• Review Article •

Exosomes in ocular graft-versus-host disease: an overview

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Abstract

● Ocular graft-versus-host disease (oGVHD) is an immune disease that occurs in the eye after allogeneic hematopoietic stem cell transplantation and seriously affects the vision and quality of life of patients. Current treatment modalities are inadequate, and new treatment options and targets are required to improve the therapeutic efficacy of oGVHD. In recent years, a large number of studies have shown that exosomes, as important mediators of cell-to-cell communication, have important research value in the pathogenesis, diagnosis, and treatment of oGVHD. This article reviews the research progress on exosomes from different sources of oGVHD.

● **KEYWORDS:** ocular graft-versus-host disease; exosomes; immunomodulation; dry eye; extracellular vesicles

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) represents one of the most effective and widely used therapeutic strategies for severe hematological disorders. However, following allogeneic hematopoietic stem cell transplantation (allo-HSCT), the incidence of graft-versus-host disease (GVHD) ranges from 30% to 70%[1-2], which elicits profound immune dysregulation and subsequent organ damage[3]. GVHD is classified into acute GVHD (aGVHD) and chronic GVHD (cGVHD) based on whether the time from the time of transplantation to the emergence of GVHD symptoms is more than 100d[4]. Both aGVHD and cGVHD can lead to ocular graft-versus-host disease (oGVHD), with an overall incidence of 40%–60%[5-6]. Specifically, the incidence of acute oGVHD is relatively low at 1.33%[7], whereas that of chronic oGVHD is substantially higher, reaching 57%[8]. Clinically, oGVHD is mainly characterized by dry eye[9-11], and additional manifestations include ocular pain, photophobia, foreign body sensation, and blurred vision[4-5]. Immunoablative chemotherapy and radiotherapy as pretreatments before allo-HSCT are considered the main risk factors for the development of oGVHD[12-13]. High-dose radiotherapy induces damage to host tissue cells, triggering the release of damage-associated molecular patterns (DAMPs) and "alarmins" [*e.g.*, heat shock proteins, interleukin-33 (IL-33)]. These released molecules then activate tissue-resident macrophages (MFs) and dendritic cells (DCs), prompting them to secrete large amounts of inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and interleukin-8 (IL-8), and upregulate the expression of major histocompatibility complex (MHC) molecules and co-stimulatory molecules [cluster of differentiation (CD) 80, CD86] on their cell membranes. These cytokines further activate host antigen-presenting cells, particularly DCs[13]. Once activated, DCs capture antigens from damaged cells, transport them to regional lymph nodes, and subsequently activate donor CD4+ T helper cells and CD8+ cytotoxic T lymphocytes (CTLs). Among these activated T cells, CD4+ type 1 T helper (Th1) cells [which secrete interferon-γ (IFN-γ)] and CD4+ type 17 T helper (Th17) cells [which secrete interleukin-17 (IL-17)] are recognized as key drivers of the pathogenesis of aGVHD[13], Specifically, interleukin-12 (IL-12) is critical for the development of Th1 cells, whereas IL-1β, IL-6, interleukin-23 (IL-23), and interleukin-17 (IL-17) collectively promote the generation of effector Th17 cells. In

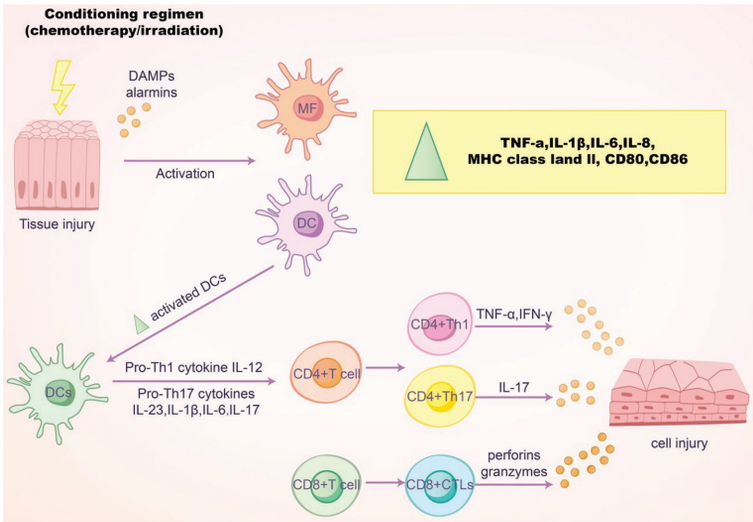

Figure 1 Long-term tissue damage mediated by Th1, Th17, and cytotoxic T cells progresses to chronic oGVHD After the activation of DAMPs, MFs and DCs are activated. These activated MFs and DCs then secrete large quantities of inflammatory cytokines, which in turn activate CD4+ T cells and CTLs. Ultimately, these immune cells mediate long-term tissue damage. Th1: Type 1 T helper cell; Th17: Type 17 T helper cell; oGVHD: Ocular graft-versus-host disease; DAMPs: Damage-associated molecular patterns; MFs: Macrophages; DCs: Dendritic cells; CTLs: Cytotoxic T lymphocytes; IL: Interleukin; TNF-α: Tumor necrosis factor-α; IFN-γ: Interferon-γ; MHC: Major histocompatibility complex; CD: Cluster of differentiation. Created with BioRender.com.

addition to CD4+ T helper cells, perforins and CTLs secrete granzyme B are also involved in tissue destruction during the progression of oGVHD. Long-term tissue destruction driven by Thl, Th17, and CTLs leads to the development of chronic oGVHD is depicted in Figure 1[13-14]. Currently, therapeutic measures such as artificial tears, autologous serum eye drops, glucocorticoids, lacrimal punctal plugs, and contact soft lenses are routinely used for oGVHD[15]. However, for atypical[16] or more advanced oGVHD cases, conventional treatment is less effective. Therefore, new treatment options and targets are urgently required to improve oGVHD treatment outcomes. Exosomes, a recent research hotspot, have shown great potential for oGVHD treatment. Summarizing the latest research progress on the origin, characteristics, mechanism, and route of medication of action of exosomes will help bring more choices and new research ideas for the treatment of oGVHD.

