

REVIEW

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Recent advances in engineered exosome-based therapies for ocular vascular disease

Jian Guan^{1†}, Fanhe Meng^{2†}, Chuanzhuo Wang^{2†}, Binglun Zhang^{2*}, Jun Chen^{1*} and Jingnan Han^{1*}

Abstract

Ocular neovascular diseases (ONDs), including corneal neovascularization (CoNV), age-related macular degeneration (AMD) and diabetic retinopathy (DR), are among the leading causes of visual impairment worldwide. Current therapeutic strategies predominantly involve intravitreal injection of anti-vascular endothelial growth factor (anti-VEGF) agents, which, despite their efficacy, present significant limitations such as drug resistance, frequent intravitreal injections, and insufficient addressing of underlying pathological mechanisms. This review critically examines recent advancements in the application of exosomes as innovative drug delivery platforms for treating ocular neovascular diseases. Exosomes, naturally occurring extracellular vesicles, exhibit superior biocompatibility, low immunogenicity, and intrinsic targeting capabilities, making them ideal carriers for bioactive molecules including proteins, RNAs, and small drugs. We explore the mechanistic roles of exosomes in modulating pathological angiogenesis, inflammation, and tissue repair within the ocular environment. Additionally, this review addresses the current challenges hindering the clinical translation of these exosomes, including large-scale production, regulatory hurdles, and safety concerns. Future perspectives highlight the potential integration of nanoparticles and exosomes with existing therapies, the development of multifunctional and personalized treatment strategies, and the necessity for standardized protocols to facilitate their transition from bench to bedside. By overcoming these challenges, exosomes hold the promise of revolutionizing the therapeutic landscape for ocular neovascular diseases, ultimately enhancing patient outcomes and quality of life.

Keywords Exosomes, Ocular neovascular diseases, Nanomedicine, Drug delivery

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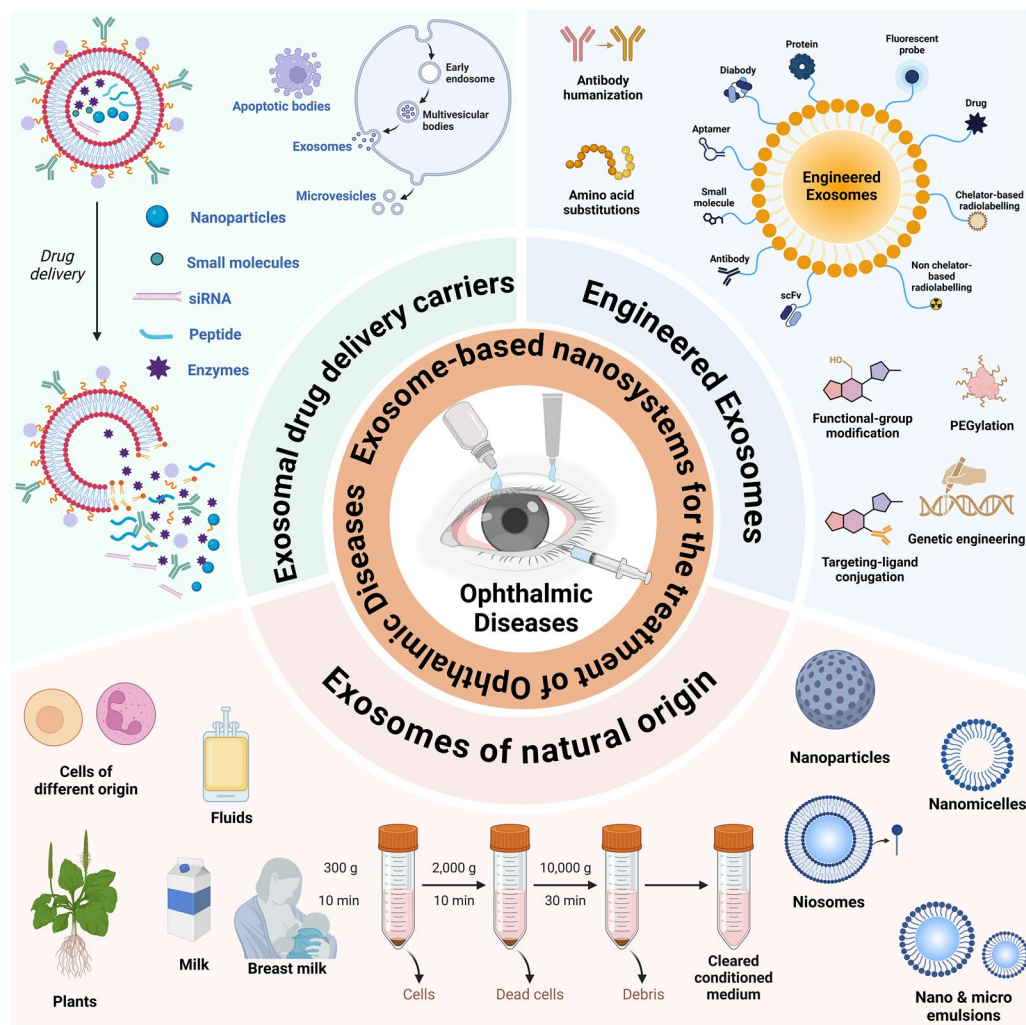
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Graphical abstract



Introduction

CoNV, AMD, and DR are known as Ocular neovascular diseases (ONVs), which has become the definitely leading causes of visual impairment [1–3]. These conditions are primarily characterized by pathological angiogenesis, inflammation, and fibrosis [4]. Specifically, the formation of pathological neovascularization plays a pivotal role in disease progression. These immature vessels disrupt the normal tissue architecture and function, and if left untreated, typically lead to permanent vision loss [5]. In wet age-related macular degeneration (wAMD), also referred to as neovascular AMD, the primary pathological hallmark is the formation of choroidal neovascularization (CNV). These immature pathological vessels penetrate Bruch's membrane and

extend into the subretinal or sub-RPE space, growing beneath the neuroretina in the macular region. This process leads to macular fluid leakage and hemorrhage, subsequently causing damage to the retinal pigment epithelium (RPE) and photoreceptors, and ultimately resulting in subretinal fibrosis [6, 7]. In DR, retinal ischemia triggers the formation of fragile new blood vessels, contributing to retinal leakage, swelling, and detachment [8]. The underlying mechanisms of these diseases are largely driven by hypoxia-inducible factors (HIFs) and vascular endothelial growth factor (VEGF), which promote endothelial cell proliferation and the formation of vessels prone to leakage and hemorrhage [1, 9, 10]. Additionally, chronic inflammation plays a pivotal role in disease progression, exacerbating retinal

damage and fostering a cycle of worsening pathology [6, 11, 12].

The current treatment strategy for neovascular ocular diseases primarily focuses on inhibiting VEGF to suppress pathological neovascularization. Intravitreal injection of anti-VEGF agents, such as ranibizumab and aflibercept, has become the first-line clinical approach for managing neovascular ocular diseases and their complications [13–16]. These therapeutic approaches have been proven to effectively reduce the formation of pathological neovascularization and stabilize visual acuity to a certain extent. [17, 18]. However, several significant limitations remain. A major challenge is drug resistance, where the retina becomes less responsive to anti-VEGF agents over time, resulting in diminished therapeutic efficacy [19–22]. Furthermore, frequent intravitreal injections are required to maintain clinical outcomes, which can be burdensome for patients and increase the risk of complications, including infection and retinal detachment [23–26]. Additionally, these therapies fail to address the underlying pathological processes of neovascularization and do not promote retinal repair [27–29]. To overcome these limitations, research is shifting toward multi-targeted therapies, sustained-release formulations, and novel drug delivery methods [30, 31]. Alternative treatments, including steroid injections, laser therapy, and topical medications, are also being explored, particularly for cases resistant to anti-VEGF therapy [32–35].

In response to these challenges, exosomes have emerged as a promising therapeutic platform. Owing to their small size and diverse surface properties, exosomes are not only utilized for targeted drug delivery but also for modulating disease mechanisms, promoting tissue repair, and enhancing therapeutic outcomes in neovascular ocular diseases [36–38]. These characteristics enhance the efficacy of anti-VEGF therapies and other agents, reducing side effects and improving patient compliance [39, 40]. Meanwhile, exosomes, naturally occurring extracellular vesicles, possess unique features such as immune evasion and targeted delivery capabilities [41, 42]. Their ability to carry bioactive molecules, such as proteins, RNA, and lipids, makes them ideal carriers for the delivery of therapeutic agents directly to the retina, overcoming some of the limitations of current treatments [43–46].

This review critically evaluates the current state of research on exosomes in the treatment of ocular neovascular diseases. We focus on their mechanisms of action, the challenges faced in clinical applications, and their potential for translation into clinical practice. By comparing these novel delivery systems with traditional therapies, we highlight their distinct advantages and explore emerging strategies for integrating exosomes

with existing treatments. This integration offers synergistic effects and more effective, personalized therapeutic options for patients with ocular neovascular diseases. A visual overview of the major exosome modalities discussed in this review is provided in Scheme 1.

Scheme 1 presents a schematic depiction of exosome-based nanosystems designed for the treatment of ophthalmic diseases. It illustrates the sources of natural exosomes (bottom left), the strategies employed for engineering and functionalizing these vesicles (upper right), and their application as versatile drug-delivery carriers (left), all converging towards targeted ocular therapy (center).

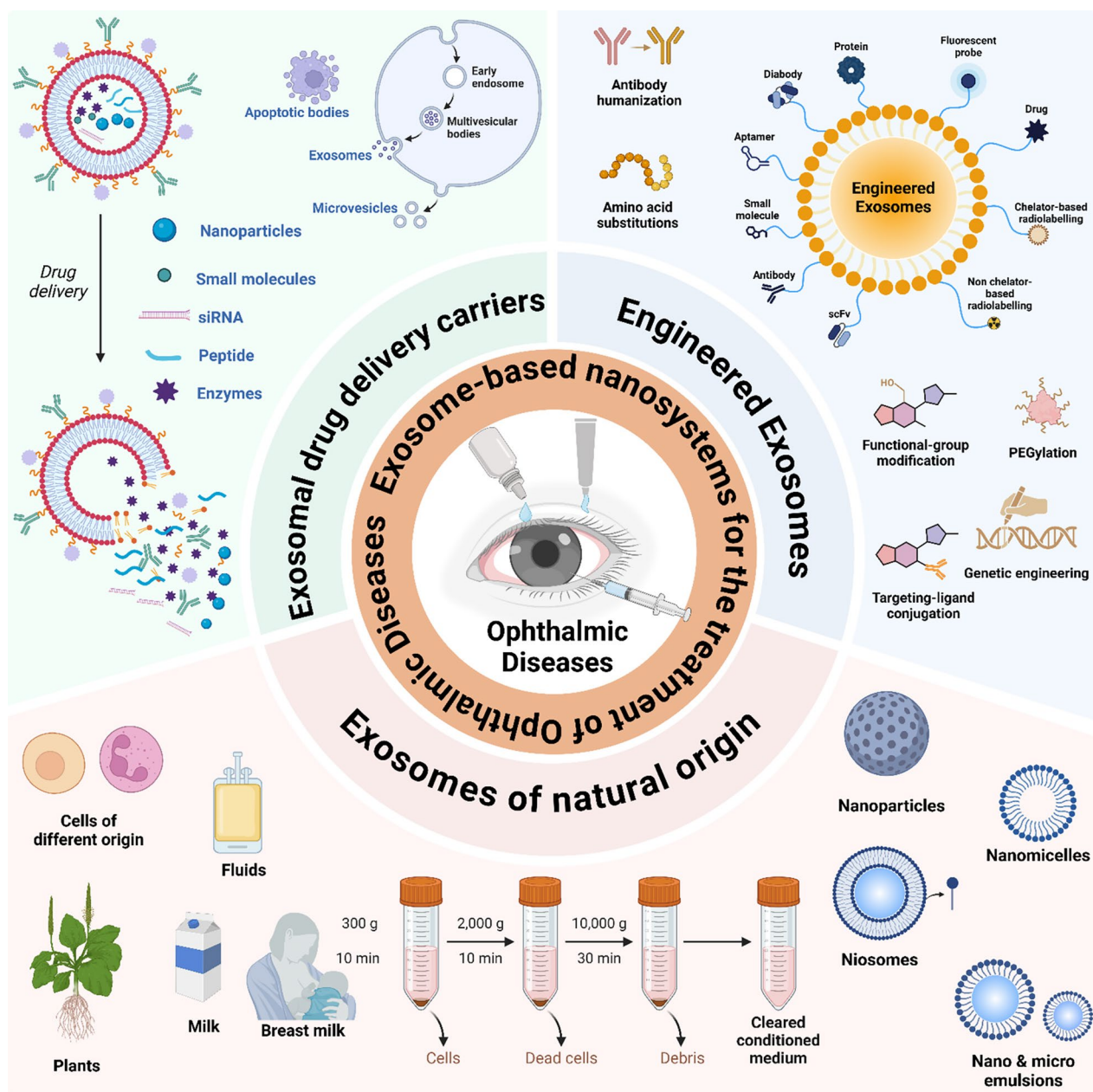
Pathophysiology and mechanisms of ocular neovascular diseases (ONDs)

ONDs are a group of pathological conditions characterized by the abnormal formation of blood vessels in the retina, cornea, choroid, and uveal tract. These diseases, including wAMD, DR, and retinal vein occlusion (RVO), often lead to vision-threatening complications such as retinal hemorrhage, macular edema, and even blindness. ONDs significantly impact patients, particularly in aging populations and those with chronic conditions like diabetes, contributing to global visual impairment [47, 48].

Ocular neovascular diseases are driven by complex molecular and cellular interactions, as illustrated in Fig. 1, which highlights the anatomical and pathological vascular changes in corneal, retinal, and choroidal tissues. The progression from normal vasculature to pathological neovascularization underscores the role of hypoxia, inflammation, and oxidative stress in disrupting ocular homeostasis [49, 50]. This section will explore the underlying pathophysiological mechanisms of ONDs, focusing on core drivers such as hypoxia and inflammation, as well as the molecular pathways contributing to ONDs. It will also introduce emerging therapeutic strategies.

Core drivers of ONDs

Ocular neovascular diseases emerge from a complex interplay of three primary molecular disturbances: tissue hypoxia, sterile inflammation, and oxidative stress. These factors collectively contribute to uncontrolled vascular proliferation and the disruption of vascular barriers. Hypoxia leads to the stabilization of hypoxia-inducible factor 1- α (HIF-1 α), which initiates vascular endothelial growth factor (VEGF)-dependent angiogenesis. Concurrently, inflammatory cells enhance this angiogenic response through the release of cytokines, while oxidative stress both triggers and sustains cellular damage, thereby exacerbating the other two pathways. Although each of these drivers operates through distinct mechanisms, they mutually reinforce one another in a positive feedback



Scheme 1 Schematic representation of an exosome nanosystem for the treatment of ophthalmic diseases

loop that accelerates pathological angiogenesis (Fig. 2). The subsequent subsections will examine these three factors individually, offering a comprehensive framework for understanding their convergence in the pathogenesis of corneal, retinal, and choroidal vascular disorders.

