Review Article

Limbal stem cell deficiency approaches and limbal niche restoration

Zahra Bibak-Bejandi, Zohre Arabpour, Alireza Razavi[1,2], Amirhossein Moghtader, Sumaiya Shahjahan, Ahmad Alzein, Jeonghyun (Esther) Kwon, Reyhaneh Bibak-Bejandi[3], Nuran Aly, Ali Djalilian

Approaches to limbal stem cell deficiency remain challenging, especially in bilateral cases, where healthy limbal stem cells are not accessible. While living-related allogeneic and allogeneic limbal stem cell sources have been utilized, their dependence on immunosuppression and its associated side effects pose significant limitations. Mucosal and mesenchymal stem cells have shown potential for differentiation into limbal stem cells and promoting corneal healing, primarily when cultured on the amniotic membrane or fibrin. However, none can fully replicate the original limbus. Innovations in surgical techniques, such as simple oral mucosal transplantation and subconjunctival or intrastromal mesenchymal stem cell injections, are emerging approaches. For successful limbal regeneration, both appropriate cells and suitable scaffolds are essential. Recent studies on decellularized and acellularized limbus models have demonstrated the potential to provide a three-dimensional native structure for cell seeding, retention, and differentiation. Creating a thin, evenly decellularized scaffold is a critical step in ensuring proper corneo-limbal slope formation, facilitating cell migration to the ocular surface. Harvesting the limbus, decellularization, and cell seeding are the three main steps in limbal reconstruction. Recent studies focus on microkeratome-assisted limbal harvesting to create a thin, even, and 360-degree limbal graft. This technique helps form an attached corneo-limbal interface, facilitating limbal stem cell migration. In the second step, acellularization is performed to preserve the extracellular matrix as much as possible, maintaining hemostasis and supporting paracrine interactions. The final steps involve recellularization and transplantation onto the eye. We summarize various limbal decellularization methods, their outcomes, and their potential in limbal reconstruction. More clinical studies are needed to validate this phase of limbal deficiency treatment.

Key words: Cell therapy, decellularized limbus, limbal niche reconstruction, limbal stem cell deficiency, mesenchymal stem cells, mucosal stem cells, native scaffolds

Access this article online

Website: https://journals.lww.com/ijo

DOI: 10.4103/IJO.IJO_464_25

Quick Response Code:

The cornea is the clear central part of the eye, surrounded by the finger-shaped structures of the limbus, which serve as a vital cell reservoir for maintaining ocular surface clarity.[1] Various etiologies, such as chemical or thermal burns, trauma, infection, and immunological diseases, can damage these cell sources.[2] Depending on the severity, partial or total limbal deficiency may occur in one or both eyes. As a result, complications such as neovascularization, conjunctivalization, fibrosis, and perforation can develop.[3] Ultimately, this can lead to total blindness.[3] When unilateral limbal stem cell deficiency (LSCD) occurs, the healthy collateral eye can serve as a source of limbal stem cells (LSCs) for transplantation. The challenging situation arises in bilateral LSCD, where no healthy partner eye exists. In these cases, allogeneic limbal stem cell transplantation techniques such as living-related conjunctival-limbal autograft (lr-CLAU),[4] Allo-simple limbal epithelial transplantation (Allo-SLET),[5] and keratolimbal allograft (KLAL) serve as alternatives.[6] Due to the risk of immunosuppression complications, alternative cell sources, including mucosal stem cells,[7] mesenchymal stem cells (MSCs),[8] dental stem cells,[9] epidermal stem cells,[10] and hair follicle stem cells, are being investigated.[11] Choosing the appropriate cell source and scaffolds are two main parts for limbal regeneration. Mucosal cells are a commonly used cell source in the literature. Three methods utilizing mucosal cells include mucosal graft transplantation (MGT),[12] simple oral mucosal transplantation (SOMT),[13] and a cultivated mucosal epithelial cells sheet (CMECS) that requires a mucosal biopsy, cell isolation, and expansion on scaffolds, with the amniotic membrane (AM) being the most commonly used.[14] However, CMECS is time-consuming, leading to the development of MGT[12] and SOMT, which bypass the culturing phase.[15] MSCs are a common cell source, derived from various tissues in the body, such as the bone marrow, adipose tissue, and umbilical cord.[16] They can be isolated and cultured in a shorter time compared to LSCs. Additionally, some studies have demonstrated that MSCs have immunomodulatory properties, allowing for their use without the need for immunosuppression.[17] It demonstrated

Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, Chicago, IL, USA, [1]Research Center for Clinical Virology, Tehran University of Medical Science, Tehran, Iran, [2]Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran, [3]Hazrat-E Fatemeh Hospital, Iran University of Medical Sciences, Tehran, Iran

Correspondence to: Dr. Ali Djalilian, Department of Ophthalmology and Visual Sciences, University of Illinois, 23 1855 W. Taylor St, STE 3.138, Chicago, IL 60612, USA. E-mail: adjalili@uic.edu

Received: 16-Feb-2025 Accepted: 24-Feb-2025
Published: 27-Mar-2025

Cite this article as: Bibak-Bejandi Z, Arabpour Z, Razavi A, Moghtader A, Shahjahan S, Alzein A, *et al.* Limbal stem cell deficiency approaches and limbal niche restoration. Indian J Ophthalmol 2025;73:468-82.

anti-inflammatory, anti-angiogenic, and healing-promoting properties, making it more desirable for limbal regeneration.[17] It can be used in both cell-based and cell-free therapies.[17] MSCs can be delivered via intrastromal and subconjunctival injection or through a carrier such as AM-Fibrin.[8,17] Various animal models have demonstrated their potential in limbal regeneration. Calonge *et al*.[18] reported a 76.5%–85.7% success rate in a clinical trial for allogeneic mesenchymal stem cell transplantation (MSCT), compared to 72.7%–77.8% for allogeneic cultivated limbal epithelial transplantation (CLET), without complications or the need for immunosuppression. AM and fibrin are two of the most common carrier scaffolds in limbal regeneration, but neither effectively mimics the limbal niche structure. For limbal reconstruction, one of the most crucial factors is creating a suitable scaffold that mimics the three-dimensional limbal niche, providing support and protection for limbal epithelial stem cells (LESCs) at the depth while promoting their differentiation into corneal epithelial cells, thereby replenishing the corneal surface.[19] Recent studies focus on the decellularized limbus to recreate the native homeostatic environment for limbal regeneration while preserving the most intact extracellular matrix (ECM). A variety of protocols have been applied to create decellularized limbal scaffolds with intact ECM, which have been successfully recellularized with adipose-derived mesenchymal stem cells (hADSCs) and limbal epithelial cell line (SIRC).[20] These promising approaches highlight the need for a multidirectional perspective in limbal reconstruction, considering appropriate cells, scaffolds, adjunct promoting factors, and gene modification. This review presents various approaches to LSCD and limbal niche reconstruction.

Limbal niche structure and homeostasis

Clarity of the cornea is crucial for vision. The corneal epithelium, which serves as an outer protective layer, undergoes continuous renewal through active repair processes. These processes are vital because the cornea constantly sheds cells, and any injury or loss of epithelial cells requires prompt repair. The corneal epithelium fully regenerates within a span of 3 to 10 days, necessitating the ongoing renewal of its cells.[21] The finger-shaped structures, limbal niche located at the periphery of the cornea, serve as a reservoir for renewal of LESCs.[22] This structure, supported by nerves, blood vessels, immune cells, and MSCs beneath the limbal niche, facilitates cell–cell interactions and maintains homeostasis through paracrine interaction.[22] Small, self-renewing LESCs in the limbal depth become more differentiated as they move upward to the superficial layer.[22] These cells also migrate in a centripetal direction and further differentiate into postmitotic cells, transient amplifying cells, and mature corneal epithelial cells, replacing shed cells on the corneal surface.[22] Additionally, basal corneal cells migrate to the superficial layer to replace lost cells. This is the physiology of ocular surface re-epithelialization in the X, Y, Z pattern.[23] The limbal niche is a multicellular environment characterized by a unique ECM and various signaling molecules that support LESCs.[24] These cells are located in the basal layer of the limbal epithelial undulations, adjacent to clusters of CD90-positive mesenchymal stem/stromal cells, which are vital for tissue homeostasis and repair.[25,26] *In vivo* confocal microscopy has revealed hyperreflective mesenchymal cell clusters in the anterior limbal niche stroma beneath the basal epithelium.[27] Mesenchymal cells interact with LESCs through various molecular substrates and signaling pathways, including aquaporin-1, vimentin,[28,29] chondroitin sulfate,[26] SDF-1/CXCR4,[30] BMP/Wnt,[31] and IL-6/STAT3.[32] There are two main subcategories of limbal niche cells: active and quiescent. Active cells are located near the cornea and undergo replication to become mature corneal epithelial cells. Quiescent cells, found in the outer limbal region, do not typically undergo replication under physiological conditions.[22,33] In pathological conditions, multidirectional collaboration occurs in the limbus—Active LSCs increase differentiation and replication beyond baseline levels, while quiescent cells become active to enhance healing.[22,33] Stromal cells, through paracrine signaling and cell–cell interactions, further promote LESCs replication and healing. In severe conditions such as chemical and thermal burns, autoimmune diseases, infections, and trauma, the LSCs reservoir can be depleted, leading to dysfunction in re-epithelialization, conjunctivalization, neovascularization, persistent epithelial defects, and ultimately vision loss.[2] Depending on the severity, either a partial or total portion of the limbus may be affected. The optimal approach is to replace lost LSCs to preserve sight. Treatment strategies vary based on whether the condition is unilateral or bilateral.

When unilateral LSCD occurs, the healthy collateral eye can serve as a reservoir of LSCs for transplantation. The challenging situation arises in bilateral LSCD, where no healthy partner eye exists. In these cases, allogeneic LSCs transplantation techniques such as lr-CLAU,[34] Allo-SLET, and KLAL[35] serve as alternatives.[36] Due to the risk of immunosuppression complications, alternative cell sources, including mucosal epithelial cells, MSCs, dental stem cells, epidermal stem cells, and hair follicle stem cells, are being investigated.[9-11,37,38]

Etiology

LSCD has been studied epidemiologically worldwide to determine the most common causes. According to a study of 738 eyes done in a single tertiary center over 14 years, there are aniridia (30.9%), chemical or thermal injury (20.6%), contact lens (16.8%), and Stevens-Johnson syndrome (10.4%).[39] As a matter of fact, chemical burns are the most common cause of LSCD among unilateral cases.[40,41] Several other causes may also contribute to the condition, such as prior surgery (most often pterygoid surgery or glaucoma surgery), drug toxicity, and mucous membrane pemphigoid (MMP).[42] Mutations in the PAX6 gene, also implicated in aniridia[43] and Peter's anomaly,[44] have been connected to LSCD.

In unilateral LSCD, the autologous LSCs source typically is taken from the contralateral eye. Allogeneic grafts from a living relative or a cadaveric donor are alternatives for bilateral limbal deficiency[45] [Fig. 1].

