The normal surface of conjunctiva epithelium. A scanning electron microscopic study

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Scanning electron microscopy (SEM) of the cellular surfaces of the rabbit conjunctiva show the monotonous appearance of fine, finger-like cytoplasmic protrusions (microvilli) covering the polygonal cells. Many of these surface cells contained a variety of intracytoplasmic vesicles, unroofed vesicles, and full-thickness holes of different sizes. These surface changes were interpreted as occurring in a sequential pattern leading to cell destruction and exfoliation. Interspersed among these light and dark polygonal cells were goblet cells representing various stages in their maturation. Specialized filopodia were found between epithelial cells only in the area just proximal to the tarsi. Duct openings of many glands were shown to enter onto the conjunctival surface. The relation of these findings to the tear film is discussed.

Key words: conjunctiva, peripheral cornea, scanning electron microscopy, exfoliation, goblet cells, filopodia, microvilli, gland orifices.

Secretions of the conjunctiva play important roles in external ocular physiology by contributing to the maintenance of an intact precorneal tear film. Conjunctival goblet cells produce a macromolecular glycoprotein which is thought to partially adsorb to the microvilli of the cornea and conjunctiva making the relatively hydrophobic epithelial surface more wettable. The middle aqueous portion of the tear film is a much thicker layer contributed by the lacrimal and accessory lacrimal glands entering through conjunctival orifices. The outer layer of the tear film, consisting of oily Meibomian gland secretions, effectively retards tear film evaporation.

Another function of the conjunctiva is that of a partial reservoir for the tear film directly on its surface, in the cul-de-sacs, and at conjunctival surface contact points. Additionally, the conjunctiva of the lids frame the precorneal tear film and also help in the distribution of the tears. This is accomplished by the congruity of the tarsal conjunctiva to the globe, assisting the blink mechanism in the even spreading of the tear film periodically over the front of the eye. In the event of total corneal epithelial cell loss, replacement occurs by sliding and then metaplasia of adjacent bulbar conjunctival epithelium. Partial or complete destruction of any of these conjunctival...
Fig. 1. Bulbar conjunctiva showing light, medium, and dark surface cells. A central immature goblet cell is present. A mound is present in the polygonal cell just above the goblet cell. Arrows show two other cells containing full-thickness holes. At the bottom, membranes of a destroyed cell lay at its periphery. x2,100.

Fig. 2. The surface of conjunctiva proximal to the tarsal plates. Note many filopodia interconnecting adjacent surface cells. x2,300.
functions results in varying degrees of corneal disease.

The present study of the surface of the conjunctiva (1) compares the pattern of corneal and conjunctival exfoliation by scanning electron microscopy (SEM), (2) shows the surface of transitional zones of conjunctiva, (3) describes the relative distribution and life cycle of surface goblet cells, and (4) demonstrates the opening of glands into the conjunctival surface.

Materials and methods

Twenty-five normal albino rabbits weighing two to three kilograms were given a lethal intravenous injection of sodium pentobarbital. As soon as the animals became drowsy, 4 per cent glutaraldehyde in Sorenson's phosphate buffer (G-S, pH = 7.2) was instilled into both eyes and the eyelids closed by clamping the upper and lower lashes together with a hemostat. The hemostat was removed and G-S irrigation of the eye was continued while pieces of bulbar conjunctiva (lower nasal quadrant), lower cul-de-sac (medial portion), upper cul-de-sac (temporal portion), nictitating membrane, upper and lower lids (midportion), limbus, and peripheral cornea were rapidly excised using forceps and scissors. The tissues were immediately transferred to a bath of 4 per cent G-S and cut into smaller pieces. Except for solution changes, all processing was performed under refrigeration. Subsequent processing of these tissues was identical to that described in a previous paper. The specimens were dried by carbon dioxide critical point apparatus. Tissues were examined in a Model IV Cambridge stereoscan.

Specimens obtained for transmission electron microscopy (TEM) were prepared using standard techniques. Tissues were embedded in Spur media and serial 0.5 μm sections examined under light microscopy. When indicated, thin sections were cut for TEM. These sections were stained with uranyl acetate and lead citrate and examined in a Philips 300 electron microscope.

Results

Conjunctiva. Under low magnification the surface conjunctival epithelium from all specimens had a polygonal cellular appearance with a shaggy texture very similar to the surface corneal epithelium (Fig. 1). The surface of tarsal conjunctival cells formed a continuous flat sheet with a frequently recurring flower petal arrangement of cells around a central core cell. Interposed between the polygonal cells were randomly distributed goblet cells. The in
in situ appearance of cul-de-sac epithelium showed large rugae which could be stretched out flat by pinning.

Two transitional conjunctival areas showed interesting local adaptations. The junction of tarsal conjunctivae with the upper and lower cul-de-sacs showed adjacent surface epithelial cells loosely interconnected with numerous filopodia (Fig. 2). At the margin of the lid, nonkeratinizing conjunctiva graded into rough keratinized epithelial cells of the skin. Goblet cells were no longer noted as conjunctiva graded into the cornea across the limbus.

