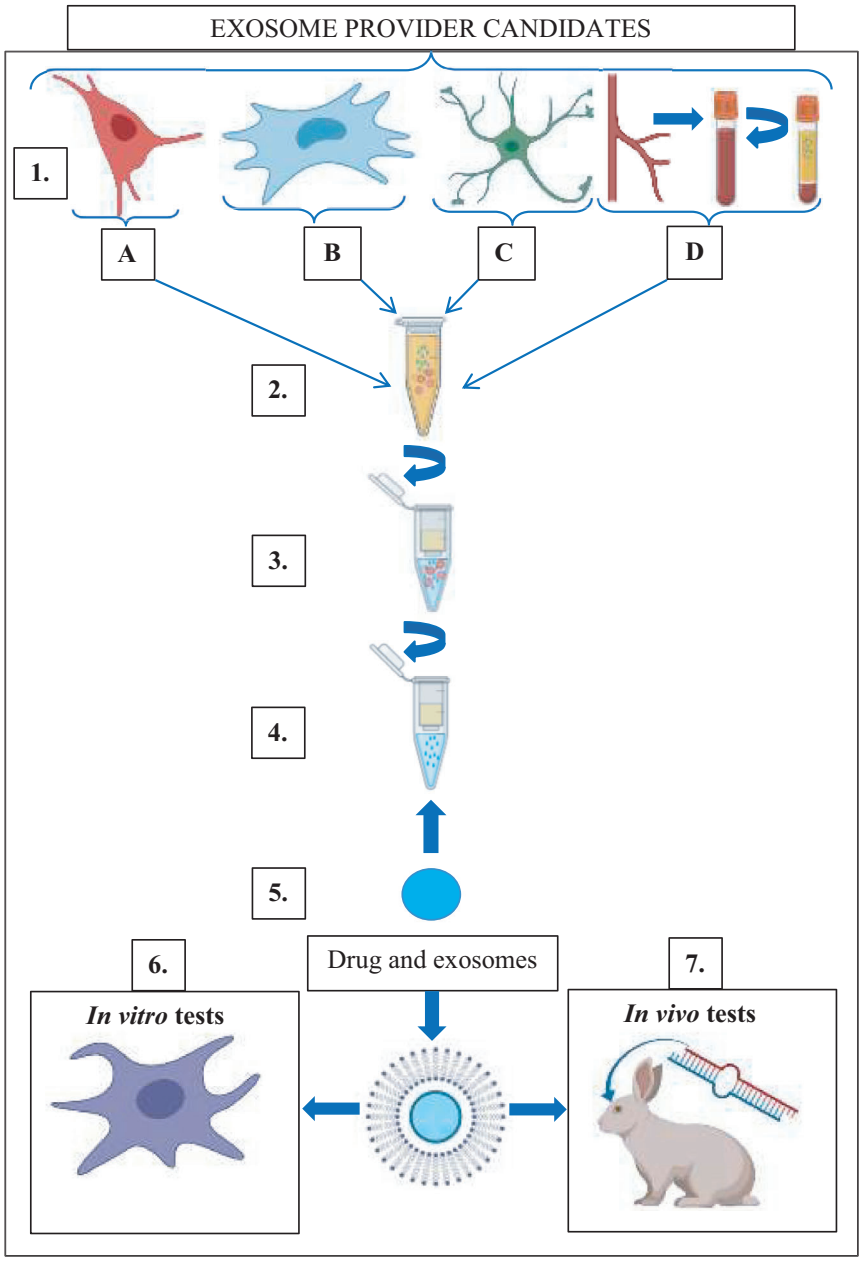

Fig. 1. Study flowchart of exosome isolation, characterization, addition of therapeutic molecules and *in vitro* and *in vivo* testing in ophthalmic diseases. 1. The exosomes are derived from either of these candidates: (A) Mesenchymal stem cells which originate from bone marrow, umbilical-cord tissue or amniotic fluid; (B) Adipose tissue mesenchymal stem cells with positive biomarkers: Sca-1, CD44, CD105, MHC I^{low}, CD71, CD73, CD90; (C) Retinal astroglial cells; (D) Cell-free blood, plasma or biofluids. 2. Exosome isolation by adding ExoQuick to the sample that contains debris, proteins, small vesicles and exosomes. 3. Ultrafiltration to remove debris proteins and small vesicles, and to extract exosomes from the sample. 4. Exosome characterization (Nanosight, microscopy, biomarkers) to ensure they are exosomes. 5. Addition of therapeutic molecules to the exosomes. 6. *In vitro* tests, for example, real-time qPCR for gene expression and cell viability. 7. *In vivo* tests, for example, local and systemic toxicity.

disease prognosis and diagnosis, particularly in glaucoma (Dismuke et al. 2015). Furthermore, other studies have established links between specific miRNA cargos and therapeutic outcomes (Safwat et al. 2018; Zhang et al. 2019).

