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Corneal stem cells niche and homeostasis impacts in regenerative medicine; concise review

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Abstract

The limbal stem cells niche (LSCN) is an optimal microenvironment that provides the limbal epithelial stem cells (LESCs) and strictly regulates their proliferation and differentiation. Disturbing the LSCN homeostasis can lead to limbal stem cell dysfunction (LSCD) and subsequent ocular surface aberrations, such as corneal stromal inflammation, persistent epithelial defects, corneal neovascularisation, lymphangiogenesis, corneal opacification, and conjunctivalization. As ocular surface disorders are considered the second main cause of blindness, it becomes crucial to explore different therapeutic strategies for restoring the functions of the LSCN. A major limitation of corneal transplantation is the current shortage of donor tissue to meet the requirements worldwide. In this context, it becomes mandatory to find an alternative regenerative medicine, such as using cultured limbal epithelial/stromal stem cells, inducing the production of corneal like cells by using other sources of stem cells, and using tissue engineering methods aiming to produce the three-dimensional (3D) printed cornea. Limbal epithelial stem cells have been considered the magic potion for eye treatment. Epithelial and stromal stem cells in the limbal niche hold the responsibility of replenishing the corneal epithelium. These stem cells are being used for transplantation to maintain corneal epithelial integrity and ultimately sustain optimal vision. In this review, we summarised the characteristics of the LSCN and their current and future roles in restoring corneal homeostasis in eyes with LSCD.

Keywords

Limbal stem cells, corneal stromal stem cells, limbus, cornea, niche, homeostasis, wound healing

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Introduction

In adult life, cell division is required to preserve the differentiated cell population at a constant level through the replacement of lost cells. The rate of cell repopulation is completely dependent on cell turnover speed. Stratified squamous epithelia, such as the corneal epithelium, have a continuous, fast cell turnover maintained by the proliferation of a specific cell subpopulation called stem cells. Stem cells are considered a novel therapeutic tool for incurable diseases. These cells have a self-renewal capacity, which is derived from their special microenvironment called "niche." The niche is an extremely dynamic environment, providing a particular structure and function for stem cells.[1–3] Three components have been identified, including stem cells, supporting cells, and extracellular matrix derived from both stem and supporting cells.[4–6]

Limbal epithelial/stromal stem cells are unipotent adult stem cells lying in an anatomically distinct stem cell niche within the limbus. Limbus, the major source of stem cells, contributes to corneal homeostasis through two subsets of stem cell populations: limbal epithelial stem cells (LESCs)[4–6] and limbal stromal stem cells (LSSCs).[7,8] These two stem cell populations, along with corneal stromal stem cells (CSSCs), have been highlighted in regenerative medicine due to their application for bioengineered corneal constructs or allogeneic transplantations (Figure 1). Induction of the immunomodulatory response of the host makes LSSCs the best candidate for ocular therapies. The maintenance of corneal transparency is dependent on three major types of stem cells residing in the limbal niche and the corneal stroma.[9,10]

This review mainly focuses on the role played by limbal stem cells (LSCs) in corneal wound repair and regeneration and how they are being used for the treatment of different ocular disorders.

Cornea

The outermost layer of the cornea is the stratified squamous epithelium, which has 4–6 layers. The integrity of corneal epithelium is continuously preserved by the population of stem cells located in the peripheral area of the cornea, called limbus.[3] The limbus is the complex sclero-corneal transitional zone that maintains nourishment of the peripheral cornea and hosts the limbal stem cell niche (LSCN).[11]

Keratocytes are mesenchyme-derived cells lying within the corneal stroma and play a vital role in corneal wound healing by producing a diversity of cellular factors, including extracellular matrix (ECM) components, growth factors, cytokines, and kinases. These factors promote corneal tissue repair and homeostasis.[12]

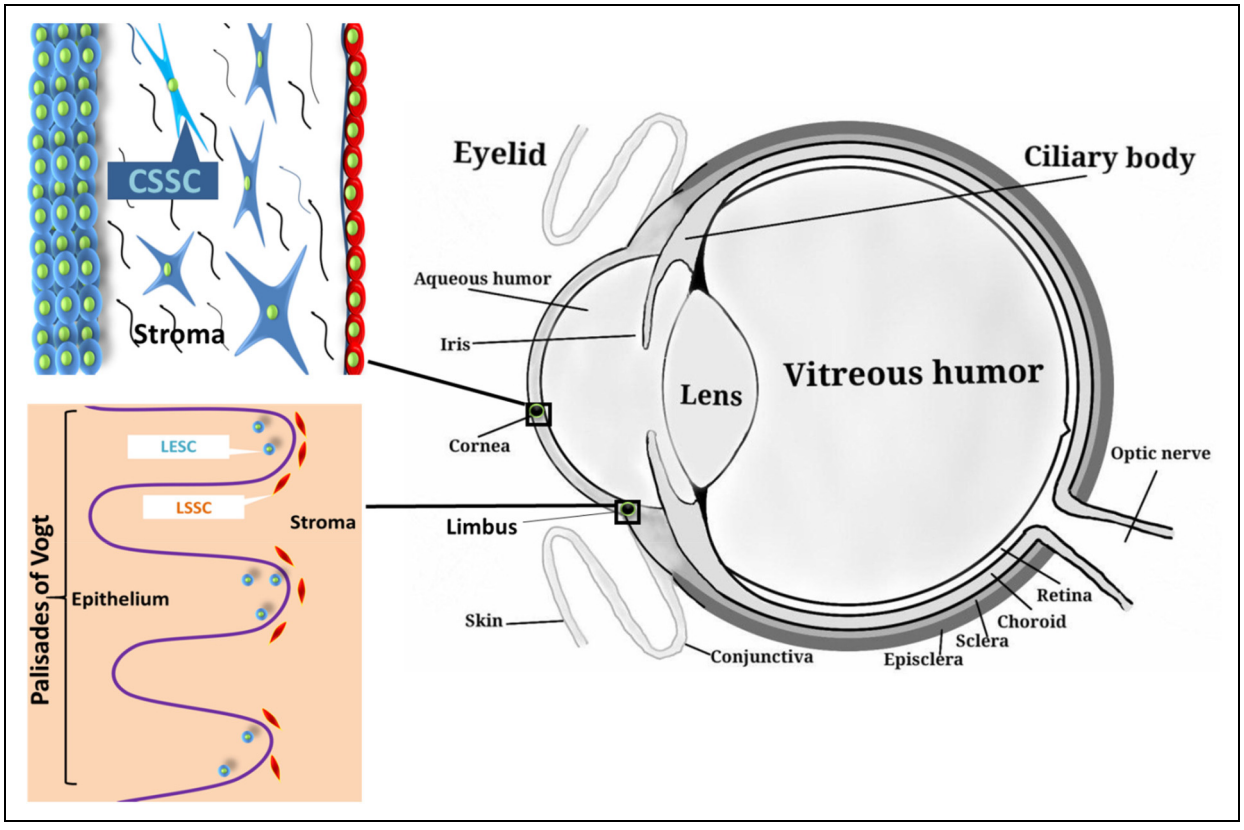

Figure 1. Human eye in cross section. Representative diagrams showing the localisation of the LESCs and LSSCs in limbal palisades of Vogt; CSSCs in the corneal stroma.

