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PERSPECTIVE

A perspective from the National Eye Institute Extracellular Vesicle Workshop: Gaps, needs, and opportunities for studies of extracellular vesicles in vision research

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Abstract

With an evolving understanding and new discoveries in extracellular vesicle (EV) biology and their implications in health and disease, the significant diagnostic and therapeutic potential of EVs for vision research has gained recognition. In 2021, the National Eye Institute (NEI) unveiled its Strategic Plan titled 'Vision for the Future

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(2021–2025),' which listed EV research as a priority within the domain of Regenerative Medicine, a pivotal area outlined in the Plan. In alignment with this prioritization, NEI organized a workshop inviting twenty experts from within and beyond the visual system. The workshop aimed to review current knowledge in EV research and explore gaps, needs and opportunities for EV research in the eye, including EV biology and applications of EVs in diagnosis, therapy and prognosis within the visual system. This perspective encapsulates the workshop's deliberations, highlighting the current landscape and potential implications of EV research in advancing eye health and addressing visual diseases.

KEYWORDS
Eye, EVs, exosomes, diagnosis, ocular, prognosis, therapy, vision

1 | INTRODUCTION

Extracellular vesicles (EVs) are heterogenous lipid membrane-bound nanoparticles (e.g., exosomes, ectosomes, microvesicles, apoptotic bodies) released by cells, facilitating cell–cell communication through their varied cargo, including RNA, DNA, proteins and lipids (Ludwig & Giebel, 2012; Ratajczak et al., 2006; Valadi et al., 2007; Van Der Pol et al., 2012; Welsh et al., 2024; Wortzel et al., 2019). Concurrently with the rapid growth of the EV field in medicine and bioengineering, EVs have emerged as significant contributors to eye health and disease. Their potential as diagnostic markers and a cell-free approach for regenerative medicine has also attracted special attention for translational applications to treat eye diseases. However, the full diagnostic and therapeutic capabilities of EVs in vision research are yet to be fully explored. Additionally, the fundamental comprehension of EV biology within the visual system is still in its early stages.

The eye, with its unique anatomy (Kels et al., 2015; Ludwig, Aslam et al., 2024; Ludwig, Jessu et al., 2024; Ludwig, Lopez et al., 2024), initially admits light through the transparent cornea, where it undergoes its first refraction. Subsequent to this, the aqueous humour and crystalline lens collaborate to further refract and focus the light onto the retina at the back of the eye. This delicate process facilitates the formation of a clear and inverted image on the retina, thus initiating the transmission of visual signals to the brain via the optic nerve. Both the cornea and the retina are composed of unique cell types and layers that contribute to their physiology (Figure 1) and thus, studying EV biogenesis, transport, release and uptake in these cells requires tailored approaches (Kolb et al., 1995). In addition to its unique anatomy and cell composition, the eye possesses several characteristics that distinguish it, including a relatively small scale, immune-privileged organ physiology with blood-ocular barriers, the presence of its own biofluid and accessibility for local drug delivery. Vision research has been at the forefront of technological advancements, exemplified by the introduction of optical coherence tomography, anti-vascular endothelial growth factor intraocular therapy and ocular gene therapy (Aiello et al., 1994; Bouma et al., 2022; Rosenfeld et al., 2006; Russell et al., 2017). Therefore, vision research holds significant potential to pioneer developments in the field of EVs, presenting a special opportunity for exploration and advancement.

In line with this aspiration, the National Eye Institute (NEI) identified EV research as a top priority in Regenerative Medicine, one of seven cross-cutting areas of emphasis in the NEI Strategic Plan: Vision for the Future (2021–2025) (Chiang, 2021; Chiang & Tumminia, 2022). As part of the implementation efforts for EV research as a priority, the NEI organized the Extracellular Vesicle Workshop, bringing together a diverse group of experts in EV research. The workshop aimed to review current EV studies, explore the utility of EVs, and identify critical knowledge gaps, needs and opportunities for studying EVs in eye health and disease.

The current perspective seeks to offer insights from an EV workshop discussion, covering three key sections: (1) EVs from biology to biomarkers, (2) EVs for regenerative medicine and (3) Tools and approaches for the rigorous study of EVs. Additionally, it aims to share a summary of the roundtable discussion on gaps, needs and opportunities. While beyond the scope of the workshop, there are numerous review articles related to EV biology (Dixson et al., 2023; van Niel et al., 2018; 2022), therapeutic implications for EVs (Carney et al., 2024; Kalluri & LeBleu, 2020; Németh et al., 2024) and EVs related to eye health and disease (Anand et al., 2022; Harrell et al., 2023; Pedersen et al., 2024) that may provide additional context. The objective of this summary is to provide guidance to current and future investigators, fostering acceleration of the advancement of EV research in vision science.

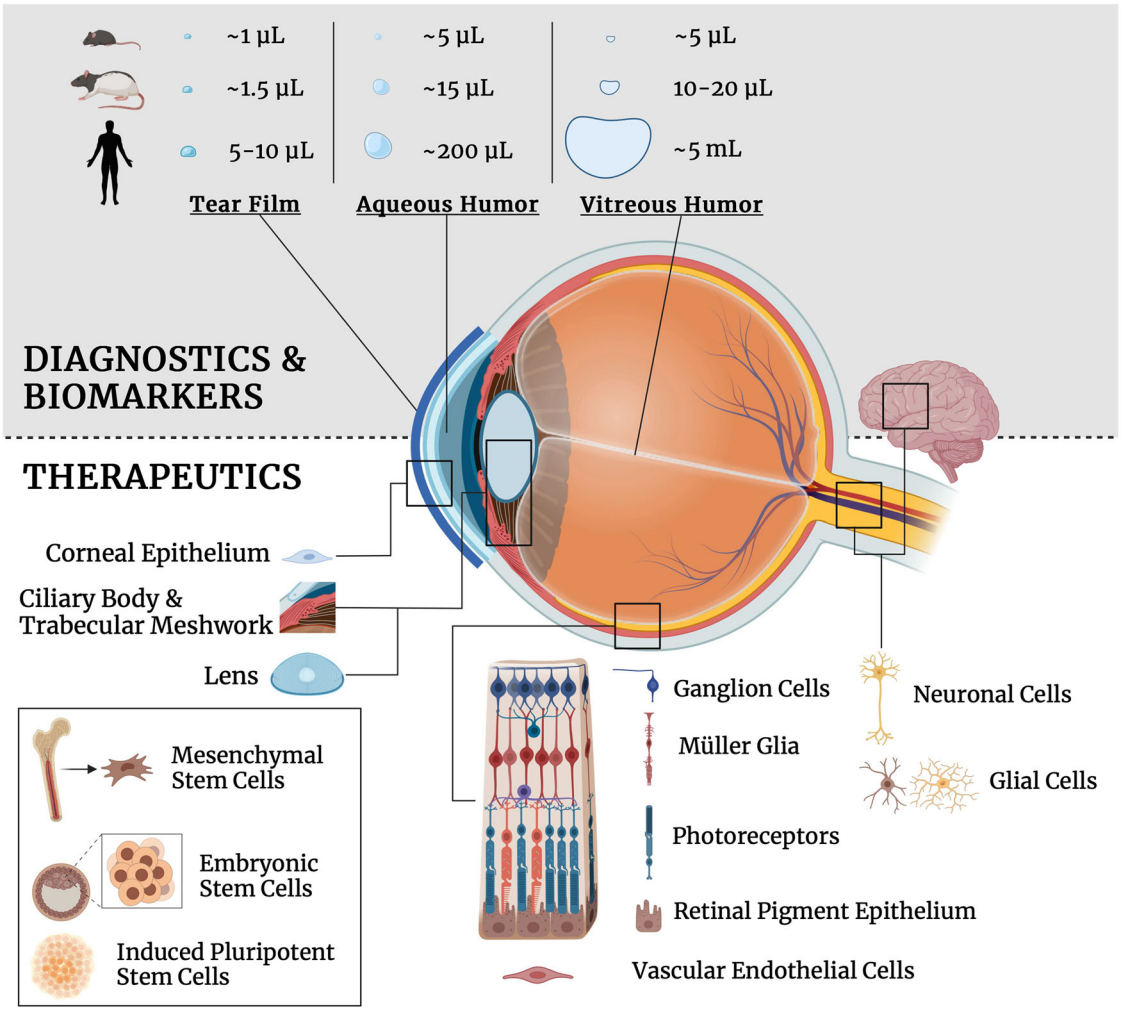

FIGURE 1 Anatomy of the eye.

2 | SECTION I: EVS FROM BIOLOGY TO BIOMARKERS

Over the last decade, substantial progress has been achieved in deepening our understanding of EV biogenesis, secretion and cellular uptake (Ludwig & Giebel, 2012; Ratajczak et al., 2006; Valadi et al., 2007; Van Der Pol et al., 2012; Welsh et al., 2024; Wortzel et al., 2019). These advancements highlight the important role played by EVs in various organ physiologies and diseases. However, a significant gap remains in understanding the regulation of diverse EV cargo loading, cellular uptake and/or interaction and cargo unloading mechanisms that influence their functional impact on target cells. This knowledge is also essential for investigating EVs as biomarkers in diverse disease processes. Additionally, a critical aspect to consider is the conservation of these mechanisms across different tissue types and organs.

Several recent publications and reviews have specifically focused on EV cargo content and function related to eye health and disease (Cioanca, Natoli et al., 2023; Cioanca, Wooff et al., 2023; Demais et al., 2022; Kalargyrou et al., 2022; Wooff et al., 2020). Nonetheless, compared to other fields, EV biology and diagnostics are at a nascent stage in vision research. Thus, this section offers insights from experts in EV biology and diagnostic biomarkers both outside of and within the vision research fields. They presented recent findings and identified gaps in our general understanding of EV biology (Sections 2.1 and 2.3). Additionally, the section features presentations on the latest advancements in EV biology and biomarkers, specifically within the context of vision research (Sections 2.2 and 2.3).

2.1 | EV biogenesis, regulation of cargo content and signalling in vivo

Alissa Weaver presented her recent work elucidating the biogenesis of RNA-containing EVs. Her research group identified endoplasmic reticulum membrane contact sites (ER MCSs) as platforms for the generation of RNA-containing EVs in cancer cells.

Additionally, the study revealed that a specific subpopulation of small EVs, highly enriched in RNA, is regulated by the ER MCS linker protein vesicle-associated membrane protein-associated protein-A (VAP-A). Lipid analysis of VAP-A-knockdown EVs demonstrated reductions in the EV biogenesis lipid ceramide. Knockdown of the VAP-A-binding ceramide transfer protein (CERT) resulted in similar defects in EV RNA content. Imaging experiments showed that VAP-A promotes luminal filling of multivesicular bodies (MVBs), CERT localizes to MVBs and the ceramide-generating enzyme neutral sphingomyelinase 2 colocalizes with VAP-A-positive endoplasmic reticulum (Barman et al., 2022). Given their findings that RNA-containing EVs are a small subpopulation of the total isolated EVs, she outlined future directions for more studies in this area, including investigating the regulatory mechanisms influencing the subset of RNA-containing EV biogenesis. Furthermore, she highlighted the need to explore cellular and tissue uptake of these RNA-containing EVs and to determine whether these systems are conserved from cancer cells to the eye.

Christie Fowler addressed the challenges associated with studying EV signalling in vivo, emphasizing the heterogeneous nature of cell-secreted EVs with diverse cargo profiles. This complexity is further compounded by complex cellular release and uptake dynamics influenced by microenvironmental changes. Existing techniques and approaches also pose limitations. To overcome these challenges, Fowler's research group developed novel transgenic animal model, ExoMap1 mouse line. This mouse line features a fluorescent marker, mNeonGreen, tagged to the EV protein CD81. The efficacy of this approach was validated by crossing the ExoMap1 mouse with a cell type-specific cre driver mouse line. Her group demonstrated that quantitative single-molecule localization microscopy, utilizing the ExoMap1 mouse, enabled the calculation of cell type-specific contributions to biofluid exosome populations. Specifically, neurons were found to contribute approximately 1% to plasma and cerebrospinal fluid exosome populations, while hepatocytes contributed around 15% to plasma exosome populations. These figures align with known vascular permeabilities of the brain and liver. With a growing demand for tools to assess EV signalling, the ExoMap1 mouse model emerges as a highly valuable resource for in-depth exploration of EV biology, especially within the eye. For instance, it could be utilized by administering AAV-cre under a cell-type-specific gene promoter, such as RPE65 (Fordjour et al., 2023).

2.2 | EVs in retinal health and disease

With the diverse array of specialized cell types unique to retina, investigating EV biogenesis, transport, release and uptake in these cells necessitates tailored methodologies. The retinal pigment epithelium (RPE) cells, for example, are highly metabolically active cells located between the photoreceptor and choroid that possess distinctive pathways for EV biogenesis. These RPE cells are highly polarized and contain separate machineries for apical and basolateral EV cargo sorting and release. Additionally, they constitute a crucial component of the outer blood-retinal barrier (Bowes Rickman et al., 2013). The RPE also has a highly active lipoprotein metabolism that intersects with pigment granule (melanosome) formation, cholesterol metabolism pathways and EV pathways, resulting in unique EVs with constituents of lipoproteins, melanosomes and EVs (Van Niel et al., 2015). **Mikael Klingeborn** and several other investigators have shown that polarized EV release from apical versus basal sides of the RPE contain tightly controlled protein cargo in both retinal health and disease (Flores-Bellver et al., 2021; Hernandez et al., 2023; Klingeborn et al., 2017). Interestingly, a recent study from the Klingeborn lab has shown that inhibition of basolateral EV release in RPE can decrease abnormal deposition of proteins and lipids (known as drusen) into the pentalaminar collagen- and elastin-rich extracellular matrix (called Bruch's membrane) in vivo, under chronic subtoxic oxidative stress conditions mimicking the earliest stages of RPE dysfunction in age-related macular degeneration (AMD) (Hernandez et al., 2023).