This review aims to give a comprehensive detailed overview of the recent studies that have utilized exosome therapies in a variety of ophthalmic diseases, including glaucoma, dry eye disease, retinal ischaemia, diabetic retinopathy, age-related macular degeneration, uveitis and optic neuropathy. We will also discuss the several challenges that would need to be overcome before exosome miRNA therapies can be translated to patients in the clinic.

Exosomes in Glaucoma

Glaucoma is the leading cause of irreversible blindness globally (Flaxman et al. 2017). It is estimated that the prevalence of glaucoma cases will rise to 112 million by 2040, with current cases affecting 76 million people in the world (Tham et al. 2014). Glaucoma is a degenerative disease that targets the optic nerve and it is often associated with elevated intraocular pressure (Henson & Thampy. 2005). The elevated intraocular pressure in glaucoma leads to the loss of retinal ganglion cells (RGCs) and blindness. RGCs convey visual information from the retina to the brain and once these cells are lost, they cannot be regenerated (Almasieh et al. 2012). Although the mechanism by which these cells degenerate is not well understood, studies have shown that potential contributors include neuroinflammation, oxidative stress, lack of neurotrophic factors, mitochondrial dysfunction and axonal transport failure (Almasieh et al. 2012; Syc-Mazurek & Libby 2019).

Exosomes extracted from ocular fluids present an opportunity to screen for notable biomarkers and can act as a prospective diagnostic test to support clinical decision-making. Dismuke et al. (2015) analysed aqueous humour samples and noted the presence of key miRNAs, which included miR-486-5p, miR-184, miR-204, miR-181a, miR-191, miR-148a, miR-26a, miR-125a-5p, let-7a and let-7b. The authors of this study also reported that the two most abundant miRNAs between exosomes extracted from aqueous humour and the conditioned media of primary human trabecular meshwork cells were miR-191 and miR-26a.

There have been many documented pathways associated with glaucoma and the impact that these can have on the progression of the disease (Gauthier & Liu 2017). Liu et al. (2016) found that the expression of miR-182 was significantly higher in exosomes derived from human trabecular meshwork cells, and stated that miR-182 may be involved in the pathogenesis of primary open-angle glaucoma through regulation of aqueous humour dynamics and intraocular pressure. A different study by Lerner et al. (2020) focussed on extracting exosomes from a non-pigmented ciliary epithelium (NPCE) cell line and studied their effects on the expression of Wnt proteins in a trabecular meshwork cell line. They cross-matched their miRNA findings with previously published aqueous humour miRNA profiles and found 77 similar miRNA profiles. The most abundant miRNAs found in the NPCE exosomes were miR-21, miR-638, let-7a, miR-100 and miR-16. Many of these miRNAs have been found to regulate Wnt signalling in the trabecular meshwork. Other identified miRNAs, such as miR-29, miR-17 and miR-21, were also found to modulate collagen production. These findings suggest that exosomes derived for NPCE can modulate the Wnt pathway in trabecular meshwork cells. However, further studies of the mechanisms by which exosomes modulate the Wnt pathway in the trabecular meshwork are needed to help identify new possible targets for therapeutic approaches to treat primary open-angle glaucoma.

Recent studies have tried to overcome the effects of glaucoma in animal models using exosomes isolated from bone marrow stem cells (BMSC) (Table 2). A study by Mead et al. (2018a) used rodents and divided them into three groups. Group 1 (n = 5) had rodents that were uninjured and untreated, whereas Group 2 (n = 30) and Group 3 (n = 35) had rodents injured using intracameral microbeads or laser photocoagulation of the trabecular meshwork, respectively, in order to induce elevated intraocular pressure. Both Groups 2 and 3 were treated with BMSC exosomes or fibroblast exosomes (control) in either a weekly or a monthly intravitreal injection plan. The authors reported that the use of weekly and monthly intravitreal BMSC exosomes promoted neuroprotection of RGCs.