Unilateral limbal stem cell deficiency approach

Conjunctival limbal autograft

- Conjunctival limbal autograft (CLAU) involves using a graft block consisting of the corneal, limbus, and conjunctiva part from the healthy, contralateral eye.[46] One of the primary benefits of CLAU is the opportunity for limbal regeneration in the affected eye using an autologous cell source, reducing the need for immunosuppression. However, depending on the graft size, there is a risk of inducing limbal deficiency in the donor eye.[46] CLAU combined with amnion-assisted conjunctival epithelial redirection (ACER) was performed

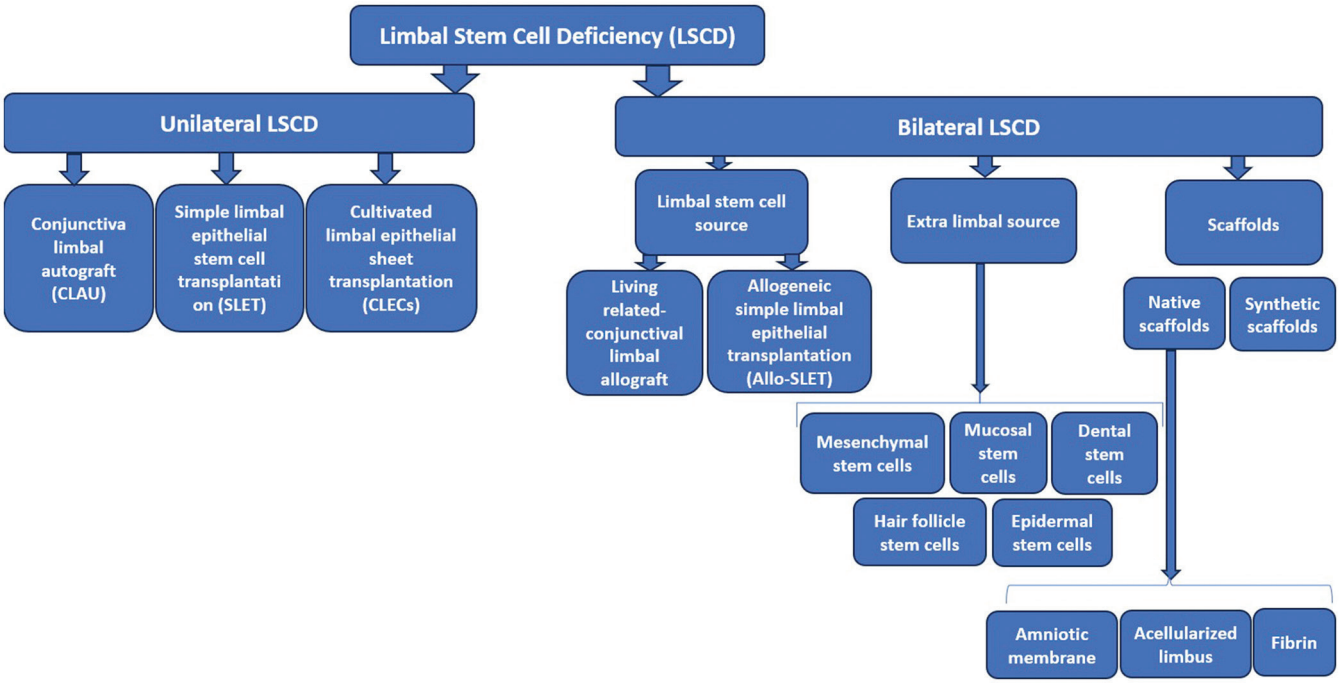

Figure 1: Approaches for treating limbal stem cell deficiency (LSCD) depend on whether the condition is unilateral or bilateral; based on this distinction, autologous or allogeneic limbal stem cell sources, as well as other extra-limbal stem cell sources, can be applied. The diagram illustrates the general alternatives for treating LSCD

in ten eyes with unilateral LSCD. The AM was placed with the epithelial side up on the cornea. The results showed epithelial healing and improvement in best-corrected visual acuity (BCVA) in all cases.[47] In another study, adding autologous platelet-rich plasma (E-PRP) to mini-conjunctival limbal autograft in ten eyes with chemical burns was considered an adjuvant therapy for corneal healing.[48] This technique is also considered for pterygium, a focal limbal deficiency, and helps reduce recurrence.[49] Various innovations in surgical techniques, such as mini-conjunctival limbal autograft, the use of healing-promoting drops, AM application, and keratoplasty, serve as alternatives for promoting ocular surface regeneration, summarized in the table. [Table 1].

Simple Limbal Epithelial Transplantation

SLET is a procedure introduced by Sangwan *et al*.[55] in 2012; it involves using fragmented pieces of limbal tissue that are adhered to the corneal surface overlaying a previously AM overlay. These alterations in limbal graft size and methods reduce the previous risk of inducing limbal deficiency in the healthy eye after CLAU.[55] Some innovative approaches, such as Gluless-SLET (G-SLET),[56] femtosecond-assisted gluless SLET (FA-G-SLET),[57] and modified SLET (M-SLET), have made these methods more favorable over time.[58] The results are summarized in Table 2. For example, in three unilateral LSCD cases, vertical oblique incisions were created using 100% FSL, and limbal epithelial segments were implanted into the incisions. Corneal regeneration was achieved within 2–3 weeks post surgery, and BCVA improved in two cases without any deterioration in the third case.[57] M-SLET, as presented by Datar *et al*.,[58] involves transplanting tissue biopsies onto the affected cornea, covering them with an AM, and securing them with a contact lens. This approach resulted in a healed and stable cornea in five patients. In severe cases, the combination of SLET and CLAU revealed sight-saving results,[70] revealing pigmentation at the 8-month follow-up posttransplantation, confirming the replication of LSCs and SLET method efficacy.[71] Although some complications, such as seroma, have been reported, the epithelialized surface appeared over 3 months without further deterioration.[72] More clinical results through these innovations are needed to evaluate their efficacy in the future.

Bilateral limbal stem cell deficiency

In bilateral limbal deficiency, there are two main cell therapy approaches [Fig. 1]:

1. Allogenic limbal stem cell transplantation:
   - Allogeneic Cultured Limbal Epithelial Transplantation (ACLET)
   - Keratolimbal Allograft (KLAL)
   - Allogeneic simple limbal epithelial transplantation (alloSLET)
2. Autologous extra-limbal cell source combined with different scaffolds for limbal reconstruction

   Extra-limbal cell source:
   - Mucosal stem cell
   - Mesenchymal stem cell

   Scaffolds:
   - Native scaffolds
   - Synthetic scaffolds

Allogeneic limbal stem cell transplantation in bilateral limbal stem cell deficiency

Allogeneic Cultured Limbal Epithelial Transplantation:

In bilateral cases, a small limbal biopsy from a living related donor is dissected, expanded on a scaffold, and transplanted onto the affected ocular surface.

Comparison of 41 cultivated oral mucosal epithelial transplantation (COMET) and 69 ACLET procedures showed a higher graft survival rate of 81.7% in ACLET, along with

Table 1: Summary of the Conjunctival Limbal Autograft (CLAU) technique, results, and complications

Procedures	Etiology	Complication	Result
Conjunctival-limbal autograft (CLAU) vs Mini-simple limbal epithelial transplantation (Mini-SLET)[50]	Pterygium	No complication in both group	2 months follow-up recurrence: CLAU: 0%/Mini-SLET: 0% 3 months follow-up recurrence: CLAU: 6.1%/Mini-SLET: 17.9% 6 months follow-up recurrence CLAu: CLAU: 8.1%/Mini-SLET: 50% 12 months follow-up recurrence CLAu: CLAU: 8.1%/Mini-SLET: 53.5%
Mini-conjunctival limbal autograft (CLAU) + platelet-rich plasma (E-PRP)[48]	Chemical burn	-	Reducing conjunctivalization/vascularization in 2 months Corneal transparency improve=7 Cases Cornea re-epithelization: Within 11–21 days BSCVA: Mini-CLAU+penetrating keratoplasty: 0.60±0.0.32 Mini- CLAU+ without penetrating keratoplasty: 0.46±0.0.25 logMAR
Mini-conjunctival limbal autograft (CLAU) + deep anterior lamellar keratoplasty (DALK)[51]	Chemical injury	Stromal rejection occurred 5 months post-surgery and was controlled with topical steroids	Corneal epithelialization Corrected visual acuity: 20/30 Vision maintenance+scleral contact lenses: Within 15 months
Conjunctival limbal autograft (CLAU) + Amniotic membrane[52]	Chemical trauma	-	Epithelial defect healing Improved VA 0.05/10 to 0.7
Conjunctival limbal transplantation +_ Amniotic membrane[53]	Chemical burn	-	Longer epithelialization: CLAU+AM Graft survival: Similar in both Failure rate: Similar in both
Conjunctival limbal allo + autograft stabilized with fibrin glue[54]			Stable ocular surface: 100% cases Epithelium healing: 10 days Mean visual acuity: 20/400 to 20/53

significant improvement in VA, compared to 60.7% in COMET, which showed no significant improvement in VA.[37] Comparison of the CLET and SLET showed the ocular surface stability over the 1 year follow-up, which was 86% and 88%, respectively.[73] The anatomical success rate in both Auto- and Allo-CLET decreased from 46.1% to 23.1% from short- to long-term evaluation.[74] A follow-up of 26.4 ± 13.6 months in varying degrees of symblepharon due to thermal and chemical burns reported complete success, partial success, and failure rates of 50%, 31.3%, and 18.8%, respectively, after ACLET.[75] Ten eyes underwent both CLET and PK with a 6-month interval, resulting in survival rates of 100% and 80% at 1-year and 3-year follow-ups, respectively.[76] Comparison of Allo and Auto CLET in 1306 eyes where 75.2% (982 eyes) included the Auto-CLET showed success and improvement in BCVA were not different between two group.[77] But systemic immunosuppression and its associated complications, renal and liver dysfunction, highlight the importance of using extra-limbal cell sources.[37,78] For example, immunosuppression-related adverse effects occurred in 13% of the 69 patients who underwent ACLET.[37]

- **Keratolimbal Allograft:**
- A 360-degree limbal strip is detached from the cadaveric globe and can be preserved in hypothermic temperature and cornea media for up to 9 days before being transferred onto the affected limbal surface.[79] The benefit of this comes from the ability to securely transplant a large number of stem cells.[80] In general, this technique involves performing a 360-degree limbal peritotomy and removing any abnormal structures to create a flat surface. After transferring the graft onto the patient's eye, the host and donor conjunctiva are approximated to ensure an even surface.[80] Limbal graft thickness is an important factor in achieving a well-attached corneo-limbal slope after transplantation. Microkeratome-assisted mechanical harvesting creates an even, smooth, and thin limbal strip for optimal limbal regeneration.[81] It is important to remember that systemic immunosuppression is needed for all patients to prevent transplant rejection and manage the postoperative inflammation.[80] For immune suppression, topical showed better results than oral immune suppression.[82] KLAL in combination with keratoplasty revealed better results than KLAL alone.[6] Results are summarized in Table 3.

Living-related conjunctival limbal allograft and Allogeneic simple limbal epithelial transplantation

- lr-CLAL was performed to reduce the need for immunosuppression. Compared to KLAL, ocular surface stability was achieved in 82.5% of patients versus 64.7%. Additionally, the failure rate was 6.3%, lower than the 15.6% observed in KLAL.[4] Allo-SLET involves harvesting a limbal biopsy from a cadaveric globe or a living-related donor. This method reduces scar formation compared to Lr-CLAL. This procedure, performed in 24 eyes with bilateral LSCD, resulted in 96% epithelialization postoperatively, which decreased to 50% over 36 months.[88] Descemet membrane anterior keratoplasty (DMAK) combined with modified Allo-SLET leads to corneal re-epithelialization, vision improvement, and pain reduction over 1.5 years.[89] In a 22-year-old female with vernal keratoconjunctivitis (VKC) and total LSCD who underwent Allo-SLET, the corrected visual acuity was 20/20 in both eyes, leading to corneal stabilization without

Table 2: Summary of the simple limbal epithelial transplantation technique, alternatives, innovations, and results