The unique corneal microstructure is responsible for preserving optimal transparency (Figure 2, Table 1). The corneal epithelium, which is the superficial layer of the cornea,[13] is composed mostly of terminally differentiated cells. These cells are recognised by corneal epithelial markers called cytokeratin (CK) −3 and −12, which are acid/base keratin pairs, expressed in the differentiated corneal epithelial cells.[13–15] Similar to the other ectoderm-derived tissues, the corneal epithelium renovates periodically every 7–12 days.[4,16,17] The corneal epithelium renewal is derived from the stem cell populations located in the basal layer of the limbal epithelial undulations, known as palisades of Vogt. These structures have a 0.31 mm length and 0.04 mm width and are mostly detectable in the superior and inferior parts of the limbal arc.[11]

The corneal epithelium and supra-basal limbal cells express the CK3 and CK12 differentiation markers, therefore, the entire corneal epithelium and limbal supra-basal cell layers are considered highly differentiated cells.[38,39] Compared to the corneal epithelium, the limbal basal layer exhibits more proliferative and differentiation potential both in-vivo and ex-vivo.[40,41] Maturity of corneal epithelial cells has a centripetal nature, which is called the "XYZ hypothesis," where X is representative for the proliferative phase of the basal epithelium, Y is for maturation and differentiation during movement toward the central cornea, and Z is for superficial desquamation. Besides the XYZ hypothesis, the regeneration of the corneal epithelium is maintained mainly through the LESCs and partially via the CSSCs. Unless stressed, as in wound healing, the trans amplifying cells (TACs) at the paracentral cornea has a significant regenerative capacity that can maintain the corneal epithelium independent of the LESCs.[40–42]

Almost 90% of the corneal thickness is made by the stroma,[42] which continues with the scleral stroma through the limbus. Unlike the scleral stroma, the corneal stroma has a distinctive arrangement of the collagen lamellae that helps maintain corneal transparency.[11,43,44] Keratocytes located in the corneal stroma secrete ECM in a highly organised fashion to preserve the corneal homeostasis.[45]

Limbus

Limbus is the corneoscleral transitional 1-mm wide ring with a highly vascularised stroma (Figure 3(a)) that provides oxygen and nutrients to the LESCs and the corneal periphery. The palisades of Vogt are the characteristic undulations of the limbal epithelium and stroma. The LSCN is a complex multicellular microenvironment with various signalling molecules that maintain the functions of LSCs. The limbal epithelium is constructed of three layers; superficial, suprabasal, and basal layers. The basal layer of the limbal epithelium is housing the LESCs,[15,46] residing deep in the crypts of the palisades of Vogt (Figure 3(b) and (c)).[47–51] These undulations provide more surface area for the LESCs within such a narrow region.

The LSCs are small, immature cells with tonofilament-enriched cytoplasm.[13,52] LSCs account for 0.5–8% of the

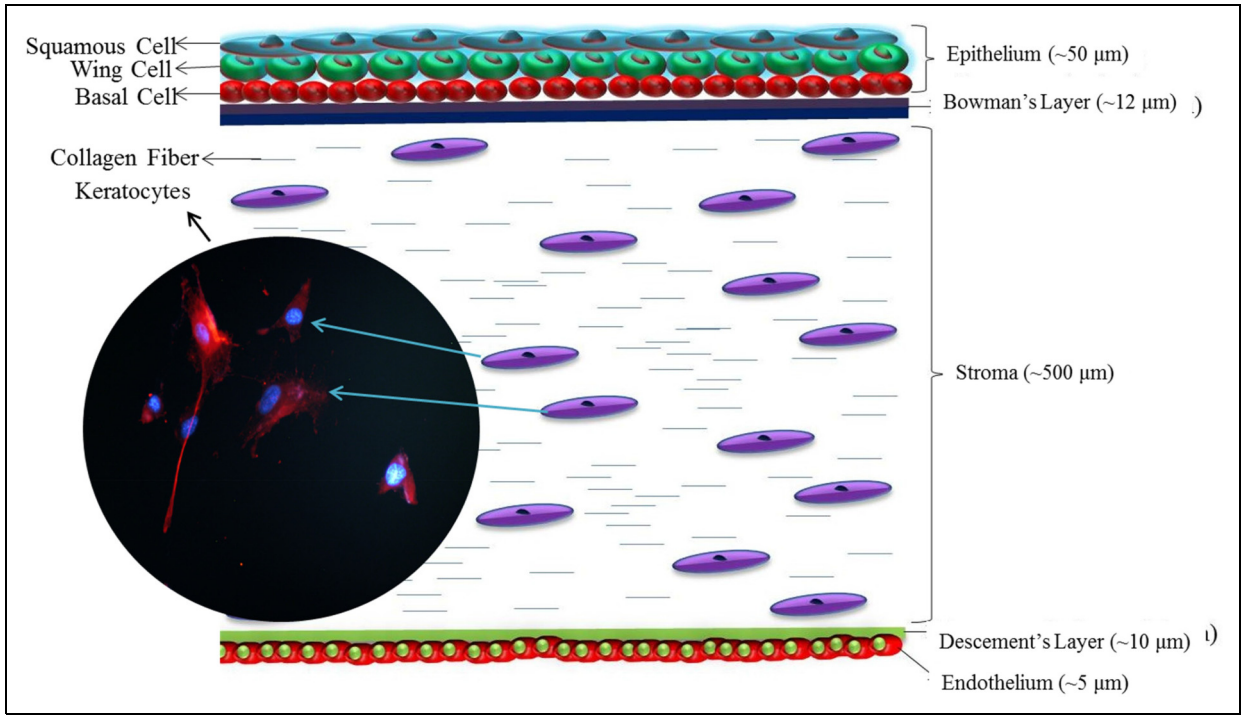

Figure 2. Structure of the cornea. Representative image of the cross-section of a human cornea, showing all the corneal layers and Corneal keratocyte cells stained positive for CD34 antibody (human corneal keratocytes marker[18]).

Table 1. Table illustrating the functions and origin of the different layers of the cornea.

