15 Mesenchymal Stem Cells for Regeneration of the Ocular Surface

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Abbreviations

CD	Cluster of differentiation
CK	Cytokeratin
CLET	Cultivated limbal epithelial transplantation
DED	Dry eye disease
EVs	Extracellular vesicles
GVHD	Graft-versus-host disease
HLA-DR	Human leukocyte antigen-DR
IL	Interleukin
LESCs	Limbal epithelial stem cells
LSCD	Limbal stem cell deficiency
MSCs	Mesenchymal stem cells
oGVHD	Ocular graft-versus-host disease
TNF-α	Tumour necrosis factor alpha
Treg	Regulatory T cells
TSG-6	Tumour necrosis factor-stimulated gene/protein-6

Key Points

- Mesenchymal stem cells (MSCs) have significant therapeutic potential to regenerate the ocular surface.
- Preclinical evidence demonstrates that MSCs can be used for the treatment of ocular surface diseases.
- MSCs have been successfully applied in clinical settings for the treatment of some ocular surface diseases.
- Work must continue to overcome the technical and scientific challenges that remain unsolved to establish the use of MSCs as a widely accepted treatment for ocular surface diseases.

Regeneration of the Ocular Surface by Mesenchymal Stem Cells

The integrity of the corneal epithelium is crucial for maintaining corneal transparency and visual function. Corneal damage due to different circumstances such as chemical or thermal burns, eye surgeries, cicatrizing-autoimmune pathologies, severe dry eye disease (DED), infections, transplant rejections, or congenital disorders can disrupt the integrity of the corneal epithelium. This type of loss is an important cause of visual impairment and blindness that affects millions of people worldwide [1]. The corneal epithelium has an extremely high turnover rate (4–7 days) that is mediated by the limbal epithelial stem cells (LESCs) located in the palisades of Vogt within the corneo-scleral limbal niche [2–4]. LESC deficiency or dysfunction and/or the destruction of the niche microenvironment produces a condition known as limbal stem cell deficiency (LSCD). LSCD reduces the regeneration and repair of the corneal epithelium, and the corneal surface is gradually replaced by conjunctival epithelium. This process is accompanied by chronic inflammation of the ocular surface, chronic pain, ulceration, and neovascularization, all of which result in corneal blindness due to the loss of corneal transparency [5].

At present, among the stem cell-based therapies, cultivated limbal epithelial cell transplantation (CLET) is the treatment of choice for LSCD. In unilateral cases of LSCD, treatment by autologous CLET is possible following acquisition of limbal tissue from the contralateral healthy eye [6–11]. However, bilateral cases of LSCD are more frequent; therefore, it is necessary to use allogeneic limbal tissue. Consequently, this requires one year of immunosuppression to avoid immune rejection, resulting in an increased risk of patient morbidity and associated medical costs [11]. To avoid this immunosuppression, it is necessary to seek either an extraocular autologous source of stem cells or a non-immunogenic allogeneic source.

In recent years, the use of mesenchymal stem cells (MSCs) has remarkably increased in the fields of cell therapy and regenerative medicine. Collectively, these stromal-derived cells retain some intrinsic developmental and differentiation features after they are derived from a variety of animal and human tissues, including bone marrow, adipose tissue, dental pulp, umbilical cord, and ocular limbal stroma, among others [12]. They are defined by their adherence to plastic substrates when cultured in standard conditions and their multipotent differentiation capacity to

form bone, cartilage, and adipose tissue in vitro. Importantly, the MSCs exhibit expression of a characteristic set of specific surface antigens, including positive expression for the cluster of differentiation (CD) 73, CD90, and CD105 [13]. However, they do not express antigens CD34, CD45, CD11b or CD14, CD19 or CD79α, or human leukocyte antigen-DR (HLA-DR) markers [13].

Moreover, MSCs present four potential advantages over LESCs with regard to their utility in cell therapy and tissue regeneration. First, acquisition of MSCs is not restricted to deceased donors or healthy eyes of living donors as they can be easily obtained from several different living tissues [12]. Second, they can be cultured in vitro to clinical scales in a short period of time, thus overcoming the limitations of LESCs, which are difficult to isolate and culture [14, 15]. Third, the stem cell phenotype is maintained even during cryopreservation [16]. Fourth, they are not immunogenic; therefore, immunosuppression is not necessary after allogeneic transplantation [17, 18].

