The conjunctiva in corneal epithelial wound healing

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Abstract

Background/aims—During the healing of corneal epithelial wounds with limbal involvement, conjunctival epithelium often migrates across the denuded limbus to cover the corneal surface. It is believed that, over a period of time, conjunctival epithelium covering the cornea assumes characteristics of corneal epithelium by a process referred to as conjunctival transdifferentiation. The purpose of this study was to examine, clinically, the fate of conjunctival epithelial cells covering the cornea and to assess the healing of corneal epithelial wounds when the conjunctival epithelium was removed or actively prevented from crossing the limbus and extending onto the cornea.

Methods—10 patients with conjunctivalisation of the cornea were followed for an average of 7.5 months. Five patients in this group had their conjunctival epithelium removed from the corneal surface and allowed to heal from the remaining intact corneal epithelium. In another four patients with corneal epithelial defects, the conjunctival epithelium was actively prevented from crossing the limbus by mechanically scraping it off.

Results—The area of cornea covered by conjunctival epithelium appeared thin, irregular, attracted new vessels and was prone to recurrent erosions. Conjunctivalisation of the visual axis affected vision. Removal of conjunctival epithelium from the cornea allowed cells of corneal epithelial phenotype to cover the denuded area with alleviation of symptoms and improvement of vision. It was also established that migration of conjunctival epithelium onto corneal surface could be anticipated by close monitoring of the healing of corneal epithelial wounds, and prevented by scraping off conjunctival epithelium before it reached the limbus.

Conclusion—This study shows that there is little clinical evidence to support the concept that conjunctival transdifferentiation per se, occurs in humans. “Replacement” of conjunctival epithelium by corneal epithelial cells may be an important mechanism by which conjunctival “transdifferentiation” may occur. In patients with partial stem cell deficiency this approach can be a useful and effective alternative to partial limbal transplantation, as is currently practised.

Figure 1 Slit lamp diffuse view of a fluorescein stained cornea of a patient with a corneal graft showing a clear demarcation between corneal and conjunctival epithelial phenotypes. The pupillary area is covered by conjunctival epithelium. Tiny “buds” of corneal epithelium can be seen along the line of contact between corneal and conjunctival epithelium (arrowheads) (×10).
**Patients and methods**

**GROUP 1**

Ten patients were included in the first part of the study. Four patients were observed prospectively during the healing of large ocular surface defects. A further six patients were recruited 2–5 years after complete ocular surface re-epithelialisation had occurred. Six patients had sustained chemical injury to the ocular surface, three were post-keratoplasty and the remaining one had conjunctivalisation of cornea following Stevens–Johnson syndrome as a child (Figs 1, 2, and 3). Patients were followed at 2–6 weekly intervals for 3–13 months with a mean of 7.5 months. All patients were examined by slit lamp biomicroscopy and fluorescein staining. Clinical photographs were taken at each visit and the area of corneal surface covered by conjunctival epithelium was estimated by planimetry as described before. Briefly, photographs of the cornea were photographed onto graph paper, the area of the abrasion and the cornea was calculated, and the area of the corneal abrasion was expressed as a percentage of the total corneal area.

In five of these patients the conjunctival epithelium was mechanically scraped off the corneal surface (as described for group 2 below) and healing of the freshly denuded surface was allowed to proceed from the adjacent corneal epithelium. In two of these patients, only the pupillary area of the cornea, rather than the whole conjunctivalised surface of the cornea, was scraped. The patients were observed daily. Clinical details of patients are given in Table 1.

**GROUP 2**

In the second part of the study, four patients with ocular surface epithelial defects secondary to chemical injury were followed daily. The advancing sheet of conjunctival epithelium was prevented from crossing the limbus by mechanically scraping it off with a No 15 surgical blade under topical anaesthesia at the slit lamp. When the conjunctival epithelium reached to within a couple of millimetres of the limbus it was scraped back to approximately 5–7 mm away from the limbus. This procedure had to be repeated twice in three patients and was required only once in the fourth patient. Clinical details of these patients is given Table 2.

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**Table 1**  
Clinical details of patients included in group 1 (conjunctival epithelium covering the cornea)

