



# An updated narrative review of treatment for limbal epithelial stem cell deficiency

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**Background and Objective:** Nearly 30 years have passed since limbal stem cell deficiency (LSCD) was first identified by pioneers and given clinical attention. LSCD remains a difficult disease to treat. It can potentially lead to blinding. At present, understanding of limbal stem cells (LSCs) has deepened and various treatment options for LSCD have been devised. The objective of this review is to summarize basic knowledge of LSCD and current treatment strategies.

**Methods:** PubMed search was performed to find studies published in English on LSCs and LSCD including original reports and reviews. Literatures published from 1989 to 2022 were reviewed.

**Key Content and Findings:** LSCs are enigmatic stem cells for which no specific marker has been discovered yet. Although LSCD is not difficult to diagnose, it is still challenging to treat. An important advancement in the treatment of LSCD is the provision of guidelines for selecting systematic surgical treatment according to the patient's condition. It is also encouraging that stem cell technologies are being actively investigated for their potential usefulness in the treatment of LSCD.

**Conclusions:** Although various treatment options for LSCD have been developed, it should be kept in mind that the best chance of treatment for LSCD is in the early stage of the disease. Every effort should be made to preserve as many LSCs as possible in the early treatment of LSCD.

**Keywords:** Limbus; stem cell; cornea; deficiency; transplantation

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## Introduction

### Background

Limbal stem cell deficiency (LSCD) is a rare disease. Its treatment is often difficult. LSCD is a condition in which normal corneal epithelization is not maintained because of the loss of limbal stem cells (LSCs) (1,2). Diseased corneal surface is covered by opaque conjunctiva with neovascularization. In severe conditions involving the visual axis, visual rehabilitation should be achieved using stepwise surgical approaches including multiple surgeries such as LSC transplantation and corneal transplantation.

### Rationale and knowledge gap

Since the pioneering works by Kenyon and Tseng who first proposed the role of LSCs in corneal epithelial homeostasis in 1989, many basic and clinical studies have been reported in this field (1). Various cell markers for LSCs have been found and innovative surgical treatment modalities have been developed (3). Recently, advanced stem cell technology has been incorporated into the development of a novel cell therapy to replace LSCs.

### Objective

The aim of this review was to summarize basic knowledge of

**Table 1** Search strategy summary

Items	Specification
Date of search	July 25 <sup>th</sup> , 2022
Databases and other sources searched	PubMed
Search terms used	Limbal, limbal stem cell, keratoprosthesis, limbal deficiency
Timeframe	May 1989 – May 2022
Inclusion and exclusion criteria	Inclusion: English publication, discusses limbal stem cell and limbal stem cell deficiency, partial LSCD, total LSCD, animal models, clinical studies, case Exclusion: non-English publication; not discussing limbal stem cell and limbal stem cell deficiency
Selection process	The author reviewed the papers involved and agreed to review them based upon the relevance of the articles

LSCD, limbal stem cell deficiency.

LSCD as clinicians and a general concept of its diagnosis and treatment. This article is presented in accordance with the Narrative Review reporting checklist (available at <https://aes.amegroups.com/article/view/10.21037/aes-22-51/rc>).

## Methods

A PubMed literature review was performed to search for relevant articles on LSCs and LSCD published in English from May 1989 to May 2022. The search strategy is presented in *Table 1*.

## Discussion

### LSCs

It has been known for a long time that stem cells for regenerating corneal epithelium exist. The exact location of these stem cells was found to be the limbus about 30 years ago. Using radio-labeling technique, Cotsarelis *et al.* (4) have verified that there are label-retaining, slow-cycling, and stem like cells at the limbus. These cells progressively lost radio-labeling as they migrated toward the central cornea.

LSCs reside in the limbal stem cell niche, a unique environment at the junction between the corneal epithelium and the conjunctival epithelium. The limbal stem cell niche has the classical architecture of the palisade of Vogt, limbal stem cell crypt, and limbal crypt or focal stromal projection near the palisade of Vogt (5). These structures are more prevalent in the superior and inferior limbus. The niche provides protection from ultraviolet light and supplies LSCs with blood vessels and various signaling molecules. Recently, there were some evidences in animals and human

to support the existence of LSCs even at the central cornea (6-8). However, it is still an established theory that most LSCs reside in the limbal area.

