Conjunctival Epithelial Wound Healing

Harry S. Geggel, Judith Friend, and Richard A. Thoft

In vivo conjunctival epithelial healing in albino rabbits was investigated by light microscopy following both n-heptanol and trephined conjunctival wounding. Reepithelialization occurred faster following n-heptanol treatment (3 days) versus trephination (6–7 days). No goblet cells were present in the migrating epithelium during reepithelialization. After 1 day of wounding, goblet cells disappeared several millimeters peripheral to the wound margin in both types of wounds. Goblet cells first reappeared peripherally 1 week after wounding before they appeared in the central wound area. These observations indicate that a large area of conjunctival epithelium surrounding a wound is involved with repair of that wound. Since the goblet cell content of conjunctival epithelium appears to change as a result of the stresses of epithelial repair, the goblet cell population may reflect the presence of reparative or proliferative processes in the ocular surface. Invest Ophthalmol Vis Sci 25:860–863, 1984

The conjunctiva occupies over 90% of the ocular surface and plays a significant role in the maintenance of the physical integrity of the eye. Although corneal wound healing has been the subject of much research, very little is known about conjunctival epithelial healing over different substrates. We do know that conjunctival epithelium growing onto the cornea transforms in an orderly fashion into corneal epithelium as it grows over denuded corneal stroma. In the work presented here, we have studied the in vivo characteristics of conjunctival epithelial healing on its own substantia propria as well as on bare sclera to document the behavior of epithelium during conjunctival wound healing.

Materials and Methods

Animal Preparation

All investigations, as described in this manuscript, conform to the ARVO Resolution on the Use of Animals in Research. New Zealand albino rabbits weighing 2 to 3 kg were anesthetized by intramuscular injection of chlorpromazine hydrochloride (25 mg) and ketamine hydrochloride (200 mg), with additional topical anesthesia with proparacaine (Ophthaine, E. R. Squibb; Princeton, NJ). The eyes were proptosed and a surgical microscope was used during wounding. Two different types of wounds were made. For the first, a 4-mm diameter filter paper disc (Whatman type I), soaked in n-heptanol and then blotted, was attached to the end of a pencil eraser and applied to the superior bulbar conjunctiva 2–3 mm from the limbus for 45 sec. The area was rubbed then with a cotton applicator and fluorescein dye was applied to outline the debrided area. This wound removed epithelial tissue leaving conjunctival stroma intact. Three to four radial cuts were made in the peripheral cornea (not extending to the limbus) pointing to the area of conjunctiva treated (Fig. 1B). For the second type of wound, a 4-mm trephine was used to outline and cut a 4-mm diameter disc of superior bulbar conjunctiva, also 2–3 mm from the limbus. This tissue, along with episclera and Tenon's capsule, was removed with Vannas scissors and forceps to expose bare sclera. Radial cuts pointing to the wound were made in the cornea as described above (Fig. 1A). Erythromycin ophthalmic ointment was used immediately after wounding.

Histologic Preparation

At 1, 2, 3, 5, 7, 14, and 21 days after wounding, the rabbits were killed by an overdose of intravenous pentobarbital. Two animals (four eyes) were used for each time point. The eyes were proptosed and fixed in situ with one-half strength Karnovsky's medium. The wounded area of bulbar conjunctiva, as well as the subjacent sclera, was then removed by excising it with a #11 Bard Parker blade (Bard Parker Co.; Rutherford, NJ). Minimal tissue distortion occurred. After fixation in one-half strength Karnovsky's medium, the tissue was bisected through the center, dehydrated, embedded in paraffin, cut into 7-μm sections and stained with periodic acid-Schiff (PAS) or hematoxylin and eosin and examined by light microscopy. PAS-
Fig. 1. A, left. Trephined wound; B, right. N-heptanol wound in experimental rabbits. Radial slits are visible in the cornea. Due to faint fluorescein staining, n-heptanol wound is demarcated poorly. Arrows outline margin of the wound (1B).

Fig. 2. Control animals (PAS stain). A, left. Normal rabbit bulbar conjunctiva with goblet cells present (X400). B, center. N-Heptanol treated conjunctival epithelium showing complete removal of epithelium centrally (X250). C, right. N-Heptanol-treated conjunctiva showing demarcation (arrow) between central denuded area and peripheral monolayer of healthy epithelium (X400).

