

Oral epithelial cells transplanted on to corneal surface tend to adapt to the ocular phenotype

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To understand the response of oral epithelial cells, transplanted on corneal surface to the ocular cues *in vivo*. The corneal button obtained after penetrating keratoplasty (PK) of an eye of a patient with total limbal stem cell deficiency (LSCD), previously treated with cultured oral mucosal epithelial transplantation (COMET) was examined by immunohistochemistry for the expression

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of keratins, p63, p75, PAX6, Ki-67, CD31, and CD34. COMET followed by optical-PK has improved visual acuity to 20/40 and rendered a stable ocular surface. The excised corneal tissue showed the presence of stratified epithelium with vasculatures. The epithelial cells of the corneal button expressed K3, K19, Ki-67, p63, p75 and the cornea-specific PAX6 and K12. This study confirms that the oral cells, transplanted to corneal surface, survive and stably reconstruct the ocular surface. They maintain their stemness at the ectopic site and acquire some of the corneal epithelial-like characters.

Key words: bilateral LCSD, eye, limbal stem cell deficiency, oral mucosa

One of the major advances made in translational research is in the field of ocular surface reconstruction using cell therapy. Limbal stem cells play an important role in the maintenance of corneal epithelium and tissue homeostasis. Several factors like chemical (alkali/acid) or thermal injury, UV and ionizing radiations, repeated surgical interventions, extensive microbial

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infection and Steven Johnson's Syndrome (SJS) may lead to LSCD. These conditions lead to corneal opacity and visual impairment apart from varying degrees of discomfort. In bilateral LSCD, allografting is done using limbal tissues from a live related donor or from cadaveric sources, which requires the continuous usage of immune suppressive drugs. To obviate the need for immune suppression in allogenic transplantations, a few groups have attempted to use autologous oral mucosal epithelial cells as an alternative to limbal epithelium for corneal surface reconstruction in humans.^[1] Patients with severe bilateral LSCD have been successfully treated with COMET, with good clinical outcomes in terms of ocular surface stabilization and marginal improvement in visual acuity.^[2] COMET, followed by an optical PK, not only provides visual rehabilitation but also provides a unique opportunity to evaluate and document the fate of transplanted cells on the excised corneal specimen. Here, we report the clinical and histological findings in a case of total LSCD that underwent COMET treatment followed by an optical-PK.

Materials and Methods

The oral mucosal biopsy, *in vitro* culture of oral mucosal epithelium and the surgical transplantations were done as reported previously. This study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board. The details were elaborated in the appendix, A1-2.

Results

Clinical outcome

On the 1 day and after 11 months of COMET for the right eye [Fig. 1b], the uncorrected visual acuity was counting fingers at 1 meter. The patient was, however, asymptomatic

with a stable ocular surface. On the 1st post-operative day of PK, the uncorrected visual acuity was 20/60 improving to 20/40. When last seen at 13 months post-PK, the best corrected visual acuity was 20/160 with a clear graft and a stable ocular surface.

Histological examination of the Post-COMET corneal tissue

H and E staining of the corneal button, excised during PK of the COMET-treated right eye, showed a 5 to 6 cell stratified epithelium with basement membrane. The hAM has integrated into the corneal stroma. Goblet cells were not observed in PAS staining, thereby suggesting the absence of conjunctivalization. Consistent with the previous reports,^[3,4] a few sub-epithelial vasculatures were also seen in close proximity to the basement membrane [Figs. 2c and d].

Immunohistochemical Examination of the Post-COMET Corneal Tissue

Both corneal and oral mucosal tissues share the expression of some of the cytokeratins like K3, K4, K13 and K15 with the conjunctival epithelium.^[5] Immunohistochemical (IHC) examination of the post-COMET corneal tissue showed K19 being expressed in all the layers of conjunctival epithelium while it was expressed only by the basal cells of the cornea, oral mucosal tissue and in the post-COMET corneal button [Figs. 3e-h]. Expression of K14 was seen only in the basal cells of the conjunctiva but not in the cornea, oral mucosa and in the post-COMET corneal tissues [Figs. 3a-d]. While the epithelial cells of all the layers of the native cornea and the PK button stained positive for the antibody that collectively recognizes the cytokeratins, K3/K12 [Fig. 3i-l], the K12-specific antibody stained only the basal cells of the PK button with a clear cytosolic expression pattern [Fig. 3m-p].

