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# Limbal Stem Cell Disease: An Ocular Regenerative Program

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**Limbal stem cells (LSCs) are principally responsible for the 7-day cycle of regeneration of the corneal epithelium. This process can be compromised by congenital, traumatic, or autoimmune factors. Management of LSC disease depends on the underlying cause and extent of damage, and can range from observation and removal of the offending agent in mild cases to complex surgical intervention. This issue of *Ophthalmology Rounds* outlines a 3-step approach to restore LSC function in serious cases: LSC transplantation, keratoprosthesis, and prosthetic replacement of the ocular surface environment.**

The ocular surface is a multifactorial self-protective system that includes the eyelashes, eyelids, tear film, and the conjunctival and corneal epithelium. All of these elements work together to provide an optimal environment to maintain the structural and functional integrity of the ocular surface. In 1966, Hanna initially described the limbal centripetal re-epithelialization of the corneal epithelium.[1] Twenty years later, Schermer,[2] Cotsarelis,[3] and Ebato[4] recognized the existence of a specific type of cell that lived at the limbus level, with longer cell cycles and a high regenerative potential.

The limbal stem cells (LSCs) are located in the basal limbal corneal epithelium; their nutrition is provided by the palisades of Vogt. They exhibit asymmetrical cell division, in which one stem cell leads to an identical daughter stem cell and to a transient amplifying cell (TAC), which will be the first cornerstone towards a differentiated corneal epithelial cell. Each TAC will subsequently differentiate into a terminally differentiated cell, which will eventually complete the differentiation process into a mature corneal epithelial cell. The corneal epithelium has the ability to fully regenerate every 7 days and the rate of epithelial sloughing must equal the rate of ocular surface epithelial regeneration.

## LSC Deficiency (LSCD)

LSCD is caused by an inability of the LSC to repopulate the corneal epithelium with healthy corneal epithelial cells, either due to their dysfunction or a sufficient decrease of the quantity of healthy LSCs. On slit lamp examination, typical findings of this condition can be the loss of palisades of Vogt, a typical pattern of late corneal staining with fluorescein where the abnormal epithelial cells absorb excess fluorescein (Figure 1), or superficial corneal neovascularization, otherwise termed conjunctivalization. Diagnosis is made by clinical examination, although histological confirmation can be achieved through the finding of conjunctival goblet cells on the corneal epithelium. These cells can be identified either by biopsy, with the use of Alcian blue and periodic acid-Schiff (PAS) stains, or by impression cytology, using a nitrocellulose filter paper pressed against the corneal surface and PAS and hematoxylin-eosin stains to identify them.

## Causes of LSCD

The causes of LSCD can be divided into 3 main categories (Table 1).

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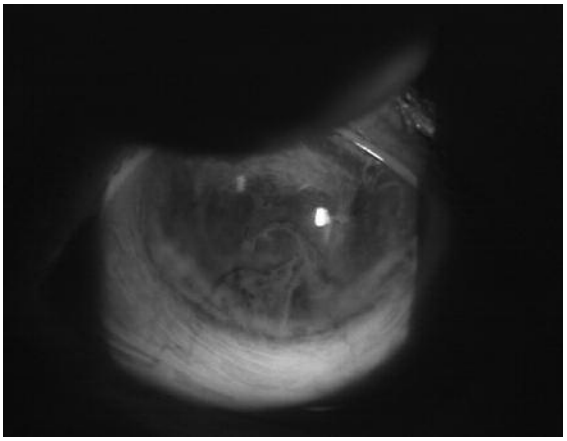
**Figure 1:** Typical late staining pattern of limbal stem cell deficiency (LSCD).



### *Congenital*

The 3 forms of congenital LSCD are aniridia (Figure 2), dominantly inherited keratitis, and ectodermal dysplasia, of which aniridia is the most common. Its spectrum ranges from stromal hypoplasia manifesting as trace iris transillumination defects[5] to total absence of iris. Depending on its severity, this condition may be associated with foveal and optic nerve hypoplasia, nystagmus, glaucoma, cataracts, zonular weakness, and aniridic keratopathy.

### *Traumatic*

Traumatic causes of LSCD include alkali and acid injury (Figure 3), thermal injury, and iatrogenic factors (eg, multiple ocular surgeries, contact lens wear).[6,7] Alkali injury is caused by saponification of fatty acids in cell membranes, leading to cell death. Severe damage may be seen with injuries occurring at a pH higher than 11.5.