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Eye</th>
<th>Injury</th>
<th>Follow up</th>
<th>Area of defect</th>
<th>Difference</th>
<th>Complications</th>
<th>Corneal buds at interface</th>
</tr>
</thead>
<tbody>
<tr>
<td>45*</td>
<td>M</td>
<td>R</td>
<td>Detergent</td>
<td>7 months</td>
<td>58.6% 56.1%</td>
<td>2.5%</td>
<td>Fine vessels</td>
<td>Present</td>
</tr>
<tr>
<td>70*</td>
<td>M</td>
<td>L</td>
<td>Post PK</td>
<td>12 months</td>
<td>35% 33.5%</td>
<td>1.5%</td>
<td>Vessels, filamentsh</td>
<td>Present</td>
</tr>
<tr>
<td>71</td>
<td>M</td>
<td>L</td>
<td>Post PK</td>
<td>13 months</td>
<td>26.4% 26.5%</td>
<td>-0.1%</td>
<td>Vessels, filamentsh</td>
<td>Present</td>
</tr>
<tr>
<td>54*</td>
<td>F</td>
<td>L</td>
<td>Detergent</td>
<td>10 months</td>
<td>28.8% 23.2%</td>
<td>5.6%</td>
<td>Vessels, filamentsh</td>
<td>Present</td>
</tr>
<tr>
<td>21</td>
<td>M</td>
<td>R</td>
<td>Chemical</td>
<td>5 months</td>
<td>15% 13%</td>
<td>2%</td>
<td>Vessels, filamentsh</td>
<td>Present</td>
</tr>
<tr>
<td>40*</td>
<td>F</td>
<td>R</td>
<td>Detergent</td>
<td>6 months</td>
<td>86.2% 85%</td>
<td>1.2%</td>
<td>Vessels, filamentsh</td>
<td>Present</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>L</td>
<td>Alcohol</td>
<td>3 months</td>
<td>18.6% 15%</td>
<td>3.6%</td>
<td>Vessels, filamentsh</td>
<td>Absent</td>
</tr>
<tr>
<td>38*</td>
<td>M</td>
<td>R</td>
<td>Alcohol</td>
<td>8 months</td>
<td>78.2% 76%</td>
<td>2.2%</td>
<td>Vessels, filamentsh</td>
<td>Present</td>
</tr>
<tr>
<td>32*</td>
<td>F</td>
<td>L</td>
<td>SJ syndrome</td>
<td>3.5 months</td>
<td>56.4% 58.7%</td>
<td>-2.3%</td>
<td>Vessels, filamentsh</td>
<td>Present</td>
</tr>
<tr>
<td>68</td>
<td>M</td>
<td>R</td>
<td>Post PK</td>
<td>4 months</td>
<td>17.3% 13%</td>
<td>4.3%</td>
<td>Fine vessels</td>
<td>Absent</td>
</tr>
</tbody>
</table>

*Patients included in the study after complete re-epithelialisation had occurred (follow up intervals for these patients are from the time they were included in the study).  
†Area of defect refers to area covered by conjunctival epithelium.
Results (Tables 1 and 2)
The area of cornea covered by conjunctival epithelium ranged from 15% to 86.2% at the outset. The area of “conjunctivalisation” had reduced by only 1.2% to 5.6% at the time of the last follow up visit. In one patient the area of conjunctivalisation had remained unchanged and in another it had increased by 2.3%. In seven of the 10 patients, with a follow up of over 3 months, tiny buds of corneal epithelium could be seen protruding into the conjunctival epithelium all along the contact line between the two epithelial phenotypes (Figs 1 and 2). These buds were always seen arising from the corneal epithelium. Four patients had a total of seven episodes of recurrent erosions during the period of follow up. The one patient with conjunctivalisation, following Stevens–Johnson syndrome, alone had over six such episodes. Nine patients had a degree of superficial vascularisation which ranged from fine vessels extending just inside the limbus to well defined vessels extending 2–5 mm inside the limbus. Four patients had filamentary keratopathy limited to the area of “conjunctivalisation”. In all patients the conjunctival epithelium covering the cornea was thinner relative to the adjacent corneal epithelium. This was evidenced by pooling of fluorescein dye (Figs 1–3). In the five patients where conjunctival epithelium covering the corneal surface was mechanically removed, the entire denuded surface, or a large proportion of it, was covered by normal corneal epithelium within a week of debridement (Figs 4–7) with alleviation of symptoms and improvement of visual acuity (see legends of figures).

Discussion
That corneal defects could heal from the conjunctival epithelium has been known for a long time. Corneal epithelial wounds are known to stimulate a proliferative response in the peripheral limbal conjunctiva, but under normal circumstances, the limbal epithelium acts as a barrier and is able to exert an inhibitory growth

Table 2 Clinical details of patients included in group 2 (healing conjunctival epithelium prevented from extending across the limbus)

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Eye</th>
<th>Injury</th>
<th>Area of defect</th>
<th>Clock hours of limbus involved</th>
<th>No of scrapes*</th>
<th>Duration to complete healing</th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td>M</td>
<td>R</td>
<td>Detergent</td>
<td>58%</td>
<td>4</td>
<td>2</td>
<td>14 days</td>
</tr>
<tr>
<td>22</td>
<td>M</td>
<td>R</td>
<td>Detergent</td>
<td>42.4%</td>
<td>4</td>
<td>2</td>
<td>12 days</td>
</tr>
<tr>
<td>28</td>
<td>M</td>
<td>L</td>
<td>Alcohol</td>
<td>60%</td>
<td>5</td>
<td>2</td>
<td>14 days</td>
</tr>
<tr>
<td>31</td>
<td>M</td>
<td>L</td>
<td>Detergent</td>
<td>45%</td>
<td>3</td>
<td>1</td>
<td>10 days</td>
</tr>
</tbody>
</table>