Furthermore, Mead et al. (2018b) conducted another study using BMSC exosomes on DBA/2J mice over a 12-month period, administering monthly intravitreal injections. They similarly found that BMSC exosomes reduced the number of degenerating axons in the optic nerve. This group also carried out an *in vitro* study using human RGCs derived from human embryonic stem cells (Mead et al. 2020). Their results confirmed their previous findings and showed that human BMSC exosomes provided significant neuroprotection to injured human RGCs and promoted RGC survival. None of the studies reported any adverse side effects with the use of BMSC exosomes.

Although the *in vitro* and *in vivo* studies have shown promising results that BMSC exosomes may be a viable therapeutic approach to treat glaucoma in the future, a major challenge is that the neuroprotective effects appear to be short-term. The duration of the first study in rodents only lasted up to 56 days (Mead et al. 2018a) and the second study's long-term results also demonstrated that RGC function deteriorated over time. Moreover, BMSC exosomes were not effective after 6 months of treatment and provided no therapeutic benefit between 9 and 12 months (Mead et al. 2018b). Repeated intravitreal injections are thus likely to be needed every few months to maintain the therapeutic levels, which would increase the risk of potential adverse effects and the number of hospital visits needed for glaucoma patients.

Mechanistically, Mead et al. (2018a) have reported that the neuroprotective effects might be related to the miRNA cargo of BMSC exosomes, namely MIR-106A-5P, MIR-486-5P, MIR-144-5P, MIR-126-5P and MIR-100-5P. Alternatively, the effects of BMSC exosomes might be due to the modulation of their mRNA cargo and TNFα priming through increased levels of pigment epithelium-derived factor (PEDF) and vascular endothelial growth factor (VEGF-A) (Mead et al. 2020). However, the miRNA neuroprotective effects on RGCs are still

Table 2. Exosome miRNA therapies in ophthalmology.

Disease	References	Species	miRNAs	Therapeutic effects	Adverse effects
Glaucoma	Mead et al. (2018a)	Rodents	The neuroprotective effects might be related to the miRNAs from BMSC exosomes, namely MIR-100-5P, MIR-106A-5P, MIR-486-5P, MIR-144-5P, MIR-126-5P, MIR-100-5P	BMSC exosomes promoted neuroprotection of RGC and reduced degenerating axons in the optic nerve	Nil
	Mead et al. (2018b)	DBA/2J mice	The effects might be related to the miRNAs from BMSC exosomes	Human BMSC exosomes provided neuroprotection to the injured RGC and promoted RGC survival	Nil
	Mead et al. (2020)	Rats	The effects of MSC exosomes might be due to the modulation of their mRNA cargo and TNFα priming through increased levels of PEDF and VEGF-AA	MSC exosomes stimulated RGC survival resulting in neuroprotective effects in rats and human RGC	Nil
Dry eye disease	Weng et al. (2012)	Humans	The precise regulation role of MSCs has not been fully defined	MScs can suppress the inflammatory and fibrous processes in dry eye in cGvHD by targeting specific $CD8^{+}CD28^{-}$ T cells	Nil
Retinal ischaemia	Mosseiev et al. (2017)	C57BL/6 mice	The effects of human MSC might be due to the paracrine factors and miRNAs	Human MSC exosomes reduced retinal thinning and neovascularization	Nil
	Hajrasouliha et al. (2013)	C57BL/6 mice	The antiangiogenic effects of RAC exosomes might be caused by their multiple molecules (proteins, lipids, mRNA, miRNA)	Exosomes from mice RAC reduced choroidal neovascularization	Nil
	Ma et al. (2020)	Sprague-Dawley rats	The effects of MSC exosomes might be due to certain mRNAs, miRNAs and proteins	MSC exosomes prevented photoreceptor apoptosis post-retinal detachment, promoted anti-inflammatory effects and suppressed inflammatory cytokine induction	Nil
Diabetic retinopathy	Safwat et al. (2018)	Rabbits	The regenerative changes in the retina might be associated with the increased expression of miRNA-222	Adipose MSC exosomes induced repair of diabetic retinal degeneration and mediated tissue repair by transporting specific miRNAs	Nil
	Zhang et al. (2019)	Rats	miRNA-126 expression in MSC exosomes might reduce retinal inflammation by downregulating the high-mobility group box 1 (HMGB1) pathway	MSC exosomes reduced the hyperglycaemia-induced retinal inflammation	Nil
Age-related macular degeneration (AMD)				Elbay et al. 2019	Humans
Wet AMD blood serum exosomes showed increased expression of miR-626 and miR-485-5p and decreased expression of miR-885-5p.	Nil		Potential increased wet AMD and choroidal neovascularization		
Uveitis	Bai et al. (2017)	Lewis rats	The effects of human MSC exosomes might be due to their cargo which is abundant in proteins and RNAs	Human MSC exosomes inhibited the autoimmune response, protected the retinal structure and rescued retinal function	Nil
	Shigemoto-Kuroda et al. (2017)	Rats	The therapeutic effects of MSCs in suppressing inflammation correlated with the TSG-6 mRNA level in MSCs	Human MSCs from different donors varied in their therapeutic effects in suppressing inflammation *in vivo,* and some MSCs failed to have any positive therapeutic effects	Nil
Optic neuropathy	Mead and Tomarev (2017)	Sprague-Dawley rats	The therapeutic effects might be caused by specific miRNAs, namely miR-17-92	BMSC exosomes promoted neuroprotection and neurogenesis of RGC. The expression of phosphatase	Nil

