The normal and abnormal human corneal epithelial surface:
a scanning electron microscope study

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The normal human corneal epithelial surface is characterized by a finely irregular surface consisting of microvilli and/or microplicae, with an adsorbed layer of mucin. This morphology is thought to contribute to the stability of the tear film. The presence of a finely irregular surface consisting of microvilli and/or microplicae, with an adsorbed layer of mucin, is thought to contribute to that stability. This morphology is of equal importance in disease processes where biological events on the corneal surface may not only affect tear film stability but also help to further define the nature and pattern of the disease process.

To examine these events, corneal specimens obtained from keratoplasty were routinely processed for SEM and, as re-
Fig. 1. Normal human corneal epithelial surface is shown in a specimen removed for central traumatic scarring. a and b are from one button, and c and d are from another. a, Epithelial cells form a polygonal mosaic on the surface of the cornea. b, Surface cells presenting only a small portion of their surface are covered with long microvilli. Adjacent large cells are covered primarily by stubby microvilli. c, Wide variety of microvilli (MV) and microplicae (MP) are present in adjacent cells in this specimen. d, Interlacing microplicae are found in a single older cell. Occasional microvilli are present between ridges.

Materials and methods

One hundred eight corneal specimens (buttons) for this study were obtained at the time of removal from patients undergoing corneal transplantation for corneal scars (12 specimens), keratoconus (43 specimens), corneal edema (48 specimens), and healed stromal herpetic keratitis (5 specimens). Most patients used no preoperative medications or appliances (contact lenses). In patients receiving medication, wearing lenses, or undergoing tonometry before surgery, correlations...
were made with SEM findings. The vast majority of keratoconus patients had not worn their contact lenses for weeks or months prior to the corneal surgery. A few patients had never worn contact lenses. External photographs were taken before surgery and correlated with oriented specimens in order to map the area of injury or disease process.

Most specimens of corneal tissue were removed from patients by a trephine without an obturator. The buttons were irrigated with physiologically balanced saline solution (BSS) during removal to prevent drying and were handled only at the edges with forceps. Although BSS may not be an ideal solution for irrigation, the excellent SEM results on scarred corneas suggested that no arti-
Fig. 3. Keratoconus specimen. a, Spherical character of the blebs and their placement at the cell margins are shown. b, Profile view of sessile blebs. c, High-power view of bleb shows coarse, irregular surface. d, Large plasma membrane holes are seen in the walls of blebs of an older cell. Micropilections are lost from all but the central cell surface.

fact was induced. They were immediately placed in warm (37°C) 4% glutaraldehyde buffered with 0.1M phosphate buffer at pH 7 and fixed overnight before sectioning for various treatments. Portions used for SEM were washed in 20% acetylcysteine (Mucomyst; Mead Johnson Laboratories, Evansville, Ind.), rinsed in 0.1M buffer, postfixed 1 hr. in 1% osmium tetroxide, dehydrated in acetone gradients, and critical-point dried with liquid carbon dioxide with the use of a Sorvall CFD unit (Ivan Sorvall, Inc., Newton, Conn.). They were then mounted on aluminum studs and sputtered with gold-palladium alloy in a Denton evaporating unit (Denton Vacuum, Inc., Cherry Hill, N. J.).

Specimen portions used for TEM were post-fixed in 1% osmium tetroxide, dehydrated in acetone, and embedded in Epon-Araldite epoxy resin. Sections were cut on a Porter-Blum ultramicrotome and stained with lead citrate and uranyl acetate. Detailed specimen preparation has been described elsewhere. Specimens for SEM
Fig. 4. Transmission electron micrograph of a surface bleb shown in Fig. 3 demonstrates unit membrane (PM) with contained 250 A granules (Gr). The cellular cortical microfibrillar skeleton (MF) is partially disrupted at the base of the bleb. The plasma membrane surface is irregular, resulting in the occasional appearance of membrane leaflets.

were examined in a Cambridge Model IV stereoscope or a field emission scanning electron microscope (Coates & Welter Instruments, Sunnyvale, Calif.). A Philips 300 electron microscope (Philips Electronic Instruments, Mt. Vernon, N. Y.) was utilized for TEM.

