

Reappearance of limbal pigmentation post-simple limbal epithelial transplant

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We report the repigmentation at the limbus in patients who underwent simple limbal epithelial transplant (SLET) for unocular chemical injury. The first case is of an 8-year-old child who presented with grade 4 chemical injury, with limbal stem cell deficiency (LSCD) corresponding to 6 o' clock till 11 o' clock. He was managed by amniotic membrane graft in the acute stage and SLET after 6 months of the initial injury. The second case is of a 15-year-old female who presented with lime injury, which

had resulted in 6 o' clock of limbal involvement (10 o' clock till 4 o' clock). The patient was managed on similar lines with amniotic membrane graft (AMG) in the acute phase and SLET after 6 months of injury. The ocular surface was stable in both the patients post-SLET. The effected limbus showed pigmentation at 8 months of follow-up which eventually became distinct and remained stable. We speculate that the pigmentation at limbus could be attributed to proliferation and movement of melanocytes from limbal biopsy in SLET. These may be capable of supporting the proliferation of limbal epithelial cells and modulation of corneal wound healing.

Key words: Chemical injury, limbal stem cell deficiency, simple limbal epithelial transplant

Various factors, including avascularity and collagen lamellar arrangement, contribute toward maintaining a transparent human cornea.^[1] Transparency is also maintained by the stem cells, which continuously replace the stratified squamous epithelium of the ocular surface, and these cells are located at the limbus in a microenvironment known as niche.^[2] Since Schofield^[3] gave the concept of stem cell niche in 1978, much work has been done to understand its role in harboring the limbal stem cells (LESC), which are clinically identified as palisades of Vogt.^[4] These palisades are identified due to pigmented melanocytes and are infiltrated with antigen presenting Langerhan's cells and suppressor T-lymphocytes.^[5]

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There are different roles melanocytes play in the human body, including protection of keratinocytes from oxidative DNA base damage from UV rays in the skin and maintaining clear and unpigmented central cornea in the palisades.^[6] Injury to these cells (mechanical or chemical) may cause limbal stem cell deficiency (LSCD). LSCD is a severe cause of corneal blindness, manifesting as corneal neovascularization and conjunctivalization, loss of palisades of Vogts, and irregularities of the corneal epithelium.^[7] Cultivated limbal epithelial transplant (CLET), as an *ex vivo* technique, has been successfully used in treating patients with ocular burns over the years.^[8] Since 2011, SLET has shown to be produce comparable results to CLET with minimal dependence on laboratory.^[9,10] But how limbal stem cell transplantation actually works, whether by stimulating the damaged or quiescent limbal stem cells or by replacing the niche with healthy stem cells, is still poorly understood. We are describing an interesting observation in patients who underwent SLET at our center, which we believe could be an area of future research.

Case Reports

Case 1

An 8-year-old boy presented to us with acute chemical burn with lime/chuna. Immediately after exposure, he developed pain and redness in his right eye. At presentation, patient had a visual acuity of 20/200. Patient was diagnosed with grade 4 chemical burn^[11] and received treatment for acute chemical injury. Amniotic membrane grafting (AMG) with

bandage contact lens was performed on day 3 of presentation. Four months post-AMG; limbal stem cell deficiency of more than 5 clock hours (6 o' clock till 11 o' clock) was noted, with conjunctivalization of the cornea and corneal scar [Fig. 1a]. Once a stable ocular surface was achieved, 6 months after the initial insult, SLET was performed using limbal biopsies from the unaffected eye. SLET was performed in a similar manner to what has been described in literature.^[9] One-month post-SLET, limbal stem cells and dissolving AMG was noted [Fig. 1b]. Interesting, at the 9th month follow-up, the stable ocular surface showed a pigmented line, which was noted from 6 o' clock to 10 o' clock limbal area, corresponding to the initial area of LSCD [Fig. 1c]. The patient maintained a best corrected visual acuity of 20/20 at the final follow-up.

Case 2

A 15-year-old female presented to the cornea clinic with 1-month history of lime injury to her right eye. She presented with pain, redness, and photophobic in the affected eye. Her unaided visual acuity was 20/80 in the right eye. Early symblepharon was noted in the superonasal quadrant with absence of limbal palisades and more than 6 o' clock hours of limbal involvement (10 o' clock till 4 o' clock) [Fig. 2a] Superior cornea was involved with stromal edema present superiorly adjacent to the area of limbal stem cell deficiency. The patient was managed with AMG and topical steroids, and was kept on regular follow-up. Eight weeks after the initial presentation, after the corneal surface stabilized, SLET was performed for the patient to address the limbal stem cell deficiency [Fig. 2b]. Over the next several months, a similar