Table 2 (Continued)

Disease	References	Species	miRNAs	Therapeutic effects	Adverse effects
				and tensin homolog (PTEN), an important suppressor of RGC axonal growth and survival, was downregulated	
	Pan et al. (2019)	Wistar rats	The most abundant miRNAs in human UMSC exosomes are miRNA-21-5p, miRNA-125b-5p, miRNA-23a-3p, miRNA-100-5p and let-7f-5p	UMSC exosomes promoted RGC survival and glial cell activation	Nil

BMSC = bone marrow stem cells; MSC = mesenchymal stem cells; RAC = retinal astroglial cells; RGC = retinal ganglion cells; UMSC = umbilical-cord mesenchymal stem cells.

largely unknown and will require further investigation. Further studies would also be beneficial regarding the long-term efficacy of exosome therapy and to determine whether these preclinical results could be translated to glaucoma patients in the future.

Exosomes in Dry Eye Disease

Dry eye disease (DED), also known as keratoconjunctivitis sicca, is a common condition associated with tear film disruption and damage of the ocular surface. Even though the symptoms can be varying, they can be generally categorized into tear-deficient dry eyes and evaporative dry eyes (Lemp et al. 2007). Current treatments vary from the administration of artificial tears for mild and moderate DED to the administration of autologous serum eye drops and anti-inflammatory drugs for severe cases (Pflugfelder et al. 2007). Chronic graft versus host disease (cGvHD) is a condition that can occur after bone marrow transplantation, as the donor cells can view the recipient's body as foreign and attack it, causing inflammation (Lee et al. 2003). The cGvHD is characterized by dry eyes, joint pain, rashes and shortness of breath, with dry eyes being the most common symptom affecting 40–76% of patients (Lee et al. 2021).

MSCs have been considered as an alternative therapeutic approach for dry eyes in cGvHD. In a 2012 study by Weng et al. (2012), the group investigated the efficacy of MSCs for the treatment of cGvHD-related dry eye disease as the most important clinical feature of MSCs is their immunomodulatory role and their ability to assist in controlling inflammatory diseases (Table 2). The authors concluded that the infusion of MSCs improved clinical symptoms in 54.6% of the patients, presenting a promising alternative treatment for dry eye disease.

Based on this study and the knowledge that exosomes derived from MSCs have many of the same immunoregulatory properties as their cells of origin, the same group registered for a phase 1/2 clinical trial in December 2019 (NCT04213248). This single-arm trial aimed to determine whether umbilical MSC (UMSC)-derived exosomes can alleviate dry eye symptoms in patients with cGvHD and to evaluate the clinical outcomes. The patients will be treated with artificial tears for 2 weeks, in order to establish a baseline, followed by 10 μg/drop of UMSC exosomes four times a day for 14 days. Disease indicators, such as the amount of tear secretion, tear break time and the degree of damage to tissues, will be recorded and assessed for 12 weeks after treatment.

To the best of our knowledge, this is the first and only clinical trial utilizing exosomes in ophthalmology. If successful, this study will be a breakthrough and proof of concept for the use of exosomes as an alternative therapeutic approach for the treatment of ocular diseases. It has to be noted that the estimated completion date for this trial was May 2020, however it still remains in the recruiting phase.