OVERVIEW OF EXOSOMES

Exosomes are small extracellular vesicles (EVs), about 40 to 100 nm in diameter. They carry RNAs and proteins and can influence recipient cells locally or at a distance. They are initially generated through direct budding from the plasma membrane[17]; alternatively, the primary biogenesis pathway involves the invagination of intracellular endosomal membranes to form multivesicular bodies (MVBs). Subsequently, exosomes are released into the extracellular space following the fusion of MVB outer membranes with the parental cell membrane. Exosomes are secreted by nearly all cell types in the human body, including mesenchymal stem cells (MSCs), immune cells, hepatocytes, endothelial cells, and fibroblasts[15]. By transferring bioactive molecules (*e.g.*, RNA and proteins) to target cells, exosomes can modulate the physiological functions of these cells either locally or remotely. They are thus involved in multiple pathophysiological processes, such as immune regulation[18] and inflammatory responses[19-20], in the organism. Against the background of oGVHD, exosomes are significantly different from other EVs. In terms of basic characteristics, exosomes, with a diameter of 30–150 nm, are generated *via* the endosomal pathway and released through exocytosis, stably express characteristic proteins such as CD63 and CD81 on their surface, and mainly contain specific microRNAs (miRNAs) and functional proteins, with stable biological activity and easy genetic engineering modification. In contrast, microvesicles, ranging from 100 to 1000 nm in diameter, bud directly from the cell membrane, lack specific surface markers, have heterogeneous contents and their activity is susceptible to environmental factors. Apoptotic bodies, with a diameter of 500–5000 nm, are fragment-like structures released after cellular apoptosis; their core function is to mediate the clearance of apoptotic substances, and they do not possess the ability to actively regulate cellular functions. The differences in biogenesis pathways, physicochemical properties and core functions among these three types of vesicles underpin their distinct roles in oGVHD. From a clinical perspective, exosomes, owing to their advantages of low immunogenicity, ability to penetrate the ocular surface barrier and precise delivery of regulatory molecules, can serve as non-invasive diagnostic biomarkers

and targeted therapeutic carriers for oGVHD. Existing studies have confirmed that exosomes can reflect the status of ocular surface immune imbalance and regulate local inflammatory responses. Although microvesicles can also carry inflammation-related factors, their high heterogeneity and unstable activity make them difficult to be developed as standardized diagnostic or therapeutic tools. Apoptotic bodies can only indirectly reflect the degree of ocular surface cell apoptosis in oGVHD, with no direct clinical application value. A consensus has been reached in most existing literature regarding the differences in basic physicochemical properties and functions among the three types of EVs. Endowed with favorable biocompatibility, stability, low toxicity, and molecular transfer capacity[21]. The study found that in multiple models, exosomes can cross biological barriers including the blood-brain barrier (BBB) and blood-ocular barrier (BOB), and interact with target cells *via* multiple pathways. Owing to their considerable potential in drug delivery and as biomarkers[22], these vesicles have gradually emerged as a research focus in the medical field.

EXOSOME SEPARATION

To date, six major classes of exosome isolation strategies have been reported, including ultracentrifugation (UC), ultrafiltration, immunocapture, charge-neutralization polymer-based precipitation, size-exclusion chromatography (SEC), and microfluidic (MF) techniques[23]. However, challenges such as the complexity of biofluids, significant overlap in biochemical properties between exosomes and other extracellular components (*e.g.*, lipoproteins, viruses, and other EVs), and inherent exosome heterogeneity result in limitations even for the gold-standard UC method, which is frequently contaminated by impurities such as proteins and lipoproteins[24]. To the extent that, for the time being, no single exosome isolation technique has been recognized as being suitable for all studies[25]. Therefore, it is currently believed that a combination of two or more techniques is required for effective exosome isolation[26-28]. In the field of oGVHD research, this combined multi-technique isolation strategy has emerged as a pivotal pathway to overcome the limitations of single methods and acquire exosomes with high purity and bioactivity. Its application rationale is closely tailored to the unique sample quality requirements inherent in oGVHD studies. From the perspective of practical research applications[29], the combination of UC and SEC, designated as UC-SEC, is the most widely adopted. Initially, differential UC is employed to preliminarily eliminate macroscopic impurities such as cell debris and large vesicles from biological fluids (patient serum, bone marrow supernatant). This is followed by coupling with SEC, which leverages the molecular sieve effect of gel particles to accurately separate exosomes from small-molecule contaminants like protein aggregates and lipoproteins. This process maximizes the preservation of the structural integrity and immunomodulatory activity of exosomes, making this combination particularly well-suited for investigating the mechanisms underlying exosome-mediated immune cell interactions in oGVHD. While being highly compatible with tear fluid and aqueous humor (AH)[30-31] specimens, its future application is constrained by shortcomings like prolonged operation time, incapability of isolating exosome subsets, and limited recovery efficiency for low-abundance samples. The combination of immunoaffinity capture (IAC) and UC, termed UC-IAC, focuses on the targeted isolation of exosomes with specific functions[32]. Taking MSC exosomes, which exert IAC in oGVHD, as an example, a crude exosome mixture is first obtained *via* UC. Subsequently, IAC is conducted using antibodies against their surface-specific markers (CD9, CD73), enabling efficient enrichment of target exosomes and minimizing interference from irrelevant subtypes. This provides highly specific samples for elucidating the role of specific exosomes in the pathogenesis or treatment of oGVHD. This method is explicitly suitable for the extraction of low-abundance specific exosomes from AH and vitreous humor, with advantages including strong targeting ability, excellent anti-interference performance, and preservation of complete biological functions, while its disadvantages are low throughput, high cost, and cumbersome operation procedures. Furthermore, the combination of MF technology and IAC, referred to as MF-IAC, demonstrates distinctive advantages[33]. MF chips facilitate efficient sample processing and precise manipulation through microchannel design. When integrated with the high specificity of IAC, they enable rapid isolation of exosomes from small-volume biological samples (*e.g.*, peripheral blood from oGVHD mouse models). This not only shortens the experimental cycle but also reduces sample loss, thereby offering a novel direction for the challenging isolation of small-volume clinical samples in oGVHD research. This technique is mainly applicable to the extraction of exosomes from micro-volume biological samples such as tear fluid and peripheral blood, but it still requires further development due to drawbacks including high equipment and consumable costs, strong reliance on specific biomarkers, and high operational thresholds. The exploration of these combined strategies is propelling the advancement of exosome isolation technology in oGVHD research toward greater efficiency, enhanced precision, and improved alignment with clinical demands (Table 1).

