Sliding of the epithelium in experimental corneal wounds*

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The corneal epithelial cell has a unique sliding capability. The epithelial cell spreads and migrates in an amebic fashion without mitotic activity when the continuity of the epithelium is broken. This movement is demonstrated both in vivo and in vitro. Prompt sliding for sealing the wound defect is apparently the first step of the wound healing of the superficial cornea. Cut edges of collagen fibers show no sign of activity towards healing the wound. The energy source of the sliding is provided mainly from stored glycogen in the epithelial cells. Sliding is inhibited by removal of glycogen from the cell or by adding glycolytic enzyme inhibitors.

Key words: cornea, corneal epithelium, wound healing, epithelial sliding, organ culture, rabbit.

A unique characteristic of the cornea is the sliding movement of the epithelium. This phenomenon was first reported in 1885 by Peters and further described by Buschke and Friedenwald, Salzer, Maumenee and Scholz, and Hanna and O'Brien. The movement of epithelial cells into corneal wounds has been reported infrequently in recent literature. Experimental wound healing as described by several authors generally involves extensive wounds and various complicating factors rather than the movement of the epithelium.

The purpose of this paper is to describe the light and electron microscopic characteristics of epithelial sliding in experimental wound healing in vivo and in vitro.

Materials and methods

New Zealand albino rabbits weighing about 2 kilograms were used in this study. Since the application of local anesthetics (tetracaine or pontocaine) was found to cause transient but severe damage to the epithelial cells, the animals were anesthetized by intravenous injections of sodium pentobarbital. A nonpenetrating shallow wound was made with the tip of a razor blade which was clamped with a pair of curved hemostats. The center of the cornea was stroked vertically with this instrument, producing a linear wound measuring about 5 mm. long and 0.2 mm. deep. The wounds were always clean, noncrushing, and were...
Incised perpendicular to the surface. The corneas were examined 0, 1, 3, 6, 12, 24, 48, and 72 hours after wounding. The animals were killed with overdose injections of pentobarbital. In order to avoid contaminating the wound with blood cells during the enucleation of the eye, the animals were exsanguinated by cardiac puncture. The enucleated eyes were briefly washed in a physiological saline solution and were fixed in 4 per cent glutaraldehyde solution in 0.15 M phosphate buffer (pH 7.2) at room temperature. One-half of the wounded cornea was studied by transmission electron microscopy and the other half by scanning electron microscopy.

For transmission electron microscopy, small pieces, including the linear wound, were excised during the 15-minute fixation period in glutaraldehyde and transferred into a 1 per cent osmium tetroxide solution prepared with phosphate buffer at 4°C. A short period of fixation in the glutaraldehyde solution and cutting (but not mincing) the cornea into small pieces before dehydration were helpful in preventing undesirable shrinkage of the tissue. The tissue was dehydrated in ethyl alcohol and embedded in epoxy resin. Semi-thin sections were stained with toluidine blue and examined by light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate and were examined by electron microscopy.

Half of the wounded cornea was trimmed into pieces measuring about 2 mm square. The pieces were transferred to a mixture of equal amounts of 4 per cent glutaraldehyde and 100 per cent neutral formalin and were kept there for 24 hours. The tissue was washed well in water with a low frequency ultrasonic vibrator and was dehydrated in a series of graded ethyl alcohols and finally in amyl acetate. The amyl acetate was removed by a critical point drying technique using liquid carbon dioxide under a pressure of 1,600 pounds per square inch at 50°C. The dried tissues were mounted on metallic specimen holders with a double-coated adhesive tape, coated with gold-palladium vapor in vacuum, and examined by scanning electron microscopy at 15 KV accelerating voltage.

Inhibition of wound healing was studied in vivo. Some conditions tested were: total removal of the epithelium before wounding, removal of glycogen from the epithelial cells by amylase, and topical administration of various enzyme inhibitors. The effects of these conditions were investigated at one- to six-hour periods.

The sliding of epithelial cells was also studied in vitro. Small pieces (2 by 3 mm) of fresh cornea were excised and incubated for varying time periods at 37°C. In a medium containing equal parts of TC 199 and rabbit serum. Al-
though the epithelial sliding was equally observed in a moist chamber, the above mixture was found to be convenient for keeping the swelling of the corneal tissue at a minimum. The corneal epithelium was viable in this organ culture for more than five days. After 48 hours of incubation the sliding epithelium was studied histologically with various experimental alterations including temperature, atmospheric gaseous phases, nutritional constituents, and addition of metabolic inhibitors.