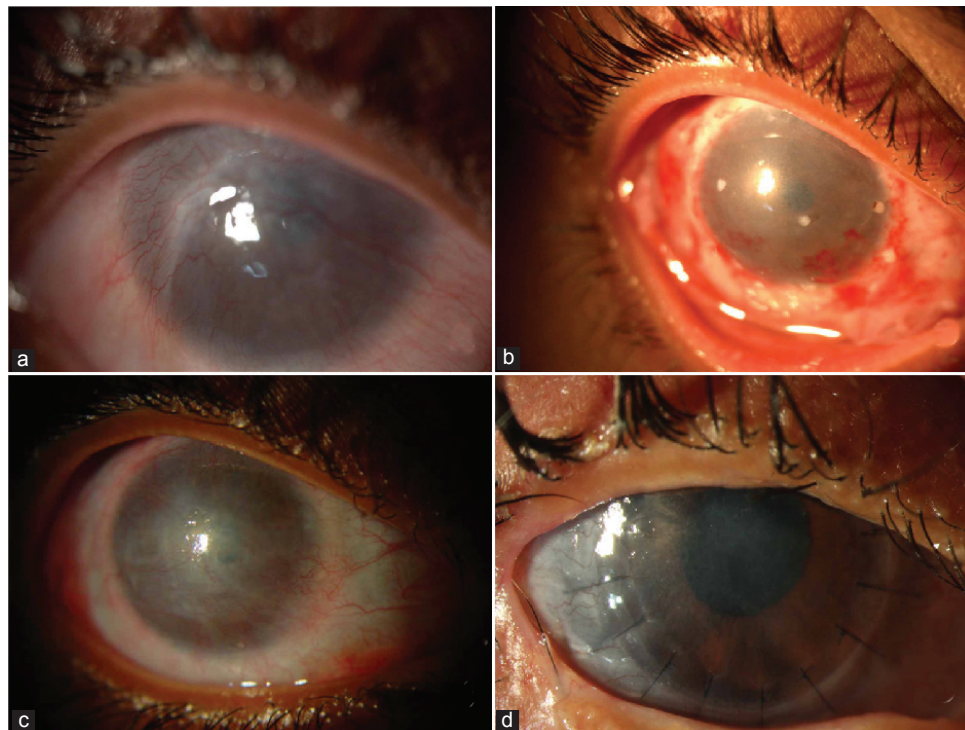


Figure 1: Slit lamp photograph of the right eye showing total vascularized cornea due to limbal stem cell deficiency (a) Slit lamp photograph, post-operative day 1 (b) and after 10 months (c) post- COMET and post COMET-PK (d) slit lamp pictures

To our surprise, we found PAX6 expression in the IHC sections of the native oral tissue (diffused and pan-nuclear pattern) as opposed to the strong nuclear staining in the corneal and conjunctival epithelium [Fig. 3m-o]. More interestingly, the basal cells of the post-COMET corneal button showed a clear increase in the nuclear PAX6 staining [Fig. 3p] and the cytosolic K12 expression.

In the post-COMET corneal button, we found that the supra-basal cells were positive for the cell proliferation marker Ki-67, similar to the native oral, corneal, and conjunctival tissues [Fig. 4a-d]. The basal and suprabasal cells of post-COMET corneal tissue showed reactivity to the epithelial stem cell marker, p63 [Fig. 4e-h] and the NGF receptor, p75⁶¹ [Fig. 4i-l], thus confirming the presence of stem cells and TA cells in the reconstructed ocular surface. Subepithelial vasculatures [Fig. 2c] endothelial cells express CD31 and CD34 [Fig. 5 and b]. Staining with Ki-67 antibody was also positive in endothelial cells [Fig. 4d] (Refer A3 for IHC summary).

Discussion

This study reports a case of alkali burn-induced bilateral total LSCD treated with autologous COMET. In our study, the histological examination of the corneal button showed that the transplanted oral cells had undergone stratification and successfully reconstructed the ocular surface. It was encouraging to note that there was no recurrence of conjunctivalization as confirmed by PAS staining, K14 and K19 expression patterns. The suprabasal cells were positive for the cell proliferation marker, Ki-67, thus confirming the presence of proliferating cells.