Procedure	Etiology	Result
Autologous simple limbal epithelial transplantation + conjunctival autograft[59]	Burn	24 months follow-up: Stable ocular surface Best-corrected visual acuity: 6/12p
Simple conjunctival epithelial transplantation + Simple limbal epithelial transplantation[60]	Chemical burn	Conjunctival epithelium reconstruction: 3 months follow-up Corneal epithelium reconstruction: 3- and 6-months follow-up Wong-Baker FACES Pain Rating Scale: Reach from 6 and 4 to 0 (2 cases) best-corrected visual acuity: 1.40 and 1.10 to 0.5 logarithm of the minimum angle of resolution
Femtosecond laser-Glueless simple limbal epithelial transplantation[57]	Chemical burn	Corneal stabilization: 6-month follow-up
Glueless simple limbal epithelial transplantation[56]	Eye burn	Visual improvement was observed in one case, while another case showed no improvement due to severe cataract
Simple limbal epithelial transplantation[61]	Chemical injury	Epithelization: 1 month follow-up Visual acuity: log MAR 1.8±1.9 to log MAR 0.5±0.4 Epithelial/stromal ratio: Reduced from 1.53±0.2 to 1.02±0.17 Densitometry: Anterior stromal: 64.52±27.4 to 53.16±20.0 Mid-stromal: 56.22±29.8 to 38.6±17.68 Posterior stromal: 47.48±33.05 to 29.44±16.73
Simple limbal epithelial transplantation without Amniotic membrane, fixed with fibrin glue[62]	Firecracker+ Chemical injuries + Squamous neoplasia excision	Corneal epithelialization=2-3 Weeks follow-up
Penetrating keratoplasty + Autologous simple limbal epithelial transplantation[63]	Carbide blast	Visual acuity: 20/100, Stable and epithelialized cornea achieved in 18 months follow-up
Modified supportive simple limbal epithelial transplantation[58]	Chemical injury + xeroderma pigmentosa	Corneal and ocular stabilization
Autologous or Allogeneic Simple limbal epithelial transplantation[64]	Chemical injury	Epithelial thickness (ET): Same as normal eye over 1 year follow-up Epithelial/stromal (ES) reflectivity ratio: 2.1±0.8 vs 1±0.2, P<0.001 over 1-year follow-up
Poly-lactic co-glycolic acid (PLGA) + Autologous simple limbal epithelial transplantation[65]	Acid burn, Chemical, Lime injury and Idiopathic	2 out of 5 patients two-line vison improvement Resurgence of pannus: 2 out of 5 patients 3 out of 5 reach epithelialized surface without defect over 12 months
Simple limbal epithelial transplantation + Cataract surgery[66]	Ocular surface squamous neoplasia	Stable ocular surface without resurgence over 18 months
Autologous simple limbal epithelial transplantation + Live related- Allogeneic simple limbal epithelial transplantation[67]	Variety of etiology	Survival rate: 2-year follow-up: 89.3% 3-year follow-up: 75.6% Visual improvement in both group
Autologous + Allogeneic modified simple limbal epithelial transplantation[68]	Thermal and chemical burn	Symblepharon, conjunctivalization, vascularization, and opacification healing Visual acuity: Two-line improvement 60% of eyes Recurrency: 23.08% of eyes Limbal and corneal structure: Detected in vivo confocal microscopy and impression cytology
Autologous simple limbal epithelial transplantation[69]	Chemical burn	Serial OCT: Increase in the corneal epithelial layer, a decrease in Amniotic membrane thickness, and enhanced incorporation with the membrane.
Combination of conjunctival-limbal autografting + Simple limbal epithelial transplantation[70]	Alkali chemical injury	Visual acuity: Hand motion initially, to 20/20 Stabel ocular surface

recurrence.[90] HLA-matched living-related Allo-SLET was performed in both eyes of a bilateral LSCD patient. In the left eye, modified Allo-SLET was performed, leading to an improvement in visual acuity from hand motion to 6/20 and corneal surface epithelialization at the 1-year follow-up. In the right eye, the sandwich-modified SLET was performed, resulting in visual improvement from light perception to counting fingers.[91] Lr Allo-SLET transplantation resulted in BCVA improving to 20/50 in the left eye over a 4-month follow-up; however, the right eye deteriorated to hand movements.[92] AM combined with penetrating keratoplasty (PKP) transplantation and an 8-week oral steroid regimen resulted in BCVA reaching 20/20, with a stable and clear cornea at a 2-year follow-up.[93] One of the main drawbacks of this method is the risk of rejection and the need for strong immunosuppression.[94]

Table 3: Summary of the keratolimbal allograft technique, innovations, and results

Procedure	Etiology	Numbers	Follow-up	Results
keratolimbal allograft (KLAL) with central lamellar keratoplasty (CLK)[83]	Delayed-onset mustard gas keratopathy	13 eyes	87.6±49.8 months	Surgical success: 93% Best-corrected visual acuity (BCVA): 1.07±0.24 to 0.63±0.30 logMAR
keratolimbal allograft or + Lamellar keratoplasty[6]	Delayed-onset mustard gas keratopathy	108 eyes	81.9±38.4 months	Success rate: 75% Duration of success: 80.6±38 months
Keratolimbal allograft + Penetrating keratoplasty[84]	Mustard gas keratopathy Herpes simplex keratitis Acid chemical burn		6.5 years	Epithelialized cornea: 88.8% Average best-corrected visual acuity: 1.89±0.18 to 1.02±0.64 logMAR
Keratolimbal allograft + _Deep anterior lamellar keratoplasty[85]	Alkali burns Acid burns Thermal burns	49 eyes	46.80±31.22 months	Survival: KLAL: 66.67%, KLAL-DALK: 76% KLAL: BCVA ≥20/200 in 48.98% KLAL-DALK: BCVA ≥20/200 in 44.0%
Keratolimbal allograft + Live related- Conjunctival limbal Autograft[86]	Stevens-Johnson syndrom Aniridia Chemical injury Atopic eye disease	8 eyes	37.3±22.7 months	Stable ocular surface: 87.5%
Keratolimbal allograft + Penetrating keratoplasty[33]	Varity etiology	43 eyes	-	Surgical success: 53% Visual acuity improvement: One or more lines Early graft rejection: 9%
Femtosecond laser-assisted keratolimbal allograft[87]	Chemical burn + Stevens-Johnson syndrome	10 eyes	16.8±7.3 months	Stable ocular surface: 90% Confocal microscopy: Dentric cells at limbus

Limbal reconstruction

For limbal reconstruction, an appropriate scaffold and cell source are essential. In bilateral LSCD, alternative extra-limbal cell sources such as mucosal,[95] mesenchymal,[18] dental,[9] hair follicle,[37] and epidermal stem cells[10] hold potential. Mucosal and mesenchymal are the most common sources.[95] New scaffolds, including acellularized limbus, provide an ideal three-dimensional structure for creating a niche environment for LESCs differentiation.[19]

Mucosal Stem Cells

In instances of total bilateral LSCD, allogeneic transplants are essential, sourced from either living-related donors or cadaveric donors. This approach necessitates long-term chronic immunosuppression, subsequently increasing the disease transmission risk.[96] This situation has motivated researchers to investigate alternative nonlimbal cells that may be utilized for autologous grafting procedures.[96,97] COMET technique was first introduced by Nishida *et al*. in 2004.[98] This procedure is called *ex vivo* cultivated oral mucosal autograft (EVOMAU).[96] Many individuals seeking relief from ocular surface issues have found hope in COMET, which has been reported to restore stability in about 43% to 67% of cases.[24] While results can vary, it is important to recognize the potential for improvement and support those navigating their eye health challenges. Kolli *et al*.[99] and Ilmarinen *et al*.[100] clearly identified that suboptimal visual outcomes after COMET are a direct result of the persistent phenotype of the oral mucosal epithelium, which is characterized by thicker and less transparent features. Post-COMET analysis reveals that peripheral corneal neovascularization occurs in the majority of patients, with a remarkable 83% of examined corneal quadrants exhibiting epithelial neovascularization as confirmed by AS-OCTA.[101-103] The long-term effects of COMET transplantation are still unknown.[1] Corneal epithelial cells are more functional than oral mucosal epithelial cells.[1] Since there is no indication of limbal niche regeneration after COMET transplantation, their transplantation may mainly aid in surface stabilization.[1] As a result, scientists are looking into using corneal epithelial cells that are produced from multipotent or pluripotent stem cells.[1] Even though these methods have been successful in maintaining the corneal surface, oral mucosa lacks the perfect properties of corneal epithelium.[1] Generally, the use of mucosal cells is based on three techniques: COMET, MGT, and SOMT. In COMET, after a mucosal biopsy and cell extraction, the cells are co-cultured with 3T3 feeder cells on AM in a culture medium to generate a cultivated epithelial cell sheet. This process takes 2 to 3 weeks. AM is the most commonly used scaffold for generating mucosal epithelial sheets.[104-106] In MGT, after mucosal dissection, the subepithelial tissue is removed and then fixed using fibrin glue and nylon sutures[105] This method is easier and more time-saving than the previous one. New studies emphasize SOMT, where chopped mucosal segments are transferred onto AM and then covered by AM.[106] These findings indicate a trend toward surgical techniques that are more time-saving and beneficial. This is summarized in Table 4.

Mesenchymal stem cells

Given its intricate cellular and molecular networks as well as its distinct mechanical and adhesion characteristics, restoration of the LSCs microenvironment is still being researched. MSCs, cytokines, growth factors, and serum/platelet-derived products are among the biological components that can be delivered.[116] A remarkable population of MSCs have the ability to differentiate into a variety of mesodermal lineage cells, such as chondroblasts, adipocytes, and osteoblasts. The amazing potential of nature's design is demonstrated by its special capacity to release a wide range of cytokines and growth factors, which promote an immunomodulatory and anti-inflammatory milieu.[38,117-119]

Table 4: Mucosal cell source, transplantation techniques, and results in limbal deficiency treatment

Cell Source and Technique	Results
Cultivated oral mucosal epithelial transplantation[37]	Elevated intraocular pressure (IOP)=22.0% Visual acuity (VA)=No change Infection=9.8% Perforation=9.8%
Cultivated oral mucosal epithelial transplantation[107]	Success rates=57.8% Survival rates=53.2%
Cultivated oral mucosal epithelial transplantation (Ocural)[14]	Stem cells markers=p63, p75 Proliferation markers=Ki-67 Differentiation markers=Keratin-3, -4, and -13
Cultivated oral mucosal epithelial transplantation[108]	IVCM=Cornea-like epithelium ICIF=CK3 and CK12 are representative markers of oral mucosal cells
Cultured autologous oral mucosal epithelial cells + Amniotic membrane[109]	Corneal epithelialization rate: 73.33%
Cultured oral mucosal epithelial cell sheets + Penetrating keratoplasty[110]	Epithelialization over 53.6 days Visual improvement (≥2 lines) K12=Corneal phenotype K4, and K13=Mucosal phenotype
Cultivated oral mucosal epithelial transplantation + Penetrating keratoplasty[111]	Epithelialized in surface without any defect Best-corrected visual acuity=Increased 2.67±0.08 LogMAR to 0.64±0.27 LogMAR
Cultivated oral mucosal epithelial transplantation + Penetrating keratoplasty[112]	Patient 1 BCVA=20/125 Patient 2 BCVA=20/100 K3 +, K12 – Basal corneal density similar to normal
Cultivated oral mucosal epithelial transplantation[102]	Stable corneal surface in 75%=No epithelial defect, fibrovascular and inflammation 1-year success rate=79.3% 4-year success rate=70.5% Visual acuity (VA)=70%
Cultivated oral mucosal epithelial transplantation[95]	Uniform epithelialized=76.4% Epithelial defect=23.5% Improved in VA=88.2%
Cultured autologous oral mucosal epithelial cells[113]	No ulcer in 22 out of 23 patients 19 decrees in severity
Simple oral mucosal epithelial transplantation[15]	Ocular surface epithelialization over 3 weeks One year follow-up visual acuity=light perception to 20/250
Simple oral mucosal epithelial transplantation[12]	Fixation (right eye)=UCsUM (left eye)=UCSM in the Visual axis clear
Simple oral mucosal epithelial transplantation[106]	Visual acuity=Counting fingers to 10/100
Autologous labial mucous membrane grafts (MMGs)[114]	Vision improved=20/160 left eye Schirmer's score=5 Clear cornea
Oral mucosal graft[115]	Pain/photophobia decreased=7 eyes Stable epithelium decreased vascularization/chronic inflammation=6 out of 7 Peripheral vascularization=5 eyes

Research on a variety of disorders, especially severe corneal diseases, has shown a great deal of interest in MSCs due to their special qualities. There are currently over 1000 registered clinical trials investigating the potential of MSCs produced from different sources, such as tooth pulp, bone marrow, fat, and umbilical cord.[96] More recently, a proof-of-concept study was carried out to compare allogeneic bone marrow MSCs transplantation with allogeneic CLET.[18] There were no negative cell product-related events in 72.7–77.8% of CLET cases and 76.5–85.7% of MSCs cases after 6–12 months.[18] In comparison to limbal epithelial cells, MSCs may offer several advantages, such as the ability to be harvested from various tissues, independence from cadaveric donors, and a quicker and less expensive procedure. Furthermore, the population of stem cells in CLET may be much smaller than that of MSCs in a transplant, where 100% of the MSCs are stem cells.[96]

