The healing of corneal epithelial abrasions in the rabbit: a scanning electron microscope study

**Roswell R. Pfister**

The morphologic appearance and time course of regenerating rabbit corneal epithelium was studied after 6.0 mm. corneal abrasions. The immediate response to injury was separation and thickening of basal and squamous epithelial cells at and near the wound margin. A variable number and distribution of polymorphonuclear leukocytes appeared on the basal lamina and regenerating epithelial edge at 3 hours, persisting up to 38 hours after abrasion. Most of the epithelial cells at the wound margin were extensively flattened after 15 hours. These flattened epithelial cells showed a wide variety of surface ruffling near their free edges. This ruffling activity often was associated with long fingerlike processes (filopodia) extending out on to the basal lamina. The method of epithelial cell movement on the basal lamina appeared to depend mainly on the ruffling and filopodial activity of squamous epithelial cells. Ruffles and filopodia projected out ahead of the cell edge, contacted the basal lamina, and appeared capable of drawing the cells forward into the area of the defect. Normal corneal re-epithelization proceeded with two or three cell layers moving as an orderly sheet over the basal lamina until the defect was closed. The corneal surface showed no defect at 55 hours and was returned to normal at 114 hours.

**Key words:** cornea, epithelium, cell movement, abrasion.

The migration of epithelial cells from wound borders has been recognized as the means by which defects were repaired on the corneal surface.1-4 This migration was initially thought to result from cellular proliferation but the issue resolved by Barfurth5 who noted no increase in cellular mitoses. Later, workers showed that increases in cellular division occurred 96 hours after large corneal epithelial wounds6 and four hours after minute injuries.7 These early studies were later confirmed in other epithelia and in tissue culture.5, 9

The nature of this epithelial migration was thought, by various investigators, to be a flattening of existing cells,8 ameboid movements of individual cells,1-5, 10 or mass movement of an epithelial sheet.8, 11 Buschke12 helped to clarify this question by recognizing "pseudopodial extensions" and...
cellular movement into the wound area from epithelial cells surrounding a small wound. The exact manner of movement was limited by the optics of light microscopy.

There is little further information on the mechanism and appearance of corneal epithelial cells as they migrate over the denuded basal lamina or epithelial cells. However, a considerable amount of data has accumulated over the past two decades on cell locomotion of various tissues in vivo and in vitro. This wealth of information has recently been extensively reviewed.13

The purpose of this paper is to examine by scanning electron microscopy (SEM) the anatomic patterns of corneal epithelial cell locomotion after simple corneal abrasion. These mechanisms are compared and correlated with known translocations of cells observed in tissue cultures and in vivo.

Materials and methods
Twenty-five normal New Zealand strain albino rabbits, weighing 2 to 3 kilograms, were anesthetized with intravenous pentobarbital. As soon as the animal became drowsy, the eyelids were closed by clamping the upper and lower lashes together with a hemostat. When the animals were completely anesthetized they were positioned under an operating microscope and eyelids were opened. An epithelial abrasion was produced in both eyes of each rabbit by lightly marking the surface centrally with a 6.0 mm. trephine and removing the enclosed epithelium with a No. 15 scalpel blade. The surface of the basement membrane was lightly scraped, but not penetrated. Immediately after the procedure both eyes were again closed by clamping the upper and lower lashes together until the animal was awake and capable of blinking normally.

To obtain corneal tissues for SEM, animals were reanesthetized, using the same techniques as noted above, and tissues were removed at 0 and 5 minutes and 1, 3, 6, 15, 24, 38, 55, and 114 hours after the corneal abrasion. The techniques of fixation and preparation of tissues for SEM are identical to those described in a previous paper.14
Fig. 2. Zero time. Individual cells separate from each other near wound margin. ×1,100.

Fig. 3. One hour after abrasion. Retraction of cells from wound edge gives smoother wound margin. ×5,600.
A JEOL-JSM 35 or Cambridge Model IV stereocanar was used to examine specimens. Stereo photographs were taken where indicated.

Light microscopy was employed in selected tissue specimens.

