CASE REPORT

Growth of corneal epithelial cells over in situ therapeutic contact lens after simple limbal epithelial transplantation (SLET)

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SUMMARY

An 11-year-old boy underwent simple limbal epithelial transplantation (SLET) from the healthy right eye to his left eye for total limbal stem cell deficiency. One month later, corneal surface epithelialised and whitish plaques overlying the transplants were seen inferiorly. Those plaques were adherent to the surface of the contact lens and underlying corneal surface had smooth elevations. Similar findings were noted in a 23-year man following cyanoacrylate glue application for corneal perforation. On histological and immunohistochemical analysis, cells lining the contact lenses were identified as corneal epithelial cells. These cases illustrate epithelial cell growth on the contact lens and epithelial hyperplasia on corresponding surface of the cornea. Exorbitant proliferation of the epithelial cells may be owing to young age; therefore, early contact lens removal after SLET in young age, can possibly avoid epithelial hyperplasia. This also reiterates the possibility of using contact lens as a scaffold to grow epithelial cells.

BACKGROUND

Simple limbal epithelial transplantation (SLET) is a novel surgical technique of limbal stem cell transplantation (LSCT), which combines the benefits of cultivated and conventional LSCT.¹ SLET obviates the need for a stem cell biology laboratory and requires minimal amount of donor limbal tissue.¹ These cases report the growth of epithelial cells on the contact lens and underlying corneal epithelial hyperplasia. These observations may add to the understanding of the behaviour of limbal stem cells onto the corneal surface and help us to monitor young patients undergoing SLET. Also it reiterates the possibility of using the contact lens as a scaffold for culture of limbal stem cells to facilitate the treatment of limbal stem cell deficiency in the future.

CASE PRESENTATION

Case 1

An 11-year-old boy presented to our ophthalmology department with left eye total limbal stem cell deficiency attributed to the chemical burns 6 months prior to presentation (figure 1A). Visual acuity in the right eye was 6/6 and light perception with accurate projection in the left eye. Ultrasound B scans of the left eye were normal. He underwent right eye limbal biopsy of 1 o'clock followed by left eye pannus resection and SLET with placement of therapeutic contact lens (silicon hydrogel lens—Senofilcon A). Postoperatively steroid eye drops in tapering doses and antibiotic drops were prescribed. After 1 week, most of the limbal explants were in place (figure 1B). At the 2-week post-operative visit, corneal surface was partially epithelialised and small whitish islands were seen overlying the limbal transplants in the periphery. The contact lens was not removed at this visit. Three weeks after surgery he had visual acuity of 20/200 with well epithelialised corneal surface superiorly. Whitish islands noted on the previous visit had grown to a larger size (figure 1C). It was hypothesised that those whitish islands might represent the hyperplastic corneal epithelial cells in the area of explants, but to our surprise, after removing the contact lens, those whitish plaques were actually adherent to the undersurface of the contact lens in contact with transplants (Figure 2A). Also, areas of cornea underlying the whitish plaques showed smooth round elevations giving bumpy appearance to the corneal surface (figure 2B). The contact lens was subjected to histological and immunohistochemical analysis, which included periodic acid-Schiff (PAS), H&E staining and P53 assay, cytokeratin-3 and 12, respectively.

Contact lens-bound epithelial cells were identified on H&E as well as PAS staining. These cells had prominent nuclei and were adherent to the

Figure 1 (A) Clinical photograph showing left eye conjunctivalised ocular surface and extensive symblepharon formation. (B) Small pieces of donor limbal explants (arrows) placed over the amniotic membrane in situ. (C) Whitish plaques seen overlying the area of limbal explants with contact lens in situ (arrow).

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Figure 2 (A) Slit lamp photograph of the contact lens removed from the same patient demonstrating whitish plaques on the under surface of the contact lens. (B) Corneal surface underlying the area of whitish plaques showing smooth areas of elevations (arrows) suggestive of epithelial hyperplasia.

contact lens. Morphologically, these cells were multilayered at some areas while monolayer was seen in some areas (figure 3A, B). Epithelial cells showed intense cyokeratin 3, 12 expressions confirming corneal epithelial origin of the cells (figure 3C). P53 reactivity was seen in some areas, especially in areas of multilayered epithelium.