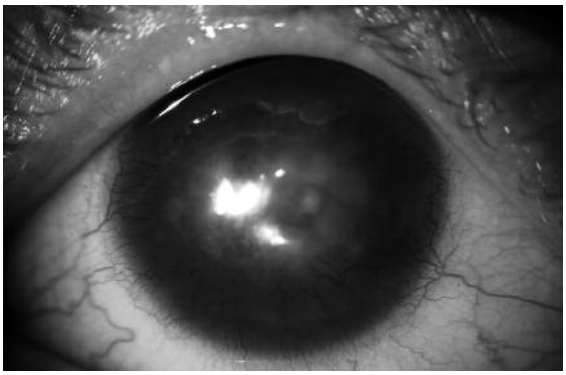
### *Autoimmune disorders*

Autoimmune disorders associated with LSCD include Stevens-Johnson syndrome (SJS) and ocular cicatricial pemphigoid (Figure 4). The acute phase of SJS occurs 1–3 weeks after the triggering exposure and lasts for about 2–4 weeks. It involves a membranous conjunctivitis frequently aggravated by secondary bacterial infection, and symblepharon formation. Corneal findings include abnormalities at the epithelium level and pannus formation. Following the acute phase, SJS evolves into a chronic condition, with variable degrees of conjunctival cicatricial changes, including fornix foreshortening and symblepharon formation. Eyelids may also be involved, with entropion and ectropion, trichiatic lashes, keratinization of the lid margins and meibomian gland dysfunction causing an inhospitable environment that further aggravates the patient's LSCD.

**Table 1:** Causes of LSCD

| |
|---|
| Congenital |
| • Aniridia |
| • Dominantly inherited keratitis |
| • Ectodermal dysplasia |
| Traumatic |
| • Alkali and acid injury |
| • Thermal injury |
| • Iatrogenic |
| Autoimmune disorders |
| • Stevens-Johnson syndrome |
| • Ocular cicatricial pemphigoid |

**Figure 2:** Severe aniridic keratopathy with corneal neovascularization and scarring.



## Staging

Staging of the patient's LSCD is important to determine which stem cell procedure is best suited for that particular individual and the patient's prognosis following LSC transplantation (Table 2).[8,9]

## Management of LSCD: Ocular Regenerative Program

The management of LSCD will depend mainly on the extent of the disease at the time of diagnosis, and

**Figure 3:** Severe LSCD with corneal neovascularization, secondary to alkali injury.

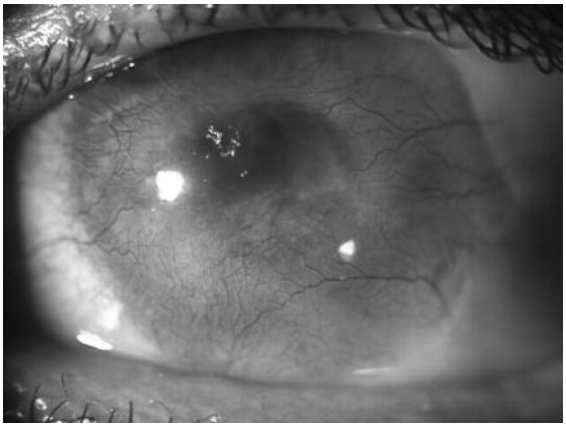


**Figure 4:** Severe scarring and neovascularization in a patient with ocular cicatricial pemphigoid (OCP).

will vary from observation and removing the offending agent in mild cases, to stabilization of the ocular surface followed by complex surgical interventions in severe disease. Eyes with chronic conjunctival inflammation are the most challenging to treat and carry the poorest prognosis. The complexity of this entity and required multidisciplinary approach inspired the creation of a hospital-supported ocular regenerative program that involves the efforts of ophthalmologists, immunologists, transplant specialists, nurses, social workers, and pharmacists. Each member of this team is responsible to individually analyze and follow-up on every patient with LSCD to provide personalized long-term immunosuppression on a case-by-case basis.