Results

Zero-time after corneal abrasion—6.0 mm. residual epithelial defect. With a full-thickness corneal abrasion the exposed basal lamina was seen as a coarse, slightly pebbled surface with a moderate amount of amorphous cellular debris. After abrasion an intact basal lamina was substantiated by transmission electron microscopy (Fig. 1). There were many long, dichotomously branching fibers, presumably nerves, left attached to the basal lamina. The edge of the epithelial defect showed some destroyed cells, with their membranes severely stretched out. The edges of the defects were rarely vertical so that often some of the basal cells were exposed at the wound edge. Other apparently viable squamous cells, at or near the edge, showed evidence of retraction from neighboring cells (Fig. 2). These injured epithelial cells had lost most of their microvilli, with the development of a very irregular appearance to the plasma membrane. There was a relatively normal appearance of light and dark cell surfaces distant from the defect.

Five minutes after corneal abrasion—6.0 mm. residual epithelial defect. Most of the epithelial cells within 0.5 to 1 ml. of the defect appeared to detach from one another but with retention of some residual contacts to the basal lamina or other epithelial cells.

One hour after corneal abrasion—6.0 mm. residual epithelial defect. The rough basal lamina was devoid of most cellular debris. The edge of the epithelial defect was smooth and slightly elevated with epithelial cells clustering at the edge (Fig. 3). There was a small amount of lacy, fibrinous material stretching between epithelial cells and the basal lamina.

Three hours after corneal abrasion—6.0 mm. residual epithelial defect. A variable...
Fig. 5. Six hours after abrasion. Variable numbers of PMN's at the wound edge and on the basal lamina. ×840.

Fig. 6. Fifteen hours after abrasion. Very flat, dark epithelial cell at advancing edge. This may represent a resting stage prior to initiation of motion. ×3,200.
Fig. 7. Fifteen hours after abrasion. Ruffles of cell margin presenting as thin membranous leaves. A few barium sulfate crystals remain from processing. Stereo, ×6,800.

number of polymorphonuclear leukocytes (PMN) at the edge of the abraded area appeared at the epithelial wound edge, often significantly elevating the rim. These cells were determined to be PMN's on the basis of light microscopy. These areas alternated randomly with very flat squamous cells at the wound margin. Many times a large discontinuous and irregular monolayer of PMN's formed portions of the wound margin, extended out on the basal lamina or were found in islands ahead of the defect margin. Almost all of these leukocytic cells were partially enmeshed in a fibrinous, lace-like network attaching to other epithelial cells and/or the basal lamina (Fig. 4).

Six hours after corneal abrasion—5.9 mm. residual epithelial defect. PMN's were still frequently found at or ahead of the wound edge (Fig. 5). Fibrinous material was still observed enmeshing the PMN's at the epithelial wound edge. For the first time ruffling was noted in some squamous epithelial cells at the edge of the defect. There was a preponderance of surface dark cells, with only a few microvilli clustered near the nucleus, just behind the area of the defect. There seemed to be less visible amorphous material covering the plasma membrane of these cells.

Fifteen hours after corneal abrasion—4.2 mm. residual epithelial defect. This appeared to be one of the most active stages in the epithelial reparative process. PMN's were still present in large numbers at and over the wound edge as well as extending out over the basal lamina.

Very thin dark squamous cells were usually noted at the wound edge. These cells had a microvillous free zone 2 μm to 10 μm at their free border. Some of the edges of these cells were smooth and relatively flat against the basal lamina (Fig. 6). This microvillous free zone often showed a wide variation of surface membrane folds known as ruffles. Ruffles were found as thin leaves of cell membrane ris-
Fig. 8. (Left) Localized, circumscribed elevation in microvillous free zone of squamous epithelial cell. ×9,400. (Right) Broad, thick ruffles appear to develop from circumscribed elevations near cell edge. ×5,500.

Fig. 9. Ruffles of different shapes extend out over the basal lamina. Note the broad, tongue-like ruffles extending out from under the top cell. A thinner edge is present on a ruffle of the overlying cell. In both cases, filopodia extend off the crests and edges of the ruffles into the defect area. Stereo, ×6,800.

ing almost perpendicular to the cell surface (Fig. 7). Other cells showed rounded and fairly circumscribed elevations near the free edge (Fig. 8, left). In some cases these rounded cellular elevations gave rise to very broad and thick ruffles projecting slightly out over the basal lamina (Fig. 8, right). The largest of these tongue-like ruffles extended out over the basal lamina for a considerable distance (Fig. 9). Some of these ruffles were found to contact the basal lamina ahead of the cell directly.
Fig. 10. Fifteen hours after abrasion. Some cells seem to move by broader, flat processes involving a large edge of a cell. This may represent a large ruffle extending over the edge of the cell as a wave. x2,700.

