The Potential Role of Exosomes in Ocular Surface and Lacrimal Gland Regeneration

Article *in* Current Eye Research · November 2024

DOI: 10.1080/02713683.2024.2424265

4 authors:

Jilu Jaffet
L V Prasad Eye Institute
13 PUBLICATIONS **92** CITATIONS
SEE PROFILE

Vivek Singh
L V Prasad Eye Institute
111 PUBLICATIONS **2,585** CITATIONS
SEE PROFILE

Stefan Schrader
Carl von Ossietzky University of Oldenburg
100 PUBLICATIONS **2,403** CITATIONS
SEE PROFILE

Sonja Mertsch
Carl von Ossietzky University of Oldenburg
53 PUBLICATIONS **942** CITATIONS
SEE PROFILE

Current Eye Research

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/icey20

The Potential Role of Exosomes in Ocular Surface and Lacrimal Gland Regeneration

Jilu Jaffet, Vivek Singh, Stefan Schrader & Sonja Mertsch

To cite this article: Jilu Jaffet, Vivek Singh, Stefan Schrader & Sonja Mertsch (07 Nov 2024): The Potential Role of Exosomes in Ocular Surface and Lacrimal Gland Regeneration, Current Eye Research, DOI: 10.1080/02713683.2024.2424265

To link to this article: https://doi.org/10.1080/02713683.2024.2424265

Published online: 07 Nov 2024.

CURRENT EYE RESEARCH
https://doi.org/10.1080/02713683.2024.2424265

The Potential Role of Exosomes in Ocular Surface and Lacrimal Gland Regeneration

Jilu Jaffet[a,b,c], Vivek Singh[b], Stefan Schrader[a] and Sonja Mertsch[a]

[a]Laboratory of Experimental Ophthalmology, Department of Ophthalmology, Pius-Hospital, Carl von Ossietzky University, Oldenburg, Germany; [b]LV Prasad Eye Institute, Centre for Ocular Regeneration, Hyderabad, Telangana, India; [c]Manipal Academy of Higher Education (MAHE), Manipal, Karnataka, India

ABSTRACT

Purpose: Dry eye disease (DED), a multifactorial disease of the lacrimal system, manifests itself in patients with various symptoms such as itching, inflammation, discomfort and visual impairment. In its most severe forms, it results in the breakdown of the vital tissues of lacrimal functional unit and carries the risk of vision loss. Despite the frequency of occurrence of the disease, there are no effective curative treatment options available to date. Treatment using stem cells and its secreted factors could be a promising approach in the regeneration of damaged tissues of ocular surface. The treatment using secreted factors as well as extracellular vesicles has been demonstrated beneficial effects in various ocular surface diseases. This review provides insights on the usage of stem cell derived exosomes as a promising therapy against LG dysfunction induced ADDE for ocular surface repair.

Methods: In order to gain an overview of the existing research in this field, literature search was carried out using the PubMed, Medline, Scopus and Web of Science databases. This review is based on 164 publications until June 2024 and the literature search was carried out using the key words "exosomes", "lacrimal gland regeneration", "exosomes in lacrimal dysfunction".

Results: The literature and studies till date suggest that exosomes and other secreted factors from stem cells have demonstrated beneficial effects on damaged ocular tissues in various ocular surface diseases. Exosomal cargo plays a crucial role in regenerating tissues by promoting homeostasis in the lacrimal system, which is often compromised in severe cases of dry eye disease. Exosome therapy shows promise as a regenerative therapy, potentially addressing the lack of effective curative treatments available for patients with dry eye disease.

Conclusion: Stem cell-derived exosomes represent a promising, innovative approach as a new treatment option for ADDE. By targeting lacrimal gland dysfunction and enhancing ocular surface repair, exosome therapy offers potential for significant advances in dry eye disease management. Future research is needed to refine the application of this therapy, optimize delivery methods, and fully understand its long-term efficacy in restoring ocular health.

ARTICLE HISTORY
Received 16 April 2024
Accepted 29 October 2024

KEYWORDS
Exosomes; lacrimal gland; ocular surface; regenerative medicine; mesenchymal stem cells

The lacrimal functional unit

The Dry Eye Disease (DED), also known as keratoconjunctivitis sicca, and its effects on the ocular integrity and vision affects millions of people worldwide and is one of the most prevalent eye conditions.[1] Currently, there are different factors contributing to DED, which are a loss of homeostasis, hyperosmolarity and instability of the tear film, damage/dysfunction of the corneal nerves, defects in the integrity and function of the corneal epithelium, an inflammation of the ocular surface. The loss of tear film homeostasis is based on dysfunction of the lacrimal functional unit (LFU).[2,3] The LFU is a complex balanced system essential for maintaining the health, integrity, and functionality of the eye. The LFU comprises of various structures, including the lacrimal glands (LG), ocular surface (cornea, conjunctiva), meibomian as well as accessory glands, the eye lids and the sensory and motor nerves. This system serves critical functions in ensuring optical clarity, protection against pathogens, and facilitating visual acuity by the production, distribution, and drainage of the tear film.[4,5] Dysfunction within this unit mainly affects the tear film homeostasis and can lead to DED, which is a multifactorial disease characterized by symptoms like itching, burning, inflammation, and vision disturbances.[6] In addition to the impact of DED on patients' quality of life, the disease also has a significant socio-economic impact, mainly through the overall cost to healthcare systems worldwide.[7]

Dry eye disease

The prevalence of DED varies between 5–50%, depending on diagnostic criteria, ethnicity and gender. The overall worldwide prevalence was estimated at approximately 12%.[8]

CONTACT Sonja Mertsch Sonja.Mertsch@uni-oldenburg.de Laboratory of Experimental Ophthalmology, Department of Ophthalmology, Pius-Hospital, Carl von Ossietzky University Oldenburg, Germany

The prevalence of DED in the Asian population is significantly higher compared to Caucasian population, which is presumable not only based on different genetic backgrounds of the populations, but also on different climatic and environmental conditions.[1] Additionally, based on the hormonal effect on ocular surface, the lacrimal and the meibomian gland, women have a higher risk to develop DED than men. This is observed predominantly during pregnancy and menopause due to hormonal disbalance, which is shown in the significantly higher 10-year age-adjusted incidence of women (25%) compared to men (17.3%).[9] Furthermore, the prevalence of DED increases linearly with age.[10]

The DED is a multifactorial disease with a high number of different causes, including environmental factors, systemic diseases, ocular diseases as well as iatrogenic causes like various medications and surgeries. To date, several systemic medications are known to cause DED, especially immune checkpoint inhibitors used in cancer therapy as well as other novel chemotherapeutic agents.[11] Other known topical medications are glaucoma eye drops as well as some benzalkonium chloride (*BAC*) containing eye drops.[12,13] Several skin diseases around or on the eye lids, for example rosacea or eczema can be a cause for DED as well as ocular allergies.[14,15] Neurogenic dry eye can be caused due to damages of the corneal nerves due to refractive surgery, damages of the trigeminal ganglia, viral infections (e.g. herpes virus) leading to reduced corneal sensation, reduced blink rate as well as epithelial damage and tear film instability.[16] Other causes of DED can be conjunctival scarring due to chemical and/or thermal burns, leading to lack of mucin due to the loss of goblet cells, and the immune inflammatory reaction caused by the keratoconjunctival epithelial damage, both resulting in an imbalance of the tear film.[17] Systemic disease, for example autoimmune disorders like Sjogren's Syndrome, Lupus or rheumatoid arthritis can lead to DED, as well as Graft-versus-host disease (GVHD).[5,18–20]

DED is widely classified into two main categories: the evaporative Dry Eye Disease (EDE), and the aqueous deficiency Dry Eye Disease (ADDE), however, numerous patients have a mixed form of these categories.[2]

In the following chapters the contributions of each layer of the tear film, underlying causes, and current treatment options for DED are discussed in detail with emphasis on ADDE:

Mucin layer of the tear film and the goblet cells

The innermost layer of the tear film mainly consists of membrane adherent/associated mucins (MAMs) and secreted mucins, which are secreted by the goblet cells of the conjunctiva. This layer interacts directly with the corneal epithelial cells and is a defence barrier of the ocular surface.[21] On one hand, this hydrogel-like layer is responsible for removing dust, bacteria and other environmental factors. On the other hand, this layer also acts as one of the sources of nutrients for corneal epithelial cells. Additionally, mucins are responsible for reduction of surface tension of the tear film and helps distribute it evenly on the ocular surface.[21] MUC5AC and MUC19 are the two main mucins secreted by the goblet cells. The meibomian and lacrimal gland also produce mucins, mainly MUC1, MUC5AC, MUC5B, MUC7, and MUC19.[22,23] Even though the mucin layer plays an important role in DED, there is no subtype of DED where only this layer is affected. The loss of goblet cells is a common feature of both forms of DED and the decrease in goblet cell density is directly proportional to the severity of the disease. It was also shown that the loss of goblet cells is accompanied by a decrease in the MUC5A, MUC1, MUC2, MUC4 and MUC16 expression.[24,25] Changes in the expression levels of certain MAMs can lead to a disruption in the glycocalyx and therefore to direct damages in the conjunctival and corneal cells. There are also studies showing a decrease expression of MUC5AC and MUC19 in patients with Sjögren's syndrome.[26,27] Taken together, both subtypes of DED enter a vicious circle of an inflammatory reaction triggered by the changes in homeostasis of the tear film. This inflammation can lead to a reduced expression of glycocalyx mucins, resulting in damages of the ocular surface epithelial cells and goblet cells, which in turn leads to a reduced expression of gel-forming mucins and a further damage of the epithelial cells. This can lead to irregularities in tear film distribution, causing dry spots on the cornea, subsequently leading to epithelial cell damage and therefore contributing to the vicious circle when left untreated.[28,29]

Lipid layer of the tear film and the meibomian glands

Embedded within the tarsal plates of the eyelids, the meibomian glands are essential for maintaining the integrity of the tear film by producing lipid layer of the tear film. These sebaceous glands secrete the meibum, which is composed of polar and nonpolar lipids, including phospholipids, wax esters and cholesterol, and form the outermost layer of the tear film.[28] This lipid layer is approximately 40–100nm thick and serves as an evaporative barrier and maintains the tear film stability.[30] It also serves to reduce surface tension of the tear film to enable its evenly distribution over the ocular surface. Deficiencies or alterations in lipid composition can lead to evaporative dry eye disease, the most common form of DED.[31] The meibomian gland dysfunction (MGD), which is characterized by a reduction in meibum secretion or a change in its composition, is the leading cause for the development of the EDE.[32–34] MGD is further described with terminal duct obstruction with or without quantitative as well as qualitative changes in the lipid secretion.[28] The prevalence of EDE/MGD based on clinical signs ranges from 38–68% in people over the age of 40.[1] One cause of MGD is the progressive keratinization of meibomian gland orifices and the loss of progenitor cells, leading to an atrophy of the glands over time and therefore to an alteration in the composition of the meibum.[34,35] These changes in the tear fluid composition can lead to an increase in tear evaporation, hyperosmolarity as well as inflammatory reactions and finally can result in ocular surface damages.[36,37] The primary treatment aim for EDE is to improve the flow of meibum, together with restoring the function of the meibomian glands leading to a normal lipid layer. The EDE is classified into three different stages depending on secretion quality, severity of symptoms and corneal staining.[34,38] Current treatment options for EDE include different

interventions such as eyelid hygiene, warming eye compresses, eyelid massage, ocular lubricants, and the use of topical as well as systemic antibiotics or corticosteroids. Even though these approaches have shown to be an effective symptomatic treatment option, they cannot cure the disease completely, especially in advanced forms of the EDE.[39] Clinical treatments include the use of thermal pulsation, intense pulsed light, manual expression and eyelid exfoliation treatment (microblepharoexfoliation (BlephEx)).[38,40] These clinical treatment options address the liquefaction of meibum, preventing tear evaporation and treating inflammation with antibiotics and/or corticosteroids, but they do not address the underlying cause of the disease. Despite the high number of different treatment options available, there is no curative treatment for EDE available so far.[31]