Layer	Function & Mechanism	Embryological Origin	References
Endothelium	Responsible for the hydration control in the cornea through the "pump-leak" mechanism. The electrochemical potential/gradient due to the activity of Na^+, K^+ ATPase, and HCO_3^-, Cl^-, carbonic anhydrase is responsible for the pump and the swelling pressure of stroma is responsible for the leak.	Neural Crest	19–25
Descemet's membrane	1. An acellular, collagen-made (type VIII), strong barrier to protect the ocular surface from injuries and infections. 2. It plays a crucial role in healing the endothelium after injury.	Neural Crest	19,25,26
Stroma	Liable for maintenance of the limbal epithelial stem cell niche and regulation of limbal epithelial stem cell survival, proliferation, and differentiation *in vivo*. It has keratocytes and nervefiberss.	Neural Crest	8,19,25,27–33
Bowman's layer(BL)	The exact function of BL is unknown. It is considered to be associated with stromal wound healing in restoring the transparency of the anterior cornea and rebuilding the corneal epithelium after injury and trauma. It also works as a barrier	Neural Crest	19,25,34,35
Epithelium	The smooth epithelium is required for the optimal transparency of the cornea. It acts as a protective layer prohibiting the infiltration of foreign bodies. Epithelium acts as a barrier with the help of the tight junction between its cells, thus preventing the movement of electrolytes, fluids, etc.	Surface Ectoderm	19,23,25,30,32,36,37

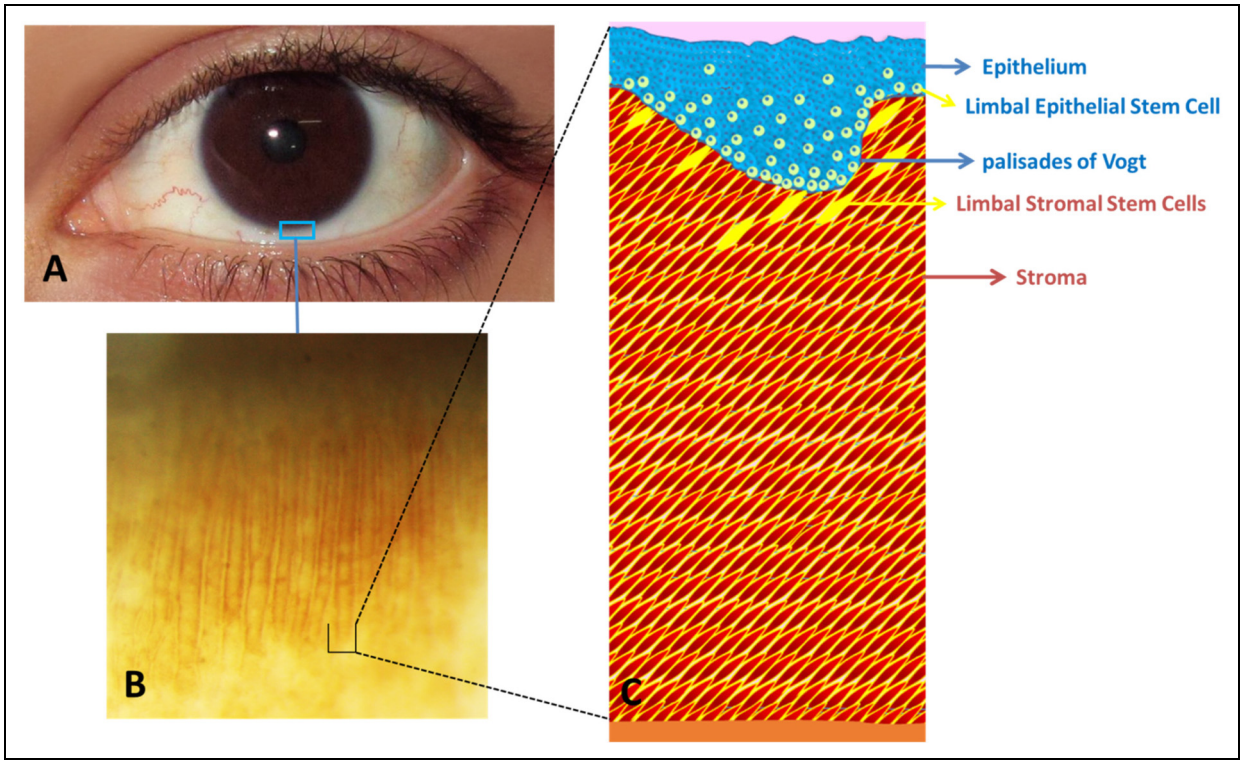

Figure 3. Palisades of Vogt in the human limbus. (a) Location of limbus on the ocular surface (b) magnified image of a portion of limbus, showing the finger-like projections: Palisades of Vogt, (c) representative diagram showing the cross-section of the limbus.

cell population present in the basal layer of the limbus. These cells express putative corneal surface markers such as CK3, CK12, CK14, and stem cell markers such as ATP-binding cassette transporter G2 (ABCG2), ABCB5, and transcription factors such as C/EBPδ, Bmi-1, and ΔNp63α.[3,19] LSCs have been cultivated/expanded *in-vitro* for more than a decade and

are considered highly successful in regenerating corneal epithelium. The homeostasis of the limbal niche is orchestrated via the coordinated crosstalk between its cellular and extracellular matrix components. LESCs maintain and promote their stemness through direct and indirect interactions with stromal mesenchymal cells, neuronal and microvascular networks, as well as ECM proteins and macromolecules.[19]

Various populations of stem cells to maintain the corneal transparency

Limbal epithelial stem cells

The limbal epithelium is formed from 7–10 layers and is held responsible for the regeneration of the corneal epithelium during either wound healing or the regular wear and tear due to blinking. The renewal of the corneal epithelium occurs in the following ways: *First*, the renewal of limbal epithelium: upon stimulation, the TACs of the limbal basal layer would divide asymmetrically to produce a limbal epithelial daughter stem cell and a limbal epithelial cell to renew the lost limbal epithelium and maintain the quiescence (7–12 days). TACs are mainly recognised by the expression of the P63 marker, and more precisely by ΔP63α. *Second*, the corneal epithelium is repaired at the time of injury: the regeneration of the lost or damaged corneal epithelium occurs through the symmetrical division of the LSCs, where the TACs of the limbus migrate to the central cornea. Then, TACs would divide multiple times to develop post-mitotic cells (PMCs) and terminally differentiate into the wing cells of the basal layer. *Third*, corneal epithelial renewal: The regular renewal (7–10 days) of corneal epithelium follows the same pattern as aforementioned, where the TACs develop into terminally differentiated cells (TDC), but with a lower rate of cell division (Figure 4(a-c)).[53,54]

Limbal stromal stem cells (LSSCs)

The limbal stroma is highly vascularised with capillaries and lymphatic vessels. It has a mixed population of fibroblast-like cells, myofibroblasts, dendritic cells, lymphocytes, mast cells, nerves, and macrophages. Unlike the corneal stroma, the limbal stroma's connective tissue is loosely and atypically adjusted.[55] Limbal stromal cells support the establishment of stratified squamous epithelial cell layers in the cornea via providing the matrix and maintaining the homeostasis of the LSCN.[56] Limbal stromal stem cells are characterised by their plasticity. These cells do not express the haematopoietic markers, but they exhibit mesenchymal characteristics and develop spindle-shaped outgrowths in the aged limbal culture.[57,58] The regenerative capacity of LSSCs has been reported previously.[57] The immunomodulatory and immunosuppressive properties of the LSSCs have been explored in various studies worldwide.[59,60] The safety of LSSCs has been assessed earlier in a mouse model of corneal injury where ex-vivo-cultivated human LSSCs were engrafted onto debrided murine corneas. Despite being a xenotransplant, limb stromal stem cells did not elicit an immune response.[61] This population of LSSCs is now being used therapeutically for patients with various corneal disorders.[10] Studies have shown that LSSCs have mesenchymal origin with the expression of Vimentin, ABCG2, BMI1, CXCR4, PAX6 and Six2, NGFR, NESTIN, CDH2, SSEA4, SOX2, REX1, NANOG, KLF4, OCT4 CD166, CD90, and CD73, CD34.[7]