*Advancing sheet of healing conjunctiva was scraped 5–7 mm away from the limbus to allow limbal healing to complete.
pressure preventing migration of conjunctival epithelial cells onto the cornea. However, when the epithelial defect involves the limbus, this barrier is lifted and conjunctival migration onto the cornea occurs. This is associated with the appearance of goblet cells and, often, new blood vessels. It is believed that conjunctival epithelium covering the cornea undergoes a slow transformation to assume characteristics resembling corneal epithelium, a process referred to as conjunctival transdifferentiation.13 This process has been extensively studied in experimental animals where it has been shown that goblet cells do not migrate onto the cornea but develop de novo from non-goblet epithelial cells which can be identified by electron microscopy. Loss of goblet cells during transdifferentiation occurs by desquamation and in situ cell death.14 Vitamin A and vascularisation of regenerated epithelium are important factors that influence the process of transdifferentiation.15–18

The consensus from most animal studies is that although complete morphological transdifferentiation is possible biochemically and functionally it is far from satisfactory. Conjunctival transdifferentiation in animal models can therefore, at best, be described as squamous metaplasia with loss of goblet cells. Moreover, it has also been suggested that, in many of the above studies, conjunctival transdifferentiation could have occurred due to incomplete removal of limbal basal epithelium,22 with the result that regenerated epithelium demonstrated both corneal and conjunctival features without one actually changing to the other.

Dua and Forrester48,23 studied the healing, in humans, of large ocular surface epithelial wounds that involved the cornea, limbus, and conjunctiva. They identified two tongue-shaped sheets of epithelium, arising from either end of the limbal defect, that showed a preferential circumferential migration along the limbus. In some patients they noted a centripetally migrating sheet of conjunctival epithelium that reached and migrated across the limbus, preventing the circumferentially migrating limbal sheets from meeting each other. As a result, varying areas of the cornea were covered by conjunctival epithelium. The epithelium in these areas was invariably thinner than adjoining normal corneal epithelium, showed a stippled stain with fluorescein, attracted new vessels, and was prone to recurrent erosions. This study confirms the above observations and demonstrates that a similar healing response also occurs in patients with corneal grafts. In all patients in this study, even several months on, the corneal surface covered by conjunctival cells remained relatively thin and irregular without clinically evident transdifferentiation. The difference in thickness sharply demarcated the area of “conjunctivalisation” from the adjacent healthy corneal epithelium and was rendered more obvious by the pooling of fluorescein dye. What was more interesting was that tiny buds of corneal epithelium could be seen protruding into the conjunctival epithelium all along the contact line between the two epithelial phenotypes. This observation has also been well illustrated in previous publications. These buds were always seen arising from the corneal epithelium and gave the impression that normal corneal epithelium was attempting to replace the conjunctival epithelium, gradually nudging it outward, towards the limbus. “Replacement” of conjunctival epithelium by normal corneal epithelium may...
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Therefore be yet another factor contributing to conjunctival “transdifferentiation”. The results of this study would tend to support the view that complete conjunctival transdifferentiation probably does not occur in humans.

On the basis of these observations Dua et al. had suggested that, although any kind of epithelial cover for the cornea may be a desirable goal in the short term, the ideal situation would be to encourage conjunctival epithelial cover for conjunctiva and corneal epithelial cover for cornea. They recommended that, in corneal epithelial defects with partial limbal involvement, conjunctival epithelialisation should be prevented from crossing the limbus until the circumferentially migrating sheets of limbal epithelium have met each other and the limbal barrier is re-established. This can be achieved by mechanically scraping the advancing conjunctival epithelial sheet and may have to be repeated two or three times because the conjunctival epithelium migrates rapidly compared to the limbal sheets. (While describing the procedure to the patients, the term “brushing” was adopted, to replace “mechanical scraping” as it sounds less invasive and was very acceptable to patients, causing much less anxiety and apprehension.) Such an approach would ensure corneal epithelial cover for the cornea and conjunctival epithelial cover for the conjunctiva. This hypothesis has been substantiated by the present study. On the other hand, in patients where conjunctival epithelium had already covered a part of the corneal surface at the time of presentation, it is easy to mechanically remove the conjunctival epithelium under topical anaesthesia at the slit lamp. This procedure was followed by rapid re-epithelisation of the cornea with corneal epithelium. Interestingly, it was the corneal epithelial sheet that rapidly advanced to cover the defect rather than conjunctival epithelium from the limbus. This observation, coupled with the presence of corneal epithelial buds described above, would suggest the presence of a constant and persistent drive in the corneal epithelial sheet to replace conjunctival epithelium. Furthermore, the observations made in this study indicate that although varying proportions of the corneal surface may be covered by conjunctival and corneal epithelium, the two phenotypes can achieve a state of equilibrium and “peacefully coexist”. As long as the two phenotypes can achieve a state of equilibrium and “peacefully coexist”, the author wishes to express his gratitude to Paddy Tighe and April Powell Richards for their help in preparing the colour illustrations. HS Dua is the Julia Duane Scholar (Wills Eye Hospital, Philadelphia).

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