The ocular surface after simple limbal epithelial transplant (SLET): A high-resolution OCT study of the early postoperative period

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Simple limbal epithelial transplantation (SLET) is an emerging technique for treating unilateral limbal stem cell deficiency. We report the high-resolution, anterior segment optical coherence tomography (OCT) features of the first 2 weeks of a patient undergoing SLET for an old acid injury of the right eye, repeatedly from postoperative day 1 through day 14. Three out of 11 explants with the subjacent human amniotic membrane (hAM) and the overlaid bandage contact lens were imaged. The hAM was intact and of the same thickness throughout the study period; the sub-hAM space increased from day 3 to 9 and disappeared by day 10; the explants started thinning from day 3 with the fibrin around them starting to decrease from day 2 and completely disappeared by day 4. Epithelialization occurred between day 8 and 14 and proceeded more rapidly towards the limbus than centrally. There was no change of the corneal stromal thickness or reflectivity. This case report uses high-definition, spectral-domain OCT to document the events on the ocular surface after a successful SLET surgery and opens up an avenue to study epithelialization in a convenient and noninvasive manner.

Key words: Amniotic membrane, epithelialization, LSCD, optical coherence tomography, simple limbal epithelial transplant

Chemical and thermal injuries of the eye were once considered to cause irreversible and untreatable blindness. However, over the past few years, the situation has changed dramatically, with the identification of limbal epithelial stem cells[1,2] and the advent of cultured stem cell transplantation (CLET).[3,4] Kenyon and Tseng's technique of conjunctival limbal autografting (CLAU)[5] had the disadvantage of needing large segments of limbal tissue which could lead to limbal stem cell deficiency (LSCD) of the healthy donor eye.[6] CLET has its origin in circumventing this very problem using small amounts of donor limbus and expanding it *ex vivo* on human amniotic membrane (hAM). However, CLET restricted the widespread acceptability of autologous limbal transplant due to its requirement of expensive and sophisticated infrastructure.[7] The last barrier – requirement of expensive cell culture laboratories – was overcome when Sangwan *et al*. reported the remarkably easy technique of simple limbal epithelial transplant (SLET).[7] SLET essentially consists of meticulous pannus dissection, harvesting of healthy limbal tissue from the fellow (unaffected) eye, dividing it, and anchoring it to the damaged cornea by fibrin glue over the hAM. The hAM acts as a scaffold for the regenerating epithelium emerging out of the limbal tissue explants and is also believed to help the limbal tissue retain its stemness.[8] There are a few emerging reports regarding the behavior of the explants in various situations after SLET. Mittal *et al*.[9] have reported the process of epithelialization of the cornea after SLET, with their technique involving removal of the bandage contact lens (BCL) and fluorescein staining on a daily basis. Yeung *et al*.[10] have demonstrated in *in vitro* experiments that fibrin glue takes about 2 weeks to dissolve and allows epithelial sheets from explants to start expanding onto the culture media. There are no reports to date on the events in the early postoperative period after SLET, especially with regard to the hAM–cornea interaction and the onset and progress of epithelialization. In this case report, we have studied the events in and around the explants and the changes in the hAM and sub-hAM space using high-definition, spectral-domain optical coherence tomography (OCT).

Case Report

The patient, a 26-year-old male, with a history of acid injury in the right eye 3 months prior had been variously treated with symblepharon release, hAM transplantation, and BCL insertion in the acute phase followed by lid reconstruction, symblepharon release, and an Ahmed glaucoma valve (AGV) for uncontrolled intraocular pressure (IOP). At presentation, he had a total LSCD with total conjunctivalization of the cornea [Fig. 1a]. There were symblephara of the upper and lower lids [Fig. 1b and c]. Vision was perception of light with inaccurate projection of rays. Digital tension was higher than normal. The left, unaffected eye was within normal limits with a healthy limbus. B-scan ultrasound of the affected eye revealed an attached retina. The technique of SLET used was as described by Sangwan *et al*.[7] – blunt dissection of the pannus

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from the corneal surface, release of all symblephara, gluing of an hAM (non-cryopreserved, fresh), and fixation of limbal explants from the contralateral eye with fibrin glue (Tisseel Kit from Baxter AG, Vienna, Austria). At the end of the procedure, a BCL was placed. Tissue harvested from the healthy eye was approximately 2 mm wide and was divided into 11 bits which were placed in two concentric circles on the right eye [Fig. 1d]. Fig. 1e shows the slitlamp photograph of the operated eye on post-operative day 2.