Mild disease may require only observation, intensive lubrication with preservative-free artificial tears and autologous serum drops, topical steroids, or minor procedures such as sectoral conjunctival epitheliectomy or amniotic membrane transplantation. More severe disease requires a staged approach. Upon improvement of lid closure abnormalities, trichiasis, glaucoma, and chronic conjunctival inflammation, focus can then be turned to re-establishment of LSC function in terms of re-epithelialization of the cornea and maintenance of its transparency.

**Table 2:** Staging of limbal stem cell deficiency based on the percent of lost stem cells and the presence or absence of conjunctival inflammation[8]

| | Normal conjunctiva (Stage a) | Abnormal conjunctiva (Stage b) |
|---|---|---|
| Partial limbal deficiency (Stage I) | Iatrogenic, CIN, contact lens (Stage Ia) | Mild SJS, OCP, mild chemical injuries (Stage Ib) |
| Total limbal deficiency (Stage II) | Aniridia, severe contact lens, and iatrogenic (Stage IIa) | Severe SJS, OCP, severe chemical injuries (Stage IIb) |

CIN = corneal intraepithelial neoplasia; SJS = Stevens-Johnson syndrome



The main cornerstones of our ocular regenerative program include 3 components: LSC transplantation, keratoprosthesis, and prosthetic replacement of the ocular surface environment (PROSE).

## *LSC transplantation*

Since its first description by Strampelli in 1963,[10] and further development by Barraquer during that same decade,[11] LSC transplantation has become one of the most important pillars in LSCD management. Several techniques for LSC transplantation have been described throughout the years.[12]

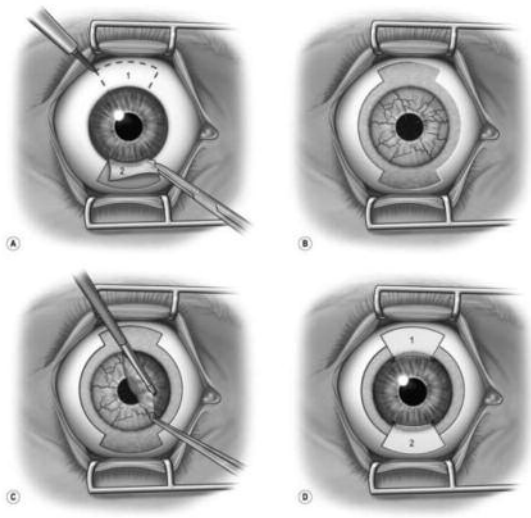
### *Conjunctival limbal autograft (CLAU; Figure 5)*

This type of transplant is used in unilateral LSCD, in which the healthy donor eye will provide a certain amount of LSCs to the affected recipient eye in order to restore the ocular surface function. Limbal tissue from the donor eye attached to a conjunctival carrier will be harvested, a superficial keratectomy is performed on the diseased eye in order to remove any pannus or scar formation and to regularize the ocular surface, and the carrier is then transplanted onto the diseased limbus. This technique is especially effective in partial LSCD; it requires an absolutely healthy contralateral eye and, since this is essentially an autograft, it has the great advantage of not requiring immunosuppression postoperatively.

### *Living-relative conjunctival limbal allograft (lr-CLAL)*

This technique is very similar to CLAU, but it differs in that the limbal tissue is harvested from a patient's living relative and then transplanted into the diseased eye. If there are more than one potential donor, it is advantageous to determine who is immunologically the best match for the recipient. This tech-

**Figure 5:** Diagram for conjunctival limbal autograft, in which donor is the fellow eye, and living-relative conjunctival limbal allograft (lr-CLAL), in which the donor is from a living relative.



nique can be used in cases of bilateral stem cell disease. The main disadvantage is that the recipient needs long-term systemic immunosuppression to prevent graft rejection.

### *Keratolimbal allograft (KLAL; Figure 6)*[13]

KLAL is generally indicated when there is no available or suitable living related donor. In this technique, limbal tissue attached to a corneal carrier is harvested from cadaver eyes and then transplanted into the affected eye. It is also intended for severe LSCD because it provides a large number of stem cells. One and a half cadaver eyes are required to provide enough limbal stem cells for one affected eye. KLAL also requires long-term immunosuppression for rejection prophylaxis.