MSCs have additional advantages over LESCs, especially for ocular surface repair. For instance, the capacity of MSCs for differentiation following transplantation enables them to undergo integration, proliferation, and differentiation in the damaged tissues, and in many cases, facilitate tissue regeneration [19–21]. MSCs may also reduce inflammation, apoptosis, and fibrosis and improve tissue regeneration by activating endogenous progenitor cells [22]. MSCs also have immunomodulatory properties that enable the regulation of T cells, B-cells, and natural killer cells, thus mitigating the secretion of inflammatory cytokines [23, 24].

Considering all, MSCs have emerged as very attractive candidates for cell-based therapies in numerous and highly varied clinical applications including the treatment of some ocular surface diseases such as LSCD, DED, or even as a potential treatment to improve corneal allograft survival [11, 25]. This chapter summarizes the main existing preclinical and clinical evidence that currently supports MSC-based therapies as safe and effective for the regeneration of the ocular surface.

Preclinical Evidence of MSC Efficacy in Ocular Surface Regeneration

Currently, there are many published preclinical studies showing the potential restorative effects of MSCs for ocular surface pathologies in experimental models [26, 27]. These studies were conducted with MSCs obtained from different sources such as bone marrow, adipose tissue, limbal stroma, umbilical cord, and others, and they were administered by different routes. The most relevant therapeutic preclinical studies that support the use of MSCs for the treatment of ocular surface diseases are described below.

MSCs for the Treatment of LSCD and Corneal Epithelial Damage

CLET is the current treatment of choice among stem cell-based interventions for LSCD. This surgical procedure aims to replace the destroyed limbal stem cell population by an autologous or allogeneic cell population with full functionality [6, 7]. However, this treatment has some limitations such as the low availability of donor tissues, or the difficulty in culturing the limbal epithelial cells [11]. Nevertheless, in recent years MSCs have been shown to be safe and effective and, therefore, good candidates for the treatment of LSCD [8, 11].

In experimental models of corneal epithelial damage and LSCD, transplantation of both bone marrow- and adipose tissue-derived MSCs reduces the clinical signs of LSCD such as neovascularization, corneal opacity, and epithelial defects (Fig. 15.1). The cells can be administered using routes such as sub-conjunctival injection [29–37], topical administration [38, 39], application of MSC-bearing amniotic membrane [28, 40–43] or MSC-bearing biopolymers [44–47], or by intravenous [48–53] and intraperitoneal injection [51]. MSCs obtained from other cell sources