Exosomes in Retinal Ischaemia

The retina is a light-sensitive tissue forming the innermost layer at the back of the eye and consists of photoreceptors that absorb light impulses. These light impulses then travel through the optic nerve to the brain to process the image seen through our eyes. For the retina to function, it requires an adequate supply of oxygen and nutrients derived from the blood supply via the central retinal artery. If the blood supply is reduced, the retinal tissues can become ischaemic, which then leads to loss of vision. Several conditions can cause retinal ischaemia, including diabetic retinopathy and retinal artery occlusion (Minhas et al. 2012).

To study the protective effects of MSC-derived exosomes in retinal ischaemia, Mosseiev et al. (2017) used 12 male C57BL/6 mice in a 2-week study and split them into three groups (n = 4 mice in each group) (Table 2). Mice in the first and second groups were placed in a closed chamber at 75% oxygen for 5 days to cause oxygen-induced retinopathy. Mice in the first group were then injected with saline, whereas mice in the second group were injected with exosomes derived from human MSC. Mice in the third group (control group) were kept in standard room air and were injected with saline. The authors reported that human MSC exosomes were well-tolerated and had a positive therapeutic effect on retinal ischaemia, leading to a significant reduction in retinal thinning and neovascularization. They also suggested that the therapeutic effects might be due to the paracrine factors and miRNAs of human MSC exosomes (Mosseiev et al. 2017).

These findings were further supported by an earlier study by Hajrasouliha et al. (2013) who used exosomes from mice retinal astroglial cells (RAC) to significantly suppress retinal vessel leakage and to inhibit choroidal neovascularization (CNV) in a laser-induced C57BL/6 mice model. These studies show that exosome therapy could provide a more targeted therapeutic approach to the damaged tissue and limit the treatment's adverse effects. The antiangiogenic effects might also be

related to the multiple molecules (proteins, lipids, mRNA, miRNA) of RAC exosomes (Hajrasouliha et al. 2013).

A more severe form of retinal ischaemia can occur if the retina becomes detached from the retinal pigment epithelium. Some of the consequences of a retinal detachment include atrophy of the photoreceptor cell layer, retinal thinning and retinal pigment epithelium alterations (Ghazi & Green 2002). A study conducted by Ma et al. (2020) used MSC exosomes from Sprague-Dawley rats to study their effects in retinal detachment. They found that MSC exosomes were able to prevent photoreceptor apoptosis following retinal detachment in the rat model. The MSC exosomes also promoted anti-inflammatory effects and suppressed inflammatory cytokine induction. Similar to the studies in glaucoma, this study showed that exosome treatment had beneficial neuroprotective effects (Mead et al. 2020).

MSC exosomes represent a promising therapy for retinal ischaemia with no adverse side effects reported in the studies. However, further dose-response studies are needed to determine the mechanism of action and any potential long-term treatment effects. Immunogenicity is also a potential concern for clinical translation in patients. Interestingly, (Mosseiev et al. (2017) used human MSC exosomes on mice in their study and found no immunogenicity Mosseiev et al. (2017). However, it is unknown if animal-derived exosomes would be compatible with human retinal disease models and whether exosomes would need to be matched to specific patients in clinical practice. Many of these preclinical studies have been conducted on animal models; however, future studies will need to test whether the same effect can be achieved when using exosome therapies in humans.

Exosomes in Diabetic Retinopathy

The prevalence of diabetes was over 3.9 million people in the UK in 2019 (Diabetes UK, 2020). Diabetic retinopathy is a common complication of diabetes and is one of the leading causes of blindness in the working-age population (Wang & Lo 2018). Diabetic retinopathy can be divided into two main types: non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). Clinical manifestations of NPDR are microaneurysms, retinal haemorrhages and intraretinal microvascular abnormalities, whereas PDR is characterized by neovascularization. Both types of diabetic retinopathy can lead to diabetic macular oedema (Stitt et al. 2016).