Results

Normal cornea. The surface appearance of epithelial cells over traumatic corneal scars was found to be identical to that of epithelium over undamaged stroma in all specimens. The epithelial surface appearance of light, intermediate, and dark cells parallels findings in the rabbit cornea. Cells with small surface area had long and relatively sparse microvilli, giving the cell a "light" SEM appearance. The majority of cells were intermediate in brightness and flatter in profile, with a variety of surface microprojections consisting of various shapes, sizes, and numbers of microvilli and curvilinear microplicae (Fig. 1). Specific cells of this type showed variations in surface appearance, from all microvilli to nearly all microplicae, including all intermediate combinations. A third appearance, seen in darker cells of largest surface area, was microprojections limited to the center...
Fig. 5. Corneal epithelial edema. a and b, Low-power views of the corneal epithelial surface show severe irregularity. Hollow squares indicate area of increased magnification. c, Surface epithelial cells are edematous, with considerable discontinuity in the cell sheet. Gaps between cells are probably partially caused by cell loss. Many loose cells are noted on the surface. Light spot in photograph represents charging artifact due to cell elevation. d, Higher-power view of edematous cells demonstrates the maintenance of relatively normal surface microprojections despite swelling.

of the cells. These cells sometimes had smooth surfaces, with degradation of the plasma membrane often noted. Rarely, lacy residuals of the plasma membrane of cells were found on the corneal surface.

Keratoconus. There were three different patterns found in the distribution of dark cells in the nipple or sagging forms of keratoconus. In the nipple type keratoconus, dark cells were clustered heavily but randomly over the entire cone (Fig. 2, a and b). In the sagging or larger cone
Fig. 6. Stromal herpetic keratitis with scarring. a, Corneal button shows the extensive appearance of loose cells on the surface and bulging of edematous epithelial cells in the top cell sheet. b, At the center of the cornea, heaped-up, swollen, and desquamating epithelial cells (Ep) and inflammatory cells (IC) form a small mound. c and d, Away from central cornea, loose cells produce an uneven surface. Hollow squares indicate areas of increased magnification.

dark cells were clustered more heavily in a broad encircling band just inside the cone base, with a lower concentration scattered over the apex (Fig. 2, c). Other large or sagging type cones showed dark cells scattered over the entire sample button.

The cells with dark appearance by SEM (Fig. 2, d) could be grouped into three general categories: (1) those with short, stubby microvilli and/or microprotrusion over the surface and some long, clublike microvilli; (2) those with characteristic bleb-like outpouchings frequently at the periph-
eral margin of the cell (Fig. 3, a to c); and (3) smooth cells often with some remnants of collapsed blebs and few if any microprojections and showing membrane disruption (Fig. 3, d).

Blebbing of dark cells was a constant feature on the surface of keratoconus buttons. Blebbing did occur occasionally in other disease processes including interstitial keratitis, epithelial edema, and ulcerating rosacea keratitis. In these latter cases blebbing was inconsistently present over the surface of the cornea, sometimes involving only small patches of cells.

The blebs observed are spherical, from 0.25 to over 3 µm, having either a sessile (Fig. 3, b) or pedunculate (Fig. 3, c) appearance. The bleb surface, as seen at high magnification, is distinctly uneven (Fig. 3, c).

By TEM a unit membrane covered the blebs, which were composed of cytoplasm containing 250 Å radiopaque glycogen-like granules (Fig. 4). The microfilament network at the base of the blebs was partially disrupted.

In a few cases contact lenses were worn over the operated cornea up to 1 hr. before the time of surgery. The SEM views of the corneal surface revealed many degenerated, poorly attached epithelial cells. Blebs were also seen in these cases.

In one case a fresh button from a keratoconus patient was immediately examined by epi-illumination at 100x. These peripheral cellular blebs were visualized in cells over the cone as identified by SEM.

**Corneal edema.** In the presence of epithelial edema the corneal surface was very irregular in all specimens (Fig. 5). This irregularity is caused by (1) many epithelial cells lying on the surface cellular sheet, (2) scattered groups of partially detached epithelial cells bulging anteriorly, or (3) occasionally large groups of rounded epithelial cells bulging anteriorly, or (3) occasional groups of rounded epithelial and inflammatory cells (Fig. 6). A heaped-up cluster of swollen epithelial cells marked the center of this specimen (Fig. 6, b). Intermixed with these epithelial cells were smaller (6 to 8 µm) inflammatory cells. Epithelial cells could be distinguished from inflammatory cells by their characteristic surface microprojections and size of 12 to 15 µm. Inflammatory cells were much smaller (6 to 8 µm) and smoother, with occasional longer microvilli. Ruffles were common, especially near attachment sites.