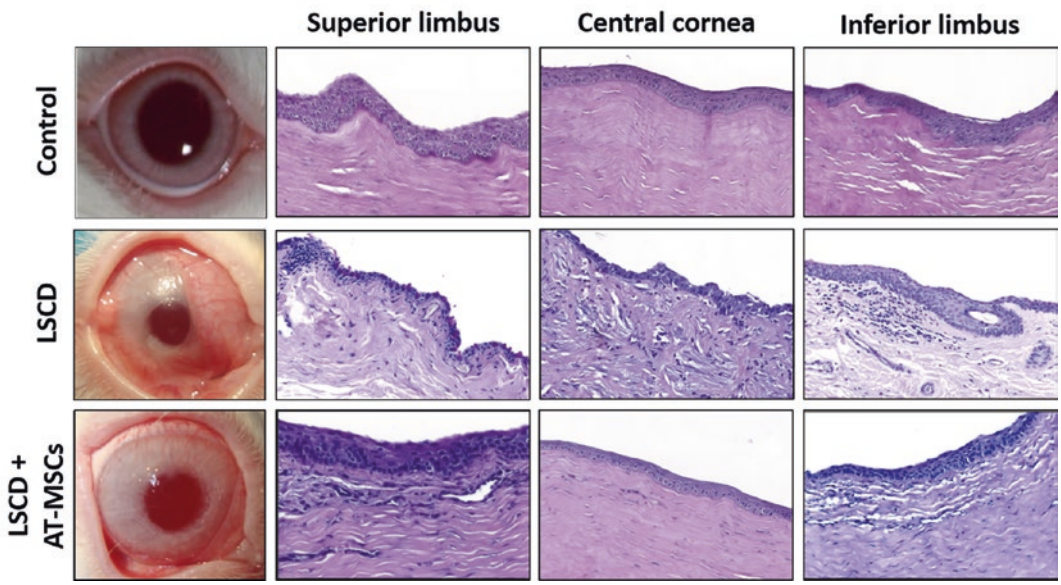

Fig. 15.1 Histological evaluation of ocular surface tissues from a rabbit model of total limbal stem cell deficiency (LSCD) treated with human adipose tissue-derived mesenchymal stem cells (AT-MSCs). Representative images of periodic acid-Schiff staining of ocular surface tissues obtained from healthy control eyes, untreated LSCD eyes, and LSCD eyes 8 weeks after being transplanted with AT-MSCs on amniotic membranes. Compared to healthy control eyes, untreated LSCD eyes had fewer epithelial layers, a disorganized corneal epithelium and stroma, and the presence of inflammatory cells (in dark purple) in the stroma of the central cornea. However, LSCD eyes transplanted with AT-MSCs showed fewer inflammatory cells and less disorganization in the epithelium and stroma of the central cornea than the untreated eyes. (Results from [28])

such as limbal stroma [35, 54, 55] or dental pulp [56] are also able to decrease these clinical signs in experimental models of LSCD. The preclinical data have also demonstrated that transplantation of MSCs to treat LSCD does not induce adverse events or toxicological effects, even with xenogeneic transplantation [28, 32, 38, 40, 41, 49, 51, 53, 54, 56, 57].

The molecular mechanism(s) of MSC-based tissue restoration is not yet fully understood. However, we do know that the transplanted cells reduce inflammation in the ocular surface of experimental models of corneal epithelial damage or LSCD, both by decreasing inflammatory infiltrates [28, 33, 38–40, 43, 57–59] and reducing proinflammatory cytokines such as tumour necrosis factor-alpha (TNF-α), IL-6, and IL-1β, among others [29–31, 34, 37, 53]. In addition, some authors have described the tumour necrosis factor-stimulated gene/protein-6 (TSG-6) as one of the molecules involved in the anti-inflammatory effect of MSCs in the cornea [29, 37, 51, 53]. Furthermore, other authors have also shown that MSCs have an antioxidant effect on the ocular surface of experimental models of corneal burns or LSCD [45–47, 49, 51]. Some authors have demonstrated migration and engraftment of the cells on the ocular surface after topical administration [28, 38–40, 42, 56], sub-conjunctival injection [29, 34, 35, 54], and intravenous injection [48, 50, 52, 58]. However, others did not observe the presence of MSCs at the area of damage after topical administration on amniotic membranes [55], or sub-conjunctival [30, 33, 37], intravenous, or intraperitoneal injections [51]. Therefore, the evidence suggests that MSCs can promote therapeutic effects at a distance from the target tissues by releasing trophic factors.

Additionally, some preclinical data showed recovery of the differentiated corneal epithelial cell markers cytokeratin (CK) 3 and CK12 [28, 41, 43, 47, 50, 56, 60] and the limbal epithelial stem cell markers p63, CK15, and ATP-binding

cassette sub-family G member 2 [28, 29, 41, 50, 56, 58, 61] in the ocular surface of the MSC-transplanted experimental LSCD models. Although transdifferentiation of MSCs into corneal and limbal epithelial cells has not been demonstrated in vivo, MSCs seem to contribute to the recovery of the corneal and limbal phenotype by secreting factors and helping resident stem cells.

MSCs for the Treatment of DED

DED is a multifactorial and inflammatory-based pathology [62] that affects between 5.5% and 35% of the world population [63]. It presents with varying severity of symptoms such as pain and blurred vision, and the most severe cases can lead to corneal ulcers, infections, and even perforations [64, 65]. DED is also characterized by an increase of inflammatory molecules and reactive oxygen species and by a decrease of anti-inflammatory and growth factors in the ocular surface [66, 67].

