Functional competence of regenerating ocular surface epithelium

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Several aspects of the ocular surface epithelium, including its clinical appearance, its effect on the strength of underlying stromal wounds, and its healing rates, have been compared between regenerating epithelium of conjunctival and that of corneal origin in rabbits. The results showed that conjunctival epithelium could not transform completely into functionally competent corneal epithelium within 6 weeks.

Key words: ocular surface epithelium, regenerating epithelium, cornea, conjunctiva, chemical injury, wound healing

Although histological studies have demonstrated that conjunctival epithelium can transform into corneal epithelium within 3 to 5 weeks as it grows over a denuded corneal stroma, clinical observations and biochemical studies have indicated that functional transformation may take longer.

The purpose of the studies reported here is to evaluate some aspects of ocular surface function after repopulation of the corneal surface with epithelium of conjunctival origin or with epithelium of corneal origin in order to see whether the biochemical alterations noted previously are correlated with abnormal surface behavior. As indicators of function we have observed the clinical appearance, including the incidence of recurrent epithelial breakdowns, measured the tensile strength of underlying stromal wounds, and determined epithelial healing rates over defects in the surface. The comparisons cover the initial 6 weeks after damage to the cornea, encompassing the period of greatest jeopardy following corneal injury.

Methods

Animals. Rabbits weighing 2 to 4 kg were used in all experiments. Sodium pentobarbital anesthesia, with local proparacaine was used, maintained with ether inhalation. When required, animals were killed by an overdose of sodium pentobarbital.

Removal of epithelium. After anesthesia, either (1) the whole corneal epithelium plus a ring of limbal conjunctival epithelium or (2) a 9 mm diameter area of central corneal epithelium was removed by iodine debridement followed by cocaine neutralization, by heptanol washing, or by scraping as described previously. In the case of total epithelial removal, the regenerating epithelium originated from the conjunctiva. In removal of an area of the central corneal epithelium, the regenerating epithelium was derived from the remaining ring of corneal cells.

Appearance. For this study, 24 eyes were totally denuded with iodine-cocaine cautery, 19 were totally scraped, and seven were totally de-
nuded with heptanol. In addition, nine had the central corneal epithelium removed by iodine-cocaine cautery, nine had the central portion scraped, and five had the central portion heptanol-washed. The 39 animals were examined with a flashlight three to five times weekly and by slit lamp at least once weekly. The occurrence of superficial punctate keratopathy, defect formation (as demonstrable with fluorescein staining), and vascularization were noted.

**Experimental.** Tensile strength and epithelial healing rates were measured as described below.

**Measurement of wound tensile strength**

**PREPARATION OF ANIMALS.** After anesthesia and (1) no epithelial removal (10 eyes), (2) total epithelial removal (15 eyes), or (3) central corneal epithelial removal (11 eyes) as described above, an 8 mm penetrating linear wound was made through the stroma, across the visual axis. Two 10-0 nylon sutures were placed 5 mm apart to secure the wound. The anterior chamber reformed spontaneously, with occasional eyes showing iris synchiae at the ends of the wounds. Erythromycin and atropine ointment were given immediately after the operation. The 19 animals were examined three to five times weekly, and antibiotics and atropine were given if indicated. After the regenerating epithelium had been healed over the stromal wound for 5 days or longer, the tensile strength was measured.

At 5 days, vascularization was grossly visible in four of 15 eyes (26%), with epithelium of conjunctival origin, and those eyes were omitted from the study. After 5 days, virtually every eye with epithelium derived from conjunctiva developed vessel ingrowth. Therefore, when the ingrowing vessels were within 3 mm of the wound, from any direction, the animal was killed and the tensile strength measured. Eyes with more severe vascularization or with synchiae to the wound were not used. Tensile strength in wounded eyes with epithelium of corneal origin was measured at times comparable to the time eyes with epithelium of conjunctival origin were measured. These eyes did not vascularize.