Aqueous layer of the tear film and the lacrimal gland

The middle aqueous layer forms the largest part of the tear film and is produced mainly by the lacrimal glands, located in the anterior, superotemporal orbit within the *Fossa glandulae lacrimalis*. A part of the aqueous secretions is from the accessory glands (glands of Krause and Wolfring) in the stroma of the conjunctival fornix as well as in the orbital border of the tarsal plate. This aqueous layer contains different electrolytes, water and various proteins, such as lactoferrin, lysozyme and immunoglobulins. These proteins contribute to the innate immune defence of the ocular surface, as for instance lysozyme hydrolyze the outer membrane of Gram-positive bacteria, and lactoferrin interfere with viral and bacterial growth by binding iron.[41,42] Furthermore, this layer is crucial for lubrication and nourishment of the ocular surface. This aqueous layer of the tear film is mainly produced by the acinar cells of the LG, which represents approximately 80% of the total cell number in the LG. The tear fluid is further processed by the ductal cells, which account for 10-12% of the total cell number.[43] Besides the acinar and duct cells, the lacrimal gland further consists of myoepithelial cells, and different stromal cells, for example fibroblasts, which secrete the extracellular matrix, and mast cells, which produce histamines and also matrix proteins and thereby providing structural and metabolic support to the tissue structure. Furthermore, Nestin-positive mesenchymal stromal/stem cells have also been identified in the lacrimal gland, which play an important role in the tissue regeneration of the gland.[44–48]

Aqueous Deficient Dry Eye, a subgroup of the DED can be developed due to multiple factors like damage of the LG tissue (caused by autoimmune dacryoadenitis, such as Sjögren's syndrome or GvHD), tumour entities in the surrounding tissue, lacrimal gland duct obstruction, a dysfunctional reflex block, age-related atrophy and fibrosis, or based on systemic drugs (the so called non-Sjögren dry eye), etc.[6,49] The ADDE occurs for approximate 1/3 of all the DED cases and results in a more severe symptoms in the patients than the more common form EDE.[1,18,50–53]

Current treatment options for ADDE, depending on the stage of the disease, mainly include artificial tears, nonsteroidal or corticosteroidal anti-inflammatory agents, immunosuppressive drugs, and punctual occlusion. These are only symptomatic treatment options and do not address the underlying cause of the disease.[2] In case of severe forms of the ADDE, these treatment options are often not sufficient to ensure a stabilization of the ocular surface homeostasis and therefore are not able to mitigate the symptoms. Hence, no curative treatment for the ADDE is available till today.[2]

As one of the main causes of ADDE is damage to functional LG tissue, a promising approach for curative treatment is to enhance the regenerative capacity of the remaining tissue *in situ*. This includes restoring the tissués function and restoring the tear film's quantity and quality. To address the regenerative capacity of the remaining tissue, there are two main lines of research: first, the use of various drugs/proteins/enzymes to enhance the activity of the remaining LG cells; and second, the implantation of stem cells capable of (a) recruiting cells, (b) enhancing the activity and growth of the remaining cells by secreting growth factors, and (c) interfering with the inflammatory response in the tissue.

Several studies showed an increase in mesenchymal stem cells (MSC) during lacrimal gland regeneration, pointing towards an important role of these cells in maintaining tissue homeostasis.[45,46,54] Thus, it seems a promising approach to use these cells to enhance tissue regeneration in the damaged LG. Additionally, MSCs are highly immunomodulatory as they promote the polarization of monocytes/macrophages toward an anti-inflammatory and immune-regulatory phenotype, and directly inhibit the differentiation into dendritic cells.[55,56] MSCs are nonhematopoietic, multipotent stem cells, which have the ability to differentiate into a variety of different cell types, such as adipocytes, osteocytes, chondrocytes and many more, depending on the conditions.[57–59] Whilst MSCs were first isolated from bone marrow, they can also be obtained from various other adult and fetal tissues, such as adipose tissue (AT), cord blood (CB), and the lacrimal gland.[44,60,61] Despite their potential for immunomodulation, they have important functions in tissue repairing. Once an injury occurs in a tissue, MSCs migrate to the site of the injury and then differentiate into the appropriate cell types and participate to the tissue repair. Furthermore, they also secrete a number of different factors, for example vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF-2), as well as exosomes, contributing to the tissue repair.

Preliminary studies in different animal models mimicking ADDE, not only showed an increase in Nestin-positive MSCs after inducing inflammation in the gland,[46] but also showed a significant increase in the tissue regeneration after transplanting LG derived MSCs into the damaged organ.[54,60,62] The use of MSCs in clinical regenerative medicine has also increased noticeably in the last decade, based on their low immunogenicity, as well as their promising tissue regenerative capacity and immunomodulation behavior. Currently, there are 11613 clinical MSC studies listed in the US National Library of Medicine by the NIH, including 42 studies using different kind of MSCs in ophthalmologic research, mainly regarding ocular surface reconstruction. MSCs are administered for regenerative treatments in diseases like myocardial infarction,[63] and muscular dystrophy.[64] They are also applied to reduce immune responses in diseases such as GvHD, arthritis and diabetes.[65,66] Currently, 177 completed clinical trials have shown that the use of autologous as well as

allogenic MSCs is effective, well tolerated and safe, which makes them promising candidates for lacrimal gland regeneration.[67]

Nevertheless, the use of cells for regenerative medicine harbors some risks, such as the potential for oncological complications. Additionally, the therapeutic effects of the MSCs are known to be based on their paracrine effects, rather than an engraftment of the transplanted cells into the damaged tissue. An alternative approach which offers similar benefits without the concerns associated with the transplantation of living cells is the use of exosomes or extracellular vesicles.[68]

Overview of exosomes, its origin and mode of action

Exosomes are nano-sized, single membrane-bound secretory vesicles of endosomal origin with an approximate diameter range of 30–200 nm.[69] Cells secrete vesicles of distinct sizes, the larger ones with mostly the same cellular composition are termed as microvesicles whereas the smaller ones with the specific cellular proteins, lipids, glycoconjugates and nucleic acids enriched within the cargo are termed as exosomes. Distinct vesicles secreted by the cells are denoted by an umbrella term "Extracellular vesicles (EVs)" and are classified based on their origin and function. The EVs secreted from the internal compartments of the cell and released out through the multivesicular bodies are termed as "exosomes", whereas the EVs secreted from the cell surface are known as "microvesicles or ectosomes".[70] Microvesicles are larger than exosomes, have a size range of ~100-1000 nm and are formed by direct budding from the plasma membrane. Largest type of EVs are apoptotic bodies that has a size range of 1–5 µm, generated during apoptosis by the disassembling cellular organelles.[72] Apoptotic bodies are secreted by the apoptotic cells whereas the exosomes are secreted by the actively dividing cells.[71] Exosomes are derived by the fusion of the intermediate endocytic compartment, multivesicular body with the plasma membrane. The intraluminal vesicles within the MVBs are released into the extracellular milieu and these are termed as exosomes.[73] They are well known for their role in intercellular communication, transfer of macromolecules as well as signals to the target cell and thus reprogram the recipient cells by regulation of cellular and physiological processes.[73]

Exosome biogenesis begins from the late endosomes, the inward budding of the endosomal membranes leads to the formation of a structure known as multivesicular body (MVB). MVBs are approximately 250–1000 nm large endosomal organelles characterized by numerous spherical or ellipsoidal vesicles enclosed within a single outer membrane.[74] The internal vesicles within the MVBs are termed as intraluminal vesicles (ILVs) and they are predominantly uniform in size, about 60-70 nm in diameter.[74] The MVBs or the matured endosomes can have three different fates; they can either fuse with lysosomes for degradation, or can deliver their contents for the development of lysosome related specialized organelles, else they can fuse with the plasma membrane of the cell to release the ILVs as "exosomes".[73] One among the other factors that determine the fate of MVBs is the localization of cholesterol with the protein, perfringolysin. The MVBs enriched with cholesterol are determined for exosome secretion whereas the ones with poor cholesterol composition are targeted for lysosomal degradation.[75] In addition, the presence of lysobisphosphatidic acid is unique to epidermal growth factor containing MVBs that are destined for lysosomal degradation.[76]

The development of ILVs can occur through activation of the endosomal sorting complex required for transport (ESCRT) machinery or through an ESCRT-independent pathway. The ESCRT protein complexes are the major contenders for the mechanism that drives MVB vesicle formation. ESCRT pathway recruits a set of cytosolic protein complexes ESCRT 0 to III, that coordinates the MVB biogenesis, vesicle formation and the protein cargo sorting.[77,78] The ESCRT 0 is involved in the initiation of the process by recognizing ubiquitinated cargo for the exosomes *via* its ubiquitin-binding subunits. The recruitment of ESCRT I and II complexes cause the cargo clustering through its ubiquitin-binding domains and leads to invagination of the membrane. The ESCRT II subunit activates the assembly of ESCRT III complex to promote vesicle maturation and neck constriction that can eventually lead to the vesicle scission. The last step in ILV biogenesis requires the activity of the ATPase Vps4 complex which dissociates ESCRT III and helps in recycling of the ESCRT machinery.[78]

The ESCRT-independent mechanism is predominantly observed in melanosomes and is mostly driven by lipids and the tetraspanins. One of the ESCRT independent pathways is through the action of lipids in cell membrane remodelling and in formation of exosomes. Various lipids such as neutral sphingomyelinase (nSMase), phospholipase D2 (PLD2), diacylglycerol kinase α (DGKα) are involved in the maturation of MVBs and the release of exosomes.[79–82] However, another ESCRT-independent mechanism is through the effect of tetraspanin proteins. Tetraspanins are a protein superfamily capable of organizing the tetraspanin enriched microdomains (TEMs) by forming clusters and interacting with multiple cytosolic and transmembrane signalling proteins.[83] One of the melanocyte specific glyco-protein Pmel17 interacts with CD63 to form a specialized domain containing pre-melanosomal membranes to enter ILVs. The action of the lipids and the Pmel17 promotes the domains to invaginate and bud from the membrane, leading to the formation of ILVs.[84] Final step in the exosome biogenesis is its release from the parent cell by various mechanisms like membrane fission, ATP-dependant contraction of actin and myosin filaments, and can also be released as response to stress-like stimuli.[85,86] The exosome secretion could also be modulated by diverse proteins like SNAP, SNARE and Rabs. Rab proteins are key regulators of exosomal transport whereas Rab5 and Rab7 are specifically involved in delivering the cargo components to the early endosomes.[87] Activation of the protein ARF6 promotes the vesicle budding from the cell plasma membrane and its release into the environment.[88] However, the exosomes interact with the recipient cell *via* the cell surface receptors and the cargo contents can be internalized through three distinct mechanisms (Figure 1); (i) fusion of the exosomes with the plasma membrane of the recipient