Molecular markers of LSCs are important for isolating and harvesting LSCs and culturing them for transplantation. For a long time, many researchers have tried to find molecular markers to characterize LSCs. However, there are no established markers for LSCs yet. At present, cytokeratins (such as cytokeratin K15),  $\Delta Np63\alpha$ , C/EBP $\sigma$ , Bmi1, ABCG2, and Notch-1 are well known candidate markers for LSCs (9).

### Limbal stem cell niche

The LSC niche is located in the Palisades of Vogt that are radially oriented, usually pigmented, and more prominent in superior and inferior limbi. Unlike the cornea, basement membrane at Palisades of Vogt is undulating with papillae, crypts, or stromal projections and fenestrated. This unique structure can shelter LSCs from physical shearing stress and provide a larger surface area to accommodate more LSCs within the confined area. Pigments of surrounding melanocyte can protect LSCs from ultraviolet damage (10). Limbal stroma underlying the Palisades of Vogt is heavily innervated and vascularized.

The interaction of stem cell with the surrounding environment such as vessel, neurons, and extracellular matrix is important in the homeostasis or activation of stem cells. How LSCs divide and maintain homeostasis has not been clarified yet. However, many studies have speculated that the unique three-dimensional structure of the limbus might play a critical role. Characteristically, the basement membrane at the Palisades of Vogt expresses laminin  $\alpha 2\beta 2$  chains, whereas the corneal basement membrane does not.

**Table 2** Etiology of limbal epithelial stem cell deficiency

Primary causes
Aniridia
Peter's anomaly
Ectrodactyly-ectodermal-dysplasia-clefting syndrome
Xeroderma pigmentosum
Turner syndrome
Dyskeratosis congenita
Multiple endocrine deficiency
Congenital erythrokeratodermia
Secondary causes
Thermal or chemical burn
Excessive contact lens wear
Ocular inflammation
Stevens-Johnson syndrome
Toxic epidermal necrolysis
Ocular cicatricial pemphigoid
Atopic keratoconjunctivitis
Herpes keratitis
Trachoma
Neurotrophic keratitis
Radiation therapy
Iatrogenic
Mitomycin C
5-FU
Cryotherapy
Extensive pterygium
5-FU, 5-fluorouracil.

In addition,  $\alpha 1$ ,  $\alpha 2$ , and  $\alpha 5$  chains of type IV collagen are present in the limbal basement membrane, while  $\alpha 3$  and  $\alpha 5$  chains are present in the central cornea. These properties may assist in sequestering and modulating concentrations of growth factors and cytokines for efficient and precise targeting to LSCs (11).

### ***Etiology of LSCD***

LSCD can be acquired or congenital. In most cases, it is acquired (12). The most common etiologies of LSCD are

chemical/thermal burns, allergic conjunctivitis, Stevens Johnson syndrome, and mucous membrane pemphigoid, whereas the most common etiology of congenital LSCD is aniridia (2). Common etiologies of LSCD are described in *Table 2*.

### ***Symptoms and signs of LSCD***

In its early stages, LSCD is usually asymptomatic. When LSCD progresses, various symptoms can occur, including blurring, ocular discomfort, photophobia, tearing, conjunctiva injection, and pain (3). Patients usually experience these symptoms months to years before diagnosis.

Typical early sign of LSCD is extension of the conjunctival epithelium (slight loss of transparency of epithelium) with fine vessels crossing the anatomic limbus. This is more common in the superior limbus. Punctate epithelial staining, tear film dysfunction, and conjunctival injection can occur when the disease progresses. At the late stage, corneal vascularization (superficial and deep) and fibrovascular pannus can develop and lead to visual loss.

### ***Diagnosis of LSCD***

The diagnosis of LSCD is primarily based on slit-lamp findings. An irregular corneal epithelium and conjunctivalization with neovascularization of the involved area suggest LSCD. Close observation of the corneal limbus may reveal disappearance of the palisade of Vogt. Fluorescein staining highlights the irregular, whorl-like pattern of the corneal epithelium (5). Sometimes, the demarcation line between the cornea epithelium and the invading conjunctival epithelium can be observed (12). Persistent epithelial defect is another important sign of LSCD. When the disease progresses further, corneal melting and perforation can occur.