Some wounded corneas were fixed in 95 per cent alcohol and paraffin sections were stained for glycogen. In addition, fresh tissue was incubated in a tetrazolium solution with lactic acid and NAD for demonstration of lactic acid dehydrogenase.

Results

Normal corneal epithelium. The fine structure of corneal tissue has been described in detail by Jakus, Hogan, Alvarado, Weddell, and others so only a few points which are relevant to the present study will be emphasized. The epithelial cells are closely attached to each other without intercellular spaces, except for the 200 A regular intermembranous spacing. Membranes are frequently joined by desmosomes, the number of which decreases toward the superficial layer. The cytoplasm consists of abundant fine fibrils and free ribosomes, moderate numbers of the rough endoplasmic reticulum and Golgi apparatus, and sparse mitochondria. The epithelial cells, especially the wing cells, contain abundant glycogen. Lactic acid dehydrogenase activity, demonstrated by tetrazolium staining, is high in all epithelial cells. Glycogen synthetic activity is also demonstrated in the corneal epithelium following incubation in a carbonate buffer (pH 7.0) containing 0.1 M glucose under 95 per cent oxygen for three hours. Glycogen is histologically demonstrated with PAS staining.

Fig. 2. One hour after wounding. Damaged epithelial cells have disappeared from the area. The epithelial cells are beginning to slide down over the cut edge of the collagen fibers and cell debris. (×). ×10,000. Inset shows smooth edges of the wound. Scanning electron microscopy, ×300.
The superficial cells are attached loosely to the underlying cells with sparse desmosomes and firmly to neighboring flat cells with gap junctions. The superficial cells are nucleated and their cytoplasm is electron dense though they are often vacuolated. The tear surface of the superficial cell has fine vermiform ridges which are about 0.3 μm high, 0.2 μm wide, and of various lengths. These ridges are slightly less abundant in the central cornea, but are uniformly distributed throughout the epithelium. At the limbus, the ridge structures gradually transform into the villi of the conjunctival epithelium. Exfoliating cells are occasionally found in the peripheral zone of the cornea.

Although Bowman's membrane is believed to be absent in the rabbit cornea, there is a thin layer consisting of irregular, short collagen fibers and fine fibrils beneath the basal lamina of the epithelium. The appearance of this layer is identical to that of Bowman's membrane in the human.

Appearance of the fresh wound. Several epithelial cells and some stromal cells are cut open and their cell substance escapes immediately following wounding (Fig. 1). The cell fluid and debris accumulate for a short while around the wound. The damaged cells disappear within a few minutes and the wound edge of the epithelium becomes rounded. Collagen fibers seem to shrink and the cut edge of the stroma becomes uneven within the first hour.

Stromal cells in the vicinity of the wound edge retract their cell processes and become round in shape. These cells have convoluted nuclei. The background stroma is moderately swollen.

Early wound healing. The earliest reaction to the wound is an invasion of leuco-
Fig. 5. Three hours after wounding. The sliding epithelium covers the cut edge tightly. Fine cell processes extend into spaces around a keratocyte which may have been damaged (arrows). ×11,500.

Fig. 6. Three hours after wounding. Fine cell processes fill the tissue defect at the cut edge of the stroma. No specific difference in structure is seen in the extending cytoplasm. ×55,000.
Fig. 7. Three hours after wounding. A, the advancing tip of the epithelium has no glycogen. PAS stain, 150X. B, lactic acid dehydrogenase is active in the sliding epithelium. Tetrazolium stain, ×200.

cytes which originate from conjunctival capillaries. Some leukocytes invade into the stroma. Details of this mechanism have been described earlier.8

The epithelial cells begin to slide into the wound about one hour after the wounding (Fig. 2). The main sliding cells are wing cells, but the flat superficial cells regain the cytoplasmic constituents of the younger cell and slide similarly. The basal cell becomes flatter in shape and slides, but to a lesser extent. The fine ridges of the epithelial surface disappear in the sliding tip (Fig. 3). The sliding cells lose their tight interdigitation, but the cells are held to each other by desmosomes of extended...
Fig. 9. Twenty-four hours after wounding. The sliding cells are well interdigitated. The surface cell has formed normal ridges (arrow and inset). ×13,000. Inset scanning microscopy, ×10,000.

Fig. 10. Two days after wounding. A linear space is present between the superficial cells of both sides of the wound. This is often found in deeper wounds. ×7,000.