Recent studies utilizing exosome miRNA therapy have shown promising results in diabetic retinopathy (Table 2). Safwat et al. (2018) induced diabetes in a rabbit model using streptozotocin intravenous injections. Exosomes were extracted from MSCs isolated from rabbits' adipose tissue and were injected in the diabetic rabbits using intravenous, subconjunctival or intraocular routes (Safwat et al. 2018). Interestingly, different routes of exosome administration resulted in different therapeutic effects on the retina. Twelve weeks post-injection, subconjunctival exosome treatment restored the retina's cellular components, whereas intraocular exosome treatment encouraged retinal regeneration and formed well-defined layers like those in a normal retina. Conversely, systemic intravenous exosome treatment increased retinal thickness with an irregular ganglion layer. The authors found an increased expression of miRNA-222 in the retinal tissue of the rabbits treated with exosomes and proposed that MSC exosomes could mediate regenerative changes in the retina by transporting specific miRNAs like miRNA-222 (Safwat et al. 2018).

Another study by Zhang et al. (2019) showed that MSC exosomes overexpressing miRNA-126 were able to reduce the retinal inflammation caused by hyperglycaemia in a rat model. MSC exosomes were isolated from the media of human umbilical cord-derived MSCs, followed by the transfer of miRNA-126. The authors reported that the administration of miRNA-126-expressing MSC exosomes significantly reduced the hyperglycaemia-induced expression of the high-mobility group box 1 (HMGB1) pathway and the activity of the NLRP3 inflammasome in human retinal endothelial cells (Zhang et al. 2019). Although the results are promising with no adverse effects reported in both studies, the role of MSC exosomes and specific miRNAs in the process is still unknown, and further studies will be needed to explore this further. A later study by Huang et al. (2018) also found that in the mouse model, there was an association with IgG-laden exosomes in diabetes that led to the activation of the classical complement pathway.

Exosomes in Age-Related Macular Degeneration

Age-related macular degeneration (AMD) is the leading cause of central vision loss in the elderly population in the western world. It was projected that in 2020, 196 million people were affected with AMD and by 2040, the number is likely to increase to 288 million (Wong et al. 2014). AMD is a progressively chronic disease on the central retina and there are 2 main forms: dry AMD and wet AMD (also known as neovascular AMD). Dry AMD is characterized by drusen formation and atrophy of the retinal pigment epithelium layer (Arya et al. 2018), whereas choroidal neovascularization in wet AMD leads to the leaking of fluids, lipids and blood that can cause fibrous scarring (Lim et al. 2012).

Studies have been carried out to investigate the exosome cargo of retinal pigment epithelium (RPE) cells in order to develop innovative therapies and to understand the mechanisms that cause this disease. A study conducted by Atienzar-Aroca et al. (2016) found that stressed RPE cells release a high quantity of exosomes containing mRNA for VEGFR-1 and VEGFR-2. These are receptors for VEGF that play a vital role in new blood vessel formation. Further work by this group identified that VEGFR-2 is activated in abnormal angiogenesis by receptors released from RPE-derived exosomes. In addition, the authors reported that exosomes released from stressed RPE cells promote autophagy, which furthers the progression of AMD (Atienzar-Aroca et al. 2018).

Kang et al. (2014) also looked into identifying biomarkers for wet AMD from exosomes derived from the aqueous humour of AMD patients and from ARPE-19 cell line and identified six proteins of interest. Elbay et al. (2019) looked into extracting exosomes from the blood serum of patients with wet AMD (n = 70) and notably found that the expression of miR-626 and miR-485-5p was increased when compared to control patients (n = 50)

(Table 2). However, an underexpression of miR-885-5p when compared to the control group was observed. Although these miRNAs could act as targets for future therapies, it cannot be confirmed whether these markers are the same for all forms of AMD. The authors pointed out that their sample size did not include subjects with early AMD, and therefore, it cannot be determined yet whether these markers could be used for patients with earlier stages of AMD or more subtle signs of the disease.

Exosomes in Uveitis

Uveitis is a disease caused by inflammation of the iris, ciliary body, vitreous, retina or choroid (Seve et al. 2017). It can lead to irreversible visual loss if not treated in a timely manner, and current treatments include immunosuppressive therapy and corticosteroids (Chen & Sheu 2017). Exosome therapy has also been explored as a potential treatment in uveitis.

In one study, Bai et al. (2017) used Lewis rats which were induced with experimental autoimmune uveoretinitis (Table 2). Human MSC exosomes were shown to inhibit autoimmune response in the rat model, thereby protecting the retinal structure and rescuing retinal function. The effects of human MSC exosomes might be due to their cargo which are abundant in RNAs and proteins (Bai et al. 2017). The mechanism through which human MSC exosomes can act as an immunosuppressant remains unclear and will need to be assessed in future studies.