RESEARCH PROGRESS AND SPECIFIC MECHANISMS OF EXOSOMES IN THE TREATMENT OF oGVHD

Immunomodulatory Effects In recent years, the immunomodulatory properties of exosomes in ocular diseases

Table 1 Comparison of yield, purity, sample volume and typical pollutants of exosomes isolated by three methods from three raw materials

Isolation method	Raw material	Yield (μg/mL)	Purity	Sample volume (mL)	Typical pollutants
UC-SEC	Tear fluid	20–80	$>1\times10^9$	0.5–2	Soluble protein, lipoprotein complexes, cell debris
UC-SEC	Aqueous humor	5–30	$>5\times10^8$	0.2–1	Soluble albumin, immunoglobulin, cell-derived microvesicles
UC-SEC	Serum	80–200	$>2\times10^9$	1–5	High-density lipoprotein, low-density lipoprotein, platelet-derived microparticles
UC-IAC	Tear fluid	15–60	$>5\times10^9$	1–3	Tear mucin, trace antibody fragments
UC-IAC	Aqueous humor	3–15	$>1\times10^9$	0.5–2	Microalbumin
UC-IAC	Serum	50–150	$>8\times10^9$	2–6	High-density lipoprotein
MF-IAC	Tear fluid	10–45	$>3\times10^9$	0.8–2.5	Cellulose fragments
MF-IAC	Aqueous humor	2–10	$>6\times10^8$	0.3–1.5	Aqueous humor albumin
MF-IAC	Serum	40–120	$>5\times10^9$	1.5–5	Fibrous matrix fragments

UC: Ultracentrifugation; SEC: Size-exclusion chromatography; IAC: Immunoaffinity capture; MF: Microfluidic.

have been well-established. Kalluri and LeBleu *et al*[34] noted that exosomes possess the capacity to modulate both adaptive and innate immune responses, while the cell model established by Liao *et al*[35] demonstrated that exosomes secreted by donor-derived immune cells can carry immune-related molecules and suppress immune responses *via* multiple pathways; the cell model developed by Hee Kim *et al*[36] demonstrated that exosomes secreted by immature DCs contribute to the attenuation of inflammation and autoimmune responses *via* MHC class II (MHC-II)-dependent pathways. Knickelbein *et al*[37] showed that retinal pigment epithelium (RPE) cells can release EVs within the size range of exosomes under various conditions to suppress immune responses. Knickelbein *et al*[37] demonstrated that exosomes derived from unstimulated human retinal pigment epithelial cell line-19 (ARPE-19) only inhibited T cell proliferation and monocyte-mediated immune responses, with no effect on cell survival. However, when ARPE-19 cells were stimulated with inflammation-associated cytokines, small EVs secreted by these activated RPE cells not only suppressed T cell proliferation but also induced monocyte death, likely as a mechanism to mitigate detrimental immune responses.

Anti-Inflammatory Effects Inflammation is considered to be one of the main causative factors of oGVHD[13]. Dry eye represents the main clinical manifestation of oGVHD. The ocular surface stress response to environmental factors, infections, endogenous stimuli, or antigens is considered the initiating mechanism of dry eye; this response triggers the activation of acute inflammatory signaling pathways, including the upregulation of pro-inflammatory cytokines, matrix metalloproteinases (MMPs), and chemokines[38]. Exosomes are also reported to exhibit potent anti-inflammatory properties[39]. From the perspective of molecular signal regulation, exosomes can directly deliver anti-inflammatory molecules or interfere with pro-inflammatory pathways through non-coding RNAs. The cell model study conducted by Anggraeni *et al*[40] has shown that the miRNAs such as miR-146a encapsulated in them can target and silence key genes in pro-inflammatory pathways like nuclear factor (NF)-κB, inhibit the transcription and release of pro-inflammatory factors including TNF-α, IL-6, and IL-1β, and block the initiation of inflammatory cascade reactions at the source. A cell model study conducted by Kang *et al*[41] found that miR-27a-3p carried by neutrophil-derived exosomes can target and inhibit the expression of *Suclg1* gene in MFs, reduce the decomposition of the anti-inflammatory metabolite itaconate, and then down-regulate inflammatory signaling pathways such as NF-κB through itaconate, thereby playing a protective role in cytokine storms. In terms of immune cell function remodeling, exosomes can precisely regulate the differentiation and activity of various inflammation-related immune cells. For MFs, they can inhibit their polarization towards the pro-inflammatory M1 type, induce their transformation into the anti-inflammatory M2 type that secretes interleukin-10 (IL-10) and transforming growth factor (TGF)-β, and reduce the secretion of inflammatory factors and phagocytic damage. For example, umbilical cord mesenchymal stromal cell-derived exosomes can significantly decrease the expression of M1 markers CD86 and human leukocyte antigen (HLA)-DR on the surface of MFs, while increasing the levels of M2 markers CD206 and CD163. For T cells, exosomes can inhibit the differentiation of CD4+ T cells into pro-inflammatory Th1 and Th17 subtypes, promote the proliferation of anti-inflammatory Treg cells (CD25+FOXP3+), and down-regulate the expression of T cell activation markers CD25 and CD69, avoiding tissue damage caused by excessive infiltration of immune cells. This mechanism has been verified in studies on knee osteoarthritis involving preclinical models and clinical models[42]. In addition, studies have found[43] that exosomes can also inhibit the chemotaxis and activation of neutrophils, reduce the release of reactive oxygen species (ROS) and proteases, thereby alleviating oxidative stress and tissue damage at the inflammatory site. From the perspective of maintaining tissue microenvironment homeostasis, exosomes have a synergistic effect of anti-inflammation and repair. In inflammation-related diseases, exosomes not only inhibit inflammatory responses through the above-mentioned