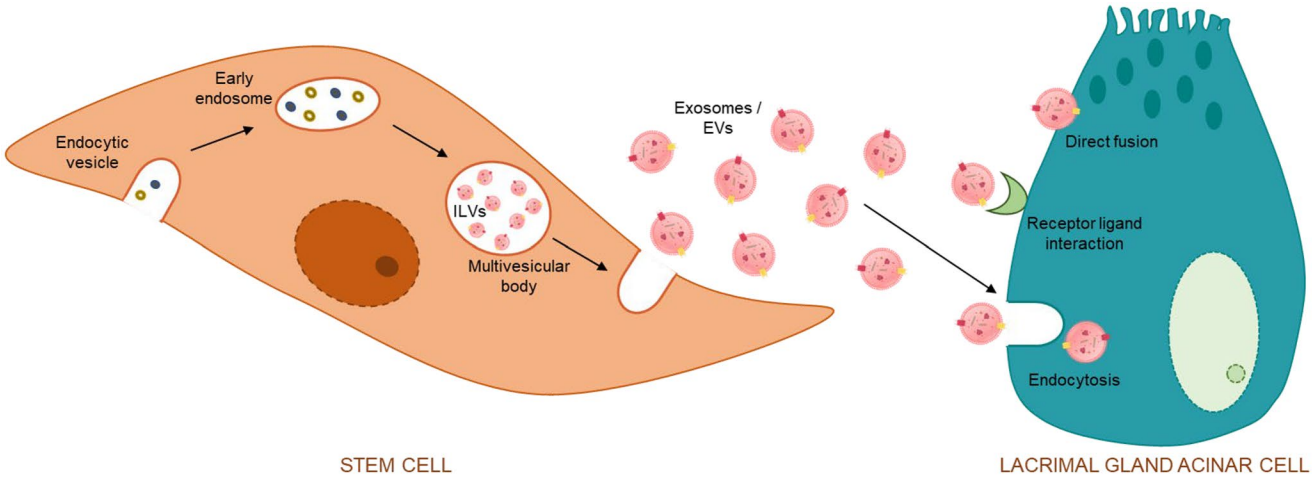

Figure 1. Exosome biogenesis and its secretion: Schematic diagram represents the formation of an exosome beginning from the invagination of donor cell plasma membrane to form endocytic vesicle, which later develops into early endosome and a multivesicular body (MVB). MVB comprises of multiple Intraluminal vesicles within them, which will be released into the extracellular environment as exosomes. The released exosomes enter the target cell *via* mechanisms like direct plasma membrane fusion, receptor-ligand mediated as well as the endocytosis.

cell, (ii) the transfer of signals *via* receptor–ligand interaction and (iii) the recipient cell uptake exosomes by various forms of endocytosis like micropinocytosis, phagocytosis, clathrin-mediated endocytosis and raft-mediated endocytosis.[89,90] Once the exosome cargo contents are internalized by the recipient cell, it can activate distinct cellular activities, physiological responses and regulation of gene expression.[87]

The major reason behind the popularity of cell-free therapy is the evidence that the therapeutic effect of the cell-based therapies is due to the biologically active molecules secreted as part of the intercellular signaling. The cellular secretions like the extracellular vesicles (EVs) and the conditioned media are the commonly used products for cell-free therapy.[91] Exosomes are considered a potential therapeutic candidate due to their very low immunogenicity, presence of a stable lipid bilayer that protects the cargo from digestive enzymes and the native immune cells, the ability to cross the blood–brain barrier and the targeted cargo delivery at the site of interest.[92,93] The exosomes enclose substances like proteins, lipids, DNA, mRNA, miRNA, and the long non-coding RNAs within their cargo and transport them to the recipient cells.[91] Exosomes have been found to be present in various tissues and in almost all body fluids in humans, denoting their significance in cell-cell communication and maintenance of tissue homeostasis.[89,94] Even though exosomes have been isolated from various cell types, tissues and even the body fluids, the detailed information regarding the source of these exosomes are lacking. The exosome from body fluids like tear, blood, urine, etc., could be a pool of vesicles secreted from varied cell types in the body. However, the cargo packed within these vesicles exhibits potential therapeutic effects in various disease conditions.

Exosomes from distinct sources, its cargo and its function

Exosomes are part of the cellular secretion from the tissues including the cells the of immune system and hence present abundantly in various body fluids like plasma, amniotic fluid, tears, breast milk, malignant ascites, cerebrospinal fluid, urine, saliva, lymph, etc.[95] Exosomes holds diverse constituents within their cargo such as metabolites, cytosolic and cell surface proteins, amino acids, DNA, RNA, and lipids that can reveal the cellular composition from where it originated.[96] The basic proteome composition of the exosomes include endosome associated proteins (GTPase, Rab, Annexins), proteins involved in MVB biogenesis (TSG101 and Alix), membrane proteins (tetraspanins), etc.[97] Apart from the variety of proteins, the exosomal membranes are enriched with saturated or monounsaturated fatty acids like cholesterol, hexosylceramides, sphingomyelin etc. Exosomes have components that are beneficial to the cellular function, however they are also known to remove the excess toxic by-products of the cells in order to maintain the cellular homeostasis.[96] The exosome contents mediate the intercellular and intracellular communication by their autocrine, paracrine and endocrine functions.[98]

Exosomes have been used as a promising drug delivery or gene delivery system due to the presence of miRNA and the mRNA within the cargo.[99–101] The exosomal contents and the quantity were found to be mostly different in the healthy and diseased cells, and therefore could be a potential source in identifying exosome-based biomarkers of the disease.[102,103] Exosomes derived from the breast cancer cells were shown to be capable of instigating the normal epithelial cells to develop tumors by inducing tumorigenesis through a Dicer-dependant pathway.[104] Similarly, the TLR3 pathway activation by the primary tumor-derived exosomal RNAs induced the metastatic niche formation and tumor formation in parent lung epithelial cells.[105] Evidences from various studies show that the exosomes from the tumor microenvironment predominantly favors the modulation of cancer cell metabolism and proliferation of tumor cells.[106] Additionally, the primary tumor-derived exosomes possess anti-tumor immunity that modulates the immune system by mediating the crosstalk between the immune cells and the tumor cells.[102] Exosomes from the cardiomyocytes were shown to

have beneficial role in improving myocardial function, promoting the cell proliferation and inhibiting apoptosis, through the effect of miR-146a.[107] Previous studies have found that the exosomes have more miRNA content compared to other small RNAs, and this could be because of a specialized sorting mechanism in cells that packs specific miRNAs within them exosomes.[108]

The functional heterogeneity of the exosomes depends on the exosomal cargo and the surface receptors within the exosomes. Depending on the cell from where it originated, exosomes may induce cell survival, promote immune modulation or may cause cell apoptosis.[96] Understanding the exosome specific cargo contents by analysing sorted exosome populations would help in better identification of biomarkers or therapeutics compared to the crude samples that contribute constituents from the entire cell.[109]

Exosomes in the treatment of various diseases

Exosomes from various sources have been used in distinct clinical applications like cell-free therapeutics, biomarker discovery, carriers for drug delivery and in the development of cancer vaccine.[110] With regard to the therapeutic potential of exosomes, they represent stem cells in its clinical and therapeutic benefits due to the secreted factors.[111] The findings from many studies have shown that the conditioned medium or the components of MSCs mediate various biological functions like tissue repair, immune modulation, wound healing, etc.[112] The paracrine secretions from the MSCs that comprises of the secreted vesicles as well as the trophic factors are attributable to the beneficial effects of MSCs.[113] Preconditioning of the MSCs using certain factors like inflammatory cytokines or hypoxia helps adjusting the cellular secretions and hence the exosomal cargo and its function.[114] Various studies have proved the therapeutic effects of exosomes in the treatment of diverse diseases spanning from regenerative medicine to cancers. Exosomes from MSCs were shown to preferably target the abnormal tumor tissues and the inflammatory sites within the body.[115] MSCs from human umbilical cord Wharton's jelly were shown to have beneficial role in accelerating the wound healing and tissue regeneration.[116] MSC derived exosomes have been used in GvHD patients in a phase I clinical trial and they observed improvement in the clinical symptoms of GvHD after the exosome treatment.[117] MSC-derived exosomes can promote the immunomodulation and the properties of the host cells from where it derived.[118] Apart from MSCs, the exosomes from $CD4^{+}CD25^{-}$ Tregs could prolong the kidney allograft survival *in vivo* and provide evidence for its immunosuppressive nature.[119] The immune regulation and the molecular transfer function of exosomes makes them a promising candidate for cancer immunotherapy. They could be used as targeted drug carriers for activation of anti-cancer immune response, or can be used as cancer vaccines as they have tumor antigens like MHC I.[120] The exosomes secreted by the amniotic fluid stem cells were found to be beneficial in delivering anti-apoptotic miR-10a to the ovarian granulosa cells and preventing the damage during chemotherapy.[121] One of the first phase I clinical trials using exosomes highlights the safety of exosome administration in 15 patients with stage III/IV melanoma. Exosome therapy did not cause any major toxicity or any delayed hypersensitivity reactions in the administered patients.[122] The exosomes from both MSCs and epithelial cells did not exhibit any signs of toxicity in mice after repeated doses of injection.[123,124] Overall, the exosomes derived from mesenchymal stem cells promote reduced inflammation and liver damage,[125] alleviate hepatic fibrosis,[126] promote wound healing,[127,128] improve cardiac function and neoangiogenesis,[129] reduce renal fibrosis,[130] etc. Although the exosomes have significant therapeutic potential, the exosome concentration required for the clinical application to achieve a good therapeutic significance is comparatively higher to the actual yield. The standardization of a high yield exosome isolation protocol or bioengineering the desired exosomal cargo is a better alternative.

Synthetic exosomes or exosome mimics are one of the emerging therapeutic tools for drug delivery because loading of the specific therapeutic component within the exosomes would be beneficial. The main advantage of modifying the exosomal cargo is that it enhances the therapeutic effects as well as the targeting capability of the exosomes. In addition, large-scale production of exosomes required for both research as well as clinical applications can be achieved through bio-engineering of exosomes. Two known mechanisms of generating engineered exosomes are either loading the exogenous cargo directly to the exosomes by methods like electroporation, sonication, etc or loading the cells with the specific exogenous cargo so that the cells secrete exosomes containing these specific cargoes.[131] Various studies have shown that the bio-mimetic exosomes are similar to the natural exosomes in terms of size distribution, morphology, immunocompatibility, stability and their function.[132–134] Engineered exosome mimetic nanovesicles with chemotherapeutic drug doxorubicin facilitated reduction of tumor growth with no systemic side effects in a mice model.[132] CD4+ T-cell membranes have been coated on to a polymeric core to generate exosome mimics termed as T-cell membrane coated nanoparticles (TNPs). TNPs were effective against HIV infection in the peripheral mononuclear blood cells and the human-monocyte-derived macrophages as they could selectively bind to the HIV envelope glycoprotein, gp120.[135] Exosome mimetic nanovesicles were generated from the cells by a series of cell extrusions through nano filters and could achieve efficient loading of the siRNA. Thus, the recipient cells could up take these siRNA loaded nanovesicles and attenuate the target gene expression.[136]

GMP manufacturing of exosomes is a pre-requisite for its usage in clinical trials. Literature shows data for five existing cell populations, that is, bone marrow-derived MSCs, human cardiac progenitor cells, monocyte-derived DCs, adipose tissue-derived stem cells, and HEK293 cells which are standardized for exosome production with GMP compliance.[137] Nevertheless, a total of 173 clinical trials using exosomes have been registered among the global researchers in various disease pathologies. However, 37 out of the 173 clinical trials have been completed and have shown the positive effects of exosome-based treatment in several diseases.

Table 1. Overview of studies using EVs/exosomes in treatment of Ocular surface diseases.

