Most experts agree that the limbal stem cells reside in a complex microenvironment at the limbus and are evident by both the clinical observation and the basic scientific research\(^1\)\(^\text{-}^2\). Clinically, it is evident by the observation that the corneal epithelial wound heals from the peripheral aspect of the cornea\(^3\). Moreover, the basic scientific research such as DNA labelling studies of mouse cornea, have identified a special group of cells in the basal layer of the corneal epithelium that invariably remains quiescent and get activated whenever there is a requirement to replenish the damaged corneal epithelial cells\(^4\). Any insult to this complex microenvironment (niche), whether congenital or acquired, leads to limbal stem cell deficiency (LSCD).

**Etiology of LSCD**

**Congenital:** Aniridia, congenital erythrokerato-dermia, keratitis associated with multiple endocrine deficiencies and epidermal dysplasia. It is the stromal microenvironment that is insufficient in these congenital causes.

**Acquired:** Ocular surface burns, prolonged contact lens wear, multiple surgeries involving the limbal region, ocular surface inflammatory disorders like Stevens Johnson syndrome, ocular cicatrical pemphigoid, chronic vernal keratoconjunctivitis, microbial infections involving limbus, radiotherapy and toxicity by topical medications like mitomycin C and 5-Fluorouracil. These acquired causes not only affect the niche but directly destroy the stem cells.

**Clinical features and Diagnosis of LSCD**

In partial limbal stem cell deficiency, where a sector of cornea is conjunctivalized, patients often remain asymptomatic. In total limbal stem cell deficiency, the entire corneal surface is conjunctivalized. This thick corneal pannus is often associated with inflammation and vascularization. Diagnosis of LSCD is mainly clinical. However, it can be confirmed by either impression cytology or by In vivo laser scanning confocal microscopy (IVCM)\(^5\).

**Management protocol**

**Ocular surface optimization**

This initial step involves lubrication of the ocular surface, suppressing the ocular surface inflammation and correction of lid structural abnormalities. Oflate, scleral contact lens is gaining popularity which help in providing ocular comfort and in resolution of kertopathy.

**Partial limbal stem cell deficiency**

Asymptomatic patients can be kept under observation. However, symptomatic patients can be treated by sequential sector conjunctival epitheliectomy (SSCE)\(^6\), amniotic membrane transplantation (hAM)\(^7\) or ipsilateral limbal translocation\(^8\). In SSCE, the conjunctival epithelium from the cornea is repeatedly scraped so as to allow the normal corneal epithelial cells to grow over the denuded surface. In ipsilateral limbal translocation, a healthy portion of limbus is transferred to the burned area of the same eye, without intervening in the contralateral eye.

**Total limbal stem cell deficiency**

The current treatment strategies include conjunctival limbal autograft (CLAU), cultivated limbal epithelial transplantation (CLET) and simple limbal epithelial transplantation (SLET) for unilateral cases. Keratolimbal allograft (KLAL), allogenic CLET, keratoprosthesis and cultivated oral mucosal transplantation (COMET) are meant for bilateral cases.
Conjunctival limbal autograft (CLAU)

In this technique, the limbal graft tissues (2 o’clock hours each) are harvested along with conjunctival carrier from the superior and inferior limbal zones at 12 and 6 o’clock positions respectively. The donor tissue is then sutured on to the recipient bed with interrupted 10-0 monofilament nylon sutures at the corneal and scleral margin. However, this procedure is associated with a potential risk of iatrogenic limbal stem cell deficiency in the donor eye.

Cultivated limbal epithelial transplantation (CLET) (Figure 1)

The main concern with CLAU is the development of donor site LSCD. To overcome this, Pellegrini et al., proposed a method in which a small limbal biopsy is harvested from the donor site followed by its ex vivo expansion in culture and then transplanted onto the limbal stem cell deficient eye. Two types of culture techniques exist i.e. ‘suspension’ and ‘explant’ culture techniques. In suspension technique, the limbal cells are separated from the limbal niche by enzyme digestion and are supported by the feeder cells (Mouse NIH3T3-J2 fibroblasts). In this technique, there is a theoretical chance of transmission of prion disease and viruses to the recipients. In explants technique, the limbal biopsy specimen is transported to laboratory in modified human corneal epithelium medium (HCE) and shredded into multiple pieces on to the hAM and cultured in HCE medium with 10% autologous serum at 37°C, 5% CO2 and 95% air. The growth of limbal stem cells is monitored daily under an inverted phase contrast microscope and the medium is changed on alternate days. A confluent monolayer is typically formed in 10 to 14 days. This cultured epithelial sheet is then transplanted onto the corneal surface after removal of pannus and made adhered to it with the help of fibrin glue. The periphery of the cultured sheet is tucked underneath the conjunctival edge.