mechanisms, but also carry growth factors [such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF)] and tissue repair-related proteins, promote the proliferation, migration and differentiation of damaged tissue cells, and accelerate tissue regeneration. A preclinical study by Duan *et al*[44] found that in inflammatory skin wounds, bone marrow mesenchymal stem cell-derived exosomes (BMSCs-Exos) can inhibit local inflammation while activating the repair pathways of skin epithelial cells and vascular endothelial cells. A preclinical study by Bui *et al*[45] discovered that in intestinal inflammation, intestinal epithelial cell-derived exosomes (IEC-Exos) can enhance the intestinal barrier function, reduce systemic inflammation caused by the entry of intestinal flora and toxins into the blood, and achieve multi-dimensional regulation of "anti-inflammation-repair-barrier reinforcement". An animal model study by Xu *et al*[46] found that in pulmonary inflammation, nebulized exosomes can carry anti-inflammatory components and repair factors to clear pathogenic microorganisms and impurities in the respiratory tract, while repairing damaged airway mucosa and alveoli, and regulating lung immune function. At present, the anti-inflammatory effect of exosomes has been verified in a variety of disease models. For instance, umbilical cord mesenchymal stromal cell-derived exosomes have shown significant anti-inflammatory effects and cartilage protective effects in patients with knee osteoarthritis, with good safety. Their therapeutic potential has also been confirmed in studies on diseases such as rheumatoid arthritis, ulcerative colitis, and acute lung injury. As indicated by prior research, the anti-inflammatory properties of exosomes have been validated across multiple disease models, excluding ocular-related conditions. In recent years, studies have found that they also play an important anti-inflammatory role in ocular diseases.

Exosomes can inhibit the release of inflammatory factors Exosomes can deliver a variety of anti-inflammatory components (*e.g.*, miRNAs) and proteins] to suppress the release of pro-inflammatory factors. The preclinical study conducted by Guo *et al*[47] demonstrated that exosomes derived from amniotic fluid mesenchymal stem cells (AF-MSC-Exos) contain the immunosuppressive TGF-β and hepatocyte growth factor (HGF). These factors can inhibit T cell proliferation and attenuate T cell-mediated inflammatory responses. The clinical sample study carried out by Li *et al*[48] demonstrated, using a suspension of exosomes derived from umbilical cord blood mesenchymal stem cells (UCB-MSC-Exos), that these exosomes can downregulate the transcription of IL-6, IL-1β, and TNF-α. By doing so, UCB-MSC-Exos participate in the regulation of both the immune system and the complement system, thereby achieving therapeutic effects in dry eye; Meanwhile, in their preclinical animal experiment, Li *et al*[48] established a rabbit model of autoimmune dry eye and demonstrated that exosomes derived from human umbilical cord mesenchymal stem cells (hUC-MSC-Exos) induce the differentiation of peripheral blood mononuclear cell-derived MFs (PBMC-derived MFs) toward an anti-inflammatory M2 phenotype. These exosomes also suppress the expression and secretion of TNF-α and IL-1β, while simultaneously upregulating the expression of TGF-β and IL-10. Collectively, these effects alleviate corneal epithelial damage in dry eye; the preclinical study conducted by Bai *et al*[49] demonstrated that MSC-Exos can alleviate corneal epithelial damage in dry eye. This effect is mediated by two key mechanisms: first, by inhibiting the expression or secretion of chemokines such as C-C motif chemokine ligand 21 (CCL21) and C-C motif chemokine ligand 2 (CCL2); second, by attenuating the pathogenic immune responses driven by Th1 and Th17 cells. Collectively, these actions also ameliorate experimental autoimmune uveitis (EAU).

Reducing inflammatory signaling pathway activation Exosomes can interfere with the activation of inflammatory signaling pathways. Previous studies have demonstrated that, during inflammatory responses, the activation of specific signaling pathways, such as the NOD-like receptor pyrin domain-containing protein 3 (NLRP3) inflammasome-IL-1β axis[50], results in the upregulated transcription and expression of a spectrum of pro-inflammatory factors. Specific components within exosomes can mitigate the initiation and progression of inflammatory responses. They protect ocular tissues from inflammatory damage by inhibiting the activation of the NLRP3-IL-1β signaling pathway. Additionally, a preclinical study by Wang *et al*[50] demonstrated that exosomes derived from adipose-derived stem cells (ADSC-Exos) can downregulate pro-inflammatory cytokine levels *via* inhibiting both the TLR4 signaling pathway and the NLRP3-IL-1β signaling pathway. Consequently, ADSC-Exos exert a notable therapeutic effect on benzalkonium chloride (BAC)-induced dry eye in a mouse model.

However, overall, oGVHD is essentially an immune imbalance where donor immune cells attack the ocular surface. The core of exosomes lies in reshaping immune homeostasis, inhibiting excessive T-cell activation, and correcting abnormal immunity at its source. Anti-inflammatory effects are merely downstream manifestations of this immune reprogramming. When key immunomodulatory molecules in exosomes (*e.g.*, miR-204) are blocked, both their anti-inflammatory and therapeutic effects are significantly diminished. Conversely, exosomes overexpressing miR-204 can restore therapeutic efficacy, demonstrating that immunomodulation is the core mechanism, with anti-inflammatory effects dependent on the initiation of immunomodulation. In the treatment of oGVHD,

the advantage of exosomes lies in addressing the root cause through immunomodulation and alleviating symptoms *via* anti-inflammatory mechanisms, making them more suitable than standalone anti-inflammatory drugs for long-term maintenance of ocular surface homeostasis and reducing the risks of recurrence and infection.

Promotes Ocular Tissue Repair

Promoting corneal epithelial cell repair The integrity of corneal epithelial cells is essential for sustaining normal ocular function, and exosomes can promote the proliferation and migration of these cells. Specifically, components such as growth factors within exosomes can activate proliferation and modulate relevant intracellular signaling pathways in corneal epithelial cells. Additionally, studies have demonstrated that ADSC-Exos enhance the viability of damaged corneal epithelial cells by inhibiting caspase-1-mediated apoptosis[37]. This not only accelerates the repair of corneal epithelial cells but also alleviates corneal epithelial damage associated with Ogvhd. Furthermore, studies have demonstrated that miR-10a-5p and miR-10b-5p derived from MSC-Exos can also prevent apoptosis of injured corneal epithelial cells[51]. Separately, another preclinical study showed that microRNA-191-5p (miR-191-5p) enhances the viability of corneal limbal stem cells, thereby alleviating corneal injury[52]; moreover, a clinical study demonstrated that eye drops formulated with exosomes derived from sheep amniotic fluid mesenchymal stem cells (sheep AF-MSC-Exos) exert beneficial effects on the repair and regeneration of damaged corneal epithelial cells[53]. In a separate *in vitro* study, scratch wound assays showed that exosomes derived from human corneal mesenchymal stem cells (hCor-MSC-Exos) can promote the proliferation and migration of corneal epithelial cells. These exosomes also modulate wound healing-related genes and signaling pathways, such as the extracellular signal-regulated kinase (ERK) and protein kinase B (AKT) pathways[54].

