BOOK: Modern Keratoplasty: Surgical Techniques and Indications

Section: Epithelial Lamellar Keratoplasty

Chapter 15: Mesenchymal Stem Cells for Regeneration of the Ocular Surface

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Abstract

Mesenchymal tissues can provide a source of stem cells (MSCs) that are readily available, non-immunogenic, and which have tremendous regenerative and anti-inflammatory properties. For these reasons, MSCs have emerged as very attractive candidates for cell-based therapies in numerous and diverse clinical applications including the treatment of ocular surface diseases such as limbal stem cell deficiency, dry eye disease, or even as a potential therapy to improve corneal allograft survival. Although some of the current preclinical evidence has already been successfully translated into clinical applications, work must continue to overcome all of the scientific and technical challenges that remain unsolved. This book chapter summarizes the main preclinical and clinical evidence that strongly supports MSC-based therapies as safe and effective treatments for the regeneration of the ocular surface.

Bullet 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.

Keywords: Mesenchymal stem cells; MSCs; Ocular surface; Cornea, Corneal epithelium; Limbal stem cell deficiency; LSCD; Dry eye disease; Corneal transplant; Keratoplasty.

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: Tumor necrosis factor alpha

Treg: Regulatory T cells

TSG-6: Tumor necrosis factor-stimulated gene/protein-6

1.1 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 the 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 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.

1.2 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 the 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. 10.1). The cells can be administered using routes such as subconjunctival injection [28–36], topical administration [37, 38], application of MSC-bearing amniotic membrane [39–43] or MSC-bearing biopolymers [44–47], or by intravenous [48–53] and intraperitoneal injection [51]. MSCs obtained from other cell sources such as limbal stroma [34, 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 [31, 37, 39, 40, 43, 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 [32, 37–39, 42, 43, 57–59] and reducing pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), IL-6, and IL-1β, among others [28–30, 33, 36, 53]. In addition, some authors have described the tumor necrosis factor-stimulated gene/protein-6 (TSG-6) as one of the molecules involved in the anti-inflammatory effect of MSCs in the cornea [28, 36, 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 [37–39, 41, 43, 56], subconjunctival injection [28, 33, 34, 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 subconjunctival [29, 32, 36], 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 [40, 42, 43, 47, 50, 56, 60] and the limbal epithelial stem cell markers p63, CK15, and ATP-binding cassette sub-family G member 2 [28, 40, 43, 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 eyedrops [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 hematopoietic 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 *in vivo* models of DED associated with oGVHD [83–86]. Subconjunctival 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 subconjunctival 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

239 results obtained so far, there are still many issues and challenges that need to be
240 overcome before the clinical application of this therapeutic approach in humans is
241 attempted. These include determination (1) if one or a few sources of MSCs produce
242 better clinical results than others, (2) the best dose and route of administration, and also
243 (3) the most effective frequency and timing of cell administration [95, 96].

244 **1.3 Clinical evidence of MSC efficacy in ocular surface pathology**

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

254 *MSCs for the treatment of LSCD*

255 The destruction or dysfunction of the stem cells residing in the limbal niche, leading to
256 LSCD, can have several etiologies: chemical injuries, immune-mediated cicatrizing
257 diseases of the ocular surface (e.g., Stevens-Johnson syndrome and its spectrum,
258 mucous membrane pemphigoid, atopic keratoconjunctivitis, ocular rosacea), sequelae of
259 infectious keratitis, or primary causes such as congenital aniridia or ectodermal
260 dysplasia. All of these conditions lead to neovascular pannus, an unstable corneal
261 surface, and eventually, visual deficit and chronic nociceptive pain [11]. Diseases
262 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 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 eyedrops to treat DED associated with chronic GVHD in 14 patients [111]. Exosomes are a subtype 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.

1.4 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 centers can be properly compared. Despite all the challenges and unknowns that remain, the future of MSCs in the ocular surface is certainly promising (Fig. 10.2).

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 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 clinic 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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References

