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# Simple Limbal Epithelial Transplantation

## *Long-Term Clinical Outcomes in 125 Cases of Unilateral Chronic Ocular Surface Burns*

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***Purpose:*** This study describes the long-term clinical outcomes of autologous simple limbal epithelial transplantation (SLET), a relatively new technique of limbal stem cell transplantation.

***Design:*** This was a single-center prospective interventional cases series.

***Participants:*** This study included 125 patients, 65 adults and 60 children who developed unilateral limbal stem cell deficiency (LSCD) after suffering with ocular surface burns and underwent SLET between 2010 and 2014.

***Methods:*** A 1-clock hour limbal biopsy sample was obtained from the unaffected eye. At the same sitting, the recipient eye was surgically prepared and the donor tissue was divided into small pieces and transplanted using an amniotic membrane scaffold with fibrin glue.

***Main Outcome Measures:*** The diagnosis and outcome in every case was validated by 5 independent masked assessors. The primary outcome measure was restoration of a completely epithelized, stable, and avascular corneal surface. The secondary outcome measure was improvement in visual acuity. Complications, risk factors for failure, and immunohistochemistry analysis of corneas that underwent SLET also were described.

***Results:*** At a median postoperative follow-up of 1.5 years (range, 1−4 years), 95 of 125 eyes (76%; 95% confidence interval, 68.5%−83.5%) maintained a successful outcome. Kaplan-Meier analysis revealed a comparable survival probability at 1 year of 80% in adults and 72% in children ($P = 0.304$). Two-line improvement in visual acuity was seen in 75.2%, and 67% of successful cases attained 20/60 or better vision ($P < 0.0001$). Progressive conjunctivalization occurred in 18.4% of eyes. The clinical factors associated with failure were identified as acid injury, severe symblepharon, SLET combined with keratoplasty, and postoperative loss of transplants ($P \leq 0.0075$). Success rates were comparable among faculty and trainees ($P = 0.71$). Immunohistochemistry revealed successful regeneration of normal corneal epithelium ($CK3^+/12^+$) without admixture of conjunctiva cells ($Muc5AC^-/CK19^-$) and replenishment of limbal stem cell ($\Delta Np63\alpha^+/ABCG2^+$) reserve.

***Conclusions:*** Autologous SLET is an effective, reliable and replicable technique for long-lasting corneal regeneration and vision restoration in unilateral chronic ocular surface burns. Simple limbal epithelial transplantation is probably preferable to other techniques of limbal stem cell transplantation, particularly where cell cultivation facilities are unavailable. *Ophthalmology 2016;123:1000-1010 © 2016 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

*Supplemental material is available at www.aaojournal.org.*

A delicately thin layer of stratified but nonkeratinized squamous epithelium covers the corneal surface. This epithelial cover is renewed continuously as younger cells migrate inward from the periphery and older cells are lost from the surface.[1] The constant source of corneal epithelial cells is believed to be the limbus, which is the annular transitional area between the cornea and the sclera.[1] Corneal epithelial stem cells have been identified deep within a protected microenvironment or niche at the limbal palisades of Vogt.[2,3] When the limbus is intact, corneal epithelial defects heal promptly. But when the limbus is damaged, either because of injury or inflammation, the normal corneal epithelial physiologic features are disrupted. Delay or failure in corneal epithelialization leads to conjunctival encroachment over the cornea, vascularization, and nonhealing epithelial defects.[4] The consequent clinical condition, termed *limbal stem cell deficiency* (LSCD), is a rare but severe cause of corneal blindness.

Fortunately, transplantation of healthy limbal tissue can reverse LSCD and restore a normal corneal surface.[5,6] In the last 3 decades, both the understanding of limbal biology and the techniques of limbal transplantation have evolved considerably.[7,8] Although conjunctival-limbal or keratolimbal grafting continues to be practiced,[9] transplantation of ex vivo−cultivated limbal epithelial sheets has become popular in many centers worldwide.[7,8] Regulatory issues



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and the expenses of maintaining a clinical-grade laboratory limit the use of ex vivo cultivation, whereas conventional limbal grafting requires no special infrastructure, but is technically demanding and carries some risk to the donor eye.[10] No head-to-head trials have been conducted, and it is unclear whether one technique is more effective than the other. Therefore, availability of resources or individual preference, rather than scientific evidence, usually determines which technique a particular surgeon or center adopts.

Having performed more than 1000 ex vivo–cultivated limbal epithelial transplantation (CLET) procedures and having reported long-term outcomes comparable with those of other groups,[10–13] we adopted a novel technique called simple limbal epithelial transplantation (SLET) in 2010.[14] Simple limbal epithelial transplantation essentially showed that direct transplantation of a tiny limbal fragment could reverse LSCD without needing ex vivo expansion.[14] After that initial report, 2 other groups independently replicated the successful outcomes in varied indications using slight modifications of the original technique.[15,16] However, for wider acceptance of any new technique, the results need to be validated in larger numbers and with longer follow-up. Therefore, in this study, we report the outcomes of autologous SLET in a large cohort of patients with unilateral LSCD after sustaining ocular surface burns.