Various objective diagnostic tools for LSCD are available. Impression cytology can reveal the presence of goblet cells in the conjunctivalized area (12). However, the absence of goblet cells does not necessarily imply intact LSCs. Additional immunocytochemistry is useful to find cells expressing conjunctival epithelial markers (such as cytokeratins 7, 13, and 19) or goblet cell markers such as mucin 5AC (13,14). Cytokeratin 12 is a corneal epithelial cell marker (15). Goblet cells and decreased density of the sub-basal nerve plexus can be detected in patients with LSCD using non-invasive *in vivo* confocal microscopy (16). LSCD can induce subtle changes in cornea

epithelial layers, such as thinning of the epithelial layer or increased reflectivity of the epithelium. These changes can be imaged using anterior segment optical coherence tomography (OCT) (17). OCT angiography can also detect limbal vascular changes in patients with LSCD (18).

### *Medical treatment of LSCD*

Except for sudden trauma such as chemical burn, diseases causing LSCD can slowly deplete LSCs. The damage is further promoted by uncontrolled inflammation, unstable tear film, and eyelid abnormalities. Therefore, treatment is needed to minimize LSC damage at an early stage of the disease to prevent further compromise of residual LSCs. Correction of the eyelid and tear film abnormalities with effective control of ocular surface inflammation may prevent the progression of LSCD to symptomatic stages.

Growth factor therapy is also effective in protecting the remaining LSCs in the limbal niche (3). Autologous serum, platelet-rich plasma, and amniotic membrane extract eyedrops contain various growth factors that can revitalize the limbal niche have the potential to reverse LSCD at an early stage (19). However, these treatments have limited efficacy for LSCD at its advanced stages.

### *Surgical procedures*

Mild degree of partial LSCD can be treated by simply removing abnormal epithelium and allowing the denuded cornea to be resurfaced with cells from the remaining intact LSCs. However, if LSCD has progressed with invasion of the visual axis, advanced surgical treatment is required. The goal of surgery is to transfer a sufficient amount of LSCs or corneal epithelial-like cells to the diseased ocular surface to promote and maintain corneal epithelialization. Although many types of limbal epithelial cell transplantation technique have been developed, none of them is universally successful.

Among various surgical methods introduced, the choice of surgical methods should be based on a thorough consultation between the patient and the surgeon. Depending on the surgical method, the patient may be at risk of damage to the healthy contralateral eye or long-term systemic immunosuppression if an allograft is used. Harvesting LSCs from the contralateral eye (e.g., conjunctival limbal autograft, CLAU) can induce iatrogenic LSCD in the donor eye (20). To avoid the risk of iatrogenic LSCD or bilateral advanced LSCD where enough autologous LSCs cannot be harvested, cell culture