Shigemoto-Kuroda et al. (2017) carried out further work to support the role of human MSC exosome therapy in rat models. They found that the therapeutic efficacy of MSCs in suppressing inflammation correlated with the mRNA level of TNF-α-simulated gene 6 (TSG-6) in those MSCs. However, they reported that a challenge with human MSC exosome therapy is that exosomes are heterogeneous depending on their source and environment. Human MSC from different donors varied in their therapeutic effects in suppressing inflammation *in vivo,* and some human MSC had no positive therapeutic effect. This could act as a major limitation of exosome therapy in humans, as exosomes derived from a specific patient may have limited or no therapeutic effect. Exosomes could potentially be donated from other patients and used for treatment, but there is uncertainty on whether this would elicit a deleterious immune response. The source of exosomes thus needs careful consideration when developing MSC exosome therapies for use in patients in the future.

Exosomes in Optic Neuropathy

Optic neuropathy or optic atrophy refers to the pathological damage of the optic nerve or its blood supply. It is most commonly caused by optic neuritis and ischaemic optic neuropathy and can eventually lead to loss of vision (Dworak & Nichols. 2014). Optic nerve crush is an experimental disease model used in research to induce optic neuropathy. It leads to the loss of RGCs, which can be useful to understand the neuronal mechanisms and to develop new therapies (Tang et al. 2011). Crushing the optic nerve in the rodent model leads to an abrupt loss of RGCs after day 5, followed by a loss of 50% and over 90% by day 7 and day 14, respectively (Berkelaar et al. 1994).

Recent studies have looked at the neuroprotective effects of exosome therapies in optic neuropathy (Table 2). Mead and Tomarev (2017) found that BMSC exosomes promoted neuroprotection and neurogenesis of RGCs in primary retinal cultures. The authors also reported similar results in their *in vivo* studies, which were done over 21 days following optic nerve crush in Sprague-Dawley rats. As part of the treatment plan, exosomes were injected intravitreally every week after the optic nerve crush. The rats treated with BMSC exosomes only had a 30% loss of RGCs, whereas untreated rats displayed 80-90% loss of RGCs after 21 days following the optic nerve crush. BMSC exosomes promoted survival of RGCs and regeneration of their axons, and partially prevented RGC axonal loss and RGC dysfunction. BMSC exosomes contain several miRNAs, particularly miR-17-92, which downregulate the expression of phosphatase and tensin homolog (PTEN) and are an important suppressor of RGC axonal growth and survival (Zhang et al. 2017).

Pan et al. (2019) built on this study and used 18 adult Wistar rats that were divided into three groups. The first group were uninjured, untreated rats. The rats in the second group underwent optic nerve crush and were treated with exosomes derived from umbilical-cord MSC (UMSC), whereas the rats in the third group underwent optic nerve crush and were treated with saline. After three treatments with an interval of 1 week, the authors found that the UMSC exosomes significantly promoted RGC survival and glial cell activation compared to saline controls. However, UMSC exosomes were not able to promote axonal regeneration in the optic nerve like the BMSC exosomes (Mead & Tomarev 2017). The authors proposed that this could be due to the differences in miRNA cargo between BMSC exosomes and UMSC exosomes. The five most abundant miRNAs in human UMSC exosomes were miRNA-21-5p, miRNA-125b-5p, miRNA-23a-3p, miRNA-100-5p and let-7f-5p (Fang et al. 2016), whereas the five most abundant miRNAs in BMSC exosomes were miR-143-3p, miR-10b-5p, miR-486-5p, miR-22-3p and miR-21-5p (Baglio et al. 2015).

These studies demonstrated that the regular administration of BMSC exosomes had a beneficial neuroprotective effect. However, further dose-response studies will be required to determine the optimum dosage and frequency of treatment. Previous work in other disease models has shown that exosomes do not exert any therapeutic effects after six months (Mead et al. 2018). Further studies to investigate whether outcomes improve after long-term treatment will need to be carried out in the future. Moreover, since these studies showed limited therapeutic effects in the form of axonal regeneration for optic nerve injuries, it would be interesting to study the effects of exosomes from alternative sources and whether they could further improve the therapeutic effects.