In this context, MSCs have been proposed as a possible treatment for patients affected by the most severe forms of DED. MSCs isolated from bone marrow [68–72], adipose tissue [73–75], or umbilical cord [76] have been therapeutically administered in experimental in vivo DED models using different routes of delivery such as topical application through eye drops [69], intraorbital injection around or directly into lacrimal glands [70, 73–75], and intraperitoneal [71] or intravenous injections [68, 72, 76, 77]. These studies have shown that MSC therapy to treat DED improves tear volume and tear film stability [69–72, 74–76], maintains corneal epithelial integrity [72, 74], increases the number of conjunctival goblet cells [69, 70], and reduces ocular surface hyperemia [74–76]. Some studies also reported lacrimal gland regeneration [72, 77]. Moreover, several authors found decreased ocular surface inflammation following MSC treatment. The reduced inflammation was associated with decreased lymphocytic foci [71, 73] or CD4+ T cell infiltration [70], maintained or increased regulatory T cell (Treg) and Th2 presence [68, 71, 72], modulation of macrophage infiltration [77] or macrophage maturation [76], decreased proinflammatory factors such as TNF-α [72, 76], IL-1 [72], or IL-6 [76], and/or increased anti-inflammatory factors such as IL-10 [72, 76] or epidermal growth factor [72].

One of the most severe forms of DED occurs in the context of chronic graft-versus-host disease (GVHD) that can develop after allogeneic haematopoietic stem cell transplantation, appearing in 60% of patients [78]. GVHD with ocular damage (oGVHD) is caused by the immune response produced by the immunocompetent cells from the donor graft that "attack" the recipient ocular surface (conjunctiva, cornea, limbus, and tear film) and all of the glands that produce tear components. This attack produces chronic ocular inflammation and ocular tissue destruction [79–83].

Because of the high immunoregulatory and immunosuppressive capacity and the ocular anti-inflammatory and ocular tissue regenerative potential of MSCs, they have been successfully tested as therapy in vivo models of DED associated with oGVHD [83–86]. Sub-conjunctival injection of bone marrow-derived MSCs in a mouse model of GVHD decreased both the presence of CD3+ T cells in corneal tissues and corneal keratinization [84, 85]. In addition, other authors showed that for mice with GHVD, MSCs can engraft into lacrimal gland tissues and secrete collagen type I that reduces the pathogenic fibrosis of the gland [86]. All of these preclinical results suggest that MSCs are a promising cell therapy to treat DED, although more studies are needed to optimize it [87–89].

MSCs Promote Corneal Allograft Survival

Corneal transplantation or keratoplasty is the most frequent type of human tissue transplant [90]. In low-risk patients, the survival rate of full-thickness corneal grafts at 1 year is around 90% (even without donor-recipient major histocompatibility complex matching). However, in high-

risk patients with corneal neovascularization and inflammation, the long-term prognosis is lower than 50% [91, 92]. Topical corticosteroids are currently the most common immunosuppressive drugs used in corneal transplantation. However, their effectiveness is lower in high-risk patients, and prolonged application can provoke numerous side effects [93, 94]. Therefore, alternative therapeutic strategies are required to improve the prognosis of long-term corneal transplantation and to diminish the adverse side effects of the current pharmacological treatments.

Preclinical studies have shown that systemic and sub-conjunctival administration of MSCs can prolong corneal allograft survival. Therefore, their administration in combination with or in the absence of immunosuppressive drugs could help prevent immune rejection of the corneal graft [95–97]. The mechanism by which MSCs modulate corneal allograft survival has not been fully elucidated yet; however, it has been associated with inhibition of antigen-presenting cell activation, change in Th1/Th2 balance, reduction of $CD4^+$ T cell infiltration, and induction of Treg proliferation [95, 96, 98, 99]. These immunomodulatory and immunosuppressive actions are related to the MSC-dependent secretion of soluble factors such as TSG-6, hepatocyte growth factor, nitric oxide, and prostaglandin E2 [100, 101]. Despite the encouraging preclinical results obtained so far, there are still many issues and challenges that need to be overcome before the clinical application of this therapeutic approach in humans is attempted. These include determination (1) if one or a few sources of MSCs produce better clinical results than others, (2) the best dose and route of administration, and also (3) the most effective frequency and timing of cell administration [95, 96].

Clinical Evidence of MSC Efficacy in Ocular Surface Pathology

Most studies of ocular surface stem cell functional failure have focused on the LESCs that reside in the corneoscleral limbal niche. However, there are several other potential stem cell niches in the ocular surface that could help maintain cellular homeostasis of the corneal stroma, conjunctiva, and meibomian glands [102]. And although the main stem cell deficiency at the ocular surface is the LSCD, causing corneal opacity, other pathologies are starting to be thought of as amenable to therapy with stem cells, as reviewed in a previous section on preclinical studies. The following are the most relevant ocular surface pathologies for which stem cell treatment, most specifically with MSCs, have already been translated into clinical practice and published.