Regulation of lacrimal and meibomian glands repair Dry keratoconjunctivitis sicca (KCS) is the hallmark and most common manifestation of chronic oGVHD, which develops when the lacrimal and meibomian glands are subjected to activated T cells, MFs, and CD34+ fibroblasts that disrupt the tear layer[55-60]. A cell model study by Dietrich *et al*[61] showed that lacrimal gland-derived MSC-Exos contain proteins such as Lcn2, prosaposin, Rac1, and signal transducer and activator of transcription 1 (STAT1), which positively affect lacrimal epithelial cell survival and may induce lacrimal regeneration, which may ameliorate ocular damage caused by oGVHD; the article by Zhang *et al*[62] mentioned that exosomes stimulate the proliferation and migration of surrounding cells by carrying substances such as growth factors or proteins, which contribute to the repair of damaged tissues; and the article on animal models by Harrell *et al*[63] mentioned that the lacrimal gland rupture time significantly improved after 21d of topical application of AF-MSC-exos source eye drops, confirming the restoration of the meibomian glands function.

Regulation of conjunctival tissue repair In conjunctival tissue, exosomes can regulate the function of conjunctival fibroblasts. In oGVHD, conjunctival tissue develops lesions such as fibrosis[64]. Exosomes may prevent excessive fibrosis, promote the normal repair of conjunctival tissues, and improve the structure and function of the ocular surface by regulating the activities of conjunctival fibroblasts, such as proliferation and collagen synthesis. Rong *et al*[65] demonstrated that transplantation of exosomes derived from BMSCs-Exos slowed the progression of liver fibrosis and attenuated inflammation in a rat model. The underlying mechanism involves the activation of the Wnt/β-catenin pathway, which upregulates the expression of WNT1 inducible signaling pathway protein 1 (WISP1) and CyclinD1. This pathway activation subsequently inhibits the activation of hepatic stellate cells (HSCs) and downregulates the expression of type I collagen, ultimately mitigating hepatic fibrosis. The cell model study by Xiao *et al*[66] demonstrated that exosomes derived from rat BMSCs-Exos can deliver miR-340 to mesenchymal stromal cells of the uterine endometrium (ESC-MSCs). miR-340 inhibits the TGF-β-induced upregulation of collagen type I α1 (Col1α1) and α-smooth muscle actin (α-SMA) proteins. This not only reduces endometrial damage and collagen deposition but also prevents endometrial fibrosis. Separately, the preclinical study by Shojaati *et al*[67] demonstrated that exosomes derived from corneal stromal stem cells (CSSC-Exos) suppress fibrotic scar formation in mouse corneas following injury. Given exosomes' established role in inhibiting fibrosis across other tissues, combined with their multiple therapeutic mechanisms in oGVHD treatment, exosomes are now recognized to hold significant potential for preventing excessive conjunctival fibrosis.

Prevention and treatment of lacrimal gland fibrosis oGVHD is a major complication of allo-HSCT, and hyperfibrosis is often observed in lacrimal gland tissues[68], with clinical manifestations of ocular inflammation, fibrosis, and neuropathic pain. In the inflammatory microenvironment formed post-transplantation, cytokines such as TGF-β can induce epithelial-mesenchymal transition (EMT) in lacrimal gland epithelial cells[69]. In turn, these lacrimal gland epithelial cells gain mesenchymal cell characteristics and differentiate into myofibroblast-like cells. These differentiated cells secrete large amounts of collagen and fibronectin, ultimately contributing to lacrimal gland tissue fibrosis. This process is thought to be associated with the upregulated expression of the *SNAI1* gene and the downregulated expression of E-cadherin

protein[70-71]. Study demonstrated[72] that ADSCs-Exos inhibited radio salivary gland fibrosis by targeting Twist1 through miR-199a-3p and regulating the TGFβ1/Smad3 axis, and the results of the study are also informative for confirming the inhibition of lacrimal gland fibrosis by exosomes, due to the similarity between salivary and lacrimal glands in terms of physiological function and structure (Table 2).

In summary, exosomes, nanoscale EVs secreted by cells, play a critical role in the treatment of oGVHD by mediating immunomodulation, anti-inflammation, and ocular tissue repair is depicted in Figure 2.

POTENTIAL OF EXOSOMES AS DIAGNOSTIC MARKERS FOR oGVHD

In recent years, exosomes and other EVs have been increasingly recognized as ideal, minimally invasive biomarkers for disease detection, monitoring, and prognosis[73]. Specifically, patients with oGVHD exhibit significant differences in the quantity and composition of exosomes in tears and AH compared to healthy individuals or transplant recipients who do not develop oGVHD[22]. Detecting specific proteins or miRNAs in exosomes can provide a basis for the early diagnosis of oGVHD. For instance, Klingeborn *et al*[74] characterized and compared exosomes isolated from ARPE-19 cell culture supernatants with those from the AH of patients with age-related macular degeneration (AMD). Their results showed that exosomes in the AH of AMD patients contained elevated levels of specific proteins[75]. This finding provides evidence that AH-derived exosomal proteins can serve as biomarkers for AMD diagnosis. Diabetic retinopathy (DR) is the most common microvascular complication of diabetes. By comparing the serum exosomal miRNA expression profiles between patients with diabetes alone and those with DR, Yang *et al*[76] demonstrated that miR-3976 holds potential as a DR biomarker. These findings have implications for oGVHD biomarker research, highlighting that exosomes could act as noninvasive biomarkers for ocular diseases. In addition, with respect to patient prognosis, exosomal biomarkers can dynamically track treatment efficacy and disease recurrence risks. However, tear or aqueous exosome signatures in oGVHD remain to be defined and propose a minimal reporting set for future studies. With respect to exosomes as diagnostic biomarkers, it is imperative to define sample details and specify the isolation protocol, with strict adherence to the three mandatory criteria for exosome identification: morphological features *via* transmission electron microscopy, particle size range determined by nanoparticle tracking analysis, and the expression of characteristic biomarkers. In addition, a comprehensive and rigorous assessment of diagnostic performance should be carried out, encompassing sensitivity, specificity, positive predictive value and related metrics.