Disease	Source	Characterization	Models tested	Efficacy	PMID
Corneal epithelial wound	Corneal Mesenchymal Stromal Cell-derived Exosomes	TEM, DLS, EXOCET assay, Western Blot	*in vitro* wound healing and mice corneal wound healing	Accelerate epithelial wound healing compared to control	30372747
Corneal wound	Corneal epithelial cell-derived exosomes	TEM, DLS, Western Blot	*in vitro* effect in corneal fibroblasts and *ex vivo* aorta ring assay	Improved corneal wound healing and neovascularization	28165027
Dry eye in cGVHD patients	Umbilical MSCs derived Exosomes	NA	*in vivo* effect in mice and ongoing clinical trial in cGVHD patients	Restoration of ocular surface homeostasis and reduction in inflammation	35020440
Corneal fibrosis	Corneal stromal stem cells derived EVs	TEM, Flow cytometry	*in vitro* (HEK cells) and *in vivo* (Mice model of corneal wound)	Block corneal scarring and initiate corneal regeneration in mice model	31290598
Corneal Stromal Fibroblast function	Adipose-derived Mesenchymal Stem Cell Exosomes	NTA, Western Blot	*in vitro* (Corneal stromal fibroblasts)	Improved corneal fibroblast viability and ECM remodelling	29521294
Corneal angiogenesis	Corneal fibroblast derived exosomes	Western Blot	*in vitro* (vascular endothelial cells)	Transport MMP14 to the endothelial cells and promote corneal angiogenesis	25015352
Corneal Scar	Corneal epithelial cell derived EVs	TEM, Western Blot, Stimulated emission depletion microscopy	*in vitro* (human corneal fibroblasts)	increase in myofibroblast differentiation	32357574

Exosomes in ocular surface diseases and its outcome

Exosomes being a newer approach, the number of studies using exosomes for ocular surface diseases are lesser compared to other areas of the disease (Table 1). However, the findings from the existing studies prove that exosome therapy could be a promising approach in future. Administration of the MSC-derived exosomes as eye drops in patients with GVHD associated dry eye disease demonstrated reduced inflammation, improved tear secretion and epithelial recovery. The miR-204 within the MSC-exosomes were identified as the potential therapeutic target in GVHD-associated dry eye in mice.[138] Exosomes isolated from human and mouse corneal epithelial cells promoted modulation of the keratocytes and bone marrow-derived progenitor cell functions in the cornea. Additionally, the results demonstrated the presence of proteins specific to wound healing and neovascularization in the mouse corneal epithelium-derived exosomes.[139] Human corneal stromal MSC-exosomes were found to be readily up taken by the corneal epithelial cells upon administration and promote cell proliferation, migration and thereby accelerating the corneal epithelial wound healing *in vitro*.[140] In addition, limbal stromal cell (LSC) derived exosomes were shown to have major role in maintaining the cell-cell communication between the LSCs and the limbal epithelial stem cells (LESC) and influence a key factor involved in the migration and proliferation of the LESC both *in vitro* and *ex vivo*.[141] Similarly, the delivery of miRNA by exosomes from corneal stromal stem cells reduced the expression of fibrotic genes (Col3a1 and Acta2), attenuated the neutrophil infiltration and maintained the corneal tissue integrity in murine corneal wound models.[142] Exosomes exhibit great potential in corneal and conjunctival wound healing, epithelial cell proliferation, response to inflammation and maintenance of the normal tissue morphology. Exosomes from the stem cells exhibit greater immunoregulatory potential as compared to the stem cells as such.[143] Synthetic short collagen-like peptides induce stable corneal and nerve regeneration in a mini-porcine model by stimulating the endogenous host cells to secrete EVs that produce matrix components.[144] This further backs the idea that the exosomes are crucial mediators in maintaining tissue homeostasis and are being used in several disease systems as cell-free therapeutics. However, the data from the registry of clinical trials (clinicaltrials.gov; keywords - Ocular diseases, Dry eye, Exosome) shows the existence of only two registered clinical trials in the treatment of ocular diseases using exosomes till date and they are still in their early phases.

Exosomes in glandular disorders

Sjogren's syndrome is an autoimmune disorder that affects the exocrine glands of the body, particularly lacrimal glands and the salivary glands. Providing treatments to regenerate both the salivary as well as lacrimal glands is inevitable in preventing the Sjogren's disease progression.[145] Exosomes from the murine olfactory ecto-MSCs (OE-MSCs-Exos) demonstrated significant attenuation of the disease progression and functional restoration of myeloid-derived suppressor cells (MDSCs) in the mice with experimental Sjögren's syndrome (SS). OE-MSCs-Exos upregulated the arginase, ROS and NO levels and thereby enhanced the immunosuppressive function of the MDSCs. In addition, the suppressive effect of OE-MSC-Exos in SS progression were also proved by the reduction of Th1 and Th17 cell responses after the adoptive transfer of OE-MSC-Exo-treated MDSCs.[146] The EVs from induced pluripotent stem cells (iPSCs) derived MSCs inhibits the interaction between salivary gland epithelial cells and the immune cells, preventing the progression of SS in an *in vitro* co culture system. Similar experiments in a NOD mice model revealed that iPSC-MSC-EVs could inhibit the lymphocytic infiltration and the production of auto-antibodies in sub mandibular glands. These effects could possibly be achieved by preventing the activation of APCs and Tfh cells in the sub mandibular glands by the downregulation of ICOSL and CD40 and the significant upregulation of IL10.[147] Similarly, labial salivary gland MSCs and the exosomes derived from these cells were shown to have effect in ameliorating the inflammatory cell infiltration and improving the salivary gland function in a Sjögren's

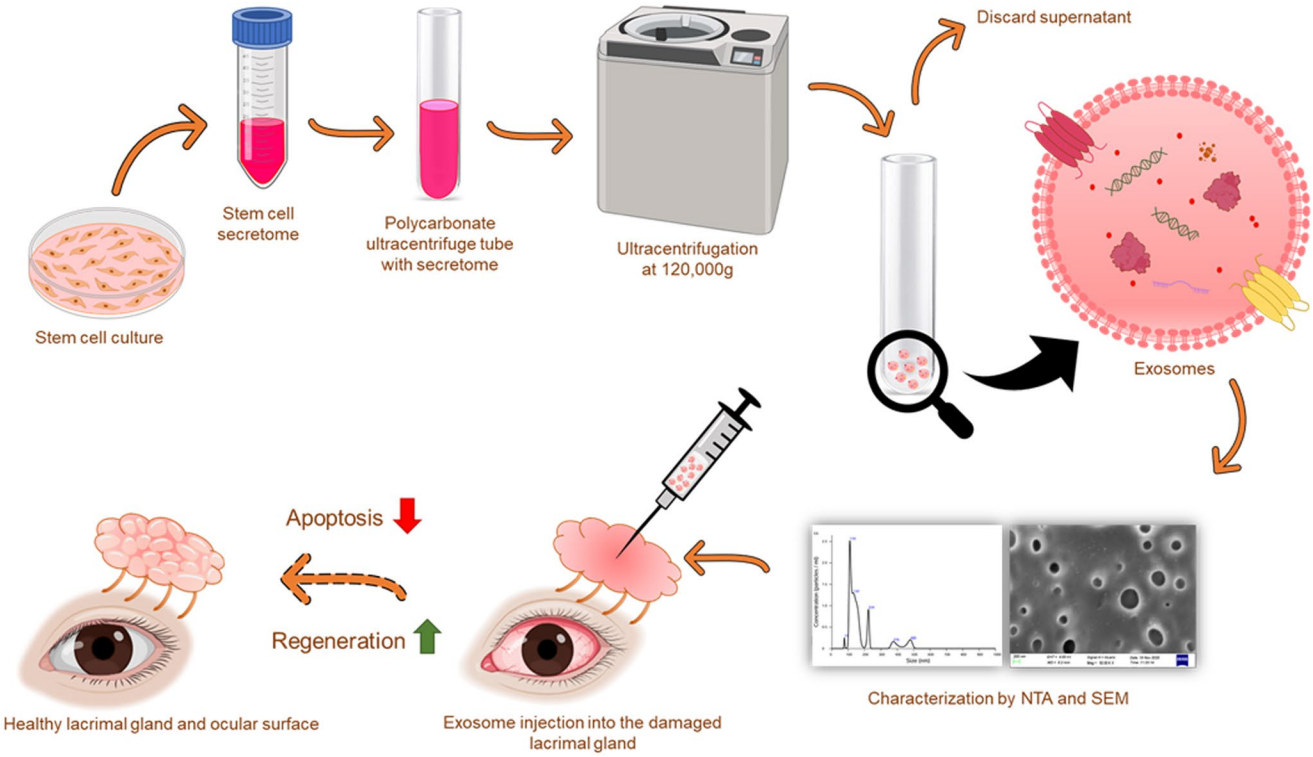

Figure 2. Exosomes from stem cells provide a platform for lacrimal gland and ocular surface repair: Exosomes can be isolated from the supernatant of the stem cell culture using ultracentrifugation and they undergo advanced characterization and quantification by electronical microscopy as well as nanoparticle tracking analysis. The characterized exosomes could be explored for its therapeutic role in LG damage as well as the ocular surface repair.

syndrome mice model. Labial salivary gland MSC treatment reduced the expression of inflammatory cytokines, IFN-γ, TNF-α, IL-6, and IL-17 and modulated the Treg cells and Th17 cells in order to suppress the autoimmune responses. Similarly, the treatment using both the MSCs and its exosomes were helpful in significant reduction of the rate of apoptosis in the salivary gland cells.[148] MSCs and its exosomes have shown its potential in the restoration of salivary gland function in order to maintain the tissue homeostasis. However, the effect of MSC-exosomes in the functional restoration or regeneration of LGs are not much studied and may have a potential in curing aqueous deficient dry eye caused due to LG dysfunction.

The existing strategies for LG regeneration include usage of adult precursor cells or MSCs within the LGs, using cultured LG epithelial cells, differentiation of human induced pluripotent stem cells and embryonic stem cells into LG cells, development of transplantable organoids or spheroids, using stem cell secretome and its components, etc.[149,150] Studies has proven that LG has significant regenerative potential, and this could be due to the inherent stem or progenitor cells within the mature human lacrimal glands.[48,151] Lacrimal gland derived mesenchymal stem cell transplantation or administration of its secretome is also shown to induce LG regeneration in mice models of LG dysfunction.[54,62] Therapeutic administration of stem cell derived exosomes could be a promising approach in the LG dysfunction as it may promote regeneration of the damaged LG tissue (Figure 2). There are no studies till date exploring the possibility of using exosomes as a treatment option for LG dysfunction induced aqueous deficient dry eye disease.

Comparing the benefits of exosomes and secretome treatment

Secretome is a broad term used for the secreted bioactive molecules like soluble proteins, nucleic acids, lipids and the extracellular vesicles from the donor cells. The secretome comprises of two differentiated components; the primary component that involves the secreted soluble factors and the other part comprises of the EVs.[152] The soluble factors in the secretome are proven to have anti-inflammatory agents, growth factors, cytokines, and various enzymes that promote cell proliferation and immunomodulation.[153] These paracrine factors present in secretome are well packed inside the extracellular vesicles are known as exosomes or microvesicles.[154] Similar to secretome, the EV fraction also has a rich cargo within that includes various mRNAs, miRNA, soluble proteins, lipids, etc that has potential roles in cell differentiation, proliferation, immunoregulation and regeneration.[155–157] The usage of secretome derivatives like conditioned medium and the EVs are considered to be beneficial as they are cell-free preparations and they could be produced readily without any storage concerns.[158] However, the isolated secretome and the exosomes exhibit batch variability based on the cell source, the pre-conditioning strategies, the growth conditions, and the donor variations etc.[152] Evidences from various studies proved that the secretome from stem cells can induce regeneration of injured or damaged tissues as effectively as the stem cells.[159] The secretome comprises of paracrine factors that mediates cell–cell communication in order to promote proliferation and differentiation of the surrounding cells. Secretome is a pool of components that has immunomodulatory, antifibrotic,

antiapoptotic, anti-inflammatory, and the proliferative properties.[160–162] However, EVs or exosomes are considered to be a concentrate of the secretome as they incorporate specific secretome components within their exosomal cargo enabling efficient remote communication and targeting. Similar to the secretome, exosomes can impart their therapeutic effect just by releasing its contents into the environment, even without undergoing internalization into the target cells.[163] Studies prove that the bioactive component in both secretome and exosomes are secreted stem cell products, which can be identified by the various proteomic approaches.