The patient was followed up daily for the first 6 days and subsequently on days 8, 10, and 14. At each visit, besides a full clinical evaluation, slit lamp photographs were taken and anterior segment OCT was done on a Cirrus™ HD-OCT (Carl Zeiss Meditec AG, Goeschwitzer Strasse 51–52, 07745 Jena, Germany), spectral-domain OCT with an axial resolution of 5 µm and a transverse resolution of 15 µm.

For the purpose of the study, three explants were identified on a clinical photograph [Fig. 1f] and labelled as 1, 2, and 3. Serial OCT was done along a radial axis for each explant reaching up to the limbus, as shown in Fig. 1f by a single investigator (MS) at each follow up day on each of the three explants. Quality of all OCT images achieved was satisfactory. The best images were obtained for explant 2 and are reproduced here.

Postoperative regime followed was prednisolone acetate drops 6 times a day, moxifloxacin drops 6 times a day, and carboxy-methyl cellulose drops 2 hourly. The BCL was retained in the operated eye for the entire duration of the study.

There was rapid resolution of conjunctival congestion and good mobility of the globe with release of the symblephara. Lid closure was good. Digital IOP was normal. Anterior chamber details could be made out hazily through the translucent cornea, with the pannus removed. Vision remained perception of light with inaccurate projection of rays. The donor fellow eye had rapid closure of the epithelial defect at the donor site.

Findings on OCT

The hAM remained of even density (OCT reflectivity) and thickness throughout the study duration [Fig. 2]. Sub-hAM space increased from day 3 and resolved completely by day 10. There was thinning of the explants from day 3 which corresponded to the sub-hAM space expansion, which may have been compressing the explants against the BCL. Fibrin around the explants started decreasing from day 2 onwards and disappeared by day 4. The hAm, even though glued to the corneal surface with fibrin glue, was not absolutely stable in the early days after SLET. The fibrin glue beneath the hAM appeared to swell, displacing the hAM anteriorly. However, this settled by day 10, and thereafter the hAM was adherent to the cornea. There were no changes in the consistency (reflectivity) and thickness of the hAM. The fibrin around the explants developed optically hollow spaces immediately adjacent the explants and started decreasing from day 2 and disappeared by day 4. Comment regarding the thinning rate of the explants is difficult as it is impossible to ensure that the OCT section is being taken at exactly the same site of the explants on each occasion. Further, the thinning of the explants appear to correlate with the initial bulging of the sub-hAM space and continued even as the hAM settled down and adhered to the cornea. There was no evidence of epithelialization up to day 8. By day 14, there was complete epithelialization on the limbal side, in contrast centripetally directed epithelialization was poor extending only about 0.24 mm on day 14. The nature of the epithelium was boggy and not the crisp reflective layer that is seen in OCT of normal corneas. There was little change in the corneal stromal thickness and lucidity on OCT.

Discussion

There is little data on the early structural changes after SLET, with most articles focusing on the long-term clinical outcomes and advantages over CLET. Some idea about epithelialization post-SLET can be obtained from the work of Mittal *et al*.[9] but the "activity" around the explants observed from the very first postoperative day may be fibrin glue around the explants. Most surgeons practicing SLET would hesitate to remove the BCL immediately after the procedure to observe what is happening to and around the explants as such a manoeuvre could dislodge the explants or disturb the process of epithelialization. In the 125 case series reported by Basu *et al*.,[8] it was found that all 7