EXPLORATION OF DRUG DELIVERY ROUTES OF EXOSOMES

Exosomes as Drug Carriers Exosomes represent promising natural drug carriers due to their structural composition, diverse biological functions, and unique biological origin[77]. Leveraging the natural properties of exosomes, anti-inflammatory and immunomodulatory drugs can be loaded into them. Through genetic engineering or membrane modification, these drug-loaded exosomes can then be targeted specifically to ocular diseased tissues[78], thereby enhancing drug efficacy and reducing systemic side effects. For instance, loading glucocorticoids into exosomes enables more efficient drug action at ocular surface inflammation sites. Studies have shown[79] that using MSC-Exos as carriers, the endogenously carried miR-204 can target the IL-6/IL-6R/STAT3 signaling pathway on the ocular surface, reprogram pro-inflammatory M1 MFs into anti-inflammatory M2 type. This significantly alleviates ocular surface damage and inflammation in GVHD-associated dry eye disease, verifying the effectiveness of exosomes as endogenous miRNA carriers in treating ocular complications of oGVHD. Studies have also found[80] that MSCs were genetically engineered to secrete exosomes with programmed death-ligand 1 (PD-L1) protein on their surface (exosomes serving as exogenous functional protein carriers). These exosomes can target and bind to activated T cells, inhibit T cell proliferation and induce the differentiation of regulatory T cells (Treg). They can be applied in immune diseases represented by GVHD to reduce pathological damage.

Vitreous Injection of Exosomes Yu *et al*[81] found that vitreous injection of miR-21a-5p-rich MSC-Exos could protect retinal nerve cells from damage, modulate immune-inflammatory responses, and protect retinal ganglion cells. Tian *et al*[82] found that vitreous injection of exosomes combined with anti-VEGF was a new strategy for the treatment of fundus neovascularization.

Exosomes Prepared into Eye Drops for Topical Application Zhou *et al*[79] demonstrated that eye drops formulated with MSC-Exos could effectively increase tear secretion and promote the recovery of corneal epithelial thickness. Additionally, these exosome-based eye drops inhibited corneal epithelial cell apoptosis and excessive proliferation, reduced corneal macrophage infiltration, and downregulated the expression of inflammatory factors, ultimately alleviating the signs and symptoms of cGVHD-associated dry eye.

Analysis of Safety Limitations and Administration Considerations for Ocular Applications of Exosomes

Safety Limitations Safety restrictions on cell sources: The cell source type shall be clearly specified. The use of tumor cells or cells with undefined risks of gene editing is prohibited to avoid tumorigenic and immunogenic risks. Rigorous quality

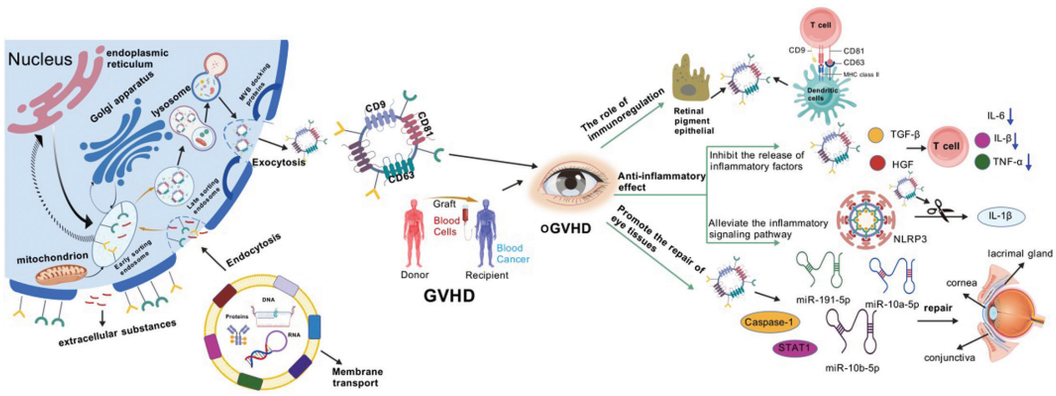

Figure 2 Mechanism of exosome therapy for oGVHD This image illustrates the roles of exosomes in the treatment of oGVHD, including immune regulation, anti-inflammation, and ocular tissue repair. oGVHD: Ocular graft-versus-host disease; MVB: Multivesicular body; GVHD: Graft-versus-host disease; TGF-β: Transforming growth factor-β; HGF: Hepatocyte growth factor; IL-6: Interleukin-6; IL-1β: Interleukin-1β; TNF-α: Tumor necrosis factor-α; NLRP3: NOD-like receptor pyrin domain-containing protein 3; STAT1: Signal transducer and activator of transcription 1; miR: microRNA; CD: Cluster of differentiation; MHC: Major histocompatibility complex. Created with BioRender.com.

Table 2 The origin, experimental models, key molecular cargo, and results of exosomes