The conditioned medium from bone marrow derived MSCs contained better therapeutic factors compared to fibroblast conditioned medium and were effective in enhancing the wound healing in a mice model of excisional wound healing. BM-MSC conditioned medium effectively regulated the migration and proliferation of the keratinocytes and the endothelial cells.[164] Similarly, the treatment using human embryonic mesenchymal stem cell-derived conditioned medium reduced the progression of chronic kidney disease (CKD) in the established rat model of CKD. The conditioned media administration has significantly increased the glomerular filtration rate and effective the renal plasma flow leading to better renal function in the rat models.[165] The administration of MSCs and MSC extracts (MSCE) were proven to be successful in protecting the secretory function of both the lacrimal and salivary glands in a NOD mice model of SS. These treatment strategies demonstrated upregulation of distinct genes responsible for proliferation, tissue regeneration, and tear/saliva secretion and downregulated genes responsible for apoptosis.[166] With respect to LG regeneration, the secretome from mice LG-derived MSCs were shown to have beneficial effect in the cell viability of ethanol-damaged LG epithelial cells *in vitro*. The STAT1 protein found within the LG MSC secretome was upregulated under inflammatory conditions and have been shown to improve the cell viability of the LG epithelial cells.[62] Likewise, identification of the active proteins, RNAs and factors responsible for the therapeutic role of the secretome as well exosomes could be beneficial for better treatment of various disease conditions. While acknowledging the positive roles of secretome and exosomes, it is crucial to recognize that their therapeutic impact predominantly stems from specific therapeutic factors present within them. Therefore, it is essential to identify these components and understand the mechanisms through which they exert their effects on damaged target cells.

In summary, research from diverse sources highlights the therapeutic promise of both the secretome and exosomes in treating various diseases. Both the secretome and exosomes play pivotal roles in showcasing the functions of stem cells and their mechanisms in diseased conditions. The secretome encompasses a wide array of factors, while exosomes are isolated components extracted from the overall secretome. Currently, there is a lack of comparative studies assessing the effectiveness of the secretome versus exosomes, making it inconclusive to determine which exhibits superior therapeutic efficiency. Identification and differentiation of EV with exosomes also need to be further validated carefully with international consent before it can be taken further for translational applications.

Conclusion and future challenges

Exosomes from different biological sources play various roles in different physiological and pathological processes in the human body. Based on function, exosomes especially derived from immune cells may be developed into nanomedicines or vaccines for patients with tumors. Currently, there is no treatment for the aqueous deficient dry eye disease, caused due to LG dysfunction. Therefore, using exosome-based therapies to aid LG repair or regeneration would benefit many patients who are now receiving symptomatic care in the clinics. Further studies on the exosome cargo sorting mechanisms would provide more insights on the molecular pathways and its role in LG regeneration and repair. Understanding the pathways would help decipher the specific therapeutic component in the exosomal cargo. Usage of this specific components for therapies would be more beneficial as it represents a potential tool for enriched therapeutics rather than the crude cargo. Currently, exosome-based therapies are still in its infancy as they are in their early phase clinical trial and there are no specific international guidelines for the production and clinical transplantation of the exosomes. Hence, it is a pre-requisite to define specific guidelines and safety standards prior to the usage of exosomes in the treatment of patients with dry eye disease.

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Vivek Singh http://orcid.org/0000-0003-3958-5635
Sonja Mertsch http://orcid.org/0000-0001-8803-9150

References

1. Stapleton F, Alves M, Bunya VY, Jalbert I, Lekhanont K, Malet F, Na KS, Schaumberg D, Uchino M, Vehof J, et al. Tfos dews ii epidemiology report. Ocul Surf. 2017;15(3):334–365. doi: 10.1016/j.jtos.2017.05.003.
2. Jones L, Downie LE, Korb D, Benitez-Del-Castillo JM, Dana R, Deng SX, Dong PN, Geerling G, Hida RY, Liu Y, et al. Tfos dews ii management and therapy report. Ocul Surf. 2017;15(3):575–628. doi: 10.1016/j.jtos.2017.05.006.
3. Aragona P, Rolando M. Towards a dynamic customised therapy for ocular surface dysfunctions. Br J Ophthalmol. 2013;97(8):955–960. doi: 10.1136/bjophthalmol-2012-302568.
4. Stern ME, Gao J, Siemasko KF, Beuerman RW, Pflugfelder SC. The role of the lacrimal functional unit in the pathophysiology of dry eye. Exp Eye Res. 2004;78(3):409–416. doi: 10.1016/j.exer.2003.09.003.
5. Veernala I, Jaffet J, Fried J, Mertsch S, Schrader S, Basu S, Vemuganti GK, Singh V. Lacrimal gland regeneration: the unmet challenges and promise for dry eye therapy. Ocul Surf. 2022;25:129–141. doi: 10.1016/j.jtos.2022.06.005.
6. Dietrich J, Schrader S. Towards lacrimal gland regeneration: current concepts and experimental approaches. Curr Eye Res. 2020;45(3):230–240. doi: 10.1080/02713683.2019.1637438.
7. Yu J, Asche CV, Fairchild CJ. The economic burden of dry eye disease in the united states: a decision tree analysis. Cornea. 2011;30(4):379–387. doi: 10.1097/ICO.0b013e3181f7f363.

8. Papas EB. The global prevalence of dry eye disease: a bayesian view. Ophthalmic Physiol Opt. 2021;41(6):1254–1266. doi: 10.1111/opo.12888.
9. Sullivan DA, Rocha EM, Aragona P, Clayton JA, Ding J, Golebiowski B, Hampel U, McDermott AM, Schaumberg DA, Srinivasan S, et al. Tfos dews ii sex, gender, and hormones report. Ocul Surf. 2017;15(3):284–333. doi: 10.1016/j.jtos.2017.04.001.
10. Zhao M, Yu Y, Ying GS, Asbell PA, Bunya VY, Dry Eye A, Management Study Research G Age associations with dry eye clinical signs and symptoms in the dry eye assessment and management (dream) study. Ophthalmol Sci. 2023;3(2):100270. doi: 10.1016/j.xops.2023.100270.
11. Kam KW, Di Zazzo A, De Gregorio C, Narang P, Jhanji V, Basu S. A review on drug-induced dry eye disease. Indian J Ophthalmol. 2023;71(4):1263–1269. doi: 10.4103/IJO.IJO_2782_22.
12. Andole S, Senthil S. Ocular surface disease and anti-glaucoma medications: various features, diagnosis, and management guidelines. Semin Ophthalmol. 2023;38(2):158–166. doi: 10.1080/08820538.2022.2094714.
13. Thacker M, Sahoo A, Reddy AA, Bokara KK, Singh S, Basu S, Singh V. Benzalkonium chloride-induced dry eye disease animal models: current understanding and potential for translational research. Indian J Ophthalmol. 2023;71(4):1256–1262. doi: 10.4103/IJO.IJO_2791_22.
14. Sobolewska B, Schaller M, Zierhut M. Rosacea and dry eye disease. Ocul Immunol Inflamm. 2022;30(3):570–579. doi: 10.1080/09273948.2021.2025251.
15. Suárez-Cortés T, Merino-Inda N, Benitez-Del-Castillo JM. Tear and ocular surface disease biomarkers: a diagnostic and clinical perspective for ocular allergies and dry eye disease. Exp Eye Res. 2022;221:109121. doi: 10.1016/j.exer.2022.109121.
16. Mastropasqua L, Barboni P, Savini G, Aragona E, D'Aloisio R, Lanzini M, Agnifili L, Galzignato A, Solimeo A, Knutsson KA, et al. Refractive surgery and dry eye. Eur J Ophthalmol. 2023:11206721231176312. doi: 10.1177/11206721231176312.
17. Jiang B, Hu Q, Li T, She M, Li C, Zhou X. Relationship between dry eye and expressions of cxcr3 and ccr5 after ocular acid burn. BMC Ophthalmol. 2022;22(1):489. doi: 10.1186/s12886-022-02678-3.
18. The definition and classification of dry eye disease: report of the definition and classification subcommittee of the international dry eye workshop (2007). Ocul Surf. 2007;5(2):75–92.
19. Barabino S, Aragona P, di Zazzo A, Rolando M, with the contribution of selected ocular surface experts from the Societa Italiana di Dacriologia e Superficie O Updated definition and classification of dry eye disease: renewed proposals using the nominal group and delphi techniques. Eur J Ophthalmol. 2021;31(1):42–48. doi: 10.1177/1120672120960586.
20. Dietrich J, Massie I, Roth M, Geerling G, Mertsch S, Schrader S. Development of causative treatment strategies for lacrimal gland insufficiency by tissue engineering and cell therapy. Part 1: regeneration of lacrimal gland tissue: can we stimulate lacrimal gland renewal in vivo? Curr Eye Res. 2016;41(9):1131–1142. doi: 10.3109/02713683.2016.1148741.
21. Willcox MDP, Argüeso P, Georgiev GA, Holopainen JM, Laurie GW, Millar TJ, Papas EB, Rolland JP, Schmidt TA, Stahl U, et al. Tfos dews ii tear film report. Ocul Surf. 2017;15(3):366–403. doi: 10.1016/j.jtos.2017.03.006.
22. Paulsen F. Cell and molecular biology of human lacrimal gland and nasolacrimal duct mucins. Int Rev Cytol. 2006;249:229–279. doi: 10.1016/S0074-7696(06)49005-7.
23. Paulsen F, Langer G, Hoffmann W, Berry M. Human lacrimal gland mucins. Cell Tissue Res. 2004;316(2):167–177. doi: 10.1007/s00441-004-0877-7.
24. Uchino Y, Uchino M, Yokoi N, Dogru M, Kawashima M, Okada N, Inaba T, Tamaki S, Komuro A, Sonomura Y, et al. Alteration of tear mucin 5ac in office workers using visual display terminals: the osaka study. JAMA Ophthalmol. 2014;132(8):985–992. doi: 10.1001/jamaophthalmol.2014.1008.
25. Corrales RM, Narayanan S, Fernández I, Mayo A, Galarreta DJ, Fuentes-Páez G, Chaves FJ, Herreras JM, Calonge M. Ocular mucin gene expression levels as biomarkers for the diagnosis of dry eye syndrome. Invest Ophthalmol Vis Sci. 2011;52(11):8363–8369. doi: 10.1167/iovs.11-7655.
26. Argüeso P, Balaram M, Spurr-Michaud S, Keutmann HT, Dana MR, Gipson IK. Decreased levels of the goblet cell mucin muc5ac in tears of patients with sjogren syndrome. Invest Ophthalmol Vis Sci. 2002;43(4):1004–1011.
27. Yu DF, Chen Y, Han JM, Zhang H, Chen XP, Zou WJ, Liang LY, Xu CC, Liu ZG. Muc19 expression in human ocular surface and lacrimal gland and its alteration in sjogren syndrome patients. Exp Eye Res. 2008;86(2):403–411. doi: 10.1016/j.exer.2007.11.013.
28. Bron AJ, de Paiva CS, Chauhan SK, Bonini S, Gabison EE, Jain S, Knop E, Markoulli M, Ogawa Y, Perez V, et al. Tfos dews ii pathophysiology report. Ocul Surf. 2017;15(3):438–510. doi: 10.1016/j.jtos.2017.05.011.
29. Portal C, Gouyer V, Gottrand F, Desseyn JL. Ocular mucins in dry eye disease. Exp Eye Res. 2019;186:107724. doi: 10.1016/j.exer.2019.107724.
30. Bai Y, Ngo W, Khanal S, Nichols JJ. Characterization of the thickness of the tear film lipid layer in meibomian gland dysfunction using high resolution optical microscopy. Ocul Surf. 2022;24:34–39. doi: 10.1016/j.jtos.2021.12.011.
31. Sheppard JD, Nichols KK. Dry eye disease associated with meibomian gland dysfunction: focus on tear film characteristics and the therapeutic landscape. Ophthalmol Ther. 2023;12(3):1397–1418. doi: 10.1007/s40123-023-00669-1.
32. Hassanzadeh S, Varmaghani M, Zarei-Ghanavati S, Heravian Shandiz J, Azimi Khorasani A. Global prevalence of meibomian gland dysfunction: a systematic review and meta-analysis. Ocul Immunol Inflamm. 2021;29(1):66–75. doi: 10.1080/09273948.2020.1755441.
33. Hom MM, Martinson JR, Knapp LL, Paugh JR. Prevalence of meibomian gland dysfunction. Optom Vis Sci. 1990;67(9):710–712. doi: 10.1097/00006324-199009000-00010.
34. Nelson JD, Shimazaki J, Benitez-del-Castillo JM, Craig JP, McCulley JP, Den S, Foulks GN. The international workshop on meibomian gland dysfunction: report of the definition and classification subcommittee. Invest Ophthalmol Vis Sci. 2011;52(4):1930–1937. doi: 10.1167/iovs.10-6997b.
35. Jester JV, Parfitt GJ, Brown DJ. Meibomian gland dysfunction: hyperkeratinization or atrophy? BMC Ophthalmol. 2015;15 Suppl 1(Suppl 1):156. doi: 10.1186/s12886-015-0132-x.
36. Chhadva P, Goldhardt R, Galor A. Meibomian gland disease: the role of gland dysfunction in dry eye disease. Ophthalmology. 2017;124(11S):S20–S26. doi: 10.1016/j.ophtha.2017.05.031.
37. Baudouin C, Messmer EM, Aragona P, Geerling G, Akova YA, Benítez-del-Castillo J, Boboridis KG, Merayo-Lloves J, Rolando M, Labetoulle M. Revisiting the vicious circle of dry eye disease: a focus on the pathophysiology of meibomian gland dysfunction. Br J Ophthalmol. 2016;100(3):300–306. doi: 10.1136/bjophthalmol-2015-307415.
38. Geerling G, Tauber J, Baudouin C, Goto E, Matsumoto Y, O'Brien T, Rolando M, Tsubota K, Nichols KK. The international workshop on meibomian gland dysfunction: report of the subcommittee on management and treatment of meibomian gland dysfunction. Invest Ophthalmol Vis Sci. 2011;52(4):2050–2064. doi: 10.1167/iovs.10-6997g.
39. Olson MC, Korb DR, Greiner JV. Increase in tear film lipid layer thickness following treatment with warm compresses in patients with meibomian gland dysfunction. Eye Contact Lens. 2003;29(2):96–99. doi: 10.1097/01.ICL.0000060998.20142.8D.
40. Ballesteros-Sánchez A, Gargallo-Martínez B, Gutiérrez-Ortega R, Sánchez-González J-M. Eyelid exfoliation treatment efficacy and safety in dry eye disease, blepharitis, and contact lens discomfort patients: a systematic review. Asia Pac J Ophthalmol (Phila). 2023;12(3):315–325. doi: 10.1097/APO.0000000000000607.
41. Kell DB, Heyden EL, Pretorius E. The biology of lactoferrin, an iron-binding protein that can help defend against viruses and

