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Minimal Conjunctival Limbal Autograft for Total Limbal Stem Cell Deficiency

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Abstract

Purpose—To report the results of one 60° conjunctival limbal autograft (CLAU) combined with amniotic membrane (AM) transplantation for an eye with total limbal stem cell deficiency (LSCD).

Methods—One eye of a patient with chronic total LSCD and symblepharon caused by chemical burn was subjected to symblepharon lysis, removal of pannus from corneal surface, AM transplantation to cover the conjunctival and corneal surfaces as a permanent graft, one 60° CLAU to the superior limbal area, and insertion of ProKera as a temporary AM patch to cover the CLAU.

Results—After surgery, corneal epithelialization over the AM was evident adjacent to the CLAU on day 6, progressed to pass the horizontal midline by day 11, and was completed by day 18. During a follow-up of 1 year, the corneal surface remained stable and smooth, and the stroma considerably regained clarity with regression of midstromal vascularization. The best-corrected visual acuity improved from 20/400 to 20/50. The conjunctival inflammation completely resolved, and the fornices were deep.

Conclusions—One 60° CLAU combined with AM transplantation as both a permanent graft and a temporary patch can restore the entire corneal surface in an eye with total LSCD caused by chemical burn.

Keywords

amniotic membrane; conjunctival limbal autograft; chemical burn; limbal stem cell deficiency

The maintenance of a healthy functional corneal epithelium under both normal and traumatic conditions is provided by a unique subpopulation of stem cells at the limbal region.^{1,2} When limbal epithelial stem cells are destroyed and/or their supporting stromal environment becomes dysfunctional, a pathologic state known as limbal stem cell deficiency (LSCD) develops.³ LSCD carries the cytologic evidence of conjunctivalization of the corneal surface by a goblet cell-containing conjunctival epithelium.⁴ Pathologically, limbal deficient corneas exhibit destruction of the basement membrane, superficial neovascularization, chronic inflammation, scarring, and poor epithelial integrity.^{3,5}

For eyes inflicted with partial LSCD, where only a part of the limbal circumference is damaged, the corneal surface can be reconstructed by debridement of conjunctivalized epithelium with^{6–8} or without⁹ transplantation of cryopreserved amniotic membrane. However, for eyes inflicted with total LSCD, where the entire limbal circumference is damaged, corneal surface

reconstruction resorts to transplantation of limbal epithelial stem cells.^{7,10} When total LSCD involves only 1 eye, successful reconstruction can be achieved by transplanting autologous limbal epithelial stem cells from the fellow eye in a procedure termed “conjunctival limbal autograft (CLAU).”¹¹ Because the source of limbal epithelial stem cells is autologous, there is no risk of immune rejection and hence no need for systemic immunosuppression. However, when total LSCD involves both eyes, keratolimbal allograft is frequently needed, which requires prolonged, if not indefinite, immunosuppression.¹²

Several studies have shown successful visual outcomes with regression of neovascularization and improvement of corneal transparency after CLAU^{13–22} since it was introduced by Kenyon and Tseng¹¹ on the basis of the concept of limbal epithelial stem cells. Traditionally, CLAU has involved removal of 2 large free grafts, each spanning from 5 to 7 mm in the limbal arc length, from the healthy fellow eye. Although variations of this technique have been developed with time, it remains unclear how small the size of limbal graft is still sufficient to restore the corneal surface with total LSCD. In this case report, we showed successful reconstruction of a corneal surface with total LSCD by using only one 60° (ie, 2 clock hours) graft together with amniotic membrane (AM) transplantation.

CASE REPORT

A 17-year-old man without any history of eye diseases suffered a work-related chemical burn in his right eye caused by cement splash. After initial washing, he was transferred to the emergency room of a local hospital, where his uncorrected visual acuity was 20/800. Anterior segment examination of the right eye showed multiple large clumps of concrete embedded in conjunctival fornices, moderate conjunctival injection, and moderate corneal edema and cloudiness. The left eye was normal. The cement particles were removed, the eye was irrigated, and oral vitamin C with topical prednisolone acetate and gatifloxacin eyedrops were prescribed. At 2–3 weeks after the injury, he presented with a total corneal epithelial defect, which took several months to heal and resulted in complete conjunctivalization of the corneal surface. Because of total LSCD, the patient was referred for CLAU ~6 months after the injury.

On presentation, the best-corrected visual acuity was 20/400 and 20/20 in the right and left eyes, respectively. Extraocular motility and intraocular pressure were normal in both eyes, and there was 4 mm of blepharoptosis in the right eye. The left eye was normal. Anterior segment examination of the right eye showed mild tarsal inflammation with a diffuse symblepharon in the superior fornix (Fig. 1A) and a focal symblepharon in the inferior fornix. The right cornea had diffuse superficial neovascularization and stromal cloudiness without any epithelial defect (Fig. 1B). A fluorescein clearance test showed normal tear secretion and drainage. Impression cytology confirmed total LSCD with the presence of goblet cells on all quadrants and the center of the right cornea (data not shown).