The fibroblastic and epithelial layers of the AM include amniotic mesenchymal cells (AMCs). These cells are essential because they produce growth factors and cytokines that are easily incorporated into the AM's ECM.[120,121] Collagen types I, III, IV, and V, laminins 1 and 5, and fibronectin are among the vital constituents of adipose tissue's ECM. It also contains important cytokines and growth factors that are essential for tissue function and repair, such as hepatocyte growth factor (HGF) and epidermal growth factor (EGF).[122] Eslani

Table 5: Brief descriptions of current and emerging nonsurgical treatments

Treatment	Study	Description	Results
Blood-Derived Therapeutics[155]	Varies	• Autologous Serum: Whole blood centrifuged to separate the serum • Platelet-rich in growth factors (PRGF): Filtration of plasma supernatants after centrifugation of the whole blood • Platelet-rich plasma (PRP): Increased concentrations of platelets with an additional centrifugation of the whole blood • Platelet lysate: Induced platelet lysis and released of growth factors using PRP	• Improved symptoms, corneal epithelial healing, squamous metaplasia and tear stability, and visual acuity in LSCD patients[164,165]
CSB-001 (NCT06452316)	Phase I	Topical ophthalmic solution 0.1% human recombinant dHGF (5-amino acid deleted hepatocyte growth factor)	
MSC	Phase II	Subconjunctival injections of human bone marrow-derived MSC	• Two of the three patients with persistent epithelial defects in the setting of lenticule-assisted limbal stem cell transplantation (LSCD) exhibited complete resolution of the on day 7 and day 90, respectively • One patient had a -97% reduction in defect size on day 90
MSC secretome	Phase II	Topical ophthalmic solution containing MSC secreted factors; promote the survival and proliferation of LSCs	• Of the eight patients, 7 experienced improvements in visual acuity, 6 reported symptom alleviation with decrease in OSDI scores, and 5 had their corneal sensitivity increased compared to the baseline
MSC exosomes	Varies	Nanosized vesicles containing similar therapeutic features as MSCs (e.g., induce cell differentiation, anti-inflammatory effects, angiogenesis)[166]	• Promoted proliferation of corneal epithelial cells, reduced fibrosis, and inflammation[15] • Lowered levels of proinflammatory cytokines (e.g., TNF-α, IL-1β, and IL-6) and increased anti-inflammatory cytokines (e.g. IL-10 and TGF-β)[15] • Subconjunctival injection in rats effectively prolonged survival of corneal allograft by inhibiting infiltration of CD4 + and CD25 + T-cells and lowering levels of IFN-γ and chemical ligand 11 (CXCL11)[167]
Anti-inflammatory eye drops (e.g., corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs))		Reduce inflammation and improve corneal wound healing, create a favorable environment for the limbal stem cells	
Amniotic Membrane Extract (AME)	In Vivo, Ex Vivo	Non-surgical, derived form to include amniotic membrane transplant's (AMT) growth factor, anti-inflammatory, and re-epithelization supporting properties	• AME eye drops were clinically and histopathologically (stromal inflammatory cell inflammation, corneal vascularization, intraepithelial edema, stromal fibrosis, and metaplastic epithelial layer thickness) better at corneal healing than AMT and control groups[168] • Effective in preventing the development of complications of LSCD[168] • Decreased epithelium healing duration in vivo and increased LSC proliferation ex vivo[169]

et al.[123] assessed the angiogenic characteristics of MSCs obtained from the cornea and found that these cells secrete low levels of VEGF-A and high quantities of antiangiogenic factors (soluble fms-like tyrosine kinase-1 and pigment epithelial growth factor). New vessel formation was significantly reduced as a result of those conditions. The proof of concept for the applicability of bone marrow-derived MSCT has been reported in 17 cases. The success rate for MSCT ranged from 76.5% to 85.7%, which was comparable to CLET.[18] A Phase IIa clinical trial reported by Los Bueis *et al*. involved the injection of 400,000 ASCs into each limbo-conjunctival quadrant, resulting in the healing of all epithelial defects over an 86.5-month follow-up period.[124]

In a Phase I clinical trial (NCT04626583), the safety of the aforementioned MSCs as evaluated. A dose-escalation design was employed, with each cohort receiving a single dose of 1 million, 3 million, or 6 million MSCs administered locally via subconjunctival injection. A total of eight participants with persistent epithelial defects were enrolled. All patients demonstrated tolerance to MSCs without any treatment-related adverse events. Of the three patients with epithelial defects in the setting of lenticule-assisted LSCD, two patients exhibited complete resolution of the defects on day 7 and day 90, respectively. One patient demonstrated a 97% reduction in the defect size on Day 90. Following the assessment of

MSCs safety, a Phase II clinical trial is currently underway to evaluate their efficacy. This multicenter, randomized, 2:1 allocation, double-masked trial involves the evaluation of MSCs in patients with corneal epithelial defects. The US sites have completed the study, and international sites are actively enrolling patients (NCT05705024).

MSC secretomes

MSCs have therapeutic effects through paracrine mechanism by secreting various factors including insulin-like growth factor-1, hepatocyte growth factor, vascular endothelial growth factor, prostaglandin E2, and various cytokines for their immunomodulatory, regenerative, antiapoptotic, and antiscarring effects.[17,125,126] While MSCs are cell-based therapies, these secreted factors, namely, secretomes, can be utilized as cell-free therapies.[17] In our Phase I study, we produced topical MSC secretomes by collecting the cultured media and cryopreserving them until prior to the dispense. A total of eight patients with chronic corneal epitheliopathy were enrolled in our study. Following the dose-escalation model, a low-dose cohort administered the topical MSC secretome eye drops twice a day. A medium dose and high dose were administered to the eye four times and six times daily, respectively. All patients in the low and medium doses did not have any treatment-related adverse events. However, in the high-dose group, two dose-limiting toxicities with the development of a new epithelial defect and a significant decrease in visual acuity were reported, which were recovered with appropriate standard of care. Our preliminary data analysis indicated that seven patients experienced improvement in visual acuity, six patients reported symptom alleviation with decrease in OSDI scores, and five patients had their corneal sensitivity increased compared to the baseline. Proving the safety of the twice a day and four times a day dosings, we are in preparation of starting a randomized, double-masked Phase II study to evaluate the efficacy of the topical MSC secretomes.

Biological scaffolds for limbal regeneration

Human amniotic membrane (HAM)

Human amniotic membrane (HAM), the innermost layer of the placenta, has found widespread use in medicine due to its natural ability to reduce inflammation, prevent scarring, and fight infections. What makes it particularly valuable for eye treatments is its structural similarity to the cornea, which provides a supportive environment for LSCs. Over the years, HAM has been used as a platform to grow these stem cells outside the body before transplantation into patients with LSCD.[127] In limbal transplantation, adding HAM addresses the disadvantages of CLAU, which involved donor eye damage. This led to SLET, where tiny limbal tissue pieces are placed on HAM and transplanted.[128] To make HAM more effective, scientists have devised ways to strengthen and preserve it through techniques such as decellularization, cross-linking, and cryopreservation. Some researchers have also experimented with folding HAM into a three-dimensional structure to better mimic the natural limbal environment, although this approach is still being tested. Despite its many advantages, HAM has challenges, including variations in donor quality, the need for careful screening to prevent infection, and high storage costs. To overcome these issues, an alternative called HC-HA/PTX3 has been developed. This water-soluble extract provides a similar supportive environment for LSCs and has already been approved by the FDA for clinical use.[129] HC-HA/PTX3, a bioactive complex derived from HAM, maintains key regenerative properties such as anti-inflammatory and anti-fibrotic effects. Clinically, it supports LSCs survival and corneal regeneration, with FDA approval reinforcing its potential for ocular surface repair. Further research is needed to optimize its formulation and assess long-term efficacy.[130]

Fibrin

Fibrin, a protein involved in blood clotting, is another popular scaffold for LSCs grafts. It has been used in medical procedures for years as a natural adhesive and biodegradable membrane, making it an excellent option for delivering stem cells to the eye. Clinical trials using fibrin-based LSC grafts have shown success rates of between 66% and 90% and have even led to the development of Holoclar, the first approved stem cell therapy in Europe for the treatment of burn-induced LSCD.[130] One of the key benefits of fibrin is its ability to maintain the activity of LSCs before transplantation, helping them form a stable, healthy corneal surface after surgery. In many cases, patients treated with fibrin-based grafts have regained their vision without the need for additional procedures. However, there are limitations: Fibrin degrades quickly, which can make it less durable, and its production often relies on animal-derived components such as bovine serum, which raises safety concerns. Some researchers are working to improve fibrin scaffolds by incorporating platelets, which release growth factors that help maintain the health and function of stem cells.[131]

Acellular and Decellularized Limbus: Native and Novel Scaffolds

Organ rejection and dependency on immune rejection are two major limitations of allogeneic organ transplantation.[132] Regenerative medicine is an emerging field aimed at restoring lost organs. The concepts of decellularization and acellularization are essential components of regenerative medicine and have shown significant potential in various applications, such as heart valve reconstruction,[133] cornea regeneration,[134] and organ repair. In the case of LSCD, it is necessary to replace the lost limbal structure, which provides the limbal niche for cell settlement.[135] Although AM is already used for this purpose, it cannot fully mimic the three-dimensional biological environment of the palisades of Vogt.[135] Additionally, the paracrine signaling of the limbal stem cells' ECM plays a crucial role.[136-141] The applicability of decellularized limbal grafts in limbal and corneal regeneration was presented by Shafiq *et al*.[142] Re-epithelialization of the de-epithelialized porcine corneo-limbal graft by human LESCs and the subsequent corneal regeneration occurred within 5 to 7 days following multiple central corneal wounds. H&E staining confirmed the organized stratification of the epithelial layers.[143] Sodium desoxychelate and DNase were used for human limbus decellularization. The results showed that the DNA content reached 0.15 ± 0.01 μg/mg and that the basement membrane and ECM components were preserved. These methods could be applicable for creating a native scaffold for limbal reconstruction.[144] A comparison of the three materials—SDS, hypertonic saline (HS), and N2 gas (NG)—for corneo-limbal decellularization showed that SDS caused irreversible destruction of the corneal structure, while NG was not applicable for limbal decellularization. Among these, HS provided a more balanced outcome in terms of limbal decellularization and ECM preservation.[145] For limbal

reconstruction, three crucial steps are required. The first step is harvesting the limbal graft as thinly as possible to achieve two main objectives: reducing the severity of decellularization while preserving the ECM, and enhancing post-transplantation healing by establishing a securely attached corneo-limbal band that promotes LESCs migration and corneal healing. The second step involves decellularization and developing a gentler method that preserves the ECM structure. Choosing the appropriate harvesting techniques can facilitate this process. According to Sánchez-Porras *et al*., treating half-thickness limbal tissue with 1% SDS yielded the best decellularization results.[20] Replacing traditional techniques with mechanical limbal harvesting via a microkeratome to create a thin and even limbal epithelial graft represents a novel approach in limbal regeneration.[81] The third and last step is the recellularization of acellularized limbus and preparing for transplantation. The recellularization with hADSCs and SIRC is promising results for future research in limbal niche restoration.[145]

Synthetic scaffolds for limbal regeneration

Synthetic polymers are becoming increasingly popular as alternatives for expanding and transplanting LESCs. Their biggest advantage lies in their customizability; scientists can design them to meet specific LSCD approaches and limbal niche restoration needs, ensuring consistent quality and large-scale production without relying on biological materials. This also eliminates concerns about disease transmission, which can be a risk with donor-derived scaffolds. Unlike natural biomaterials, synthetic polymers can be fine-tuned to mimic the ECM, control how quickly they break down in the body, and enhance their mechanical strength, making them a promising option for limbal regeneration. Silk fibroin,[146] poly (lactic-co-glycolic acid),[147] polyethylene glycol (PEG),[148] gelatin methacrylate (GelMA),[149] and polycaprolactone (PCL)[150] are some synthetic materials candidates for limbal regeneration.