bacteria. Front Immunol. 2020;11:1221. doi: 10.3389/fimmu.2020.01221.
42. Hanstock HG, Edwards JP, Walsh NP. Tear lactoferrin and lysozyme as clinically relevant biomarkers of mucosal immune competence. Front Immunol. 2019;10:1178. doi: 10.3389/fimmu.2019.01178.
43. Tóth-Molnár E, Ding C. New insight into lacrimal gland function: role of the duct epithelium in tear secretion. Ocul Surf. 2020;18(4):595–603. doi: 10.1016/j.jtos.2020.07.002.
44. Roth M, Spaniol K, Kordes C, Schwarz S, Mertsch S, Häussinger D, Rotter N, Geerling G, Schrader S. The influence of oxygen on the proliferative capacity and differentiation potential of lacrimal gland-derived mesenchymal stem cells. Invest Ophthalmol Vis Sci. 2015;56(8):4741–4752. doi: 10.1167/iovs.14-15475.
45. You S, Kublin CL, Avidan O, Miyasaki D, Zoukhri D. Isolation and propagation of mesenchymal stem cells from the lacrimal gland. Invest Ophthalmol Vis Sci. 2011;52(5):2087–2094. doi: 10.1167/iovs.10-5686.
46. Zoukhri D. Mechanisms involved in injury and repair of the murine lacrimal gland: role of programmed cell death and mesenchymal stem cells. Ocul Surf. 2010;8(2):60–69. doi: 10.1016/s1542-0124(12)70070-8.
47. Zoukhri D, Fix A, Alroy J, Kublin CL. Mechanisms of murine lacrimal gland repair after experimentally induced inflammation. Invest Ophthalmol Vis Sci. 2008;49(10):4399–4406. doi: 10.1167/iovs.08-1730.
48. Jaffet J, Mohanty A, Veernala I, Singh S, Ali MJ, Basu S, Vemuganti GK, Singh V. Human lacrimal gland derived mesenchymal stem cells - isolation, propagation, and characterization. Invest Ophthalmol Vis Sci. 2023;64(10):12. doi: 10.1167/iovs.64.10.12.
49. Pflugfelder SC. Prevalence, burden, and pharmacoeconomics of dry eye disease. Am J Manag Care. 2008;14(3 Suppl):S102–S106.
50. Lemp MA, Crews LA, Bron AJ, Foulks GN, Sullivan BD. Distribution of aqueous-deficient and evaporative dry eye in a clinic-based patient cohort: a retrospective study. Cornea. 2012;31(5):472–478. doi: 10.1097/ICO.0b013e318225415a.
51. Albietz JM. Prevalence of dry eye subtypes in clinical optometry practice. Optom Vis Sci. 2000;77(7):357–363. doi: 10.1097/00006324-200007000-00010.
52. Bhatt K, Singh S, Singh K, Kumar S, Dwivedi K. Prevalence of dry eye, its categorization (dry eye workshop ii), and pathological correlation: a tertiary care study. Indian J Ophthalmol. 2023;71(4):1454–1458. doi: 10.4103/IJO.IJO_2591_22.
53. Donthineni PR, Kammari P, Shanbhag SS, Singh V, Das AV, Basu S. Incidence, demographics, types and risk factors of dry eye disease in india: electronic medical records driven big data analytics report i. Ocul Surf. 2019;17(2):250–256. doi: 10.1016/j.jtos.2019.02.007.
54. Dietrich J, Ott L, Roth M, Witt J, Geerling G, Mertsch S, Schrader S. Msc transplantation improves lacrimal gland regeneration after surgically induced dry eye disease in mice. Sci Rep. 2019;9(1):18299. doi: 10.1038/s41598-019-54840-5.
55. Deng Y, Zhang Y, Ye L, Zhang T, Cheng J, Chen G, Zhang Q, Yang Y. Umbilical cord-derived mesenchymal stem cells instruct monocytes towards an il10-producing phenotype by secreting il6 and hgf. Sci Rep. 2016;6(1):37566. doi: 10.1038/srep37566.
56. Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, Yu XD, Mao N. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood. 2005;105(10):4120–4126. doi: 10.1182/blood-2004-02-0586.
57. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284(5411):143–147. doi: 10.1126/science.284.5411.143.
58. Sohni A, Verfaillie CM. Mesenchymal stem cells migration homing and tracking. Stem Cells Int. 2013;2013:130763–130768. doi: 10.1155/2013/130763.
59. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. Cytotherapy. 2006;8(4):315–317. doi: 10.1080/14653240600855905.
60. Dietrich J, Schlegel C, Roth M, Witt J, Geerling G, Mertsch S, Schrader S. Comparative analysis on the dynamic of lacrimal gland damage and regeneration after interleukin-1alpha or duct ligation induced dry eye disease in mice. Exp Eye Res. 2018;172:66–77. doi: 10.1016/j.exer.2018.03.026.
61. Liu Y, Hirayama M, Kawakita T, Tsubota K. A ligation of the lacrimal excretory duct in mouse induces lacrimal gland inflammation with proliferative cells. Stem Cells Int. 2017;2017:4923426–4923429. doi: 10.1155/2017/4923426.
62. Dietrich J, Roth M, König S, Geerling G, Mertsch S, Schrader S. Analysis of lacrimal gland derived mesenchymal stem cell secretome and its impact on epithelial cell survival. Stem Cell Res. 2019;38:101477. doi: 10.1016/j.scr.2019.101477.
63. Chen Y, Teng X, Chen W, Yang J, Yang Z, Yu Y, Shen Z. Timing of transplantation of autologous bone marrow derived mesenchymal stem cells for treating myocardial infarction. Sci China Life Sci. 2014;57(2):195–200. doi: 10.1007/s11427-013-4605-y.
64. Sohn RL, Gussoni E. Stem cell therapy for muscular dystrophy. Expert Opin Biol Ther. 2004;4(1):1–9. doi: 10.1517/14712598.4.1.1.
65. Li H, Shen S, Fu H, Wang Z, Li X, Sui X, Yuan M, Liu S, Wang G, Guo Q. Immunomodulatory functions of mesenchymal stem cells in tissue engineering. Stem Cells Int. 2019;2019:9671206. doi: 10.1155/2019/9671206.
66. Fiorina P, Jurewicz M, Augello A, Vergani A, Dada S, La Rosa S, Selig M, Godwin J, Law K, Placidi C, et al. Immunomodulatory function of bone marrow-derived mesenchymal stem cells in experimental autoimmune type 1 diabetes. J Immunol. 2009;183(2):993–1004. doi: 10.4049/jimmunol.0900803.
67. Health USNIo. 2022.
68. Zhang K, Cheng K. Stem cell-derived exosome versus stem cell therapy. Nat Rev Bioeng. 2023:1:608–609. doi: 10.1038/s44222-023-00064-2.
69. Pegtel DM, Gould SJ. Exosomes. Annu Rev Biochem. 2019;88(1):487–514. doi: 10.1146/annurev-biochem-013118-111902.
70. Welsh JA, Goberdhan DCI, O'Driscoll L, Buzas EI, Blenkiron C, Bussolati B, Cai H, Di Vizio D, Driedonks TAP, Erdbrugger U, et al. Minimal information for studies of extracellular vesicles (misev2023): from basic to advanced approaches. J Extracell Vesicles. 2024;13(2):e12404. doi: 10.1002/jev2.12404.
71. Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol. 2002;2(8):569–579. doi: 10.1038/nri855.
72. Kim HI, Park J, Zhu Y, Wang X, Han Y, Zhang D. Recent advances in extracellular vesicles for therapeutic cargo delivery. Exp Mol Med. 2024;56(4):836–849. doi: 10.1038/s12276-024-01201-6.
73. Edgar JR. Q&a: what are exosomes, exactly? BMC Biol. 2016;14(1):46. doi: 10.1186/s12915-016-0268-z.
74. Von Bartheld CS, Altick AL. Multivesicular bodies in neurons: distribution, protein content, and trafficking functions. Prog Neurobiol. 2011;93(3):313–340. doi: 10.1016/j.pneurobio.2011.01.003.
75. Möbius W, Ohno-Iwashita Y, van Donselaar EG, Oorschot VMJ, Shimada Y, Fujimoto T, Heijnen HFG, Geuze HJ, Slot JW. Immunoelectron microscopic localization of cholesterol using biotinylated and non-cytolytic perfringolysin o. J Histochem Cytochem. 2002;50(1):43–55. doi: 10.1177/002215540205000105.
76. White IJ, Bailey LM, Aghakhani MR, Moss SE, Futter CE. Egf stimulates annexin 1-dependent inward vesiculation in a multivesicular endosome subpopulation. Embo J. 2006;25(1):1–12. doi: 10.1038/sj.emboj.7600759.
77. Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: biogenesis, biologic function and clinical potential. Cell Biosci. 2019;9(1):19. doi: 10.1186/s13578-019-0282-2.
78. Henne WM, Buchkovich NJ, Emr SD. The escrt pathway. Dev Cell. 2011;21(1):77–91. doi: 10.1016/j.devcel.2011.05.015.
79. Stuffers S, Sem Wegner C, Stenmark H, Brech A. Multivesicular endosome biogenesis in the absence of escrts. Traffic. 2009;10(7):925–937. doi: 10.1111/j.1600-0854.2009.00920.x.

80. Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, Schwille P, Brügger B, Simons M. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. Science. 2008;319(5867):1244–1247. doi: 10.1126/science.1153124.
81. Laulagnier K, Grand D, Dujardin A, Hamdi S, Vincent-Schneider H, Lankar D, Salles JP, Bonnerot C, Perret B, Record M. Pld2 is enriched on exosomes and its activity is correlated to the release of exosomes. FEBS Lett. 2004;572(1-3):11–14. doi: 10.1016/j.febslet.2004.06.082.
82. Mazzeo C, Calvo V, Alonso R, Mérida I, Izquierdo M. Protein kinase d1/2 is involved in the maturation of multivesicular bodies and secretion of exosomes in t and b lymphocytes. Cell Death Differ. 2016;23(1):99–109. doi: 10.1038/cdd.2015.72.
83. Xie S, Zhang Q, Jiang L. Current knowledge on exosome biogenesis, cargo-sorting mechanism and therapeutic implications. Membranes (Basel). 2022;12(5):498. doi: 10.3390/membranes12050498.
84. Babst M. Mvb vesicle formation: escrt-dependent, escrt-independent and everything in between. Curr Opin Cell Biol. 2011;23(4):452–457. doi: 10.1016/j.ceb.2011.04.008.
85. D'Souza-Schorey C, Clancy JW. Tumor-derived microvesicles: shedding light on novel microenvironment modulators and prospective cancer biomarkers. Genes Dev. 2012;26(12):1287–1299. doi: 10.1101/gad.192351.112.
86. Pasquet JM, Dachary-Prigent J, Nurden AT. Calcium influx is a determining factor of calpain activation and microparticle formation in platelets. Eur J Biochem. 1996;239(3):647–654. doi: 10.1111/j.1432-1033.1996.0647u.x.
87. Wei H, Chen Q, Lin L, Sha C, Li T, Liu Y, Yin X, Xu Y, Chen L, Gao W, et al. Regulation of exosome production and cargo sorting. Int J Biol Sci. 2021;17(1):163–177. doi: 10.7150/ijbs.53671.
88. Schlienger S, Campbell S, Claing A. Arf1 regulates the rho/mlc pathway to control egf-dependent breast cancer cell invasion. Mol Biol Cell. 2014;25(1):17–29. doi: 10.1091/mbc.E13-06-0335.
89. Muthu S, Bapat A, Jain R, Jeyaraman N, Jeyaraman M. Exosomal therapy-a new frontier in regenerative medicine. Stem Cell Investig. 2021;8:7–7. doi: 10.21037/sci-2020-037.
90. Liu J, Jiang F, Jiang Y, Wang Y, Li Z, Shi X, Zhu Y, Wang H, Zhang Z. Roles of exosomes in ocular diseases. Int J Nanomedicine. 2020;15:10519–10538. doi: 10.2147/IJN.S277190.
91. Chen LY, Kao TW, Chen CC, Niaz N, Lee HL, Chen YH, Kuo CC, Shen YA. Frontier review of the molecular mechanisms and current approaches of stem cell-derived exosomes. Cells. 2023;12(7):1018. doi: 10.3390/cells12071018.
92. Cho E, Nam GH, Hong Y, Kim YK, Kim DH, Yang Y, Kim IS. Comparison of exosomes and ferritin protein nanocages for the delivery of membrane protein therapeutics. J Control Release. 2018;279:326–335. doi: 10.1016/j.jconrel.2018.04.037.
93. György B, Hung ME, Breakefield XO, Leonard JN. Therapeutic applications of extracellular vesicles: clinical promise and open questions. Annu Rev Pharmacol Toxicol. 2015;55(1):439–464. doi: 10.1146/annurev-pharmtox-010814-124630.
94. Boukouris S, Mathivanan S. Exosomes in bodily fluids are a highly stable resource of disease biomarkers. Proteomics Clin Appl. 2015;9(3-4):358–367. doi: 10.1002/prca.201400114.
95. Keller S, Ridinger J, Rupp AK, Janssen JW, Altevogt P. Body fluid derived exosomes as a novel template for clinical diagnostics. J Transl Med. 2011;9(1):86. doi: 10.1186/1479-5876-9-86.
96. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science. 2020;367(6478):eaau6977. doi: 10.1126/science.aau6977.
97. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol. 2013;200(4):373–383. doi: 10.1083/jcb.201211138.
98. Saber SH, Ali HEA, Gaballa R, Gaballah M, Ali HI, Zerfaoui M, Abd Elmageed ZY. Exosomes are the driving force in preparing the soil for the metastatic seeds: lessons from the prostate cancer. Cells. 2020;9(3):564. doi: 10.3390/cells9030564.
99. Bunggulawa EJ, Wang W, Yin T, Wang N, Durkan C, Wang Y, Wang G. Recent advancements in the use of exosomes as drug delivery systems. J Nanobiotechnology. 2018;16(1):81. doi: 10.1186/s12951-018-0403-9.
100. Saari H, Turunen T, Lõhmus A, Turunen M, Jalasvuori M, Butcher SJ, Ylä-Herttuala S, Viitala T, Cerullo V, Siljander PRM, et al. Extracellular vesicles provide a capsid-free vector for oncolytic adenoviral DNA delivery. J Extracell Vesicles. 2020;9(1):1747206. doi: 10.1080/20013078.2020.1747206.
101. O'Loughlin AJ, Woffindale CA, Wood MJ. Exosomes and the emerging field of exosome-based gene therapy. Curr Gene Ther. 2012;12(4):262–274. doi: 10.2174/156652312802083594.
102. Wang J, Sun X, Zhao J, Yang Y, Cai X, Xu J, Cao P. Exosomes: a novel strategy for treatment and prevention of diseases. Front Pharmacol. 2017;8:300. doi: 10.3389/fphar.2017.00300.
103. Logozzi M, De Milito A, Lugini L, Borghi M, Calabrò L, Spada M, Perdicchio M, Marino ML, Federici C, Iessi E, et al. High levels of exosomes expressing cd63 and caveolin-1 in plasma of melanoma patients. PLoS One. 2009;4(4):e5219. doi: 10.1371/journal.pone.0005219.
104. Melo SA, Sugimoto H, O'Connell JT, Kato N, Villanueva A, Vidal A, Qiu L, Vitkin E, Perelman LT, Melo CA, et al. Cancer exosomes perform cell-independent microrna biogenesis and promote tumorigenesis. Cancer Cell. 2014;26(5):707–721. doi: 10.1016/j.ccell.2014.09.005.
105. Liu Y, Gu Y, Han Y, Zhang Q, Jiang Z, Zhang X, Huang B, Xu X, Zheng J, Cao X. Tumor exosomal rnas promote lung pre-metastatic niche formation by activating alveolar epithelial tlr3 to recruit neutrophils. Cancer Cell. 2016;30(2):243–256. doi: 10.1016/j.ccell.2016.06.021.
106. Zhao H, Yang L, Baddour J, Achreja A, Bernard V, Moss T, Marini JC, Tudawe T, Seviour EG, San Lucas FA, et al. Tumor microenvironment derived exosomes pleiotropically modulate cancer cell metabolism. Elife. 2016;5:e10250. doi: 10.7554/eLife.10250.
107. Ibrahim AG-E, Cheng K, Marbán E. Exosomes as critical agents of cardiac regeneration triggered by cell therapy. Stem Cell Reports. 2014;2(5):606–619. doi: 10.1016/j.stemcr.2014.04.006.
108. Goldie BJ, Dun MD, Lin M, Smith ND, Verrills NM, Dayas CV, Cairns MJ. Activity-associated mirna are packaged in map1b-enriched exosomes released from depolarized neurons. Nucleic Acids Res. 2014;42(14):9195–9208. doi: 10.1093/nar/gku594.
109. Schey KL, Luther JM, Rose KL. Proteomics characterization of exosome cargo. Methods. 2015;87:75–82. doi: 10.1016/j.ymeth.2015.03.018.
110. Rezaie J, Feghhi M, Etemadi T. A review on exosomes application in clinical trials: perspective, questions, and challenges. Cell Commun Signal. 2022;20(1):145. doi: 10.1186/s12964-022-00959-4.
111. Han C, Sun X, Liu L, Jiang H, Shen Y, Xu X, Li J, Zhang G, Huang J, Lin Z, et al. Exosomes and their therapeutic potentials of stem cells. Stem Cells Int. 2016;2016(1):7653489. doi: 10.1155/2016/7653489.
112. Yu B, Zhang X, Li X. Exosomes derived from mesenchymal stem cells. Int J Mol Sci. 2014;15(3):4142–4157. doi: 10.3390/ijms15034142.
113. Hade MD, Suire CN, Suo Z. Mesenchymal stem cell-derived exosomes: applications in regenerative medicine. Cells. 2021;10(8):1959. doi: 10.3390/cells10081959.
114. Nikfarjam S, Rezaie J, Zolbanin NM, Jafari R. Mesenchymal stem cell derived-exosomes: a modern approach in translational medicine. J Transl Med. 2020;18(1):449. doi: 10.1186/s12967-020-02622-3.
115. Fitts CA, Ji N, Li Y, Tan C. Exploiting exosomes in cancer liquid biopsies and drug delivery. Adv Healthc Mater. 2019;8(6):e1801268. doi: 10.1002/adhm.201801268.
116. Bakhtyar N, Jeschke MG, Mainville L, Herer E, Amini-Nik S. Acellular gelatinous material of human umbilical cord enhances wound healing: a candidate remedy for deficient wound healing. Front Physiol. 2017;8:200. doi: 10.3389/fphys.2017.00200.
117. Kordelas L, Rebmann V, Ludwig AK, Radtke S, Ruesing J, Doeppner TR, Epple M, Horn PA, Beelen DW, Giebel B. Msc-derived exosomes: a novel tool to treat therapy-refractory