Case 2

A 23-year-old man presented to our clinic with left eye corneal melt presumed to be necrotising stromal keratitis of herpetic origin. He underwent cyanoacrylate glue along with contact lens (silicon hydrogel lens—Senofilcon A) application on the corneal surface on the same day. He was prescribed oral antiviral, topical steroids in tapering dose and antibiotic drops. He missed the follow-up at 1 week and presented 1 month later with similar whitish plaques adherent to the undersurface of the contact lens as seen in the previous case (figure 4A,B). In this case also after removal of the contact lens multiple smooth elevations were seen giving bumpy appearance to the cornea (figure 4C,D). Again, histological and immunohistochemical analysis revealed epithelial cells lining the contact lens surface with positive reactivity to cyokeratin 3 and 12, and positive P53 in some areas.

DISCUSSION

LSCT is currently the standard of care for treatment of limbal stem cell deficiency. First described by Kenyon and Tseng,² the surgical technique of limbal transplantation has since evolved from conjunctival-limbal grafting to ex vivo—cultivated limbal epithelial transplantation, introduced by Pellegrini *et al.*³ Authors have reported results of allogeneic cultivated limbal epithelial transplantation and it can successfully restore the ocular

surface and improve vision in patients with corneal blindness owing to bilateral LSCD.⁴ SLET, recently described by our group involves in vivo expansion of limbal stem cells over the amniotic membrane placed over the surface of the cornea.¹ However, as our understanding of the biology of the limbal epithelial cells is evolving, behaviour of the limbal explants and epithelial cells on the surface of the cornea after SLET in different clinical scenarios is being explored. The present case illustrates the growth of epithelial cells on the undersurface of the contact lens as well as epithelial hyperplasia on the corresponding surface of the cornea. In the first case, as cells had grown in the area where limbal transplants were placed, it is possible that limbal explants had exorbitant proliferative potential which lead to growth of epithelial cells on the contact lens as well as epithelial hyperplasia on the corneal surface. However, in the second case it is possible that epithelium which was scraped off in the area around the perforation, might have got implanted on the contact lens followed by further proliferation of cells. The young age of both patients might explain the excessive proliferative potential of the epithelial cells. Retrospectively, we feel that after performing SLET in young age, it would be better to follow them closely and remove the contact lens at the earliest following corneal epithelialisation to avoid epithelial hyperplasia which can cause visual disturbances.

The idea of growing epithelial cells on therapeutic contact lens was generated after the similar observation made by Girolamo *et al*⁵ in pterygium cases. Epithelial cells were grown on the therapeutic contact lens in their study and they proposed that therapeutic contact lenses can act as a scaffold for culturing limbal epithelial cells and may provide a protective barrier while cells transfer on to and replenish the ocular surface.⁵ Clinical observation in our cases also reiterates the possibility of using

Figure 3 Histological and immunohistochemical assessment of whole-mounted contact lenses. Morphological appearance of epithelial cells adherent to the contact lens. (A) H&E staining showing monolayer of epithelial cells (arrow). (B) periodic acid-Schiff staining showing monolayer of epithelial cells with prominent nuclei (arrow). (C) Cytokeratin 12 expression by the epithelial cells (arrow). Original magnification $\times 40$ (A and C), $\times 100$ (B).

Figure 4 Clinical photograph of left eye which had undergone cyanoacrylate glue and contact lens application. (A) With contact lens in situ and showing whitish plaques (arrows) surrounding the area of cyanoacrylate glue (arrowhead) (B) High-magnification view. (C) After removal of the contact lens, corneal surface showed smooth epithelial elevations (arrows) suggestive of epithelial hyperplasia. (D) Better delineated with fluorescein staining of the corneal surface.

the contact lens as a scaffold to grow limbal epithelial cells. It is also possible that use of younger donor for culture of limbal stem cells on any scaffold would increase the chances of epithelial cell growth.

In conclusion, these observations may add to the understanding of the biology of limbal stem cells and help us to monitor young patients undergoing SLET. The possibility of contact lens as a scaffold for culture of limbal stem cells needs to be explored further.

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Learning points

- Simple limbal epithelial transplantation (SLET) is a viable option for the treatment of limbal stem cell deficiency.
- It is necessary to recognise early signs of epithelial hyperplasia in young patients after SLET and removal of the contact lens at the earliest after corneal epithelialisation is completed.
- Use of young donor for culture of limbal stem cells on any scaffold would increase the chances of epithelial cell growth.
- Growth of corneal epithelial cells on contact lens is possible and it can serve as scaffold for culture and transfer of epithelial cells onto the cornea.

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