Hypoxia-inducible pathways (HIF-1 α /VEGF Axis)

Hypoxia is a hallmark of ischemic retinopathies, often triggered by vascular insufficiency or tissue damage. Under hypoxic conditions, the hypoxia-inducible

factor (HIF) pathway is activated, leading to the upregulation of pro-angiogenic factors such as VEGF [51, 52]. VEGF induces endothelial cell proliferation, migration, and the formation of new, often leaky, blood vessels, thereby contributing to pathological ONDs [53]. In the retina, hypoxia stabilizes HIF-1 α , which in turn upregulates VEGF-A and drives neovascularization in diseases such as DR and wet age-related macular degeneration (wAMD) [54, 55]. Notably, tissue-specific HIF isoforms elicit differential responses:

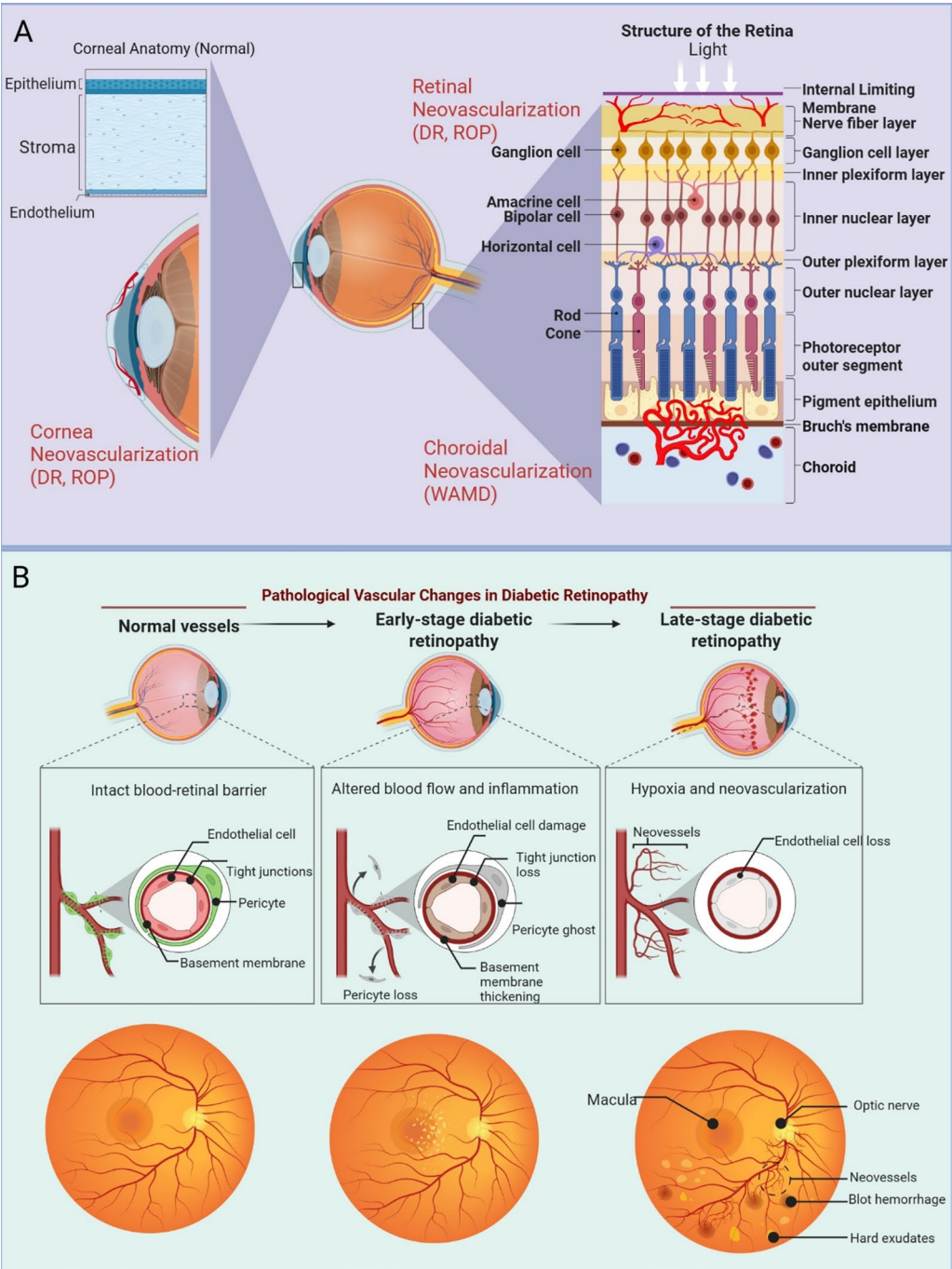


Fig. 1 Mechanisms of angiogenesis in ocular neovascular diseases. **A** Anatomical and pathological vascular changes in ocular tissues. **B** Progression of vascular pathology in DR

HIF-1 α promotes VEGF-A-mediated vascular leakage in the retina, while HIF-2 α in the choroid contributes to complement activation in AMD [48, 56–58].

Inflammatory cascades and immune cell crosstalk
Inflammation exacerbates pathological neovascularization by recruiting immune cells and activating

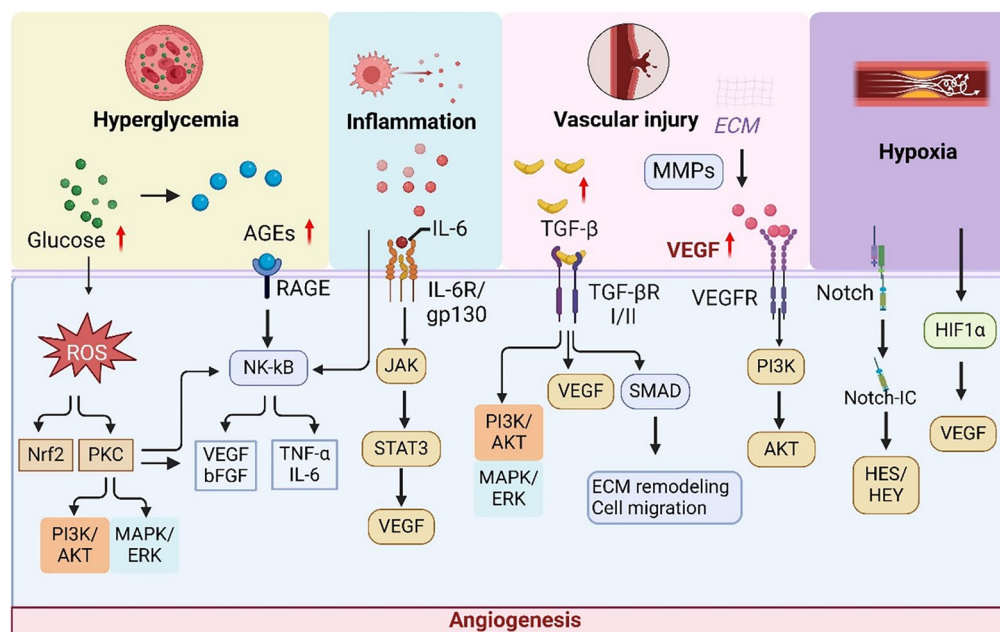


Fig. 2 Molecular mechanisms of hyperglycemia, hypoxia, vascular injury, and inflammation-driven angiogenesis in ocular neovascular diseases. This schematic illustrates the signaling pathways underlying pathological angiogenesis in conditions such as DR and wAMD

pro-inflammatory cytokines. Macrophages, T-cells, microglia, and neutrophils release cytokines such as TNF- α , IL-1 β , and IL-6, which amplify endothelial cell activation and drive angiogenesis [59, 60]. These cytokines disrupt endothelial cell junctions, compromise the integrity of the blood-retinal barrier (BRB), and contribute to fluid leakage, edema, and disease progression. In retinal diseases like DR, inflammatory cytokines further stimulate VEGF production, perpetuating the pathological angiogenic loop [61]. In the cornea, macrophages contribute to CoNV by secreting VEGF and chemotactic factors that recruit additional immune cells. Chronic inflammation in the cornea can lead to excessive neovascularization, resulting in scarring and visual impairment [62, 63].

Oxidative stress

Oxidative stress is a critical mechanism underlying the development and progression of ocular neovascular diseases (ONDs) [64]. Under normal physiological conditions, a dynamic balance is maintained between cellular oxidation and antioxidant systems. However, this equilibrium is disrupted under pathological conditions such as hypoxia and aging, leading to excessive production of reactive oxygen species (ROS) that overwhelm the body's antioxidant defense mechanisms, thereby inducing oxidative stress [64–66]. In the eye, oxidative stress promotes neovascularization through multiple pathways. Excessive ROS damage intracellular macromolecules, particularly

mitochondrial function, impairing energy metabolism and leading to cellular dysfunction and death [67]. This damage activates intracellular signaling pathways, such as nuclear factor- κ B (NF- κ B) and mitogen-activated protein kinase (MAPK) pathways, which induce the expression of pro-inflammatory cytokines (e.g., TNF- α , IL-1 β , and IL-6) and chemokines. These factors not only exacerbate local inflammatory responses but also promote the production of VEGF, driving neovascular formation [68]. Oxidative stress serves as a central pathological factor in ONDs, bridging pathogenic factors such as hypoxia, aging, and inflammation [65, 66, 68]. Under hypoxic conditions, the accumulation of ROS activates hypoxia-inducible factor (HIF-1 α), which upregulates VEGF expression and promotes neovascularization [69]. During aging, decreased cellular antioxidant capacity exacerbates ROS accumulation, further worsening cellular damage and inflammatory responses. Additionally, pro-inflammatory cytokines released by inflammatory cells further induce VEGF production via oxidative stress pathways, creating a vicious cycle that drives disease progression. Thus, oxidative stress is not only a key pathological link in ONDs but also a bridge connecting multiple pathogenic factors, playing a crucial role in the onset and progression of these diseases.

Pathogenic “vicious cycle” in ONDs

In conclusion, hypoxia, oxidative stress, and inflammation interact in a pathogenic cycle that perpetuates

ocular neovascular diseases. Hypoxic conditions in retinal tissue, often resulting from ischemia, lead to the stabilization of hypoxia-inducible factors such as HIF-1 α , which subsequently triggers an upregulation of pro-angiogenic factors like VEGF and pro-inflammatory cytokines [70–72]. This cascade results in pathological neovascularization and the recruitment of inflammatory cells into the retina [73]. The resultant inflammation exacerbates oxidative stress, as activated microglia and infiltrating leukocytes release reactive oxygen species (ROS), thereby intensifying tissue damage. Excessive ROS and oxidative damage further compromise retinal cell and vessel integrity, exemplified by the breakdown of the blood-retinal barrier and capillary dropout, which exacerbates ischemia and intensifies hypoxia. Consequently, once initiated, hypoxia, inflammation, and oxidative stress mutually reinforce each other in a self-sustaining loop, perpetuating chronic inflammation, ongoing oxidative damage, and aberrant neovascularization in ocular neovascular diseases [72, 74, 75].

Tissue-specific mechanisms of ONDs

Corneal neovascularization

The cornea is an avascular transparent tissue with a capillary network surrounding the limbus. Under physiological conditions, they maintain an immune-privileged state. The normal physiological functions of the cornea depend on its transparency and avascularity. However, under pathological conditions, which is characterized by the growth of new vessels from the surrounding capillaries that extend beyond the limbus into the clear corneal zone [76], such as hypoxia, inflammation, or trauma, oxidative stress can activate corneal stromal cells and infiltrating immune cells, inducing the release of pro-angiogenic factors, such as VEGF and placental growth factor (PlGF) [62, 63]. These factors stimulate the formation of new blood vessels in the normally avascular cornea. Although corneal neovascularization is usually a response to trauma, excessive and chronic neovascularization can impair corneal transparency, leading to visual impairment and scar formation. CoNV is commonly associated with ocular conditions, such as infectious keratitis, graft rejection following corneal transplantation, and diabetic keratopathy. The pathogenesis of CoNV is driven by multiple factors, including dysregulation of angiogenic and inflammatory signaling pathways. Pro-angiogenic factors, such as VEGF, fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF), are often upregulated in response to corneal injury or inflammation, promoting the sprouting of new blood vessels from the limbal vasculature.

Retinal neovascularization (RNV)

In retinal neovascularization, VEGF-A and other angiogenic factors play a central role in the formation of abnormal blood vessels. Müller cells and microglia in the retina release pro-angiogenic factors in response to hypoxia, further exacerbating endothelial cell proliferation and migration [77]. Pericytes are essential in the development and progression of retinal neovascularization. As integral components of the retinal neurovascular unit, pericytes interact with endothelial cells to maintain the structural integrity and stability of retinal capillaries [78, 79]. In a healthy state, pericytes support the formation and maintenance of the blood-retinal barrier (BRB) by regulating endothelial cell junctions and suppressing factors that promote vascular permeability. However, in the hyperglycemic environment of diabetes, pericytes are highly susceptible to damage. Their loss results in increased vascular permeability and compromised BRB function, which are key features of early DR. Moreover, pericytes play a significant role in angiogenesis regulation. Their depletion in the diabetic retina leads to uncontrolled neovascularization, a hallmark of proliferative diabetic retinopathy (PDR) [79]. Additionally, pericytes modulate the retinal inflammatory response. The loss of pericytes exacerbates the inflammatory cascade triggered by hyperglycemia, further destabilizing retinal blood vessels. This multifaceted involvement of pericytes in both vascular and inflammatory processes underscores their critical role in retinal neovascularization [80].

Choroidal neovascularization

In wet age-related macular degeneration (wAMD), the onset of choroidal neovascularization is initiated when the accumulation of age-related lipofuscin and the deposition of drusen compromise the retinal pigment epithelium (RPE) and thicken Bruch's membrane, leading to localized hypoxia and oxidative stress. These conditions stabilize hypoxia-inducible factors within RPE and choroidal endothelial cells, resulting in an upregulation of vascular endothelial growth factor (VEGF) and other pro-angiogenic mediators. The established gradient induces endothelial tip cells in the choriocapillaris to undergo sprouting, proliferation, and ultimately penetrate the compromised Bruch's membrane, invading the sub-RPE or subretinal space [81]. The newly formed vessels are immature and exhibit high permeability, leading to the accumulation of plasma exudation and microhemorrhages beneath the neurosensory retina, which contributes to macular edema and early visual distortion. Persistent leakage, in conjunction with complement-mediated inflammation and ongoing oxidative stress,

sustains the overexpression of VEGF and recruits microglia and macrophages, thereby perpetuating angiogenesis. Over time, this chronic inflammatory environment promotes fibrovascular proliferation, resulting in the formation of a subretinal scar that damages photoreceptors and leads to irreversible central vision loss in wAMD [82].

Molecular networks and therapeutic targets

Classic pathways: VEGF/VEGFR, angiopoietin/Tie2, Notch/DLL

Pathological angiogenesis in ONDs is regulated by several molecular cascades, with the VEGF/VEGFR axis playing a central role. In this pathway, the binding of VEGF-A to its primary receptor, VEGFR2, promotes endothelial cell proliferation, migration, and survival. Importantly, the activation of VEGFR2 also leads to an acute increase in vascular permeability [83, 84]. This process involves the tyrosine phosphorylation of VEGFR2, which recruits Src-family kinases and PLC- γ , resulting in the phosphorylation and internalization of VE-cadherin and occludin. Consequently, the disassembly of adherens and tight junctions occurs, weakening endothelial barriers and contributing to the exudation observed in diabetic macular edema and the accumulation of subretinal fluid in wAMD [85, 86]. In addition to the role of vascular endothelial growth factor (VEGF), the Angiopoietin/Tie2 signaling pathway is crucial in the pathophysiology of ONDs by modulating vascular stability. Angiopoietin-1 (Ang-1) is the primary Tie2 agonist that maintains endothelial quiescence and vessel integrity, whereas Ang-2 serves as a context-dependent antagonist that disrupts this balance [87]. Mechanistically, Ang-2 competes with Ang-1 for binding to the Tie2 receptor on endothelial cells. However, unlike Ang-1, Ang-2 does not effectively activate Tie2 signaling [87]. Instead, the competitive binding of Ang-2 inhibits Ang-1-mediated Tie2 phosphorylation, resulting in reduced Tie2 activity and a subsequent loss of endothelial junction stability [88]. This inhibition by Ang-2 leads to pericyte dropout and compromises the vascular barrier, making blood vessels more permeable and abnormally responsive to pro-angiogenic factors such as VEGF. In diabetic retinopathy (DR) and wet age-related macular degeneration (wAMD), pathological stimuli—such as chronic hyperglycemia-induced hypoxia or inflammation—induce upregulation of Ang-2, thereby shifting the Ang-1/Ang-2 ratio in favor of Ang-2 and promoting vascular destabilization [88]. In these optic nerve disorders (ONDs), elevated levels of Angiopoietin-2 (Ang-2) competitively inhibit the vessel-stabilizing effects of Angiopoietin-1 (Ang-1), leading to the formation of fragile and permeable blood vessels, thereby promoting vascular endothelial growth factor

(VEGF)-mediated neovascular sprouting. Importantly, Ang-2 and VEGF frequently act synergistically within the retinal environment, collectively exacerbating vascular leakage and pathological angiogenesis. These findings have substantial clinical implications, as the Ang-2/Tie2 signaling pathway has emerged as a promising therapeutic target for the treatment of ONDs [89].

Notch/DLL signaling also regulates endothelial cell differentiation and vascular remodeling. Dysregulation of Notch signaling contributes to abnormal vessel formation, characteristic of pathological neovascularization. VEGF and NOTCH signaling pathways exhibit a synergistic interaction during angiogenesis. NOTCH acts as a downstream effector of VEGF, modulating its activity through negative feedback mechanisms [90]. Specifically, VEGFA activates VEGFR2 in retinal vascular endothelial tip cells, leading to the induction of Delta-like ligand 4 (DLL4) expression [91]. Elevated DLL4 expression in a single tip cell engages NOTCH receptors on adjacent endothelial cells. The resulting cleavage and nuclear translocation of the Notch intracellular domain induce target genes such as Hes1 and Hey1, which suppress VEGFR2 and DLL4 in those neighbors, thereby converting them into stalk (quiescent) endothelial cells and preventing ectopic tip-cell formation [91, 92]. At subsequent fusion points where sprouting vessels anastomose, these stalk cells can re-acquire tip-like behaviour if Jagged1 (JAG1) outcompetes DLL4, relieving Notch inhibition and locally expanding the tip-cell pool to extend or remodel the network [93, 94]. This dynamic tip–stalk plasticity orchestrated by VEGF and Notch ensures orderly vessel branching; dysregulation—common in diabetic retinopathy and wAMD—leads to disorganised, leaky vasculature [95].

In more technical terms, VEGF designates a single endothelial "tip cell" to guide the sprouting process by upregulating DLL4. DLL4 subsequently activates the NOTCH signaling pathway in adjacent cells, compelling them to assume a "stalk-cell" phenotype, thus inhibiting the excessive formation of tip cells [96, 97]. This lateral inhibition mechanism ensures the orderly development of new blood vessels and mitigates the formation of disorganized, permeable networks characteristic of DR and wAMD. Therefore, the dynamic feedback loop between VEGF and NOTCH signaling orchestrates the rearrangement of vascular endothelial tip cells, ensuring robust retinal blood vessel formation. The interplay between hyperglycemia, inflammation, and hypoxia in driving pathological angiogenesis is summarized in Fig. 2.

