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CASE REPORT

Simple limbal epithelial transplantation in limbal stem cell deficiency after chemical eye injury

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Abstract

To present a pediatric patient with unilateral limbal stem cell deficiency (LSCD) after acetone burn, managed by simple stem cell transplantation simple limbal epithelial transplantation (SLET) surgery and to review the literature on limbal stem cell transplantation techniques. A 12-year-old boy was admitted to the emergency department for acetone burn on his left eye. Following acute management of the chemical injury and amniotic membrane transplantation, the cornea healed with extensive conjunctivalization. He suffered severe photophobia and visual acuity (VA) loss up to 0.16 Snellen lines. Because of severe clinical findings of LSCD, SLET surgery was performed. He had dramatic improvement in corneal epithelialization, stromal transparency, and disappearance of photophobia 2 weeks after the surgery. At 1 year postoperatively, his VA was 0.7 with a stable epithelial surface and minimal corneal haze and he had returned to normal life. SLET is a viable alternative technique in the management of unilateral LSCD and should be present in the armamentarium of all corneal surgeons.

Keywords: Chemical eye injury; limbal stem cell deficiency; simple limbal epithelial transplantation.

Chemical burn is a leading cause of corneal blindness. Ocular surface injuries lead to 19 million unilateral and 1.6 million bilateral visual losses annually.[1] Incidence of blindness due to trauma and corneal ulceration is approximately 2 million cases per year.[2] Among all ocular injuries, the rate of chemical burn is 1.5–22.1%.[3,4] Inadequate management of the acute burn or late sequela may lead to severe dry eye syndrome, limbal stem cell deficiency (LSCD), corneal neovascularization, and corneal opacities. Eyelid disorders, trichiasis, symblepharon, ankyloblepharon, corneal keratinization, subsequent corneal infections, or glaucoma require life-long follow-up of the patients, probable additional interventions, and eventually loss of labor of the patient and may cause great economic impact.

Chemical eye burn is a major cause of LSCD. Corneal scraping, amniotic membrane transplantation, conjunctival limbal autograft (CLAU) or allograft, keratolimbal allograft, and *ex vivo* cultivated limbal stem cell transplantation have been used for the treatment of LSCD. Simple limbal epithelial transplantation (SLET) is a recently introduced technique for LSCD. Its advantages include need for a small limbal biopsy, being repeatable due to low risk of iatrogenic damage at the healthy fellow eye and being applicable at low-budget facilities. Immunosuppressive

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treatment is not required as there is essentially no risk of immune rejection.

Herein, we present a pediatric case whose unilateral LSCD due to acetone burn was successfully treated by SLET; as well as a review of the literature on the treatment of LSCD. Consent and permission upon publication of the medical data was obtained from the patient and his parents.

Case Report

A 12-year-old male patient was admitted to Dokuz Eylul University, Department of Ophthalmology, after blasting eye injury with an acetone bottle. His eye was rinsed with saline solution at the emergency department, before referring to our clinic. At the initial admission, his visual acuities were 1.0 at the right eye and 0.5 at the left eye, in Snellen lines. Slit-lamp examination revealed wide corneal epithelial defect, 360° limbal ischemia, and chemosis on the left eye (Fig. 1). Right eye examination revealed normal findings.

In our clinic, his injured eye was rinsed deliberetely again with ringer lactate solution. A silicone hydrogel bandage contact lens (balafilcon A, PureVision®, Bausch & Lomb, USA) was fitted and topical preservative-free dexamethasone qid, moxifloxacin tid, trehalose - sodium hyaluronate qid, cyclopentolate tid, polivinil alcohol/povidone tears, and (PO) 500 mg vitamin C were prescribed. On the 3rd day of the injury, slit-lamp examination revealed initiation of corneal epithelization. However at the 4th week, only less than a quarter of corneal epithelium has healed and amniotic membrane transplantation was performed to decrease ocular inflammation and improve epithelialization (Fig. 2a and b). At the 2nd week of surgery, the amniotic membrane has dissolved and the epithelium has completely healed leaving stromal haze, vascularization, and conjunctivalization implying LSCD (Fig. 3a and b). His visual acuity (VA) initially improved to 0.4 with +0.25(−1.25 at 140), but severe photophobia was restricting his life and he quitted attending his school. The healed epithelium itself was loose, displaying recurrent erosions with associated pain and discomfort. At the postoperatively 3rd month, VA worsened to 0.16 levels and stayed stabile in the following visits.