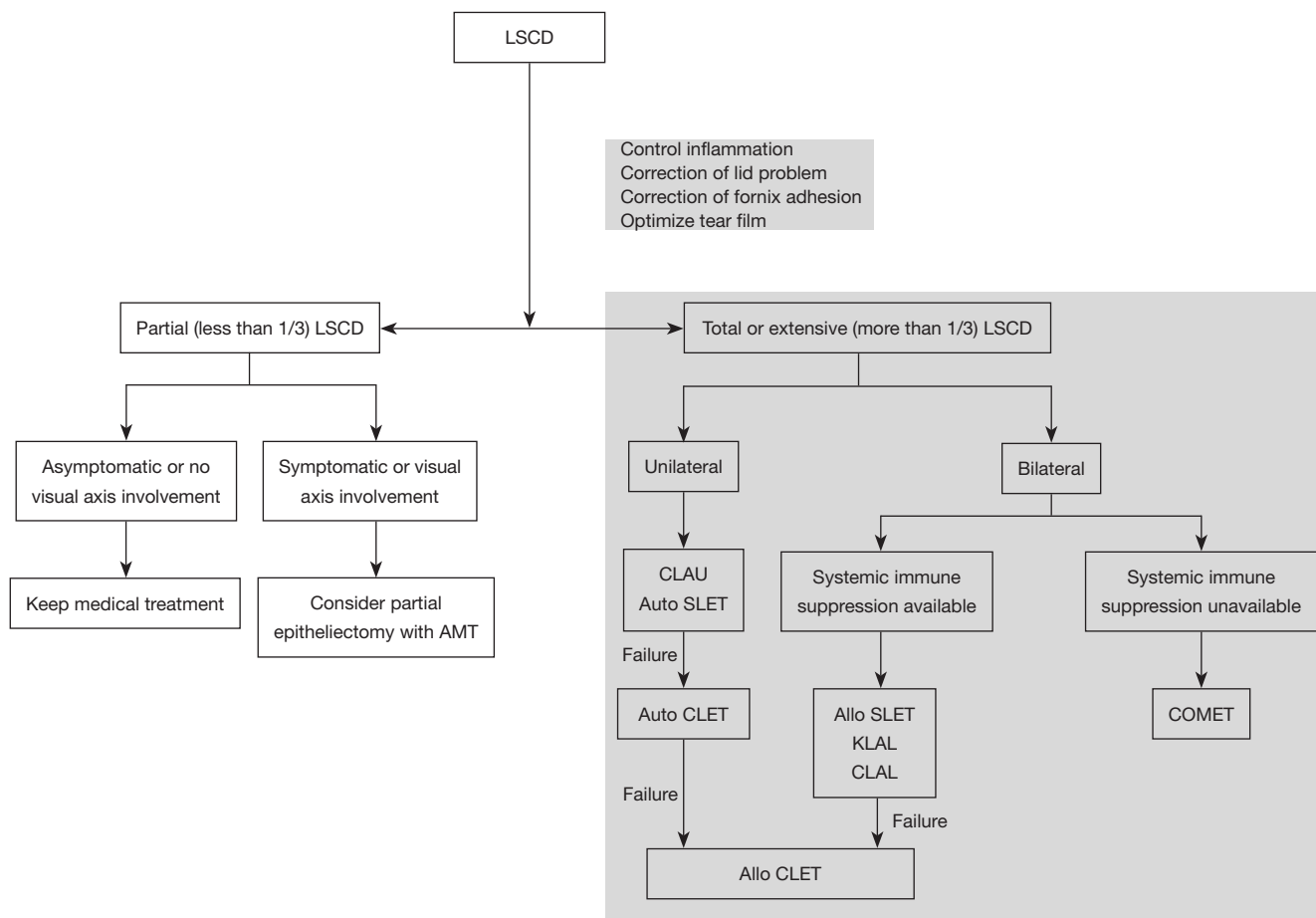
methods such as autologous cultivated limbal epithelial transplantation (CLET) can be used (21). Alternatively, simple limbal epithelial transplantation (SLET) recently introduced that requires only the use of 2×2 mm-sized small limbal block from the healthy donor eye can be used to minimize the risk of iatrogenic LSCD (22).

SLET deserves further discussion. Other surgical methods developed so far can transplant the harvested graft onto the limbus, the primary area of disease. However, in SLET, harvested limbal graft is cut into 6–10 pieces. These pieces are placed in the mid-periphery of cornea in a concentric pattern using fibrin glue (23). In some cases, human amniotic membrane graft is applied first followed by SLET. The hypothesis supporting SLET is that LSCs in each piece of graft can proliferate and form epithelial clump or island, which can then merge with each other to reconstruct corneal epithelial layer. LSCs may remain around the grafted area or slowly migrate toward the limbus (24). In a long-term (median: 1.5 years, range: 1 to 4 years) outcome analysis of 125 cases of SLET in unilateral ocular surface burn, 76% of eyes maintained successful outcomes and 67% of successful cases achieved 20/60 or better vision (25). In addition, analysis of 10 cases underwent penetrating keratoplasty for further visual gain revealed that basal cells expressing limbal stem cell markers [ $\Delta$ Np63 $\alpha$ (+)/ABCG2(+)] were observed in the trephined cornea after undergoing SLET (25).

In general, autografts have high success rates and better long-term prognosis. However, when LSCs cannot be obtained from the patient because of advanced bilateral LSCD, the use of allogeneic cells may be considered if the patient can tolerate systemic immunosuppression. The major limitation of an allograft is the risk of graft rejection because of the high vascular nature at the limbus. Allogeneic limbal stem cells can be used for conjunctival limbal allograft (CLAL), allo-SLET, allo-CLET, and keratolimbal allograft (KLAL).

If patients cannot tolerate long-term immunosuppressive treatment owing to their general conditions, surgical methods using cells from other body parts of the patient, even those without LSCs, should be considered. Cultivated oral mucosal epithelial transplantation (COMET) can be used to harvest autologous oral mucosal cells, to culture cells *in vitro* on carriers such as the amniotic membrane, or as a cell sheet for transplanting on the diseased ocular surface (26,27).

Cabral *et al.* (28) have analyzed clinical outcomes of COMET studies published from 2004 to 2019. Among



**Figure 1** Flowchart of current surgical therapeutic options for LSCD. LSCD, limbal stem cell deficiency; AMT, amniotic membrane transplantation; CLAU, conjunctival limbal autograft; SLET, simple limbal epithelial transplantation; CLET, cultivated limbal epithelial transplantation; KLAL, keratolimbal allograft; CLAL, conjunctival limbal allograft; COMET, cultivated oral mucosal epithelial transplantation.