**TENSILE STRENGTH MEASUREMENTS.** At the appropriate time after the regenerating epithelium was healed (see preceding paragraph), the rabbits were killed and the eyes enucleated. With the use of a method described previously, a 4 mm wide strip, including the wound with the sutures removed, was cut out and suspended between two clamps (Fig. 1). Weights were added to the bucket at increments of 5 gm every 10 sec until the corneal strip broke at the wound. The combined weight of the lower clamp, bucket, and weights was used as the tensile strength which is expressed as grams per 4 mm strip.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of eyes developing</th>
<th>Epithelial abnormalities*</th>
<th>Vascularization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine-cocaine</td>
<td>24</td>
<td>21 (88%)</td>
<td>19 (79%)</td>
</tr>
<tr>
<td>Scaping</td>
<td>19</td>
<td>13 (68%)</td>
<td>17 (81%)</td>
</tr>
<tr>
<td>Heptanol washing</td>
<td>7</td>
<td>6 (86%)</td>
<td>7 (100%)</td>
</tr>
<tr>
<td>Iodine-cocaine</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Scaping</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Heptanol washing</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*These included irregular surface, recurrent epithelial breakdowns, and superficial punctate keratopathy and were substantially more common in eyes with regenerating epithelium of conjunctival origin.
regenerated epithelium within an area defined by a gently marked trephine ring. No eyes with more than a 1 to 2 mm fringe of vessels from the limbus and no eyes which had demonstrated delayed healing after the initial epithelium removal were used.

The healing rates were then determined by the method of Ho and Elliot.6 Briefly, the progressive decrease in the area of the epithelial defects was determined by measurement with planimetry of standardized, serial, color photographs of the defects. By plotting the change in wound area against time, a slope for each eye could be calculated with the linear regression method. The slopes represent the healing rates, which were then averaged for each experimental group. Healing rates in square millimeters per hour were thus obtained for (1) corneal epithelium and (2) conjunctival epithelium healing the primary corneal defect, (3) regenerated epithelium of corneal origin at 1 day and 2 and 6 weeks, and (4) regenerated epithelium of conjunctival origin at 1 day and 2 and 6 weeks healing a secondary defect.

Results

Appearance. Eyes with regenerating epithelium of conjunctival origin had poorer healing than eyes with regenerating epithelium of corneal origin, regardless of the method initially used to remove the epithelium (Table I and Fig. 2). The eyes with conjunctivally derived epithelium had frequent epithelial breakdown. This was manifested by delays or regressions in healing, occurrence of secondary defects after initial healing (Fig. 2, A and B), and varying degrees of superficial punctate keratopathy (demonstrated with fluorescein staining). These eyes also had a higher frequency of corneal vascularization. In most cases, the vascularization
Table II. Ocular surface epithelium healing rates (mm²/hr)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age</th>
<th>Source of epithelium</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cornea</td>
<td>Conjunctiva</td>
</tr>
<tr>
<td>Healing rates over primary defects:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scraping</td>
<td>1 day healed</td>
<td>0.27 ± 0.02 (8)*</td>
<td>0.13 ± 0.03 (6)</td>
</tr>
<tr>
<td>Iodine-cocaine cautery</td>
<td>1 day healed</td>
<td>0.35 ± 0.02 (6)</td>
<td>0.09 ± 0.03 (7)</td>
</tr>
<tr>
<td>Healing rates over secondary defects:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scraping</td>
<td>2 weeks</td>
<td>0.33 ± 0.03 (4)</td>
<td>0.18 ± 0.04 (5)</td>
</tr>
<tr>
<td>Iodine</td>
<td>2 weeks</td>
<td>0.35 ± 0.02 (4)</td>
<td>0.15 ± 0.03 (7)</td>
</tr>
<tr>
<td>Scraping</td>
<td>6 weeks</td>
<td>0.35 ± 0.01 (4)</td>
<td>0.35 ± 0.01 (6)</td>
</tr>
<tr>
<td>Iodine</td>
<td>6 weeks</td>
<td>0.37 ± 0.01 (4)</td>
<td>0.21 ± 0.02 (6)</td>
</tr>
</tbody>
</table>

*Mean ± S.E.M., with the number of determinations in parentheses.
†p values derived by comparing the healing rates of conjunctivally derived epithelium to the corresponding rate for corneally derived epithelium. Values are given if p < 0.05 by the Student t test.