Discussion

In recent years, there has been a growing interest in developing exosome therapies in ophthalmology. They are considered natural novel therapeutic agents largely due to their internal contents, in particular, their unique miRNA cargo. The studies discussed

in this review highlight the therapeutic effects of using exosomes extracted from various sources and their ability to induce desirable effects in overcoming common eye diseases. However, the studies did not conduct any experiments to assess toxicity, which is an important factor we must consider. We have limited information on whether there is any toxicity noted when administering exosome therapies in these studies. Zhu et al. (2017) reported minimal toxicity and immunogenicity when administering sustained doses of exosomes derived from HEK293T cells in mice. However, it cannot be concluded whether this would be the same for all exosomes from various sources.

While there are current alternative therapies looking into the use of lipid nano-molecules and gene-based therapies, one of the many challenges in creating a new drug complex is creating an efficient delivery system. Fortunately, exosomes offer the ability to be modified to allow specificity and due to their small size, they can overcome biological barriers that conventional medications and gene therapies are not able to do. It has been noted that exosomes are able to penetrate the most difficult of barriers, including the blood-brain barrier (Batrakova & Kim, 2015). However, a paper by Riau et al. (2019) highlighted the concerns regarding the sustained release of administered exosomes. When exosomes were injected, they were cleared from the blood circulation and accumulated in the liver, spleen, lung and gastrointestinal tract after only two hours. Topical application of exosomes poses the same concerns but are likely to clear at a much rapid rate due to the fluid turnover of tears or sweat. Moreover, with the variation in exosome extraction and purification, it may not be possible to mass produce exosomes that are of good quality to allow for continued administration to induce therapeutic effects (Ayala-Mar et al. 2019). Therefore, to facilitate treatment with smaller quantities of exosomes, it has been suggested to embed exosomes into hydrogel matrices to facilitate a sustained release and to prevent premature clearance, leading to a more localized and concentrated dosage at the target site. This can act as a promising solution to overcome the large quantities of exosomes required to elicit a therapeutic response (Riau et al. 2019).

As exosome technology is emerging, there is an element of experimental approach when extracting exosomes from various sources. Ludwig et al. (2019) concluded that currently there is no gold standard in exosome isolation, therefore, variation in isolation technique may influence the findings of studies using exosome therapies. In addition, there is also an unmet need for standardizing exosome quality and minimum standards of requirement before it can be clinically administered for treatment in patients (Xu et al. 2020).

Furthermore, underpinning the exact mechanisms by which many of these ocular diseases progress is imperative so that drugs can be created to target specific pathways. As exosomes are critical in cell-to-cell communication, understanding how the miRNA cargo differs in healthy and affected individuals may highlight key differences and potential prominent pathways of action. Hence, the approach to extract exosomes to identify key biomarkers could determine whether it may be beneficial to upregulate or downregulate specific pathways. Many ocular diseases are polygenic and are, therefore, influenced by many pathways. In glaucoma, some of the key pathways include TGF-β signalling, Phosphatase and Tensin Homologue (PTEN) Pathway and Rho Kinase, which can be activated by a variety of factors including Wnt (Gauthier & Liu, 2017). While the studies discussed in this review highlighted the impact of naturally occurring miRNAs, exosomes present an opportunity to be modified and used as an organic nanovesicle. There is a potential to create exosomes with modifications, such as additional regulating molecules like siRNAs, to further enhance the existing benefits of exosome therapies (Fernando et al. 2018; Gupta et al. 2021; Sanghani et al. 2021). Another approach could also be to create nanovesicles with targeting moieties (Tagalakis et al. 2017) and the addition of natural glycosides (Weng et al. 2015) that could increase the uptake of exosomes in the cells of interest and promote the release of their cargos into the cytoplasm.

While interest in this field continues to grow, we require further in-depth *in vivo* studies that may translate into human clinical trials. As highlighted above, to our knowledge, there is currently only one clinical trial underway but we expect this number to grow in the future. It would also be interesting to compare if the elicited therapeutic effects of exosome therapies are an improvement to current treatment options available to patients and which treatment option offers the best long-term prospects in reducing ocular diseases.

Conclusion

Currently, the possibility of exploring exosome therapies to overcome ophthalmological diseases proves to be a promising field. However, it is yet to be determined whether these therapeutic effects can be replicated in human clinical trials. If these prove successful, it should be investigated whether they will have the same long-term desirable outcomes and minimal adverse effects. There is also a need to standardize the techniques associated with exosome extraction and to determine a universal standard of exosome quality before any form of exosome therapy can be offered to patients with ocular diseases.

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