Adjutant therapy in corneal and limbal healing

Platelet-Rich Plasma

Eye drops provide a convenient, noninvasive method for delivering therapeutic agents directly to the ocular surface. They encompass a range of formulations, including artificial tears, growth factors, and anti-inflammatory agents, aimed at supporting the survival and proliferation of the corneal LSCs. Among these, blood-derived products (e.g., autologous serum eye drops (ASEs) and platelet-derived preparations) have been used for promoting ocular surface healing.[151] ASEs and platelet-derived preparations share similar compositions, including growth factors, cytokines, vitamins, and minerals that mimic the properties of tears for supporting corneal epithelial homeostasis and growth.[152] These properties make them promising therapeutics for restoring the LSCN.[153] ASEs have demonstrated efficacy in clinical studies for patients with persistent epithelial defects following LSCD.[154] By revitalizing the ocular surface, ASEs are routinely used in clinical practice for managing various ocular surface disorders such as dry eye disease, graft-versus-host disease (GVHD), and Sjögren disease.[1] However, ASEs lack platelets, which promote wound healing by prolonged release of growth factors, distinguishing it as a potentially superior alternative.[154]

Platelet-derived preparations, including PRP, plasma rich in growth factors (PRGF), and platelet lysate, are obtained from the supernatant of anti-coagulated whole blood through specialized centrifugation protocols.[155] These preparations are enriched with EGF, transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (bFGF), and pigment epithelium-derived factor (PEDF), which are important regulators of corneal wound repair and highlight platelet-derived preparations usefulness in limbal niche regeneration.[156] The platelet-derived products have demonstrated efficacy in reconstructive and regenerative ability in *in vivo* and clinical studies, including for dry eye disease and ocular chemical injuries.[157-159] With PRP containing a 5- to 6-fold higher concentration of growth factors and 8-fold higher concentration of platelets compared to peripheral blood, it has been effective in supporting the vitality of limbal transplantation by supporting the disturbed microenvironment in LSCD.[160,161] A North American multicenter study reported the safety and efficacy of PRGF in treating significant ocular surface diseases, with 6 out of 10 LSCD patients showing partial improvements in objective outcomes.[162] Platelet-rich fibrin lysates (PRF lysates) have also shown potential for limbal niche regeneration. An *in vivo* study in rabbits demonstrated that PRF lysates significantly increased LSCs proliferation and restored the LSCs niche in a chemical trauma model resembling LSCD.[163] Despite their therapeutic potential, a key challenge with eye drops lies in optimizing the limited bioavailability of therapeutic agents. Additionally, the varying severity and underlying causes of LSCD necessitate personalized, tailored treatment approaches. Promoting nonsurgical treatments is summarized in Table 5.

Gene modification associated with limbal regeneration

Gene modification is a promising area of research that can promote corneal and limbal regeneration. Some results suggest alternative genes that could open a new chapter in therapeutic design.[170] Single-cell analysis identified CREB-5 as a factor in corneal injury. Its downregulation reduced LESCs migration and corneal epithelial cell regeneration.[171] Xihong Lan *et al*.[172] showed that reducing IFNβ impaired corneal healing. MMP13 promotes IFNβ expression, supporting LSCs proliferation and corneal repair. Using human unrestricted somatic stem cells (hUSSCs) instead of 3T3 feeder cells in LESCs culture increased stemness and decreased differentiation gene expression. It may serve as an alternative for limbal and ocular surface regeneration.[173] LSCs are modified to silence the HLA expression and prevent the T-cell immune activation in consequence; this method will reduce the immune rejection in LSCs allotransplantation.[174] Bone morphogenetic protein 4 (BMP4) and LSCs media increase the expression of LSCs putative markers ABCG2 and ΔNp63α and promote the differentiation of iPS cells into LSCs.[175] Cisd2 knockout can impair corneal healing and may be a suitable target for drug design in ocular surface regeneration.[176] The secretome of AdMSCs reduces epithelial-mesenchymal transition (EMT) through the inhibition of TGF-β.[177] Fibronectin promotes rLESC renewal and proliferation by activating Wnt11-Fzd7 interaction and upregulating ROCK1 and ROCK2, respectively.[178] The 44-mer has shown a role in LSCs regeneration in the injured limbal area. Both SHh and ATGL inhibitors can impair 44-mer activity and LSCs proliferation.[179] It can also reduce neovascularization and goblet cell migration on the ocular surface.[180] Transfecting LSCs to block or express ΔNp63α can promote or inhibit LESCs proliferation, respectively.[181] After 21 days of transfection, AdMSCs expressing PAX6 exhibit corneal epithelial morphology and markers (cytokeratin 3/12 and E-cadherin), leading to the

formation of a stratified corneal layer and reconstruction.[182] Frizzled (Fz) is upregulated in putative LSCs, helping maintain their undifferentiated state, while its downregulation leads to the loss of stemness.[183]

Conclusion

Limbal niche reconstruction is a challenging process that requires a multidirectional approach. The three main aspects of limbal regeneration are limbal graft harvesting, decellularization, and recellularization with limbal or extra-limbal cell sources. Replacing manual limbal harvesting with mechanical techniques allows for the creation of thin, even, and uniform limbal grafts suitable for regeneration. Selecting a minimal decellularization process to preserve the ECM while ensuring an appropriate and potent cell source for regeneration is another crucial aspect of limbal reconstruction. Additionally, gene modification to regulate differentiation and the development of therapeutic drops to enhance healing and proliferation represent emerging strategies in limbal restoration.

Financial support and sponsorship: Nil.

Conflicts of interest: There are no conflicts of interest.

References

1. Yazdanpanah G, Haq Z, Kang K, Jabbehdari S, Rosenblatt M, Djalilian A. Strategies for reconstructing the limbal stem cell niche. Ocul Surf 2019;17:230-40.
2. Moshirfar M, Masud M, Harvey DH, Payne C, Bruce E, Ronquillo Y, *et al*. The multifold etiologies of limbal stem cell deficiency: A comprehensive review on the etiologies and additional treatment options for limbal stem cell deficiency. J Clin Med 2023;12:4418.
3. Sejpal K, Bakhtiari P, Deng S×. Presentation, diagnosis and management of limbal stem cell deficiency. Middle East Afr J Ophthalmol 2013;20:5-10.
4. Cheung AY, Eslani M, Kurji KH, Wright E, Sarnicola E, Govil A, *et al.* Long-term outcomes of living-related conjunctival limbal allograft compared with keratolimbal allograft in patients with limbal stem cell deficiency. Cornea 2020;39:980-5.
5. Basu S, Sureka SP, Shanbhag SS, Kethiri A, Singh V, Sangwan V, *et al.* Simple limbal epithelial transplantation: Long-term clinical outcomes in 125 cases of unilateral chronic ocular surface burns. Ophthalmology 2016;123:1000-10.
6. Karimian F, Jafarinasab MR, Feizi S, Javadi M, Ansari M, Hassanpour K. Long-term clinical outcomes of keratolimbal allograft alone or in combination with keratoplasty in chronic and delayed-onset mustard gas keratopathy. Cornea 2023;42:536-43.
7. Satake Y, Higa K, Tsubota K, Shimazaki J. Long-term outcome of cultivated oral mucosal epithelial sheet transplantation in treatment of total limbal stem cell deficiency. Ophthalmology 2011;118:1524-30.
8. Soleimani M, Mirshahi R, Cheraqpour K, Baharnoori S, Massoumi H, Chow C, *et al.* Intrastromal versus subconjunctival injection of mesenchymal stem/stromal cells for promoting corneal repair. Ocul Surf 2023;30:187-95.
9. Patil S, D'Souza C, Patil P, Patil V, Prabhu M, Bargale A, *et al.* Culture and characterization of human dental pulp-derived stem cells as limbal stem cells for corneal damage repair. Mol Med Rep 2019;20:4688-94.
10. Zhang P, Ma XY, Huang DT, Yang XY. The capacity of goat epidermal adult stem cells to reconstruct the damaged ocular surface of total LSCD and activate corneal genetic programs. J Mol Histol 2020;51:277-86.
11. Meyer-Blazejewska EA, Call MK, Yamanaka O, Liu H, Schlötzer-Schrehardt U, Kruse F, *et al.* From hair to cornea: toward the therapeutic use of hair follicle-derived stem cells in the treatment of limbal stem cell deficiency. Stem Cells 2011;29:57-66.
12. Umfress AC, Mawn LA, Joos KM, Donahue S, Schmitt A, Shieh C, *et al.* Surgical management of large bilateral epibulbar dermoids with autologous oral mucous membrane transplantation. Am J Ophthalmol Case Rep 2020;20:100982.
13. Inamochi A, Tomioka A, Kitamoto K, Miyai T, Usui T, Aihara M, *et al.* Simple oral mucosal epithelial transplantation in a rabbit model. Sci Rep 2019;9:18088.
14. Toshida H, Kasahara T, Kiriyama M, Iwasaki Y, Sugita J, Ichikawa K, *et al.* Early clinical outcomes of the first commercialized human autologous *ex vivo* cultivated oral mucosal epithelial cell transplantation for limbal stem cell deficiency: Two case reports and literature review. Int J Mol Sci 2023;24:8926.
15. Doctor MB, Rajagopal RN, Basu S. Simple oral mucosal epithelial transplantation (SOMET) for ocular surface reconstruction in Stevens-Johnson syndrome: A case report. Int J Surg Case Rep 2023;110:108643.
16. Costela-Ruiz VJ, Melguizo-Rodríguez L, Bellotti C, Illescas-Montes R, Stanco D, Arciola C, *et al.* Different sources of mesenchymal stem cells for tissue regeneration: A guide to identifying the most favorable one in orthopedics and dentistry applications. Int J Mol Sci 2022;23:6356.
17. Kobal N, Marzidovšek M, Schollmayer P, Maličev E, Hawlina M, Marzidovšek ZL. Molecular and cellular mechanisms of the therapeutic effect of mesenchymal stem cells and extracellular vesicles in corneal regeneration. Int J Mol Sci 2024;25:11121.
18. Calonge M, Pérez I, Galindo S, Nieto-Miguel T, López-Paniagua M, Fernández I, *et al.* A proof-of-concept clinical trial using mesenchymal stem cells for the treatment of corneal epithelial stem cell deficiency. Transl Res 2019;206:18-40.
19. Polisetti N, Roschinski B, Schlötzer-Schrehardt U, Maier P, Schlunck G, Reinhard T. A decellularized human limbal scaffold for limbal stem cell niche reconstruction. Int J Mol Sci 2021;22:10067.
20. Sánchez-Porras D, Caro-Magdaleno M, González-Gallardo C, García-García Ó, Garzón I, Carriel V, *et al.* Generation of a biomimetic substitute of the corneal limbus using decellularized scaffolds. Pharmaceutics 2021;13:1718.
21. Lim P, Fuchsluger TA, Jurkunas UV. Limbal stem cell deficiency and corneal neovascularization. In Seminars in Ophthalmology. Taylor and Francis; 2009.
22. Bibak-Bejandi Z, Djalilian AR. The ocular surface. In: Reference Module in Neuroscience and Biobehavioral Psychology. Elsevier; 2024. DOI: 10.1016/B978-0-443-13820-1.00212-7.
23. Yoon JJ, Ismail S, Sherwin T. Limbal stem cells: Central concepts of corneal epithelial homeostasis. World J Stem Cells 2014;6:391-403.
24. Elhusseiny AM, Soleimani M, Eleiwa TK, ElSheikh R, Frank C, Naderan M, *et al*. Current and emerging therapies for limbal stem cell deficiency. Stem Cells Transl Med 2022;11:259-268.
25. Massie I, Dziasko M, Kureshi A, Levis H, Morgan L, Neale M, *et al*. Advanced imaging and tissue engineering of the human limbal epithelial stem cell niche. Stem Cell Protocols 2015:179-202.
26. Yamada K, Young RD, Lewis PN, Shinomiya K, Meek KM, Kinoshita S, *et al*. Mesenchymal–epithelial cell interactions and proteoglycan matrix composition in the presumptive stem cell niche of the rabbit corneal limbus. Mol Vis 2015;21:1328-39.
27. Mathews S, Chidambaram JD, Lanjewar S, Mascarenhas J, Prajna N, Muthukkaruppan V, *et al. In vivo* confocal microscopic analysis of normal human anterior limbal stroma. Cornea 2015;34:464-70.
28. Higa K, Kato N, Yoshida S, Ogawa Y, Shimazaki J, Tsubota K, *et al.* Aquaporin 1-positive stromal niche-like cells directly interact with N-cadherin-positive clusters in the basal limbal epithelium. Stem Cell Res 2013;10:147-55.
29. Masood F, Chang J-H, Akbar A, Song A, Hu W, Azar D, *et al.* Therapeutic strategies for restoring perturbed corneal epithelial homeostasis in limbal stem cell deficiency: current trends and future directions. Cells 2022;11:3247.
30. Xie H-T, Chen S-Y, Li G-G, Tseng SC. Limbal epithelial stem/progenitor cells attract stromal niche cells by SDF-1/CXCR4 signaling to prevent differentiation. Stem Cells 2011;29:1874-85.
31. Han B, Chen S-Y, Zhu Y-T, Tseng SC. Integration of BMP/Wnt signaling to control clonal growth of limbal epithelial progenitor cells by niche cells. Stem Cell Res 2014;12:562-73.
32. Notara M, Shortt AJ, Galatowicz G, Calder V, Daniels JT. IL6 and the human limbal stem cell niche: A mediator of epithelial–stromal interaction. Stem Cell Res 2010;5:188-200.
33. Robertson SYT, Roberts JS, Deng SX. Regulation of limbal epithelial stem cells: Importance of the niche. Int J Mol Sci 2021;22:11975.
34. Daya SM. Conjunctival-limbal autograft. Curr Opin Ophthalmol 2017;28:370-6.
35. Krysik K, Dobrowolski D, Tarnawska D, Wylegala E, Lyssek-Boroń A. Long-term outcomes of allogeneic ocular surface reconstruction: Keratolimbal Allograft (KLAL) followed by Penetrating Keratoplasty (PK). J Ophthalmol 2020;2020:5189179.
36. Shanbhag SS, Chanda S, Donthineni PR, Basu S. Surgical management of unilateral partial limbal stem cell deficiency: Conjunctival autografts versus simple limbal epithelial transplantation. Clin Ophthalmol 2021;15:4389-97.