1. Flaxman SR, Bourne RRA, Resnikoff S, Ackland P, Braithwaite T, Cicinelli M V., Das A, Jonas JB, Keeffe J, Kempen J, Leasher J, Limburg H, Naidoo K, Pesudovs K, Silvester A, Stevens GA, Tahhan N, Wong T, Taylor H, Arditi A, Barkana Y, Bozkurt B, Bron A, Budenz D, Cai F, Casson R, Chakravarthy U, Choi J, Congdon N, Dana R, Dandona R, Dandona L, Dekaris I, Del Monte M, Deva J, Dreer L, Ellwein L, Frazier M, Frick K, Friedman D, Furtado J, Gao H, Gazzard G, George R, Gichuhi S, Gonzalez V, Hammond B, Hartnett ME, He M, Hejtmancik J, Hirai F, Huang J, Ingram A, Javitt J, Joslin C, Khairallah M, Khanna R, Kim J, Lambrou G, Lansingh VC, Lanzetta P, Lim J, Mansouri K, Mathew A, Morse A, Munoz B, Musch D, Nangia V, Palaiou M, Parodi MB, Pena FY, Peto T, Quigley H, Raju M, Ramulu P, Reza D, Robin A, Rossetti L, Saaddine J, Sandar M, Serle J, Shen T, Shetty R, Sieving P, Silva JC, Sitorus RS, Stambolian D, Tejedor J, Tielsch J, Tsilimbaris M, van Meurs J, Varma R, Virgili G, Wang YX, Wang NL, West S, Wiedemann P, Wormald R, Zheng Y (2017) Global causes of blindness and distance vision impairment 1990–2020: a systematic review and meta-analysis. The Lancet Global Health 5:e1221–e1234. https://doi.org/10.1016/S2214-109X(17)30393-5
2. Cotsarelis G, Cheng SZ, Dong G, Sun TT, Lavker RM (1989) Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. Cell 57:201–209. https://doi.org/10.1016/0092-8674(89)90958-6
3. Li W, Hayashida Y, Chen Y-T, Tseng SC (2007) Niche regulation of corneal epithelial stem cells at the limbus. Cell Research 17:26–36. https://doi.org/10.1038/sj.cr.7310137
4. Schermer A, Galvin S, Sun TT (1986) Differentiation-related expression of a major 64K corneal keratin in vivo and in culture suggests limbal location of corneal epithelial stem cells. Journal of Cell Biology 103:49–62. https://doi.org/10.1083/jcb.103.1.49
5. Dua HS, Saini JS, Azuara-Blanco A, Gupta P (2000) Limbal stem cell deficiency: concept, aetiology, clinical presentation, diagnosis and management. Indian Journal of Ophthalmology 48:83–92
6. Rama P, Matuska S, Paganoni G, Spinelli A, De Luca M, Pellegrini G (2010) Limbal Stem-Cell Therapy and Long-Term Corneal Regeneration. New England Journal of Medicine 363:147–155. https://doi.org/10.1056/NEJMoa0905955
7. Ramírez BE, Sánchez A, Herreras JM, Fernández I, García-Sancho J, Nieto-Miguel T, Calonge M (2015) Stem Cell Therapy for Corneal Epithelium Regeneration following Good Manufacturing and Clinical Procedures. BioMed Research International 2015:1–19. https://doi.org/10.1155/2015/408495
8. Calonge M, Pérez I, Galindo S, Nieto-Miguel T, López-Paniagua M, Fernández I, Alberca M, García-Sancho J, Sánchez A, Herreras JM (2019) A proof-of-concept clinical trial using mesenchymal stem cells for the treatment of corneal epithelial stem cell deficiency. Translational Research 206:18–40. https://doi.org/10.1016/J.TRSL.2018.11.003
9. Behaegel J, Zakaria N, Tassignon M-J, Leysen I, Bock F, Koppen C, Ní Dhubhghaill S (2019) Short- and Long-Term Results of Xenogeneic-Free Cultivated Autologous and Allogeneic Limbal Epithelial Stem Cell Transplantations. Cornea 38:1543–1549. https://doi.org/10.1097/ICO.0000000000002153
10. Shimazaki J, Satake Y, Higa K, Yamaguchi T, Noma H, Tsubota K (2020) Long-term outcomes of cultivated cell sheet transplantation for treating total limbal stem cell deficiency. The Ocular Surface 18:663–671. https://doi.org/10.1016/j.jtos.2020.06.005
11. Calonge M, Nieto-Miguel T, de la Mata A, Galindo S, Herreras JM, López-Paniagua M (2021) Goals and challenges of stem cell-based therapy for corneal blindness due to limbal deficiency. Pharmaceutics 13:1483. https://doi.org/10.3390/pharmaceutics13091483
12. Rohban R, Pieber TR (2017) Mesenchymal Stem and Progenitor Cells in Regeneration: Tissue Specificity and Regenerative Potential. Stem Cells International 2017:1–16. https://doi.org/10.1155/2017/5173732
13. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F., Krause DS, Deans RJ, Keating A, Prockop DJ, Horwitz EM (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8:315–317. https://doi.org/10.1080/14653240600855905
14. Zhang L, Coulson-Thomas VJ, Ferreira TG, Kao WWY (2015) Mesenchymal stem cells for treating ocular surface diseases. BMC Ophthalmology 15:155. https://doi.org/10.1186/s12886-015-0138-4
15. O'Callaghan AR, Daniels JT (2011) Concise Review: Limbal Epithelial Stem Cell Therapy:

Controversies and Challenges. Stem Cells 29:1923–1932. https://doi.org/10.1002/stem.756
16. Luetzkendorf J, Nerger K, Hering J, Moegel A, Hoffmann K, Hoefers C, Mueller-Tidow C, Mueller LP (2015) Cryopreservation does not alter main characteristics of Good Manufacturing Process–grade human multipotent mesenchymal stromal cells including immunomodulating potential and lack of malignant transformation. Cytotherapy 17:186–198. https://doi.org/10.1016/j.jcyt.2014.10.018
17. Ho MSH, Mei SHJ, Stewart DJ (2015) The Immunomodulatory and Therapeutic Effects of Mesenchymal Stromal Cells for Acute Lung Injury and Sepsis. Journal of Cellular Physiology 230:2606–2617. https://doi.org/10.1002/jcp.25028
18. Griffin MD, Ritter T, Mahon BP (2010) Immunological Aspects of Allogeneic Mesenchymal Stem Cell Therapies. Human Gene Therapy 21:1641–1655. https://doi.org/10.1089/hum.2010.156
19. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH (2001) Multilineage Cells from Human Adipose Tissue: Implications for Cell-Based Therapies. Tissue Engineering 7:211–228. https://doi.org/10.1089/107632701300062859
20. Phinney DG, Prockop DJ (2007) Concise Review: Mesenchymal Stem/Multipotent Stromal Cells: The State of Transdifferentiation and Modes of Tissue Repair—Current Views. Stem Cells 25:2896–2902. https://doi.org/10.1634/stemcells.2007-0637
21. Kuo TK, Ho JH, Lee OK (2009) Mesenchymal Stem Cell Therapy for Nonmusculoskeletal Diseases: Emerging Applications. Cell Transplantation 18:1013–1028. https://doi.org/10.3727/096368909X471206
22. Joe AW, Gregory-Evans K (2010) Mesenchymal Stem Cells and Potential Applications in Treating Ocular Disease. Current Eye Research 35:941–952. https://doi.org/10.3109/02713683.2010.516466
23. Chamberlain G, Fox J, Ashton B, Middleton J (2007) Concise Review: Mesenchymal Stem Cells: Their Phenotype, Differentiation Capacity, Immunological Features, and Potential for Homing. Stem Cells 25:2739–2749. https://doi.org/10.1634/stemcells.2007-0197
24. Ren G, Chen X, Dong F, Li W, Ren X, Zhang Y, Shi Y (2012) Concise Review: Mesenchymal Stem Cells and Translational Medicine: Emerging Issues. Stem Cells Translational Medicine 1:51–58. https://doi.org/10.5966/sctm.2011-0019
25. Nieto-Miguel T, Galindo S, López-Paniagua M, Pérez I, Herreras JM, Calonge M (2019) Cell Therapy Using Extraocular Mesenchymal Stem Cells. In: Alió, J., Alió del Barrio, J., Arnalich-Montiel F (ed) Corneal Regeneration . Essentials in Ophthalmology. Springer, Cham., pp 231–262
26. Beeken LJ, Ting DSJ, Sidney LE (2021) Potential of mesenchymal stem cells as topical immunomodulatory cell therapies for ocular surface inflammatory disorders. Stem Cells Translational Medicine 10:39–49. https://doi.org/10.1002/sctm.20-0118
27. Galindo S, de la Mata A, López-Paniagua M, Herreras JM, Pérez I, Calonge M, Nieto-Miguel T (2021) Subconjunctival injection of mesenchymal stem cells for corneal failure due to limbal stem cell deficiency: state of the art. Stem Cell Research & Therapy 12:60. https://doi.org/10.1186/s13287-020-02129-0
28. Di G, Du X, Qi X, Zhao X, Duan H, Li S, Xie L, Zhou Q (2017) Mesenchymal Stem Cells Promote Diabetic Corneal Epithelial Wound Healing Through TSG-6–Dependent Stem Cell Activation and Macrophage Switch. Investigative Opthalmology & Visual Science 58:4344. https://doi.org/10.1167/iovs.17-21506
29. Yao L, Li Z, Su W, Li Y, Lin M, Zhang W, Liu Y, Wan Q, Liang D (2012) Role of Mesenchymal Stem Cells on Cornea Wound Healing Induced by Acute Alkali Burn. PLoS ONE 7:e30842. https://doi.org/10.1371/journal.pone.0030842
30. Ke Y, Wu Y, Cui X, Liu X, Yu M, Yang C, Li X (2015) Polysaccharide Hydrogel Combined with Mesenchymal Stem Cells Promotes the Healing of Corneal Alkali Burn in Rats. PLOS ONE 10:e0119725. https://doi.org/10.1371/journal.pone.0119725
31. Lin H-F, Lai Y-C, Tai C-F, Tsai J-L, Hsu H-C, Hsu R-F, Lu S-N, Feng N-H, Chai C-Y, Lee C-H (2013) Effects of cultured human adipose-derived stem cells transplantation on rabbit cornea regeneration after alkaline chemical burn. The Kaohsiung Journal of Medical Sciences 29:14–18. https://doi.org/10.1016/j.kjms.2012.08.002
32. Ghazaryan E, Zhang Y, He Y, Liu X, Li Y, Xie J, Su G (2016) Mesenchymal stem cells in corneal neovascularization: Comparison of different application routes. Molecular Medicine Reports 14:3104–3112. https://doi.org/10.3892/mmr.2016.5621
33. Shukla S, Mittal SK, Foulsham W, Elbasiony E, Singhania D, Sahu SK, Chauhan SK (2019) Therapeutic efficacy of different routes of mesenchymal stem cell administration in corneal injury. The Ocular Surface 17:729–736. https://doi.org/10.1016/j.jtos.2019.07.005