Emerging regulators: the role of non-coding RNAs in pathological angiogenesis

Beyond protein-coding signaling cascades, non-coding RNAs have emerged as novel regulatory layers in ONDs. Long non-coding RNAs and microRNAs play a crucial role in the regulation of VEGF, HIF-1 α , and junctional proteins, thereby influencing endothelial cell proliferation and maintaining barrier integrity [98, 99]. A notable example is miR-126-3p, an endothelial-specific "vascular guardian," whose reduction is associated with increased vascular permeability and abnormal neovascularization, as observed in diabetic retinopathy and wAMD [100, 101]. miR-126-3p not only directly inhibits VEGFR2 translation but also targets PIK3R2/p85 β and SPRED1, thereby alleviating the negative regulation of the PI3K/AKT and Ras-ERK/MAPK signaling pathways [102–104]. This dual regulatory mechanism allows for precise modulation of VEGFR-2 downstream signaling, maintaining endothelial tight-junction integrity while facilitating controlled angiogenic sprouting under physiological conditions. Pathological down-regulation of miR-126-3p disrupts these pathways, leading to decreased expression of occludin and claudin, and increased vascular permeability. The therapeutic potential of miR-126-3p is highlighted by studies demonstrating that its restorative delivery, via exosome or viral vectors, can re-establish the blood-retinal barrier and inhibit neovascular tuft formation in diabetic mouse models [102, 105].

Overview of exosomes

Definition and basic structure of exosomes

Exosomes are nanosized extracellular vesicles, typically ranging from 30 to 150 nm, secreted by a variety of cell types, including epithelial and immune cells. Exosomes are naturally derived extracellular vesicles that mediate intercellular communication and cargo delivery. As depicted in Fig. 3, their biogenesis involves endosomal sorting and secretion, enabling transport of bioactive molecules to modulate angiogenesis and inflammation in ocular tissues. These vesicles play a crucial role in intercellular communication by carrying a range of bioactive molecules—such as proteins, lipids, mRNAs, and microRNAs which can influence various biological processes in recipient cells [106–108]. The core-shell structure of exosomes is key to their function as natural carriers and facilitators of these processes. The core of exosomes encapsulates a diverse array of biomolecules, which are critical for regulating cellular functions upon their transfer to target cells. Meanwhile, the lipid bilayer shell ensures structural integrity, protecting the cargo from degradation and enabling precise targeting of specific recipient cells [108]. Exosomes contribute to cellular homeostasis and facilitate diverse physiological

processes, including immune responses, tumor progression, and intercellular communication [109–111]. For example, immune cells release exosomes that present antigens to stimulate immune responses, while exosomes secreted by cancer cells can modify the tumor microenvironment to promote neovascularization [112, 113]. The unique structural versatility and functional capacity of exosomes underpin their potential as therapeutic tools, particularly in the context of drug delivery and gene therapy.

In conclusion, as depicted in Fig. 3, exosomes are formed intracellularly via the endosomal maturation pathway. Initially, early endosomes, which contain internalized cargo, undergo maturation into late endosomes or multivesicular bodies (MVBs). These MVBs encapsulate intraluminal vesicles (ILVs) that are enriched with specific proteins, lipids, and nucleic acids. The subsequent fusion of MVBs with the plasma membrane results in the release of these ILVs as exosomes into the extracellular milieu. Upon release, exosomes can engage with recipient cells through various mechanisms, such as endocytic uptake or direct membrane fusion, thereby facilitating the transfer of their molecular cargo to target cells. Figure 3 illustrates this process of exosome biogenesis and the diverse mechanisms of intercellular vesicle transfer.

Exosome formation pathways

Exosomes are derived from the endosomal compartment of cells through a series of complex biogenesis steps. The process is initiated by the invagination of the endosomal membrane, leading to the formation of early endosomes [114, 115]. These early endosomes mature into multivesicular bodies (MVBs), which contain intraluminal vesicles (ILVs)—the precursors of exosomes. Specific cargo is sorted into ILVs under the strict regulation of key proteins such as TSG101 and Alix, which are involved in the ESCRT (endosomal sorting complexes required for transport) mechanism. This mechanism ensures the selective packaging of proteins, lipids, and RNA into exosomes. Subsequently, when MVBs fuse with the plasma membrane, exosomes are released into the extracellular space. This biogenesis process is conserved but varies among different cell types, featuring specialized exosome secretion pathways. For example, in epithelial cells, the ALIX-Syntenin1-Syndecan1 mechanism and ceramide production mediate the polarized release of exosomes [116]. In immune cells, stimuli such as infection can enhance exosome release, making them crucial for immune regulation and intercellular signaling [117, 118]. Additionally, Rab GTPases, particularly Rab27a and Rab11, play a key role in the transport of MVBs to the plasma membrane, facilitating the release of exosomes

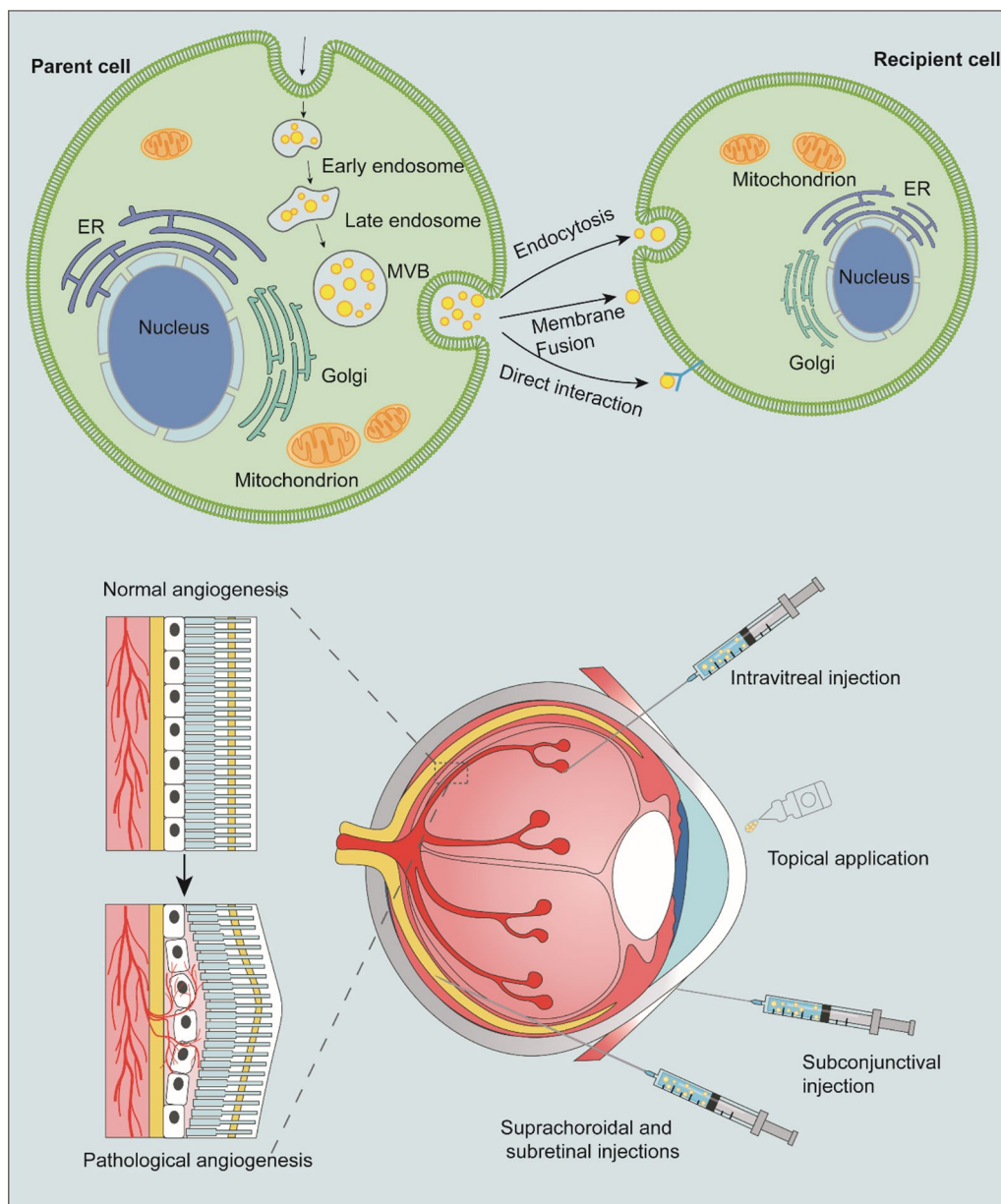


Fig. 3 Schematic illustration of exosome biogenesis, intercellular transport, and application in ocular pathologic neovascularization. Top: Exosome biogenesis occurs within a producer cell through the endosomal pathway, where early endosomes undergo invagination to form intraluminal vesicles within multivesicular bodies (MVBs). These MVBs subsequently fuse with the plasma membrane, facilitating the release of these vesicles as nano-sized exosomes into the extracellular milieu. Right: The intercellular transfer of exosomes involves their travel to recipient cells, where they are internalized via mechanisms such as receptor-mediated endocytosis or direct membrane fusion. This process results in the delivery of their cargo, including proteins and RNAs, thereby modulating the behavior of the target cell. Bottom: In the context of ocular therapeutic applications, exosomes are illustrated as drug-delivery nanocarriers capable of traversing ocular tissues to target sites of pathological neovascularization. By transporting anti-angiogenic factors or genetic regulators, therapeutic exosomes can effectively inhibit abnormal blood vessel growth in conditions that threaten vision

into the extracellular space [119]. The biogenesis of exosomes is also influenced by the lipid composition of the membrane, with sphingolipids, ceramide, and cholesterol playing important roles in membrane budding and vesicle release. This lipid composition allows exosomes to

be stable in circulation and capable of efficiently interacting with target cells [120, 121].

Membrane composition and encapsulated cargo

Exosomes, which are nanoscale extracellular vesicles, are characterized by a complex membrane structure that plays a crucial role in their stability, functionality, and interaction with target cells. The exosomal membrane is rich in a variety of proteins and lipids, which are essential for their biogenesis, cargo packaging, and therapeutic potential.

Membrane composition

A significant component of the exosomal membrane is the tetraspanin family, including CD9, CD63, and CD81, which are integral membrane proteins that regulate exosome formation, internalization, and cell-to-cell communication. These tetraspanins serve not only as markers for exosome identification but also play a vital role in stabilizing the vesicle, supporting fusion with recipient cell membranes, and facilitating the sorting of cargo proteins. Integrins are another important family of proteins found on the exosomal surface. They are responsible for mediating cellular adhesion and signal transduction, allowing exosomes to engage with specific cell types, thus enabling targeted delivery of the enclosed biomolecules. These integrins also can be exploited for engineering purposes to further enhance the specificity of exosomes for ocular tissues, where integrin receptors are often upregulated in diseases such as DR and AMD. Beyond proteins, the lipid bilayer of exosomes is composed of a diverse range of lipids, such as phospholipids, sphingomyelin, cholesterol, and ceramide, which contribute to the structural integrity, fluidity, and biocompatibility of exosomes. The lipid composition ensures the stability of exosomes during circulation and protects the internal cargo from degradation by enzymes or oxidative stress. Cholesterol, for instance, maintains the membrane's rigidity and promotes the encapsulation of bioactive molecules by facilitating the proper formation of the lipid bilayer. The presence of sphingomyelin and ceramide not only helps to modulate membrane fluidity but also influences the exosome's ability to interact with and penetrate biological barriers, a key feature for their use in drug delivery.

Encapsulated cargo

Exosomes encapsulate a diverse array of bioactive molecules that reflect the physiological or pathological state of their parent cells. These molecules have the capacity to modulate various cellular processes in recipient cells, including immune modulation and tissue repair. Exosomes transport a wide range of proteins, such as enzymes, cytokines, growth factors, and signaling molecules, which are integral to cellular communication, immune responses, angiogenesis, and

tissue regeneration [122, 123]. For instance, exosomes derived from mesenchymal stem cells (MSCs) contain proteins and growth factors that promote vascular regeneration and wound healing in ischemic tissues. Specifically, research has shown that MSC-derived exosomes facilitate advantageous vascular remodeling, enhance tissue perfusion, and expedite wound healing in ischemic ocular models, such as corneal injury and retinal ischemia, where controlled and regulated revascularization is essential for tissue repair and functional restoration [124–126]. Exosomes are abundant in diverse RNA species, including microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and messenger RNAs (mRNAs). These RNA molecules play pivotal roles in the regulation of gene expression and cellular behavior [127, 128]. Notably, miRNAs serve as crucial regulators of post-transcriptional gene expression. For instance, miRNAs such as miR-29b have been associated with the regulation of angiogenesis, where they downregulate VEGF expression to mitigate pathological blood vessel proliferation in conditions like diabetic retinopathy (DR). The capacity of exosomes to deliver miRNAs that target angiogenic pathways, including the VEGF/VEGFR axis, positions them as promising candidates for anti-angiogenic therapy in ocular neovascular diseases.

Exosomes not only transport proteins and RNA but also carry bioactive lipids that contribute to cellular signaling and membrane dynamics. These lipids can modulate pathways involved in vascular remodeling, cell migration, and inflammation [129, 130]. For instance, exosomes enriched in sphingolipids may promote endothelial cell stability, an important factor in maintaining the integrity of newly formed retinal vasculature in diseases like wAMD. Moreover, exosomes can be engineered to carry therapeutic molecules, such as small-molecule drugs, peptides, and nucleic acids (e.g., siRNAs or anti-VEGF RNA). This capacity enables precise drug delivery to tissues affected by neovascularization or vascular leakage, which significantly increases local drug concentration at the target site [131, 132]. In the context of ONDs, engineered exosomes can encapsulate anti-inflammatory agents or gene silencing agents to specifically target pathological cells in the retina, thereby reducing side effects commonly associated with systemic drug delivery. For ocular neovascular diseases like AMD and DR, exosomes offer the potential to deliver highly targeted therapeutic agents directly to affected tissues, overcoming many of the barriers associated with conventional drug delivery systems. This unique capability to modulate both immune responses and angiogenesis makes exosomes a versatile platform for the treatment of multifactorial retinal diseases.

Biophysical properties relevant to ocular delivery

One of the major advantages of exosomes for ocular drug delivery is their nanoscale size, which is critical for traversing highly restrictive biological barriers, such as the blood-retinal barrier (BRB) [111, 133, 134]. The BRB consists of tightly sealed endothelial cells and forms a physical barrier that limits the uptake of systemic drugs into the retina, particularly for diseases involving retinal neovascularization or inflammation. Exosomes, due to their small size, are able to navigate through these restrictive barriers and target the retinal tissue more effectively than larger drug carriers [135]. Exosomes exhibit low immunogenicity, which is a key feature for their ocular application. Unlike synthetic nanoparticles, which may provoke immune responses and lead to unwanted side effects such as inflammation, exosomes are derived from cellular membranes and thus present a more natural profile that is better tolerated by the immune system. Their biocompatibility is particularly beneficial for intraocular injections, where minimizing immune activation is critical to avoid complications such as endophthalmitis or retinal detachment. This biocompatibility also enhances their circulatory stability, allowing them to maintain functional integrity while circulating in the bloodstream or eye fluids for extended periods, ensuring a sustained release of therapeutic agents at the site of action [136, 137].

Additionally, the lipid bilayer not only protects the exosomal cargo from degradation but also facilitates specific receptor interactions on the surface of retinal cells. The fluidity of the lipid bilayer in exosomes also contributes to their ability to undergo endocytosis or membrane fusion when interacting with recipient cells [111, 138–140]. Exosomes can readily enter cells by fusion with the plasma membrane or internalization via receptor-mediated endocytosis. This is particularly advantageous for delivering therapeutic agents directly to target retinal cells, such as endothelial cells in neovascular tissue or retinal pigment epithelium cells involved in AMD [141]. This interaction is crucial for effective ocular therapy, as the retina is highly sensitive to off-target effects due to its delicate architecture and functionality [142]. The membrane fusion process allows for the direct release of the exosome's contents into the cytoplasm, leading to effective drug delivery with enhanced therapeutic outcomes. The ability of exosomes to carry surface proteins, such as tetraspanins and integrins, further aids in targeting specific cell types [108]. Exosome surface modification allows for the engineered targeting of retinal cells, enhancing their localization to sites of pathological neovascularization or inflammation [131, 143]. For example, targeting ligands such as RGD peptides can be conjugated to exosomes, enabling them to bind to integrin receptors that are upregulated on retinal endothelial cells involved

in neovascular diseases. These targeted interactions significantly improve therapeutic specificity, reducing off-target effects and enhancing the therapeutic index.

In summary, exosomes possess several biophysical properties that make them highly suitable for ocular drug delivery. The combination of these properties positions exosomes as a promising next-generation platform for delivering both small-molecule drugs and genetic therapies to the retina, potentially transforming the treatment landscape for conditions like AMD and DR [133, 140].

Exosome isolation and characterization approaches

Exosome isolation and characterization are critical steps in ensuring the functionality, purity, and suitability of exosomes for both research and therapeutic applications. The methods employed for isolating exosomes can vary significantly based on the intended use, and each comes with its own set of advantages and limitations that can influence downstream experimental results. In the context of ONDs, obtaining exosomes with high purity, functional integrity, and minimal contamination is paramount for ensuring that therapeutic efficacy is not compromised.