Study type	Source	Experimental model	Key molecular cargo	Results
Basic experimental research	Rabbit's aqueous humor[35]	*In vitro* cell experiments	–	This aqueous humor-derived exosome has significant immunosuppressive function
Basic experimental research	Genetically modified DC cells that are specifically transferred and stably express the Fas ligand (FasL) gene[36]	Cellular models associated with ocular immunity	FasL protein	Exosomes are involved in reducing inflammation and autoimmune responses through MHC-II-dependent pathways
Basic experimental research	Exosome-derived RPE cells[37]	Cellular models associated with ocular immunity	TGF-β, PD-L1, miRNA	Exosomes can inhibit the activation and proliferation of T lymphocytes and reduce the secretion of pro-inflammatory cytokines
Basic experimental research	BMSCs, UC-MSCs, AD-MSCs, and other sources[40]	Viral infection-associated inflammatory cell models	miR-146a	MiRNAs in exosomes can inhibit the transcription and release of pro-inflammatory factors
Clinical observational study (cross-sectional study)	BMSCs, UC-MSCs, AD-MSCs, and other sources[48]	Ocular surface epithelial cell injury model, mouse dry eye model	HGF, TGF-β	AF-MSC-exos can inhibit T cell proliferation and attenuate T cell-mediated inflammation
Basic experimental research	BMSCs, UC-MSCs, AD-MSCs, and other sources[49]	Autoimmune rat model	miRNA, TGF-β, IL-10, PD-L1, HLA-G	MSC-exo can improve experimental autoimmune uveitis
Narrative review	BMSCs, UC-MSCs, AD-MSCs, and other sources[52]	*In vitro* cell models, *in vivo* animal models	miR-10a-5p, miR-10b-5p	MSC-exo-derived can prevent apoptosis of damaged corneal epithelial cells
Narrative review	BMSCs, UC-MSCs, AD-MSCs, and other sources[53]	*In vitro* cell models *vs in vivo* animal models	miR-191-5p	Exosomes carry miR-191-5p to improve corneal damage by promoting the cellular viability of limbal stem cells
Basic experimental research	Neutrophils[41]	Neutrophil and macrophage co-culture system, LPS-induced mouse cytokine storm model, and bacterial infection-induced hyperinflammation model	Itaconic acid synthesis related enzymes	MiR-27a-3p carried by neutrophil-derived exosomes can play a protective role in cytokine storms
Basic experimental research	Neutrophils[43]	*In vitro* cell model	CXCL8, CD66b, CD63, miR-146a, CD81, Alix	Exosomes can reduce oxidative stress and tissue damage at the site of inflammation
Narrative review	AD-MSCs[52]	BAC-induced mouse dry eye model, BAC-induced mouse corneal epithelial cell injury model	EGF, HGF, TGF-β, miR-146a, miR-21, SOD, CAT	ADSCs-exos may have a significant therapeutic effect on benzalkonium chloride-induced dry eye in mice
Clinical review	CSSCs[69]	Corneal fibroblast activation model, corneal alkali burn model, corneal mechanical injury model	miRNA	MSC-exo from CSSCs can prevent fibrotic scarring after corneal injury in mice by delivering miRNAs

DC: Dendritic cell; MHC: Major histocompatibility complex; RPE: Retinal pigment epithelium; TGF-β: Transforming growth factor-β; PD-L1: Programmed death-ligand 1; miRNA: microRNA; BMSC: Bone marrow mesenchymal stem cell; UC-MSC: Umbilical cord mesenchymal stem cell; AD-MSC: Adipose-derived mesenchymal stem cell; AF-MSC: Amniotic fluid mesenchymal stem cell; HGF: Hepatocyte growth factor; IL-10: Interleukin-10; HLA-G: Human leukocyte antigen-G; LPS: Lipopolysaccharide; CXCL8: C-X-C motif chemokine ligand 8; CD: Cluster of differentiation; Alix: ALG-2 interacting protein X; EGF: Epidermal growth factor; SOD: Superoxide dismutase; CAT: Catalase; BAC: Benzalkonium chloride; CSSC: Corneal stromal stem cell.

testing of cells is required: free of mycoplasma and viral contamination, without chromosomal karyotype abnormalities, and the number of *in vitro* passage generations must be controlled within a safe range. Safety restrictions on exosome preparation and quality control: Standardized isolation and purification methods shall be adopted to ensure exosome purity. The stability of exosome biological activity needs to be tested, and storage conditions shall be defined to prevent non-

specific effects caused by exosome rupture and content release. Strictly control the endotoxin content: The endotoxin level of exosome preparations for intraocular administration must comply with the national standards for ophthalmic preparations to avoid inducing endophthalmitis.

Administration considerations Selection and adaptation of administration routes: Ocular surface administration: suitable for corneal and conjunctival diseases. It is necessary to optimize the ocular surface residence time of exosomes (*e.g.*, adding adhesives), while avoiding ocular surface irritation caused by preservatives. Intraocular administration: Suitable for retinal and vitreous diseases. Minimally invasive injection techniques should be adopted to reduce the risks of retinal hemorrhage and detachment; the injection volume must be strictly controlled (generally ≤0.1 mL for intravitreal injection). Local injection administration: Suitable for moderate intraocular inflammation or optic nerve diseases. It can prolong the action time of exosomes in the eye and reduce systemic exposure. Optimization of dosage and administration frequency: The minimum effective dose and maximum tolerated dose shall be determined through animal experiments. Combined with the metabolic half-life of exosomes in the eye, a reasonable administration frequency shall be formulated to avoid ocular tissue damage caused by frequent administration.

CLINICAL APPLICABILITY OF EXOSOMES

Exosomes exhibit remarkable clinical value in the diagnosis and treatment of oGVHD. The feasibility of their application has been preliminarily verified by basic research, yet clinical translation still encounters multiple barriers that are urgent to overcome.

In terms of clinical significance, oGVHD is a severe ocular complication following HSCT, and its early diagnosis is often delayed due to overlapping symptoms with ordinary dry eye. Endowed with the property of being “information carriers”, exosomes offer a novel avenue to resolve this dilemma. Exosomes derived from immune cells in ocular surface secretions or serum can be enriched with biomarkers closely linked to the pathological progression of oGVHD (*e.g.*, miR-146a, TNF-α, IL-6). These molecules can specifically reflect the degree of ocular surface immune imbalance and the status of epithelial damage, which not only enables early screening and differential diagnosis of the disease, but also allows dynamic monitoring of biomarker changes to evaluate treatment responses, thereby providing evidence for adjusting individualized therapeutic regimens. In the therapeutic field, the clinical significance of exosomes is reflected in their dual regulatory effects: on one hand, MSC-Exos can inhibit abnormally activated T cells and MFs in oGVHD and alleviate ocular surface inflammatory infiltration by delivering anti-inflammatory factors and functional proteins; on the other hand, the repair-related miRNAs they carry can promote corneal epithelial cell proliferation and stromal remodeling, ameliorate clinical symptoms such as ocular xerosis and foreign body sensation, and provide an alternative therapeutic strategy for patients with poor responses to traditional immunosuppressants.

From the perspective of feasibility, the inherent biological properties of exosomes lay a solid foundation for their ocular application. First, compared with cell therapy, exosomes are smaller in size (30–150 nm), allowing them to penetrate the corneal epithelial barrier. They also have extremely low immunogenicity, which rarely triggers graft-versus-host reaction or allergic response, thus matching the particularity of ocular tissues. Second, exosomes can precisely act on lesion sites through local administration routes such as topical instillation and subconjunctival injection, avoiding systemic adverse effects caused by systemic medication and improving treatment safety. Third, modern separation and purification technologies have enabled efficient enrichment of exosomes, and genetic engineering modification can enhance their targeting ability to damaged ocular surface tissues, further improving diagnostic sensitivity and therapeutic efficacy. In addition, existing basic research has confirmed that MSC-Exos can significantly reduce ocular surface inflammation scores and improve corneal epithelial integrity in oGVHD animal models, providing critical preclinical evidence for clinical translation.