**Herpetic keratitis.** The epithelial surface was irregular even a year or more after stromal herpes simplex infection. This irregularity was caused by (1) many epithelial cells lying on the surface cellular sheet, (2) scattered groups of partially detached epithelial cells bulging anteriorly, or (3) occasional large groups of rounded epithelial and inflammatory cells (Fig. 6). A heaped-up cluster of swollen epithelial cells marked the center of this specimen (Fig. 6, b). Intermixed with these epithelial cells were smaller (6 to 8 µm) inflammatory cells. Epithelial cells could be distinguished from inflammatory cells by their characteristic surface microprojections and size of 12 to 15 µm. Inflammatory cells were much smaller (6 to 8 µm) and smoother, with occasional longer microvilli. Ruffles were common, especially near attachment sites.

**Discussion**

The human corneal surface showed microprojections similar to those previously noted in animals. In the present paper, high resolution SEM helped to establish that both microvilli and microplicae exist in variable numbers in adjacent cells. These variations of cell surfaces may well represent a spectrum of morphological change during the maturation process at the corneal surface. The most prominent difference between the human and rabbit corneal surfaces is the absence in humans of numerous epithelial holes previously associated with the normal pattern of exfoliation or other metabolic processes in rabbits. In the human cornea, large, dark cells having few or no microprojections did demonstrate extensive disruption of surface membranes. Despite the fact that some cells showed plasma membrane destruction to a lacy residuum, it was difficult to be sure that this process was a manifestation of normal exfoliation.
Specific disease processes were clearly identified by distinct morphology shown by SEM. The process of cellular blebbing found in keratoconus was characteristic, often delineating the size and extent of the cone. These outpouchings of the plasma membrane are not artifact since (1) fixatives used were isosmotic; (2) the phenomenon was observed almost exclusively in keratoconus specimens, (3) the outpouchings were not associated with the specific use of contact lenses, (4) blebs have never been found on the surface of normal animal or human specimens prepared identically, and (5) blebs could be found on the surface of a keratoconus button in the fresh state by epi-illumination techniques.

A central question, as yet unexplained, is the nature of these blebs and their occurrence in keratoconus. A destructive process originating in the epithelium was suggested from degenerative changes in basal epithelial cells, basal lamina fragmentation, Bowman's membrane and stromal digestion occurring over the cone. It is noteworthy that in advanced cases of keratoconus involving large areas of thinning, the predominance of blebbing is near the periphery of the cone. This gives some support for the idea that the loss or remodeling of stromal tissue might be linked in some way with the observed blebbing process. The concept that epithelial-collagen-keratocyte interactions could trigger the ectasia is hypothetical at this time. Finally, blebbing of the surface cells may simply represent accelerated cellular breakdown and exfoliation over the cone. Evidence for this is the finding of extensive plasma membrane disruption in some cells with broken blebs.

Epithelial irregularity, cellular edema, and loosely attached surface cells were typical findings associated with corneal edema. It is not surprising that in corneal edema the epithelial surface is uneven with premature desquamation, for the punctate erosion patterns seen with fluorescein and the irregular astigmatism are common. Although continuously worn soft lenses did not improve the surface appearance, it is reassuring that no apparent damage was seen.

Active herpes simplex infections in rabbit cornea are reported to show "pocks" of full-thickness epithelial loss surrounded by rounded and partially detached epithelial and inflammatory cells. In our cases, even a year after subsidence of stromal herpetic keratitis, it appeared that large numbers of epithelial cells were detached from the surface layer, with or without localized areas of heaped-up epithelial and inflammatory cells. Such surface findings are consistent with the persistently unstable tear film usually found in this disease process.

This study shows that the high resolving power of SEM, especially when used in conjunction with TEM, provides additional significant insights into some common human corneal disease processes.

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REFERENCES