In addition to the example above of the role of EV biology in RPE cell health and AMD, there are a number of recent publications and reviews which describe new methods, approaches, understanding and discourse on EV isolation, characterization, uptake pathways, role in drusen formation and communication in the retina in both health and degeneration (Cioanca, Natoli et al., 2023; Cioanca, Wooff et al., 2023; Demais et al., 2022; Kalargyrou et al., 2022; Wooff et al., 2020; Martins et al., 2024).

2.3 | EV-based diagnostics and biomarkers

For a long time, detecting RNA in biofluids like plasma and urine seemed implausible due to RNA's instability and the presence of RNAses. However, a breakthrough came when researchers found tumour-derived RNA in serum and plasma EVs from cancer patients, even after long storage. **Johan Skog** highlighted that this discovery led to a shift in RNA-based liquid biopsies, along with advancements in EV isolation and characterization (Skog et al., 2008). In 2016, the world saw the first EV-based diagnostic, using three RNA targets in urine EVs to predict prostate cancer likelihood (Mckiernan et al., 2016). Subsequently, EV-based diagnostics gained clinical acceptance, securing insurance coverage and becoming part of cancer care guidelines (Moses et al., 2023). Beyond carrying RNA, EVs contain a diverse mix of multi-omic content, including proteins, lipids, carbohydrates, DNA and metabolites. This unique feature has driven the development of second-generation EV-based assays, offering a comprehensive understanding of molecular changes in various diseases. The evolution of EV-based diagnostics, from biomarker discoveries to influencing clinical applications, represents a transformative journey.

Within the cardiovascular system, **Saumya Das** and his team have previously shown strong connections between plasma microRNA (miR) levels, particularly miR-30d and cardiac function (Danielson et al., 2018). While miR-30d is found in small extracellular vesicles (sEVs) and has higher levels associated with cardioprotection, its transfer between cells may play a role in disease development. Their work highlights the role of cardiomyocyte miR-30d in protecting against adverse cardiac remodelling after a heart attack, acting on both cardiomyocytes and fibroblasts. This suggests that EVs and small RNAs could be considered 'functional biomarkers' in cardiovascular diseases (Li et al., 2021, 2022; Melman et al., 2015; Murillo et al., 2019).

Their study also found that analysis of EV nucleotide cargo including mRNA and LncRNAs revealed distinct patterns in different heart failure subtypes, reflecting specific pathways related to cardiomyocytes in heart failure with reduced ejection fraction and more diverse pathways centred around metabolism and immune response in heart failure with preserved ejection fraction. Mapping these transcripts to single nuclear RNAseq datasets further demonstrated their potential as a liquid biopsy for understanding dynamic changes in cellular stress and disease pathways (Gokulnath et al., 2023, 2024). Future development of methods to isolate tissue-specific EVs in human diseases may have significant value as high fidelity markers of disease progression in many human diseases, including ocular diseases.

Sarah Hamm-Alvarez introduced her current research studying EVs in tears. The tear fluid contains EVs, which are relatively abundant per volume of tears (Grigor'eva et al., 2016). EVs may reach the tears through different pathways including secretion and transcytosis from the epithelial cells lining the ocular surface and from the lacrimal gland. By transmission electron microscopy evaluation of mouse lacrimal gland, her team has documented the presence of intralumenal vesicles in subapical MVBs in mouse lacrimal gland acinar cells. They have also identified EVs in the lumena formed by adjacent apices of lacrimal acinar cells that collect secreted material which is then drained through intra- and interlobular ducts to the ocular surface. These findings suggest that EVs are actively secreted from lacrimal gland acinar cells into tears. Other ocular surface epithelia and supporting cells which provide other content to the tears may also secrete EVs to tears (Dartt & Willcox, 2013; Hodges & Dartt, 2013; Van Haeringen, 1981; Willcox et al., 2017). Tear EVs have been shown to contain neuronal and glial cell markers of origin, suggesting that circulating EVs can reach tears (Pieragostino et al., 2019). Tear collection from human subjects is non-invasive and typically uses Schirmer's strips or glass capillaries (Pieczyński et al., 2021). Although EV purification from tears uses established methods for EV isolation from other biofluids, specialized devices such an iTEARS has recently been developed to purify and concentrate tear EVs (Hu et al., 2022). The number of studies evaluating changes in tear EV abundance, content and cargo in association with disease development is so far limited, but studies have yielded promising data identifying specific EV surface and luminal cargo proteins and miRNAs as putative biomarkers for diverse ocular and systemic diseases (Inubushi et al., 2020; Shi et al., 2022; Zhang et al., 2020).

2.4 | Gaps, needs and opportunities

During the discussion, it was noted that the study of EVs in vision research lags behind, as evidenced by the numbers of publications and clinical trials, as also discussed in depth in a recent publication (Dhodapkar et al., 2024). In addition to collective acknowledgement of the general gaps, needs and opportunities across the EV field, specific considerations were discussed for vision research. For instance, tears provide an easily accessible biofluid offering valuable biological information relevant to numerous anterior segment ocular diseases, including dry eye disease, keratoconjunctivitis, Sjögren's syndrome, meibomian gland dysfunction and ocular graft versus host disease (OGVHD) (Tamhane et al., 2019). Recent studies have demonstrated that tears contain a myriad of molecules, including lipids, electrolytes, proteins, peptides and various small molecule metabolites, originating from multiple sources such as the lacrimal glands, Meibomian glands, goblet cells and ocular surface epithelial and nerve cells (Zhou & Beuerman, 2012). Nevertheless, challenges persist in studying EVs in tears and other ocular biofluids such as aqueous humour and vitreous humour. These challenges include limited sample volumes leading to restricted EV abundance for discovery studies, variability in experimental results, a lack of knowledge regarding specific markers indicating the origin of EVs from ocular cells and tissues and uncertainties surrounding the functions of altered EVs in ocular disease (Figure 1). Additionally, the extent to which systemically circulating EVs are relevant to eye diseases remains to be determined.

Due to these gaps in the knowledge about EVs in the vision field, some opportunities were felt to lie in leveraging insights from other EV fields. For example, since the blood-retinal barrier is similar to the blood-brain barrier, data from this area in neuroscience could be applied to the study of the retina. Nonetheless, the field must recognize unique characteristics of specialized cell types within the eye. Establishing collaborations to cross-validate EVs from various ocular tissues could potentially expedite the understanding of the distinctiveness of EV involvement in eye-related pathologies. This endeavor must be achieved through a reproducible framework for EV isolation and characterization that can reflect the structural and molecular heterogeneity of naturally cell-secreted EVs.

TABLE 1 Presented studies of EV therapeutics for ocular and non-ocular diseases.

Investigator	Cell source or bioengineering of EV	Testing tissue/species	Endpoint
Ali Djalilian (An et al., 2023; Samaeekia et al., 2018)	hBM-MSC	Cornea (mouse and human)	Safety Corneal wound healing
Ben Mead (Mead, Ahmed et al., 2018; Mead, Amaral et al., 2018; Mead & Tomarev, 2017)	hBM-MSC	Traumatic optic neuropathy and glaucoma (mouse and rat)	RGC survival
Sun Young Lee (Pollalis, Georgescu et al., 2024; Pollalis, Nair et al., 2024; Pollalis et al., 2022)	hESC-RPE Bioengineered EV	Retina degeneration (rat) Chorioretinal neovascularization (mouse)	PR cell rescue and in vivo function Active targeting
Charles Egwuagu (Kang et al., 2020; Kang et al., 2023; Zhou et al., 2022)	IL-35- or IL-27- Breg Cell hUC-MSC	Uveitis/GVHD (mouse) K GVHD (human)	Inflammation in uveitis Corneal healing in GVHD
Sriram Ravindran (Huang, Kang, Lu et al., 2020; Huang, Kang, Narayanan et al., 2020; Kang et al., 2022)	hBM-MSC (Hypoxia preconditioned)	Retina Ischemia (rat)	Neuroinflammation, neuroprotection Functional recovery
Michael Chopp (Wang et al., 2019; Ding et al., 2022; Venkat et al., 2019; Zhang et al., 2022)	Cerebroendothelial cell (rat)	Neural stem cell (diabetic rat)	Neurogenesis Cognition
Tara Moore (Go et al., 2020; McCann et al., 2023; Medalla et al., 2020; Zhou et al., 2023)	(NHP) BM-MSC	Cortical injury (NHP)	Motor function
Ashok K. Shetty (Attaluri et al., 2023; Madhu et al., 2024; Upadhya et al., 2020)	hiPSC-NSC	Alzheimer's disease (mouse)	Neuronal penetration via intranasal treatment Transcriptomic analysis Amyloid-beta (Aβ) plaques Cognitive function
Christopher Cutler (Elashiry et al., 2020; Elashiry et al., 2021)	TGFb1 and IL-10 treated dendritic cell (mouse)	Periodontitis (mouse)	Periodontitis
Jeff Bulte (Han et al., 2021)	hiPSC	Acute kidney injury heart ischemic and reperfusion injury (mouse)	Inflammation

Abbreviations: GVHD, graft versus host diseases; hBM-MSC, human bone-marrow mesenchymal stem cell; hESC-RPE, human embryogenic stem cell-retinal pigment epithelium; hiPSC, human induced pluripotent stem cell; hUC-MSC, human umbilical cord mesenchymal stem cell; TGF, transforming growth factor.

3 | SECTION II: EVS FOR REGENERATIVE MEDICINE

In this section, investigators presented their recent and ongoing work aimed at developing EV therapeutics for ocular diseases and other conditions affecting the nervous system (Table 1).

Ali Djalilian presented the potential therapeutic role of EVs derived from human bone marrow derived mesenchymal stem cells (hBM-MSC) in wound repair in cornea. Djalilian's group previously demonstrated the safety of hBM-MSC and potential therapeutic benefit in non-healing corneal wounds and acute chemical injuries during a Phase 1 clinical trial (Clinicaltrials.gov ID #NCT04626583) (Chang et al., 2023). Furthermore, his group demonstrated that human MSC-derived EVs can facilitate corneal epithelial regeneration, nerve regeneration and enhance corneal endothelial cell survival by topical application in a mouse model (An et al., 2023; Samaeekia et al., 2018). Expanding on these promising pre-clinical studies, a new Phase 1 clinical trial (Clinicaltrials.gov ID #NCT05204329) sponsored by NIH is currently underway to assess the safety of MSC secreted eye drops containing EVs as the active ingredient. The study aims to evaluate safety and explore efficacy in patients with chronic corneal surface diseases, including limbal stem cell deficiency and neurotrophic keratitis.

Ben Mead presented the potential therapeutic role of intravitreally delivered hBM-MSC derived EVs in mouse and rat models of traumatic optic neuropathy and glaucoma (Mead, Ahmed et al., 2018; Mead, Amaral et al., 2018; Mead & Tomarev, 2017).

The treatment aims to protect retinal ganglion cells (RGCs) from degeneration, thereby preserving visual function. Indeed, intravitreal delivery of hBM-MSC derived EVs, compared to fibroblast derived EVs, promotes regeneration of RGCs and restores RGC function in a model of optic nerve crush. Further, Mead's group also demonstrated that miRNA serves as a crucial mediator of the therapeutic effect of hBM-MSC-derived EVs utilizing the knockdown of Argonaute-2, a key miRNA effector molecule (Mead & Tomarev, 2017). One of the many important unanswered questions in the field is what is driving the therapeutic effect of EVs, and Mead's work provides insight into this potential mechanism with evidence that miRNAs are at least partially responsible for exerting a meaningful therapeutic effect on retinal tissue.

Sun Young Lee proposed two distinctive therapeutic strategies for ocular EV therapy in treating retinal diseases: (1) using stem cell derived sEVs as a cell free regenerative therapy, and (2) employing bioengineered sEVs to modulate tissue targeting and drug loading. Lee's group observed that the sEVs secreted from human embryonic stem cells derived fully polarized RPE cells, could rescue photoreceptor cells and their function in a retinal degeneration animal model. sEVs treatment was administered by intravitreal injection to the Royal College of Surgeons rats, which develop retinal degeneration due to a mutation in the *MerTK* gene in RPE cells. Transcriptomic studies revealed detrimental alterations in gene expression in Royal College of Surgeons rats, including essential RPE functions such as phototransduction, retinol metabolism and lipid metabolism were partially reversed. Defective photoreceptor outer segment engulfment due to intrinsic MerTK mutation was partially ameliorated (Pollalis, Georgescu et al., 2024; Pollalis, Nair et al., 2024). It was emphasized that RPE-secreted small EVs may play a functional role similar to that of RPE cells. This approach aims to realign multiple RPE functions simultaneously, offering a potential avenue for therapeutic intervention with broad applications for dysfunctional RPE in various retinal diseases. Secondly, Lee's group previously demonstrated that when intravitreally administered, surface-decorated EVs with Arginylglycylaspartic acid (RGD), a ligand targeting a chorioretinal neovascularization (CNV), effectively delivers EV to the areas of CNV. This EV bioengineering strategy can be utilized as a drug delivery system that actively targets CNV, which is a pathological feature of neovascular AMD (Pollalis et al., 2022).

Charles Egwuagu presented therapeutic benefit of exosomes derived from regulatory B-cells that produce IL-35 (i35-Bregs) or IL-27 (i27-Bregs) and suppress neuroinflammation in mouse models of uveitis (experimental autoimmune uveitis) and encephalomyelitis (experimental autoimmune encephalomyelitis). The Breg cells secreting IL-35-containing exosomes (i35-exosomes) or i27-exosomes suppress uveitis (Kang et al., 2020, 2023) or graft versus host disease (GVHD; unpublished). They suppress inflammation by inhibiting proinflammatory Th17/Th1 lymphocytes, upregulating checkpoint inhibitors (PD-1, LAG-3) that induce T-cell exhaustion and inducing bystander lymphocytes to produce immunosuppressive cytokines. Egwuagu also presented collaborative work showing that exosomes containing miRNA-204 (MSC-exo) from mesenchymal stromal cells, when administered as eye drops, alleviated GVHD-associated dry eye disease in a prospective clinical trial involving 28 eyes with refractory GVHD-dry eye disease (Zhou et al., 2022). The patients experienced substantial relief after treatment as evidenced by reduced fluorescein scores, longer tear-film breakup time, increased tear secretion and lower Ocular Surface Disease Index scores. Mechanistically, MSC-exo reprogrammed proinflammatory M1-macrophages into immunosuppressive M2-macrophages through targeting of IL-6/IL-6R/Stat3 pathway (Zhou et al., 2022).