Confirming the diagnosis of LSCD by clinical findings, SLET surgery was performed at the 10th month following chemical burn, by taking the limbal donor tissue taken from the fellow eye (İD, CAU) (Fig. 4a and b). Initially, 2 mm×2 mm area on the donor eye limbus was marked and a conjunctival fleb was dissected toward limbus to prepare the graft tissue. Conjuctival graft was cut into 14 small pieces. The conjunctiva that has grown onto the cornea with LSCD was gently dissected and the corneal surface was covered with

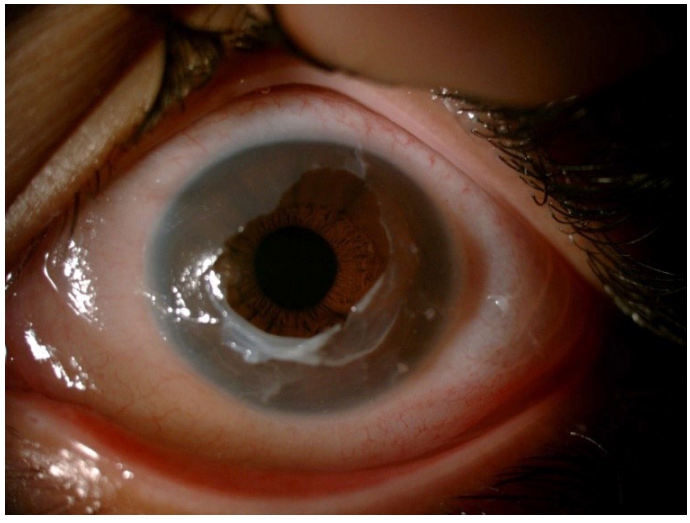


Fig. 1. Large corneal epithelial defect, chemozis, and limbal ischemia immediately after ocular acetone burn.

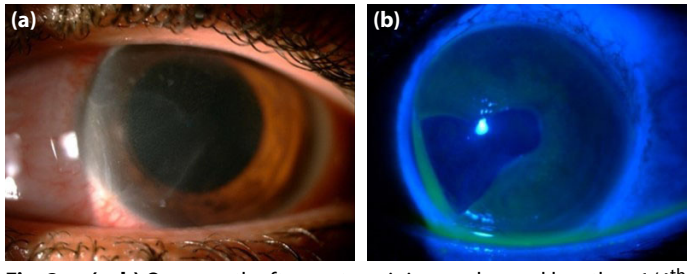


Fig. 2. **(a, b)** One month after acetone injury, only nasal less than 1/4th cornea has re-epithelialized.

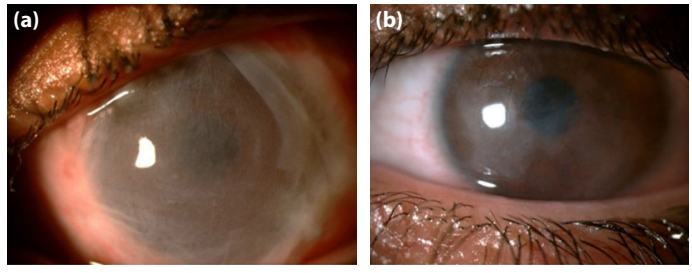


Fig. 3. **(a, b)** Following amniotic membrane transplantation, corneal epithelium healed with corneal haze, vascularization, conjunctivalization, and scarring.