Most of the studies have stated a favorable outcome following CLET. However, the factors affecting the outcomes have been sparingly described. These factors could be clinical or histological. Etiology of LSCD and age has been slated as the clinical factors in the literature. Of late, a retrospective study by Sati and associates, has found that the presence of either calcific deposits or hyperplasia in the excised corneal pannus indicate poor prognosis.

The strengths of this procedure, besides the clinical success are twofold. Firstly, it does not induce LSCD in donor eyes. Secondly, a repeat CLET can be easily performed. The limitation lies in establishing the expensive culture laboratory facilities.

Simple limbal epithelial transplantation (SLET) (Figure 2)

This is the latest technique for unilateral LSCD and is being introduced by Sangwan and associates. Its advantages over CLET is that it avoids the need of expensive laboratory facilities. In this technique, a 2x2 mm limbal graft is harvested from the unaffected eye and is then divided into multiple small pieces. Amniotic membrane is made to adhere to the recipient cornea with the help of fibrin glue after removal of pannus. The limbal graft pieces (10 to 12 in number) are then placed on the amniotic membrane in a double concentric ring pattern without involving the visual axis and secured by fibrin glue. A Bandage contact lens (BCL) is then placed over the grafted material. In the pioneered published series from L V Prasad Eye Institute, Hyderabad, it is observed that this technique was quite effective in six patients who underwent this procedure for unilateral LSCD for ocular surface burns.

Keratolimbal allograft (KLAL)

This procedure involves the transplantation of limbal tissue with a corneal carrier from the cadaver to the stem cell.
deficient eye. The success of this procedure relies on, in part, the administration of systemic immunosuppression, though the prolonged administration of immunosuppression has its own limitations. Moreover, the graft survival following KLAL has been reported to be inferior to autologous transplantation\(^\text{17}\).

**Living Related Conjunctival Limbal Allograft (Ir-CLAL)**

Unlike CLAU, this procedure is meant for bilateral cases and involves transplantation of limbal tissue with conjunctival carrier from the live related donor to the stem cell deficient eye. Unlike CLAU, this technique requires prolonged immunosuppression to reduce the risk of rejection. Moreover, unlike KLAL, a limited amount of limbus can be harvested resulting in transplantation of fewer stem cells.

**Allogenic cultivated limbal epithelial transplantation (CLET)**

Considering the low graft survivability of KLAL and a low proliferative potential of cadaveric donor tissue both in vivo and ex vivo, a live related allogenic CLET has gained wider acceptance for patients with bilateral LSCD. Though the success rate is quite variable, the issues that are bothersome include the requirement of expensive laboratory facilities and the need of long term immunosuppression with its adverse effects.

**Cultivated oral mucosal transplantation (COMET)**

The concept of using cultivated oral mucosal cells was pioneered by Nakamura and associates\(^\text{18}\).

The technique begins with the collection of oral mucosal biopsy specimens, each measuring 2 mm\(^2\), from the patient.
and is divided into multiple small explants after removing the submucosal connective tissue. These explants are then immersed thrice in phosphate buffered saline solution followed by treatment with trypsin-EDTA 0.05% solution. The separated oral mucosal cells are then spread onto the denuded amniotic membrane, present at the bottom of culture inserts thereby forming the culture sheets. This culture sheet is then made adhered to the cornea with the help of fibrin glue. Though this technique has found to be quite effective, the biggest challenge is its long term effectiveness that has been found to be poorer due to persistent epithelial defect (PED) and a tendency to develop a varying degree of vascularisation with time.

**Boston keratoprosthesis (BKPro) (Figure 3)**

Boston type I keratoprosthesis (Massachusetts Eye and Ear Infirmary, Boston, MA) is an emerging option for patients with bilateral LSCD with wet ocular surface. The other types of prosthesis i.e. osteoodontokeratoprosthesis and Boston type 2 keratoprosthesis are reserved for patients with bilateral LSCD with minimal or no tear function.

**Emerging therapies**

Since each of the above mentioned ocular surface reconstructive strategy has limitation, so a search is on for a more idealistic treatment strategy. In this regard, both the clinicians and the scientists are working on the tissue engineering approaches along with stem cell based regenerative therapies and the ocular surface regenerative techniques using embryonic, induced pluripotent and mesenchymal stem cells. Such strategies are expected to enhance the outcomes of limbal stem cell transplantation in the future.

**Conclusion**

Amongst the above mentioned techniques, the most favourable strategy for both the unilateral or bilateral cases, is a matter of debate. So far, literature is scant on comparative evaluation between the different strategies. Moreover, the actual mechanism by which limbal transplantation works is also debated. It is unclear whether the treatment revives the surviving stem cells by improving the complex microenvironment or replenishes the stem cell reserve.

**References**