34. Li G, Zhang Y, Cai S, Sun M, Wang J, Li S, Li X, Tighe S, Chen S, Xie H, Zhu Y (2018) Human limbal niche cells are a powerful regenerative source for the prevention of limbal stem cell deficiency in a rabbit model. Scientific Reports 8:6566. https://doi.org/10.1038/s41598-018-24862-6
35. Pan J, Wang X, Li D, Li J, Jiang Z (2019) MSCs inhibits the angiogenesis of HUVECs through the miR-211/Prox1 pathway. The Journal of Biochemistry 166:107–113. https://doi.org/10.1093/jb/mvz038
36. Zhang N, Luo X, Zhang S, Liu R, Liang L, Su W, Liang D (2021) Subconjunctival injection of tumor necrosis factor-α pre-stimulated bone marrow-derived mesenchymal stem cells enhances anti-inflammation and anti-fibrosis in ocular alkali burns. Graefe's Archive for Clinical and Experimental Ophthalmology 259:929–940. https://doi.org/10.1007/s00417-020-05017-8
37. Zeppieri M, Salvetat ML, Beltrami AP, Cesselli D, Bergamin N, Russo R, Cavaliere F, Varano GP, Alcalde I, Merayo J, Brusini P, Beltrami CA, Parodi PC (2013) Human Adipose-Derived Stem Cells for the Treatment of Chemically Burned Rat Cornea: Preliminary Results. Current Eye Research 38:451–463. https://doi.org/10.3109/02713683.2012.763100
38. Oh JY, Kim MK, Shin MS, Lee HJ, Ko JH, Wee WR, Lee JH (2008) The Anti-Inflammatory and Anti-Angiogenic Role of Mesenchymal Stem Cells in Corneal Wound Healing Following Chemical Injury. Stem Cells 26:1047–1055. https://doi.org/10.1634/stemcells.2007-0737
39. Ma Y, Xu Y, Xiao Z, Yang W, Zhang C, Song E, Du Y, Li L (2006) Reconstruction of chemically burned rat corneal surface by bone marrow-derived human mesenchymal stem cells. Stem cells (Dayton, Ohio) 24:315–21. https://doi.org/10.1634/stemcells.2005-0046
40. Rohaina CM, Then KY, Ng AMH, Wan Abdul Halim WH, Zahidin AZM, Saim A, Idrus RBH (2014) Reconstruction of limbal stem cell deficient corneal surface with induced human bone marrow mesenchymal stem cells on amniotic membrane. Translational Research 163:200–210. https://doi.org/10.1016/j.trsl.2013.11.004
41. Pınarlı FA, Okten G, Beden U, Fışgın T, Kefeli M, Kara N, Duru F, Tomak L (2014) Keratinocyte growth factor-2 and autologous serum potentiate the regenerative effect of mesenchymal stem cells in cornea damage in rats. International journal of ophthalmology 7:211–9. https://doi.org/10.3980/j.issn.2222-3959.2014.02.05
42. Jiang T-S, Cai L, Ji W-Y, Hui Y-N, Wang Y-S, Hu D, Zhu J (2010) Reconstruction of the corneal epithelium with induced marrow mesenchymal stem cells in rats. Molecular vision 16:1304–16. https://doi.org/144 [pii]
43. Galindo S, Herreras JM, López-Paniagua M, Rey E, de la Mata A, Plata-Cordero M, Calonge M, Nieto-Miguel T (2017) Therapeutic Effect of Human Adipose Tissue-Derived Mesenchymal Stem Cells in Experimental Corneal Failure Due to Limbal Stem Cell Niche Damage. Stem Cells 35:2160–2174. https://doi.org/10.1002/stem.2672
44. Cejkova J, Trosan P, Cejka C, Lencova A, Zajicova A, Javorkova E, Kubinova S, Sykova E, Holan V (2013) Suppression of alkali-induced oxidative injury in the cornea by mesenchymal stem cells growing on nanofiber scaffolds and transferred onto the damaged corneal surface. Experimental Eye Research 116:312–323. https://doi.org/10.1016/j.exer.2013.10.002
45. Cejka C, Cejkova J, Trosan P, Zajicova A, Sykova E, Holan V (2016) Transfer of mesenchymal stem cells and cyclosporine A on alkali-injured rabbit cornea using nanofiber scaffolds strongly reduces corneal neovascularization and scar formation. Histology and histopathology 31:969–80. https://doi.org/10.14670/HH-11-724
46. Cejka C, Holan V, Trosan P, Zajicova A, Javorkova E, Cejkova J (2016) The Favorable Effect of Mesenchymal Stem Cell Treatment on the Antioxidant Protective Mechanism in the Corneal Epithelium and Renewal of Corneal Optical Properties Changed after Alkali Burns. Oxidative Medicine and Cellular Longevity 2016:1–12. https://doi.org/10.1155/2016/5843809
47. Holan V, Trosan P, Cejka C, Javorkova E, Zajicova A, Hermankova B, Chudickova M, Cejkova J (2015) A Comparative Study of the Therapeutic Potential of Mesenchymal Stem Cells and Limbal Epithelial Stem Cells for Ocular Surface Reconstruction. Stem Cells Translational Medicine 4:1052–1063. https://doi.org/10.5966/sctm.2015-0039
48. Mittal SK, Omoto M, Amouzegar A, Sahu A, Rezazadeh A, Katikireddy KR, Shah DI, Sahu SK, Chauhan SK (2016) Restoration of Corneal Transparency by Mesenchymal Stem Cells. Stem Cell Reports 7:583–590. https://doi.org/10.1016/j.stemcr.2016.09.001
49. Lee RH, Yu JM, Foskett AM, Peltier G, Reneau JC, Bazhanov N, Oh JY, Prockop DJ (2014) TSG-6 as a biomarker to predict efficacy of human mesenchymal stem/progenitor cells (hMSCs) in modulating sterile inflammation in vivo. Proceedings of the National Academy of Sciences 111:16766–16771. https://doi.org/10.1073/pnas.1416121111
50. Lan Y, Kodati S, Lee HS, Omoto M, Jin Y, Chauhan SK (2012) Kinetics and Function of

Mesenchymal Stem Cells in Corneal Injury. Investigative Opthalmology & Visual Science 53:3638. https://doi.org/10.1167/iovs.11-9311

51. Roddy GW, Oh JY, Lee RH, Bartosh TJ, Ylostalo J, Coble K, Rosa RH, Prockop DJ (2011) Action at a Distance: Systemically Administered Adult Stem/Progenitor Cells (MSCs) Reduce Inflammatory Damage to the Cornea Without Engraftment and Primarily by Secretion of TNF-α Stimulated Gene/Protein 6. Stem Cells 29:1572–1579. https://doi.org/10.1002/stem.708

52. Ye J, Yao K, Kim JC (2006) Mesenchymal stem cell transplantation in a rabbit corneal alkali burn model: engraftment and involvement in wound healing. Eye 20:482–490. https://doi.org/10.1038/sj.eye.6701913

53. Yun YI, Park SY, Lee HJ, Ko JH, Kim MK, Wee WR, Reger RL, Gregory CA, Choi H, Fulcher SF, Prockop DJ, Oh JY (2017) Comparison of the anti-inflammatory effects of induced pluripotent stem cell–derived and bone marrow–derived mesenchymal stromal cells in a murine model of corneal injury. Cytotherapy 19:28–35. https://doi.org/10.1016/j.jcyt.2016.10.007

54. Acar U, Pinarli FA, Acar DE, Beyazyildiz E, Sobaci G, Ozgermen BB, Sonmez AA, Delibasi T (2015) Effect of Allogeneic Limbal Mesenchymal Stem Cell Therapy in Corneal Healing: Role of Administration Route. Ophthalmic Research 53:82–89. https://doi.org/10.1159/000368659

55. Nili E, Li FJ, Dawson RA, Lau C, McEwan B, Barnett NL, Weier S, Walshe J, Richardson NA, Harkin DG (2019) The Impact of Limbal Mesenchymal Stromal Cells on Healing of Acute Ocular Surface Wounds Is Improved by Pre-cultivation and Implantation in the Presence of Limbal Epithelial Cells. Cell Transplantation 28:1257–1270. https://doi.org/10.1177/0963689719858577

56. Gomes JÁP, Geraldes Monteiro B, Melo GB, Smith RL, Cavenaghi Pereira da Silva M, Lizier NF, Kerkis A, Cerruti H, Kerkis I (2010) Corneal Reconstruction with Tissue-Engineered Cell Sheets Composed of Human Immature Dental Pulp Stem Cells. Investigative Opthalmology & Visual Science 51:1408. https://doi.org/10.1167/iovs.09-4029

57. Espandar L, Caldwell D, Watson R, Blanco-Mezquita T, Zhang S, Bunnell B (2014) Application of Adipose-Derived Stem Cells on Scleral Contact Lens Carrier in an Animal Model of Severe Acute Alkaline Burn. Eye & Contact Lens: Science & Clinical Practice 40:243–247. https://doi.org/10.1097/ICL.0000000000000045