Interestingly, we found that the basal cells of the post-COMET corneal button showed a clear increase in nuclear PAX6 and cytoplasmic K12 expression. A recent report^[7] have shown full thickness K12 expression only in a peripheral zone of a COMET-treated patient. Wherein, the increased nuclear PAX6 and the cytoplasmic K12 expression in the basal cells of the entire post-COMET, PK button of our total LSCD case is very encouraging. This suggests that the basal oral cells are responding to the signals from local corneal niche *in vivo* and are slowly acquiring the corneal phenotype.

Most of the basal and supra basal cells of the post-COMET corneal tissue stained positive for p63 antibody against all 3 isoforms of p63. The basal cells also expressed p75, similar to the limbal and oral epithelium. Though it confirms that the stem cells do survive and maintain the reconstructed tissue, they continue to reside in the basal layers of the post-COMET corneal tissue, similar to the native oral tissue.

In keeping with earlier reports,^[4] we observed subepithelial vasculatures in the corneal button that were positive for the endothelial markers, CD31 and CD34. The post-operative inflammation and the vasculatures that persist post-debridement could have triggered the initial neovascularization until the ocular surface is stably reconstructed by the oral cells. The presence of blood cells in the vasculatures and Ki-67 positive endothelial cells suggest that they are active vasculatures [Fig. 4d, arrow]. A recent study reported trans-differentiation of hair follicle stem cells into corneal epithelial-like cells in limbal microenvironment.^[8] Therefore, it is possible that these subepithelial vasculatures could slowly regress over time as the oral cells respond to the native corneal cues.

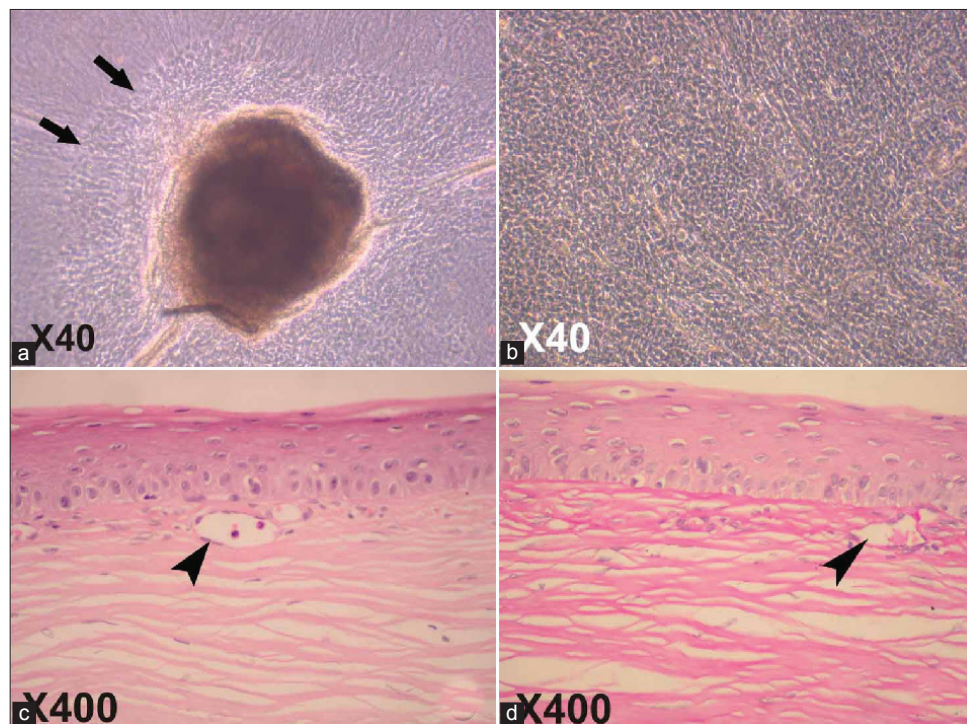


Figure 2: Growth pattern of cultivated oral mucosal epithelial cells on denuded hAM and histological pictures of post-COMET cornea. (a) Phase contrast images taken at day 2 in culture showing the initiation of cellular outgrowth from the explant tissue and monolayer formation (arrows). (b) Confluent monolayer formed after 9 days in culture. (c) Hematoxylin and Eosin, PAS staining on post COMET PK tissue showing hyperplasia of epithelial cells. Sub epithelial vasculatures with blood cells (arrow head) can also be seen. (d) PAS staining shows no goblet cells in the stratified epithelium

We conclude that post-COMET visual outcome depends on the severity of damage and the extent of stromal scarring, and can be further improved by an optical PK. The transplanted oral mucosal epithelial cells do survive and proliferate on the wounded corneal surface. While they maintain their native morphology and marker expression profiles at the ectopic site, they tend to respond

to corneal cues *in vivo* and upregulate cornea-specific PAX6 and K12 expression in the basal epithelial cells.