Common isolation methods

Exosome isolation typically involves separating extracellular vesicles based on size, density, or specific surface markers. Below are the most commonly employed methods for exosome isolation, each with its advantages and drawbacks:

Ultracentrifugation is the most commonly used method for exosome isolation. This technique separates exosomes based on size and density through a series of centrifugation steps. It is widely employed because of its simplicity and effectiveness, and it is particularly useful for processing larger volumes. However, ultracentrifugation has several drawbacks, including the risk of co-isolating non-exosomal contaminants such as protein aggregates and apoptotic bodies [144, 145]. These contaminants can interfere with the biological activity and therapeutic efficacy of isolated exosomes. Moreover, the process is time-consuming, and repeated centrifugation can cause exosome degradation, diminishing their functional integrity. While ultracentrifugation remains a standard method for exosome isolation, it often results in a heterogeneous exosome population that may require further purification [145].

Size exclusion chromatography (SEC) has emerged as an effective alternative to ultracentrifugation. By separating exosomes based on their size, SEC minimizes contamination from smaller particles such as proteins and lipoproteins. This method typically results in higher purity exosome preparations, which is essential

for applications requiring high-quality exosomes [146]. SEC is faster and less damaging to exosomes compared to ultracentrifugation, but it generally produces a lower yield of exosomes, making it less suitable for large-scale isolations. Nevertheless, SEC is increasingly favored for applications that demand highly purified exosomes, such as in molecular characterization or targeted delivery studies [147]. Another prominent method is immunoaffinity isolation, which employs antibodies specific to exosomal surface markers such as CD63, CD9, and CD81. This technique allows for the selective isolation of exosomes based on their protein composition, providing highly specific isolation of certain subpopulations. Immunoaffinity isolation is particularly valuable when exosomes need to be sorted based on specific markers for tissue-specific applications or for functional assays [137, 146]. However, this method also has its limitations. The cost and availability of high-quality antibodies can be a constraint, and the antibody binding process may alter the surface protein composition of the exosomes, potentially affecting their functionality. Despite these challenges, immunoaffinity isolation is widely used for obtaining high-purity exosomes that are suited for studies on exosome-mediated intercellular communication and therapeutic applications [147].

Microfluidic technologies represent a newer approach to exosome isolation. These platforms integrate isolation and detection on a single device, offering high precision, low sample volume requirements, and the ability to process samples quickly. Microfluidic platforms are increasingly being used in clinical diagnostics, where rapid and reproducible results are crucial. These systems are capable of isolating exosomes with high efficiency and minimal contamination. However, their use is still somewhat limited by scalability challenges for large-volume clinical applications. Despite these limitations, microfluidics holds great promise for future clinical applications due to its precision and potential for high-throughput processing [148, 149].

The choice of isolation technique has a significant impact on the downstream applications of exosomes [147, 150]. Different methods can influence the purity, biological activity, and cargo integrity of exosomes. For instance, while ultracentrifugation is effective for obtaining larger quantities of exosomes, it may result in the co-isolation of unwanted cellular debris that can compromise the quality of the exosome preparation. On the other hand, techniques like SEC and immunoaffinity isolation provide higher purity exosomes but may offer lower yields. The integrity of the exosomal surface proteins and cargo is also essential for their functionality, especially when used for therapeutic purposes such as targeted drug delivery. Harsh isolation methods, such

as ultracentrifugation, may degrade sensitive cargo like RNA, thereby reducing the therapeutic potential of the exosomes. SEC and microfluidic platforms, by minimizing exposure to extreme conditions, tend to preserve the integrity of the exosomal cargo, making them more suitable for applications that involve genetic material or other sensitive therapeutic agents.

Characterization methods

After isolation, exosomes must be characterized to confirm their identity and functionality. Several techniques are commonly used to assess exosome size, morphology, surface protein composition, and molecular content.

TEM is considered the gold standard for visualizing the morphology of exosomes. It provides high-resolution images that reveal the characteristic cup-shaped structure of exosomes and is particularly useful for confirming their size and shape. However, TEM is labor-intensive and does not provide quantitative data on exosome concentration. For size and concentration analysis, nanoparticle tracking analysis (NTA) is commonly used. NTA allows for the real-time measurement of the size distribution and concentration of exosomes by tracking the Brownian motion of individual particles. This method is particularly useful in determining exosome populations but can be affected by the presence of contaminants [151].

Dynamic light scattering (DLS) is another widely used technique for determining the size distribution of exosomes, although it may not be as effective for detecting smaller vesicles or aggregates that fall below the detection threshold [151]. For molecular characterization, techniques like Western blotting are employed to detect specific exosomal protein markers, ensuring that the isolated vesicles are indeed exosomes [152]. Mass spectrometry can provide a more detailed analysis of the exosomal protein content and overall molecular composition, offering deeper insights into their functional properties. Flow cytometry, while also used to analyze surface markers and size distribution, may have limitations in detecting smaller exosomes and is often less effective for heterogeneous samples [150].

In conclusion, the choice of exosome isolation and characterization methods must be tailored to the specific needs of the intended application. While ultracentrifugation remains the most widely used method, newer techniques such as SEC, immunoaffinity methods, and microfluidic platforms offer distinct advantages in terms of specificity, purity, and high throughput. These methods should be carefully selected based on the required exosome yield, purity, and functional integrity to ensure optimal results in both research and clinical applications. The continuous development of more efficient isolation

and characterization technologies will enhance the ability to harness the therapeutic potential of exosomes in treating complex diseases, including ocular neovascular diseases.

Engineering exosomes for therapy

Exosome engineering has become a pivotal strategy for enhancing the therapeutic potential of exosomes, particularly in the context of targeted drug delivery [122, 153]. By modifying the exosome surface and optimizing cargo loading techniques, researchers have significantly improved the ability of exosomes to deliver specific therapeutic agents to targeted tissues, especially in diseases such as ONDs, where precise delivery and efficacy are crucial.

Surface modification strategies

One of the primary methods to optimize the targeting capacity of exosomes is through surface modification. This process involves the introduction of specific ligands or homing peptides onto the exosomal membrane, enabling exosomes to recognize and bind to receptors that are overexpressed in target tissues. Surface modification strategies can enhance the precision of drug delivery, ensuring that the therapeutic payload is delivered specifically to the affected tissue while minimizing off-target effects. For example, rabies virus glycoprotein (RVG) peptides have been successfully incorporated into

exosomes to facilitate selective targeting of neuronal cells, allowing for more efficient delivery to the brain [154]. Similarly, the use of RGD peptides, which bind to integrins that are overexpressed on tumor cells, has been explored to target glioblastoma cells and enhance exosome internalization into the tumor microenvironment, thereby improving the uptake of therapeutic agents [155].

In the context of ocular diseases, such as AMD and DR, mesenchymal stem cell (MSC)-derived exosomes have shown great promise in targeted delivery to the retina. These exosomes, when modified with appropriate targeting peptides, have the potential to deliver therapeutics directly to retinal cells, which are often difficult to reach using traditional drug delivery methods [142]. For example, exosomes derived from human umbilical cord MSCs have demonstrated the ability to mitigate inflammation and promote healing in experimental models of autoimmune uveoretinitis [156]. Additionally, exosomes enriched with miR-124, a microRNA known for its neuroprotective effects, have been investigated for the treatment of retinal degeneration, illustrating how exosomes can carry RNA-based therapies to protect retinal cells [157]. By incorporating targeting peptides such as RGD or others that recognize receptors on the retinal cells, the specificity of these exosome-based treatments can be further enhanced, ensuring efficient drug delivery while minimizing systemic side effects. Figure 4 illustrates innovative strategies such as electroporation for cargo

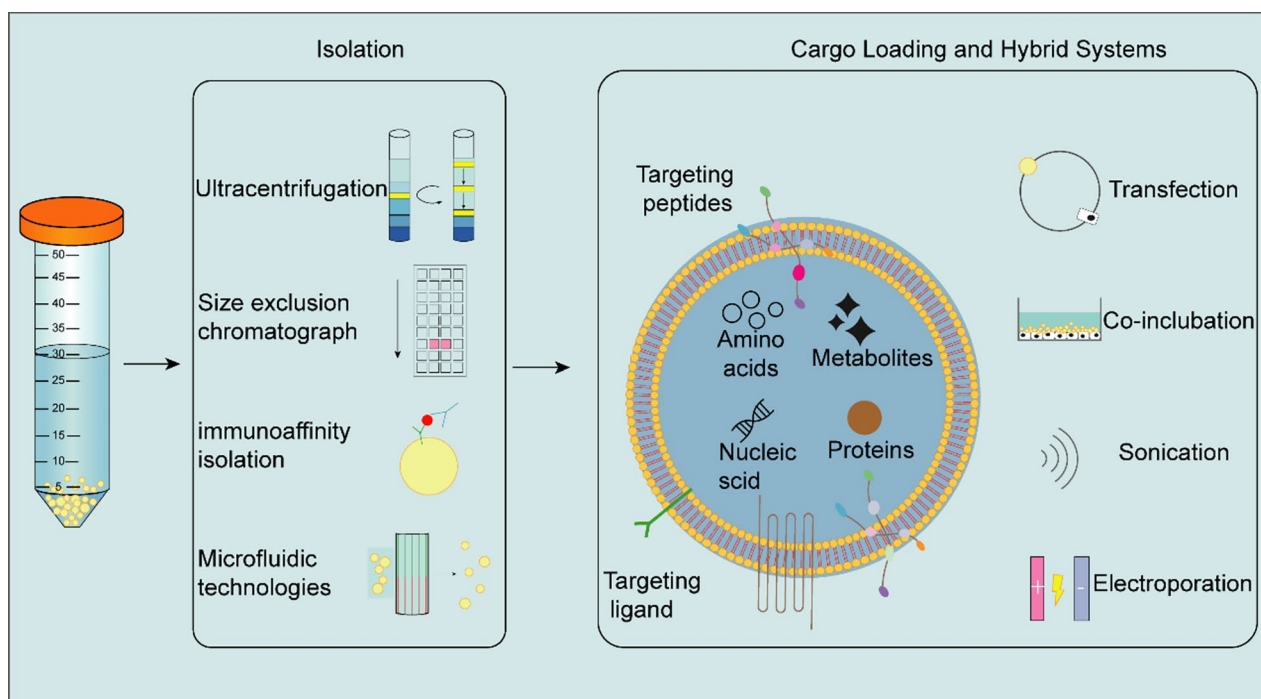


Fig. 4 Schematic illustration of exosome isolation, cargo loading, and hybrid systems

loading and exosome-liposome hybrids, which improve stability and targeting efficiency.

Cargo loading and hybrid systems

Another critical aspect of exosome bioengineering is the optimization of cargo loading strategies. These techniques are designed to improve the stability, release profiles, and therapeutic efficacy of encapsulated agents, ensuring that the therapeutic molecules remain intact and active until they reach their target. Common strategies for loading therapeutic agents into exosomes include electroporation, sonication, and chemical conjugation [158, 159]. Electroporation involves applying an electrical field to the exosome membrane, temporarily creating pores that allow for the encapsulation of small molecules, proteins, or nucleic acids [160]. This technique is widely used for loading RNA-based therapies, such as siRNA or miRNAs, into exosomes, providing an effective way to target specific genes or molecular pathways in retinal cells [80]. Sonication, on the other hand, uses high-frequency sound waves to facilitate the loading of hydrophobic molecules into exosomes. While effective, sonication can sometimes result in the disruption of exosome membranes if not carefully controlled. Chemical conjugation involves the use of covalent bonds to attach therapeutic molecules to exosomal membranes, ensuring stable loading and release over time [158].

One particularly promising strategy in the context of ocular drug delivery is the development of hybrid systems, where exosomes are combined with other drug delivery vehicles, such as liposomes. These hybrid systems combine the advantages of both exosomes and liposomes to overcome the limitations of each individual platform. Exosome-liposome hybrids have been developed to enhance the stability and circulation time of exosomes, as well as to improve their ability to cross biological barriers. The combination of liposomes, which can carry a larger payload of drugs or RNA, with exosomes, which possess high targeting efficiency and low immunogenicity, results in a highly effective delivery system for ocular therapies. Such hybrid systems can also help to overcome challenges like rapid clearance by the mononuclear phagocyte system (MPS), which often limits the effectiveness of traditional drug delivery vehicles. By prolonging the circulation time of exosomes, these hybrids enhance the targeting efficiency and therapeutic outcomes, while reducing systemic toxicity.

In addition to hybrid systems, cationic-motif-modified exosomes are being explored to improve ocular drug delivery. Cationic motifs, such as arginine-rich peptides, are added to the exosomal surface to enhance their transport and retention within ocular tissues. These modifications leverage electrostatic interactions between the

positively charged exosomes and the negatively charged components of ocular tissues, such as the cornea and vitreous humor [161]. By incorporating these modifications, exosomes are better able to penetrate ocular barriers and achieve higher uptake by retinal cells. This approach has significant potential for improving gene delivery to the retina, as it allows for the non-invasive delivery of therapeutic agents, which is particularly important in diseases like wet AMD and diabetic macular edema (DME), where frequent injections are not ideal [161]. Cationic-motif-modified exosomes represent a promising advancement in non-invasive ocular therapies, enabling the precise and efficient delivery of therapeutics to the retina without the need for invasive procedures.

When comparing different exosome loading methods, electroporation and sonication are two commonly used techniques. Electroporation uses brief electrical pulses to create pores in the exosome membrane, enabling highly efficient drug loading with yields typically exceeding 70%. However, this method may cause some damage to the exosome membrane, affecting its integrity and stability [162]. In contrast, sonication uses mechanical vibrations to load drugs into exosomes. Although its loading efficiency is slightly lower than electroporation (about 50–70%), it causes less damage to the exosome membrane [163]. Nevertheless, sonication may cause aggregation of exosomes, affecting their dispersibility [164]. When choosing a loading method, it is necessary to balance loading efficiency with exosome membrane integrity [165, 166]. For applications requiring high loading efficiency, electroporation may be a better choice; while for those needing to maintain exosome integrity, sonication may be more appropriate [166].

Administration methods and ocular barriers

Engineered exosomes can be administered to the eye through various routes, each presenting distinct challenges [167]. Topical application, such as through eye drops, is attractive due to its non-invasive nature; however, the tight junctions of the corneal epithelium significantly restrict penetration into intraocular tissues [168]. Innovative approaches, including the modification of exosome surfaces with cell-penetrating peptides like TAT, have demonstrated potential in enhancing corneal penetration, thereby enabling topically applied exosomes to reach the posterior segment of the eye [169, 170]. Systemic administration, such as intravenous delivery, bypasses the ocular surface but must overcome the BRB. The BRB, consisting of the retinal vascular endothelium and the RPE with tight junctions, rigorously controls the entry of molecules into the retina, posing a significant obstacle to most systemically delivered therapeutics [171]. Notably, exosomes, which are vesicles

approximately 30–150 nm in size, have shown the capability to traverse certain biological barriers, including reports of crossing the BRB [172, 173]. Nevertheless, effective retinal delivery via systemic routes likely necessitates active targeting strategies, such as ligand display, or a compromised BRB, as observed in certain disease states [174]. In practice, the most direct route is *intravitreal injection*, depositing exosomes into the vitreous humor adjacent to the retina. Intravitreal delivery bypasses the cornea and BRB, achieving higher local concentrations at target retinal tissues. This approach is already routine in treating neovascular AMD and DR [175]. Engineered exosomes could leverage the same route to deliver drugs or genes, with the potential advantage of reduced dosing frequency if they prolong intraocular retention or provide sustained release [176]. The viscous vitreous itself can pose a barrier, impeding diffusion of larger particles; however, nanoscale exosomes are relatively small and stable, which may facilitate their dispersion through the vitreous matrix [177]. Indeed, one study demonstrated that intravitreally injected RGD-peptide engineered exosomes homed to choroidal neovascular lesions with excellent tissue penetration. These findings suggest that, by careful route selection and surface engineering, exosome-based delivery can overcome ocular barriers to reach therapeutic targets in the eye.

Target cells and delivery efficiency

A significant advantage of utilizing exosome carriers lies in their ability to achieve cell-specific targeting within ocular tissues. Exosomes are endowed with membrane proteins and lipids that inherently facilitate their direction towards receptor cells, thereby enhancing the efficiency of delivery to target cell populations [175, 181]. In the context of ocular neovascular disorders, the primary targets encompass the retinal pigment epithelium RPE, vascular endothelial cells, and retinal neurons, such as ganglion cells [178, 179, 180]. The engineering of exosomes to present targeting ligands can further enhance this specificity. For instance, the incorporation of integrin-binding RGD motifs on the surfaces of exosomes can promote preferential uptake by neovascular endothelial cells in cases of choroidal neovascularization [181]. The capability to deliver therapeutic cargo, such as anti-angiogenic factors, small siRNAs, or microRNAs, directly to pathological endothelial cells or the RPE is critical for the treatment of diseases such as wAMD and proliferative diabetic retinopathy [182]. Preclinical models provide promising evidence; for example, exosomes derived from retinal astrocytes have been demonstrated to reduce pathological vascular leakage and inhibit choroidal neovascularization in an AMD model [172, 183]. RPE cell-derived exosomes have the capacity to modulate

the immune milieu and confer protection to photoreceptors, thereby demonstrating the potential of targeting RPE and adjacent cells to simultaneously inhibit neovascular proliferation and maintain retinal integrity [184]. In models of diabetic retinopathy, exosomes derived from MSCs have been shown to attenuate retinal inflammation induced by high glucose levels, likely through the delivery of regulatory molecules to retinal endothelial cells and glial cells [185]. Furthermore, exosomes can be directed towards retinal ganglion cells and other neuronal populations; for example, exosomes derived from intraocular stem cells have exhibited neuroprotective effects on retinal ganglion cells and photoreceptors under stress conditions [186]. The effective uptake of exosomal cargo by these target cells underscores their therapeutic potential. By modifying exosomal surface markers or engineering their cargo, researchers aim to optimize delivery to diseased cells while minimizing effects on non-target tissues, thereby enhancing the efficacy of treatments for ocular neovascular and degenerative disorders [187].