243 eyes from 24 studies, 70.8% of eyes achieved a stable ocular surface. Visual acuity improvement was observed in 63.5% of eyes. It is known that the presence of pre-operative epithelial defect and poor tear production can increase the failure rate of COMET. Therefore, these abnormalities should be corrected prior to surgery for better outcome. Although several studies reported that the oral mucosal cells grafted with COMET could transdifferentiate into corneal epithelial phenotype expressing cytokeratin such K12 (29,30) without expressing MUC5AC, it is still difficult to definitively determine whether cultivated oral mucosal epithelial cells have transdifferentiated to the corneal lineage or whether the presence of corneal epithelial cells indicates expansion and migration of the remaining corneal cells.

With advances in stem cell technology, other stem cell

populations can be transdifferentiated to mimic corneal epithelial cells. Although this is still at the laboratory level, it is a promising option for future treatment of LSCD.

Figure 1 shows an example of how to select an appropriate surgical method according to various patient conditions. The flow chart was modified from suggestions of two recent publications (3,20).

**Alternative cells to replace LSCs**

With the advancement of stem cell technology, various attempts to use alternative cell populations to replace allogeneic cells are underway. These alternative cell populations can be utilized as limbal stem cell substitutes for ocular surface reconstruction. Pluripotent stem cells, oral

**Table 3** Alternative cell populations for treating LSCD

Alternative cells	Description	Stage	References
Oral mucosal cells	Autologous oral mucosal cells are cultured on amniotic membrane or as a cell sheet and transplanted (COMET)  Clinical evidence of efficacy has been accumulated. However, oral mucosal cells are thicker and less transparent than corneal epithelial cells. Transplanted oral mucosal epithelial cells have been reported to survive up to 4 years after surgery	Clinical	(26,27,31)
Human pluripotent stem cells	Human embryonic stem cells differentiate into corneal epithelial progenitor cells in conditions mimicking the LSC niche. Conditioned medium, feeder cells, amniotic membrane, and growth factors have been used to produce and LSC niche-mimicking environment  Human embryonic stem cell-derived corneal epithelial-like cells and endothelial-like cells were co-cultured on an acellular porcine corneal matrix and transplanted in rabbits. The intact and transparent graft was maintained for 8 weeks  Corneal epithelial cells were generated from induced pluripotent stem cells derived from human dermal fibroblasts. The cell expressed specific corneal epithelium-related genes such as K12, K3, and Pax6	Under clinical trials	(32-36)
Mesenchymal stem cells	MSCs play immunomodulatory roles. They migrate to the injured area of the cornea and decrease inflammatory cell infiltration and cytokine production in animal models  MSCs can be delivered to the ocular surface either by MSC containing carriers transplantation or direct subconjunctival injection  Subconjunctival injection of MSCs was effective in the acute phase of severe ocular burn	Under clinical trials	(37-39)
Amniotic epithelial cells	Amniotic epithelial cells can differentiate into corneal epithelial-like cells when they are co-cultured with human corneal epithelial cells	Preclinical	(40,41)
Hair follicle cells	A LSC niche-mimicking environment induces a cornea epithelial phenotype in stem cells isolated from the hair follicles of mice	Preclinical	(42)
Dental pulp stem cell	Dental pulp stem cells labeled with green fluorescent Qtracker 525 were seeded onto a pretreated contact lens and delivered to an <i>ex vivo</i> debrided human cornea. Dental pulp stem cells transdifferentiated into corneal epithelial progenitors and expressed keratin 12	Preclinical	(43)

LSCD, limbal epithelial stem cell deficiency; COMET, cultivated oral mucosal epithelial transplantation; LSC, limbal epithelial stem cell; MSCs, mesenchymal stem cells.

mucosa cells, dental pulp stem cells, and hair follicle cells can be cultured on the carrier substrate and transplanted to the eye with severe LSCD. The implanted cell population can transdifferentiate into more corneal epithelial-like cells and maintain ocular surface integrity and transparency. Although the usefulness of these cells is still being verified in the preclinical stages, corneal limbus reconstruction using these cells is possible in the future (*Table 3*).