58. Ahmed SK, Soliman AA, Omar SMM, Mohammed WR (2015) Bone Marrow Mesenchymal Stem Cell Transplantation in a Rabbit Corneal Alkali Burn Model (A Histological and Immune Histo-chemical Study). International Journal of Stem Cells 8:69–78. https://doi.org/10.15283/ijsc.2015.8.1.69

59. Almaliotis D, Koliakos G, Papakonstantinou E, Komnenou A, Thomas A, Petrakis S, Nakos I, Gounari E, Karampatakis V (2015) Mesenchymal stem cells improve healing of the cornea after alkali injury. Graefe's Archive for Clinical and Experimental Ophthalmology 253:1121–1135. https://doi.org/10.1007/s00417-015-3042-y

60. Gu S, Xing C, Han J, Tso MOM, Hong J (2009) Differentiation of rabbit bone marrow mesenchymal stem cells into corneal epithelial cells in vivo and ex vivo. Molecular Vision 15:99–107. https://doi.org/10 [pii]

61. Reinshagen H, Auw-Haedrich C, Sorg R V., Boehringer D, Eberwein P, Schwartzkopff J, Sundmacher R, Reinhard T (2011) Corneal surface reconstruction using adult mesenchymal stem cells in experimental limbal stem cell deficiency in rabbits. Acta Ophthalmologica 89:741–748. https://doi.org/10.1111/j.1755-3768.2009.01812.x

62. Craig JP, Nichols KK, Akpek EK, Caffery B, Dua HS, Joo C-K, Liu Z, Nelson JD, Nichols JJ, Tsubota K, Stapleton F (2017) TFOS DEWS II Definition and Classification Report. The Ocular Surface 15:276–283. https://doi.org/10.1016/j.jtos.2017.05.008

63. Gayton J (2009) Etiology, prevalence, and treatment of dry eye disease. Clinical Ophthalmology 405. https://doi.org/10.2147/OPTH.S5555

64. Calonge M, Enríquez-de-Salamanca A, Diebold Y, González-García MJ, Reinoso R, Herreras JM, Corell A (2010) Dry Eye Disease as an Inflammatory Disorder. Ocular Immunology and Inflammation 18:244–253. https://doi.org/10.3109/09273941003721926

65. Wei Y, Asbell PA (2014) The Core Mechanism of Dry Eye Disease Is Inflammation. Eye & Contact Lens: Science & Clinical Practice 40:248–256. https://doi.org/10.1097/ICL.0000000000000042

66. Hagan S, Martin E, Enríquez-de-Salamanca A (2016) Tear fluid biomarkers in ocular and systemic disease: potential use for predictive, preventive and personalised medicine. EPMA Journal 7:15. https://doi.org/10.1186/s13167-016-0065-3

67. Choi SW, Cha BG, Kim J (2020) Therapeutic Contact Lens for Scavenging Excessive Reactive Oxygen Species on the Ocular Surface. ACS Nano 14:2483–2496.

https://doi.org/10.1021/acsnano.9b10145
68. Xu J, Wang D, Liu D, Fan Z, Zhang H, Liu O, Ding G, Gao R, Zhang C, Ding Y, Bromberg JS, Chen W, Sun L, Wang S (2012) Allogeneic mesenchymal stem cell treatment alleviates experimental and clinical Sjögren syndrome. Blood 120:3142–3151. https://doi.org/10.1182/blood-2011-11-391144
69. Beyazyıldız E, Pınarlı FA, Beyazyıldız Ö, Hekimoğlu ER, Acar U, Demir MN, Albayrak A, Kaymaz F, Sobacı G, Delibaşı T (2014) Efficacy of Topical Mesenchymal Stem Cell Therapy in the Treatment of Experimental Dry Eye Syndrome Model. Stem Cells International 2014:1–9. https://doi.org/10.1155/2014/250230
70. Lee MJ, Ko AY, Ko JH, Lee HJ, Kim MK, Wee WR, Khwarg SI, Oh JY (2015) Mesenchymal Stem/Stromal Cells Protect the Ocular Surface by Suppressing Inflammation in an Experimental Dry Eye. Molecular Therapy 23:139–146. https://doi.org/10.1038/mt.2014.159
71. Aluri HS, Samizadeh M, Edman MC, Hawley DR, Armaos HL, Janga SR, Meng Z, Sendra VG, Hamrah P, Kublin CL, Hamm-Alvarez SF, Zoukhri D (2017) Delivery of Bone Marrow-Derived Mesenchymal Stem Cells Improves Tear Production in a Mouse Model of Sjögren's Syndrome. Stem Cells International 2017:1–10. https://doi.org/10.1155/2017/3134543
72. Abughanam G, Elkashty OA, Liu Y, Bakkar MO, Tran SD (2019) Mesenchymal Stem Cells Extract (MSCsE)-Based Therapy Alleviates Xerostomia and Keratoconjunctivitis Sicca in Sjogren's Syndrome-Like Disease. International Journal of Molecular Sciences 20:4750. https://doi.org/10.3390/ijms20194750
73. Park SA, Reilly CM, Wood JA, Chung DJ, Carrade DD, Deremer SL, Seraphin RL, Clark KC, Zwingenberger AL, Borjesson DL, Hayashi K, Russell P, Murphy CJ (2013) Safety and immunomodulatory effects of allogeneic canine adipose-derived mesenchymal stromal cells transplanted into the region of the lacrimal gland, the gland of the third eyelid and the knee joint. Cytotherapy 15:1498–1510. https://doi.org/10.1016/j.jcyt.2013.06.009
74. Villatoro AJ, Fernández V, Claros S, Rico-Llanos GA, Becerra J, Andrades JA (2015) Use of Adipose-Derived Mesenchymal Stem Cells in Keratoconjunctivitis Sicca in a Canine Model. BioMed Research International 2015:1–10. https://doi.org/10.1155/2015/527926
75. Bittencourt MKW, Barros MA, Martins JFP, Vasconcellos JPC, Morais BP, Pompeia C, Bittencourt MD, Evangelho KDS, Kerkis I, Wenceslau C V. (2016) Allogeneic Mesenchymal Stem Cell Transplantation in Dogs with Keratoconjunctivitis Sicca. Cell Medicine 8:63–77. https://doi.org/10.3727/215517916X693366
76. Lu X, Li N, Zhao L, Guo D, Yi H, Yang L, Liu X, Sun D, Nian H, Wei R (2020) Human umbilical cord mesenchymal stem cells alleviate ongoing autoimmune dacryoadenitis in rabbits via polarizing macrophages into an anti-inflammatory phenotype. Experimental Eye Research 191:107905. https://doi.org/10.1016/j.exer.2019.107905
77. Dietrich J, Ott L, Roth M, Witt J, Geerling G, Mertsch S, Schrader S (2019) MSC Transplantation Improves Lacrimal Gland Regeneration after Surgically Induced Dry Eye Disease in Mice. Scientific Reports 9:18299. https://doi.org/10.1038/s41598-019-54840-5
78. Li F, Zhao S (2016) Control of cross talk between angiogenesis and inflammation by mesenchymal stem cells for the treatment of ocular surface diseases. Stem Cells International 2016:1–8. https://doi.org/10.1155/2016/7961816
79. Ogawa Y, Shimmura S, Dogru M, Tsubota K (2010) Immune Processes and Pathogenic Fibrosis in Ocular Chronic Graft-Versus-Host Disease and Clinical Manifestations after Allogeneic Hematopoietic Stem Cell Transplantation. Cornea 29:S68–S77. https://doi.org/10.1097/ICO.0b013e3181ea9a6b
80. Ogawa Y, Okamoto S, Wakui M, Watanabe R, Yamada M, Yoshino M, Ono M, Yang H-Y, Mashima Y, Oguchi Y, Ikeda Y, Tsubota K (1999) Dry eye after haematopoietic stem cell transplantation. British Journal of Ophthalmology 83:1125–1130. https://doi.org/10.1136/bjo.83.10.1125
81. Shikari H, Antin JH, Dana R (2013) Ocular Graft-versus-Host Disease: A Review. Survey of Ophthalmology 58:233–251. https://doi.org/10.1016/j.survophthal.2012.08.004
82. Ogawa Y, Kawakami Y, Tsubota K (2021) Cascade of Inflammatory, Fibrotic Processes, and Stress-Induced Senescence in Chronic GVHD-Related Dry Eye Disease. International Journal of Molecular Sciences 22:6114. https://doi.org/10.3390/ijms22116114
83. Shimizu S, Sato S, Taniguchi H, Shimizu E, He J, Hayashi S, Negishi K, Ogawa Y, Shimmura S (2021) Observation of Chronic Graft-Versus-Host Disease Mouse Model Cornea with In Vivo Confocal Microscopy. Diagnostics 11:1515. https://doi.org/10.3390/diagnostics11081515
84. Sanchez-Abarca LI, Hernandez-Galilea E, Lorenzo R, Herrero C, Velasco A, Carrancio S, Caballero-Velazquez T, Rodriguez-Barbosa JI, Parrilla M, Del Canizo C, San Miguel J, Aijon J,