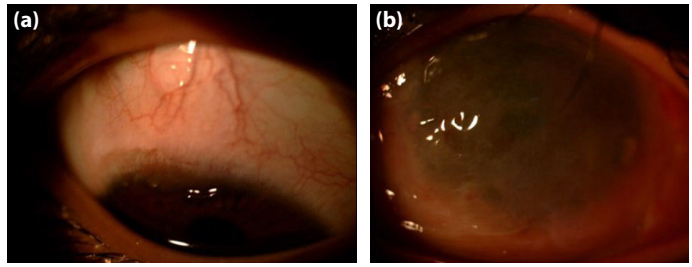


Fig. 4. **(a, b)** Simple limbal epithelial transplantation surgery in conjunction with amniotic membrane transplantation was performed with superior limbal biopsy from the healthy fellow eye.

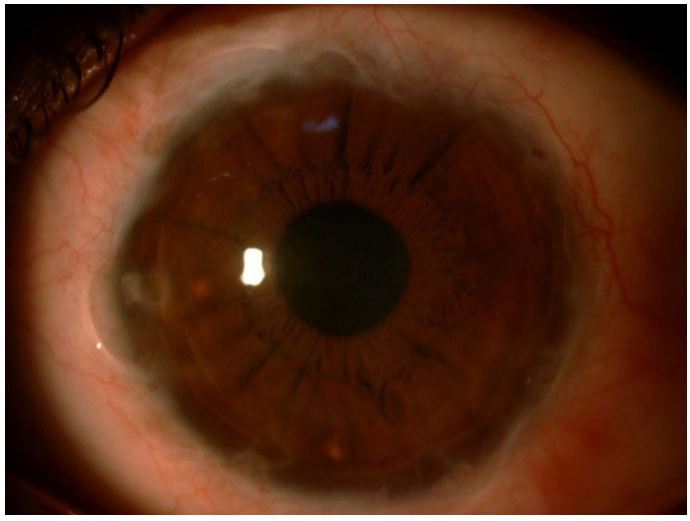


Fig. 5. Postoperatively, the corneal stroma was transparent. There was no epithelial defect, neovascularization, and limbal deficiency. Limbal stem cell grafts can be seen circumferentially.

amniotic membrane using tissue fibrin sealant (Tisseel, Baxter Healthcare, US). Small limbal stem cell grafts were placed circumferentially for 360° on the limbus intermittently, over amniotic membrane with fibrin sealant. A 14.0 mm diameter silicone hydrogel contact lens (balafilcon A, PureVision®, Bausch & Lomb, USA) was placed at the end of the surgery.

Postoperatively, topical treatment of moxifloxacin qid, preservative-free dexamethasone qid, and 0.15% sodium hyaluronate artificial tears frequently was commenced. On the post-operative 2nd week, upon melting and dissolving of the amniotic membrane, clear stroma has appeared. His VA improved to 0.3 uncorrected and 0.5 with −0.50 (−1.50@100) D. Photophobia improved dramatically, and the patient could return his normal life and school. At the slit-lamp examination, minimal cornea haze was present with no epithelial defect or neovascularization (Fig. 5). Topical treatment was switched to cyclosporine 0.05% qid and preservative-free 0.15% sodium hyaluronate qid.

At the 1st year follow-up, his corneal stroma had only minimal haze with regular epithelial surface. The uncorrected and corrected VAs were 0.6 and 0.7 with −0.50 (−1.50 at 100), respectively. The patient is still under medical treatment with cyclosporine 0.05% bid and preservative-free artificial tears, as needed.