37. Elalfy M, Elsawah K, Maqsood S, Jordan N, Hassan M, Zaki A, *et al.* Allogenic cultured limbal epithelial transplantation and cultivated oral mucosal epithelial transplantation in limbal stem cells deficiency: A comparative study. Ophthalmol Ther 2025;14:413-32.
38. Berebichez-Fridman R, Montero-Olvera PR. Sources and clinical applications of mesenchymal stem cells: State-of-the-art review. Sultan Qaboos Univ Med J 2018;18:e264-77.
39. Cheung AY, Sarnicola E, Denny MR, Sepsakos L, Auteri N, Holland E. Limbal stem cell deficiency: demographics and clinical characteristics of a large retrospective series at a single tertiary referral center. Cornea 2021;40:1525-31.
40. Vazirani J, Nair D, Shanbhag S, Wurity S, Ranjan A, Sangwan V. Limbal stem cell deficiency—demography and underlying causes. Am J Ophthalmol 2018;188:99-103.
41. Cartes C, Lako M, Figueiredo FC. Referral patterns of patients with limbal stem cell deficiency to a specialized tertiary center in the United Kingdom. Ophthalmol Ther 2021;10:535-45.
42. Hu JC, Trief D. A narrative review of limbal stem cell deficiency and severe ocular surface disease. Ann Eye Sci 2023;8:13.
43. Skeens HM, Brooks BP, Holland EJ. Congenital aniridia variant: minimally abnormal irides with severe limbal stem cell deficiency. Ophthalmology 2011;118:1260-4.
44. Hatch KM, Dana R. The structure and function of the limbal stem cell and the disease states associated with limbal stem cell deficiency. Int Ophthalmol Clin 2009;49:43-52.
45. Kate A, Basu S. A review of the diagnosis and treatment of limbal stem cell deficiency. Front Med 2022;9:836009.
46. Genna VG, Maurizi E, Rama P, Pellegrini G. Biology and medicine on ocular surface restoration: Advancements and limits of limbal stem cell deficiency treatments. Ocul Surf 2025;35:57-67.
47. Yao TY, Wang JS, Geng W, Xie H-T, Zhang M-C. Conjunctival limbal autograft combined with amnion-assisted conjunctival epithelial redirection for unilateral total limbal stem cell deficiency after severe chemical burn. J Clin Med 2023;12:6235.
48. Baradaran-Rafii A, Heidari-Keshel S, Behnaz N, Alemzadeh-Ansari M, Feizi S, Hassanpour K, *et al.* Mini-Conjunctival Limbal Autograft (Mini-CLAU) using Platelet-Rich Plasma Eye Drops (E-PRP): A case series. Cornea 2023;42:1116-23.
49. Masters JS, Harris DJ Jr. Low recurrence rate of pterygium after excision with conjunctival limbal autograft: A retrospective study with long-term follow-up. Cornea 2015;34:1569-72.
50. Oliva-Biénzobas V, Nava-Castañeda A, Jimenez-Corona A, Kahuam-López N, Ramirez-Miranda A, Navas A, *et al.* Comparison of mini-simple limbal epithelial transplantation and conjunctival-limbal autograft for the treatment of primary pterygium: A randomised controlled trial. Br J Ophthalmol 2023;107:1776-81.
51. Kate A, Basu S. Mini-conjunctival autograft combined with deep anterior lamellar keratoplasty for chronic sequelae of severe unilateral chemical burn: A case report. Int J Surg Case Rep 2021;88:106508.
52. Cankaya C. Conjunctival limbal autograft combined with amniotic membrane transplantation to treat a moderate chemical eye injury. Beyoglu Eye J 2019;4:46-50.
53. Barreiro TP, Santos MS, Vieira AC, de Nadai Barros J, Hazarbassanov R, Gomes J. Comparative study of conjunctival limbal transplantation not associated with the use of amniotic membrane transplantation for treatment of total limbal deficiency secondary to chemical injury. Cornea 2014;33:716-20.
54. Welder JD, Pandya HK, Nassiri N, Djalilian AR. Conjunctival limbal autograft and allograft transplantation using fibrin glue. Ophthalmic Surg Lasers Imaging 2012;43:323-7.
55. Sangwan VS, Basu S, MacNeil S, Balasubramanian D. Simple limbal epithelial transplantation (SLET): A novel surgical technique for the treatment of unilateral limbal stem cell deficiency. Br J Ophthalmol 2012;96:931-4.
56. Malyugin BE, Gerasimov MY, Borzenok SA. Glueless simple limbal epithelial transplantation: The report of the first 2 cases. Cornea 2020;39:1588-91.
57. Malyugin B, Svetlana K, Fabian M, Werner B, Boris K, Maksim G. Femtosecond laser-assisted autologous glueless simple limbal epithelial transplantation in unilateral limbal stem cell deficiency: 12-month outcome of the first clinical cases. Cornea 2025;44:262-70.
58. Datar SG, Godse GN. Modified Supportive Simple Limbal Epithelial Transplantation (M-SLET): A surgical technique modified for limbal stem cell deficiency. Indian J Ophthalmol 2022;70:4434-7.
59. Mazumdar S, Chauhan R, Chaudhary S. Autologous simple limbal stem cell transplantation with conjunctival autograft in chronic unilateral total LSCD with severe symblepharon due to severe alkali burn. BMJ Case Rep 2024;17:e262267.
60. Bonacci E, Fasolo A, Pagnacco C, Bosello F, Marchini G, Pedrotti E. Simple epithelial transplantation for ocular surface reconstruction after severe ocular burn injury. Cornea 2024. doi: 10.1097/ICO.0000000000003726.
61. Jain M, Gupta N, Lohchab M, Gour A, Sangwan V, Singh B. Corneal stromal changes following simple limbal epithelial transplantation on Scheimpflug densitometry: Early results. Indian J Ophthalmol 2025;73:77-82.
62. Jain N, Mittal V, Sanandiya D. Outcomes of simple limbal epithelial transplantation without amniotic membrane grafting in unilateral limbal stem cell deficiency: A case series of 6 patients. Cornea 2025;44:80-5.
63. Sharma S, Singh S, Shanbhag SS. Case Report: Simultaneous penetrating keratoplasty with autologous simple limbal epithelial transplantation as an alternative to keratoprosthesis. F1000Res 2023;12:488.
64. Kate A, Mudgil T, Basu S. Longitudinal changes in corneal epithelial thickness and reflectivity following simple limbal epithelial transplantation: An optical coherence tomography-based study. Curr Eye Res 2022;47:336-42.
65. Ramachandran C, Deshpande P, Ortega I, Sefat F, McKean R, Srivastava M, *et al.* Proof-of-concept study of electrospun PLGA membrane in the treatment of limbal stem cell deficiency. BMJ Open Ophthalmol 2021;6:e000762.
66. Polania-Baron EJ, Graue-Hernandez EO, Ramirez-Miranda A, Amescua G, Navas A. Visual rehabilitation with limbal autologous stem cells transplant and cataract surgery in a patient with ocular surface squamous neoplasia. Am J Ophthalmol Case Rep 2021;23:101167.
67. Prabhasawat P, Chirapapaisan C, Ngowyutagon P, Ekpo P, Tangpagasit W, Lekhanont K, *et al.* Efficacy and outcome of simple limbal epithelial transplantation for limbal stem cell deficiency verified by epithelial phenotypes integrated with clinical evaluation. Ocul Surf 2021;22:27-37.
68. Wang Y, Hu X, Yang K, Zhang Y, Deng S, Wang Z, *et al.* Clinical outcomes of modified simple limbal epithelial transplantation for limbal stem cell deficiency in Chinese population: A retrospective case series. Stem Cell Res Ther 2021;12:259.
69. Kate A, Shanbhag SS, Goyal R, Basu S. Serial anterior segment optical coherence tomography post autologous simple limbal epithelial transplantation. BMJ Case Rep 2020;13:e236692.
70. Panthier C, Bouvet M, Debellemaniere G, Gatinel D. Conjunctival limbal autografting (CLAU) combined with customised simple limbal epithelial transplantation (SLET) in a severe corneal chemical burn: Case report. Am J Ophthalmol Case Rep 2020;20:100906.
71. Gupta N, Farooqui JH, Dziasko MA, Daniels J, Umang M, Sangwan V, *et al.* Reappearance of limbal pigmentation post-simple limbal epithelial transplant. Indian J Ophthalmol 2020;68:927-9.
72. Sah R, Patel N, Sangwan VS. Seroma under the amniotic membrane following simple limbal epithelial transplantation. BMJ Case Rep 2025;18:e261530.
73. Sharma N, Venugopal R, Mohanty S, Priyadarshini K, Nagpal R, Singhal D, *et al.* Simple limbal epithelial transplantation versus cultivated limbal epithelial transplantation in ocular burns. The Ocular Surface 2024;34:504-9.
74. Behaegel J, Zakaria N, Tassignon MJ, Leysen I, Bock F, Koppen C, *et al.* Short- and long-term results of xenogeneic-free cultivated autologous and allogeneic limbal epithelial stem cell transplantations. Cornea 2019;38:1543-9.
75. Cheng J, Zhai H, Wang J, Duan H, Zhou Q. Long-term outcome of allogeneic cultivated limbal epithelial transplantation for symblepharon caused by severe ocular burns. BMC Ophthalmol 2017;17:8.
76. Xue JF, Li DF, Wang YN, Chen C, Yang R, Zhou Q, *et al.* Midterm outcomes of penetrating keratoplasty following allogeneic cultivated limbal epithelial transplantation in patients with bilateral limbal stem cell deficiency. Int J Ophthalmol 2021;14:1690-9.
77. Mishan MA, Yaseri M, Baradaran-Rafii A, Kanavi MR. Systematic review and meta-analysis investigating autograft versus allograft cultivated limbal epithelial transplantation in limbal stem cell deficiency. Int Ophthalmol 2019;39:2685-96.
78. Yin J, Jurkunas U. Limbal stem cell transplantation and complications. Semin Ophthalmol 2018;33:134-41.
79. Porter RJ, Peretz D, Alsarhani WK, Chan C, Cheung A, Holland E. Donor tissue preservation time impact on keratolimbal allograft outcomes. Cornea 2024. doi: 10.1097/ICO.0000000000003746.
80. Cheung AY, Holland EJ. Keratolimbal allograft. Curr Opin Ophthalmol 2017;28:377-81.
81. Bibak-Bejandi Z, Mohammadi SF, Davoudi M, *et al.* A New Technique for Harvesting Limbal Stem Cell Tissue for Transplantation Using an Automated Microkeratome and a Novel Globe-Fixation System. Cornea, 2022: p. 10.1097.
82. Tran TM, Azher TN, Miller CJ, Hou JH. Topical tacrolimus compared with oral tacrolimus for postoperative immunosuppression in primary keratolimbal allograft. Cornea 2024;43:333-42.