Perez-Simon JA (2015) Human bone marrow stromal cells differentiate into corneal tissue and prevent ocular graft-versus-host disease in mice. Cell Transplant 24:2423–2433. https://doi.org/10.3727/096368915x687480

85. Martínez-Carrasco R, Sánchez-Abarca LI, Nieto-Gómez C, Martín García E, Sánchez-Guijo F, Argüeso P, Aijón J, Hernández-Galilea E, Velasco A (2019) Subconjunctival injection of mesenchymal stromal cells protects the cornea in an experimental model of GVHD. The Ocular Surface 17:285–294. https://doi.org/10.1016/j.jtos.2019.01.001

86. Rusch RM, Ogawa Y, Sato S, Morikawa S, Inagaki E, Shimizu E, Tsubota K, Shimmura S (2021) MSCs Become Collagen-Type I Producing Cells with Different Phenotype in Allogeneic and Syngeneic Bone Marrow Transplantation. International Journal of Molecular Sciences 22:4895. https://doi.org/10.3390/ijms22094895

87. Al-Jaibaji O, Swioklo S, Connon CJ (2019) Mesenchymal stromal cells for ocular surface repair. Expert Opinion on Biological Therapy 19:643–653. https://doi.org/10.1080/14712598.2019.1607836

88. Dietrich J, Schrader S (2020) Towards Lacrimal Gland Regeneration: Current Concepts and Experimental Approaches. Current Eye Research 45:230–240. https://doi.org/10.1080/02713683.2019.1637438

89. Baiula M, Spampinato S (2021) Experimental Pharmacotherapy for Dry Eye Disease: A Review. Journal of Experimental Pharmacology Volume 13:345–358. https://doi.org/10.2147/JEP.S237487

90. Gain P, Jullienne R, He Z, Aldossary M, Acquart S, Cognasse F, Thuret G (2016) Global Survey of Corneal Transplantation and Eye Banking. JAMA Ophthalmology 134:167–73. https://doi.org/10.1001/jamaophthalmol.2015.4776

91. Williams KA, Esterman AJ, Bartlett C, Holland H, Hornsby NB, Coster DJ (2006) How Effective Is Penetrating Corneal Transplantation? Factors Influencing Long-Term Outcome in Multivariate Analysis. Transplantation 81:896–901. https://doi.org/10.1097/01.tp.0000185197.37824.35

92. Alio JL, Montesel A, El Sayyad F, Barraquer RI, Arnalich-Montiel F, Alio Del Barrio JL (2021) Corneal graft failure: an update. British Journal of Ophthalmology 105:1049–1058. https://doi.org/10.1136/bjophthalmol-2020-316705

93. Tahvildari M, Amouzegar A, Foulsham W, Dana R (2018) Therapeutic approaches for induction of tolerance and immune quiescence in corneal allotransplantation. Cellular and Molecular Life Sciences 75:1509–1520. https://doi.org/10.1007/s00018-017-2739-y