MSCs for the Treatment of LSCD

The destruction or dysfunction of the stem cells residing in the limbal niche, leading to LSCD, can have several aetiologies: chemical injuries, immune-mediated cicatrizing diseases of the ocular surface (e.g., Stevens-Johnson syndrome and its spectrum, mucous membrane pemphigoid, atopic keratoconjunctivitis, ocular rosacea), sequelae of infectious keratitis, or primary causes such as congenital aniridia or ectodermal dysplasia. All of these conditions lead to neovascular pannus, an unstable corneal surface, and eventually, visual deficit and chronic nociceptive pain [11]. Diseases leading to LSCD are difficult to manage, requiring complex medical and surgical approaches. Upon the development of LSCD, the problem becomes unsolvable unless new stem cells can be provided in the correct location [103]. Since the first transplantations of autologous limbal tissue in 1989 [104] and the cultivated autologous limbal cells in 1997 [105] to the more recent techniques of delivering limbal tissue (simple limbal epithelial transplantation) in 2012 [106] or the cultivation of autologous and allogeneic stem cells (reviewed in [11]), many cases have been successfully treated.

There is still a big need for the development of safer, more accessible techniques that avoid the necessity of immunosuppression when the source of tissue or cells must be allogeneic, as it is often the case in bilateral diseases. This can be achieved

with MSCs due to their many beneficial properties, especially the absence of immunogenicity. The use of allogeneic bone marrow-derived MSCs has already been applied in the clinic. A randomized controlled clinical trial demonstrated the benefits of this stem cell type, which was assessed to be comparable and slightly superior to CLET in the management of LSCD [8]. This methodology avoids the use of immunosuppression but can only be applied in places where a Cell Processing Unit that complies with the accepted standards of good manufacturing procedures [107] is available. Therefore, work must progress to find solutions that are more accessible and that perhaps can do more to replace the damage limbal niche instead of just providing stem cells.

MSCs for the Treatment of DED

The most severe forms of DED are still difficult to manage with current therapies. Undoubtedly, DED associated with chronic GVHD is one of the most, if not the most, severe form of DED. It can be devastating with unbearable pain, photophobia, and reduced quality of life [108]. The therapeutic efficacy of MSCs in the treatment of DED was first reported in a 2012 clinical study of 22 chronic GVHD patients with refractory DED. The patients were intravenously transfused with allogeneic MSCs, and 55% achieved clinical improvement that was attributed to the generation of CD8+CD28-Tcells [109].

In 2020, 7 patients with severe Sjögren's syndrome-associated DED were treated with adipose tissue-derived MSCs that were delivered by a single transconjunctival injection into the main lacrimal gland. The treatment was well tolerated, and patients showed great improvement that lasted up to 16 weeks [110].

In 2022, a clinical trial demonstrated the beneficial effects of exosomes from human umbilical cord MSCs that were administered as eye drops to treat DED associated with chronic GVHD in 14 patients [111]. Exosomes are a sub-type of extracellular vesicles (EVs) of endosomal origin with a size range of ~30 to ~200 nm in diameter. EVs are lipid-encapsulated membranous vesicles that are released by cells into the extracellular spaces and contain components (protein, DNA, and RNA) from the cells that release them. While that trial was run for only 14 days, the signs and symptoms of the GVHD-dependent DED were significantly mitigated. Thus, this cell-free approach for delivering MSC components to treat DED in general and specifically DED associated with chronic GVHD is promising. The long-term effects and safety remain to be demonstrated, and MSC exosome-based therapy still faces challenges such as determining the stability during storage and transport, and determination of the heterogeneity of the exosome composition.

Conclusions and Future Perspectives

MSC-based therapies for ocular surface pathology, from corneal blindness due to LSCD, to immune-based inflammatory diseases such as DED, or to corneal transplantation, show great potential to reduce the onset of vision loss. Current preclinical evidence has already been partially translated into clinical applications. These studies, of course, still need to be confirmed with larger controlled clinical trials, and some questions and technical problems remain to be solved. Among them, it should be elucidated if some MSC sources are better than others, and what are the safest and most clinically effective MSC doses and routes of administration. In addition, it is essential to develop standardized protocols for the culture and characterization of MSCs so that the results obtained in different preclinical and clinical centres can be properly compared. Despite all the challenges and unknowns that remain, the future of MSCs in the ocular surface is certainly promising (Fig. 15.2).