### *Cincinnati procedure (Figure 7)*

This technique combines an lr-CLAL and a KLAL to maximize the advantages of both. Two 3 o'clock hours of limbal tissue on a conjunctival carrier are harvested from a living related donor and then implanted superiorly and inferiorly on the recipient's eye. The rest of the affected limbus is then replaced by cadaveric donor tissue. This technique requires long-term immunosuppression as well.[14] A variation of this technique, termed the modified Cincinnati procedure, uses limbal tissue from a patient's fellow healthy eye secured at 12 and 6 o'clock and keratolimbal allograft tissue at 3 and 9 o'clock for eyes with unilateral severe limbal stem cell deficiency with significant conjunctival deficiency.[15]

### *Immunosuppression*

Similar to other types of transplants, and due to their proximity to the vascularized conjunctiva, limbal stem cells have a high risk of rejection in a foreign host, even under the best of circumstances.[16] Systemic immunosuppression is the main pillar in avoiding graft rejection, thereby prolonging tissue survival and graft success rate. In this context, a multidisciplinary approach is optimal in the pre- and postoperative management of patients with limbal stem cell disease who require systemic immunosuppression. Patients are usually younger and otherwise healthy with no contraindications to systemic immunosuppression and thus have few associated adverse events.[17]

Our immunosuppressive protocol involves initiating systemic treatment with 3 different types of immunosuppressive drugs: tacrolimus, mycophenolate mofetil, and prednisone. Topical immunosuppression includes use of cyclosporine 0.05% and prednisolone acetate 1% or difluprednate ophthalmic emulsion. Tacrolimus is adjusted monthly based on plasma levels and is tapered off after 12–18 months depending on the level of ocular surface inflammation. Mycophenolate mofetil is maintained as monotherapy for at least 24–36 months depending on the inflammation level and systemic tolerance of the patient. Oral prednisone is typically used only for a few months in order to avoid the known adverse effects of systemic steroids.

**Figure 6:** Diagram for keratolimbal allograft (KLAL). A: Limbal tissue from one-and-a-half cadaver eyes is harvested. B,C: The pannus is excised from the recipient. D: Limbal segments are secured to the recipient's bed.

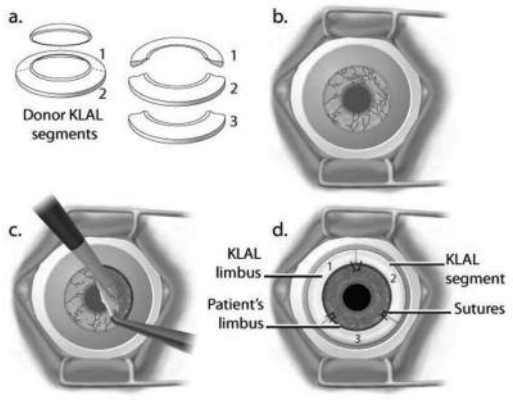
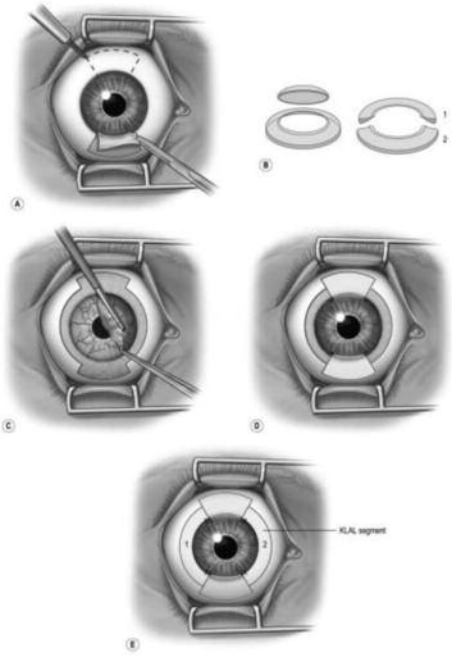



**Figure 7:** Diagram for the Cincinnati procedure, in which lr-CLAL and KLAL are combined.



### *Keratoprosthesis*

Another important cornerstone of our ocular regenerative program is the use, in appropriate

settings, of artificial corneas. Keratoprosthesis is primarily used in severe LSCD secondary to either multiple corneal allograft failures, chemical injuries, or autoimmune-related corneal opacities and ulcerations. It is especially useful in elderly patients who may not be able to tolerate the adverse effects of systemic immunosuppression or when there is a medical contraindication for the long-term use of systemic immunosuppression.