Among alternative cells, mesenchymal stem cells (MSCs) deserve further explanation. In general, MSCs can be obtained from bone marrow or adipose tissues of patients. Transplanted MSCs can differentiate into corneal epithelial cells (44). Although a sufficient number of MSCs do not

differentiate into corneal epithelial-like cells, transplanted MSCs are known to have anti-angiogenic and anti-inflammatory properties (45). In addition, the secretomes of cultured limbal MSCs can promote corneal epithelial regeneration (46). MSCs found in the normal limbal stem cell niche are believed to be able to crosstalk with LSCs and control LSC proliferation and differentiation (3,47). Therefore, an exogenous supply of MSCs is expected to secrete trophic and growth factors to stimulate residual LSCs, to control inflammation preventing further damage to LSCs, and finally to improve the limbal niche microenvironment. The non-immunogenic nature of MSCs allows them to be used allogeneically without eliciting



immunosuppression of the recipient. Several routes of MSCs application in LSCD are possible. Intravenous injection of MSC in acute phase of cornea damage can alleviate ocular signs such as opacity and neovascularization in animal models (48,49). Various carriers including amniotic membrane, contact lens, and biocompatible polymers can be used to deliver MSCs on the ocular surface (50-52). However, the actual number of transplanted MSCs in this method is known to be small compared to injection methods. Subconjunctival injection of MSCs has also been proven to be effective in LSCD (37). MSCs can migrate to the inflammatory area from the subconjunctival space and modulate inflammation and tissue damage. There is still debate whether injected MSCs can change to LSCs.

Reprogrammed cells called induced pluripotent stem cells (iPSCs) are also promising stem cell sources to replace LSCs (32,53,54). iPSCs can be induced by culture in a cocktail of signaling factors. LSCs induced from iPSCs may allow personalized therapy according to the patient's need without the risk of allogeneic rejection. Recently, iPSC derived corneal epithelial sheet have been transplanted to a patient suffering bilateral LSCD in Japan (55). Although clinical results have not yet been reported, this clinical trial shows clear advances in LSCD treatment.

### ***Keratoprosthesis for advanced bilateral LSCD***

When bilateral LSCD is combined with severe dry eye, fornix adhesion, lid abnormality, or previous failures of LSC transplantation, the success of LSC transplantation is limited and the use of keratoprotheses can be considered (12,56). Keratoprotheses do not require long-term systemic immunosuppression. Early visual rehabilitation is another advantage of keratoprotheses. However, risks of retinal detachment, endophthalmitis, glaucoma, and implant extrusion are serious limitations (56). In keratoprosthesis surgery, epithelialization of the corneal surface by the corneal epithelium is abandoned. Instead, an implant is inserted to replace the optically transparent cornea, allowing light to reach the retina. After keratoprosthesis surgery, the ocular surface is totally covered with the conjunctival epithelium except for a small optical opening in the implant. The most popular keratoprotheses are Boston KPro, AutoKPro, LV Pradad KPro, and modified osteo-odonto-keratoprosthesis (MOOKP). Among these, Boston KPro type 1 is the most commonly used keratoprosthesis. It has an optical cylinder with a skirt of donor tissue. Good outcomes of visual rehabilitation have been reported with

Boston KPro type. However, retroprosthetic membrane and corneal melting are common complications (57,58). In a retrospective analysis of 23 eyes receiving Boston type 1 keratoprosthesis, postoperative corrective visual acuity of 20/50 or better was obtained in 67% of eyes at a 3-year follow up and persistent corneal epithelial defect and corneal necrosis were observed in 56.5% and 30% of eyes, respectively (59).

### ***Limitations of this review***

This review described basic knowledge of LSC and various treatment options for LSCD. Since the purpose of this review was to convey knowledge that a general ophthalmologist or ophthalmology resident could understand, contents that were too specialized or experimental had to be excluded. In addition, only studies published in English and full-length articles retrieved from PubMed search were included in this review. This approach has a limitation in that it does not include latest research studies presented as abstracts in recent academic conferences.

### **Conclusions**

Our understanding of LSCs and LSCD has deepened over the past years. However, much details of LSCs or LSCs niche remains unknown as interesting future topics of research. Despite the enigmatic identity of LSCs, various treatments have been devised and proven effective in clinical practice. However, it should be kept in mind that the best chance of treating LSCD is in the early stages of the disease. In some cases, initial treatment is difficult, especially for hereditary cases, cases with frequent relapses, and cases with intractable underlying etiologies. Once LSCD has progressed, treatment becomes challenging. Nevertheless, in the early treatment of LSCD, every effort should be made to preserve as many LSCs as possible.

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