Corneal stromal stem cells (CSSCs)

Approximately 90% of the cornea consists of stroma, which is a collagenous mesenchymal, connective tissue derived from the neural crest.[9] The CSSCs reside in the periphery of the cornea, adjacent to the limbal stroma, which is known for being the niche for CSSCs. Although CSSCs are obviously distinguished from corneal epithelial stem cells, these two cell populations may have some interactions because of their proximity in vivo. The corneal stroma is maintained by keratocytes, which account for about 3% of the stromal volume.[9] They retain proteoglycans and collagen lamellae that are crucial for corneal transparency.[47,62–65] Many studies reported that the keratocytes are multipotent mesenchymal stromal cells (MSCs) expressing both haematopoietic and mesenchymal markers (Table 2).[57,64–66] When the cornea is inflamed or injured, keratocytes (normally quiescent cells) are transformed into the fibroblast-like cells and produce various signalling molecules that facilitate the migration and reconstruction of the damaged part of the cornea (Figure 5, Table 3).[9]

Impact of limbal niche

The stem cell niche is a special multicellular microenvironment with a distinctive ECM and several signalling molecules that are essential to conserving a subpopulation of cells with both regenerative and differentiation capacities. Within the palisades of Vogt, the basal epithelial cells are reported to be expressing a few stem cell markers, such as Connexion 43, Δp63a, Fzd7, N-cadherin and ABCG2, and cytokeratin 19.It is assumed that the limbus provides an epitomised stromal micro-environment to maintain and support the corneal epithelial stem cells.[114]

The limbal stromal tissue is also highly vascularised and contains unique ECM components such as α1 and α2 collagen IV, β2 laminin, and vitronectin compared to the corneal stroma. Moreover, it has been reported that there are direct biomechanical interactions between limbal stromal cells and epithelial cells in the palisades of Vogt, which are crucial to maintain the stemness property of

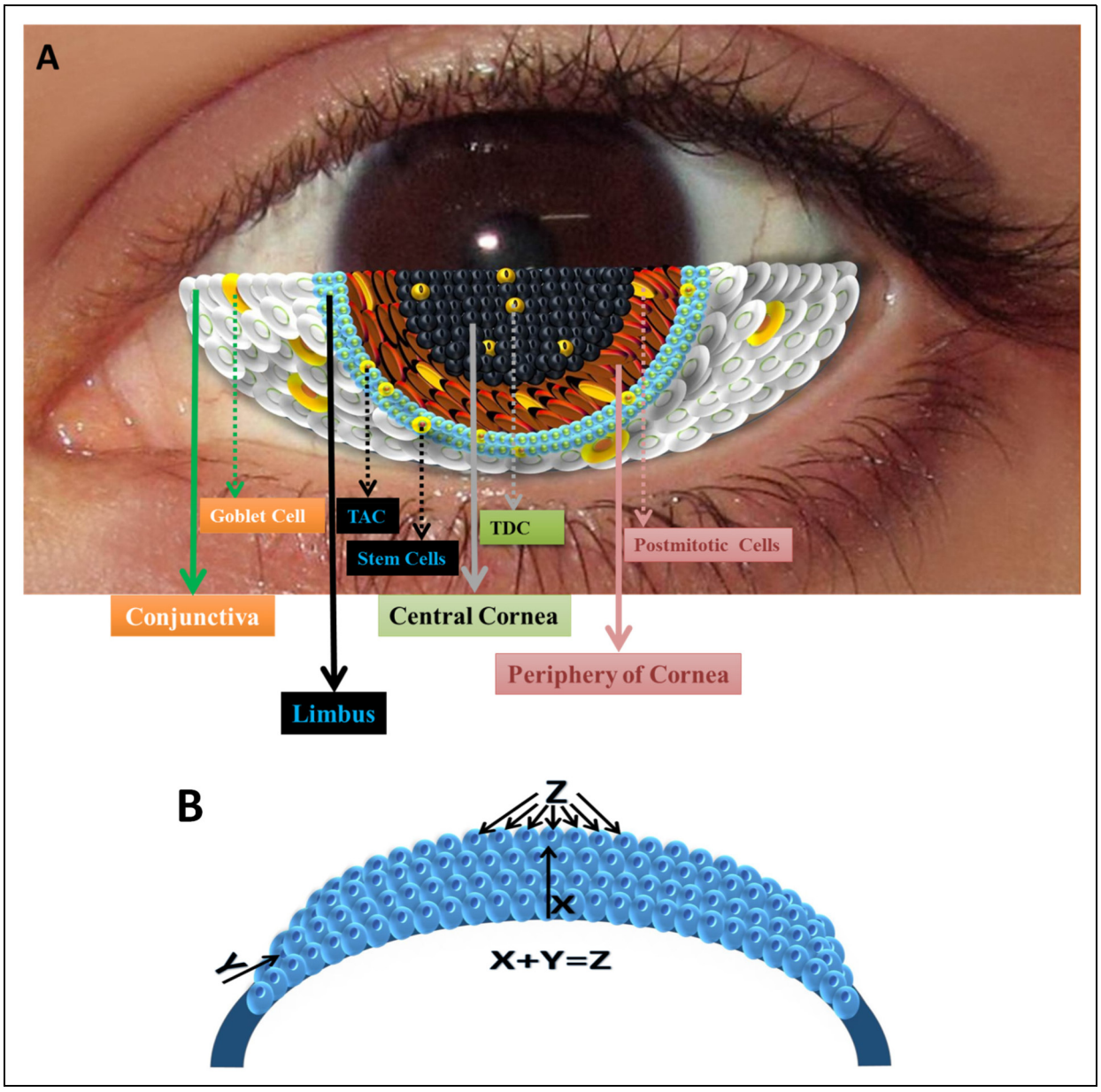

Figure 4. Cell fate of limbal epithelial stem cells to renew the corneal epithelium. (a) Migration of the LESCs from limbal epithelium to the central corneal epithelium, (b) stimulation of limbal stem cells for a symmetrical division and subsequent production of TDC in the central cornea according to XYZ hypothesis - Proliferated cells in basal layer of limbus (X) leading to the terminally differentiated cells (Z) with cell migration (Y) toward the center.