graft-versus-host disease. Leukemia. 2014;28(4):970–973. doi: 10.1038/leu.2014.41.
118. Zheng Q, Zhang S, Guo WZ, Li XK. The unique immunomodulatory properties of msc-derived exosomes in organ transplantation. Front Immunol. 2021;12:659621. doi: 10.3389/fimmu.2021.659621.
119. Aiello S, Rocchetta F, Longaretti L, Faravelli S, Todeschini M, Cassis L, Pezzuto F, Tomasoni S, Azzollini N, Mister M, et al. Extracellular vesicles derived from t regulatory cells suppress t cell proliferation and prolong allograft survival. Sci Rep. 2017;7(1):11518. doi: 10.1038/s41598-017-08617-3.
120. Xu Z, Zeng S, Gong Z, Yan Y. Exosome-based immunotherapy: a promising approach for cancer treatment. Mol Cancer. 2020;19(1):160. doi: 10.1186/s12943-020-01278-3.
121. Xiao GY, Cheng CC, Chiang YS, Cheng WT, Liu IH, Wu SC. Exosomal mir-10a derived from amniotic fluid stem cells preserves ovarian follicles after chemotherapy. Sci Rep. 2016;6(1):23120. doi: 10.1038/srep23120.
122. Escudier B, Dorval T, Chaput N, André F, Caby M-P, Novault S, Flament C, Leboulaire C, Borg C, Amigorena S, et al. Vaccination of metastatic melanoma patients with autologous dendritic cell (dc) derived-exosomes: results of thefirst phase i clinical trial. J Transl Med. 2005;3(1):10. doi: 10.1186/1479-5876-3-10.
123. Mendt M, Kamerkar S, Sugimoto H, McAndrews KM, Wu CC, Gagea M, Yang S, Blanko EVR, Peng Q, Ma X, et al. Generation and testing of clinical-grade exosomes for pancreatic cancer. JCI Insight. 2018;3(8). doi: 10.1172/jci.insight.99263.
124. Zhu X, Badawi M, Pomeroy S, Sutaria DS, Xie Z, Baek A, Jiang J, Elgamal OA, Mo X, Perle K, et al. Comprehensive toxicity and immunogenicity studies reveal minimal effects in mice following sustained dosing of extracellular vesicles derived from hek293t cells. J Extracell Vesicles. 2017;6(1):1324730.
125. Jiao Y, Xu P, Shi H, Chen D, Shi H. Advances on liver cell-derived exosomes in liver diseases. J Cell Mol Med. 2021;25(1):15–26. doi: 10.1111/jcmm.16123.
126. Qu Y, Zhang Q, Cai X, Li F, Ma Z, Xu M, Lu L. Exosomes derived from mir-181-5p-modified adipose-derived mesenchymal stem cells prevent liver fibrosis via autophagy activation. J Cell Mol Med. 2017;21(10):2491–2502. doi: 10.1111/jcmm.13170.
127. Zhang B, Wu X, Zhang X, Sun Y, Yan Y, Shi H, Zhu Y, Wu L, Pan Z, Zhu W, et al. Human umbilical cord mesenchymal stem cell exosomes enhance angiogenesis through the wnt4/beta-catenin pathway. Stem Cells Transl Med. 2015;4(5):513–522. doi: 10.5966/sctm.2014-0267.
128. Zhang J, Guan J, Niu X, Hu G, Guo S, Li Q, Xie Z, Zhang C, Wang Y. Exosomes released from human induced pluripotent stem cells-derived mscs facilitate cutaneous wound healing by promoting collagen synthesis and angiogenesis. J Transl Med. 2015;13(1):49. doi: 10.1186/s12967-015-0417-0.
129. Bian S, Zhang L, Duan L, Wang X, Min Y, Yu H. Extracellular vesicles derived from human bone marrow mesenchymal stem cells promote angiogenesis in a rat myocardial infarction model. J Mol Med (Berl). 2014;92(4):387–397. doi: 10.1007/s00109-013-1110-5.
130. Chen L, Wang Y, Li S, Zuo B, Zhang X, Wang F, Sun D. Exosomes derived from gdnf-modified human adipose mesenchymal stem cells ameliorate peritubular capillary loss in tubulointerstitial fibrosis by activating the sirt1/enos signaling pathway. Theranostics. 2020;10(20):9425–9442. doi: 10.7150/thno.43315.
131. Li SP, Lin ZX, Jiang XY, Yu XY. Exosomal cargo-loading and synthetic exosome-mimics as potential therapeutic tools. Acta Pharmacol Sin. 2018;39(4):542–551. doi: 10.1038/aps.2017.178.
132. Jang SC, Kim OY, Yoon CM, Choi D-S, Roh T-Y, Park J, Nilsson J, Lötvall J, Kim Y-K, Gho YS. Bioinspired exosome-mimetic nanovesicles for targeted delivery of chemotherapeutics to malignant tumors. ACS Nano. 2013;7(9):7698–7710. doi: 10.1021/nn402232g.
133. Jo W, Jeong D, Kim J, Cho S, Jang SC, Han C, Kang JY, Gho YS, Park J. Microfluidic fabrication of cell-derived nanovesicles as endogenous rna carriers. Lab Chip. 2014;14(7):1261–1269. doi: 10.1039/c3lc50993a.
134. Hu CM, Zhang L, Aryal S, Cheung C, Fang RH, Zhang L. Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. Proc Natl Acad Sci U S A. 2011;108(27):10980–10985. doi: 10.1073/pnas.1106634108.
135. Wei X, Zhang G, Ran D, Krishnan N, Fang RH, Gao W, Spector SA, Zhang L. T-cell-mimicking nanoparticles can neutralize hiv infectivity. Adv Mater. 2018;30(45):e1802233. doi: 10.1002/adma.201802233.
136. Lunavat TR, Jang SC, Nilsson L, Park HT, Repiska G, Lässer C, Nilsson JA, Gho YS, Lötvall J. Rnai delivery by exosome-mimetic nanovesicles - implications for targeting c-myc in cancer. Biomaterials. 2016;102:231–238. doi: 10.1016/j.biomaterials.2016.06.024.
137. Chen YS, Lin EY, Chiou TW, Harn HJ. Exosomes in clinical trial and their production in compliance with good manufacturing practice. Ci Ji Yi Xue Za Zhi. 2020;32(2):113–120. doi: 10.4103/tcmj.tcmj_182_19.
138. Zhou T, He C, Lai P, Yang Z, Liu Y, Xu H, Lin X, Ni B, Ju R, Yi W, et al. Mir-204-containing exosomes ameliorate gvhd-associated dry eye disease. Sci Adv. 2022;8(2):eabj9617. doi: 10.1126/sciadv.abj9617.
139. Han KY, Tran JA, Chang JH, Azar DT, Zieske JD. Potential role of corneal epithelial cell-derived exosomes in corneal wound healing and neovascularization. Sci Rep. 2017;7(1):40548. doi: 10.1038/srep40548.
140. Samaeekia R, Rabiee B, Putra I, Shen X, Park YJ, Hematti P, Eslani M, Djalilian AR. Effect of human corneal mesenchymal stromal cell-derived exosomes on corneal epithelial wound healing. Invest Ophthalmol Vis Sci. 2018;59(12):5194–5200. doi: 10.1167/iovs.18-24803.
141. Leszczynska A, Kulkarni M, Ljubimov AV, Saghizadeh M. Exosomes from normal and diabetic human corneolimbal keratocytes differentially regulate migration, proliferation and marker expression of limbal epithelial cells. Sci Rep. 2018;8(1):15173. doi: 10.1038/s41598-018-33169-5.
142. Shojaati G, Khandaker I, Funderburgh ML, Mann MM, Basu R, Stolz DB, Geary ML, Dos Santos A, Deng SX, Funderburgh JL. Mesenchymal stem cells reduce corneal fibrosis and inflammation via extracellular vesicle-mediated delivery of mirna. Stem Cells Transl Med. 2019;8(11):1192–1201. doi: 10.1002/sctm.18-0297.
143. Li SF, Han Y, Wang F, Su Y. Progress in exosomes and their potential use in ocular diseases. Int J Ophthalmol. 2020;13(9):1493–1498. doi: 10.18240/ijo.2020.09.23.
144. Jangamreddy JR, Haagdorens MKC, Mirazul Islam M, Lewis P, Samanta A, Fagerholm P, Liszka A, Ljunggren MK, Buznyk O, Alarcon EI, et al. Short peptide analogs as alternatives to collagen in pro-regenerative corneal implants. Acta Biomater. 2018;69:120–130. doi: 10.1016/j.actbio.2018.01.011.
145. Huang Y, Li R, Ye S, Lin S, Yin G, Xie Q. Recent advances in the use of exosomes in sjogren's syndrome. Front Immunol. 2020;11:1509. doi: 10.3389/fimmu.2020.01509.
146. Rui K, Hong Y, Zhu Q, Shi X, Xiao F, Fu H, Yin Q, Xing Y, Wu X, Kong X, et al. Olfactory ecto-mesenchymal stem cell-derived exosomes ameliorate murine sjogren's syndrome by modulating the function of myeloid-derived suppressor cells. Cell Mol Immunol. 2021;18(2):440–451. doi: 10.1038/s41423-020-00587-3.
147. Hai B, Shigemoto-Kuroda T, Zhao Q, Lee RH, Liu F. Inhibitory effects of ipsc-mscs and their extracellular vesicles on the onset of sialadenitis in a mouse model of sjogren's syndrome. Stem Cells Int. 2018;2018:2092315–2092310. doi: 10.1155/2018/2092315.
148. Li B, Xing Y, Gan Y, He J, Hua H. Labial gland-derived mesenchymal stem cells and their exosomes ameliorate murine sjogren's syndrome by modulating the balance of treg and th17 cells. Stem Cell Res Ther. 2021;12(1):478. doi: 10.1186/s13287-021-02541-0.
149. Liu CY, Hirayama M, Ali M, Shah D, Aakalu VK. Strategies for regenerating the lacrimal gland. Curr Ophthalmol Rep. 2017;5(3):193–198. doi: 10.1007/s40135-017-0142-3.

150. Daniel MG, Wu AY. Applications of stem cell biology to oculoplastic surgery. Curr Opin Ophthalmol. 2016;27(5):428–432. doi: 10.1097/ICU.0000000000000288.
151. Garg A, Zhang X. Lacrimal gland development: From signaling interactions to regenerative medicine. Dev Dyn. 2017;246(12):970–980. doi: 10.1002/dvdy.24551.
152. Munoz-Perez E, Gonzalez-Pujana A, Igartua M, Santos-Vizcaino E, Hernandez RM. Mesenchymal stromal cell secretome for the treatment of immune-mediated inflammatory diseases: latest trends in isolation, content optimization and delivery avenues. Pharmaceutics. 2021;13(11):1802. doi: 10.3390/pharmaceutics13111802.
153. Keshtkar S, Azarpira N, Ghahremani MH. Mesenchymal stem cell-derived extracellular vesicles: novel frontiers in regenerative medicine. Stem Cell Res Ther. 2018;9(1):63. doi: 10.1186/s13287-018-0791-7.
154. Sun DZ, Abelson B, Babbar P, Damaser MS. Harnessing the mesenchymal stem cell secretome for regenerative urology. Nat Rev Urol. 2019;16(6):363–375. doi: 10.1038/s41585-019-0169-3.
155. Eleuteri S, Fierabracci A. Insights into the secretome of mesenchymal stem cells and its potential applications. Int J Mol Sci. 2019;20(18):4597. doi: 10.3390/ijms20184597.
156. Driscoll J, Patel T. The mesenchymal stem cell secretome as an acellular regenerative therapy for liver disease. J Gastroenterol. 2019;54(9):763–773. doi: 10.1007/s00535-019-01599-1.
157. Wu H, Fan H, Shou Z, Xu M, Chen Q, Ai C, Dong Y, Liu Y, Nan Z, Wang Y, et al. Extracellular vesicles containing mir-146a attenuate experimental colitis by targeting traf6 and irak1. Int Immunopharmacol. 2019;68:204–212. doi: 10.1016/j.intimp.2018.12.043.
158. Jenner F, Ribitsch I. Secretome, extracellular vesicles, exosomes. In Filardo G, Mandelbaum BR, Muschler GF, Rodeo SA, Nakamura N (Eds). Orthobiologics (pp. 155–166). Cham: Springer, 2020. doi: 10.1007/978-3-030-84744-9_12.
159. Foo JB, Looi QH, Chong PP, Hassan NH, Yeo GEC, Ng CY, Koh B, How CW, Lee SH, Law JX. Comparing the therapeutic potential of stem cells and their secretory products in regenerative medicine. Stem Cells Int. 2021;2021:2616807–2616830. doi: 10.1155/2021/2616807.
160. Geiger A, Walker A, Nissen E. Human fibrocyte-derived exosomes accelerate wound healing in genetically diabetic mice. Biochem Biophys Res Commun. 2015;467(2):303–309. doi: 10.1016/j.bbrc.2015.09.166.
161. Kusuma GD, Carthew J, Lim R, Frith JE. Effect of the microenvironment on mesenchymal stem cell paracrine signaling: opportunities to engineer the therapeutic effect. Stem Cells Dev. 2017;26(9):617–631. doi: 10.1089/scd.2016.0349.
162. Gemayel J, Chaker D, El Hachem G, Mhanna M, Salemeh R, Hanna C, Harb F, Ibrahim A, Chebly A, Khalil C. Mesenchymal stem cells-derived secretome and extracellular vesicles: perspective and challenges in cancer therapy and clinical applications. Clin Transl Oncol. 2023;25(7):2056–2068. doi: 10.1007/s12094-023-03115-7.
163. Lee B-C, Kang I, Yu K-R. Therapeutic features and updated clinical trials of mesenchymal stem cell (MSC)-derived exosomes. J Clin Med. 2021;10(4):711. doi: 10.3390/jcm10040711.
164. Chen L, Tredget EE, Wu PY, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. PLoS One. 2008;3(4):e1886. doi: 10.1371/journal.pone.0001886.
165. van Koppen A, Joles JA, van Balkom BW, Lim SK, de Kleijn D, Giles RH, Verhaar MC. Human embryonic mesenchymal stem cell-derived conditioned medium rescues kidney function in rats with established chronic kidney disease. PLoS One. 2012;7(6):e38746. doi: 10.1371/journal.pone.0038746.
166. Abughanam G, Elkashty OA, Liu Y, Bakkar MO, Tran SD. Mesenchymal stem cells extract (mscse)-based therapy alleviates xerostomia and keratoconjunctivitis sicca in Sjogren's syndrome-like disease. Int J Mol Sci. 2019;20(19):4750. doi: 10.3390/ijms20194750.