Dear Editor,

In the treatment of unilateral total limbal stem cell deficiency (LSCD), conjunctival limbal autograft (CLAU) surgery taken from the healthy fellow eye with or without amniotic membrane transplantation (AMT) is one of the main therapeutic alternatives.\[1\] There are some controversies regarding the optimal size of the limbal graft necessary for complete and stable epithelialization of the cornea.\[2,3\] Traditionally, two 60° limbal grafts have been used.\[4\] Few attempts have been made to reduce the overall size of the donor graft and it has been suggested that combining CLAU and AMT as a graft may minimize the required size of the limbal graft.\[5]\[5\] In a single case report, a group of authors noted that corneal epithelialization using one 60° block of CLAU was complete by day 18 after surgery.\[5\] They attributed this achievement to the use of amniotic membrane (AM) as a permanent graft underneath the CLAU and as a temporary patch over it. It has already been shown that in partial LSCD, one segment CLAU may suffice.\[6\] Rao et al successfully used one 60-90° limbal block for treating eyes with partial LSCD. They found it insufficient to treat 2 other eyes with total LSCD.\[7\] Moldovan et al also found that one 90-100° limbal block could restore eyes with total LSCD but that of 80° block surgery was unsuccessful.\[8\]

In the present study, patients with total unilateral LSCD received a single 60° limbal block of CLAU, and we observed that the procedure was insufficient for stable and permanent ocular surface epithelialization by corneal phenotype epithelium. Medical charts of patients, who had received a single 60° block of CLAU or had inadvertently lost one of their blocks from previous conventional CLAU, were evaluated (March 2011 to April 2013). All patients suffered from total unilateral LSCD due to chemical injury with total conjunctivalized/vascularized corneal opacity and poor epithelial integrity (persistent or recurrent epithelial defects) experiencing chronic irritation, redness, tearing and decreased visual acuity. A fluorescein clearance test showed normal tear secretion and drainage. Diagnosis of total LSCD in all patients was based on characteristic clinical findings and confirmed by classic impression cytology. Integrity of the ocular surface epithelium was evaluated by fluorescein staining and the pattern of epithelial healing was evaluated. Findings were documented using digital corneal photography (Imagent; Topcon SL-8Z, Tokyo, Japan) at all follow-up visits. All risks and benefits were clearly explained, and informed consent was obtained from all participants. All operations were performed by one surgeon (AB).

Failure was defined as the appearance of persistent epithelial defect (PED) (nonhealing epithelial defect for more than 2 weeks) with progressive corneal conjunctivalization/vascularization and thinning. Appearance of a peripheral superficial vascularized corneal pannus over subsequent transplanted cornea was considered as corneal conjunctivalization which was supported by late fluorescein staining and confirmed by impression cytology.

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Five eyes of 5 patients with total unilateral LSCD were included. All subjects had sustained injury by acidic \((n = 3)\) or alkaline chemical agents \((n = 2)\). Mean age of the patients was 32.3 ± 6.4 (range, 23-42) years and the time interval between ocular surface damage and the limbal transplantation was more than 12 months in all cases. Patients had received classic CLAU from their healthy fellow eyes. The exposed ocular surface including total corneal surface was thoroughly covered by cryopreserved AM measuring approximately 3 cm × 3 cm (sticky stroma side down). One 60° arc of limbal graft was harvested from the 12-o’clock position, starting with lamellar dissection in the cornea 1 mm anterior and extending 2 mm posterior to the limbus, leaving behind the Tenon capsule as much as possible. A 5-mm conjunctival mantle was simultaneously harvested to help fornix reconstruction in the injured eye. If necessary, subsequent penetrating keratoplasty (PKP) was performed at least 3 months after stem cell transplantation. After surgery, oral prednisolone 1 mg/kg per daily was initiated and tapered off by 6-8 weeks along with decreasing inflammation. Furthermore, topical antibiotic (chloramphenicol 0.5%) and steroid drops (betamethasone 0.1%) 4 times a day were administered after surgery. The former drop was discontinued when corneal epithelialization was complete, whereas the latter was tapered off according to ocular surface inflammation. Lubrication of the ocular surface was continuously performed using topical preservative-free artificial tears and lubricating gels (Liposic; Bausch and Lomb, Rochester, NY, USA). If elevated intraocular pressure was diagnosed, topical antiglaucoma medications were used.