83. Karimian F, Hassanpour K, Arzaghi M, Karjou Z. "En bloc" combined 270-degree keratolimbal allograft with central lamellar keratoplasty for severe limbal stem cell deficiency secondary to mustard gas exposure. Ocul Surf 2024;34:510-5.
84. Karimian F, Hassanpour K. En bloc keratolimbal allograft and central penetrating keratoplasty: A novel surgical technique in severe limbal stem cell deficiency. Cornea 2023;42:656-61.
85. Li Z, Yang K, Zhou Y, Wu T, Zhang H, Yang Q, *et al.* Outcome of keratolimbal allograft transplantation with deep anterior lamellar keratoplasty for bilateral limbal stem cell deficiency. Front Med (Lausanne) 2022;9:986194.
86. Mimouni M, Cole E, Kim SJ, Schiff J, Cardella C, Tinckam K, *et al.* Outcomes of keratolimbal allograft from ABO compatible donors for severe bilateral limbal stem cell deficiency. The Ocular Surface 2023;27:48-53.
87. Qi X, Duan F, Li X, Zhang X, Li N, Liu M, *et al.* Femtosecond laser-assisted keratolimbal allograft transplantation for the treatment of total limbal stem cell deficiency. Cornea 2019;38:1280-5.
88. Riedl JC, Wasielica-Poslednik J, Giers BC, Buonfiglio F, Pfeiffer N, Musayeva A, *et al.* Midterm results after allogeneic simple limbal epithelial transplantation from deceased-donor eyes in patients with persistent corneal epithelial defects due to limbal stem cell deficiency. Acta Ophthalmol 2024;103:e125–35.
89. Cheung AY, Reinisch CB, Hou JH. Decellularized descemet membrane anterior keratoplasty with allogeneic simple limbal epithelial transplantation for partial limbal stem cell deficiency following partial keratolimbal allograft failure. Cornea 2025;44:108-12.
90. Jain N, Kate A, Chaudhary S, Basu S. Allogeneic simple limbal epithelial transplantation for bilateral limbal stem cell deficiency in chronic vernal keratoconjunctivitis: A case report. Int J Surg Case Rep 2022;94:106968.
91. Chang YS, Chan TY, Jan RL, Tseng SH. Case report: Allogenic simple limbal epithelial transplantation from a human leukocyte antigen-matched living related donor to treat bilateral corneal chemical burns post laser-assisted *in situ* keratomileusis. Front Med (Lausanne) 2022;9:849791.
92. Kaur A, Jamil Z, Priyadarshini SR. Allogeneic simple limbal epithelial transplantation: An appropriate treatment for bilateral stem cell deficiency. BMJ Case Rep 2021;14:e239998.
93. Kunapuli A, Fernandes M. Successful outcome of simultaneous allogeneic simple limbal epithelial transplantation with therapeutic penetrating keratoplasty (PKP) for limbal stem cell deficiency and sterile keratolysis after chemical injury. Cornea 2021;40:780-2.
94. Vazirani J, Basu S, Sangwan VS. Allograft rejection after living-related simple limbal epithelial transplantation. Indian J Ophthalmol 2021;69:433-5.
95. Dobrowolski D, Orzechowska-Wylegala B, Wowra B, Wroblewska-Czajka E, Grolik M, Szczubialka K, *et al.* Cultivated oral mucosa epithelium in ocular surface reconstruction in aniridia patients. Biomed Res Int 2015;2015:281870.
96. Chen K, Soleimani M, Koganti R, Cheraqpour K, Habeel S, Djalilian A. Cell-based therapies for limbal stem cell deficiency: A literature review. Ann Eye Sci 2023;8:6. doi: 10.21037/aes-22-55.
97. O'Callaghan AR, Dziasko MA, Sheth-Shah R, Lewis M, Daniels J. Oral mucosa tissue equivalents for the treatment of limbal stem cell deficiency. Adv Biosyst 2020;4:e1900265.
98. Nishida K, Yamato M, Hayashida Y, Watanabe K, Yamamoto K, Adachi E, *et al.* Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. N Engl J Med 2004;351:1187-96.
99. Kolli S, Ahmad S, Mudhar HS, Meeny A, Lako M, Figueiredo F. Successful application of *ex vivo* expanded human autologous oral mucosal epithelium for the treatment of total bilateral limbal stem cell deficiency. Stem Cells 2014;32:2135-46.
100. Ilmarinen T, Laine J, Juuti-Uusitalo K, Numminen J, Seppänen-Suuronen R, Uusitalo H, *et al.* Towards a defined, serum-and feeder-free culture of stratified human oral mucosal epithelium for ocular surface reconstruction. Acta Ophthalmol 2013;91:744-50.
101. Kiritoshi S, Oie Y, Nampei K, Sato S, Morota M, Nishida K. Anterior segment optical coherence tomography angiography in patients following cultivated oral mucosal epithelial transplantation. Am J Ophthalmol 2019;208:242-250.
102. Prabhasawat P, Ekpo P, Uiprasertkul M, Chotikavanich S, Tesavibul N, Pornpanich K, *et al.* Long-term result of autologous cultivated oral mucosal epithelial transplantation for severe ocular surface disease. Cell Tissue Bank 2016;17:491-503.
103. Sasamoto Y, Sasamoto N, Tran J, Mishra A, Ksander BR, Frank MH, *et al.* Investigation of factors associated with ABCB5-positive limbal stem cell isolation yields from human donors. Ocul Surf 2020;18:114-20.
104. Cabral JV, Jackson CJ, Utheim TP, Jirsova K. *Ex vivo* cultivated oral mucosal epithelial cell transplantation for limbal stem cell deficiency: A review. Stem Cell Res Ther 2020;11:301.
105. Choe HR, Yoon CH, Kim MK. Ocular surface reconstruction using circumferentially-trephined autologous oral mucosal graft transplantation in limbal stem cell deficiency. Korean J Ophthalmol 2019;33:16-25.
106. Kara N, Dogan L. Simple oral mucosal epithelial transplantation in a patient with bilateral limbal stem cell deficiency. Eye Contact Lens 2021;47:65-7.
107. Kengpunpanich S, Chirapapaisan C, Ngowyutagon P, Chotikavanich S, Sikarinkul R, Taetrongchit N, *et al.* Comparative analysis of long-term results of three epithelial cell transplantation procedures for treating limbal stem cell deficiency. Ocul Surf 2024;32:71-80.
108. Prabhasawat P, Chirapapaisan C, Jiravarnsirikul A, Ekpo P, Uiprasertkul M, Thamphithak R, *et al.* Phenotypic Characterization of corneal epithelium in long-term follow-up of patients post-autologous cultivated oral mucosal epithelial transplantation. Cornea 2021;40:842-50.
109. Gao M, Chen Y, Zhai F, Liu Z, Liu Q, Wang Z. The effect of cultured autologous oral mucosal epithelial cells on ocular surface reconstruction. Arch Med Sci 2021;20:813.
110. Kim YJ, Lee HJ, Ryu JS, Kim Y, Jeon S, Oh J, *et al.* Prospective clinical trial of corneal reconstruction with biomaterial-free cultured oral mucosal epithelial cell sheets. Cornea 2018;37:76-83.
111. Baradaran-Rafii A, Delfazayebaher S, Aghdami N, Taghiabadi E, Bamdad S, Roshandel D. Midterm outcomes of penetrating keratoplasty after cultivated oral mucosal epithelial transplantation in chemical burn. Ocul Surf 2017;15:789-94.
112. Inatomi T, Nakamura T, Kojyo M, Koizumi N, Sotozono C, Kinoshita S. Ocular surface reconstruction with combination of cultivated autologous oral mucosal epithelial transplantation and penetrating keratoplasty. Am J Ophthalmol 2006;142:757-64.
113. Burillon C, Huot L, Justin V, Nataf S, Chapuis F, Decullier E, *et al.* Cultured autologous oral mucosal epithelial cell sheet (CAOMECS) transplantation for the treatment of corneal limbal epithelial stem cell deficiency. Invest Ophthalmol Vis Sci 2012;53:1325-31.
114. Sudana P, Basu S, Shanbhag SS. Oral mucous membrane grafts for total symblepharon and lid margin keratinisation post Stevens-Johnson syndrome. BMJ Case Rep 2020;13:e239383.
115. Liu J, Sheha H, Fu Y, Giegengack M, Tseng S. Oral mucosal graft with amniotic membrane transplantation for total limbal stem cell deficiency. Am J Ophthalmol 2011;152:739-47.e1.
116. Bonnet C, González S, Deng SX. Ocular surface regeneration by limbal stem cells therapies: State of the art, challenges, and perspectives. Stem Cells Transl Med 2023;12:714-9.
117. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, *et al.* Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315-7.
118. Liang L, Luo X, Zhang J, Su W, Zhu W, Xie Y, *et al.* Safety and feasibility of subconjunctival injection of mesenchymal stem cells for acute severe ocular burns: A single-arm study. The Ocular Surface 2021;22:103-9.
119. Colby K, Dana R. Foundations of Corneal Disease: Past, Present and Future. Springer; 2019.
120. Niknejad H, Yazdanpanah G. Anticancer effects of human amniotic membrane and its epithelial cells. Med Hypotheses 2014;82:488-9.
121. Niknejad H, Yazdanpanah G, Mirmasoumi M, Abolghasemi H, Peirovi H, Ahmadiani A. Inhibition of HSP90 could be possible mechanism for anti-cancer property of amniotic membrane. Med Hypotheses 2013;81:862-5.
122. Niknejad H, Yazdanpanah G, Ahmadiani A. Induction of apoptosis, stimulation of cell-cycle arrest and inhibition of angiogenesis make human amnion-derived cells promising sources for cell therapy of cancer. Cell Tissue Res 2016;363:599-608.
123. Eslani M, Putra I, Shen X, Hamouie J, Afsharkhamseh N, Besharat S, *et al.* Corneal mesenchymal stromal cells are directly antiangiogenic via PEDF and sFLT-1. Invest Ophthalmol Vis Sci 2017;58:5507-17.
124. Boto de Los Bueis A, Vidal Arranz C, Del Hierro-Zarzuelo A, et al., Long-Term Effects of Adipose-Derived Stem Cells for the Treatment of Bilateral Limbal Stem Cell Deficiency. Curr Eye Res, 2024. 49(4): p. 345-353.
125. da Silva Meirelles L, Fontes AM, Covas DT, Caplan AI. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. Cytokine Growth Factor Rev 2009;20:419-27.
126. Bhujel B, Oh S-H, Kim C-M, Yoon Y, Kim Y, Chung H, *et al.* Mesenchymal stem cells and exosomes: A novel therapeutic approach for corneal diseases. Int J Mol Sci 2023;24:10917.
127. Di Girolamo N. Biologicals and biomaterials for corneal regeneration and vision restoration in limbal stem cell deficiency. Adv Mater 2024;36:2401763.