## Methods

### Study Approval, Design, and Subjects

The Ethics Committee of the L. V. Prasad Eye Institute, Hyderabad, India, prospectively approved this study. After evaluating the results of the initial pilot trial involving 6 patients,[14] the committee approved SLET as an alternative option to ex vivo CLET for the treatment of LSCD. This study was conducted in strict adherence to the tenets of the Declaration of Helsinki. All adults and legal guardians of children who underwent SLET gave informed written consent for all procedures described in this study.

All 163 consecutive patients who underwent SLET between October 1, 2010, and March 31, 2014, were considered for analysis. Of 163 patients, 125 patients met the following inclusion criteria: (1) a documented history of chemical or thermal burns, and (2) presence of unilateral (defined as no history or clinical signs of ocular surface disease in the other eye) LSCD (defined as total or partial superficial corneal vascularization, punctate fluorescein staining of the corneal surface with or without persistent epithelial defects, conjunctivalization of the corneal surface, and absence of limbal palisades of Vogt). The 39 cases that were excluded from this study included 12 cases with unknown cause of LSCD, 11 cases of bilateral LSCD, 5 cases of primary or recurrent pterygium, 5 cases of LSCD occurring after ocular surface tumor excision, 3 cases of LSCD occurring after radiotherapy for intraocular tumors, and 3 cases of LSCD without visual potential in which SLET was performed for cosmetic correction. Patients with untreated concurrent ocular problems, such as severe dry eye disease (Schirmer's test I measure of less than 10 mm of wetting at 5 minutes), entropion, trichiasis, lagophthalmos, glaucoma, and infection, were not considered for surgery.

### Outcome Measures of Efficacy

In recipient eyes, the primary outcome measure was the success of SLET, defined clinically as a completely epithelized, clinically stable, and avascular corneal surface (Fig 1A–J). Failure was defined as the occurrence of progressive conjunctivalization of the cornea encroaching onto the central 8 mm, occurrence of persistent epithelial defects, or both (Fig 1P–T). Occurrence of microbial keratitis and need for repeat surgery were additional criteria for failure. Survival time was calculated in months from the date of SLET to the date of failure or the date of last follow-up, depending on the clinical outcome. The secondary outcome measure of efficacy was the change in best-corrected visual acuity (BCVA) at each postoperative follow-up visit.

### Outcome Measures of Safety

The outcome measures of safety were intraoperative and postoperative complications of both limbal biopsy and SLET in the donor and recipient eye.

### Surgical Technique of Simple Limbal Epithelial Transplantation

We followed the surgical technique that has been described previously for total LSCD[14] with certain modifications for partial LSCD cases (Supplemental Appendix 1 and Supplemental Fig 1, available at www.aaojournal.org). All tissue samples excised during SLET or keratoplasty during or after SLET were processed in a standardized fashion for histopathologic and immunohistochemical analysis, as described in Supplemental Appendix 2 and Supplemental Table 1 (available at www.aaojournal.org).

### Postoperative Care and Follow-up Schedule

All patients underwent comprehensive ophthalmic examinations of both eyes at every follow-up visit. Patients were seen on days 1, 7, 30 (at 1 month) or day 42 (at 6 weeks), 90, and at 3-month intervals thereafter. For the entire duration of the first year after surgery, patients were contacted by telephone if they missed a scheduled visit, and the next earliest possible appointment was arranged for them. This was done proactively to ensure that all patients completed at least 1 year of follow-up after the procedure. Patients were prescribed ciprofloxacin 0.3% eye drops (Cipla India, Mumbai, India) 4 times daily for 1 week and prednisolone acetate 1% eye drops (Alcon Laboratories Pvt. Ltd, Bangalore, India) 6 times daily tapered weekly over 6 weeks in both eyes. The bandage contact lens (BCL) was removed from the recipient eye on day 7 and carboxymethyl cellulose 0.5% eye drops (Allergan India Pvt. Ltd, Bangalore, India) were added in the recipient eye.

### Data Collection

Data were collected at every visit in a predesigned format and the completed form was filed in the medical record. These data included patient age and gender, type and date of injury, details of prior ocular procedures, Snellen BCVA, intraocular pressure, presence or absence of lid abnormalities, dry eye disease, symblepharon, degree of limbal involvement, intraoperative surgical details, postoperative complications, duration of follow-up, and status of the ocular surface at each visit (slit-lamp findings including fluorescein staining).

### Validation of Diagnosis and Outcome by Independent Masked Assessors

Five fellowship-trained cornea specialists (3 ocular surface disease specialists and 2 refractive surgeons) volunteered as assessors to validate the investigator's assessment of the diagnosis of LSCD and the outcome of treatment in every case based on 5 objective