(Fig. 10). More commonly, filopodia were found to extend off the crests and edges of ruffles toward the denuded basal lamina (Fig. 9).

Filopodia of various sizes appeared poised above the basal lamina while other cells had very long tapering, finger-like filopodia stretched out with their peripheral fingers contacting the basal lamina (Fig. 11). Some cells showed shorter filopodia in contact with the basal lamina over their entire length. Filopodia were of various lengths, usually present at irregular but closely spaced intervals along the advancing edge of the cell (Fig. 12). Two adjacent cells at the edge of the defect were often noted to touch one another with ruffles and filopodia (Fig. 13). When one adjacent cell had overlapped another, only the top cell was noted to ruffle.

A second and sometimes third layer of cells advanced over the first and second layers, respectively, such that the more peripheral portion of the underlying cell was partially overlapped (Fig. 14). In some cases, the intensely ruffling edge of the bottom cell could be seen just peeking out from under the second layer.

Extremely long (100 μ) cytoplasmic processes occasionally extended from the edge of the defect onto the basal lamina (Fig. 15). These cytoplasmic processes often terminated in a pad of cytoplasm similar to the growth cone of a neuronal axon. Small specialized enlargements of various shapes along the course of the process appeared directly attached to the basement membrane. These processes did not appear attached along the full course as evidenced by small "humps" off the basement membrane and by the presence of other strands and cells under these processes.

Twenty-four hours after corneal abrasion—3.3 mm. residual epithelial defect. At this time there was only the appearance
Fig. 11. Filopodia extend out on the basal lamina. Note proximal portion of the filopodia and cell edge are not in contact with the basal lamina. \( \times 7,200 \).

Fig. 12. Fifteen hours after abrasion. A combination of ruffles and filopodia are present in cells at the wound edge. \( \times 4,800 \).
Fig. 13. Fifteen hours after abrasion. Moderately long filopodia, some coming from ruffles, extend out toward the basal lamina while others appear to "sense" other cells with ruffles laterally. ×7,800.

Fig. 14. Fifteen hours after abrasion. Ruffles of a broader nature near edge of basal advancing cell. Second and third cell layers overlap the basal layer, respectively. ×6,600.
Fig. 15. Fifteen hours after abrasion. Collage of a cytoplasmic process, presumably an axon, extending from under the edge of the epithelial defect out on to the basal lamina. Note the specializations along the course of the axon and the growth cone at its tip. x2,300.

of thin, dark cells at the margin of the defect. Most cells were actively ruffling with or without associated filopodia. There were many dark cells in the area of the recovered defect (Fig. 16). There were very few microvilli on dark cells covering the recently abraded area. Those few that did exist were present particularly around the nucleus. Very few holes in this covering layer of cells were noted.

Small numbers of PMN’s were still present along the wound edge.

Thirty-eight hours after corneal abrasion—0.5 mm. residual epithelial defect. There was less ruffling and filopodial activity at the wound edge than that noted at 24 hours. Occasional PMN’s were found scattered at and over the wound edge.

Fifty-five hours after corneal abrasion—no epithelial defect. The area of the original defect could not be identified. The surface cells in the abraded area had a slightly higher proportion of dark cells but their surfaces were otherwise normal. The number of surface epithelial holes was less than normal. No PMN’s were noted.

One hundred fourteen hours after corneal abrasion—no epithelial defect. There was a relatively normal appearance of surface epithelial cells with a proportionate number of holes.

Discussion

There appear to be three early responses to the simple trauma of abrasion identified in these studies. Basal and squamous epi-
Fig. 16. Twenty-four hours after abrasion. The surface of the epithelial cells which have just covered the defect are predominantly large and dark in appearance. x590.

The epithelial cells, surviving at or near the margin of the defect retract and become thicker by breaking their tight attachments to adjacent cells.

PMN's found on the abraded basal lamina probably represent leukocytic chemotaxis to traumatized tissues, since no bacteria were found on the corneal surface and no clinical ocular infection was apparent. Fine strands enmeshing the cells at the wound edge, presumably fibrin originating from leaking conjunctival blood vessels, may initially assist the adherence of PMN's and epithelial cells to the basal lamina.