Over the last few years, EVs derived from MSCs have strongly emerged as a potential alternative to MSC treatment. EVs appear to replicate many of the therapeutic effects of MSCs but without most of the safety risks and

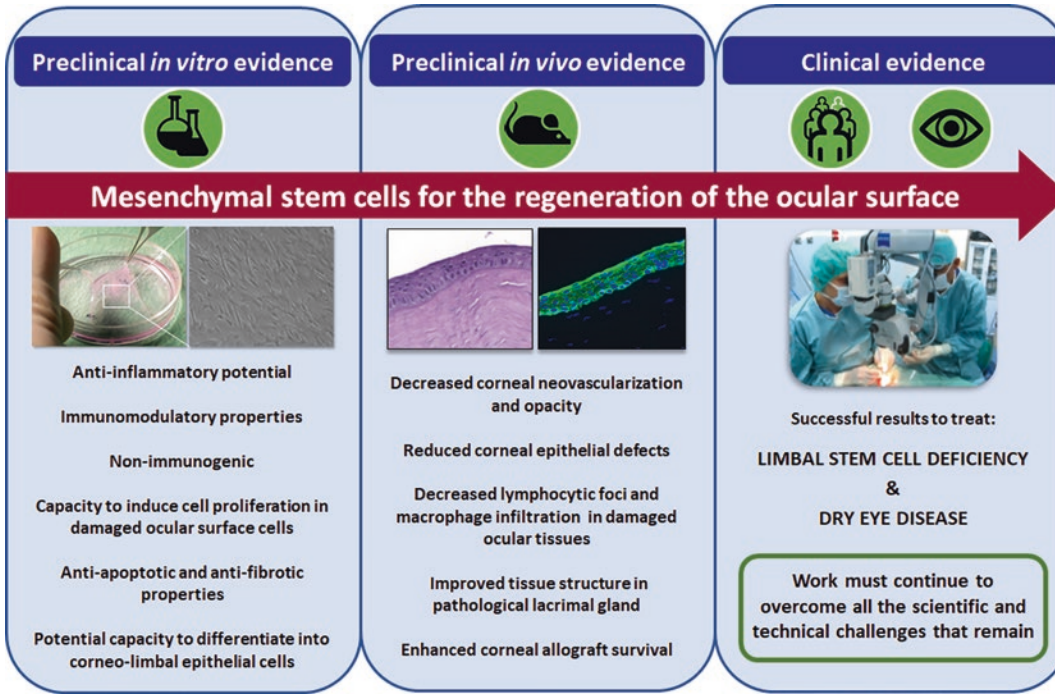

Fig. 15.2 Mesenchymal stem cells for the regeneration of the ocular surface: from preclinical to clinical evidence

regulatory issues related to live cell therapies [112, 113]. As a consequence, MSC-derived EVs could represent a safer and more cost-effective alternative than cell therapies with live MSCs. Currently, a lot of preclinical evidence supports the idea that MSC-derived EV application in corneal disease models induces anti-fibrotic, anti-apoptotic, and anti-inflammatory effects, and that it promotes corneal epithelial cell proliferation. These observations are consistent with the induction by EVs of accelerated corneal epithelial wound healing and reduced corneal epithelial defects [114, 115]. The therapeutic development of EVs is still at an early stage, and the EV mechanism of action in ocular surface diseases remains to be fully elucidated. Nevertheless, the solid evidence obtained from preclinical studies strongly suggests that, in the near future, isolated MSC-derived EVs could become a new therapeutic strategy for patients suffering from ocular surface diseases.

Take Home Notes

- MSC-based treatments for ocular surface pathology have shown potential therapeutic value.
- Preclinical studies have revealed that MSCs can prolong corneal allograft survival.
- Preclinical evidence supporting the use of MSCs for treating LSCD and DED has already been translated into clinical practice.
- Although the results obtained so far on the use of MSCs for ocular surface pathology are very encouraging, more preclinical and clinical studies are needed to confirm them.
- The clinical future of MSC-based therapy, and potentially MSC-derived EV therapy, in the ocular surface, is undoubtedly very promising.

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