stem cell population.[3] Due to the dome-like topography of the human cornea, the distribution of the mechanical forces varies in different parts of the cornea, which may favour stem cell maintenance at the superior and inferior limbus.[115] The niche also preserves appropriate metabolic conditions for stem cell maintenance, including nutrient availability and oxygen concentration.[116] Limbal stromal mesenchymal cells have been reported to secrete factors such as, BMPs, FGFs, TGF-β, noggin, and Sonic hedgehog (Shh) that have been found to regulate stem cell proliferation and activation.[117–119]

Aberrant function of corneal stem cell niche

A lack of proper response from corneal epithelial stem cells (CESCs) to the ocular surface injury can lead to dysfunction of the LSCN. For instance, uveitis or systemic autoimmune diseases can eventually lead to limbal stem cell deficiency. The abnormal immune responses can induce profound changes in the niche microenvironment where the CESCs reside, thus impairing their maintenance or even survival.[120–122] The recruitment of inflammatory cells at the limbus leads to reduced anti angiogenic and colony-forming

Table 2. Comparison of four major cell populations playing roles in transparency and maintenance of the cornea.

Types of Cells	Source/ Origin	Expression Markers for related stem cells	Major function and location	Importance of wound healing, etc.	Reference
Limbal epithelial cells	• Surface Ectoderm	ΔNp63α, ABCG2, Integrand α9, N-adhering and, neural stem cell markers like NGF, GDNF, TrkA, GDNF, Notch1.	Production and maintenance of limbal epithelial stem cells from the basal layer of the limbus in palisades of Vogt which generally these stem cells by asymmetrical division produce the TACs and by sending them to the cornea doing the routine homeostasis to maintain the corneal transparency.	During the corneal epithelial injury. The limbal epithelial stem cells and TACs along with growth factors (IGF2, HGF, KGF, PEDF) renew the corneal epithelium	72–79
Limbal stromal cells	Neural Crest	Express mesenchymal markers, CD29, CD54, CD71, CD90, SH2 (CD105), CD106, CD166, SH3, or SH4 (CD73 and STRO-1).	Because of the rich extracellular matrix, stromal cells in limbus act as a niche for limbal epithelial cells and the other hand the importance of stroma in limbus would be needed for cellular differentiation in limbal endothelium.	After the corneal stromal injury, keratocytes activated in form of fibroblast and then transformed to moveable myofibroblast along with transforming growth factor (TGF)-β. These motile fibroblasts, which are positive for alpha-SMA, will remodel the ECM to change the wound's basement to heal the injury.	9,57,58,72
Corneal Epithelial cells	Ectoderm	K3, Connexin 43, Connexin 50, InvolucrinEGFR	The TDCs reside from limbus renew the corneal epithelium every 7–12 days as a normal homeostasis activity.	Without corporation of limbal epithelial stem cells can not heal the corneal epithelial injury.	9,53,80–84
Corneal stromal cells	Neural Crest	Integrin β1,haematopoietic markers (CD133, CD34 and CD11b) and also mesenchymal markers (CD105, CD90, CD73, CD56, CD44, CD29 and CD13) PAX6, p63	In corneal stroma, keratocytes (fibroblasts) and lamella of collagen fibers by secretion of an extracellular matrix, comprising collagen and proteoglycans. it produces crystalline proteins, maintain the corneal transparency.	Without corporation of limbal epithelial stem cells can not heal the corneal stromal injury.	9,18,57,63,64,85–89

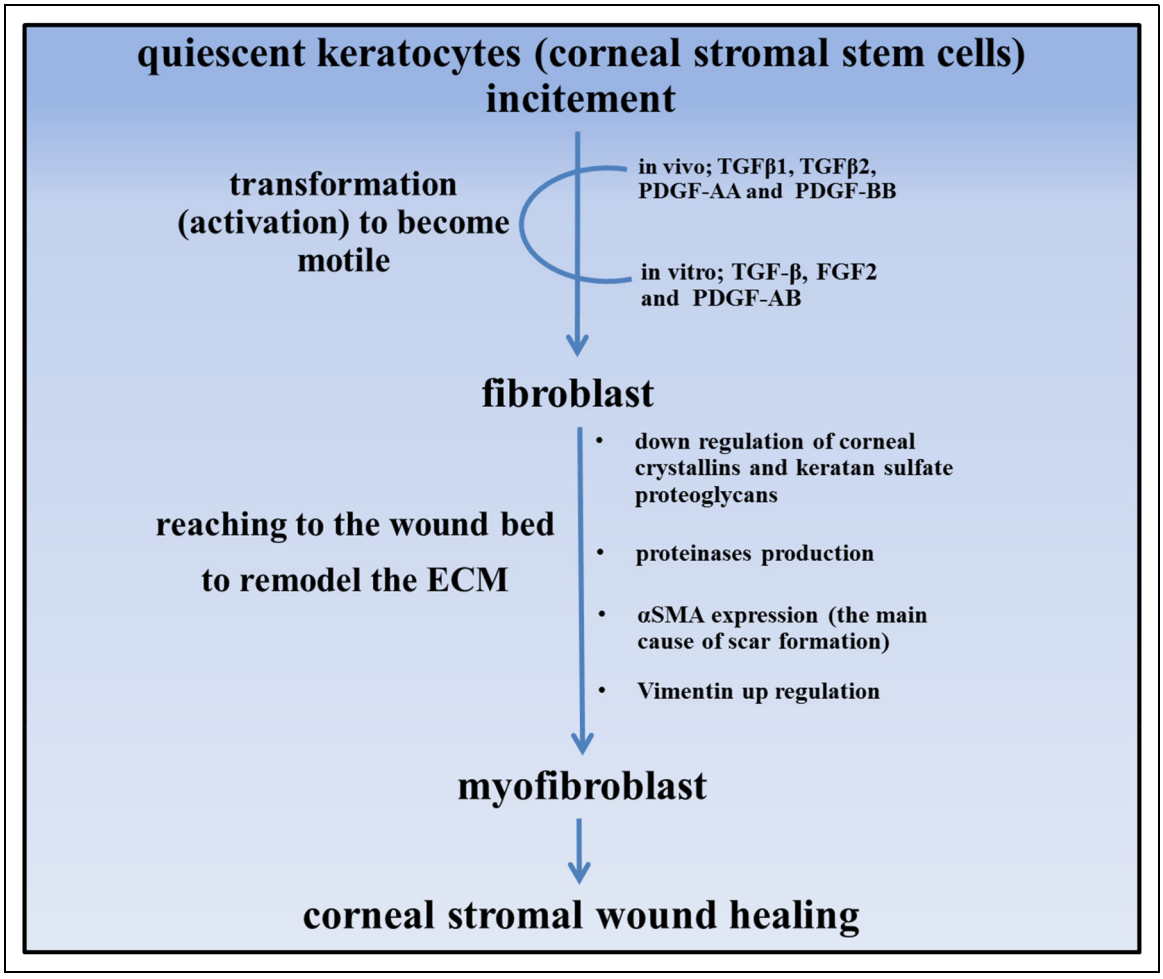

Figure 5. Schematic diagram describing the natural process of wound healing in corneal stroma which leads to scar formation. Upon injury to the corneal stroma, injured keratocytes of cornea undergo apoptotic pathway, stimulating the corneal stromal stem cells. The quiescent keratocytes proliferate and transform into fibroblast and ultimately to the form of highly motile contractile myofibroblasts.[62,67–71] To solve the corneal stromal haze and scar treatment, LSSCs (corneal stromal like cells) are being used in various clinical trials. Our group is involved in the similar clinical trials where LSSCs are being used for different corneal diseases.