Fig. 11. Three days after wounding. Exfoliation of epithelial cells is seen along the wound, which has been filled up completely. ×300.

cytoplasm and never become free from the underlying cells (Fig. 4). New desmosomes are formed by the contact of cytoplasmic protrusions. The moving cells extend cytoplasmic processes into the tissue defects in an amebic fashion. This phenomenon is
most clearly demonstrated in wounds three to six hours old (Figs. 5 and 6). The sliding cells maintain the normal structure of cytological constituents except for the disappearance of glycogen particles. Despite the depletion of glycogen, the cytoplasm maintains normal glycolytic enzyme activity (Fig. 7). The cells that have slid to the stroma surface attach firmly to the adjacent cells and to the connective tissue. Some more distant cells slide over these already settled cells.

After three hours the wound opening has widened and about one-half of the wound edge is covered by the thin layer of the epithelium (Fig. 8). The sliding cells reach the bottom of the wound in about six hours. The V-shaped wound defect is filled gradually with epithelial cells. No mitosis is noted in the vicinity of the wound for about three days. However, mitosis becomes active in the epithelium at a considerable distance from the wound 24 hours after wounding. These proliferating epithelial cells appear to push the whole epithelium toward the wound, but the active sliding of individual cells also continues until the third day. The moving of the cells into the wound causes a sudden decrease in the thickness of the epithelium around the wound edge. Mitosis begins to show in the wound three days after wounding and the thickness of the epithelium returns to normal. Also, the surface of the wound becomes even with the surrounding epithelium.

The epithelial cells at the base of the wound increase in height in 24 hours and then begin to reveal the characteristic appearance of basal cells. However, a new basement membrane is not noted until the third day. The epithelial cells in the wound are firmly interdigitated with each other and the superficial cells begin to show fine ridges (Fig. 9). In slightly deeper wounds, the apical surfaces of the epithelial cells of the wound edges often form a linear space which lasts for about 24 hours (Fig. 10). The fine ridges appear to engage and eventually form interdigitations. The epithelial cells in the wound begin to accumulate glycogen particles. Exfoliation of the superficial epithelial cells along the wound begins around the third day (Fig. 11).

There is no sign that the cut edges of the collagen fibers are brought closer during the wound healing (Fig. 12). The cut edges of the collagen fibers remain unchanged for a long period of time. An increase of fine fibrils among the stumps...
Fig. 13. One hour incubation of excised corneal tissue. Iodoacetic acid (10^{-5}M) has been added in the incubation medium. Cells have lost their coherence. No sliding is observed. Hematoxyl-eosin, ×300.

Fig. 14. Diagram of the healing mechanism of a superficial corneal wound. See the explanation in the text. Fine dots in the epithelium indicate glycogen particles.

of the collagen fibers is noted, but no appreciable scar formation is apparent.

Similar wounds which were produced after removal of the epithelium did not heal for a few days until the corneal surface was covered with epithelium. The stroma was severely swollen and infiltrated by leukocytes.

**Epithelial sliding in vitro.** Sliding of the epithelium is observed in excised corneal tissue in the same manner as in vivo. The epithelium at the cut edge starts to slide toward the denuded stroma after a latent period. In many cases this latent period is about two to three hours. The speed of the movement of the advancing front line of the epithelium is calculated by measuring the length of the newly covered area in paraffin sections. The epithelium slides at a speed of 26 μm per hour at 37° C. in the TC-199 and serum mixture under atmospheric air. The advance of the epithelium is at a uniform speed. This is confirmed by time-lag cinemicrophotography. This speed appears to be constant for three days or longer if the incubation medium is suitable for the tissue. The sliding continues until the cells of the moving front meet other living epithelium or endothelium. No mitosis occurs in the present experimental condition. The sliding cells cover not only the denuded corneal tissue, but also any material continuously present on the denuded stroma, i.e., necrotic debris and foreign material. When the block is embedded in an agar plate the epithelium spreads onto the surface of the agar plate. The sliding speed is also 26 μm per hour. The covering epithelium, including the original epithelium, becomes a thin monolayer after a 24-hour incubation period.