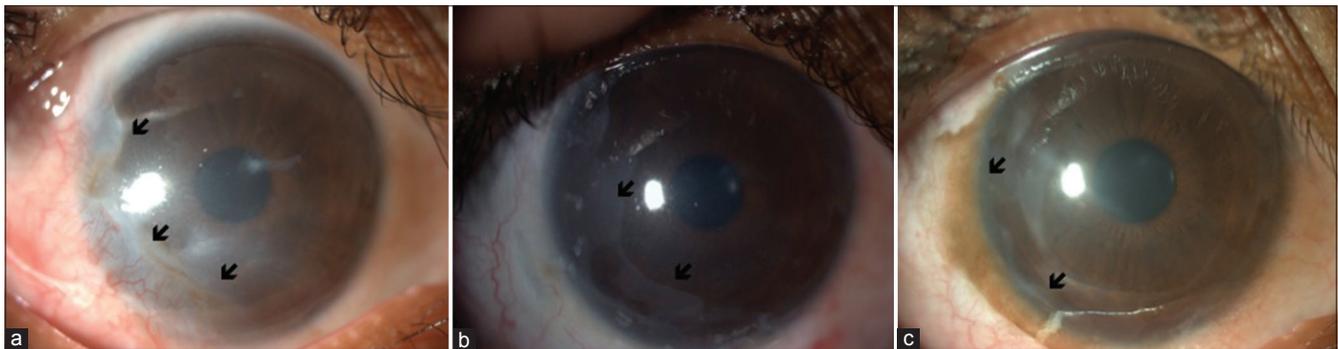


Figure 1: (a) Slit-lamp photo of the affected eye showing limbal stem cell deficiency from 6 o' clock till 11 o' clock (black arrows) with conjunctivalization of the cornea, 4 months after initial insult. (b) One month post-SLET photo showing limbal stem cells and dissolving AMG (black arrows). (c) Ninth month follow-up, showing stable ocular surface with a pigmented line, noted from 6 o' clock to 10 o' clock (black arrows) limbal area, corresponding to the initial area of LSCD



Figure 2: (a) Symblepharon in the superonasal quadrant with absence of limbal palisades and more than 6 o' clock hours of limbal involvement (10 o' clock till 4 o' clock; black arrows). (b) Slit-lamp photo post-SLET showing limbal biopsies in the superior aspect. (c) Eighth month post-SLET, showing pigmented line from 10 o' clock till 4 o' clock limbal area (black arrows), corresponding to the area of LSCD

pigmented line was noted as was seen in Case 1. This line was seen in the area of corneal wound healing and was seen most prominently around the 8th month period extending from 10 o' clock till 3 o' clock [Fig. 2c].

Discussion

As clinicians, our observation in successful SLET patients prompted us to report these cases. We observed appearance of pigmentation at the involved limbus over a period of 8–12 months post-SLET and that remained stable thereafter.

SLET preserves the epithelial–mesenchymal microenvironment in the transplanted biopsies and this probably helps recreating the stem cell niche.^[9] We speculate that probably the functional involvement of melanocytes in the microenvironment of the transplanted biopsies promotes wound healing or may be the wound healing promotes melanocyte migration, at the limbus. This requires further research.

This, of course, is fundamentally different from other methods of LSCT, like CLET. Evidence shows that limbal epithelial stem cells account for only 2–9% of the entire limbal epithelial population *in vivo*,^[12] and hence, we believe, it is likely that not all cells of the niche on *ex vivo* cultured limbal epithelial grafts are stem cells. Therefore, it is possible, since the stem cell niche is not transplanted in total; the movement of melanocytes is probably not noted.

Very few studies have actually dealt with characterizing melanocytes in human cornea, *vis-à-vis* the skin where their role has been described more extensively.^[13] However, both in cornea and skin, two types of melanin are present—brownish black eumelanin (pigmented melanin) and reddish yellow pheomelanin (non-pigmented).^[14] In the human ocular surface, melanocytes can be observed around the limbal circumference. Interestingly, these cells concentrate within the basal layer of the limbal crypts (downwards projections of the limbal epithelium into the limbal stroma between the palisades of Vogt) that also concentrate limbal epithelial stem cells.^[2] Laboratory studies have developed an *in vitro* of the limbal stem cell niche in which mitotically active limbal melanocytes have been used as feeders for the expansion of limbal epithelial cells. In fact, limbal melanocytes are capable to support the proliferation of limbal epithelial cells that maintain stem cell properties (morphological aspects, high proliferative potential, expression of putative stem cell markers).^[4] These observations suggested that limbal melanocytes, more than providing protection against UV rays by the release of melanin, could also act as niche cells for the functional maintenance of the progenitors in their native environment.

Lastly, we would like to acknowledge the limitations while reporting this observation. There are no current investigative (biopsy or scan) modalities to prove the movement of melanocytes clinically. The role of anterior segment Optical Coherence Tomography (AS-OCT) has not been defined for studies of the limbus, pigmented lesions, or for lesions less than 5 microns in size.^[15,16] Other possibility that could be considered is denatured hemoglobin from subconjunctival hemorrhage that also collects in the area of hyperpigmentation, however this is less likely and would be difficult to prove. These observations of reappearance of pigmentation at the limbus have been observed with few of the SLET cases operated upon at our center. Whether melanocytes have a bigger role to play in corneal wound healing and if this

can lay a foundation for understanding the mechanism of SLET is something only time (and research) will tell.

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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