Michael Chopp presented the biphasic role of EVs in neurovascular injury, discussing the detrimental effects of EVs generated post neural injury, such as in stroke and diabetic vascular dementia, which provoke vascular endothelial cells to become pro-inflammatory, pro-thrombotic, pro-coagulant and permeable (Chen et al., 2019; Li et al., 2021; Wang et al., 2019; Zhang & Chopp, 2016). In addition, these post stroke and neural injury EVs induce secondary organ, such as heart and liver, dysfunction (An et al., 2021; Li et al., 2018; Venkat et al., 2018, 2023; Yan et al., 2020). Conversely, he emphasized the potential therapeutic effects of sEVs from healthy vascular endothelial cells in promoting vascular and neurological recovery post neural injury and diabetic vascular dementia (Ding et al., 2022; Venkat et al., 2019). Healthy EVs derived from cerebral vascular endothelial cells were shown to reverse vascular dysfunction and enhance neurological recovery (Wang et al., 2019). Chopp demonstrated that sEVs derived from primary rat cerebral endothelial cells of aged diabetic rats suppress neurogenesis in rat neural stem cells. Further, treatment with small EVs derived from healthy cerebral endothelial cells increased neurogenesis in neural stem cells. Consequently, the administration of sEVs from healthy cerebral endothelial cells improved cognitive function in aged diabetic rats (Zhang et al., 2022). Based upon his study in the extraocular nervous system, he concluded that vascular endothelial cell-derived EVs may have potential therapeutic applications for eye diseases.

Tara Moore presented that MSC-derived EVs accelerate and enhance recovery of motor function following cortical injury in aged monkeys. In a histologic study, compared to vehicle-treated monkeys, EV-treated monkeys exhibited greater frequencies of ramified microglia labelled with Iba1 and P2Y12, a purigenic receptor important for motility in the homeostatic state. EV treated monkeys had lower densities of pro-inflammatory hypertrophic microglia expressing LN3+, a marker for MHC II receptors upregulated with immune activation suggesting suppressed microglial activation by EV treatment. EV-treated monkeys exhibited full recovery by 3–5 weeks post-injury and untreated monkeys reached a plateau in recovery by 8–12 weeks post-injury (Go et al., 2020; McCann et al., 2023; Medalla et al., 2020; Zhou et al., 2023).

Ashok K. Shetty demonstrated the beneficial effects of human induced pluripotent stem cell-neural stem cell-derived EVs (hiPSC-NSC-EVs) in an early-stage mouse model of Alzheimer's disease (3-month-old 5xFAD mice). Weekly intranasal administration for two weeks led to improvements in proficiency for hippocampus-dependent cognitive and memory tasks (Attaluri et al., 2023). Additionally, there were reductions observed in astrocyte hypertrophy, microglia density, plaque-associated microglia

(microglial clusters), microglia presenting inflammasomes, the expression of disease-associated microglia genes, oxidative stress markers and multiple proinflammatory cytokines in the hippocampus (Attaluri et al., 2023). The hiPSC-NSC-EV treatment also contributed to a decrease in amyloid-beta load and phosphorylated tau concentrations while maintaining a higher level of neurogenesis in the Alzheimer's disease mice (Attaluri et al., 2023). Shetty's group also demonstrated that intranasal administration leads to the delivery of EVs directly into neurons and microglia in virtually all regions of the Alzheimer's disease brain (Madhu et al., 2024). The team has also identified specific naturally enriched miRNAs (e.g., miR-21-5p, miRNA-26a, miRNA-103a, miRNA-181) contained in hiPSC-NSC-EVs, known for their involvement in neuroprotection, anti-inflammatory responses, long-term potentiation (LTP) and cognition/memory enhancement effects (Upadhya et al., 2020).

Christopher W. Cutler introduced immunomodulatory therapeutic effects of small EVs derived from dendritic cells (DCs), known as the 'directors' of the immune response. Building upon the promise of DC derived exosomes for treatment of diseases of aging, most notably cancer (Chen et al., 2020; Pitt et al., 2016), autoimmune diseases (Yin et al., 2013) and severe COVID-19 (Elashiry et al., 2021), Cutler's group has been studying the therapeutic benefits of DC-derived EVs in periodontitis. Periodontitis is oral degenerative bone disease affecting 50% of Americans and has been linked with Alzheimer's disease (Dominy et al., Jan 2019; Teixeira et al., 2017), cancer (Michaud et al., 2017) and other chronic conditions such as AMD (Arjunan et al., 2020). Cutler's laboratory has demonstrated that EVs derived from engineered mouse DCs exposed to TGFb1 and IL-10 (Regulatory DCs) are effective in promoting a T regulatory response to prevent or resolve periodontitis, or a Th17 response to potentiate cytotoxic T cell responses in a mouse model when delivered by local injection. In contrast, EVs derived from DCs exposed to LPS (stimulatory DCs) promoted inflammatory bone loss (Elashiry et al., 2020, 2021). Additionally, Cutler's team has shown that EVs derived from autologous human monocyte-derived DCs exhibit similar efficacy in vitro (Elsayed et al., 2023).

3.1 | Gaps, needs and opportunities

During the round table discussion, investigators agreed on the significant therapeutic potential of EVs for eye diseases. Given their ability to bypass the blood-brain barrier and blood-retinal barrier, the biocompatibility of both native (unmodified) and bioengineered (modified) EVs holds promise as multi-molecular therapeutics and targeted drug delivery cargos. EVs represent the potential next-generation of cell-free regenerative and gene therapies. Feasible local delivery to the eye could enhance the therapeutic potential of EVs by bypassing entrapment sites such as the liver and spleen, thus achieving higher therapeutic concentrations. Furthermore, there was agreement that heterogeneous EV particles cannot be defined by a single set of molecules and require characterization through comprehensive approaches. Delineating the key mechanisms of action, which are likely multiple, and identifying bioactive molecules, also likely multiple, to optimize EV treatment, remains to be fully elucidated. Currently, it is also challenging to correlate the effects derived from multiple molecules across various pathways. A better strategy for multidimensional and multitudinal analyses is necessary. The progress in AI-based computation analyses may aid in addressing these challenges. All of these efforts are critical for establishing potency markers, release criteria and streamlining the manufacturing process, all of which are necessary steps toward scaling up production and establishing regulatory guidelines.

4 | SECTION III: TOOLS AND APPROACHES FOR THE RIGOROUS STUDY OF EVS

In this section, investigators presented their recent and ongoing work aimed at developing new tools and approaches to study and characterize EVs in ocular diseases and other conditions affecting the nervous system.

Kenneth Witwer began this section by clarifying that he represents the current perspectives of the EV community on behalf of International Society of Extracellular Vesicles (ISEV). He introduced the goals and aims of the 'Minimal information for the studies of EVs' (MISEV) guidelines, that were recently updated (Welsh et al., 2024). MISEV aims to provide researchers with an updated overview of available approaches, their advantages and limitations for production, separation and characterization of EVs from multiple sources, including cell culture, body fluids and solid tissues as well as the latest state-of-art principles of EV research. The main ideas include using nomenclature that convey concepts and information, while avoiding dogma, and steering clear of potentially misleading EV subpopulation nomenclature due to the evolving understanding of EV biogenesis (Witwer & Théry, 2019). MISEV advocates for a strategic approach to selecting EV separation and characterization methods (Tables 2 and 3). Each method has its own set of advantages and disadvantages, and it is important to provide description in sufficient detail, ensuring alignment with the experimental goals.

Witwer further discussed how many published studies of EVs lack scientific rigor and rationale due to inadequate EV separation methods, such as reliance on certain commercial kits. Although advertised as EV-specific, these kits may rely on undisclosed principles, such as polyethylene glycol-based precipitation, and thus co-concentrate many non-EV extracellular particles. Even prevalent methods like ultracentrifugation are not highly EV-specific. The focus on 'EVs' is often unjustified, based on mistaken assumptions about EV biogenesis and its relationship to size, morphology and phenotype. He also highlighted concerns about contamination of the EV literature with fraudulent papermill products and the misinterpretation of in vitro and in vivo studies.

TABLE 2 A summary of the most relevant characteristics of common EV isolation methods for use in ocular tissues.

Isolation methods					
Method	**Yield**	**EV purity**	**Optimal input volume**	**Biological activity[a]**	**Preferred analysis[b]**
DUC	++	++	Medium—Large	++	EM, NTA, WB, FC/dFC
PEG	+++	+	Small—Large	+	2nd isolation method required
DGUC	+	+++	Medium—Large	++	EM, WB, NTA, MS, NGS, LA, FC/dFC
IAC	++	++/+++	Small	+	WB, MS, NGS, LA
SEC	+/++	++	Small—Medium	+++	EM, NTA, WB, MS, NGS, LA, FC/dFC
TFF	++	++	Large	+++	EM, NTA, WB, MS, NGS, LAFC/dFC
HiMEX	+	++/+++	Small	+	N/A; combines isolation and analysis

[a]Biological activity is impacted by different isolation methods by altering exosome integrity. Examples of biological activity includes exosome binding and uptake, enzyme activity, and other protein and nucleic acid-induced activity measured in target cells.
[b]Analyses that the resulting EV preparation is most suited/optimal for based on purity and physical integrity of the exosomes and small EVs. Abbreviations: DUC, differential ultracentrifugation; DGUC, density gradient ultracentrifugation; EM, electron microscopy; FC/dFC, (digital) Flow Cytometry; HiMEX, high-throughput magneto-electrochemical extracellular vesicles system; IAC, immunoaffinity capture; LA, lipidomic analysis; MS, mass spectrometry; NGS, next-generation sequencing; NTA, nanoparticle tracking analysis; PEG, polyethylene glycol precipitation; SEC, size exclusion chromatography; TFF, tangential flow filtration; WB, western blotting.

TABLE 3 A summary of the most relevant characteristics of EV analysis methods for use in ocular tissues.

	Biophysical		**Cargo composition**			
Method	**EV Concentration**	**EV Size**	**Protein**	**RNA**	**DNA**	**Lipid**
EM	Yes	Yes	Yes	Yes	Yes	Yes
NTA	Yes	Yes	Yes	Yes	Yes	Yes
ExoView	Yes	Yes	Yes	No	No	No
TRPS	Yes	Yes	No	No	No	No
WB	Semiquant	No	Yes	No	No	No
ELISA	Semiquant	No	Yes	Yes	Yes	Yes
MS	Semiquant	No	Yes	No	No	See LA
NGS	Semiquant	No	No	Yes	Yes	No
LA	Semiquant	No	No	No	No	Yes
FC/dFC	Yes	Yes	Yes	Yes	Yes	Yes
HiMEX	Semiquant	No	Yes	Yes	No	No

Abbreviations: EM, electron microscopy; FC/dFC, (digital) flow cytometry; HiMEX, high-throughput magneto-electrochemical extracellular vesicles system; LA, lipidomic analysis; MS, mass spectrometry; NGS, next-generation sequencing; NTA, nanoparticle tracking analysis; TRPS, tunable resistive pulse sensing; WB, western blotting; ELISA, enzyme-linked immunosorbent assay.

For example, in vitro, molecules often reported to be specifically packaged into 'EVs' (e.g., miRNAs such as liver-specific miR-122 and red blood cell-specific miR-451a) may actually originate from bovine serum and other cell culture media additives, leading to misconceptions. Similarly, confusing secondary effects, such as liver damage, with primary causes (e.g., mistaking miR-122 as a biomarker of non-liver diseases when it is actually indicative of liver damage) can result in erroneous conclusions.

Witwer concluded by suggesting that the future of EV studies should adhere to expert consensuses guidance, rather than inherited wisdom, no matter how often it is repeated in review articles. Finally, he emphasized the importance of critically assessing whether EVs are truly responsible for observed effects or functions, advising researchers to be pragmatic about ascribing effects to EVs versus the 'EV preparation' in general, or even to different fractions of the secretome when necessary.

Yutao Liu described EV isolation and characterization in studying glaucoma, which is an optic neuropathy characterized by progressive RGC loss and optic nerve degeneration, leading to irreversible visual impairment (Weinreb et al., 2016, Youngblood et al., 2019, 2023). EVs containing miRNAs, proteins and lipids have been isolated and characterized in the aqueous humour, vitreous humour, tears and other ocular tissues related to glaucoma (Mead & Tomarev, 2017; An et al., 2022; Dismuke et al., 2015; Hardy et al., 2005; Hefley et al., 2022; Locke et al., 2014; Mueller et al., 2023; You et al., 2023). EVs from glaucoma-relevant tissues/cells, including trabecular meshwork, ciliary body and retinal glial cells, may contain specific contents related to glaucoma pathogenesis and serve as potential biomarkers for glaucoma. EVs from the MSCs and other stem cells have been successfully applied to prevent or treat glaucoma in animal models of glaucoma via targeting the retina or the trabecular meshwork (Mead, Ahmed et al., 2018; Mead, Amaral et al., 2018; Mead & Tomarev, 2017; Pan et al., 2019; Seong et al., 2023; Yu et al., 2023). EVs

have also been identified to function as communicators within the retinal cells or the outflow pathway (Demais et al., 2022; Aires et al., 2020; Lerner et al., 2017, 2020; Shah et al., 2018; Tabak, 2021; Takahashi et al., 2021). Specific EV contents such as proteins, miRNAs and lipids have been identified as potential markers for glaucoma diagnosis or treatment by the Liu lab and others (An et al., 2022; Mueller et al., 2023; Drewry et al., 2018; Liu et al., 2013, 2016). Liu also highlighted that despite the success, it remains unknown: (1) how aging and biological sex affects EV production and transportation in the eye, (2) how EVs interact with the extracellular matrix and basement membranes in the outflow pathway, (3) how circulating EVs could be enriched as biomarkers for glaucoma diagnosis, progression and treatment response. Simple and more efficient isolation and characterization techniques are necessary to investigate the role of EVs in glaucoma pathogenesis. Liu suggested that engineered EVs could be optimized to repopulate trabecular meshwork cells, lower intraocular pressure, regenerate/preserve RGCs and delay the aging effect. The technical innovations in EV isolation and characterization are expected to significantly promote the translational potential of EVs in glaucoma diagnosis, prevention, progression monitoring and treatment follow-up (Weinreb et al., 2016; Klingeborn et al., 2017; Leung et al., 2024; Mead & Tomarev, 2020).