94. Renfro L, Snow JS (1992) Ocular effects of topical and systemic steroids. Dermatologic clinics 10:505–12

95. Murphy N, Lynch K, Lohan P, Treacy O, Ritter T (2016) Mesenchymal stem cell therapy to promote corneal allograft survival. Current Opinion in Organ Transplantation 21:559–567. https://doi.org/10.1097/MOT.0000000000000360

96. Oh JY, Kim E, Yun YI, Lee RH (2021) Mesenchymal stromal cells for corneal transplantation: Literature review and suggestions for successful clinical trials. The Ocular Surface 20:185–194. https://doi.org/10.1016/j.jtos.2021.02.002

97. Treacy O, Lynch K, Murphy N, Chen X, Donohoe E, Canning A, Lohan P, Shaw G, Fahy G, Ryan AE, Ritter T (2021) Subconjunctival administration of low-dose murine allogeneic mesenchymal stromal cells promotes corneal allograft survival in mice. Stem Cell Research & Therapy 12:227. https://doi.org/10.1186/s13287-021-02293-x

98. Lynch K, Treacy O, Chen X, Murphy N, Lohan P, Islam MN, Donohoe E, Griffin MD, Watson L, McLoughlin S, O'Malley G, Ryan AE, Ritter T (2020) TGF-β1-Licensed Murine MSCs Show Superior Therapeutic Efficacy in Modulating Corneal Allograft Immune Rejection In Vivo. Molecular Therapy 28:2023–2043. https://doi.org/10.1016/j.ymthe.2020.05.023

99. Murphy N, Treacy O, Lynch K, Morcos M, Lohan P, Howard L, Fahy G, Griffin MD, Ryan AE, Ritter T (2019) TNF-α/IL-1β—licensed mesenchymal stromal cells promote corneal allograft survival via myeloid cell-mediated induction of Foxp3 + regulatory T cells in the lung. The FASEB Journal 33:9404–9421. https://doi.org/10.1096/fj.201900047R

100. Oh JY, Lee RH, Yu JM, Ko JH, Lee HJ, Ko AY, Roddy GW, Prockop DJ (2012) Intravenous Mesenchymal Stem Cells Prevented Rejection of Allogeneic Corneal Transplants by Aborting the Early Inflammatory Response. Molecular Therapy 20:2143–2152. https://doi.org/10.1038/mt.2012.165

101. Mittal SK, Foulsham W, Shukla S, Elbasiony E, Omoto M, Chauhan SK (2019) Mesenchymal Stromal Cells Modulate Corneal Alloimmunity via Secretion of Hepatocyte Growth Factor. Stem Cells Translational Medicine 8:1030–1040. https://doi.org/10.1002/sctm.19-0004

102. Ramos T, Scott D, Ahmad S (2015) An Update on Ocular Surface Epithelial Stem Cells: Cornea

and Conjunctiva. Stem Cells International 2015:1–7. https://doi.org/10.1155/2015/601731
103. Elhusseiny AM, Soleimani M, Eleiwa TK, ElSheikh RH, Frank CR, Naderan M, Yazdanpanah G, Rosenblatt MI, Djalilian AR (2022) Current and Emerging Therapies for Limbal Stem Cell Deficiency. Stem Cells Translational Medicine 11:259–268. https://doi.org/10.1093/stcltm/szab028
104. Keivyon KR, Tseng SCG (1989) Limbal Autograft Transplantation for Ocular Surface Disorders. Ophthalmology 96:709–723. https://doi.org/10.1016/S0161-6420(89)32833-8
105. Pellegrini G, Traverso CE, Franzi AT, Zingirian M, Cancedda R, De Luca M (1997) Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium. The Lancet 349:990–993. https://doi.org/10.1016/S0140-6736(96)11188-0
106. Sangwan VS, Basu S, MacNeil S, Balasubramanian D (2012) Simple limbal epithelial transplantation (SLET): a novel surgical technique for the treatment of unilateral limbal stem cell deficiency. British Journal of Ophthalmology 96:931–934. https://doi.org/10.1136/bjophthalmol-2011-301164
107. López-Paniagua M, De La Mata A, Galindo S, Blázquez F, Calonge M, Nieto-Miguel T (2021) Advanced Therapy Medicinal Products for the Eye: Definitions and Regulatory Framework. Pharmaceutics 13:347. https://doi.org/10.3390/pharmaceutics13030347
108. Jacobs R, Tran U, Chen H, Kassim A, Engelhardt BG, Greer JP, Goodman SG, Clifton C, Lucid C, Vaughan LA, Savani BN, Jagasia M (2012) Prevalence and risk factors associated with development of ocular GVHD defined by NIH consensus criteria. Bone Marrow Transplantation 47:1470–1473. https://doi.org/10.1038/bmt.2012.56
109. Weng J, He C, Lai P, Luo C, Guo R, Wu S, Geng S, Xiangpeng A, Liu X, Du X (2012) Mesenchymal Stromal Cells Treatment Attenuates Dry Eye in Patients With Chronic Graft-versus-host Disease. Molecular Therapy 20:2347–2354. https://doi.org/10.1038/mt.2012.208
110. Møller-Hansen M, Larsen A-C, Toft PB, Lynggaard CD, Schwartz C, Bruunsgaard H, Haack-Sørensen M, Ekblond A, Kastrup J, Heegaard S (2021) Safety and feasibility of mesenchymal stem cell therapy in patients with aqueous deficient dry eye disease. The Ocular Surface 19:43–52. https://doi.org/10.1016/j.jtos.2020.11.013
111. Zhou T, He C, Lai P, Yang Z, Liu Y, Xu H, Lin X, Ni B, Ju R, Yi W, Liang L, Pei D, Egwuagu CE, Liu X (2022) miR-204–containing exosomes ameliorate GVHD-associated dry eye disease. Science Advances 8:eabj9617. https://doi.org/10.1126/sciadv.abj9617
112. Rani S, Ryan AE, Griffin MD, Ritter T (2015) Mesenchymal Stem Cell-derived Extracellular Vesicles: Toward Cell-free Therapeutic Applications. Molecular Therapy 23:812–823. https://doi.org/10.1038/mt.2015.44
113. Fuloria S, Subramaniyan V, Dahiya R, Dahiya S, Sudhakar K, Kumari U, Sathasivam K, Meenakshi DU, Wu YS, Sekar M, Malviya R, Singh A, Fuloria NK (2021) Mesenchymal Stem Cell-Derived Extracellular Vesicles: Regenerative Potential and Challenges. Biology 10:172. https://doi.org/10.3390/biology10030172
114. Deng SX, Dos Santos A, Gee S (2020) Therapeutic Potential of Extracellular Vesicles for the Treatment of Corneal Injuries and Scars. Translational Vision Science & Technology 9:1. https://doi.org/10.1167/tvst.9.12.1
115. McKay TB, Yeung V, Hutcheon AEK, Guo X, Zieske JD, Ciolino JB (2021) Extracellular Vesicles in the Cornea: Insights from Other Tissues. Analytical Cellular Pathology 2021:1–12. https://doi.org/10.1155/2021/9983900