Table 3. List of factors involved in corneal stromal wound healing.

Growth Factors	EGF, FGF, HGF, TGFα, TGFβ, TGFβ1, TGFβ2, PDGF, aFGF, bFGF, IGF-1	Keratocytes conversion to fibroblasts and myofibroblasts in cell culture mediated by TGFβ, FGF2, and PDGF-AB and in vivo mediated by TGFβ1, TGFβ2, PDGF-AA and PDGF-BB	29,62,67–71,90–106
Immune system	Cytokines (IL1α, IL1β, IL6, IL8, TNF-α), T cells, NK cells, macrophages, monocytes, PMN leucocytes	IL1β, IL6, and TNF-α are required as pro-inflammatory response for corneal stromal healing	70,72,90,100,107–110
Protease	Collagenase, Gelatinases matrix metalloproteinase (MMP2 and -9), Stromelysins (MMP-3 and -10)	ECM degradation enzymes are needed for ECM remodeling	70,72,110
Collagen components	COLIII, COLVIII, COLXIV and COLXVIII	Collagen components expression in corneal stroma is a complex procedure and it is vital for ECM remodeling	29,62,72,111–113

capacities of the LESCs, with subsequent loss of their stem cell markers. The ocular metabolic conditions, such as oxygen concentration, availability of nutrients, up-down regulation of growth factors, and cytokines, can directly affect the niche homeostasis and eventually affect the LSSCs' function.[116,121,123]

Chemical or thermal damage and genetic disorders such as aniridia (related to PAX6 gene deficiency) disrupt the interaction between CESCs and stromal stem cells in the limbus and may also change stem cell function through niche alteration. Chronic inflammation can induce metaplasia in the cornea by remodelling normal LSCN into abnormal LSCN (mechanotransduction of corneal stem cells).[124]

Different corneal pathologies and related treatments

Several Aetiologies, such as chemical injury, direct damages secondary to numerous ocular surgeries, contact lens wear, and prolonged usage of preserved eye drops, and also systemic medications or immune-related diseases, may lead to limbal stem cell deficiency (LSCD) and even possess the potential to injure the corneal stem cells (Figure 6). In this condition, impaired homeostasis of the limbal niche can be expected, and subsequently, the corneal epithelium and/or stroma would lose the capacity to regenerate,[4,125] with inevitable scar formation, corneal conjunctivalization, and limbal niche dysfunction.[4,51,52] It should be mentioned that the severity of the insult is a determining factor for the ocular surface response and the course of disease; in minor damages, the provoked inflammation has a regulatory effect and leads to epithelium regeneration and restoration of limbal niche homeostasis, whereas severe injuries would lead to compromising the limbal niche and impaired wound repair through a vicious cycle via prolonged secretion of pro-inflammatory materials.[125]

Diagnosis of LSCD

Although the diagnosis of LSCD is mainly clinically based on slit-lamp findings, impression cytology is the gold standard diagnostic method. Impression cytology in such conditions would reveal goblet cells on the corneal surface, expression of mucin5AC, mucin1, CK7, and CK19.[51,126,127] The other useful diagnostic tools for LSCD include confocal scanning, AS-OCT, and AS-OCTA.[125,127] Providing information at the cellular level is a considerable advantage of confocal scanning, which can record the progression of LSCD. This modality may show a reduction in sub-basal nerve plexus density, abnormal architecture of the limbus, goblet cells, epithelial cells with blurred borders, and sub-basal fibrosis. It has been shown that quantitative measurements via AS-OCT such as limbal epithelial thickness and central corneal epithelial thickness may be correlated with LSCD. Also, reflectivity of corneal epithelium and stroma may be altered and clear transition between corneal epithelium and conjunctival epithelium can be disappeared. Besides these, documentation of the architecture and density of limbal vessels can be provided via AS-OCT-A, as well as corneal neovascularisation depth and severity.[125,127]

Clinical treatments for limbal stem cell deficiency

Several therapeutic methods for LSCD treatment are available[128–132] including the following:

Non-surgical interventions

Eye lubrication: Ocular surface lubrication blocks the adhesion of epithelial to the tarsal conjunctiva and decreases shear stress. It's of note that eye drops should be non-preservative.[51,133]

Therapeutic soft contact lens: Therapeutic lens encourages healing of persistent epithelial defects (PED).[51,133,134]

Therapeutic scleral lens: Scleral lenses are employed to enhance the vision. They reduce pain and light sensitivity for people suffering from LSCD. Fluid-ventilated, gas-permeable scleral contact lenses can be helpful in the management of these patients.[51,134,135]

Biological factors: The local administration of exogenous biological factors is a non-invasive trend to support the LSCN restoration. Autologous serum-derived eye drops (ASE) have been reported to enhance ocular surface health in keratoconjunctivitis and Sjogren's disease.[136] Also, autologous serum drops have been often advised for dry eye disease, persistent corneal epithelial defects (PED), and LSCD. These agents may facilitate the proliferation and migration of healthy epithelium.[137,138] Like human tears, these drops are rich in various growth factors, cytokines, and epitheliotropic factors necessary for the physiologic maintenance of the ocular surface.[139] Also, platelet-derived formulations such as platelet releasate (PR), plasma rich in growth factors (PRGF), and platelet-rich plasma (PRP) showed similar reconstructive capabilities as ASE based on preliminary studies. Pigment epithelium-derived factor (PEDF) and nerve growth factor (NGF) possess anti-inflammatory, anti-inflammatory, and neuroprotective characteristics that have been shown to enhance corneal healing and promote LESC function.[140,141]

It should be mentioned that the only treatment for LSCD is limbal stem cell transplantation. Nevertheless, mild cases of LSCD can be managed medically without the need for further interventions. Moreover, appropriate medical management can completely affect the success rate of surgical interventions and limbal graft survival. Given this issue, dry eye and adnexal conditions should be addressed properly. However, a detailed discussion on medical management is outside the scope of this review.