Safety and translational considerations

The utilization of exosomes as drug delivery vehicles in ophthalmic applications presents several safety advantages. As endogenous nanoparticle carriers, exosomes are typically well-tolerated by the immune system. They lack the capacity for replication and can be derived autologously, thereby minimizing the risk of immune rejection or inflammation. Exosomes exhibit an "immune-tolerant" profile; for instance, exosomes originating from ocular tissue can convey immunomodulatory signals—RPE-derived exosomes contribute to the regulation of retinal immune responses—potentially fostering an immune-privileged microenvironment within the eye [188]. In comparison to cell-based therapies, such as MSC transplantation, exosome-based strategies mitigate concerns related to ectopic tissue formation or vascular occlusion. Furthermore, exosomes generally demonstrate negligible cytotoxicity; an *in vivo* study involving intravitreal administration of engineered exosomes reported no retinal toxicity or stress responses, indicating excellent biocompatibility within ocular tissues [189, 190]. Additionally, exosomes naturally express "don't eat me" signals, such as CD47, which enable them to evade phagocytic clearance. [171], potentially prolonging their circulation or residence time [191–193]. This extended retention may result in prolonged therapeutic windows within the ocular environment; however, it also necessitates an assessment of potential long-term accumulation [194]. Nevertheless, comprehensive long-term safety data are required. Current studies indicate no chronic inflammation or immune sensitization with repeated exosome administration, yet meticulous monitoring in

clinical trials remains crucial [195]. From a translational perspective, the favorable safety profile of exosomes complements their therapeutic efficacy [196]. By facilitating active targeting and sustained drug release, exosome-based ocular therapies have the potential to decrease the frequency of invasive procedures and enhance patient adherence [197]. For instance, experts envisage that an exosome-based intraocular drug delivery system could transform the treatment paradigm from the current frequent, passive anti-VEGF monotherapy to an “active-targeting, multi-drug” approach with extended efficacy [181, 198]. This underscores the significant clinical relevance of exosome engineering, as it provides a versatile, biocompatible platform for the treatment of retinal neovascular diseases, such as AMD and DR.

Applications of exosomes

Mechanisms of exosome action in ocular neovascular diseases (ONDs)

Exosomes have garnered significant attention for their role in mediating cellular communication and influencing various pathological processes, including ocular neovascular diseases. Their biogenesis, cargo composition, and capacity to modulate retinal angiogenesis and immune responses make them critical players in the pathophysiology of these diseases.

Mechanisms of exosome uptake

Exosome-mediated cellular communication relies on the uptake of exosomes by target cells including retinal endothelial cells, pericytes, microglia, and immune cells. Several uptake mechanisms have been identified, each of which contributes to the specificity and efficiency of exosome internalization.

Endocytosis is the most common mechanism through which exosomes are internalized by cells [106]. Exosomes interact with cell-surface receptors, leading to their engulfment into endocytic vesicles. The receptors involved in this process include tetraspanins (e.g., CD9 and CD63) and integrins, which mediate the attachment of exosomes to the target cells [199]. Furthermore, exosomes can also be internalized via lipid raft-mediated endocytosis, a process that involves clathrin-independent internalization of membrane domains enriched in sphingolipids and cholesterol. This pathway is particularly important in endothelial cells and contributes to the uptake of exosomal cargo that influences angiogenesis [200].

Phagocytosis is another method of exosome uptake that is very common in immune cells. In immune cells such as macrophages and microglia, exosomes can be internalized through phagocytosis [201]. This process is particularly relevant in the retina, where exosomes carry

immune-related cargo and modulate the local immune response in diseases such as DR and wAMD.

Once internalized, exosomal cargo is delivered to the cytoplasm or nucleus, where it modulates gene expression and cellular function [106]. This capacity for inter-cellular communication is essential for the regulation of retinal angiogenesis and immune response.

Regulation of angiogenesis by exosomes

Angiogenesis, the process of new blood vessel formation, is a hallmark of several ocular neovascular diseases. Exosomes are important regulators of angiogenesis under both physiological and pathological conditions [202]. Pathological angiogenesis, characterized by abnormal and leaky blood vessels, is a critical feature of many ocular diseases including wet AMD and DR. Given their ability to carry therapeutic cargo, exosomes have emerged as potential candidates for the suppression of pathological angiogenesis. Exosomes secreted from endothelial cells and their microenvironment cells can modulate the local microenvironment, enhance or suppress angiogenesis, depending on the molecular cargo. Exosome-based therapies that deliver angiogenesis-regulating molecules have the potential to enhance or inhibit blood vessel formation under various ocular conditions, offering novel avenues for treatment [202, 203]. These interactions are vital for maintaining a balance between normal and pathological angiogenesis.

Exosomes play a key role in promoting angiogenesis. Exosomes released by endothelial cells, RPE cells, and immune cells can carry a variety of pro-angiogenic factors, such as VEGF, angiopoietins, fibroblast growth factors (FGFs), and matrix metalloproteinases (MMPs). These factors play crucial roles in endothelial cell proliferation, migration, and tube formation, which are key steps in angiogenesis. Exosomes derived from RPE cells or inflammatory cells, such as macrophages, can carry VEGF, which binds to VEGF receptors on endothelial cells, leading to endothelial cell proliferation and migration. This process is essential in the pathogenesis of diseases like wAMD and DR, where abnormal blood vessel growth occurs. Exosomes can carry MMPs, which degrade the extracellular matrix (ECM) and allow endothelial cells to migrate and form new blood vessels. This function is crucial for both normal and pathological angiogenesis in the eye.

Exosomes also have the potential to inhibit pathological angiogenesis, and can be engineered to carry anti-VEGF agents, such as small interfering RNA (siRNA), or specific miRNAs that target VEGF expression. These exosome-mediated delivery systems effectively reduce VEGF-induced endothelial cell proliferation and migration, which are critical steps in pathological angiogenesis.

For instance, exosome-mediated delivery of miR-126 has been shown to inhibit VEGF expression and reduce pathological neovascularization in animal models of DR. On the other hand, chronic inflammation is also a key driver of pathological angiogenesis in eye diseases, and regulating disease progression by inhibiting the inflammatory response has also become a major therapeutic avenue. Exosomes derived from anti-inflammatory cells or engineered to carry anti-inflammatory cytokines (e.g., IL-10) can modulate immune responses, reduce inflammation, and limit the formation of abnormal blood vessels.

Exosome-mediated immune modulation

Exosomes have a profound impact on the immune micro-environment of the retina, particularly in ocular neovascular diseases. These vesicles can modulate immune cell function by transferring bioactive molecules that influence macrophage polarization, T-cell differentiation, and microglial activation. In diseases like DR and AMD, exosomes can influence the polarization of macrophages toward a pro-inflammatory (M1) or anti-inflammatory (M2) phenotype. For example, exosomes derived from activated retinal endothelial cells may promote M1 polarization, exacerbating inflammation and tissue damage, whereas exosomes from MSCs or RPE cells can induce M2 polarization, promoting tissue repair and the resolution of inflammation. Exosomes can also modulate the differentiation of T-cells in the retina, promoting either a pro-inflammatory Th1 response or a regulatory T-cell (Treg) response. Dysregulation of the immune system contributes to retinal damage and neovascularization, which are particularly important in the pathogenesis of AMD.

Furthermore, microglia, the resident immune cells of the retina, play a central role in retinal inflammation and neurodegeneration. Exosomes derived from retinal endothelial or immune cells can activate microglia, triggering the release of pro-inflammatory cytokines that further promote angiogenesis and exacerbate retinal damage. Conversely, exosomes carrying anti-inflammatory cytokines or miRNAs can suppress microglial activation, thereby offering potential therapeutic avenues for controlling inflammation in ONDs.

In summary, exosomes play a critical role in the pathophysiology of ocular neovascular diseases by regulating retinal angiogenesis and modulating the immune micro-environment. Through their complex cargo composition, including angiogenesis-related proteins, cytokines, and miRNAs, exosomes facilitate intercellular communication, which can either promote or inhibit disease progression. Understanding the molecular mechanisms underlying exosome action in ONDs holds great promise for the development of novel diagnostic and therapeutic

strategies aimed at controlling neovascularization and inflammation in the retina.

Recent progress in the application of exosomes in neovascular eye diseases

CoNV, DR, and wAMD exhibit common pathological mechanisms, particularly chronic hypoxia, inflammation, and oxidative stress, which collectively promote aberrant angiogenesis [204]. In each of these conditions, hypoxia induces the upregulation of pro-angiogenic factors, notably VEGF, along with inflammatory cytokines, resulting in the formation of fragile new blood vessels that leak and damage ocular tissues [205, 206]. Despite these shared mechanisms, the diseases differ in their etiology and affected tissue contexts. CoNV typically results from corneal injury, infection, or hypoxic events such as prolonged contact lens wear, leading to the invasion of blood vessels from the limbus into the normally avascular cornea [207]. In contrast, DR is a complication of chronic hyperglycemia associated with diabetes, where retinal capillaries experience pericyte loss and ischemia, disrupting the BRB and promoting retinal neovascularization and edema [208]. wAMD is an age-related degenerative disorder characterized by CNV that breaches Bruch's membrane into the subretinal space, driven by cumulative oxidative damage and inflammation within the RPE-choroid complex [209]. Despite these differences, neovascularization in all three diseases is driven by similar molecular pathways, such as HIF-1 α /VEGF signaling and inflammatory cascades. This suggests that therapeutic strategies targeting these shared mechanisms, including anti-VEGF and anti-inflammatory approaches, could have broad applicability across ONDs.

Engineered exosomes are emerging as versatile nanotherapeutics that can be tailored to the unique context of each disease while addressing their common pathological mechanisms. In anterior-segment diseases such as CoNV, exosomes can be administered topically, for instance, as eye drops, or via local subconjunctival injection, facilitating direct delivery to corneal tissues [186]. In contrast, for posterior-segment diseases like DR and wAMD, exosomes must reach intraocular retinal cells, typically through intravitreal injection to traverse the BRB [203]. The therapeutic payloads and targets are customized accordingly. In CoNV, exosomal therapy may aim to suppress pathological corneal angiogenesis by delivering anti-angiogenic factors or, in certain scenarios, to promote regenerative angiogenesis and tissue repair following an insult, all while modulating the local immune response. In DR, exosomes are being engineered to mitigate retinal inflammation and vascular leakage, thereby strengthening endothelial junctions, and to inhibit ischemia-induced angiogenic signaling [210]. In

wAMD, exosome strategies are concentrated on the targeted delivery of anti-angiogenic and anti-inflammatory agents to the choroid and retina [211, 212]. For example, exosome-mediated suppression of VEGF in CNV lesions aims to provide sustained, localized therapy that could reduce the frequency of injections. Collectively, these strategies underscore the potential of exosome-based therapies to be tailored to each ONDs, exploiting their common pathogenic pathways, such as VEGF and inflammation, while accommodating the distinct ocular anatomy and disease triggers [213].

The application of exosomes in CoNV

Conventional therapies for corneal neovascularization (anti-VEGF agents, steroids, laser or surgical ablation) often yield suboptimal long-term outcomes, with high recurrence and collateral tissue damage [213]. Exosome-based treatments offer a novel means to deliver therapeutics directly to the cornea and modulate the angiogenic and inflammatory processes driving CoNV [214]. One approach is to harness exosomes to inhibit pathological corneal angiogenesis. For example, exosomes engineered to carry potent anti-angiogenic molecules have significantly reduced corneal neovascular growth in preclinical studies. In one model, exosomes loaded with the angiogenesis inhibitor endostatin markedly suppressed corneal neovessel formation, indicating that exosomal delivery can improve the bioavailability and targeting of anti-angiogenic proteins at the corneal surface [214]. Similarly, exosomes enriched with anti-angiogenic microRNAs (such as miR-126, which downregulates the VEGF pathway) effectively curtailed pathological corneal vessel growth. These findings highlight the potential of exosome carriers to directly target the VEGF-driven neovascular pathways in CoNV [215]. Conversely, in situations where promoting vascularization and healing is beneficial (e.g. during corneal wound repair), exosomes can be loaded with pro-angiogenic and regenerative cargo to support tissue recovery. Mesenchymal stem cell (MSC)-derived exosomes have been shown to carry microRNAs like miR-21 and miR-155 that enhance endothelial cell proliferation and migration [166]. In a study by Zhang et al. (2018), applying MSC-derived exosomes rich in these miRNAs accelerated corneal re-vascularization and wound healing in a mouse CoNV model. This suggests that carefully engineered exosomes could help re-establish a healthy microvasculature after corneal injury, thereby enhancing tissue repair while avoiding the unchecked angiogenesis that impairs corneal transparency [166, 216].

In addition to angiogenesis, exosome therapies in CoNV target the vigorous inflammatory response that often accompanies corneal neovascular growth.

Inflammatory cells (macrophages, neutrophils) and their cytokines (TNF- α , IL-1 β , IL-6, etc.) can exacerbate corneal angiogenesis and edema [217]. Exosomes derived from anti-inflammatory sources such as MSCs have demonstrated the ability to dampen corneal inflammation: they can inhibit macrophage and dendritic cell activation, thereby reducing the local release of pro-angiogenic cytokines. For instance, MSC-exosomes were shown to suppress NF- κ B signaling in corneal immune cells, resulting in lower levels of TNF- α and IL-6 and a corresponding reduction in neovascularization and corneal edema. Furthermore, engineered exosomes can deliver combination payloads to attack multiple pathogenic processes simultaneously [218, 219]. In one strategy, exosomes were loaded with both an anti-VEGF agent and an anti-inflammatory microRNA (miR-146a); this dual-action exosome significantly suppressed corneal neovascularization *and* reduced inflammatory markers in treated corneas, leading to improved corneal clarity. Such multifunctional exosome designs are especially promising for CoNV, which often involves a feedback loop between angiogenesis and inflammation [220].

Exosome-based therapy has also shown benefit in corneal tissue regeneration, which is critical when CoNV is associated with ulcers or trauma [221]. Corneal epithelial cells rely on growth factors and matrix signals to heal properly; exosomes can deliver these signals in a targeted fashion. Studies have found that exosomes derived from corneal epithelial or retinal pigment epithelium (RPE) cells carry pro-regenerative factors like EGF and HGF, which markedly accelerate corneal epithelial wound closure and reduce scar formation in animal models [222]. Such exosome treatments not only speed re-epithelialization but also help stabilize the underlying stroma and endothelium, indirectly mitigating further neovascular stimulus. To enhance the tissue-specific delivery, investigators are functionalizing exosome surfaces with cornea-homing peptides. For example, conjugating integrin-binding ligands or extracellular matrix fragments onto exosome membranes has improved their adhesion and uptake by corneal cells, resulting in more efficient delivery of therapeutic cargo and faster wound healing [223]. A recent innovative example of a cornea-targeted vesicle is the use of platelet-derived extracellular vesicles (PEVs) as a topical drug carrier [224]. Liu et al. loaded PEVs with the anti-inflammatory flavonoid kaempferol and applied them as eye drops in an alkali-burn CoNV model [224]. The PEVs greatly improved drug retention on the ocular surface and, when combined with kaempferol, significantly inhibited corneal neovascularization while lowering pro-angiogenic and pro-inflammatory cytokine expression in the cornea. This approach leverages a naturally biocompatible vesicle to achieve potent

anti-angiogenic and anti-inflammatory effects in CoNV [224].

Finally, exosomes can be exploited to modulate the immune mechanisms that drive certain forms of corneal neovascularization. Notably, in the context of corneal transplantation (where graft rejection is often accompanied by neovascularization), an immunosuppressive exosome strategy has shown promise. Tumor cell-derived exosomes from B16-F10 melanoma cells (B16-Exo) were recently used to prevent corneal allograft rejection [225]. Rather than targeting VEGF directly, these exosomes delivered immunomodulatory proteins (e.g. a high level of JAK2 from the JAK/STAT pathway) that expanded myeloid-derived suppressor cells and inhibited T-cell proliferation in the graft microenvironment [225]. Figure 5 highlights the use of B16-Exo to suppress neovascularization and immune rejection, as evidenced by reduced CD31 staining and improved graft survival in experimental models [225]. Treated corneal grafts exhibited significantly less CD31⁺ neovascularization and maintained greater transparency compared to controls [225]. This finding underscores that exosome cargo can be tailored not only for angiogenic pathways but also to create an anti-inflammatory, anti-rejection milieu. By suppressing the immune triggers of neovascularization, such approaches can indirectly reduce corneal NV and improve graft survival, opening a new avenue for managing high-risk corneal transplants. In summary, exosome-based therapies for CoNV present a multi-pronged platform—capable of delivering anti-angiogenic, anti-inflammatory, and pro-regenerative signals—that holds considerable promise for improving outcomes in this challenging condition.