Hakho Lee emphasized that analyzing EVs in bodily fluids holds promise for improving disease detection and enabling serial patient monitoring during therapy. Research communities have begun tackling technical hurdles such as manual processes for EV enrichment, limited sensitivity and throughput of existing tools and the high cost of test equipment or assays, in efforts to establish clinical EV tests by developing EV-tailored tools (Hu et al., 2023; Im et al., 2014; Jeong et al., 2016; Kilic et al., 2022; Park et al., 2021; Reátegui et al., 2018; Yang et al., 2017). One notable example is the high-throughput magneto-electrochemical extracellular vesicles (HiMEX) system developed by the Lee lab, which combines EV isolation and detection into a single, streamlined assay format (Park et al., 2021). HiMEX utilizes immunomagnetic beads to enrich target-specific EVs and quantifies their protein levels via an electrochemical reaction. This innovative approach enables rapid and sensitive EV detection directly from clinical samples. Moreover, the assay is readily scalable for parallel detection, such as in a 96-well format. Developing such integrated assay systems will significantly accelerate the assessment of the clinical validity and utility of EVs, ultimately paving the way for establishing routine clinical EV analyses (Liu et al., 2019; Yan et al., 2023). Furthermore, new EV sensors can be optimized for the small sample volumes and amounts from ocular tissues and fluids, addressing a significant impediment to EV studies in the eye.

Daniel Chiu highlighted that EVs play a central role in liquid biopsy, intercellular communication, and disease transmission and progression and are emerging therapeutic tools. To better understand the biology of EVs and fully unlock their diagnostic and therapeutic potential, it is critical to access quantitative information regarding their concentration, composition, size and biological heterogeneities. To address this need, the Chiu lab has developed a single-molecule sensitive flow platform, which uses a high-throughput 12-colour channel flow analyser that detects every fluorescent molecule flowing through a microfluidic channel. It enables multiparameter characterization of EVs, including single-EV particle phenotyping, sizing and the absolute quantitation of EV concentrations and biomarker copy numbers (Andronico et al., 2021; Jiang et al., 2021). Chiu emphasized that this new flow technology holds a potential for a broad range of applications, from analysis of single particles such as EVs or RNA-binding protein complexes, to characterization of therapeutic lipid nanoparticles, viruses and proteins. Additionally, it provides absolute quantitation of non-EV samples such as dyes, beads and antibody-dye conjugates. This new technology is well-poised for studies of ocular health and disease.

Jeff Bulte shared that non-invasive, serial in vivo tracking of EVs is expected to play a key role in assessing the clinical efficacy of EV therapy for treatment various medical conditions including cancer, neurodegenerative diseases and many more. This tracking can address critical questions such as: (1) What is the blood half-life of EVs? (2) Do EVs reach their intended target, and if so, when and to what extent? (3) For how long do EVs remain in which organ? (4) Is there undesired accumulation at off-target sites, and if so, where, when and how much? (5) What are the excretion pathways? Previously, similar clinical questions pertained to tracking their parental cells instead of EVs (Bulte & Daldrup-Link, 2018).

Various imaging methods are available to track the fate of EVs in vivo (Arifin et al., 2022), including magnetic resonance imaging (MRI), x-ray computed tomography, magnetic particle imaging, single-photon emission computed tomography, positron emission tomography and optical imaging (fluorescence and bioluminescence imaging) (Table 4). Bulte and colleagues have successfully employed MRI imaging to localize EVs labelled with magnetic nanoparticles in pre-clinical animal models, such as mice and rats, in studies on acute kidney diseases and myocardial ischemia, respectively (Han et al., 2021). These approaches hold significant promise for studies in the eye, as illustrated in Figure 2. MRI is uniquely suited as it has an excellent spatial resolution (~50 μm using pre-clinical high-field systems) and soft-tissue contrast. Ultimately, the goal is to utilize imaging biomarkers to predict the success or failure of EV therapy and adjust injection parameters including route, dose, speed and volume in a personalized manner.

Sriram Ravindran introduced two engineering strategies to develop human mesenchymal stem cell (hMSC)-derived EV therapeutics. First, by utilizing DICER and AGO2 knockdown, the Ravindran lab demonstrated that the miRNA cargo within EVs was specifically responsible for the functionality of hMSC EVs (Huang, Kang, Lu et al., 2020; Huang, Kang, Narayanan et al., 2020; Narayanan et al., 2018). His team showed that altering the hMSC state can modify the EV miRNA cargo, resulting in enhanced regenerative or immunomodulatory function (Huang, Kang, Lu et al., 2020; Huang, Kang, Narayanan et al., 2020; Kang et al., 2022). Secondly, through miRNA sequencing and an informatics-based approach, they identified changes to hMSC EV miRNA

TABLE 4 A summary of the most relevant characteristics of EV tracking methods for use in ocular tissues in vivo.

In vivo tracking methods						
Method	**Resolution**	**Sensitivity**	**Tissue depth**	**Cost**	**Speed**	**Reference**
MRI	High	High	Deep	High	Slow	Han et al. (2021)
CT	High	Low	Deep	High	Slow	Cohen et al. (2021)
MPI	Low	High	Deep	High	Slow	Jung et al. (2018)
SPECT	Low	High	Deep	High	Slow	Jung et al. (2020)
PET	Low	High	Deep	High	Slow	Jung et al. (2020)
Bioluminescence	Low	Medium	Shallow	Medium	Medium	Lai et al. (2014)
Fluorescence fundoscopy	High	Medium	Medium	Medium	Fast	Pollalis et al. (2022)

Abbreviations: CT, (x-ray) computed tomograpy; MPI, magnetic particle imaging; MRI, magnetic resonance imaging; PET, positron emission tomography; SPECT, single-photon emission computed tomography.

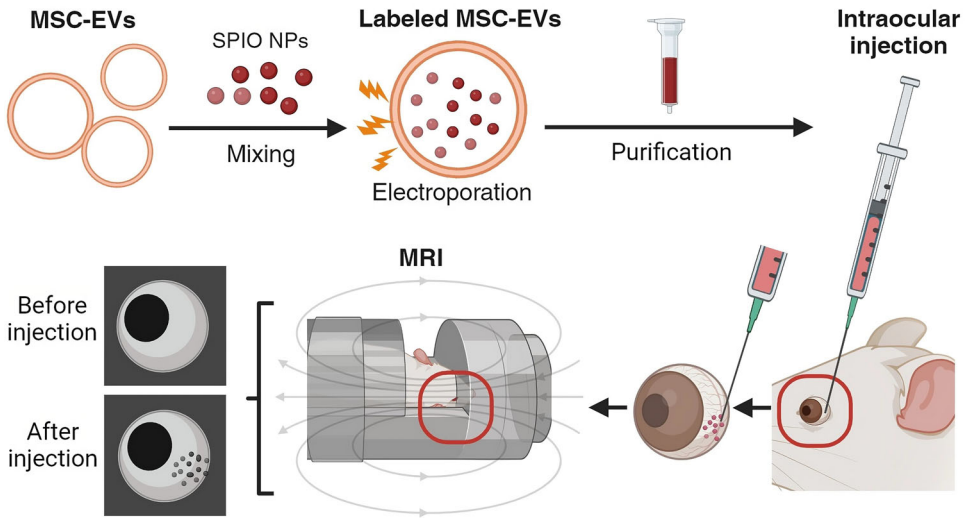

FIGURE 2 Workflow for magnetic labelling of (MSC)-EVs for detection by MRI. EVs are mixed with superparamagnetic iron oxide NPs used as MRI contrast agent and then loaded in EVs by electroporation. Following purification to remove non-bound SPIOs, labelled EVs are injected at the target site. The SPIO-EVs will disturb the local magnetic field, leading to loss of MRI signal. By comparing MRI scans made before and after injection the biodistribution of EVs can be determined by their appearance as black spots. SPIO NPs are fully biocompatible and once taken up by host cells, will slowly biodegrade with the elemental iron eventually recycled into the normal body iron pool. Figure courtesy of Dr. Shreyas Kuddannaya. EV, extracellular vesicle; NP, nanoparticle; MRI, magnetic resonance imaging.

composition in response to a hypoxic environment, reflecting changes in hMSC state. Employing this approach, they identified miR-424-5p as a candidate miRNA capable of enhancing the anti-inflammatory and regenerative properties of hMSC EVs. The mature miRNA was specifically expressed in hMSC EVs by stably transfecting parental MSCs with a plasmid that constitutively expresses this miRNA, with a GGAG tag attached to the 5p strand facilitating its shuttling specifically to the EVs (Villarroya-Beltri et al., 2013). The efficacy of these EVs was evaluated in a rat retinal ischemia model regarding neuroinflammation, neuroprotection and functional recovery. Results indicated that the engineered EVs exhibited enhanced activity both in vitro and in vivo in a pathway-specific manner directly linked to the targets of miR-424-5p. In the retinal ischemia model, they observed improved recovery and reduced inflammation post-ischemia. These results indicate that MSC EVs can be engineered to possess enhanced levels of specific mature miRNAs to elicit pathway-specific activity (Mathew et al., 2023).

4.1 | Gaps, needs and opportunities

During the discussion, panellists emphasized the importance of rigor and standardization in the EV field and introduced various tools, techniques and approaches for studying EVs. Necessary studies in multi-omics, transcriptomics and EV bioengineering, as well as single-particle analyses, sorting and visualization, highlight the demand for a multidisciplinary approach for rigor in EV research. Fortunately, ongoing research across EV fields is rapidly evolving to develop these tools and techniques, which could

significantly benefit EV research in the vision field. Proper understanding of the limitations of each technology is also necessary for maintaining study rigor.

5 | DISCUSSION

Promising data from various disciplines highlighted the diagnostic and therapeutic potential of EVs, alongside advancements in EV research technologies. Particularly noteworthy is the demonstration that EVs derived from stem cells and other cell types possess anti-inflammatory, regenerative, neuroprotective and pro-survival properties. Additionally, ongoing technological progress in bioengineering EVs, including surface modification and cargo loading, holds promise for novel ocular therapeutics.

Nonetheless, significant obstacles were recognized in translating EV research into effective diagnostics and therapeutics. Foremost among these is the limited understanding of which EV cargoes are important for driving or treating ocular diseases and their functional delivery mechanisms in both physiological and pathological conditions. Addressing these challenges also requires overcoming technological barriers in EV isolation and characterization with high sensitivity, as well as achieving single-vesicle level characterization and sorting, and enabling in vivo high-resolution EV tracking.

These gaps present an opportunity for fundamental biological studies that can drive translation and advance clinical applications centred around EVs. In parallel, the integration of novel technologies and resources developed in the broader EV field holds promise for application in ocular research which may catalyze innovations to address the distinctive fluids or cell types in the visual system. Thus, interdisciplinary collaborations create opportunities to propel the field forward, fostering synergy among investigators with diverse expertise and perspectives.

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REFERENCES

Aiello, L. P., Avery, R. L., Arrigg, P. G., Keyt, B. A., Jampel, H. D., Shah, S. T., Pasquale, L. R., Thieme, H., Iwamoto, M. A., Park, J. E., Nguyen, H. V., Aiello, L. M., Ferrara, N., & King, G. L. (1994). Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *New England Journal of Medicine*, *331*(22), 1480–1487. https://doi.org/10.1056/NEJM199412013312203

Aires, I. D., Ribeiro-Rodrigues, T., Boia, R., Catarino, S., Girão, H., Ambrósio, A. F., & Santiago, A. R. (2020). Exosomes derived from microglia exposed to elevated pressure amplify the neuroinflammatory response in retinal cells. *Glia*, *68*(12), 2705–2724. https://doi.org/10.1002/glia.23880

An, H. J., Cho, H. K., Song, D. H., & Kee, C. (2022). Quantitative analysis of exosomes in the aqueous humor of Korean patients with pseudoexfoliation glaucoma. *Scientific Reports*, *12*(1), 12875. https://doi.org/10.1038/s41598-022-17063-9

An, L., Chopp, M., Zacharek, A., Shen, Y., Chen, Z., Qian, Y., Li, W., Landschoot-Ward, J., Liu, Z., & Venkat, P. (2021). Cardiac dysfunction in a mouse vascular dementia model of bilateral common carotid artery stenosis. *Frontiers in Cardiovascular Medicine*, *8*, 681572. https://doi.org/10.3389/fcvm.2021.681572

An, S., Anwar, K., Ashraf, M., Lee, H., Jung, R., Koganti, R., Ghassemi, M., & Djalilian, A. R. (2023). Wound-healing effects of mesenchymal stromal cell secretome in the cornea and the role of exosomes. *Pharmaceutics*, *15*(5), 1486. https://doi.org/10.3390/pharmaceutics15051486

Anand, S., Trounce, I. A., & Gangoda, L. (2022). Role of extracellular vesicles in mitochondrial eye diseases. *Iubmb Life*, *74*(12), 1264–1272. https://doi.org/10.1002/iub.2687

Andronico, L. A., Jiang, Y., Jung, S. R., Fujimoto, B. S., Vojtech, L., & Chiu, D. T. (2021). Sizing extracellular vesicles using membrane dyes and a single molecule-sensitive flow analyzer. *Analytical Chemistry*, *93*(14), 5897–5905. https://doi.org/10.1021/acs.analchem.1c00253

Arifin, D. R., Witwer, K. W., & Bulte, J. W. M. (2022). Non-invasive imaging of extracellular vesicles: Quo vaditis in vivo? *Journal of Extracellular Vesicles*, *11*(7), e12241. https://doi.org/10.1002/jev2.12241

Arjunan, P., Swaminathan, R., Yuan, J., Al-Shabrawey, M., Espinosa-Heidmann, D. G., Nussbaum, J., Martin, P. M., & Cutler, C. W. (2020). Invasion of human retinal pigment epithelial cells by porphyromonas gingivalis leading to vacuolar/cytosolic localization and autophagy dysfunction in-vitro. *Scientific Reports*, *10*(1), 7468. https://doi.org/10.1038/s41598-020-64449-8