Under general anesthesia, the eye received a 360° limbal peritomy, symblepharon lysis in the inferior and superior fornices, and removal of the subjacent subconjunctival fibrovascular tissue. The conjunctival edges were recessed to the fornices and anchored by two 4-0 silk sutures to the skin in the superior fornix. The corneal pannus was removed by using blunt superficial keratectomy, and the remaining corneal surface was polished with a dental burr. Cryopreserved AM from Bio-Tissue (Miami, FL) was secured to the corneal surface, the bare sclera, and the fornices by using fibrin glue from Baxter (Irvine, CA). A CLAU consisting of a limbal arc length of 6 mm (ie, 2 clock hours or 60°), with 1 mm of peripheral cornea and 8 mm of the conjunctiva, was removed from the superior limbus of the left eye as previously described.¹¹ The resulting bare area was covered by cryopreserved AM using fibrin glue. This free graft was stretched and secured to the superior limbal area of the right eye by interrupted 10-0 nylon sutures over the pre-placed AM as previously reported.¹⁴ Finally, a 16-mm ProKera

(ie, a Food and Drug Administration–approved medical device consisting of a symblepharon ring fastened with cryopreserved AM from Bio-Tissue), was inserted into the right eye to cover the cornea and the CLAU.

After surgery, the patient received eyedrops of 1% prednisolone acetate 4 times a day and ofloxacin 3 times a day. The anchoring sutures were removed at day 6. At this time, early corneal epithelialization over the AM was evident superiorly adjacent to the CLAU (Figs. 1C, D). Epithelialization progressed to cover the corneal surface passing the horizontal midline by day 11 (Figs. 1E, F) and was completed by day 18 after surgery (Figs. 1G, H), without any growth of conjunctival epithelium over the cornea. At that time, there was still corneal cloudiness with midstromal neovascularization. With completion of epithelial healing, the ProKera was replaced by a therapeutic bandage contact lens. In the left donor eye, epithelialization over the AM was complete by day 11 after surgery. During 12 months of follow-up, the corneal surface of the right eye remained stable and smooth, and the stroma regained considerable clarity with regression of the midstromal vascularization (Figs. 1I, J). The best-corrected visual acuity improved to 20/50. The conjunctival inflammation completely resolved, and the fornices were deep without recurrence of symblepharon. No complications were found in the donor eye.

DISCUSSION

Since its introduction in 1989, multiple studies have testified the success of the CLAU to restore the corneal surface in patients with unilateral partial and total LSCD.^{11,13–22} Although relatively rare, several complications have been noted in donor eyes. They include localized haze,²³ pseudopterygium,^{24,25} filamentary keratitis,²² microperforation during surgery,²⁶ abnormal epithelium,²⁷ and corneal depression.²⁸ Moreover, because 2 large grafts are removed from the remaining normal fellow eye, there is always a concern of potential risks to the donor eye to develop LSCD, as suggested in experimental rabbit studies.^{29,30}

Therefore, although the overall risk is low if the donor eye is truly healthy (ie, without long-term contact lens wear or subclinical exposure to original trauma) and if <6 clock hours of limbal tissue is removed,³¹ few attempts were made to reduce the overall size of the donor CLAU. Rao et al²¹ successfully used 1 CLAU of 60–90° for treating eyes with partial LSCD but found it insufficient to treat 2 eyes with total LSCD. Moldovan et al³² found that 1 CLAU of 90 or 100° could restore eyes with total LSCD but that of 80° was unsuccessful. Herein, we noted that 1 CLAU of 60° could successfully restore a stable, smooth, avascular, and clearer cornea in an eye with total LSCD (Fig. 1). We attributed such a success to the use of AM as a permanent graft underneath the CLAU and as a temporary patch over the CLAU. Previously, Dua³³ suggested that partial LSCD with <90° of intact limbus could not be treated with simple epithelial debridement without AM transplantation. In contrast, AM as a permanent graft has been shown to restore the entire corneal surface in eyes with partial LSCD with as little as 30–60° of intact limbus left.^{6–8} Furthermore, AM as a permanent graft has also been shown to restore donor corneas after the CLAU is harvested^{14,18,34} and also helped transplantation of autologous^{18,34,35} or allogeneic¹⁰ limbal cells in recipient eyes. As a temporary patch, AM can reduce inflammation and promote expansion of remaining limbal epithelial stem cells in acute chemical burns^{36–38} and in acute Stevens–Johnson syndrome.^{39–42} Therefore, a combination of AM transplantation as a permanent graft and as a temporary patch might act together to secure an environment favorable for regeneration of limbal stem cells. This notion was shown by rapid epithelialization passing the visual axis by day 11 and reaching the opposite limbus by day 18 after surgery (Fig. 1), and is consistent with recent approaches using ex vivo expanded limbal epithelial progenitor cells from a small limbal biopsy cultivated on AM.⁴³ On the other hand, factors such as reduction in active conjunctival inflammation, good lid

closure, and good tear function might have also contributed to the successful outcome in this case.

We realize that it is not possible to extrapolate a general guideline from 1 case report. However, the success in restoring an eye with total LSCD by one 60° CLAU warrants others to consider such an option when considering the risk in the donor eye during the treatment of unilateral LSCD.

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FIGURE 1.

Extensive superior symblepharon with total conjunctivalization was noted before surgery (A and B). Early corneal epithelialization over AM was noted superiorly adjacent to the CLAU on day 6 (C and D), passed the horizontal midline on day 11 (E and F), and was completed on day 18 after surgery (G and H). Twelve months after surgery, the eye regained a smooth and stable corneal surface with improved stromal clarity and regressed vascularization (I and J).