In most biologic systems, cellular motility is known to be closely related to ruffling and filopodial extensions. Cell ruffling in tissue culture is demonstrable as a ridge of plasma membrane, often of considerable length and height, near the edge of the moving cell [16]. Thin sections of these ruffles show fine microfilaments similar to those thought to represent contractile elements [17].

Chick embryo epithelial cells, like fibroblasts cultured in vitro, showed ruffling membrane activity associated with movement across glass substrates. Membrane activity of the advancing edge of cells seemed to be closed related to the greater strength of the cell—substratum adhesion; nonmarginal cells appearing to be non-adherent to the substratum.

Observation of the wound edge in this study suggested a particular pattern and dynamic process of cell movement. Cellular motility appeared to be initiated by ruffling at or near the free edge of squamous epithelial cells, both on basal lamina and deeper epithelial cells. Filopodia could be seen projecting from the crests of ruffles extending out toward the defect. Extensions of these filopodia gave a spread-out, finger-like appearance ahead of the edge of many cells on to the basal lamina. Contact of only the distal portion of these long filopodia suggested the capability of the cell to move forward by contraction of these filopodia toward a fixed forward.
Filopodia are therefore considered to provide the motive force in cell migration on the cornea. In some cases it is possible that the ruffling activity alone may crest over the leading edge of the cell like a wave, thereby creating a newly advanced leading edge. These extremely flat cells had the SEM appearance of actively motile cells, as contrasted with the rounded appearance of cells undergoing division. The smooth edge present in some flat epithelial cells at the wound margin suggested the cell had moved forward in many cases, probably absorbing its own filopodia. This may represent its resting stage prior to subsequent activity. Recently, a pattern of cell movement was noted in tissue culture which is very similar to some of the findings presented in this study. In vivo observations of fundulus during the blastula and gastrula phases show deep cells undergoing locomotor activity also similar to those described in this paper. These cells appeared to move partially by flowing their cytoplasm through the filopodial extensions. Typically, the filopodial tip remains strongly adherent to the substratum. When the filopodium shortened, the cell moved toward the tip, sometimes with a sudden jump. The authors emphasize that moving cells can interconvert ruffling and filopodial activity. These filopodia are probably able to shorten by the contractile ability of their contained microfilaments.

Thin sections of perforating wounds of rabbit cornea showed fibrin clots in the anterior and posterior lips of the wound at 1.5 hours, epithelial migration and polymorphonuclear (PMN) response at 3.5 hours, and epithelial cytoplasmic processes projecting into the wound in three to four days. Those findings which are compatible with this study are the development of surface fibrin (one to three hours after abrasion), and epithelial migration with a PMN response (three to six hours after abrasion). The small tissue samples obtained with transmission electron microscopy could explain the delayed appearance of cytoplasmic processes (filopodia) as compared to this SEM abrasion study (six to fifteen hours).

It has been shown previously that a unique mechanism of surface cellular exfoliation exists in a normal rabbit cornea which is thought to be conducive to tear film stability. Disappearance of the hydrophilic dark cell by hole formation was thought to be in synchrony with gradually increasing wettability of the underlying surfacing light cell. The present study shows that by SEM most of the advancing squamous cells are quite dark with an increase in the number of dark cells in the surface of the epithelium in recently recovered abrasions. Cells like these which have very few microvilli appear dark under SEM because few secondary electrons are generated from surfaces having a uniformly low profile. It is probable that most of these cells are dark under SEM because of the extensive flattening which they undergo. These dark cells are, therefore, not mature nor hypermature, as may be expected on the normal rabbit cornea, but merely represent extensively flattened cells. This may be reflected in the relatively fewer number of holes in these surface dark cells.

Under normal circumstances, with periodic resurfacing of the tear film over the corneal surface, a stable intact film rests on a stable surface from one blink to the next. In the absence of epithelium it is clinically recognized that the tear film breaks up quite rapidly in that area. Migrating cells at the margin and behind the defect do not form a smooth continuous layer, are devoid of a normal microvillus surface, and apparently lack significant mucin adsorption to their surface. Therefore, newly migrated epithelium would not be expected to hold a stable tear film.

Last, epithelial migration on the corneal surface may serve as an excellent easily visualized model of cell locomotion in vivo. Technical assistance was provided by Vivian Johnson and Beverly Eck. C. A. Paterson and N. Burstein are thanked for manuscript review.
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REFERENCES