Surgical interventions

Amniotic membrane transplantation: amniotic membrane is an extensively studied ECM scaffold for ocular surface reconstruction. AMT has a role in the management of various acute conditions like PED, partial LSCD, pterygium excisions, conjunctival defects, and keratitis. Moreover, AMT promotes faster re-epithelialization and

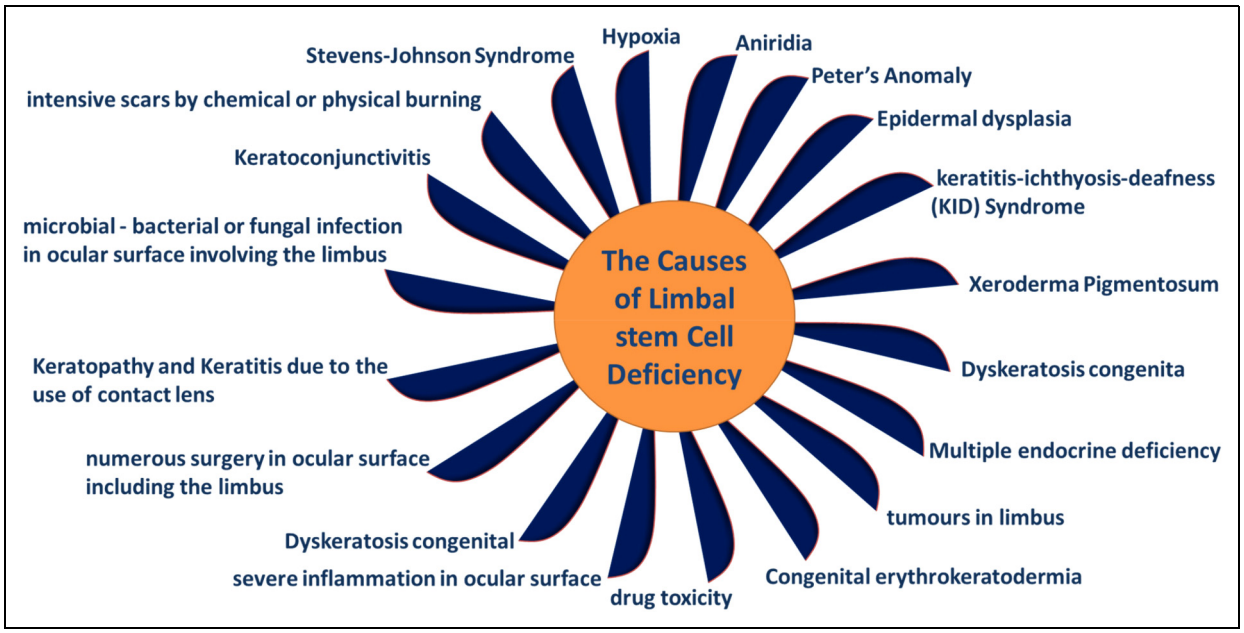

Figure 6. Causes of LSCD. Diagram showing various potential causes leading to LSCD.

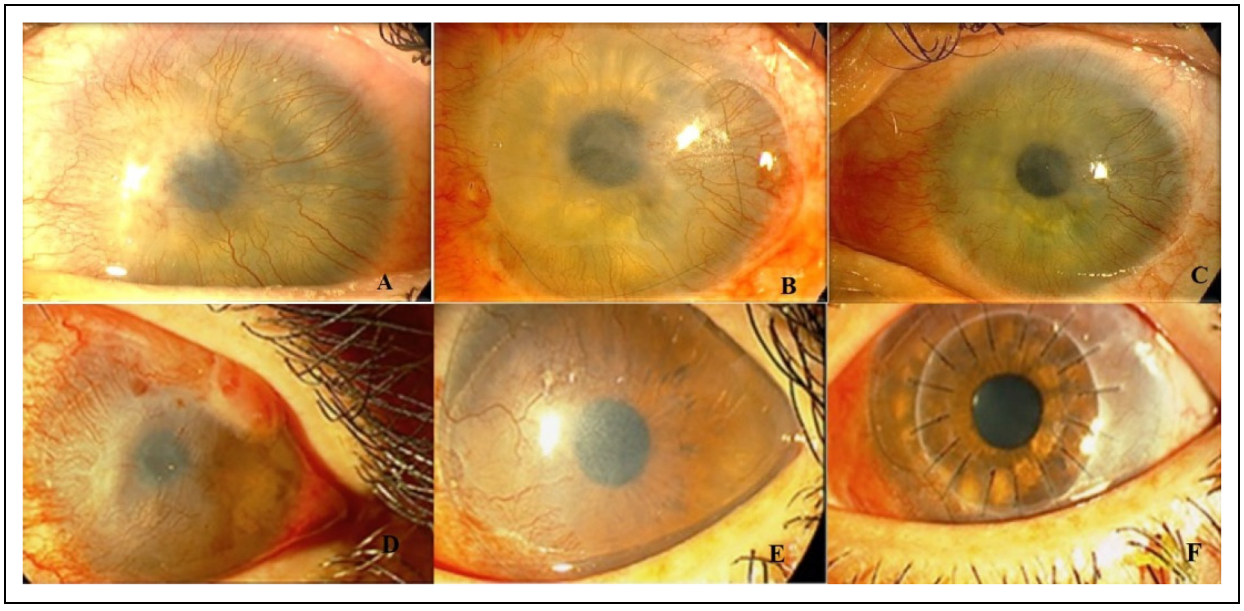

Figure 7. Clinical photographs of the two CLET techniques. A-C: CLET, D-F: *In vivo* CLET. (a) Eye with full LSCD due to chemical burn. (b) One week after transplanting the limbal epithelial monolayer, cultivated on the amniotic membrane. (c) Two months after CLET; Note the reduction of neovascularisation and increased corneal transparency. (d) Eye with full LSCD after chemical burn. (e) Improvement in corneal surface, decrease corneal vascularisation and opacity 3 months after "*In vivo* CLET". (f) After Sequential penetrating keratoplasty, corneal epithelium was clear and stable.

provides pain relief properties like a bio-bandage, for mild to moderate ocular burns.[142,143] Low transparency and rapid digestion after grafting are among the disadvantages of this material.[142] Other ECM scaffolds, including decellularized corneal matrix, hydrogels, fibrin, collagen, siloxane hydrogen contact lenses, silk fibroin, and bio-printed three-dimensional LSCN models, are promising therapies for LSCD.[144–148]

Kerato-limbal allograft (KLAL): Kerato-limbal allograft involves the transplantation of anterior halves and crescents taken from the allogeneic limbus along with corneoscleral rims, attached to the recipient's corneal bed

with the help of fibrin glue and secured with nylon sutures. This surgical method is a prominent intervention for the patient with bilateral LSCD but may require extended immunosuppression and comprehensive postoperative management.[149]

Living related-conjunctival limbal allograft (lr-CLAL): Conjunctival limbal allograft involves the transplantation of conjunctival limbal fornices with bulbar tissue included from a donor with ≥50% histocompatibility.[150] This surgical intervention is usually performed in patients with bilateral LSCD and one-eyed patients with LSCD.[133] This technique requires extensive immunosuppression and rigorous postoperative care.