Attaluri, S., Jaimes Gonzalez, J., Kirmani, M., Vogel, A. D., Upadhya, R., Kodali, M., Madhu, L. N., Rao, S., Shuai, B., Babu, R. S., Huard, C., & Shetty, A. K. (2023). Intranasally administered extracellular vesicles from human induced pluripotent stem cell-derived neural stem cells quickly incorporate into neurons and microglia in 5xFAD mice. *Frontiers in Aging Neuroscience*, *15*, 1200445. https://doi.org/10.3389/fnagi.2023.1200445

Barman, B., Sung, B. H., Krystofiak, E., Ping, J., Ramirez, M., Millis, B., Allen, R., Prasad, N., Chetyrkin, S., Calcutt, M. W., Vickers, K., Patton, J. G., Liu, Q., & Weaver, A. M. (2022). VAP-A and its binding partner CERT drive biogenesis of RNA-containing extracellular vesicles at ER membrane contact sites. *Developmental Cell*, *57*(8), 974–994.e8. https://doi.org/10.1016/j.devcel.2022.03.012

Bouma, B. E., de Boer, J. F., Huang, D., Jang, I. K., Yonetsu, T., Leggett, C. L., Leitgeb, R., Sampson, D. D., Suter, M., Vakoc, B., Villiger, M., & Wojtkowski, M. (2022). Optical coherence tomography. *Nature Reviews Methods Primers*, *2*, 79. https://doi.org/10.1038/s43586-022-00162-2

Bowes Rickman, C., Farsiu, S., Toth, C. A., & Klingeborn, M. (2013). Dry age-related macular degeneration: Mechanisms, therapeutic targets, and imaging. *Investigative Ophthalmology & Visual Science*, *54*(14), ORSF68–ORSF80. https://doi.org/10.1167/iovs.13-12757

Bulte, J. W. M., & Daldrup-Link, H. E. (2018). Clinical tracking of cell transfer and cell transplantation: Trials and tribulations. *Radiology*, *289*(3), 604–615. https://doi.org/10.1148/radiol.2018180449

Carney, R. P., Mizenko, R. R., Bozkurt, B. T., Lowe, N., Henson, T., Arizzi, A., Wang, A., Tan, C., & George, S. C. (2024). Harnessing extracellular vesicle heterogeneity for diagnostic and therapeutic applications. *Nature Nanotechnology*, https://doi.org/10.1038/s41565-024-01774-3

Chang, A. Y., Soleimani, M., Jung, R., Tu, G., Dana, R., Jeng, B. H., Hematti, P., Mahmud, N., Tu, E. Y., Joslin, C. E., & Djalilian, A. R. (2023). Phase I study on the safety of locally delivered allogeneic mesenchymal stem cells for promoting corneal repair. *Investigative Ophthalmology & Visual Science*, *64*(8), 3136–3136.

Chen, Y. S., Lin, E. Y., Chiou, T. W., & Harn, H. J. (2019). Exosomes in clinical trial and their production in compliance with good manufacturing practice. *Tzu Chi Medical Journal*, *32*(2), 113–120. https://doi.org/10.4103/tcmj.tcmj_182_19

Chen, Z., Chopp, M., Zacharek, A., Li, W., Venkat, P., Wang, F., Landschoot-Ward, J., & Chen, J. (2019). Brain-derived microparticles (BDMPs) contribute to neuroinflammation and lactadherin reduces BDMP induced neuroinflammation and improves outcome after stroke. *Frontiers in Immunology*, *10*, 2747. https://doi.org/10.3389/fimmu.2019.02747

Chiang, M. F. (2021). The 2021 national eye institute strategic plan: Driving innovation in eye and vision research. *Investigative Ophthalmology & Visual Science*, *62*(14), 2. https://doi.org/10.1167/iovs.62.14.2

Chiang, M. F., & Tumminia, S. J. (2022). The 2021 National Eye Institute strategic plan: Eliminating vision loss and improving quality of life. *Ophthalmology*, *129*(1), 12–14. https://doi.org/10.1016/j.ophtha.2021.09.012

Cioanca, A. V., Natoli, R., & Wooff, Y. (2023). Proteomics of retinal extracellular vesicles: A review into an unexplored mechanism in retinal health and AMD pathogenesis. *Advances in Experimental Medicine and Biology*, *1415*, 87–94. https://doi.org/10.1007/978-3-031-27681-1_14

Cioanca, A. V., Wooff, Y., Aggio-Bruce, R., Sekar, R., Dietrich, C., & Natoli, R. (2023). Multiomic integration reveals neuronal-extracellular vesicle coordination of gliotic responses in degeneration. *Journal of Extracellular Vesicles*, *12*(12), e12393. https://doi.org/10.1002/jev2.12393

Cohen, O., Betzer, O., Elmaliach-Pnini, N., Motiei, M., Sadan, T., Cohen-Berkman, M., Dagan, O., Popovtzer, A., Yosepovich, A., Barhom, H., Michaeli, S., & Popovtzer, R. (2021). 'Golden' exosomes as delivery vehicles to target tumors and overcome intratumoral barriers: In vivo tracking in a model for head and neck cancer. *Biomaterials Science*, *9*(6), 2103–2114. https://doi.org/10.1039/d0bm01735c

Danielson, K. M., Shah, R., Yeri, A., Liu, X., Camacho Garcia, F., Silverman, M., Tanriverdi, K., Das, A., Xiao, C., Jerosch-Herold, M., Heydari, B., Abbasi, S., Van Keuren-Jensen, K., Freedman, J. E., Wang, Y. E., Rosenzweig, A., Kwong, R. Y., & Das, S. (2018). Plasma circulating extracellular RNAs in left ventricular remodeling post-myocardial infarction. *EBioMedicine*, *32*, 172–181. https://doi.org/10.1016/j.ebiom.2018.05.013

Dartt, D. A., & Willcox, M. D. (2013). Complexity of the tear film: Importance in homeostasis and dysfunction during disease. *Experimental Eye Research*, *117*, 1–3. https://doi.org/10.1016/j.exer.2013.10.008

Demais, V., Pohl, A., Wunderlich, K. A., Pfaller, A. M., Kaplan, L., Barthélémy, A., Dittrich, R., Puig, B., Giebel, B., Hauck, S. M., Pfrieger, F. W., & Grosche, A. (2022). Release of VAMP5-positive extracellular vesicles by retinal Muller glia in vivo. *Journal of Extracellular Vesicles*, *11*(9), e12254. https://doi.org/10.1002/jev2.12254

Dhodapkar, R. M., Jung, E., & Lee, S. Y. (2024). An eye on extracellular vesicles: Trends and clinical translations in vision research. *Ophthalmology Science*, *5*(1), 100619.

Ding, G., Li, L., Zhang, L., Chopp, M., Davoodi-Bojd, E., Li, Q., Li, C., Wei, M., Zhang, Z., & Jiang, Q. (2022). MRI metrics of cerebral endothelial cell-derived exosomes for the treatment of cognitive dysfunction induced in aging rats subjected to type 2 diabetes. *Diabetes*, *71*(5), 873–880. https://doi.org/10.2337/db21-0754

Dismuke, W. M., Challa, P., Navarro, I., Stamer, W. D., & Liu, Y. (2015). Human aqueous humor exosomes. *Experimental Eye Research*, *132*, 73–77. https://doi.org/10.1016/j.exer.2015.01.019

Dixson, A. C., Dawson, T. R., Di Vizio, D., & Weaver, A. M. (2023). Context-specific regulation of extracellular vesicle biogenesis and cargo selection. *Nature Reviews Molecular Cell Biology*, *24*(7), 454–476. https://doi.org/10.1038/s41580-023-00576-0

Dominy, S. S., Lynch, C., Ermini, F., Benedyk, M., Marczyk, A., Konradi, A., Nguyen, M., Haditsch, U., Raha, D., Griffin, C., Holsinger, L. J., Arastu-Kapur, S., Kaba, S., Lee, A., Ryder, M. I., Potempa, B., Mydel, P., Hellvard, A., Adamowicz, K., … Potempa, J. (2019). Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. *Science Advances*, *5*(1), eaau3333. https://doi.org/10.1126/sciadv.aau3333

Drewry, M. D., Challa, P., Kuchtey, J. G., Navarro, I., Helwa, I., Hu, Y., Mu, H., Stamer, W. D., Kuchtey, R. W., & Liu, Y. (2018). Differentially expressed microRNAs in the aqueous humor of patients with exfoliation glaucoma or primary open-angle glaucoma. *Human Molecular Genetics*, *27*(7), 1263–1275. https://doi.org/10.1093/hmg/ddy040

Elashiry, M., Elashiry, M. M., Elsayed, R., Rajendran, M., Auersvald, C., Zeitoun, R., Rashid, M. H., Ara, R., Meghil, M. M., Liu, Y., Arbab, A. S., Arce, R. M., Hamrick, M., Elsalanty, M., Brendan, M., Pacholczyk, R., & Cutler, C. W. (2020). Dendritic cell derived exosomes loaded with immunoregulatory cargo reprogram local immune responses and inhibit degenerative bone disease in vivo. *Journal of Extracellular Vesicles*, *9*(1), 1795362. https://doi.org/10.1080/20013078.2020.1795362

Elashiry, M., Elsayed, R., & Cutler, C. W. (2021). Exogenous and endogenous dendritic cell-derived exosomes: Lessons learned for immunotherapy and disease pathogenesis. *Cells*, *11*(1), 115. https://doi.org/10.3390/cells11010115

Elashiry, M., Elsayed, R., Elashiry, M. M., Rashid, M. H., Ara, R., Arbab, A. S., Elawady, A. R., Hamrick, M., Liu, Y., Zhi, W., Lucas, R., Vazquez, J., & Cutler, C. W. (2021). Proteomic characterization, biodistribution, and functional studies of immune-therapeutic exosomes: implications for inflammatory lung diseases. *Frontiers in Immunology*, *12*, 636222. https://doi.org/10.3389/fimmu.2021.636222

Elsayed, R., Elashiry, M., Tran, C., Yang, T., Carroll, A., Liu, Y., Hamrick, M., & Cutler, C. W. (2023). Engineered human dendritic cell exosomes as effective delivery system for immune modulation. *International Journal of Molecular Sciences*, *24*(14), 11306. https://doi.org/10.3390/ijms241411306

Flores-Bellver, M., Mighty, J., Aparicio-Domingo, S., Li, K. V., Shi, C., Zhou, J., Cobb, H., Mcgrath, P., Michelis, G., Lenhart, P., Bilousova, G., Heissel, S., Rudy, M. J., Coughlan, C., Goodspeed, A. E., Becerra, S. P., Redenti, S., & Canto-Soler, M. V. (2021). Extracellular vesicles released by human retinal pigment epithelium mediate increased polarised secretion of drusen proteins in response to AMD stressors. *Journal of Extracellular Vesicles*, *10*(13), e12165. https://doi.org/10.1002/jev2.12165

Fordjour, F. K., Abuelreich, S., Hong, X., Chatterjee, E., Lallai, V., Ng, M., Saftics, A., Deng, F., Carnel-Amar, N., Wakimoto, H., Shimizu, K., Bautista, M., Phu, T. A., Vu, N. K., Geiger, P. C., Raffai, R. L., Fowler, C. D., Das, S., Christenson, L. K., … Gould, S. J. (2023). Exomap1 mouse: A transgenic model for in vivo studies of exosome biology. *Extracellular Vesicle*, *2*(100030),. https://doi.org/10.1016/j.vesic.2023.100030

Go, V., Bowley, B. G. E., Pessina, M. A., Zhang, Z. G., Chopp, M., Finklestein, S. P., Rosene, D. L., Medalla, M., Buller, B., & Moore, T. L. (2020). Extracellular vesicles from mesenchymal stem cells reduce microglial-mediated neuroinflammation after cortical injury in aged Rhesus monkeys. *Geroscience*, *42*(1), 1–17. https://doi.org/10.1007/s11357-019-00115-w

Gokulnath, P., Spanos, M., Lehmann, H. I., Sheng, Q., Rodosthenous, R., Chaffin, M., Varrias, D., Chatterjee, E., Hutchins, E., Li, G., Daaboul, G., Rana, F., Wang, A. M., Van Keuren-Jensen, K., Ellinor, P. T., Shah, R., & Das, S. (2023). Plasma extracellular vesicle transcriptome as a dynamic liquid biopsy in acute heart failure. *medRxiv*, https://doi.org/10.1101/2023.02.17.23285936

Gokulnath, P., Spanos, M., Lehmann, H. I., Sheng, Q., Rodosthenous, R., Chaffin, M., Varrias, D., Chatterjee, E., Hutchins, E., Li, G., Daaboul, G., Rana, F., Wang, A. M., Van Keuren-Jensen, K., Ellinor, P. T., Shah, R., & Das, S. (2024). Distinct plasma extracellular vesicle transcriptomes in acute decompensated heart failure subtypes: A liquid biopsy approach. *Circulation*, *149*(14), 1147–1149. https://doi.org/10.1161/CIRCULATIONAHA.123.065513

Grigor'eva, A. E., Tamkovich, S. N., Eremina, A. V., Tupikin, A. E., Kabilov, M. R., Chernykh, V. V., Vlassov, V. V., Laktionov, P. P., & Ryabchikova, E. I. (2016). Exosomes in tears of healthy individuals: Isolation, identification, and characterization. *Biochem Moscow Suppl Ser B*, *10*, 165–172. https://doi.org/10.1134/S1990750816020049

Han, Z., Liu, S., Pei, Y., Ding, Z., Li, Y., Wang, X., Zhan, D., Xia, S., Driedonks, T., Witwer, K. W., Weiss, R. G., Van Zijl, P. C. M., Bulte, J. W. M., Cheng, L., & Liu, G. (2021). Highly efficient magnetic labelling allows MRI tracking of the homing of stem cell-derived extracellular vesicles following systemic delivery. *Journal of Extracellular Vesicles*, *10*(3), e12054. https://doi.org/10.1002/jev2.12054