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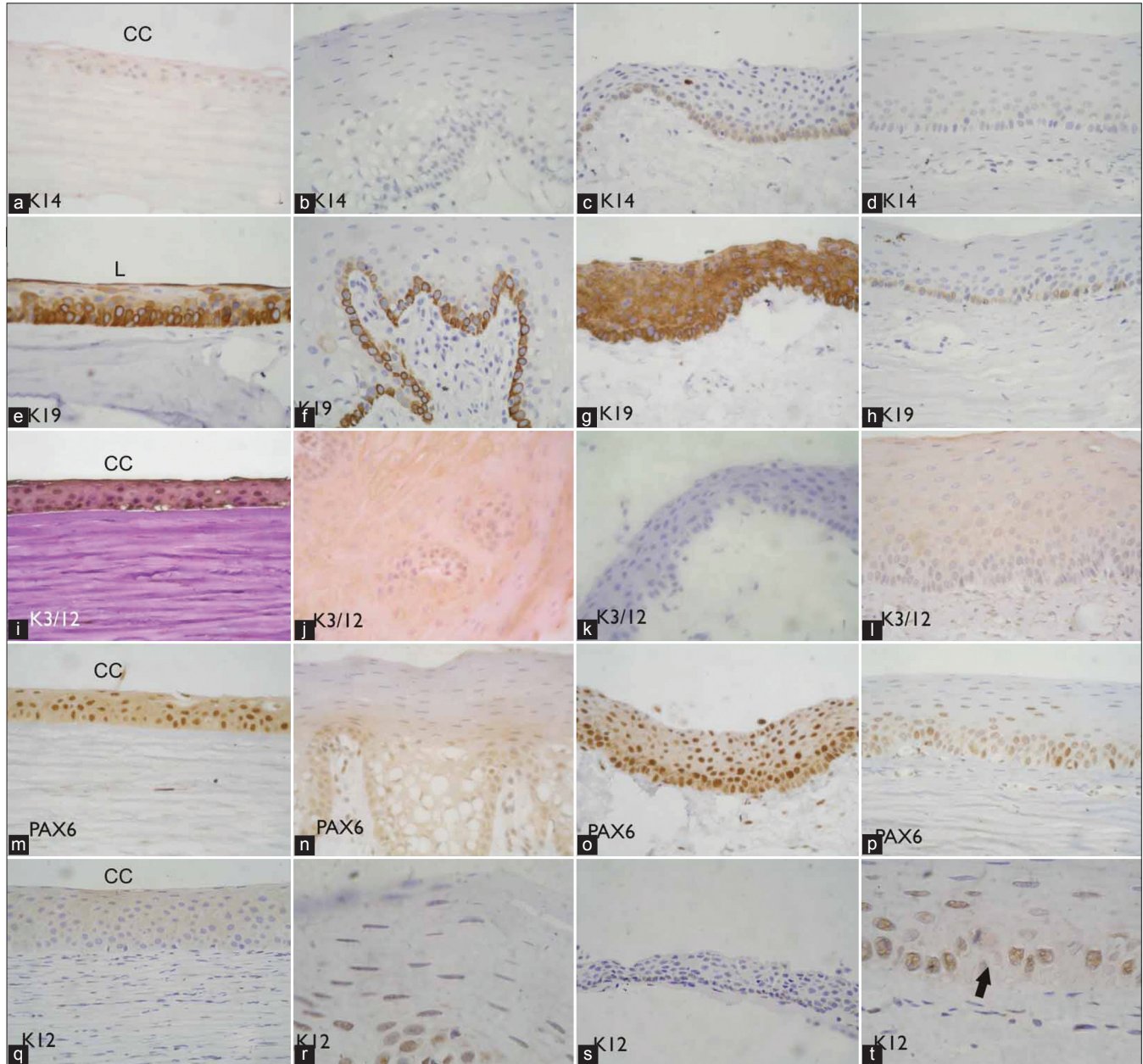