Conjunctival limbal autograft (CLAU): The technique of CLAU mostly involves the removal of two 60° conjunctivo-limbal lenticules from the healthy contralateral eye, which are transplanted to the eye with LSCD.[151] This technique requires no immunosuppressants, but it often results in complications like PED[152] associated with delayed epithelial healing, keratitis,[153] conjunctivalization, and haze. The other considerable disadvantage of this technique is the risk of iatrogenic LSCD in the donor eye. However, this rate seems to be low.[152]

Cincinnati procedure: This technique is a combination of lr-CLAL and KLAL for cases of bilateral LSCD requiring reconstruction of the conjunctiva. Although this technique provides a 360° limbus restoration, the risk of graft rejection is high. Also, combined CLAU and KLAL have been introduced as modifications of this technique. In both Cincinnati and modified Cincinnati procedures immunosuppressive regimen is required.[154]

Keratoprosthesis: An artificial cornea, or keratoprosthesis, is an alternative option for allograft transplantation, that can bypass the use of immunosuppressive therapy and related complications. The Boston type 1 keratoprosthesis can be used in LSCD patients with acceptable tear function whereas the Boston type 2 keratoprosthesis or osteo-odonto keratoprosthesis are useful for eyes with compromised tear function.[125]

Cultivated Limbal Epithelial Transplantation (CLET): The limbal epithelium of a 2-mm^2 graft extracted from the patient's healthy eye has been expanded *in vitro* as a monolayer with the use of a human amniotic membrane (hAM) and 3T3 mouse fibroblast cells (Figure 7(a–c)). These cells have polygonal morphology and positive expression for E-cadherin, CK19, CK3, CK12, and ABCG2. The transplantation of hAM-expanded monolayers from the LESC population is known as CLET. The cultural period is about 2 weeks. Conjunctivalized tissues are completely removed through superficial keratectomy followed by transplantation of a carrier scaffold onto the ocular surface. CLET is performed in patients with LSCD and PED, such as ocular burns. In cases with bilateral LSCD, CLET involves the *in vitro* expansion of LSCs derived from the next closest kin.[51,155–159] The surgical technique of CLET has a success rate of 70–80% but is limited by the frequent requirement of re-surgeries.

Simple Limbal Epithelial Transplantation (SLET): A simplified and efficient *in vivo* version of CLET is the simple limbal epithelial transplantation or SLET (Figure 7(d-f)). The technique of SLET involves the *in vivo* expansion of a 1-clock-hour limbal tissue block from the contralateral healthy eye on the debrided cornea, where the recipient eye itself acts as an incubator. SLET is a relatively less expensive and less complicated surgical technique. It has a high success rate up to 80%. Over the past decade and a half, SLET has evolved into a highly practised and impactful innovation,[160,161] with a success rate up to 84%.[162] However, both CLET and SLET techniques cannot address limbal regeneration and LSCN restoration.

Cultured Limbal Stromal Stem Cell Transplantation (CLSSCT): Severe insults to the corneal epithelium and Bowman's layer may induce a corneal scar. After the corneal stromal injury, because of the disorganised ECM, a fibrotic scar forms, leading to corneal opacity and ultimately blindness. Animal studies have shown that the LSCs' application aids regeneration as well as restoring corneal transparency by preventing fibrosis. The technique involves the transplantation of the ex vivo cultivated LSCs derived from cadaveric limbal rims. Unlike CLET, where a monolayer of limbal epithelial expanded on hAM is transplanted, CLSSCT involves transplantation of the single-cell suspension of LSSCs.[125]

Cultivated oral mucosal epithelial transplant (COMET) or ex vivo cultivated oral mucosal autograft (EVOMAU): Owing to the risk of immune rejection and the limited availability of allogenic grafts, oral mucosa autografts have been investigated as a potential alternative option in cases of bilateral LSCD, with a 72% success rate. The drawbacks include peripheral corneal neovascularisation and suboptimal visual outcomes.[163–165]

Ex vivo-cultivated conjunctival epithelial transplantation (CjET): This was first described in 2013 with an 86% success rate at an average follow-up of 18.5 months in patients with total LSCD.[166]

Mesenchymal stem cells (MSCs) are multipotent stromal cells found in adipose tissue, bone marrow, and the corneal limbus. MSCs are capable of producing an extracellular matrix in three-dimensional cultures, secreting anti-inflammatory, and growth factors. Animal studies have shown the ability of the cultivated MSCs to transdifferentiate into corneal epithelium-like cells, promote corneal epithelialization, and reduce corneal opacification and neovascularisation.[167] Calonge et al. compared allogeneic bone marrow MSC transplantation versus CLET and reported comparable safety and efficacy profiles after a 12-month follow-up.[168]

Conclusion

It is well known that the limbal stem cell niche provides an appropriate microenvironment to maintain the multipotency and self-renewal abilities of LESCs and LSCs. The

niche is a three-dimensional structure that can provide a suitable function through physical factors, cellular and molecular interactions. During severe corneal damage, part of a limbus or the entire limbus along with a limbal niche may get damaged; therefore, the remaining amount of stem cells may not be sufficient to renew the entire corneal epithelium or stromal layers, which results in worsening of the condition. In such cases, the treatment relies on the donor tissue, however, because of limited supply and graft rejections, regenerative medicine techniques have been developing and are under various stages of pre-cliniclan and clinical trials.

Stem cell therapy for corneal pathologies is a promising technique, that has been studied for over 30 years now. Among all the available stem cell sources, LSCs show better results in corneal wound healing. Limbal stromal stem cells have anti-inflammatory properties that aid in healing. Various biomolecules involved in corneal tissue repair are addressed, and the association of LESCs, LSCs, and CSSCs to renew and heal the injuries in both the corneal epithelium and stroma is summarised. Periodic renovation and wound healing are the main functions of these LSCs. Randomised controlled clinical trials are crucial for advancing the clinical understanding and therapeutic strategy for LSCD. Long-term graft survival reports are unveiling the limits of current surgical options.[154] Novel therapies such as oral mucosa and conjunctiva epithelial-based transplantation, and extracellular matrix scaffolds are being explored for improved graft survival.[169]

In conclusion, the importance of stem cells in corneal epithelial and stromal wound healing in disease, injury, and post-surgical conditions can be understood with the available data. Human limbal-derived stem cell transplantations have opened up the gateway to the treatment of different corneal pathologies, which are leading to blindness.

Declaration of conflicting interests

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Method of literature

The authors attempted to cover almost the last ten years of the literature in a review of about 5000 words (in the main body part), in which the major focus was to provide a detailed summary of the different stem cell niches and their role in the healing of various corneal pathologies and their natural function in maintaining the homeostasis of the cornea. In this work, various sources (e.g. EMBASE, ISI, and MEDLINE) were used to collect valuable studies. In this regard, only articles written in English were collected by using some relevant keywords/phrases such as "stem cells niche," "limbal stem cell dysfunction," "cell therapy for ocular disease," "repair of corneal epithelial tissue," and "regeneration of corneal epithelial tissue." Fifty articles were found and included in this review.

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