Discussion

Chemical burn is one of the leading causes of permanent visual loss in the opthalmic emergencies.[5] Treatment and prognosis vary according to severity of the chemical damage, depth and extend of area affected at the central cornea and limbal stem cell area. The exposure time and area of the ocular surface and type, concentration, temperature, and pH of the chemical also affect the prognosis.[6,7] Irreversible chemical damage of limbal basal epithelial cells that are known to have vital roles for epithelialization,[8–15] may cause LSCD. The clinical picture of LSCD may present in a broad spectrum, from undulating finger-shaped epithelial irregularities with stippled corneal fluorescein staining in vortex pattern and late fluorescein staining that extend from the limbus to the center, up to severe and total conjunctivalization of the corneal surface. Eventually, LSCD may cause serious corneal problems such as permanent conjunctivalization, basal membrane destruction, and fibrous tissue growth over cornea.[16,17]

Biomicroscopic findings of LSCD include irregular corneal surface varying in terms of depth and transparency. Severe LSCD results in fibrovascular pannus, chronic keratitis, cicatrization, and calcification.[18] Since the conjunctivalized corneal epithelium is more permeable, it stains with fluorescein irregularly, as compared to normal corneal epithelium.[19] Conjunctivalized corneal epithelium is thinner, disorganized, and stains in a punctate pattern.[20,21] In partial LSCD, a demarcation line between damaged and normal corneal area can be seen. Fluorescein stain tends to pool on the conjunctivalized area, where the epithelium is thinner.[22,23] In severe cases, persistant epithelial defects, corneal melting, and even perforation can be seen.[18] LSCD can be diagnosed histologically by showing goblet cells in the conjunctivalized corneal epithelium with impression cytology.[24] This diagnosis has vital importance to exclude conventional corneal transplantation as a treatment option.[18]

In case of a chemical injury of the eye, main goal of acute management is to suppress the inflammation, prevent progression of epithelial and stromal defects and induce epithelialization.[25] Partial LSCD that does not affect the corneal center could be managed by topical medications to improve lubrication by artifical tears, suppress inflammation by steroid and non-steroid eye-drops, and support epithelialization by autologous serum eye drops.[26–29] Autologous serum eye drops aim at providing healthy epithelial proliferation and migration and preventing corneal adhesion to tarsal conjunctiva leading to symblephora.[30–32] Therapeutic contact lenses and scleral lenses may prevent formation of new corneal epithelial defects, aid in healing persistant epithelial defects, decreasing pain, and photophobia. Lubrication prevents epithelial adhesion to tarsal conjunctiva but unlike autologous serum eye drops, artificial eye drops do not induce limbal stem cell prolifera-

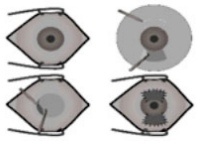
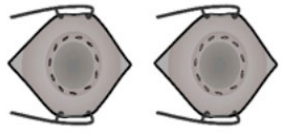
tion.[33,34] In the presence of severe LSCD, (i.e., 360° corneal vascularization, conjunctivalization, and severe visual loss) limbal stem cell transplantation surgery is the only treatment approach. SLET is a new generation technique in this context.[35]