Figure 3: Cyokeratin and eye specific marker profile. K14 was not expressed by the epithelial cells of the (a) central cornea,⁽⁹⁾ (b) oral mucosal epithelium and (d) post-COMET corneal tissue, but were expressed by the (c) basal conjunctival epithelial cells (arrow head). K19 is expressed in all the layers of the limbal (l) (arrow) (e) and conjunctival epithelium (g) but not expressed in the central cornea (data not shown). The basal cells of oral mucosal epithelium (arrow) (f) and the post COMET PK tissue showed K19 expression. K 3/12 staining is present in central corneal epithelium (i), oral mucosal epithelium (j) and post-COMET corneal epithelium (l) but not present in conjunctiva (k). PAX6 was clearly expressed in nucleus of the central corneal epithelium (m) and conjunctival epithelium (o). The oral mucosal epithelium (N) showed faint pan nuclear staining for PAX6. The Post COMET PK tissue (p) showed a clear increased PAX6 nuclear staining in the basal (arrow) and supra-basal cells. K12 is expressed throughout the central corneal epithelium (q) and is expressed faintly in the cytoplasm of the basal cells (arrow in 3t) of the post-COMET corneal tissue (t), Oral mucosal tissue (r) and conjunctival epithelium do not show any cytoplasm staining. Consistent nuclear reactivity (may be non-specific) was also noted with K12 antibody only in the oral and COMET tissues (All pictures magnification- x400, 3t and 3r-x1000)