Hardy, K. M., Hoffman, E. A., Gonzalez, P., McKay, B. S., & Stamer, W. D. (2005). Extracellular trafficking of myocilin in human trabecular meshwork cells. *Journal of Biological Chemistry*, *280*(32), 28917–28926. https://doi.org/10.1074/jbc.M504803200

Harrell, C. R., Djonov, V., Antonijevic, A., & Volarevic, V. (2023). NLRP3 inflammasome as a potentially new therapeutic target of mesenchymal stem cells and their exosomes in the treatment of inflammatory eye diseases. *Cells*, *12*(18), 2327. https://doi.org/10.3390/cells12182327

Hefley, B. S., Deighan, C., Vasini, B., Khan, A., Hjortdal, J., Riaz, K. M., Liu, Y., & Karamichos, D. (2022). Revealing the presence of tear extracellular vesicles in Keratoconus. *Experimental Eye Research*, *224*, 109242. https://doi.org/10.1016/j.exer.2022.109242

Hernandez, B. J., Skiba, N. P., Plössl, K., Strain, M., Liu, Y., Grigsby, D., Kelly, U., Cady, M. A., Manocha, V., Maminishkis, A., Watkins, T., Miller, S. S., Ashley-Koch, A., Stamer, W. D., Weber, B. H. F., Bowes Rickman, C., & Klingeborn, M. (2023). Polarized Desmosome and hemidesmosome shedding via small extracellular vesicles is an early indicator of outer blood-retina barrier dysfunction. *Journal of Extracellular Biology*, *2*(10), e116. https://doi.org/10.1002/jex2.116

Hodges, R. R., & Dartt, D. A. (2013). Tear film mucins: Front line defenders of the ocular surface; comparison with airway and gastrointestinal tract mucins. *Experimental Eye Research*, *117*, 62–78. https://doi.org/10.1016/j.exer.2013.07.027

Hu, L., Zhang, T., Ma, H., Pan, Y., Wang, S., Liu, X., Dai, X., Zheng, Y., Lee, L. P., & Liu, F. (2022). Discovering the secret of diseases by incorporated tear exosomes analysis via rapid-isolation system: ITEARS. *ACS Nano*, *16*(8), 11720–11732. https://doi.org/10.1021/acsnano.2c02531

Hu, M., Brown, V., Jackson, J. M., Wijerathne, H., Pathak, H., Koestler, D. C., Nissen, E., Hupert, M. L., Muller, R., Godwin, A. K., Witek, M. A., & Soper, S. A. (2023). Assessing breast cancer molecular subtypes using extracellular vesicles' mRNA. *Analytical Chemistry*, *95*(19), 7665–7675. https://doi.org/10.1021/acs.analchem.3c00624

Huang, C.-C., Kang, M., Lu, Y., Shirazi, S., Diaz, J. I., Cooper, L. F., Gajendrareddy, P., & Ravindran, S. (2020). Functionally engineered extracellular vesicles improve bone regeneration. *Acta Biomaterialia*, *109*, 182–194. https://doi.org/10.1016/j.actbio.2020.04.017

Huang, C.-C., Kang, M., Narayanan, R., Dipietro, L. A., Cooper, L. F., Gajendrareddy, P., & Ravindran, S. (2020). Evaluating the endocytosis and lineage-specification properties of mesenchymal stem cell derived extracellular vesicles for targeted therapeutic applications. *Frontiers in Pharmacology*, *11*, 163. https://doi.org/10.3389/fphar.2020.00163

Im, H., Shao, H., Park, Y. II, Peterson, V. M., Castro, C. M., Weissleder, R., & Lee, H. (2014). Label-free detection and molecular profiling of exosomes with a nano-plasmonic sensor. *Nature Biotechnology*, *32*(5), 490–495. https://doi.org/10.1038/nbt.2886

Inubushi, S., Kawaguchi, H., Mizumoto, S., Kunihisa, T., Baba, M., Kitayama, Y., Takeuchi, T., Hoffman, R. M., & Sasaki, R. (2020). Oncogenic miRNAs identified in tear exosomes from metastatic breast cancer patients. *Anticancer Research*, *40*(6), 3091–3096. https://doi.org/10.21873/anticanres.14290

Jeong, S., Park, J., Pathania, D., Castro, C. M., Weissleder, R., & Lee, H. (2016). Integrated magneto-electrochemical sensor for exosome analysis. *ACS Nano*, *10*(2), 1802–1809. https://doi.org/10.1021/acsnano.5b07584

Jiang, Y., Andronico, L. A., Jung, S.-R., Chen, H., Fujimoto, B., Vojtech, L., & Chiu, D. T. (2021). High-throughput counting and superresolution mapping of tetraspanins on exosomes using a single-molecule sensitive flow technique and transistor-like semiconducting polymer dots. *Angewandte Chemie (International ed in English)*, *60*(24), 13470–13475. https://doi.org/10.1002/anie.202103282

Jung, K. O., Jo, H., Yu, J. H., Gambhir, S. S., & Pratx, G. (2018). Development and MPI tracking of novel hypoxia-targeted theranostic exosomes. *Biomaterials*, *177*, 139–148. https://doi.org/10.1016/j.biomaterials.2018.05.048

Jung, K. O., Kim, Y.-H., Chung, S.-J., Lee, C.-H., Rhee, S., Pratx, G., Chung, J.-K., & Youn, H. (2020). Identification of lymphatic and hematogenous routes of rapidly labeled radioactive and fluorescent exosomes through highly sensitive multimodal imaging. *International Journal of Molecular Sciences*, *21*(21), 7850. https://doi.org/10.3390/ijms21217850

Kalargyrou, A. A., Guilfoyle, S. E., Smith, A. J., Ali, R. R., & Pearson, R. A. (2022). Extracellular vesicles in the retina-Putative roles in physiology and disease. *Frontiers in Molecular Neuroscience*, *15*, 1042469. https://doi.org/10.3389/fnmol.2022.1042469

Kalluri, R., & LeBleu, V. S. (2020). The biology, function, and biomedical applications of exosomes. *Science*, *367*(6478), eaau6977. https://doi.org/10.1126/science.aau6977

Kang, M., Choi, J. K., Jittayasothorn, Y., & Egwuagu, C. E. (2020). Interleukin 35-producing exosomes suppress neuroinflammation and autoimmune uveitis. *Frontiers in Immunology*, *11*, 1051. https://doi.org/10.3389/fimmu.2020.01051

Kang, M., Huang, C.-C., Gajendrareddy, P., Lu, Y., Shirazi, S., Ravindran, S., & Cooper, L. F. (2022). Extracellular vesicles from TNFalpha preconditioned MSCs: Effects on immunomodulation and bone regeneration. *Frontiers in Immunology*, *13*, 878194. https://doi.org/10.3389/fimmu.2022.878194

Kang, M., Yadav, M. K., Mbanefo, E. C., Yu, C. R., & Egwuagu, C. E. (2023). IL-27-containing exosomes secreted by innate B-1a cells suppress and ameliorate uveitis. *Frontiers in Immunology*, *14*, 1071162. https://doi.org/10.3389/fimmu.2023.1071162

Kels, B. D., Grzybowski, A., & Grant-Kels, J. M. (2015). Human ocular anatomy. *Clinics in Dermatology*, *33*(2), 140–146. https://doi.org/10.1016/j.clindermatol.2014.10.006

Kilic, T., Cho, Y. K., Jeong, N., Shin, I.-S., Carter, B. S., Balaj, L., Weissleder, R., & Lee, H. (2022). Multielectrode spectroscopy enables rapid and sensitive molecular profiling of extracellular vesicles. *ACS Central Science*, *8*(1), 110–117. https://doi.org/10.1021/acscentsci.1c01193

Klingeborn, M., Dismuke, W. M., Bowes Rickman, C., & Stamer, W. D. (2017). Roles of exosomes in the normal and diseased eye. *Progress in Retinal and Eye Research*, *59*, 158–177. https://doi.org/10.1016/j.preteyeres.2017.04.004

Klingeborn, M., Dismuke, W. M., Skiba, N. P., Kelly, U., Stamer, W. D., & Bowes Rickman, C. (2017). Directional exosome proteomes reflect polarity-specific functions in retinal pigmented epithelium monolayers. *Scientific Reports*, *7*(1), 4901. https://doi.org/10.1038/s41598-017-05102-9

Kolb, H., Fernandez, E., & Nelson, R. (1995). Webvision: The organization of the retina and visual system. In H. Kolb, E. Fernandez, & R. Nelson, eds. *Webvision: The organization of the retina and visual system*.

Kowal, J., Arras, G., Colombo, M., Jouve, M., Morath, J. P., Primdal-Bengtson, B., Dingli, F., Loew, D., Tkach, M., & Théry, C. (2016). Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(8), E968–E977. https://doi.org/10.1073/pnas.1521230113

Lai, C. P., Mardini, O., Ericsson, M., Prabhakar, S., Maguire, C., Chen, J. W., Tannous, B. A., & Breakefield, X. O. (2014). Dynamic biodistribution of extracellular vesicles in vivo using a multimodal imaging reporter. *ACS Nano*, *8*(1), 483–494. https://doi.org/10.1021/nn404945r

Lerner, N., Avissar, S., & Beit-Yannai, E. (2017). Extracellular vesicles mediate signaling between the aqueous humor producing and draining cells in the ocular system. *PLoS ONE*, *12*(2), e0171153. https://doi.org/10.1371/journal.pone.0171153

Lerner, N., Schreiber-Avissar, S., & Beit-Yannai, E. (2020). Extracellular vesicle-mediated crosstalk between NPCE cells and TM cells result in modulation of Wnt signalling pathway and ECM remodelling. *Journal of Cellular and Molecular Medicine*, *24*(8), 4646–4658. https://doi.org/10.1111/jcmm.15129

Leung, J., Pollalis, D., Nair, G. K. G., Bailey, J. K., Pennington, B. O., Khan, A. I., Kelly, K. R., Yeh, A. K., Sundaram, K. S., Clegg, D. O., Peng, C.-C., Xu, L., & Lee, S. Y. (2024). Isolation and characterization of extracellular vesicles through orthogonal approaches for the development of intraocular EV therapy. *Investigative Ophthalmology & Visual Science*, *65*(3), 6. https://doi.org/10.1167/iovs.65.3.6

Li, C., Wang, C., Zhang, Y., Alsrouji, O. K., Chebl, A. B., Ding, G., Jiang, Q., Mayer, S. A., Lu, M., Kole, M. K., Marin, H. L., Zhang, L., Chopp, M., & Zhang, Z. G. (2021). Cerebral endothelial cell-derived small extracellular vesicles enhance neurovascular function and neurological recovery in rat acute ischemic stroke models of mechanical thrombectomy and embolic stroke treatment with tPA. *Journal of Cerebral Blood Flow and Metabolism*, *41*(8), 2090–2104. https://doi.org/10.1177/0271678x21992980

Li, J., Salvador, A. M., Li, G., Valkov, N., Ziegler, O., Yeri, A., Yang Xiao, C., Meechoovet, B., Alsop, E., Rodosthenous, R. S., Kundu, P., Huan, T., Levy, D., Tigges, J., Pico, A. R., Ghiran, I., Silverman, M. G., Meng, X., Kitchen, R., … Das, S. (2021). Mir-30d regulates cardiac remodeling by intracellular and paracrine signaling. *Circulation Research*, *128*(1), e1–e23. https://doi.org/10.1161/CIRCRESAHA.120.317244

Li, J., Sha, Z., Zhu, X., Xu, W., Yuan, W., Yang, T., Jin, B., Yan, Y., Chen, R., Wang, S., Yao, J., Xu, J., Wang, Z., Li, G., Das, S., Yang, L., & Xiao, J. (2022). Targeting miR-30d reverses pathological cardiac hypertrophy. *EBioMedicine*, *81*, 104108. https://doi.org/10.1016/j.ebiom.2022.104108

Li, W., Li, L., Chopp, M., Venkat, P., Zacharek, A., Chen, Z., Landschoot-Ward, J., Yan, T., & Chen, J. (2018). Intracerebral hemorrhage induces cardiac dysfunction in mice without primary cardiac disease. *Frontiers in Neurology*, *9*, 965. https://doi.org/10.3389/fneur.2018.00965

Liu, C., Zhao, J., Tian, F., Cai, L., Zhang, W., Feng, Q., Chang, J., Wan, F., Yang, Y., Dai, B., Cong, Y., Ding, B., Sun, J., & Tan, W. (2019). Low-cost thermophoretic profiling of extracellular-vesicle surface proteins for the early detection and classification of cancers. *Nature Biomedical Engineering*, *3*(3), 183–193. https://doi.org/10.1038/s41551-018-0343-6

Liu, Y., Allingham, R. R., Qin, X., Layfield, D., Dellinger, A. E., Gibson, J., Wheeler, J., Ashley-Koch, A. E., Stamer, W. D., & Hauser, M. A. (2013). Gene expression profile in human trabecular meshwork from patients with primary open-angle glaucoma. *Investigative Ophthalmology & Visual Science*, *54*(9), 6382–6389. https://doi.org/10.1167/iovs.13-12128

Liu, Y., Bailey, J. C., Helwa, I., Dismuke, W. M., Cai, J., Drewry, M., Brilliant, M. H., Budenz, D. L., Christen, W. G., Chasman, D. I., Fingert, J. H., Gaasterland, D., Gaasterland, T., Gordon, M. O., Igo, R. P., Kang, J. H., Kass, M. A., Kraft, P., Lee, R. K., … Wiggs, J. L. (2016). A common variant in MIR182 is associated with primary open-angle glaucoma in the NEIGHBORHOOD Consortium. *Investigative Ophthalmology & Visual Science*, *57*(10), 4528–4535. https://doi.org/10.1167/iovs.16-19688

Locke, C. J., Congrove, N. R., Dismuke, W. M., Bowen, T. J., Stamer, W. D., & McKay, B. S. (2014). Controlled exosome release from the retinal pigment epithelium in situ. *Experimental Eye Research*, *129*, 1–4. https://doi.org/10.1016/j.exer.2014.10.010

Ludwig, A. K., & Giebel, B. (2012). Exosomes: Small vesicles participating in intercellular communication. *International Journal of Biochemistry & Cell Biology*, *44*(1), 11–15. https://doi.org/10.1016/j.biocel.2011.10.005

Ludwig, P. E., Aslam, S., & Czyz, C. N. (2024). *Anatomy, head and neck: eye muscles*. StatPearls.