Fig. 3. Healing of n-heptanol-treated conjunctival wounds (PAS stain). A, top left. After 1 day, a monolayer of migrating epithelium is present centrally. No goblet cells are visible (X400). B, top center. After 1 day, goblet cells are still present 5-6 mm from the central wound (X400). C, top right. After day 3, the central wound is covered by an intact, layered epithelium devoid of goblet cells (X400). D, bottom left. After 1 week, the central epithelium is intact but still lacks a normal complement of goblet cells (X400). E, bottom center. After 1 week, normal number of goblet cells are present 3 mm from the wound margin (X250). F, bottom right. After 2 weeks, central epithelium appears normal and has a normal complement of goblet cells (X400).

Fig. 4. Healing of trephined conjunctival wounds (PAS stain). A, top left. After 1 day, epithelium has migrated under retracted episclera. No cells are present on the sclera (arrow). No goblet cells are present in migrating epithelium (X250). B, top center. After 1 day, goblet cells are present 2 mm from the leading edge (X400). C, top right. After 5 days, the central wound is almost closed (X250). D, bottom left. After 1 week, the wound is covered by epithelium, but no goblet cells are present centrally. The conjunctiva at the wound edge (arrow) shows acanthosis (X250). E, bottom center. After 1 week, goblet cells are present 3 mm from the wound margin (X400). F, bottom right. After 2 weeks, goblet cells are present centrally but in reduced number (X400).
positive goblet cells were counted (#GC/mm) using a calibrated micrometer.

**Results**

Normal rabbit conjunctival epithelium consists of two to three cell layers with 15–20 goblet cells/mm (Fig. 2A). The n-heptanol treatment removed all epithelial cells centrally (Fig. 2B). The edge of the treated area was one cell layer thick (Fig. 2C), but a well-circumscribed defect was present. The trephined wounds exposed bare sclera.

Grossly, the n-heptanol treated wounds showed minimal vascularization during reepithelialization. Microscopically, the following observations, documented in Figures 3A-F, were made. After 1 day (Fig. 3A), a single layered leading edge of conjunctival epithelium started to migrate toward the center of the wound. Goblet cells were absent from this leading edge. However, a few goblet cells were seen 5–6 mm back from the wound edge (Fig. 3B). After 2 days, the conjunctival epithelium continued to migrate with a well-defined leading edge. The cells in this leading edge appeared flattened. No goblet cells were present in the leading edge or in a 6-mm area surrounding the wound. By the third day post-wounding (Fig. 3C), the surface was reepithelialized completely with one to two cell layers. No goblet cells were present centrally with only rare goblet cells seen in a 6 mm area around the wound. By 1 week (Figs. 3D, E), the epithelium was two to three cell layers thick. Goblet cells were present peripherally in normal numbers (12–15 GC/mm) but were decreased centrally (1 GC/mm). The conjunctival epithelium appeared similar to the control at 2- and 3-week intervals (Fig. 3F).

The trephined wounds also showed minimal vascularization during reepithelialization. Microscopically, after 1 day, the epithelium migrated as a single layer, and sometimes appeared underneath retracted episcleral tissue before migrating onto the sclera (Fig. 4A). Goblet cells were not present in the leading edge but were seen 1–2 mm from the wound edge (Fig. 4B). At 3 days, a single layered leading edge continued to migrate on prolapsed Tenon’s tissue and sclera. No goblet cells were present in the wound or in a 5-mm area around the wound margin. At 5 days, the wound was almost closed centrally (Fig. 4C). Rare goblet cells were present 3–4 mm from the wound edge but were otherwise absent. At 1 week, the wound was reepithelialized completely with 1–2 cell layers. The wound edge in some specimens showed acanthosis (six to eight cell layers) (Fig. 4D). Goblet cells were absent centrally but present 3 mm from the original wound margin in normal numbers (16–18 GC/mm) (Fig. 4E). At 2 weeks, the central area was two to three cell layers with one-half the normal complement of goblet cells (9–10 GC/mm) (Fig. 4F). The peripheral area had the normal number of goblet cells. The conjunctival epithelium appeared normal at 3-weeks.