Our program uses the Boston Keratoprosthesis (KPro) Type 1, which was designed at the Massachusetts Eye and Ear Infirmary and has been commercially available since 1992 (Figure 8). It has undergone several design upgrades over the years and is currently an alternative in the management of severe LSCD. It consists of a collarbutton-shaped polymethyl methacrylate (PMMA) structure, in which a donor corneal allograft is sandwiched between the 2 plates, one of which carries the PMMA central optic.

The surgical technique involves the creation of a 3-mm central hole on a 9-mm donor cornea, which is then placed between the optic stem and the 8.5-mm back plate and secured with a titanium-locking ring. Nylon sutures are used to secure the corneal carrier to the host corneal tissue, and a large bandage contact lens is placed over the ocular surface. A soft contact lens is required indefinitely to prevent epithelial desiccation and to decrease the likelihood of corneal melt. Long-term antibiotic prophylaxis is also required to prevent infectious keratitis and endophthalmitis, considering that there will be a permanent foreign body inside the eye and that the ocular surface and the intraocular environment will be connected through the prosthesis stem-corneal junction. Anatomical retention of this device ranges around 95% at the 9-month mark and patients achieve visual acuities (VAs) of 20/200 in around 50% of the cases, and 20/40 in 23% of cases at the 1-year mark. Our experience with the Boston KPro includes 45 eyes of 44 patients, with a follow-up of 2–57 months. Our retention rate at last follow-up reached 96% with a best-achieved median VA of 20/100; 36% of patients achieved VA of >20/40 at some point during their postoperative course. At last follow-up, median VA was 20/400 and the most common complications were retroprosthetic membrane formation (23.5% of cases) and elevated intraocular pressure (10.2%).

Many ophthalmologists, including our surgical team, consider keratoprosthesis as a "last resort" in the management of severe LSCD in patients who are not candidates for LSC transplantation. These patients have bilateral severe visual impairment and are often suffering from ocular pain. For this group of patients, there is no other viable surgical alternative. Although keratoprosthesis often offers significant sight improvement along with alleviation of ocular pain and improvement in quality of life, there are significant and possibly sight-threatening long-term complications, such as glaucoma progression, corneal melts, and endophthalmitis, which need to be considered and discussed with each patient on an individualized basis.[18]

### *PROSE lens (Figure 9)*

The third key element in our ocular regenerative program has been gaining popularity as a nonsurgical alternative for severe LSCD. The PROSE is a gas-permeable, plastic, customized, transparent dome that rests on the sclera and has the ability to vault over the damaged cornea, creating a smooth optical surface over the irregular, damaged cornea and to act as an artificial tears reservoir. Data on the PROSE lens system show that it is effective in improving vision, allevi-

**Figure 8:** Boston Keratoprosthesis Type 1. Notice the central polymethyl methacrylate optic included in the front plate.

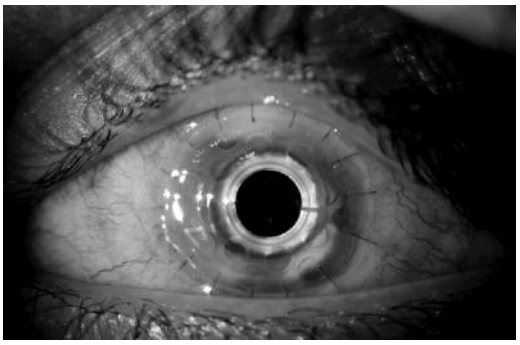


**Figure 9:** The Boston PROSE Lens. Photo courtesy of Deborah Jacobs, MD, Boston Foundation for Sight.

ating pain, supports healing of the ocular surface and improves LSCD patients' quality of life.[19]

## Summary

Patients with LSCD can be extremely challenging. A staged approach is frequently required to optimize the patient's ocular surface for long-term success of LSC transplantation. Systemic immunosuppression is required whenever allograft tissue is used. Keratoprosthesis is associated with rapid visual recovery; however, it requires long-term follow-up to monitor for potential associated complications. Finally, the PROSE lens is an excellent nonsurgical alternative. These elements of the ocular regenerative program require a multidisciplinary team to offer these patients available treatment options to improve their vision and quality of life.

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