Surgical treatment options for LSCD aim at restoring the healthy corneal epithelial surface and transparent stroma (Table 1). Corneal scraping procedure aims at removing conjunctival tissue over the cornea to help re-epithelization of corneal surface.[36] Amniotic membrane transplantation is frequently performed to induce residual limbal stem cell islands' proliferation and migration, at the early phase of chemical injury. It helps healing corneal surface, improves VA, reduces pain, and particularly photophobia. Amniotic membrane has low immunogenic, high anti-inflammatory, anti-angiogenic, antifibrinogenic, antimicrobial, and anti-apoptotic properties. After removing conjunctival overgrowth on the cornea, amniotic membrane is fixed using tissue fibrin sealants and/or sutures.[32–34,36,37] However, in terms of severe LSCD, it does not allow transparent epithelialization of the cornea. CLAU is another technique, where limbal graft from the healthy fellow eye is taken using conjunctiva as a carrier tissue. However, due to the size of the harvested graft, the technique carries inherent LSCD risk for the donor healthy eye. Conjunctival limbal allograft can be excised from alive relative or cadaver using conjunctiva as a carrier tissue. In this case, systemic immunosuppression is mandatory and risks of infection and neoplasia, as well LSCD risk as in CLAU technique, are present. Keratolimbal allograft is another technique of limbal stem cell transplant from cadavers, using cornea as carrier tissue. Larger tissue is transplanted comparing to other transplants. Its risks also include risks of systemic immunosuppression including infection.[33,38–40] *Ex vivo* cultivated stem cell transplantation (CLET) is a technique, where autologous or allogenic limbal stem cells are grown in the culture media over amniotic membrane or various carriers, and then transplanted. Main advantages include low risk of LSCD in the donor eye and low immunologic rejection risk as Langerhans cells do not reside in the composite graft.[39,41,42] Finally, simple oral mucosal epithelial transplantation (SOMET) can be used when no limbal stem cells are available in bilateral LSCD cases, to decrease ocular surface inflammation and corneal neovascularization. SOMET has particular advantage in improving photophobia and preparing ocular surface for future CLET.

SLET technique is one of the recent advances for monocular LSCD cases. In 2012, Sangwan et al.[43] presented autologous SLET surgery as a new technique combined with amniotic membrane transplantation. Basu et al.[44] analyzed long-term consequences of SLET, and reported successful results in 125 eyes of 95 patients with ocular chemical burn, at post-operative 1.5 years follow-up. Vazirani et al.[45] analyzed outcomes of SLET in 68 eyes at eight centers in three countries; and reported successful results in 57 eyes at the end of 1 year follow-up.

For successful SLET, limbal biopsy should be excised from a healthy limbal area. The biopsy size 2 mm×2 mm is adequate. A larger limbal biopsy may create a risk of LSCD in the healthy eye. Advantages of SLET include being repeatable due to low risk of iatrogenic damage at healthy fellow eye with a small biopsy requirement. In repeated SLET surgeries, biopsy might be harvested close to former biopsy area but they should not overlap. Of note, SLET is

Table 1. Comparison of limbal stem cell transplantation techniques

	CLAU	CLET	SOMET	SLET
				
Donor tissue size	10–20 mm	2×2 mm	3×4 mm	2×2 mm
Laboratory need	No	Yes	No	No
Amnion use	No	Yes	Yes	Yes
Repeatability	No	Yes	Yes	Yes
Donor eye LSCD risk	Yes	No	No	Yes
Cost	Low	High	Low	Low
Preference in unilateral LSCD	Rare	Yes	No	Yes
Preference in bilateral LSCD	Yes	No	Yes	No

CLAU: Conjunctival limbal autograft; CLET: Ex vivo cultivated stem cell transplantation; SOMET: Simple oral mucosal epithelial transplantation; SLET: Simple limbal epithelial transplantation; LSCD: Limbal stem cell deficiency.

applicable at low-budget facilities, as no laboratories for cultivating limbal stem cells are needed. SLET does not require immunosuppressive medication, as risk of immune rejection essentially does not exist.[46] Unfortunately, the autologous SLET is not applicable for bilateral LSCD cases. Allograft SLET can be an option for bilateral LSCD cases, but the rate of surgical success may be lower with this technique. The presence of symblephora might also decrease the rate of surgical success. Per-operative symblephora excision and use of amniotic membrane can be a solution.[47] To transplant the stem cell niches onto a quiet ocular surface with minimal to no inflammation, at least 4–6 months medical treatment after the chemical burn would improve the rate of post-operative success.[43]

To sum up, SLET technique is a viable and minimally invasive alternative in monocular LSCD to improve corneal epithelialization and final VA, as well as to resolve photophobia.

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Informed Consent: Written informed consents were obtained from the parents for publication of this case report and accompanying images.

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Conflict of Interest: None declared.

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