Ludwig, P. E., Jessu, R., & Czyz, C. N. (2024). *Physiology, Eye*. StatPearls.

Ludwig, P. E., Lopez, M. J., & Sevensma, K. E. (2024). *Anatomy, head and neck, eye cornea*. StatPearls.

Madhu, L. N., Kodali, M., Upadhya, R., Rao, S., Shuai, B., Somayaji, Y., Attaluri, S., Kirmani, M., Gupta, S., Maness, N., Rao, X., Cai, J., & Shetty, A. K. (2024). Intranasally administered EVs from hiPSC-derived NSCs alter the transcriptomic profile of activated microglia and conserve brain function in an Alzheimer's model. *BioRxiv*, *13*, e12519. https://doi.org/10.1101/2024.01.18.576313

Martins, B., Pires, M., Ambrósio, A. F., Girão, H., & Fernandes, R. (2024). Contribution of extracellular vesicles for the pathogenesis of retinal diseases: Shedding light on blood-retinal barrier dysfunction. *Journal of Biomedical Science*, *31*(1), 48. https://doi.org/10.1186/s12929-024-01036-3

Mathew, B., Acha, L. G., Torres, L. A., Huang, C.-C., Liu, A., Kalinin, S., Leung, K., Dai, Y., Feinstein, D. L., Ravindran, S., & Roth, S. (2023). MicroRNA-based engineering of mesenchymal stem cell extracellular vesicles for treatment of retinal ischemic disorders: Engineered extracellular vesiclesand retinal ischemia. *Acta Biomaterialia*, *158*, 782–797. https://doi.org/10.1016/j.actbio.2023.01.014

McCann, R. P., Zhou, Y., & Mojica, C. A. (2023). Extracellular vesicles derived from mesenchymal stromal cells improving clearance of damage and dampen microglial inflammation in a monkey model of cortical injury. Presented at: Neurotrauma Annual Meeting; (2023). San Antonio, TX.

Mckiernan, J., Donovan, M. J., O'neill, V., Bentink, S., Noerholm, M., Belzer, S., Skog, J., Kattan, M. W., Partin, A., Andriole, G., Brown, G., Wei, J. T., Thompson, I. M., & Carroll, P. (2016). A novel urine exosome gene expression assay to predict high-grade prostate cancer at initial biopsy. *JAMA Oncology*, *2*(7), 882–889. https://doi.org/10.1001/jamaoncol.2016.0097

Mead, B., Ahmed, Z., & Tomarev, S. (2018). Mesenchymal stem cell-derived small extracellular vesicles promote neuroprotection in a genetic dba/2j mouse model of glaucoma. *Investigative Ophthalmology & Visual Science*, *59*(13), 5473–5480. https://doi.org/10.1167/iovs.18-25310

Mead, B., Amaral, J., & Tomarev, S. (2018). Mesenchymal stem cell-derived small extracellular vesicles promote neuroprotection in rodent models of glaucoma. *Investigative Ophthalmology & Visual Science*, *59*(2), 702–714. https://doi.org/10.1167/iovs.17-22855

Mead, B., & Tomarev, S. (2017). Bone marrow-derived mesenchymal stem cells-derived exosomes promote survival of retinal ganglion cells through miRNA-dependent mechanisms. *STEM CELLS Translational Medicine*, *6*(4), 1273–1285. https://doi.org/10.1002/sctm.16-0428

Mead, B., & Tomarev, S. (2020). Extracellular vesicle therapy for retinal diseases. *Progress in Retinal and Eye Research*, *79*, 100849. https://doi.org/10.1016/j.preteyeres.2020.100849

Medalla, M., Chang, W., Calderazzo, S. M., Go, V., Tsolias, A., Goodliffe, J. W., Pathak, D., De Alba, D., Pessina, M., Rosene, D. L., Buller, B., & Moore, T. L. (2020). Treatment with mesenchymal-derived extracellular vesicles reduces injury-related pathology in pyramidal neurons of monkey perilesional ventral premotor cortex. *Journal of Neuroscience*, *40*(17), 3385–3407. https://doi.org/10.1523/JNEUROSCI.2226-19.2020

Melman, Y. F., Shah, R., Danielson, K., Xiao, J., Simonson, B., Barth, A., Chakir, K., Lewis, G. D., Lavender, Z., Truong, Q. A., Kleber, A., Das, R., Rosenzweig, A., Wang, Y., Kass, D. A., Singh, J. P., & Das, S. (2015). Circulating MicroRNA-30d is associated with response to cardiac resynchronization therapy in heart failure and regulates cardiomyocyte apoptosis: a translational pilot study. *Circulation*, *131*(25), 2202–2216. https://doi.org/10.1161/CIRCULATIONAHA.114.013220

Michaud, D. S., Fu, Z., Shi, J., & Chung, M. (2017). Periodontal disease, tooth loss, and cancer risk. *Epidemiologic Reviews*, *39*(1), 49–58. https://doi.org/10.1093/epirev/mxx006

Moses, K. A., Sprenkle, P. C., Bahler, C., Box, G., Carlsson, S. V., Catalona, W. J., Dahl, D. M., Dall'era, M., Davis, J. W., Drake, B. F., Epstein, J. I., Etzioni, R. B., Farrington, T. A., Garraway, I. P., Jarrard, D., Kauffman, E., Kaye, D., Kibel, A. S., Lagrange, C. A., … Freedman-Cass, D. A. (2023). NCCN guidelines(R) insights: prostate cancer early detection, version 1.2023. *Journal of the National Comprehensive Cancer Network*, *21*(3), 236–246. https://doi.org/10.6004/jnccn.2023.0014

Mueller, A., Anter, A., Edwards, G., Junk, A. K., Liu, Y., Ziebarth, N., & Bhattacharya, S. K. (2023). Glaucomatous aqueous humor vesicles are smaller and differ in composition compared to controls. *Experimental Eye Research*, *234*, 109562. https://doi.org/10.1016/j.exer.2023.109562

Murillo, O. D., Thistlethwaite, W., Rozowsky, J., Subramanian, S. L., Lucero, R., Shah, N., Jackson, A. R., Srinivasan, S., Chung, A., Laurent, C. D., Kitchen, R. R., Galeev, T., Warrell, J., Diao, J. A., Welsh, J. A., Hanspers, K., Riutta, A., Burgstaller-Muehlbacher, S., Shah, R. V., … Milosavljevic, A. (2019). exRNA atlas analysis reveals distinct extracellular RNA cargo types and their carriers present across human biofluids. *Cell*, *177*(2), 463–477.e15. https://doi.org/10.1016/j.cell.2019.02.018

Narayanan, K., Kumar, S., Padmanabhan, P., Gulyas, B., Wan, A. C. A., & Rajendran, V. M. (2018). Lineage-specific exosomes could override extracellular matrix mediated human mesenchymal stem cell differentiation. *Biomaterials*, *182*, 312–322. https://doi.org/10.1016/j.biomaterials.2018.08.027

Németh, K., Kestecher, B. M., Ghosal, S., Bodnár, B. R., Kittel, Á., Hambalkó, S., Kovácsházi, C., Giricz, Z., Ferdinandy, P., Osteikoetxea, X., Burkhardt, R., Buzas, E. I., & Orsó, E. (2024). Therapeutic and pharmacological applications of extracellular vesicles and lipoproteins. *British Journal of Pharmacology*, *181*(23), 4733–4749. https://doi.org/10.1111/bph.17336

Pan, D., Chang, X., Xu, M., Zhang, M., Zhang, S., Wang, Y., Luo, X., Xu, J., Yang, X., & Sun, X. (2019). UMSC-derived exosomes promote retinal ganglion cells survival in a rat model of optic nerve crush. *Journal of Chemical Neuroanatomy*, *96*, 134–139. https://doi.org/10.1016/j.jchemneu.2019.01.006

Park, J., Park, J. S., Huang, C.-H., Jo, A., Cook, K., Wang, R., Lin, H.-Y., Van Deun, J., Li, H., Min, J., Wang, L., Yoon, G., Carter, B. S., Balaj, L., Choi, G.-S., Castro, C. M., Weissleder, R., & Lee, H. (2021). An integrated magneto-electrochemical device for the rapid profiling of tumour extracellular vesicles from blood plasma. *Nature Biomedical Engineering*, *5*(7), 678–689. https://doi.org/10.1038/s41551-021-00752-7

Pedersen, C., Chen, V. T., Herbst, P., Zhang, R., Elfert, A., Krishan, A., Azar, D. T., Chang, J.-H., Hu, W.-Y., Kremsmayer, T. P., Jalilian, E., Djalilian, A. R., Guaiquil, V. H., & Rosenblatt, M. I. (2024). Target specification and therapeutic potential of extracellular vesicles for regulating corneal angiogenesis, lymphangiogenesis, and nerve repair. *The Ocular Surface*, *34*, 459–476. https://doi.org/10.1016/j.jtos.2024.10.005

Pieczyński, J., Szulc, U., Harazna, J., Szulc, A., & Kiewisz, J. (2021). Tear fluid collection methods: Review of current techniques. *European Journal of Ophthalmology*, *31*(5), 2245–2251. https://doi.org/10.1177/1120672121998922

Pieragostino, D., Lanuti, P., Cicalini, I., Cufaro, M. C., Ciccocioppo, F., Ronci, M., Simeone, P., Onofrj, M., Van Der Pol, E., Fontana, A., Marchisio, M., & Del Boccio, P. (2019). Proteomics characterization of extracellular vesicles sorted by flow cytometry reveals a disease-specific molecular cross-talk from cerebrospinal fluid and tears in multiple sclerosis. *Journal of Proteomics*, *204*, 103403. https://doi.org/10.1016/j.jprot.2019.103403

Pitt, J. M., André, F., Amigorena, S., Soria, J.-C., Eggermont, A., Kroemer, G., & Zitvogel, L. (2016). Dendritic cell-derived exosomes for cancer therapy. *Journal of Clinical Investigation*, *126*(4), 1224–1232. https://doi.org/10.1172/JCI81137

Pollalis, D., Georgescu, C., Wren, J. D., Tombulyan, G., Leung, J. M., Lo, P. A., Bloemhof, C. M., Lee, R. H., Bae, E., Bailey, J. K., Pennington, B. O., Khan, A. I., Kelly, K. R., Yeh, A. K., Sundaram, K. S., Humayun, M., Louie, S., Clegg, D. O., & Lee, S. Y. (2024). Rescuing photoreceptors in RPE dysfunction-driven retinal degeneration: The role of small extracellular vesicles secreted from retinal pigment epithelium. *BioRxiv*, 2024:2024.04.09.588773. https://doi.org/10.1101/2024.04.09.588773

Pollalis, D., Kim, D., Nair, G. K. G., Kang, C., Nanda, A. V., & SY, L. (2022). Intraocular RGD-engineered exosomes and active targeting of choroidal neovascularization (CNV). *Cells*, *11*(16), 00–00. https://doi.org/10.3390/cells11162573

Pollalis, D., Nair, G. K. G., Leung, J., Bloemhof, C. M., Bailey, J. K., Pennington, B. O., Kelly, K. R., Khan, A. I., Yeh, A. K., Sundaram, K. S., Clegg, D. O., Peng, C.-C., Xu, L., Georgescu, C., Wren, J. D., & Lee, S. Y. (2024). Dynamics of microRNA secreted via extracellular vesicles during the maturation of embryonic stem cell-derived retinal pigment epithelium. *Journal of Extracellular Biology*, *3*(9), e70001. https://doi.org/10.1002/jex2.70001

Ratajczak, J., Wysoczynski, M., Hayek, F., Janowska-Wieczorek, A., & Ratajczak, M. Z. (2006). Membrane-derived microvesicles: Important and underappreciated mediators of cell-to-cell communication. *Leukemia*, *20*(9), 1487–1495. https://doi.org/10.1038/sj.leu.2404296

Reátegui, E., Van Der Vos, K. E., Lai, C. P., Zeinali, M., Atai, N. A., Aldikacti, B., Floyd, F. P., Khankhel, H. A., Thapar, V., Hochberg, F. H., Sequist, L. V., Nahed, B. V., Carter, S. B., Toner, M., Balaj, L., Ting, T. D., Breakefield, X. O., & Stott, S. L. (2018). Engineered nanointerfaces for microfluidic isolation and molecular profiling of tumor-specific extracellular vesicles. *Nature Communications*, *9*(1), 175. https://doi.org/10.1038/s41467-017-02261-1

Rosenfeld, P. J., Brown, D. M., Heier, J. S., Boyer, D. S., Kaiser, P. K., Chung, C. Y., & Kim, R. Y. (2006). Ranibizumab for neovascular age-related macular degeneration. *New England Journal of Medicine*, *355*(14), 1419–1431. https://doi.org/10.1056/NEJMoa054481

Russell, S., Bennett, J., Wellman, J. A., Chung, D. C., Yu, Z.-F., Tillman, A., Wittes, J., Pappas, J., Elci, O., Mccague, S., Cross, D., Marshall, K. A., Walshire, J., Kehoe, T. L., Reichert, H., Davis, M., Raffini, L., George, L. A., Hudson, F. P., … Maguire, A. M. (2017). Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: A randomised, controlled, open-label, phase 3 trial. *Lancet*, *390*(10097), 849–860. https://doi.org/10.1016/S0140-6736(17)31868-8

Samaeekia, R., Rabiee, B., Putra, I., Shen, X., Park, Y. J., Hematti, P., Eslani, M., & Djalilian, A. R. (2018). Effect of human corneal mesenchymal stromal cell-derived exosomes on corneal epithelial wound healing. *Investigative Ophthalmology & Visual Science*, *59*(12), 5194–5200. https://doi.org/10.1167/iovs.18-24803