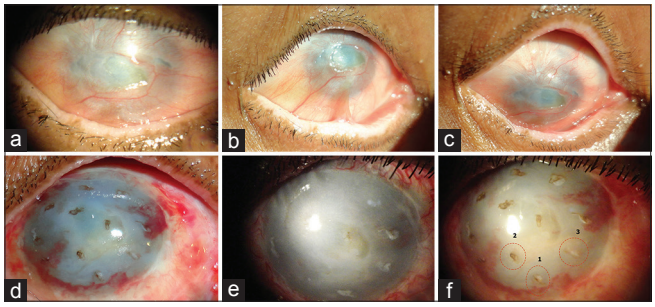

Figure 1: Pre-operative and post-operative slitlamp photographs. (a) Shows the 360 degree LSCD. (b and c) show the superior and inferior symblephara. (d) First post-operative day. (e) Second post-operative day. (f) Identification of explants for serial OCT. The lines identify the axis along which OCT cuts were taken

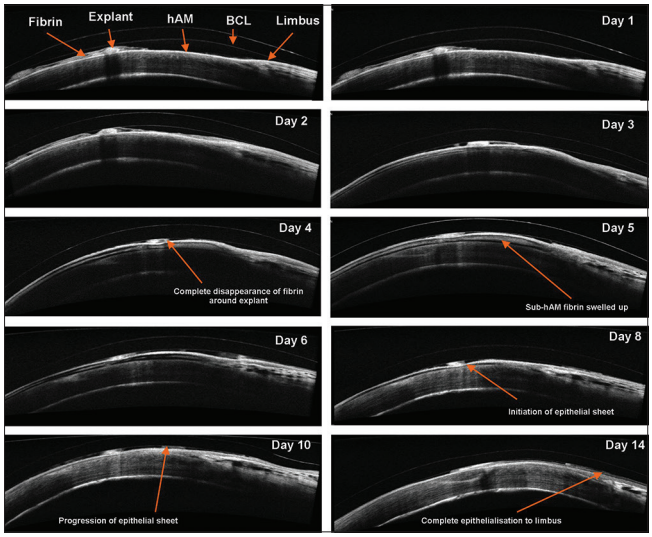

Figure 2: High resolution OCT images of explant no. 2. The number in the top left corner shows the post-operative day number. The image at the top left corner identifies the various structures on the ocular surface and the BCL

of 125 eyes, with partial loss of explants, were associated with the loss of BCL within 7 days after SLET.

We distinctly found no evidence of epithelial proliferation on OCT up to day 8. Unfortunately, in our case there was a 4-day gap (day 10 and 14) during which we do not have OCT data. It is during this period that centrifugal migration of epithelial cells occurred in this patient. Of note is the observation that centripetal epithelialization markedly lagged behind. The behavior of the hAM and the space between the corneal stroma and the hAM in the early postoperative period is largely unknown. It has been suggested by Basu *et al*.[8] that the hAM is retained on a long-term basis proven by PAS-positive basement membrane in post-SLET corneal buttons excised for penetrating keratoplasty. Amescua *et al*.[11] have also suggested the same based on high-resolution OCT. We found that the sub-hAM space showed activity between day 2 and day 10 with initial expansion of the space. This finding possibly indicates an active process involved in removing the fibrin glue from this space. After day 10, the hAM appeared to be well adhered to the subjacent stroma. We found that the hAM remained unaltered both in thickness and in optical reflectivity throughout the study period.

Our finding that the fibrin around the explants starts disintegrating as early as day 2 is contradictory to that reported by Yeung *et al*.,[10] although their observation was *in vitro* where phagocytic activity of the ocular surface was absent. We also noted that fibrin in the two compartments – sub-hAM and around explants – behave differently with the latter disintegrating much faster.

In summary, this single case report is the first, to our knowledge, that uses high-definition, spectral-domain OCT to document the events on the ocular surface after a successful SLET surgery and opens up an avenue to study epithelialization in a convenient and noninvasive manner. Similar studies and confocal microscopy are required for further elucidation of the mechanism of SLET surgery and the healing pattern.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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