The application of exosomes in DR

DR is one of the leading causes of vision impairment and blindness in individuals with diabetes mellitus. It is characterized by retinal microvascular damage, increased permeability, and neovascularization, which can lead to vision-threatening complications, such as DME and PDR [226, 227]. The pathogenesis of DR is complex and involves chronic hyperglycemia-induced inflammation, oxidative stress, endothelial dysfunction, and abnormal vascular remodeling [227]. Current treatments for DR, such as laser photocoagulation, anti-VEGF therapy, and corticosteroids, primarily aim to reduce retinal edema and prevent neovascularization [228, 229]. However, these treatments have limitations, including the need for repeated injections and risk of complications. Consequently, there is an urgent need for novel therapies that can more effectively target the underlying pathophysiological mechanisms of DR.

DME represents a principal etiology of vision impairment in DR, characterized by pathological fluid accumulation within the macular neurosensory retina secondary to vascular hyperpermeability [230]. This pathophysiological process is mediated through multifactorial mechanisms encompassing dysregulated vascular permeability, chronic low-grade inflammation, and compromised endothelial integrity, with persistent hyperglycemia serving as a critical exacerbating factor [231]. The molecular pathogenesis involves intricate interplay between inflammatory cascade activation, VEGF-mediated angiogenic signaling, and exosomal modulation of cellular homeostasis [232]. The therapeutic potential of exosomes in DME is primarily attributed to their cargo, which encompasses bioactive molecules such as proteins, lipids, microRNAs (miRNAs), and other RNA species that can specifically target pathways implicated in DME pathogenesis [233].

Inflammation is a major driver of retinal vascular leakage in DME patients. Activated retinal microglial cells and macrophages release pro-inflammatory cytokines, including tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1 β), which increase endothelial permeability and promote edema formation [234]. Exosomes can transport anti-inflammatory miRNAs, such as miR-146a and miR-155, which target inflammatory pathways and suppress cytokine secretion. Exosomes derived from stem cells, particularly MSCs, have shown significant therapeutic potential in preclinical studies for the treatment of DR [220]. These MSC-derived exosomes are enriched in regenerative factors and can promote retinal tissue repair, reduce inflammation, and restore endothelial function [235]. For instance, in a study by Chen et al. (2021), MSC-derived exosomes were shown to attenuate retinal inflammation, improve retinal vascular integrity, and enhance neuronal survival in a streptozotocin-induced DR mouse model [235]. These findings suggest that stem cell-derived exosomes may offer a more sustainable and non-invasive alternative to traditional stem cell therapies for DR [236]. Moreover, exosomes derived from MSCs or RPE cells have been shown to mitigate retinal inflammation by inhibiting the activation of nuclear factor-kappa B (NF- κ B) and mitogen-activated protein kinase (MAPK) signaling pathways. This leads to reduced levels of pro-inflammatory cytokines and improved retinal function in animal models of DME. This anti-inflammatory effect can help prevent or reduce the development of retinal edema in DME [237]. Exosomes loaded with anti-angiogenic peptides, such as EXOKV11, effectively suppress retinal neovascularization in oxygen-induced retinopathy (OIR) models [212]. Figure 6 demonstrates their ability to

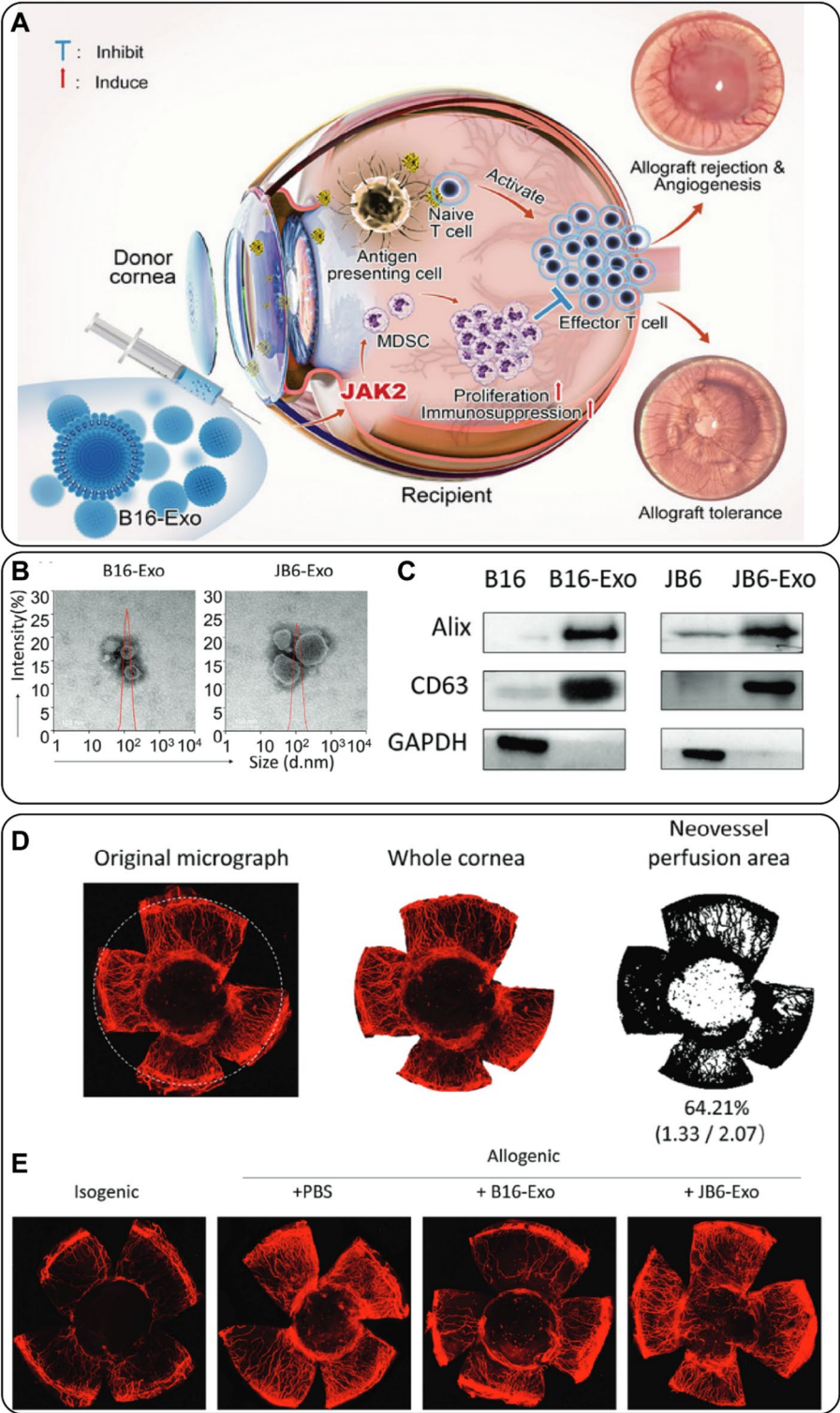


Fig. 5 The application of exosomes in corneal neovascularization. **A** B16-Exo mediated immunomodulatory regimen for corneal transplantation: Shown here is subconjunctival injection of B16Exo, which modulates the immune response during corneal transplantation. **B** Representative transmission electron microscopy (TEM) images and size distributions of B16-Exo and JB6-Exo. **C** Western blotting analysis of exosome markers in different exosomes. **D** Image processing for neovascularization assessment in graft-bearing corneas. **E** Representative immunofluorescent micrographs of CD31 staining (for neovessels) in graft-bearing corneas on day 18 post-transplant. (Adapted from [225], copyright 2025 Adv Sci (Weinh), Inc.)

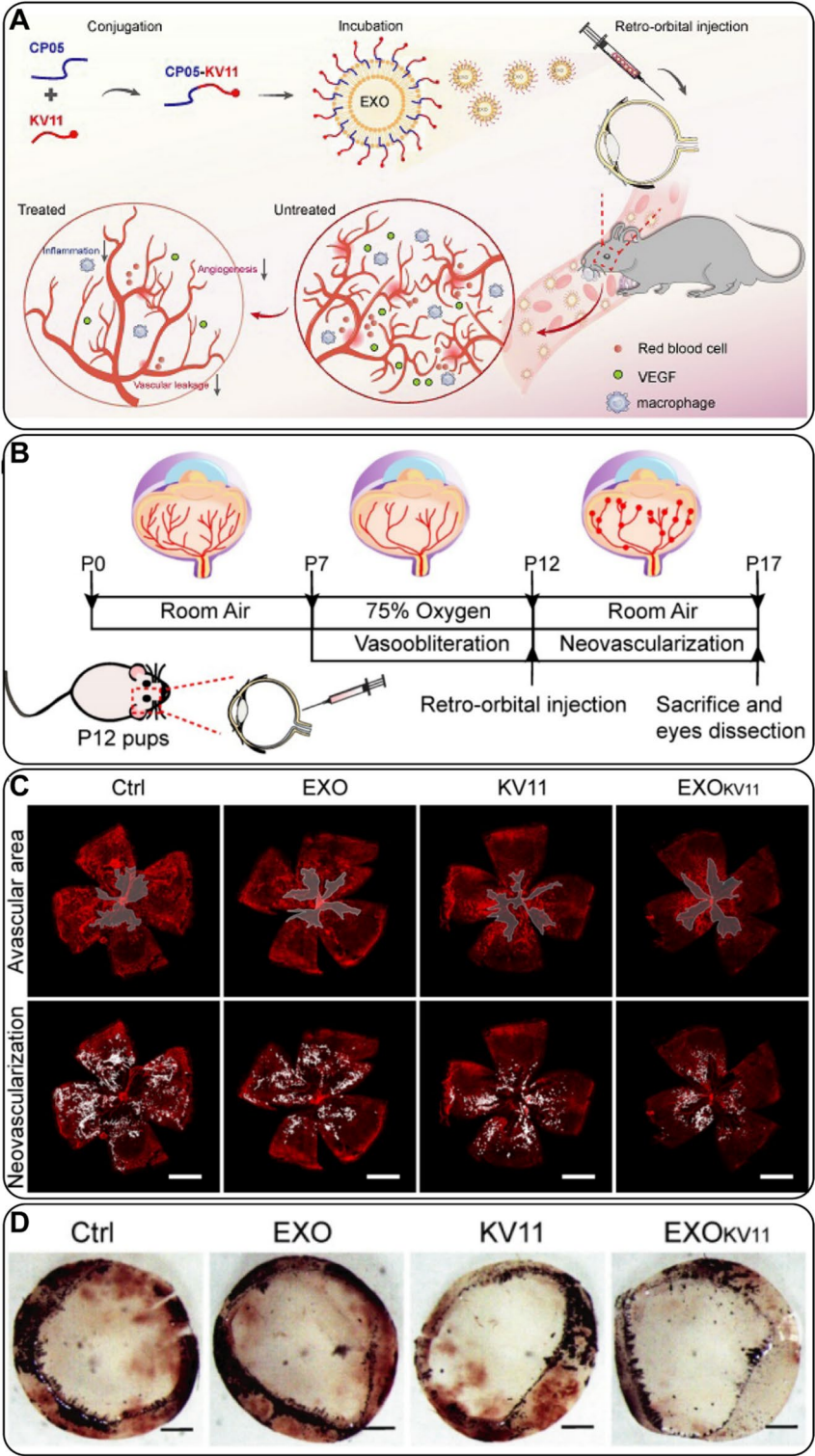


Fig. 6 The application of exosomes in retina neovascularization. **A** Schematic illustration of the preparation of EXOKV11 by coating anti-angiogenic peptide (KV11) on exo by exosome-anchored peptide CP05 and its use in the treatment of ocular pathological retinal angiogenesis in mice. **B** Schematic depiction of the mouse OIR model. Pups were placed in 75% oxygen from P7 to P12, and then returned to normal oxygen conditions. Saline vehicle (Ctrl), EXO, KV11, or EXOKV11 were injected retro-orbitally at P12. Pups were sacrificed and eyes were dissected at P17. **C** Representative confocal images of retina vasculature stained with IsoB4 in OIR retinas of saline vehicle (Ctrl)-, EXO-, KV11-, and EXOKV11-treated pups at P17. Upper panels indicate the avascular area and lower panels show the neovascular tufts. **D** Images of the inner surface of the OIR retinal cup at P17. Blood island formation indicates hemorrhages in the retinas. (Adapted from [212], copyright 2021 Theranostics, Inc.)

reduce avascular areas and neovascular tufts, highlighting their therapeutic potential in DR [118].

Vascular permeability resulting from endothelial dysfunction is a hallmark of DME and directly contributes to the disruption of the BRB. Exosomes can modulate endothelial cell function by delivering bioactive molecules that regulate tight junction proteins, such as zonula occludens-1 (ZO-1) and vascular endothelial cadherin (VE-cadherin), which are essential for maintaining BRB integrity [238]. In preclinical models, MSC-derived exosomes have been shown to reduce retinal vascular leakage by restoring the expression of these tight junction proteins, thereby improving BRB integrity and preventing further fluid accumulation [239]. Additionally, exosomes can carry miRNAs or proteins that target VEGF or other growth factors implicated in vascular permeability, offering a novel therapeutic strategy for controlling retinal edema [213, 240]. For example, exosomes loaded with anti-VEGF siRNA have been demonstrated to reduce VEGF expression in retinal endothelial cells and suppress retinal vascular leakage and edema in diabetic animal models [241]. Furthermore, exosomes derived from RPE cells or MSCs can deliver VEGF inhibitors, which have been shown to decrease pathological angiogenesis and inflammation contributing to DME [242]. This dual role of exosomes in both regulating vascular permeability and inhibiting abnormal angiogenesis renders them an attractive therapeutic option for the treatment of DME.

PDR is defined by the formation of new blood vessels on the retinal surface secondary to retinal ischemia. Neovascularization at the optic disc and elsewhere in the retina is highly susceptible to rupture, leading to vitreous hemorrhage [242]. These abnormal vessels are prone to fibrosis and contraction, and similar fibroproliferative processes can result in the formation of epiretinal membranes and retinal tears, thereby causing severe visual impairment [243]. Exosomes, which are critical mediators of intercellular communication, have been shown to influence various pathological processes in PDR, including inflammation, angiogenesis, and vascular permeability [244]. In PDR, endothelial cell dysfunction and pathological angiogenesis are key drivers of disease progression [245]. Exosomes can transport angiogenic factors such as VEGF, fibroblast growth factor (FGF), and hepatocyte growth factor (HGF), which can either promote or inhibit angiogenesis depending on their specific cargo [244]. For instance, exosomes derived from MSCs have been demonstrated to enhance endothelial cell proliferation and migration, potentially facilitating the repair of damaged retinal vasculature. Conversely, exosomes engineered to deliver anti-angiogenic molecules, such as endostatin or thrombospondin-1, can effectively suppress pathological neovascularization, a hallmark of PDR.

Moreover, the combination of exosome-based therapies with other treatment modalities for PDR holds significant promise for improving therapeutic outcomes [134, 246]. For example, exosome-loaded therapies can be used in conjunction with conventional anti-VEGF treatments to enhance the inhibition of pathological angiogenesis and potentially reduce the frequency of injections [246]. Additionally, exosomes can serve as delivery vehicles for gene therapies or small molecules that modulate the activity of growth factors, matrix metalloproteinases, and other signaling pathways implicated in diabetic retinopathy. Preliminary studies suggest that exosome-based combination therapies may provide synergistic effects in treating DR and preventing disease progression [247].

The application of exosomes in wet age-related macular degeneration

wAMD is marked by the growth of abnormal choroidal blood vessels beneath the retina, a leading cause of vision loss among the elderly, characterized by the growth of abnormal blood vessels beneath the retina, termed CNV. These aberrant vessels are prone to leakage of fluid and blood, resulting in retinal damage, scarring, and significant impairment of central vision. The pathogenesis of wAMD involves complex interactions between RPE cells, endothelial cells, immune cells, and various signaling molecules, with VEGF playing a central role in driving pathological angiogenesis [232, 248]. Although current treatment options, such as anti-VEGF therapies, have revolutionized the management of wAMD, challenges persist, including the need for frequent intravitreal injections and the development of drug resistance [249, 250].