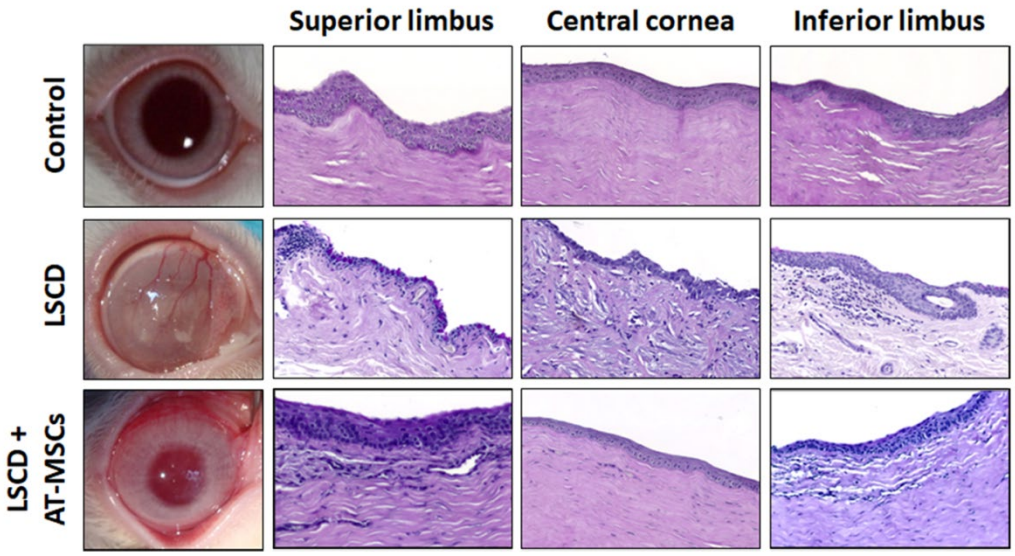

Fig. 15.1 Histological evaluation of ocular surface tissues from a rabbit model of total limbal stem cell deficiency (LSCD) treated with human adipose tissue-derived mesenchymal stem cells (AT-MSCs). Representative images of periodic acid-Schiff staining of ocular surface tissues obtained from healthy 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 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 Galindo et al. 2017)

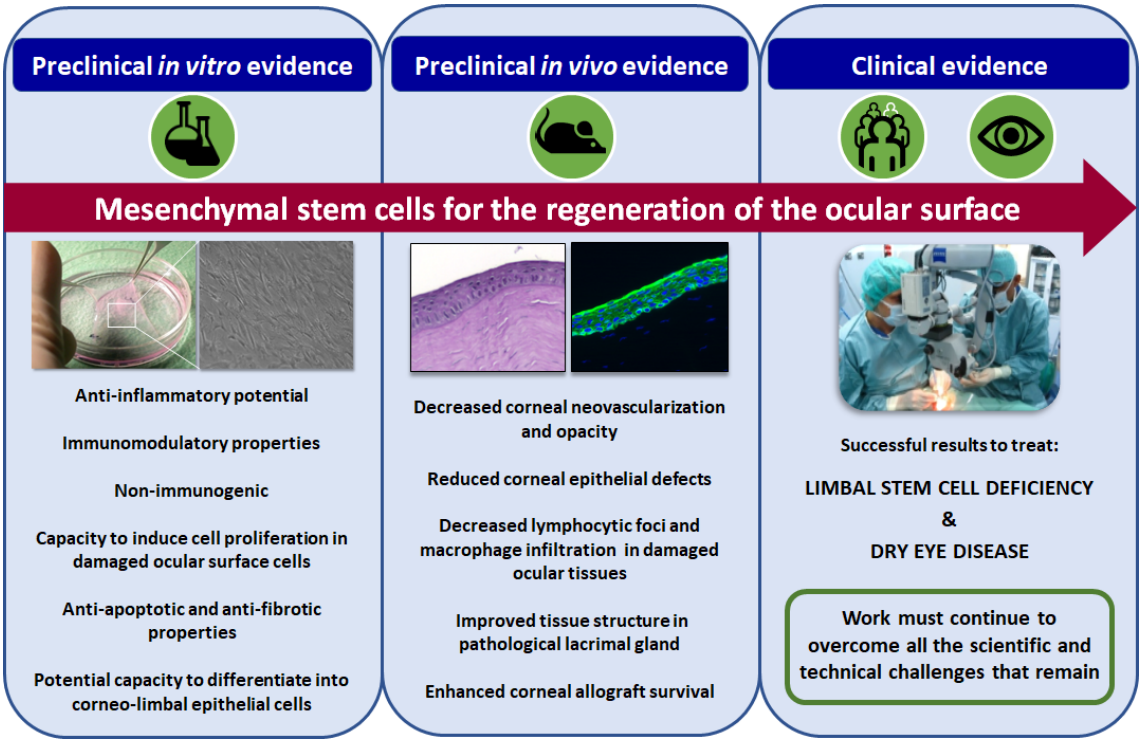

Fig 15.2 Mesenchymal stem cells for the regeneration of the ocular surface: from preclinical to clinical evidence