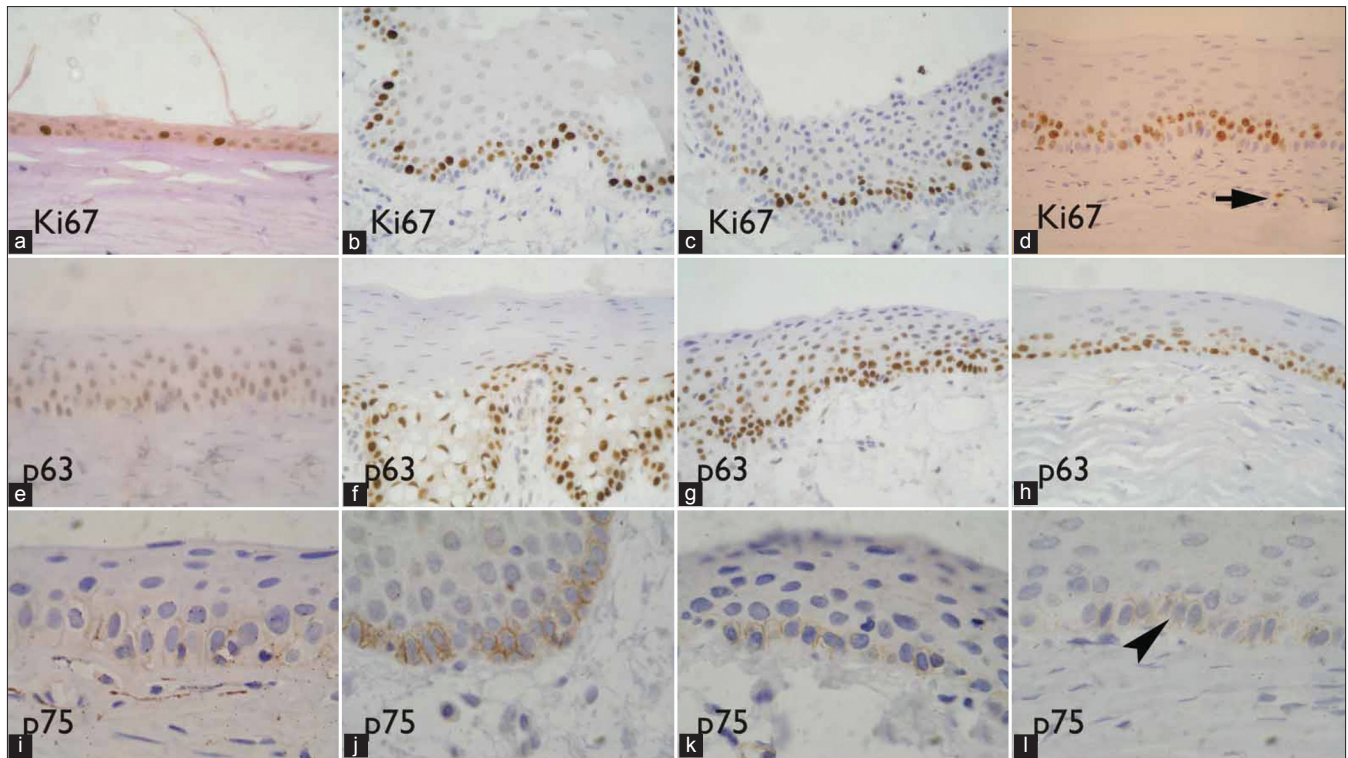


Figure 4: Immunohistochemistry for proliferative and stem cell markers. Immunohistochemistry for Ki 67 in corneal epithelium (a), oral mucosal epithelium (b), conjunctival epithelium (c), post COMET PK corneal (d) tissue showed clear nuclear expression by the proliferating supra-basal cells. In the post COMET PK corneal tissue, Ki67 expression was also noted in the nucleus of some of the endothelial cells lining the sub epithelial vasculatures (arrow in d). p63 immunostaining showed nuclear staining in the basal and supra-basal cells of all the tissues tested (e-h). p75 immunostaining showed membrane staining only in the basal epithelial cells of the cornea (i), oral mucosa (j), conjunctiva (k) and post COMET PK tissue (l). (Magnification for Ki 67 and p63- $\times 400$, p75- $\times 1000$)

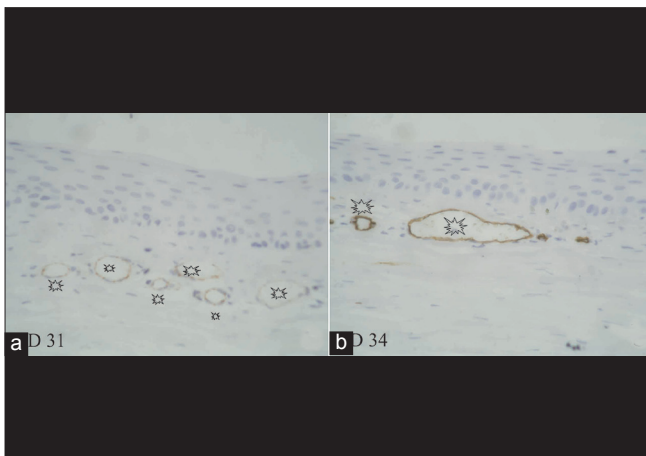


Figure 5: Endothelial markers for blood capillaries. Immunohistochemistry for CD31 (a), CD34 (b) of post COMET PK tissue showed positivity in all subepithelial vasculature (stars) (Magnification $\times 400$)

References

1. Thoft RA. Conjunctival transplantation. *Arch Ophthalmol* 1977;95:1425-7.
2. Nishida K, Yamato M, Hayashida Y, Watanabe K, Yamamoto K, Adachi E, *et al*. Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. *N Engl J Med* 2004;351:1187-96.
3. Nakamura T, Inatomi T, Sotozono C, Amemiya T, Kanamura N,

- Kinoshita S. Transplantation of cultivated autologous oral mucosal epithelial cells in patients with severe ocular surface disorders. *Br J Ophthalmol* 2004;88:1280-4.
4. Inatomi T, Nakamura T, Koizumi N, Sotozono C, Yokoi N, Kinoshita S. Midterm results on ocular surface reconstruction using cultivated autologous oral mucosal epithelial transplantation. *Am J Ophthalmol* 2006;141:267-75.
5. Madhira SL, Vemuganti G, Bhaduri A, Gaddipati S, Sangwan VS, Ghanekar Y. Culture and characterization of oral mucosal epithelial cells on human amniotic membrane for ocular surface reconstruction. *Mol Vis* 2008;14:189-96.
6. Nakamura T, Endo K and Kinoshita S. Identification of human oral keratinocyte stem/progenitor cells by neurotrophin receptor p75 and the role of neurotrophin/p75 signaling. *Stem Cells* 2007;25:628-38.
7. Chen HC, Chen HL, Lai JY, Chen CC, Tsai YJ, Kuo MT, *et al*. Persistence of transplanted oral mucosal epithelial cells in human cornea. *Invest Ophthalmol Vis Sci* 2009;50:4660-8.
8. Blazejewski EA, Schlotzer-Schrehardt U, Zenkel M, Bachmann B, Chankiewicz E, Jacobi C, *et al*. Corneal limbal microenvironment can induce transdifferentiation of hair follicle stem cells into corneal epithelial-like cells. *Stem Cells* 2009;27:642-52.
9. Kwitko S, Marinho D, Barcaro S, Bocaccio F, Rymer S, Fernandes S, *et al*. Allograft conjunctival transplantation for bilateral ocular surface disorders. *Ophthalmology* 1995;102:1020-5.

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