Recent research has increasingly focused on alternative therapeutic strategies, with exosome-based therapies emerging as a promising approach due to their capacity to deliver bioactive molecules directly to target cells with high specificity and minimal systemic toxicity. In recent years, exosome-based therapies for wAMD have garnered significant attention, with several preclinical studies demonstrating the therapeutic potential of engineered exosomes in inhibiting CNV and promoting retinal healing [251, 252]. The mechanisms underlying the therapeutic effects of exosomes in wAMD are multifaceted, encompassing the regulation of angiogenesis, suppression of inflammation, and modulation of immune responses. Notably, engineered exosomes offer several advantages over traditional therapeutic approaches, such as BPQDs@RGD-EXO (BREs) and ESIONPs@EXO, demonstrate significant potential in targeting choroidal neovascularization [251–254]. As shown in Fig. 7, these systems combine RNA-seq and metabolomics to validate

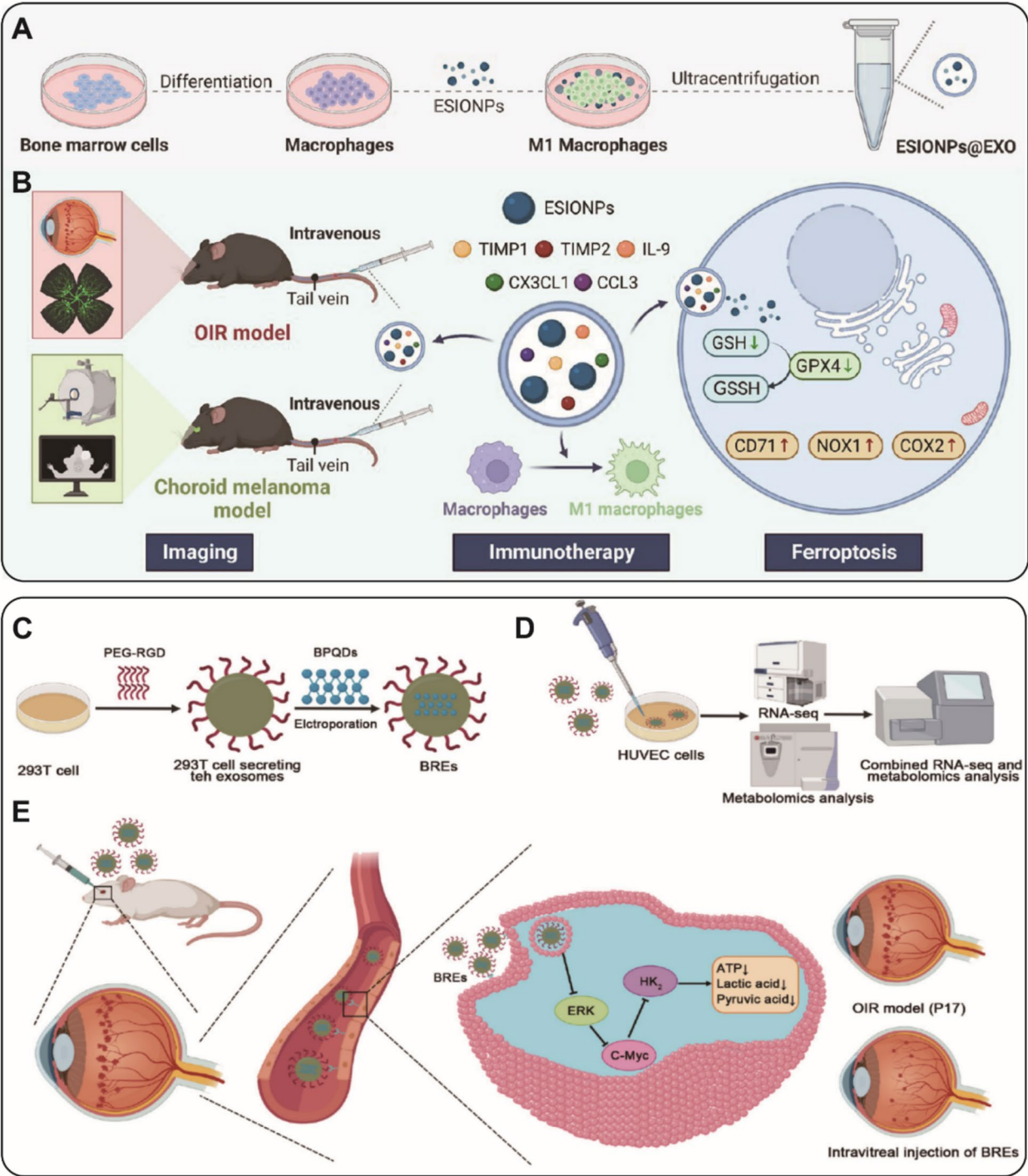


Fig. 7 Schematic illustration of the preparation and application of Engineered Exosomes. Illustration of ESIONPs@EXO preparation (**A**) and targeted pathological angiogenesis therapy (**B**). Schematic illustration of the preparation and application of BPQDs@RGD-EXO (BREs). **C** The construction of BREs. **D** The schematic illustration of combined RNA-seq and metabolomics. **E** The therapeutic outcomes and underlying mechanism of BREs treated group. (Adapted from [203], copyright 2024 ACS Nano, Inc; Adapted from [255], copyright 2023 Mater Today Bio, Inc.)

therapeutic outcomes, offering sustained anti-angiogenic effects in preclinical models [203, 255].

One of the most prominent therapeutic strategies for wAMD involves the use of anti-VEGF therapies to inhibit pathological angiogenesis. Under pathological conditions, particularly in hypoxic microenvironments, exosomes secreted by ocular cells can promote angiogenesis. For instance, exosomes derived from RPE cells or endothelial progenitor cells have been shown to enhance endothelial cell proliferation and migration *in vitro* [256, 257]. Based on these findings, exosomes engineered to deliver anti-VEGF molecules, such as anti-VEGF antibodies, VEGF-specific siRNA, or anti-VEGF microRNAs (miRNAs), have shown significant promise in preclinical models. For example, mesenchymal stem cell (MSC)-derived exosomes engineered to carry siRNA targeting VEGF have been demonstrated to reduce CNV and retinal edema in animal models of wAMD [251]. These exosome-based systems provide a targeted and sustained delivery mechanism, potentially reducing the frequency of injections compared to conventional anti-VEGF therapies [252, 258]. Additionally, several other angiogenic factor inhibitors have been explored in the context of exosome-based treatments for AMD. Exosomes engineered to deliver angiogenesis inhibitors, such as endostatin or thrombospondin-1, have been shown to suppress the growth of aberrant blood vessels, as demonstrated in a rabbit model of CNV [252, 259]. Inflammation is a pivotal factor in the pathogenesis of wAMD, contributing to vascular leakage and the progression of CNV. Exosomes can carry anti-inflammatory cytokines such as interleukin-10 (IL-10) or transforming growth factor-beta, which can modulate the immune response within the retinal microenvironment [260, 261]. For instance, MSC-derived exosomes have been shown to attenuate inflammation and promote retinal tissue repair in animal models. Furthermore, exosomes can alter the polarization of macrophages, shifting them from a pro-inflammatory (M1) phenotype to an anti-inflammatory (M2) phenotype, which has been associated with reduced CNV and retinal damage [253, 262, 263]. Targeted exosome systems, such as rEXS-cL-aV, synergistically inhibit CNV by suppressing VEGF and macrophage infiltration. Figure 8 validates their efficacy through fluorescein angiography and histological analysis, demonstrating reduced lesion size and vascular leakage.

Another emerging area of research involves the use of exosomes in combination with other therapeutic modalities. Combining exosome-based delivery systems with current anti-VEGF treatments or gene therapy approaches may enhance overall therapeutic outcomes [264]. For example, exosomes carrying miRNAs targeting VEGF signaling pathways can be used in conjunction

with anti-VEGF injections to provide a multi-pronged approach to treating CNV [251, 265]. Additionally, exosomes can be co-delivered with small molecules or cytokines that promote retinal tissue repair and regeneration, thereby improving the long-term prognosis for wAMD patients. One of the primary challenges in the development of exosome-based therapies for wAMD is achieving precise delivery of exosomes to the relevant retinal cells [252]. Recent advancements have focused on enhancing the targeting specificity of exosomes through innovative strategies. For example, modifying the exosome surface with ligands that bind to specific receptors on retinal cells, such as integrins or markers on endothelial cells, has been demonstrated to significantly improve targeting efficiency [266, 267]. Additionally, the utilization of exosomes derived from RPE cells or MSCs which inherently interact with retinal tissue—has been explored as a means to augment therapeutic efficacy in wAMD treatment [268]. While preclinical investigations have yielded encouraging results, the clinical translation of exosome-based therapies for wAMD remains an ongoing challenge. To date, several early-phase clinical trials have initiated explorations into the safety and efficacy of such therapies. For instance, a Phase I clinical trial evaluating the use of exosome-loaded MSCs for the treatment of retinal diseases, including wAMD, has highlighted the potential of exosomes as a novel therapeutic modality, with no significant adverse effects reported. However, additional studies are necessary to comprehensively assess the long-term safety, efficacy, and optimal dosing regimens of exosome-based therapies in patients with wAMD [269, 270].

The application of exosomes and engineered exosomes in other ocular diseases

In addition to their well-documented roles in ocular neovascular diseases such as corneal neovascularization, diabetic retinopathy and wet age-related macular degeneration, exosomes have also garnered attention as potential therapeutic agents for a variety of other ocular conditions. Leveraging their capacity to modulate inflammation, promote tissue regeneration, and facilitate targeted drug delivery, exosomes are being increasingly explored in the context of several ophthalmic disorders, including retinal vein occlusion (RVO), pathological myopia-related choroidal neovascularization (PM-CNV), corneal graft rejection, and retinal degenerative diseases. Moreover, the advent of engineered exosomes, which are modified to enhance their cargo loading and targeting efficiency, offers an innovative strategy to address current challenges in ophthalmic drug delivery. This section provides a comprehensive

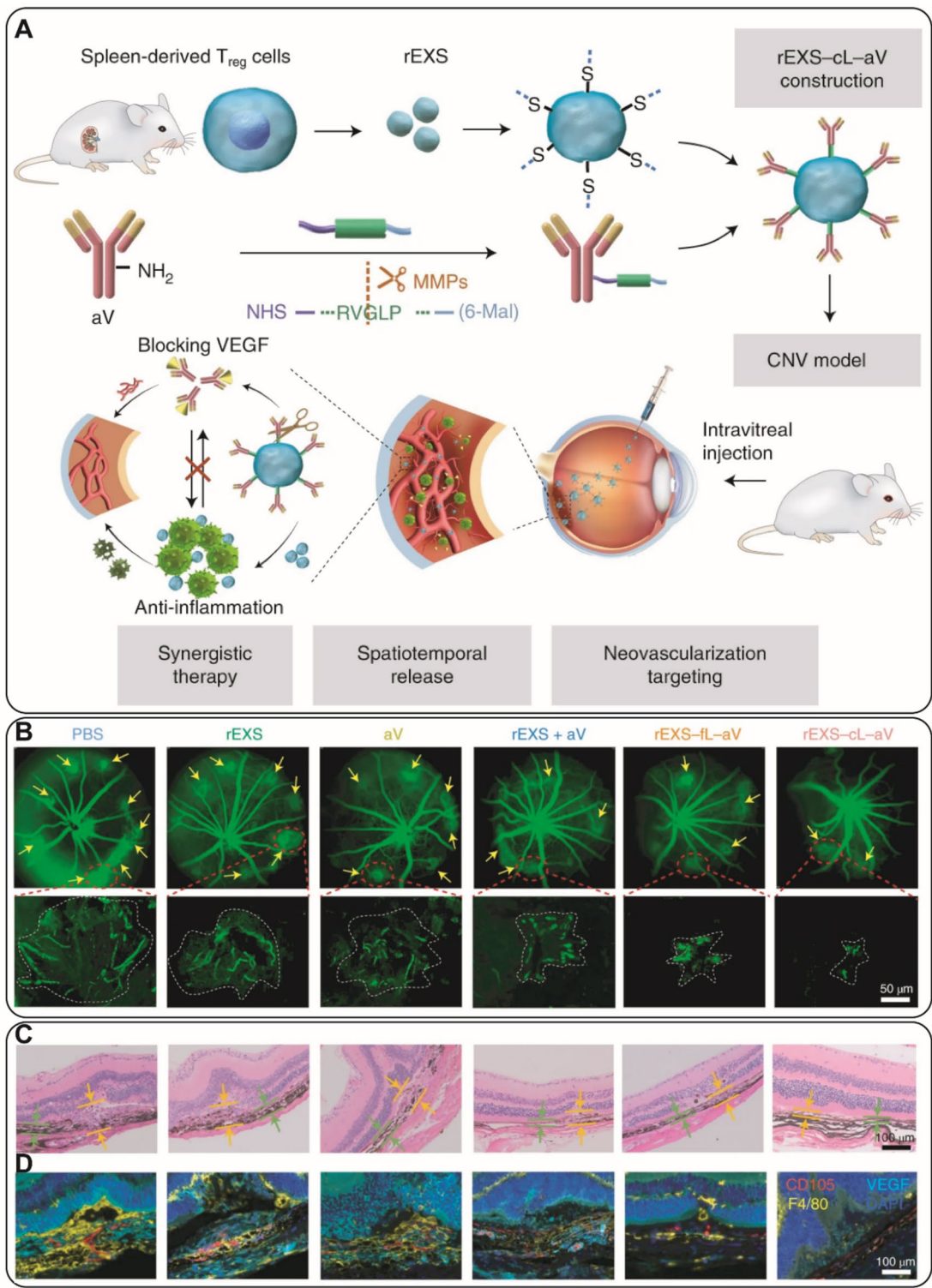


Fig. 8 The application of exosomes in choroidal neovascularization. **A** Schematic illustration of the preparation of rEXS-cL-aV, and the experimental design of a subsequent synergistic therapeutic study in a mouse model of CNV. **B** The assessment of CNV lesions by FFA. Top: retinal arterioles and venules were visualized with fluorescein. Sites of CNV lesions are highlighted with yellow arrows. Bottom: enlarged view of outlined regions in the top images, showing the neovascular sites. **C** H&E staining of retinal slices for assessment of CNV lesions. Green, normal choroid thickness; yellow, neovascular site thickness. **D** Representative images of vascular endothelial cells (red, CD105), macrophages (yellow, F4/80) and VEGF (cyan) of choroidal tissue sections by immunofluorescence staining (blue, DAPI-labelled cell nuclei). (Adapted from [141], copyright 2021 Nat Biomed Eng, Inc.)

overview of recent advancements in the application of both native and engineered exosomes in these diverse ocular diseases.

Exosomes in corneal graft rejection

Corneal transplantation is currently the primary treatment for corneal opacities; however, immune-mediated graft rejection remains a significant challenge. Given their potent immunomodulatory properties, exosomes have emerged as a promising therapeutic strategy to enhance graft survival. Exosomes derived from MSCs have been shown to suppress T-cell activation, inhibit dendritic cell maturation, and promote macrophage polarization toward an anti-inflammatory (M2) phenotype, thereby effectively prolonging corneal graft survival in animal models. In addition, engineered exosomes have also been utilized to mitigate immune rejection. For instance, exosomes carrying immunosuppressive microRNAs (e.g., miR-21, miR-150) have been employed to modulate alloreactive T-cell responses, offering a targeted and specific approach to preventing graft rejection.

Exosomes in retinal vein occlusion (RVO)

Retinal vein occlusion (RVO) is a leading cause of vision loss globally, primarily resulting from the obstruction of retinal veins, which leads to hemorrhage, ischemia, and macular edema. Current therapeutic strategies, including anti-VEGF injections and corticosteroids, are limited by their transient efficacy and potential complications. Consequently, exosome-based therapies have emerged as a potential alternative for targeting ischemia-induced damage and vascular dysfunction in RVO. Exosomes derived from MSCs have demonstrated significant potential in restoring vascular integrity in ischemic retinal diseases, including RVO. Studies have shown that MSC-derived exosomes promote endothelial cell survival, reduce oxidative stress, and enhance retinal perfusion by delivering pro-angiogenic factors such as VEGF-A, HIF-1 α , and angiopoietin-1. Given the critical role of inflammation in the pathogenesis of RVO, exosomes loaded with anti-inflammatory microRNAs (e.g., miR-126, miR-146a) have been investigated for their ability to suppress pro-inflammatory cytokines (e.g., TNF- α , IL-6) and mitigate secondary retinal damage. Furthermore, nanoparticle-modified exosomes have been developed to enhance targeted drug delivery to the ischemic retina. For instance, exosomes functionalized with integrin-binding peptides have demonstrated improved retinal targeting in RVO models, thereby facilitating the delivery of neuroprotective agents.

Exosomes in pathological myopia-associated choroidal neovascularization (PM-CNV)

Pathological myopia (PM) is a major cause of CNV, characterized by excessive axial elongation of the eyeball and subsequent choroidal thinning. Although anti-VEGF therapy remains the cornerstone of treatment for PM-associated CNV (PM-CNV), emerging studies suggest that exosomes may offer additional therapeutic benefits by targeting multiple pathways involved in disease progression. For instance, exosomes derived from RPE cells have been shown to influence choroidal angiogenesis by modulating the expression of VEGF and PEDF. Additionally, MSC-derived exosomes engineered to carry anti-VEGF siRNA have demonstrated significant inhibition of CNV growth in animal models of myopia. Beyond their anti-angiogenic effects, exosomes have also been explored for their potential to preserve retinal neurons in PM. Exosomes derived from neural progenitor cells, carrying brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor, have shown promise in preventing retinal ganglion cell degeneration in experimental myopia. As drug delivery carriers, exosomes have demonstrated several advantages in sustained drug delivery for PM-CNV, which can encapsulate a variety of therapeutic molecules while maintaining stability, making them an attractive platform for sustained drug release. For example, engineered exosomes co-loaded with anti-VEGF and anti-fibrotic agents have shown prolonged suppression of CNV activity and reduced subretinal fibrosis in preclinical models.