After 6 weeks was only a 1 to 2 mm fringe of vessels extending beyond the limbus. However, in approximately 14% of the eyes with conjunctivally derived regenerating epithelium either after scraping or after chemical injury, a heavy vascular ingrowth developed within 6 weeks (Fig. 2, C).

Peripheral scraping or iodine-cocaine debridement of a 4 mm band of epithelium at the limbus did not lead to vascularization or superficial punctate staining. Eyes with corneally derived regenerating epithelium demonstrated no healing difficulties.

Functional observations

Tensile strength. Tensile strength of stromal wounds covered with regenerating epithelium of corneal origin was the same as that of wounds covered with normal corneal epithelium at comparable times.

Stromal wound strength under the epithelium of conjunctival origin, regardless of the method used to remove the epithelium, was substantially lower than normal or than that under regenerating epithelium of corneal origin if the regenerating epithelial layers were the same age (p < 0.05) (Fig. 3).

Epithelial healing rates. Healing curves of six to 12 points were obtained for each eye. Table II shows the healing rates of ocular surface epithelium.

Conjunctivally derived epithelium growing over the cornea sometimes showed regression of healing. Therefore, for these studies, only eyes whose healing curves were linear (with linear correlation coefficients >0.80) were used for comparison of healing rates, excluding those eyes (13 of 55) in which spontaneous enlargement of the defect occurred. Linear correlation coefficients for the healing rates of epithelium of corneal origin were all between 0.76 and 0.99.

Primary defect healing. Epithelium of corneal origin had a faster healing rate than epithelium of conjunctival origin when the rates for the initial resurfacing of the cornea after scraping or iodine cautery were compared (Table II).

Secondary defect healing. After the initial defect had been healed 1 day or 2 or 6 weeks, a second 8 mm diameter defect was made, and the epithelial healing rates over that secondary defect were measured.

Epithelium of corneal origin covered a secondary defect faster than corneal epithelium covered the primary defect (p < 0.01 for all cases).

At 1 day and 2 weeks, regenerating epithelium of conjunctival origin after scraping had slower healing rates than regenerating epithelium of corneal origin. After iodine-cocaine cautery, regenerating epithelium of conjunctival origin had slower healing rates...
Discussion

The work reported here documents the functional abnormalities associated with resurfacing the cornea with regenerating epithelium of conjunctival origin. Although the corneal epithelial healing rates reported here are somewhat lower than those reported by Ho and Elliot,5 the most interesting observation is that conjunctivally derived epithelium growing over the denuded surface has substantially slower healing rates than corneally derived epithelium. These slower healing rates of the conjunctival epithelium are consistent with the findings of Kuwabara et al.7 that depletion of glycogen by amylase in vitro retards sliding of epithelial cells, and our previous report of low glycogen in cells of conjunctival origin.3

The healing difficulties found in eyes with conjunctivally derived epithelium but not in eyes with corneally derived epithelium are similar to those seen clinically. Patients with chemical or thermal burns, where the regenerating epithelium originates in the conjunctiva, experience similar episodes of recurring epithelial defects and eventual vascularization as characteristics of the clinical course. These experimental observations indicate that the obligate resurfacing of the cornea following total corneal epithelial loss requires biochemical and functional transformation which takes several weeks to occur. During this initial period of cellular conversion, frequent epithelial breaks may occur, leading to increased jeopardy from proteolytic enzymes and infections.

Stromal wound strength with the conjunctivally derived epithelium was less than with the corneally derived epithelium. In this study, wounds with epithelium of conjunctival origin had only about two-thirds the strength of those with corneally derived epithelium when the regenerating epithelial layers were the same age. Since wounding and epithelial removal were done at the same time and it takes about 1 week longer for the epithelium of conjunctival origin to cover the larger defect, the wounds under the conjunctivally derived epithelium were approximately 1 week older than those under epithelium of corneal origin. Therefore those wounds might have been expected to be somewhat stronger. However, it has been known for some time that stromal wounds require epithelium to heal,4 and these results indicate that the type of ocular surface epithelium available may also play a role in stromal wound healing. In addition, this decrease in stromal wound strength probably reflects altered rates of collagen synthesis which might have a significant effect on the maintenance of stromal integrity, even after repopulation of the stroma with healthy keratocytes.

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REFERENCES

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