**Discussion**

This study demonstrates the morphologic wound healing of conjunctival epithelium healing over its normal substrate and bare sclera. Studies on corneal epithelial healing have demonstrated initial cell sliding followed by mitosis. It appears from the light microscopic observations that conjunctival epithelium also migrates, as expected, by cell sliding. In both types of wounds, initial cell flattening and expansion in the leading edge, characteristics of sliding cells were observed. It also appears that the n-heptanol method, originally devised to remove rabbit corneal epithelium, works equally well on the rabbit conjunctival epithelium.

Exact healing rates could not be reliably measured for a variety of reasons. The n-heptanol treated wounds stained only faintly with fluorescein after a few hours of wounding and could not be adequately photographed (Fig. 1). Richardson’s stain was not used due to its adverse effect on epithelial migration. The conjunctival edges of the trephined wounds could have retracted or collapsed slightly from the initial wound edge once the proptosed eye was returned to its normal position in the orbit making reliable measurements difficult. However, a rough estimate of conjunctival healing in the n-heptanol treated wound of 0.2–0.3 mm²/hr could be made, since a 4-mm defect closed in 60–72 hr. This estimated healing rate is lower than that observed for corneal epithelial wound healing6–10 and agrees with previous estimates of conjunctival healing rates,8 which were found to be reduced from corneal rates. It also appears from these studies that conjunctival epithelial wounds heal faster if the epithelium moves over its normal substrate (n-heptanol treated) rather than bare sclera (trephined wounds).

The fate of the goblet cells during wound repair is of the utmost clinical interest. From previous studies,2–4 it is known that goblet cells drop out as conjunctiva initially resurfaces a denuded cornea (stage 1), followed by a later blossoming of goblet cells resulting in an epithelium with apparently far more goblet cells than normal conjunctiva (stage 3), and then disappearance of goblet cells as the conjunctival epithelium transforms into corneal epithelium (stage 5).4 It is apparent from this study that in normal wound repair of conjunctival defects, goblet cells also disappear during the migration of the epithelium. The goblet cells reappear only after the surface has been reepithelialized as is the case in conjunctival resurfacing of the cornea. In conjunctival
healing, however, once the goblet cells reappear, they remain, with no tendency to show an excessive number of these cells after healing.

The source of these new goblet cells is not obvious. Although goblet cells first reappear peripheral to the central wounded area, it could not be definitively determined from this study if the goblet cells within the wounded area had migrated in from the periphery, or whether they arose as a result of differentiation from stem cells present in the reepithelialized central area.

These observations show that healing of conjunctiva is associated with the histologic disappearance of goblet cells from the epithelial sheet several millimeters distant from the wound itself. This alteration in the normal cell population of the surrounding epithelial mass indicates that a rather large area of the conjunctiva becomes involved in the healing of conjunctival wounds. The surrounding conjunctiva appears to serve as the initial source of cells for epithelial sliding, with this sliding mass of cells showing a few goblet cells that apparently are carried along in the sliding epithelium.

If goblet cells represent terminal differentiation of epithelial cells, epithelial cell sliding and division necessitated by wound healing might be expected to halt formation of new goblet cells. The observations reported here are consistent with this hypothesis. A few goblet cells seem to persist for a time after their formation stops. These seem to be carried along with the migrating mass of cells explaining the occasional goblet cell seen in early stages of wound healing. These cells apparently discharge their mucus and are, then, no longer identifiable. Finally, new goblet cells appear first at the periphery as wound healing is completed.

An alternate explanation for goblet cell origin is that there are two separate populations of cells, regular epithelial cells and goblet cells, with the latter unable to synthesize histologically identifiable amounts of mucin until the sheet stops migrating and dividing. The actual origin of goblet cells cannot be determined in these experiments so that neither explanation for the disappearance of goblet cells during conjunctival healing can be verified.

However, in clinical situations in which goblet cell numbers are reduced (e.g., ocular cicatricial pemphigoid, acute chemical injury, radiation kerato-conjunctivitis, vitamin A deficiency), it may be pertinent to determine whether the ocular environment stresses the epithelium leading to increased cell division and sliding with a concurrent failure to produce goblet cells. The goblet cell population might well reflect the reparative or proliferative processes in such disorders, with the goblet cell population and its reduction serving as an index of ocular surface well being.

Key words: conjunctival, wound healing, rabbit, goblet cells

Acknowledgments

The authors thank Ilene Gipson, PhD, for helpful discussion and Pat Pearson for preparation of the histology.

References