The patients were followed for at least 6 months. Two cases had primarily received one-block CLAU; two other cases had inadvertent trephination of one limbal block during subsequent optical PKP, and the last case had lost one of his blocks due to early suture loosening and removal.

Cases with primary one block surgery developed PED which was refractory to intensive conservative management [Figure 1a and b]. 3 weeks later, the second block was applied to the ocular surface as classic CLAU. The epithelial defect completely healed after 1-week. In one of the cases, PKP was done after 3 months. The cornea was clear 6 months later without any conjunctivalization [Figure 1c]. In cases with inadvertent graft trephination during subsequent PKP, PED occurred in both grafts [Figure 2a]. Despite conservative and surgical management (tarsorrhaphy and AMT), corneal perforation occurred in both eyes and tectonic keratoplasty was performed for them. PED recurred over the grafts; in one case the condition was compensated by severe sectoral conjunctivalization [Figure 2b]. In the other case, because of refractory PED and perforation, an additional tectonic keratoplasty was performed but eventually underwent an oral mucosal graft in order to save the globe. In the last case with conventional two-block CLAU, despite primary complete epithelialization of the cornea, the superior limbal block was lost due to early suture loosening and removal; this was followed by refractory PED which was resistant to medical management and tarsorrhaphy. Ultimately, the patient underwent cultivated limbal epithelial transplantation (CLET) to provide enough stem cell reserve for stable re-epithelialization of the cornea.

Amniotic membrane may be a suitable niche for transplanted stem cells. It facilitates epithelial

**Figure 1.** Incomplete corneal epithelial healing after single block CLAU and AMT for total LSCD 2 weeks after surgery (a and b). Second segment CLAU was performed to heal the epithelium. The transplanted cornea is clear 6 months after PKP (c). CLAU, conjunctival limbal autograft; AMT, amniotic membrane transplantation; LSCD, limbal stem cell deficiency; PKP, penetrating keratoplasty.

**Figure 2.** Persistent epithelial defect after PKP in an eye with inferior cut limbal graft due to 1 mm inferior decentration of an 8.0 mm trephine (a). Late vascularization and conjunctivalization after PKP in the other eye with inferior cut limbal graft (b). PKP, penetrating keratoplasty.
proliferation, decreases ocular surface inflammation, vascularization and the amount of underlying corneal scar, and also increases corneal transparency. For permanent and stable epithelialization of the ocular surface, a minimum mass of limbal stem cells may be required even in the presence of AM with potentiating effect. We found that one 60° block of CLAU with AMT as a graft is not sufficient for permanent and stable epithelialization of the cornea. In one block CLAU, PED occurs over the diseased cornea or subsequent corneal transplantation. We have already shown that even small graft size may lead to PED, sectoral conjunctivalization, thinning and perforation after CLAU or subsequent PKP. Even in conventional CLAU with two 60° limbal grafts, progressive conjunctivalization in the horizontal exposure zone may occur. Moreover, punctate epithelial erosions and keratitis, particularly after PKP, are common findings after conventional CLAU.

In summary, although CLAU surgery is an effective procedure in unilateral total LSCD with favorable visual results, conventional two block surgery is necessary to achieve long term visual and anatomical success. Our experience with single block was disappointing in terms of adequate epithelialization and all cases required additional interventions. Therefore, in unilateral total LSCD with complete conjunctivalization and vascularization of the cornea, in lieu of decreasing the size of limbal transplantation, alternative procedures including cultivated limbal epithelial transplantation (CLET), cultivated oral mucosal epithelial transplantation (COMET), or keratolimbal allograft (KLAL) surgery should be considered.

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