Nevertheless, the translation of exosomes from basic research to clinical application in oGVHD still needs to surmount multiple translational barriers. First, the standardization issue is particularly prominent: significant heterogeneity exists in exosome sources and separation methods, leading to substantial variations in particle size distribution, biomarker expression and biological activity. At present, there is no unified quality control standard, making it difficult to ensure the stability and repeatability of clinical application. Second, evidence for safety and efficacy is insufficient: although basic research has demonstrated favorable safety of exosomes, the particularity of ocular tissues results in the lack of large-sample and long-term follow-up clinical data to support the long-term retention efficiency, potential off-target effects and long-term safety of exosomes in humans. Meanwhile, key clinical parameters such as the optimal dosage, administration frequency and treatment window of exosomes for oGVHD remain undefined, which restricts the precise exertion of their therapeutic effects. Third, technical and cost bottlenecks are distinct: large-scale production of clinical-grade exosomes needs to address the problems of low yield and high cost, and existing separation technologies cannot meet the demands of large-scale clinical application. Moreover, as biological agents, exosomes pose

additional challenges for clinical application due to strict requirements for storage conditions, transportation stability and shelf life. Finally, the regulatory system is incomplete: the clinical classification of exosome-based products has not been clearly defined, and there is a lack of targeted guidance on relevant approval procedures and ethical norms, which also brings policy-related barriers to their clinical translation.

In the future, multi-center collaborations are required to establish a unified quality evaluation system for exosomes, conduct large-sample and long-term follow-up clinical trials to clarify their efficacy and safety, and improve relevant regulatory policies. Only in this way can we promote the clinical translation and application of exosomes in the diagnosis and treatment of oGVHD.

CHALLENGES AND PROSPECTS

While exosomes have shown promise in oGVHD research, several challenges remain. First, methods for exosome isolation, purification, and identification require further standardization to ensure the accuracy and comparability of study results. Second, the complex composition of exosomes increases the difficulty of mechanistic investigations. As a natural nanocarrier, it has become a hotspot in the field of therapy due to advantages such as low immunogenicity and high targeting ability. However, in clinical translation, two major safety issues, off-target immune effects and pro-tumor signals, require focused attention. The complexity of exosome sources and contents makes them prone to off-target immune effects. When exosomes are derived from allogeneic cells (*e.g.*, allogeneic MSCs), the MHC molecules carried on their surface may be recognized as "foreign substances" by the host immune system, activating natural killer (NK) cells or T cells and triggering rejection reactions[83]. Additionally, the cargo carried by exosomes may also induce immune abnormalities. A cell experiment by Kodidela *et al*[84] demonstrated that pro-inflammatory factors (such as IL-β) naturally contained in some exosomes can act on astrocytes and neurons, increasing their IL-1β levels and thereby causing neuroinflammation. A cell study by Lehrich *et al*[85] showed that non-EVs fetal bovine serum introduced due to preparation process contamination during drug loading may non-specifically activate immune cells, leading to systemic or local immune disorders and adverse reactions such as fever and tissue edema. The "bidirectionality" of exosomes causes the biomolecules they carry to potentially activate pro-tumor pathways accidentally. They can promote tumor occurrence and development by delivering carcinogenic cargo. For example, if exosomes derived from tumor cells are mixed into therapeutic exosomes (*e.g.*, cell contamination during preparation), the oncogenic miRNAs they carry (such as miR-335-5p and miR-21) may induce malignant transformation of normal cells or accelerate the proliferation and metastasis of existing micro-tumor foci[86]. A preclinical study by Long *et al*[87] showed that exosomes derived from colorectal cancer (CRC) cells regulate VEGFA through the JAK/STAT3 pathway in endothelial cells, promoting CRC tumor angiogenesis. Furthermore, the immunosuppressive properties of exosomes may be exploited by tumors. Therapeutic exosomes (*e.g.*, MSC-Exo), in order to exert anti-inflammatory and immune-regulating effects, often carry immunosuppressive molecules such as PD-L1 and TGF-β. While these molecules inhibit abnormal immune responses, they may also weaken the body's immune surveillance against tumor cells, leading to tumor immune escape. This risk is particularly noteworthy for individuals with potential tumor risks among the treated population. A study by Poggio *et al*[88], through experiments such as constructing a mouse pancreatic cancer model resistant to PD-1 inhibitors, proved that exosomal PD-L1 can inhibit the activity of T cells in draining lymph nodes, leading to tumor immune escape[89]. Even if PD-L1 on the surface of tumor cells is blocked, exosomal PD-L1 can still enable tumor cells to evade immune system recognition and killing. In the future, it is necessary to conduct more in-depth research on the mechanism of exosomes in oGVHD, strictly screen exosome donor cells, optimize loading processes (such as precise targeted modification and impurity removal), enhance the specificity of cargo delivery, reduce the probability of off-target effects, and develop new exosome-based diagnostic and therapeutic technologies, so as to provide more effective means for the clinical management of oGVHD.

Exosomes can be isolated from tear fluid, AH, and vitreous humor using various methods, owing to their relative stability[90-94]. Given that they contain tissue-, cell-, or disease-specific proteins and nucleic acids, their application in ocular disease diagnosis holds considerable promise. In terms of disease treatment, for oGVHD, exosome-mediated paracrine effects can overcome the limitations and risks of stem cell transplantation. Their cell-free nature reduces autoimmune responses and rejection[95-102], while genetic modification of exosomes enables the achievement of optimal therapeutic affinity. Regarding drug delivery, exosomes act as natural nanoscale drug carriers. Similar to liposomes, they can shuttle both lipophilic and hydrophilic cargo[101,103-109]. Moreover, they can cross the BBB and deliver encapsulated molecules to target cell membranes. Compared with synthetic nanomaterials, exosomes offer significant advantages in targeting ability, safety, and pharmacokinetics, including reduced drug clearance[110-112]. Exosomes show promise for oGVHD. Clinical translation remains challenging because of production standardization, ocular delivery barriers, and limited long-term safety data.

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