Risks and challenges in exosome-based therapies

Exosomes, owing to their promising therapeutic potential in treating ocular diseases, face several challenges that must be overcome for their clinical application. These challenges primarily relate to safety concerns, preclinical research hurdles, and stability/storage issues, which must be carefully addressed to ensure the safe and effective translation of exosome-based therapies from research settings to clinical practice.

Safety issues

While exosomes are naturally derived from biological processes, they carry bioactive molecules (proteins, nucleic acids, lipids) that could potentially trigger immune responses. The immunogenicity of exosomes depends on their source and composition. For example, exosomes derived from human induced pluripotent stem cells (hiPSCs) have demonstrated therapeutic potential but also raised concerns regarding their immunogenicity [271]. In ocular therapy, the prolonged exposure of exosomes to ocular tissues could lead to adverse immune

responses, highlighting the need for extensive safety studies to assess long-term immunogenic effects in sensitive organs like the eye [272].

Another significant safety issue is the risk of contamination during exosome isolation and purification. Exosome isolation, often carried out through ultracentrifugation, can result in the co-isolation of unwanted contaminants such as cellular debris, proteins, or other extracellular vesicles. These contaminants can interfere with the therapeutic efficacy of exosome-based treatments and introduce unpredictable biological responses [165]. This contamination can also affect the purity of exosome preparations, leading to variability in clinical outcomes. Therefore, achieving standardized isolation protocols is crucial for ensuring high-quality exosome preparations for clinical use [273].

Ensuring efficacy and safety in clinical trials requires the development of rigorous trial designs. Comprehensive protocols for exosome isolation, characterization, and administration must be established to ensure consistency and reproducibility. In vivo monitoring of exosome behavior, such as biodistribution, targeting efficiency, and safety profiling, is essential but challenging due to their small size and the complexity of the biological environment [274, 275]. This complexity further hinders the smooth transition of exosome therapies into clinical settings. Exosome-based therapies face stringent regulatory requirements, as they are classified as biologics. Regulatory agencies, such as the FDA and EMA, must establish clear guidelines for the manufacturing, quality control, and safety of exosome preparations. Navigating these regulatory challenges remains a significant barrier to widespread clinical use [276, 277].

Exosome mass production and storage problems

The transition of exosome-based therapies from the laboratory to clinical applications involves significant challenges. A major hurdle is large-scale production, as exosomes exhibit heterogeneity in their size, composition, and functionality, which complicates the establishment of standardized production protocols. Variability in exosome preparations complicates quality control and poses a barrier to achieving consistent therapeutic results [278].

Exosome stability is a significant challenge affecting the therapeutic efficacy of these treatments. Exosomes are highly sensitive to changes in environmental conditions, such as temperature, pH, and oxidative stress, which can degrade their cargo and compromise their functional integrity. Developing strategies to enhance exosome stability during both storage and administration is crucial for their successful clinical application [279]. Bioengineering approaches, such as modifying

exosome membranes or incorporating stabilizing agents, are being explored to enhance exosome stability. These modifications can help protect exosomes from degradation, allowing them to maintain their therapeutic potential during storage and after administration [280]. Furthermore, developing optimal storage conditions to prevent exosome aggregation and preserve their bioactivity will be critical for ensuring consistent therapeutic outcomes [281].

Challenges in preclinical and clinical translation

Anatomical barriers

A significant challenge in ocular drug delivery, particularly concerning exosome-based therapies, stems from the eye's distinct anatomical and physiological barriers. Foremost among these is the blood-retinal barrier (BRB), characterized by tightly connected endothelial cells within the retinal capillaries [282, 283]. This barrier significantly restricts the penetration of therapeutic agents, thereby necessitating that exosomes and other nanoparticle-based carriers possess an optimally small size, generally less than 150 nm, along with suitable surface characteristics to facilitate effective retinal permeability [284]. Similarly, the corneal epithelium acts as a formidable barrier to topical ocular therapies, complicating efforts to achieve adequate bioavailability in the deeper corneal layers. Therefore, overcoming these ocular barriers requires the precise engineering of exosomes to enhance their targeting specificity, improve their cellular uptake efficiency, and optimize their capacity to circumvent physiological constraints.

Manufacturing and regulatory challenges

Manufacturing and Regulatory Challenges: Beyond biological limitations, significant challenges persist in the manufacturing, scalability, and standardization of exosome-based therapies. Exosomes derived from biological sources frequently display heterogeneity in terms of size, cargo content, and functionality, which complicates the attainment of consistent therapeutic quality. Scalable production techniques that preserve exosome integrity, potency, and cargo loading efficiency are currently inadequate. Furthermore, the development of standardized characterization and quality control methods is necessary to ensure batch-to-batch consistency and regulatory compliance. From a regulatory standpoint, definitive guidelines for the classification and approval processes of exosome-based therapeutics have yet to be fully established, leading to uncertainties in clinical translation and commercialization [285, 286].

Safety and immunogenicity

Safety considerations represent a significant challenge in the clinical translation of exosome-based therapies. Although exosomes are generally acknowledged for their inherent biocompatibility due to their endogenous origin, engineering modifications—such as surface functionalization, cargo loading, and hybridization with synthetic nanoparticles—may potentially alter their immunological profile [287–289]. Comprehensive preclinical evaluations are crucial to assess immunogenic responses, biodistribution, long-term stability, and potential off-target effects. Additionally, rigorous toxicological studies are required to confirm the safety and efficacy of engineered exosomes in appropriate ocular disease models prior to progression into clinical trials [137, 290, 291]. These anatomical, manufacturing, regulatory, and safety challenges collectively necessitate interdisciplinary collaboration among researchers, clinicians, industry stakeholders, and regulatory authorities to expedite the safe and effective translation of exosome-based therapies for ocular neovascular diseases [292].

Future perspectives in exosome-based therapies

Exosome-based therapies are on the verge of revolutionizing ocular disease treatments, offering exciting new possibilities in targeted drug delivery, gene therapy, and tissue regeneration. As research progresses, several advancements promise to overcome existing challenges and expand the scope of exosome applications. This section will explore the clinical translation of exosome therapies, highlight technological innovations in ophthalmology, and discuss innovative drug delivery strategies that leverage the unique properties of exosomes.

Clinical translation and innovative exosome-based drug delivery strategies

The transition of exosome-based therapies from pre-clinical research to clinical application constitutes a significant yet formidable challenge. Although preclinical studies have yielded promising results, several critical issues must be resolved to fully harness the therapeutic potential of exosomes [235]. Foremost among these challenges are the scalability of manufacturing processes, the pathways for regulatory approval, the validation of safety, and the assurance of consistent therapeutic efficacy across different production batches and patient populations. To address these challenges, researchers have investigated advanced strategies, including the engineering of exosomes for enhanced targeting, improved cellular uptake, and increased delivery of therapeutic payloads (refer to section “[Engineering exosomes for therapy](#)” for a comprehensive technical discussion) [235,

293, 294]. A particularly promising avenue is the development of hybrid exosome delivery systems, which integrate exosomes with nanoparticles, liposomes, or other nanocarriers. These hybrid systems exploit the inherent biocompatibility and targeting capabilities of exosomes, while simultaneously harnessing the stability, enhanced cargo-loading capacity, and extended circulation times afforded by synthetic nanoparticles [295–297]. Such hybrid constructs not only enhance targeting efficiency to ocular tissues but also mitigate rapid immune clearance, thereby facilitating the effective delivery of a wider range of therapeutic agents and substantially improving treatment outcomes in ocular neovascular diseases [298, 299].

Future advancements in exosome-based therapies should prioritize personalized approaches, customizing exosome vehicles to align with specific patient profiles and disease characteristics. The incorporation of precision medicine into the design of exosome-based therapies could result in treatments that are both more effective and safer, thus expediting the transition from research to clinical application. Realizing these advancements will necessitate interdisciplinary collaboration among biomedical researchers, nanotechnology experts, clinical practitioners, and regulatory authorities to refine technologies and elucidate regulatory frameworks.

Ultimately, surmounting these translational challenges will require sustained innovation, comprehensive clinical validation, and a well-defined regulatory framework to facilitate the safe, effective, and practical implementation of exosome-based therapies for ocular neovascular diseases.

Technological advancements in exosome bioengineering

Technological innovations in exosome bioengineering are unlocking new potential for ophthalmology. Research is not only focused on drug delivery but also on optic nerve protection and ocular gene therapy, which represent exciting avenues for treating vision loss and retinal degeneration. One promising development is the use of cationic-motif-modified exosomes for optic nerve protection. These engineered exosomes, capable of carrying mRNA or therapeutic agents, improve uptake by retinal cells and enhance transfection efficiency [161]. This approach could prove invaluable for treating conditions like glaucoma and optic neuropathy, where protecting the optic nerve is crucial. Furthermore, exosomes can be engineered to deliver therapeutic siRNAs or gene-editing tools, providing a novel approach to treating inherited retinal diseases or correcting genetic defects [297]. These technologies have the potential to transform the treatment of retinal degenerative diseases, allowing for targeted gene therapy.

Combination therapy strategies

Combining exosome-based therapies with existing treatments, such as anti-VEGF therapies and laser treatments, offers a promising approach to overcoming current therapeutic limitations. For example, integrating exosomes with anti-VEGF drugs like Ranibizumab or Aflibercept can enable sustained drug release, improved bioavailability, and enhanced targeting of VEGF receptors, thereby improving disease control and reducing the frequency of injections [300]. Moreover, nanoparticles can act as carriers for anti-inflammatory agents or antioxidants, addressing the inflammatory and oxidative stress components of ocular neovascular diseases. Exosomes delivering RNA molecules or siRNA can silence genes implicated in disease progression, further enhancing the efficacy of combination therapies [301, 302]. Additionally, combining laser therapy with exosome-based drug delivery may enhance therapeutic effects while minimizing tissue damage, offering a more targeted and less invasive treatment option for advanced ocular conditions [303–305].

Personalized medicine and precision therapies

Personalized medicine is set to transform ocular disease treatment by utilizing genetic, molecular, and biomarker profiling to tailor therapeutic strategies. Personalized exosome-based drug delivery systems can be customized based on a patient's specific genetic mutations, polymorphisms, and biomarkers, enhancing treatment efficacy and reducing side effects [159, 306]. For instance, targeting specific genetic variants in VEGF pathways can inform the design of exosomes that deliver drugs precisely to retinal tissues, addressing individual disease mechanisms [111, 307]. Moreover, optimizing dosing regimens based on patient responses can further personalize treatment, ensuring optimal therapeutic outcomes. The integration of precision medicine with exosome-based therapies underscores the potential for highly individualized care, improving management and prognosis for patients with ocular neovascular diseases.

Discussion

The landscape of ocular therapy is witnessing a paradigm shift with the advent of exosome-based treatments for neovascular diseases such as CoNV, DR, and AMD [308]. These diseases, which are leading causes of irreversible vision loss globally, have been traditionally managed with anti-VEGF agents. However, these treatments are not without limitations, including drug resistance, the burden of frequent intravitreal injections, and an insufficient addressal of underlying pathological mechanisms [309, 310]. Exosomes have emerged as a revolutionary therapeutic platform for ONDs, addressing the limitations of

existing anti-VEGF therapies [111, 311]. Their inherent ability to deliver drugs in a targeted manner make them versatile tools for influencing processes like angiogenesis, inflammation, and tissue repair [235, 312, 313]. Pre-clinical studies focusing on conditions such as corneal neovascularization, DR, and wAMD have shown that exosomes can effectively inhibit abnormal blood vessel growth through mechanisms like miRNA-mediated silencing of VEGF, specifically using miRNAs such as miR-126 and miR-146a [314–317]. Additionally, they have been found to restore the integrity of the blood-retinal barrier (BRB) and promote the polarization of macrophages towards anti-inflammatory states [318]. The potential of engineered exosomes is further amplified through surface modifications, such as the incorporation of integrin-targeting RGD peptides, and the development of hybrid systems that combine exosomes with liposome carriers [255].

Exosome-based nanotherapies offer significant potential for the treatment of ONDs, however, their clinical application faces substantial challenges, including issues related to ocular delivery (such as the restrictive nature of the blood-retinal barrier), potential immune responses, variability in manufacturing processes, and concerns regarding safety and regulatory compliance. These challenges are stimulating the development of innovative strategies aimed at overcoming these obstacles, thereby shifting the focus towards prospective solutions that facilitate the transition from laboratory research to clinical practice. A prominent strategy involves the bio-engineering of exosomes to enhance their therapeutic specificity and efficacy. Researchers are actively engaged in modifying the surfaces of exosomes with tissue-specific targeting ligands, such as peptides or aptamers, to improve the targeting and uptake by retinal cells. These engineered exosomes are better equipped to navigate ocular barriers and deliver therapeutic agents directly to neovascular lesions, thereby addressing delivery challenges. Additionally, hybrid nanoparticle-exosome platforms are being investigated to enhance stability and loading capacity by integrating the inherent advantages of exosomes with the customizable properties of synthetic carriers. These engineering advancements collectively aim to develop exosome formulations that are stable during circulation, specifically target ocular tissues, and safely induce therapeutic effects.

Another promising avenue is the integration of exosome therapies with existing ophthalmic treatments, leveraging synergy through combination approaches. Instead of considering exosome delivery as a standalone replacement, it can be utilized in conjunction with anti-VEGF biologics, steroids, or even laser therapy to improve overall outcomes. For instance, incorporating

current anti-VEGF drugs into exosomes or co-administering exosomes with anti-VEGF agents may facilitate more sustained intraocular drug release and enhanced bioavailability, thereby maintaining therapeutic intraocular levels for extended periods and reducing the frequency of injections. Similarly, exosomes can be enriched with anti-inflammatory agents or antioxidative molecules to simultaneously address inflammation and oxidative stress, which are two critical pathological factors often inadequately managed by anti-VEGF monotherapy. Concurrently, the exosome-mediated delivery of siRNAs or microRNAs capable of silencing pro-angiogenic genes, such as VEGF or its downstream effectors, could augment existing therapies by inhibiting neovascular signals at the molecular level. Additionally, the adjunctive use of exosomes with laser photocoagulation is proposed: exosomes, when applied in conjunction with laser therapy, may aid in tissue repair or protection post-laser treatment, thereby reducing collateral damage and facilitating more precise targeting of neovascular lesions. These combination strategies highlight an innovative paradigm in which exosomes enhance and extend the efficacy of established therapies rather than compete with them [319, 320].

Personalized medicine represents an emerging frontier in which exosome-based therapies could be specifically tailored to the unique profiles of individual patients. Ocular neovascular diseases exhibit considerable variability in their molecular foundations among different patients, indicating that a uniform treatment approach may be inadequate. By integrating genomic, transcriptomic, and proteomic data, future clinicians could potentially customize the exosomal cargo to target the specific disease drivers of each patient. For example, if a subset of patients is found to possess distinct polymorphisms or exhibit hyperactive signaling within a VEGF-related pathway, exosomes could be engineered to deliver inhibitors or RNA interference molecules that specifically target these aberrations. Additionally, dosing regimens could be modified based on a patient's pharmacogenomic markers or real-time therapeutic response, thereby maximizing efficacy while minimizing adverse effects. Adopting such precision therapy has the potential to significantly enhance treatment outcomes, as therapeutics would be precisely aligned with the individual pathological mechanisms of each patient, advancing ophthalmology towards a paradigm of highly individualized care.

To translate these forward-looking innovations into clinical practice, it is imperative to combine scientific creativity with the pragmatic development of standards and collaborative efforts. Ensuring scalable manufacturing and rigorous quality control of exosomes is crucial to maintain batch-to-batch consistency and achieve

regulatory approval. Concurrently, comprehensive safety evaluations must persist, focusing on long-term effects and potential toxicity or immune reactions in ocular tissues. Establishing standardized protocols for the isolation, characterization, and storage of exosomes will be essential for conducting multi-center trials and facilitating eventual commercialization. Regulatory agencies are in the process of drafting guidelines for biologics such as exosomes, and ongoing dialogue among scientists, clinicians, and regulators will facilitate the progression towards first-in-human trials in ophthalmology. Notably, many challenges associated with exosome therapy, such as traversing the blood-ocular barriers and achieving durable therapeutic effects—are being addressed through engineered solutions and combination approaches as previously outlined. Each incremental success in overcoming these obstacles enhances confidence in the safe integration of exosome-based treatments.

In conclusion, the prospects for exosome therapy in ophthalmology appear highly promising. Through the integration of engineering advancements, synergistic treatment regimens, and personalized design, exosome-based nanomedicine is well-positioned to overcome existing challenges and significantly enhance the management of neovascular eye diseases. These approaches have the potential to transform ocular therapeutics, providing safer, more effective, and patient-specific solutions that surpass the capabilities of traditional modalities. Realizing this vision will necessitate interdisciplinary innovation and meticulous translation, yet the current momentum in this field indicates that exosome therapeutics may soon become a transformative component of next-generation ophthalmic care.

Author contributions

J. G and F. M wrote the manuscript. C. W and B. Z processed the pictures. J. C and J. H revised and supervised the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

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Consent for publication

All authors have consented to publication.

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The authors declare no competing interests.

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