128. Jackson CJ, Myklebust Ernø IT, Ringstad H, Tønseth KA, Dartt DA, Utheim TP. Simple limbal epithelial transplantation: Current status and future perspectives. Stem Cells Transl Med 2020;9:316-27.
129. Tseng SCG, Chen S-Y, Mead OG, Tighe S. Niche regulation of limbal epithelial stem cells: HC-HA/PTX3 as surrogate matrix niche. Exp Eye Res 2020;199:108181.
130. Gui Y, He Y, Wang D, Wang S, Zhang Y. Advances in cell transplantation therapy for limbal stem cell deficiency. Current Stem Cell Res Ther 2024;19:933-41.
131. Dereli Can G, Akdere ÖE, Can ME, Aydın B, Cagil N, Gümüşderelioğlu M. A completely human-derived biomaterial mimicking limbal niche: Platelet-rich fibrin gel. Exp Eye Res 2018;173:1-12.
132. Hostetter, T.H., Chronic transplant rejection. Kidney international, 1994. 46: p. 266-279.
133. Yan G, Fan M, Zhou Y, Xie M, Shi J, Dong N, *et al*., Chondroitin Sulfate Derivative Cross-Linking of Decellularized Heart Valve for the Improvement of Mechanical Properties, Hemocompatibility, and Endothelialization. ACS Appl Mater Interfaces, 2024. 16(28): p. 35936-35948.
134. Li Q, Sun Y, Zhao H, Gao Z, Zhai D. Structure and properties of the acellular porcine cornea irradiated with electron beam and its in-situ implantation. J Biomed Mater Res B Appl Biomater, 2023. 111: p. 2013-24.
135. Amin S, Jalilian E, Katz E, Frank C, Yazdanpanah G, Guaiquil VH, *et al*. The Limbal Niche and Regenerative Strategies. Vision (Basel), 2021. 5.
136. Huang M, Li N, Wu Z, Wan P, Liang X, Zhang W, *et al.* Using acellular porcine limbal stroma for rabbit limbal stem cell microenvironment reconstruction. Biomaterials 2011;32:7812-21.
137. Guo X, Xiao Y, Xu A, Duan C. Effects of exosomal microRNAs on oral mucosal epithelial cells cocultured with limbal niche cells. Contrast Media Mol Imaging 2022;2022:9794950.
138. Duan C-Y, Xie H-T, Zhao X-Y, Zhang M-C. Limbal niche cells: A novel feeder cell for autologous cultivated oral mucosal epithelial transplantation. Regen Med 2019;14:49-62.
139. Duan CY, Xie HT, Zhao XY, Xu W, Zhang M. Limbal niche cells can reduce the angiogenic potential of cultivated oral mucosal epithelial cells. Cell Mol Biol Lett 2019;24:3.
140. Zhao XY, Xie HT, Duan CY, Li J, Zhang M. Rat limbal niche cells can induce transdifferentiation of oral mucosal epithelial cells into corneal epithelial-like cells *in vitro*. Stem Cell Res Ther 2018;9:256.
141. Polisetti N, Schmid A, Schlötzer-Schrehardt U, Maier P, Lang S, Steinberg T, *et al.* A decellularized human corneal scaffold for anterior corneal surface reconstruction. Sci Rep 2021;11:2992.
142. Shafiq MA, Milani BY, Djalilian AR. *In vivo* evaluation of a decellularized limbal graft for limbal reconstruction. Int J Tissue Eng 2014;2014. doi: 10.1155/2014/754245.
143. Notara M, Schrader S, Daniels JT. The porcine limbal epithelial stem cell niche as a new model for the study of transplanted tissue-engineered human limbal epithelial cells. Tissue Engineering Part A, 2011. 17(5-6): p. 741-750.
144. Spaniol K, Witt J, Mertsch S, Borrelli M, Geerling G, Schrader S. Generation and characterisation of decellularised human corneal limbus. Graefes Arch Clin Exp Ophthalmol 2018;256:547-57.
145. Isidan A, Liu S, Chen AM, Zhang W, Li P, Smith L, *et al.* Comparison of porcine corneal decellularization methods and importance of preserving corneal limbus through decellularization. PLoS One 2021;16:e0243682.
146. Torsahakul C, Israsena N, Khramchantuk S, Ratanavaraporn J, Dhitavat S, Rodprasert W, *et al.* Bio-fabrication of stem-cell-incorporated corneal epithelial and stromal equivalents from silk fibroin and gelatin-based biomaterial for canine corneal regeneration. PLoS One 2022;17:e0263141.
147. Jafar H, Ahmed K, Rayyan R, Sotari S, Buqain R, Ali D, *et al.* Plasma-treated electrospun PLGA nanofiber scaffold supports limbal stem cells. Polymers 2023;15:4244.
148. Guo Y, Xian H, Shereen T, Qiang F, Jin X, Daniel M, *et al*. Feasibility of corneal epithelial transplantation with polyethylene glycol hydrogel membrane as a carrier for limbal stem cell deficiency. Chinese J Exp Ophthalmol 2022.
149. Zhong Z, Balayan A, Tian J, Xiang Y, Hwang H, Wu X, *et al.* Bioprinting of dual ECM scaffolds encapsulating limbal stem/progenitor cells in active and quiescent statuses. Biofabrication 2021;13:044101.
150. Zhou Z, Long D, Hsu C-C, Liu H, Chen L, Slavin B, *et al.* Nanofiber-reinforced decellularized amniotic membrane improves limbal stem cell transplantation in a rabbit model of corneal epithelial defect. Acta Biomater 2019;97:310-20.
151. Mustafa S, Mehandzhiyski N. Application of platelet-rich plasma (PRP) in corneal lesions-a review. Tradit Mod Vet Med 2021;6:55-64.
152. Kim KM, Shin Y-T, Kim HK. Effect of autologous platelet-rich plasma on persistent corneal epithelial defect after infectious keratitis. Jpn J Ophthalmol 2012;56:544-50.
153. Semeraro F, Forbice E, Braga O, Bova A, Di Salvatore A, Azzolini C, *et al.* Evaluation of the efficacy of 50% autologous serum eye drops in different ocular surface pathologies. BioMed Res Int 2014;2014:826970.
154. Mallone F, Marenco M, Giustolisi R, Plateroti R, Lambiase A. Platelet-Rich Plasma (PRP) to promote corneal healing in firework-related ocular burn and total limbal stem cell deficiency (LSCD). Eur J Ophthalmol 2023;33:NP18-22.
155. Han SB, Liu YC, Mohamed-Noriega K, Mehta JS. Application of novel drugs for corneal cell regeneration. J Ophthalmol 2018;2018:1215868.
156. Soleimani M, Cheraqpour K, Koganti R, Baharnoori SM, Djalilian AR. Concise review: Bioengineering of limbal stem cell niche. Bioengineering 2023;10:111.
157. Soni NG, Jeng BH. Blood-derived topical therapy for ocular surface diseases. Br J Ophthalmol 2016;100:22-7.
158. Avila MY, Igua AM, Mora AM. Randomised, prospective clinical trial of platelet-rich plasma injection in the management of severe dry eye. Br J Ophthalmol 2019;103:648-53.
159. Freire V, Andollo N, Etxebarria J, Hernáez-Moya R, Durán J, Morales M. Corneal wound healing promoted by 3 blood derivatives: An *in vitro* and *in vivo* comparative study. Cornea 2014;33:14-20.
160. Panda A, Jain M, Vanathi M, Velpandian T, Khokhar S, Dada T. Topical autologous platelet-rich plasma eyedrops for acute corneal chemical injury. Cornea 2012;31:89-93.
161. Alvarado-Villacorta R, García-Carmona KP, Martínez-Pardo ME, Vázquez-Maya L. Allogeneic limbal epithelial transplantation modified with solid platelet-rich plasma for bilateral limbal stem cell deficiency. Cornea 2020;39:311-4.
162. Soifer M, Tovar A, Wang M, Mousa H, Yennam S, Sabater A, *et al.* A multicenter report of the use of plasma rich in growth factors (PRGF) for the treatment of patients with ocular surface diseases in North America. Ocul Surf 2022;25:40-8.
163. Prabawati WE, Suhendro G, Retnowati E. A first step to novel approach for treating alkali injury of the cornea: Effect of platelet rich fibrin lysates on cultured rabbit (oryctolagus cuniculus) limbal stem cell proliferation exposed by sodium hydroxide. Folia Med Indones 2022;58:150-5.
164. Wang Y, Yousefi M, Fard MRG, Masihpour N, Ali Khiavi P, Yousefi K, *et al.* Revitalizing vision: Eye drops and corneal limbal stem cells survival. Stem Cell Rev Rep 2025. doi: 10.1007/s12015-025-10841-8.
165. Giannaccare G, Versura P, Buzzi M, Primavera L, Pellegrini M, Campos EC. Blood derived eye drops for the treatment of cornea and ocular surface diseases. Transfu Apher Sci 2017;56:595-604.
166. 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;10:711.
167. Jia Z, Lv Y, Zhang W, Zhang X, Li F, Lu X, *et al.* Mesenchymal stem cell derived exosomes-based immunological signature in a rat model of corneal allograft rejection therapy. Front Biosci (Landmark Ed) 2022;27:86.
168. Tas MD, Gurdal M, Kocamanoglu M, Korkmaz I, Arici M, Yaman B, *et al.* Topical application of amniotic membrane extract at a clinically correlated dose is effective in limiting complications in an experimental ocular alkaline burn model. Exp Eye Res 2025;252:110259.
169. Asl NS, Nejat F, Mohammadi P, Nekoukar A, Hesam S, Ebrahimi M, *et al*. Amniotic membrane extract eye drop promotes limbal stem cell proliferation and corneal epithelium healing. Cell J 2018;20:459-68.
170. Xiao YT, Xie HT, Liu X, Duan C, Qu J, Zhang M, *et al.* Subconjunctival injection of transdifferentiated oral mucosal epithelial cells for limbal stem cell deficiency in rats. J Histochem Cytochem 2021;69:177-90.
171. Gurdal M, Ercan G, Barut Selver O, Aberdam D, Zeugolis DI, *et al.* Development of biomimetic substrates for limbal epithelial stem cells using collagen-based films, hyaluronic acid, immortalized cells, and macromolecular crowding. Life (Basel) 2024;14:1552.
172. Lan X, Zhang W, Zhu J, Huang H, Mo K, Guo H, *et al.* dsRNA induced IFNβ-MMP13 axis drives corneal wound healing. Invest Ophthalmol Vis Sci 2022;63:14.
173. Shirzadeh E, Heidari Keshel S, Ezzatizadeh V, Jabbehdari S, Baradaran-Rafii A. Unrestricted somatic stem cells, as a novel feeder layer: *Ex vivo* culture of human limbal stem cells. J Cell Biochem 2018;119:2666-78.
174. Valdivia E, Bertolin M, Breda C, Carvalho Oliveira M, Salz A, Hofmann N, *et al.* Genetic modification of limbal stem cells to decrease allogeneic immune responses. Front Immunol 2021;12:747357.
175. Lee HS, Mok J, Joo CK. Bone morphogenetic protein 4 (BMP4) enhances the differentiation of human induced pluripotent stem cells into limbal

progenitor cells. Curr Issues Mol Biol 2021;43:2124-2134.

176. Sun CC, Lee SY, Kao CH, Chen L-H, Shen Z-Q, Lai C-H, *et al.* Cisd2 plays an essential role in corneal epithelial regeneration. EBioMedicine 2021;73:103654.
177. Shibata S, Hayashi R, Okubo T, Kudo Y, Baba K, Honma Y, *et al.* The secretome of adipose-derived mesenchymal stem cells attenuates epithelial-mesenchymal transition in human corneal epithelium. Regen Ther 2019;11:114-22.
178. Zheng M, Tian C, Fan T, Xu B. Fibronectin regulates the self-renewal of rabbit limbal epithelial stem cells by stimulating the Wnt11/Fzd7/ROCK non-canonical Wnt pathway. Exp Eye Res 2019;185:107681.
179. Fan NW, Ho TC, Wu CW, Tsao YP. Pigment epithelium-derived factor peptide promotes limbal stem cell proliferation through hedgehog pathway. J Cell Mol Med 2019;23:4759-69.
180. Yeh SI, Ho TC, Chen SL, Chen C, Cheng H, Lan Y, *et al.* Pigment epithelial-derived factor peptide facilitates the regeneration of a functional limbus in rabbit partial limbal deficiency. Invest Ophthalmol Vis Sci 2015;56:2126-34.
181. Yao JY, Chen JK. Over-expression of ΔNp63α facilitates rat corneal wound healing *in vivo*. Biosci Biotechnol Biochem 2017;81:2279-84.
182. Sun J, Liu WH, Deng FM, Luo Y, Wen K, Zhang H, *et al.* Differentiation of rat adipose-derived mesenchymal stem cells into corneal-like epithelial cells driven by PAX6. Exp Ther Med 2018;15:1424-32.
183. Mei H, Nakatsu MN, Baclagon ER, Deng S×. Frizzled 7 maintains the undifferentiated state of human limbal stem/progenitor cells. Stem Cells 2014;32:938-45.