Seong, H.-R., Noh, C. H., Park, S., Cho, S., Hong, S.-J., Lee, A.-Y., Geum, D., Hong, S.-C., Park, D., Kim, T. M., Choi, E.-K., & Kim, Y.-B. (2023). Intraocular pressure-lowering and retina-protective effects of exosome-rich conditioned media from human amniotic membrane stem cells in a rat model of glaucoma. *International Journal of Molecular Sciences*, 8073, *24*(9). https://doi.org/10.3390/ijms24098073

Shah, N., Ishii, M., Brandon, C., Ablonczy, Z., Cai, J., Liu, Y., Chou, C. J., & Rohrer, B. (2018). Extracellular vesicle-mediated long-range communication in stressed retinal pigment epithelial cell monolayers. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1864*(8), 2610–2622. https://doi.org/10.1016/j.bbadis.2018.04.016

Shi, T.-T., Zhao, R.-X., Xin, Z., Hou, Z.-J., Wang, H., Xie, R.-R., Li, D.-M., & Yang, J.-K. (2022). Tear-derived exosomal biomarkers of Graves' ophthalmopathy. *Frontiers in Immunology*, *13*, 1088606. https://doi.org/10.3389/fimmu.2022.1088606

Skog, J., Würdinger, T., Van Rijn, S., Meijer, D. H., Gainche, L., Curry, W. T., Carter, B. S., Krichevsky, A. M., & Breakefield, X. O. (2008). Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nature Cell Biology*, *10*(12), 1470–1476. https://doi.org/10.1038/ncb1800

Tabak, S., Feinshtein, V., Schreiber-Avissar, S., & Beit-Yannai, E. (2021). Non-pigmented ciliary epithelium-derived extracellular vesicles loaded with SMAD7 siRNA attenuate Wnt signaling in trabecular meshwork cells in vitro. *Pharmaceuticals*, *14*(9), 858. https://doi.org/10.3390/ph14090858

Takahashi, E., Saruwatari, J., Fujimoto, T., Tanoue, Y., Fukuda, T., & Inoue, T. (2021). The effects of exosomes derived from trabecular meshwork cells on Schlemm's canal endothelial cells. *Scientific Reports*, *11*(1), 21942. https://doi.org/10.1038/s41598-021-01450-9

Tamhane, M., Cabrera-Ghayouri, S., Abelian, G., & Viswanath, V. (2019). Review of biomarkers in ocular matrices: Challenges and opportunities. *Pharmaceutical Research*, *36*(3), 40. https://doi.org/10.1007/s11095-019-2569-8

Teixeira, F. B., Saito, M. T., Matheus, F. C., Prediger, R. D., Yamada, E. S., Maia, C. S. F., & Lima, R. R. (2017). Periodontitis and Alzheimer's disease: A possible comorbidity between oral chronic inflammatory condition and neuroinflammation. *Frontiers in Aging Neuroscience*, *9*, 327. https://doi.org/10.3389/fnagi.2017.00327

Upadhya, R., Madhu, L. N., Attaluri, S., Gitaí, D. L. G., Pinson, M. R., Kodali, M., Shetty, G., Zanirati, G., Kumar, S., Shuai, B., Weintraub, S. T., & Shetty, A. K. (2020). Extracellular vesicles from human iPSC-derived neural stem cells: MiRNA and protein signatures, and anti-inflammatory and neurogenic properties. *Journal of Extracellular Vesicles*, *9*(1), 1809064. https://doi.org/10.1080/20013078.2020.1809064

Valadi, H., Ekström, K., Bossios, A., Sjöstrand, M., Lee, J. J., & Lötvall, J. O. (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nature Cell Biology*, *9*(6), 654–659. https://doi.org/10.1038/ncb1596

Van Niel, G., Bergam, P., Di Cicco, A., Hurbain, I., Lo Cicero, A., Dingli, F., Palmulli, R., Fort, C., Potier, M. C., Schurgers, L. J., Loew, D., Levy, D., & Raposo, G. (2015). Apolipoprotein E regulates amyloid formation within endosomes of pigment cells. *Cell reports*, *13*(1), 43–51. https://doi.org/10.1016/j.celrep.2015.08.057

Van Der Pol, E., Böing, A. N., Harrison, P., Sturk, A., & Nieuwland, R. (2012). Classification, functions, and clinical relevance of extracellular vesicles. *Pharmacological Reviews*, *64*(3), 676–705. https://doi.org/10.1124/pr.112.005983

Van Haeringen, N. J. (1981). Clinical biochemistry of tears. *Survey of Ophthalmology*, *26*(2), 84–96. https://doi.org/10.1016/0039-6257(81)90145-4

van Niel, G., Carter, D. R. F., Clayton, A., Lambert, D. W., Raposo, G., & Vader, P. (2022). Challenges and directions in studying cell-cell communication by extracellular vesicles. *Nature Reviews Molecular Cell Biology*, *23*(5), 369–382. https://doi.org/10.1038/s41580-022-00460-3

van Niel, G., D'Angelo, G., & Raposo, G. (2018). Shedding light on the cell biology of extracellular vesicles. *Nature Reviews Molecular Cell Biology*, *19*(4), 213–228. https://doi.org/10.1038/nrm.2017.125

Venkat, P., Chen, J., & Chopp, M. (2018). Exosome-mediated amplification of endogenous brain repair mechanisms and brain and systemic organ interaction in modulating neurological outcome after stroke. *Journal of Cerebral Blood Flow and Metabolism*, *38*(12), 2165–2178. https://doi.org/10.1177/0271678x18782789

Venkat, P., Cui, C., Chopp, M., Zacharek, A., Wang, F., Landschoot-Ward, J., Shen, Y., & Chen, J. (2019). MiR-126 mediates brain endothelial cell exosome treatment-induced neurorestorative effects after stroke in type 2 diabetes mellitus mice. *Stroke*, *50*(10), 2865–2874. https://doi.org/10.1161/STROKEAHA.119.025371

Venkat, P., Gao, H., Findeis, E. L., Chen, Z., Zacharek, A., Landschoot-Ward, J., Powell, B., Lu, M., Liu, Z., Zhang, Z., & Chopp, M. (2023). Therapeutic effects of CD133 + Exosomes on liver function after stroke in type 2 diabetic mice. *Frontiers in Neuroscience*, *17*, 1061485. https://doi.org/10.3389/fnins.2023.1061485

Villarroya-Beltri, C., Gutiérrez-Vázquez, C., Sánchez-Cabo, F., Pérez-Hernández, D., Vázquez, J., Martin-Cofreces, N., Martinez-Herrera, D. J., Pascual-Montano, A., Mittelbrunn, M., & Sánchez-Madrid, F. (2013). Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. *Nature Communications*, *4*, 2980. https://doi.org/10.1038/ncomms3980

Wang, C., Huang, R., Li, C., Lu, M., Emanuele, M., Zhang, Z. G., Chopp, M., & Zhang, L. (2019). Vepoloxamer enhances fibrinolysis of tPA (tissue-type plasminogen activator) on acute ischemic stroke. *Stroke*, *50*(12), 3600–3608. https://doi.org/10.1161/STROKEAHA.119.026049

Weinreb, R. N., Leung, C. K., Crowston, J. G., Medeiros, F. A., Friedman, D. S., Wiggs, J. L., & Martin, K. R. (2016). Primary open-angle glaucoma. *Nature reviews Disease primers*, *2*, 16067. https://doi.org/10.1038/nrdp.2016.67

Welsh, J. A., Goberdhan, D. C. I., O'driscoll, L., Buzas, E. I., Blenkiron, C., Bussolati, B., Cai, H., Di Vizio, D., Driedonks, T. A. P., Erdbrügger, U., Falcon-Perez, J. M., Fu, Q.-L., Hill, A. F., Lenassi, M., Lim, S. K., Mahoney, M. G., Mohanty, S., Möller, A., Nieuwland, R., … Witwer, K. W. (2024). Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. *Journal of Extracellular Vesicles*, *13*(2), e12404. https://doi.org/10.1002/jev2.12404

Willcox, M. D. P., Argüeso, P., Georgiev, G. A., Holopainen, J. M., Laurie, G. W., Millar, T. J., Papas, E. B., Rolland, J. P., Schmidt, T. A., Stahl, U., Suarez, T., Subbaraman, L. N., Uçakhan, O. Ö., & Jones, L. (2017). TFOS DEWS II tear film report. *The Ocular Surface*, *15*(3), 366–403. https://doi.org/10.1016/j.jtos.2017.03.006

Witwer, K. W., & Théry, C. (2019). Extracellular vesicles or exosomes? On primacy, precision, and popularity influencing a choice of nomenclature. *Journal of Extracellular Vesicles*, *8*(1), 1648167. https://doi.org/10.1080/20013078.2019.1648167

Wooff, Y., Cioanca, A. V., Chu-Tan, J. A., Aggio-Bruce, R., Schumann, U., & Natoli, R. (2020). Small-medium extracellular vesicles and their miRNA cargo in retinal health and degeneration: Mediators of homeostasis, and vehicles for targeted gene therapy. *Frontiers in Cellular Neuroscience*, *14*, 160. https://doi.org/10.3389/fncel.2020.00160

Wortzel, I., Dror, S., Kenific, C. M., & Lyden, D. (2019). Exosome-mediated metastasis: Communication from a distance. *Developmental Cell*, *49*(3), 347–360. https://doi.org/10.1016/j.devcel.2019.04.011

Yan, H., Wen, Y., Tian, Z., Hart, N., Han, S., Hughes, S. J., & Zeng, Y. (2023). A one-pot isothermal Cas12-based assay for the sensitive detection of microRNAs. *Nature Biomedical Engineering*, *7*(12), 1583–1601. https://doi.org/10.1038/s41551-023-01033-1

Yan, T., Chen, Z., Chopp, M., Venkat, P., Zacharek, A., Li, W., Shen, Y., Wu, R., Li, L., Landschoot-Ward, J., Lu, M., Hank, K.-H., Zhang, J., & Chen, J. (2020). Inflammatory responses mediate brain-heart interaction after ischemic stroke in adult mice. *Journal of Cerebral Blood Flow and Metabolism*, *40*(6), 1213–1229. https://doi.org/10.1177/0271678x18813317

Yang, K. S., Im, H., Hong, S., Pergolini, I., Del Castillo, A. F., Wang, R., Clardy, S., Huang, C.-H., Pille, C., Ferrone, S., Yang, R., Castro, C. M., Lee, H., Del Castillo, C. F., & Weissleder, R. (2017). Multiparametric plasma EV profiling facilitates diagnosis of pancreatic malignancy. *Science Translational Medicine*, *9*(391), eaal3226. https://doi.org/10.1126/scitranslmed.aal3226

Yin, W., Ouyang, S., Li, Y., Xiao, B., & Yang, H. (2013). Immature dendritic cell-derived exosomes: A promise subcellular vaccine for autoimmunity. *Inflammation*, *36*(1), 232–240. https://doi.org/10.1007/s10753-012-9539-1

You, J., Wu, Q., Xu, G., Gu, C., Allen, E., Zhu, T., & Chen, L. (2023). Exosomal MicroRNA profiling in vitreous humor derived from pathological myopia patients. *Investigative Ophthalmology & Visual Science*, *64*(1), 9. https://doi.org/10.1167/iovs.64.1.9

Youngblood, H., Hauser, M. A., & Liu, Y. (2019). Update on the genetics of primary open-angle glaucoma. *Experimental Eye Research*, *188*, 107795. https://doi.org/10.1016/j.exer.2019.107795

Youngblood, H., Schoenlein, P. V., Pasquale, L. R., Stamer, W. D., & Liu, Y. (2023). Estrogen dysregulation, intraocular pressure, and glaucoma risk. *Experimental Eye Research*, *237*, 109725. https://doi.org/10.1016/j.exer.2023.109725

Yu, F., Wang, Y., Huang, C. Q., Lin, S. J., Gao, R. X., & Wu, R. Y. (2023). Neuroprotective effect of mesenchymal stem cell-derived extracellular vesicles on optic nerve injury in chronic ocular hypertension. *Neural Regeneration Research*, *18*(10), 2301–2306. https://doi.org/10.4103/1673-5374.369121

Zhang, L., Li, C., Huang, R., Teng, H., Zhang, Y., Zhou, M., Liu, X., Fan, B., Luo, H., He, A., Zhao, A., Lu, M., Chopp, M., & Zhang, Z. G. (2022). Cerebral endothelial cell derived small extracellular vesicles improve cognitive function in aged diabetic rats. *Frontiers in aging neuroscience*, *14*, 926485. https://doi.org/10.3389/fnagi.2022.926485

Zhang, T., Hu, L., Ma, H., Ni, F., Liu, F., & Chen, H. (2020). Detection of tear components using matrix-assisted laser desorption ionization/time-of-flight mass spectrometry for rapid dry eye diagnosis. *Journal of Proteome Research*, *19*(9), 3644–3651. https://doi.org/10.1021/acs.jproteome.0c00138

Zhang, Z. G., & Chopp, M. (2016). Exosomes in stroke pathogenesis and therapy. *Journal of Clinical Investigation*, *126*(4), 1190–1197. https://doi.org/10.1172/JCI81133

Zhou, L., & Beuerman, R. W. (2012). Tear analysis in ocular surface diseases. *Progress in Retinal and Eye Research*, *31*(6), 527–550. https://doi.org/10.1016/j.preteyeres.2012.06.002

Zhou, T., He, C., Lai, P., Yang, Z., Liu, Y., Xu, H., Lin, X., Ni, B., Ju, R., Yi, W., Liang, L., Pei, D., Egwuagu, C. E., & Liu, X. (2022). miR-204-containing exosomes ameliorate GVHD-associated dry eye disease. *Science Advances*, *8*(2), eabj9617. https://doi.org/10.1126/sciadv.abj9617

Zhou, Y., Bhatt, H., Mojica, C. A., Xin, H., Pessina, M. A., Rosene, D. L., Moore, T. L., & Medalla, M. (2023). Mesenchymal-derived extracellular vesicles enhance microglia-mediated synapse remodeling after cortical injury in aging Rhesus monkeys. *J Neuroinflammation*, *20*(1), 201. https://doi.org/10.1186/s12974-023-02880-0

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