The sliding phenomenon is not greatly sensitive to the temperature of the incubation medium. Only a slight enhancement of the sliding is noted at higher temperatures; between 24 and 38° C. the speed is found to be about the same. No sliding of the epithelium is observed at temperatures greater than 42° C or lower than 4° C. Sliding is equally observed in most common incubation media for tissue culture. The sliding occurs also in plain physiologic saline solution or a simple moist chamber and is maintained for about 24 hours.
The sliding of the epithelium appears to require energy of which the source may be glucose or glycogen. The native intracellular glycogen is most important in the initiation of sliding. Epithelium from which glycogen has been extracted by a weak amylase solution (5x diluted human saliva) before incubation shows extreme retardation of sliding. The deglycogenated epithelium is unable to initiate the sliding and undergoes degeneration. The sliding is reactivated in a glucose-rich medium. The sliding epithelial cells show a high lactic acid dehydrogenase activity. The sliding is equally active whether the atmospheric gases are oxygen, nitrogen, carbon dioxide, or air. In addition, variation between pH 6 and 8 does not reveal much difference in the sliding ability of the cells.

Several metabolic poisons were used to demonstrate the inhibition of sliding. Para-hydroxymercuribenzoate (10^{-4}M) and iodoacetic acid (10^{-4}M) were the only effective inhibitors. Iodoacetate causes marked loosening of the epithelium (Fig. 13). Other poisons, including cyanide (10^{-3}M), did not show an appreciable effect even at high doses.

**Discussion**

Sliding of the epithelium is a common occurrence in corneal wound healing. The present study has shown the cytological details of the sliding cells and has emphasized the fact that epithelial sliding is the earliest step in the wound healing of this nonvascular tissue. The findings are summarized in a schematic drawing (Fig. 14).

Although no specific cytoplasmic constituents supporting cell movement are present, the epithelial cell moves in an amebic fashion immediately after the wounding of the corneal surface. The filaments and microtubules in the cytoplasm do not show any alteration in their arrangement or density during their sliding. Fine ridges of the surface cell membrane, however, disappear during the sliding. The flattening of finely infolded cell membranes may contribute greatly to extending the cytoplasm, but the actively movable wing cells have no ridges. Besides the actual sliding at the wounded area, all the epithelial cells of the wounded cornea appear to move as a tide toward the wound. Cells at some distance from the wound flatten and proliferate. Nonetheless, it is the prompt sealing of the tissue defect that is the apparent purpose of the epithelial sliding. Edema and leukocytic infiltration in the stroma at the wound disappear when the defect is covered by the epithelium. Maintenance of transparency of the stroma, the most important function of the cornea, is rapidly restored by this mechanism. The epithelial cells of the conjunctiva and other mucous membranes do not slide in similar wound or in vitro conditions.

The sliding movement to cover the tissue defect appears to be a unique nature of the corneal epithelial cell. Similar endless spreading of the corneal epithelium is observed in epithelial down growth in the anterior chamber, an undesirable postsurgical complication. The biochemical and physiological behavioral patterns of the corneal epithelial cells are similar to those of tumor cells. The corneal epithelial cell is characterized by sparse mitochondria and its glucose metabolic pathway differs somewhat from that of other cells. In addition, the corneal epithelial cells lose their polar orientation and reorient themselves by rolling down into the fissure of the wound. In certain pathologic conditions corneal epithelial cells lose their orientation and form aberrant basement membranes within the epithelium.

An exudate substance of the epithelium which was demonstrated in prick wounds by Friedenwald, Buschke, and Crowell may correspond to the minute degenerating debris of the damaged cells of the corneal wound. The cut edge of the epithelium becomes round by eliminating degenerated cells within a very short period of time. No exudate has been demonstrated in this study.

The intracellular glycogen seems to be the energy source for the movement of the cell. The sliding action is prevented by removal of glycogen or administration of...
glycolytic and sulfhydryl enzyme inhibitors. The inhibitory effects on cell movement of similar enzyme poisons have also been reported by Buschke and Friedenwald.19, 20 The sliding cells at the tip of the wound may have an increased membrane permeability; a topical application of amylase solution readily removes the intracellular glycogen particles. The sliding cell maintains a high activity of lactic acid dehydrogenase. A high activity of oxidase has been similarly demonstrated in the wound epithelium by Weimar and Haraguchi.21 Depletion of the epithelial glycogen is a common finding in various pathologic conditions and has been reported in experimental conditions.22, 23 This glycogen depletion may have a retarding effect on wound healing.

The cut edges of the collagen fibers remain far apart and no sign of adhesion of the collagen was observed in the superficial wound. The wound becomes shallower with time and the thickness of the epithelium becomes normal also.

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REFERENCES