Conjunctival surface epithelial cells, like surface corneal cells, could be separated into light, medium, and dark cells on the basis of their brightness as detected by the scanning electron microscope (Fig. 1). These differences were much less pronounced than those in the cornea. The surface of the light cells had about 20 to 30 per cent fewer microvilli per square micron as compared to the dark cells. No specific microvillous length difference could be appreciated between light and dark cells. However, there were more mucin strands and amorphous material on the surfaces of the dark cells compared to the light cells.

Conjunctiva and peripheral cornea. Changes in the surface cells similar to those noted in the central cornea, appeared with greater frequency in conjunctival specimens from all areas (Fig. 1). These changes were noted primarily in dark cells, less frequently in medium cells, and least frequently in light cells. One alteration noted was a mound or elevation of the surface plasma membrane (Fig. 1). Most mounds were about 3 μm in diameter, of round or slightly ovoid shape, lacking microvilli.

Occasionally a mound was seen in the cell surface with a hole through its apex, presumably an opening into the intracellular cyst (Fig. 3). A TEM photograph of a large (3 μm) double membrane-lined cyst is shown in Fig. 4. The anterior portion of
Fig. 5. Partial unroofing of the anterior cyst wall exposed the posterior cyst wall and cavity. Stereo, ×13,000.

Fig. 6. TEM section through an unroofing cyst shows a complete double membrane lining with degenerative changes in the cell. Arrow indicates original cyst cavity. ×9,900.
the cyst membrane is discontinuous as is the cell membrane anterior to the cyst. Loss of intracellular organelles and degenerative changes of the cell cytoplasm were noted. Partial unroofing of a cyst revealed the posterior cyst wall (Fig. 5). TEM of an unroofed cyst showed a complete double membrane lining (Fig. 6). Fig. 7 shows total unroofing of an intra-epithelial cyst. Occasionally a hole through the posterior cyst wall and the posterior cellular membranes revealed the second layer cell microvilli (Fig. 8).

A variety of mounds and full-thickness cellular holes were found together or separately in a single dark cell. There appeared to be more cells with holes in the superficial layer of the conjunctiva and the peripheral cornea as compared to the central cornea. In both cases, the rim of the full-thickness hole was continuous with the cell surface but elevated off the second layer cell. The rim was about 1 μm in thickness and devoid of microvilli.

When a superficial cell contained a moderately large hole, the remaining surface of that cell was remarkably normal in microvillous appearance and apparent tightness of intercellular junctions (Fig. 8). When a hole in a surface cell was so large as to be very close to most intercellular junctions, then that cell appeared completely degenerated (bottom of Fig. 1).

The second layer cell at the base of the hole usually had 20 to 30 per cent fewer microvilli than the overlying hypermature cell. When the surface cell was completely degenerated, the second layer light cell was fully surfaced.

**Limbus.** Goblet cells disappeared from the surface conjunctival epithelium at the junction of bulbar conjunctiva with cornea. The conjunctival pattern of cellular desquamation continued onto the peripheral cornea for several millimeters. The width of the zone on the cornea where the desquamation patterns change was less than one-half a millimeter.
Goblet cells. Goblet cells were found in conjunctiva from all areas, interspersed in clumps among the greater number of polygonal surface cells. Rabbit bulbar conjunctiva showed the fewest number of goblet cells, followed in increasing order by upper and lower cul-de-sac, portions of nictitating membrane, and tarsal conjunctiva. Tarsal goblet cells appeared larger than goblet cells from other areas with the heaviest concentration near the free edge. A heavier concentration of goblet cells was noted on the outer surface of the nictitating membrane. No goblet cells were seen between the anterior free edge and the row of gland orifices. Goblet cells also appeared less frequently nearer the attachment of the nictitating membrane. Goblet cells of bulbar and tarsal conjunctiva were a part of the continuous epithelial surface, filling in between adjacent flatter surface epithelial cells. In the nictitating membrane they appeared to be recessed in shallow crypts.

Small goblet cells were found with relatively flat surfaces and elliptical outlines with short, sparse, microvilli (Fig. 9). Mature goblet cells were more globular and elevated further above the surrounding surface epithelium showing small surface strands and globules, presumably mucin (Fig. 10). Other goblet cells, appearing hypermatured, presented a globular form devoid of microvilli. These goblet cells were covered by a shaggy, uneven, amorphous material having a high secondary electron density (very bright surface). Strands of mucus from discharging goblet cells frequently were noted attached to other goblet cells or to other surface epithelium (Fig. 11). A discharged, collapsed goblet cell retracted beneath the surface leaving a surface defect between cells apparently made smaller by spreading of adjacent surface epithelial cells (Fig. 12). The ragged holes in the apical membrane of the exhausted goblet cell are readily apparent.

Accessory glands. Certain conjunctival surfaces also showed the orifices of accessory glands. Glands opened in a row about one millimeter from the anterior free edge of the rabbit nictitating membrane. Harder's gland had a large opening angling downward into the inferior portion of the nictitating membrane near its attachment. Intra-epithelial net-like glands had numer-
Fig. 9. An immature goblet cell slightly elevated above the surrounding surface with sparse microvilli, ×10,000.

Fig. 10. A mature goblet cell highly elevated above the surface epithelium with small mucous excrecences. Note two red blood cells adjacent to goblet cell. Stereo, ×5,250.
Fig. 11. A strand of mucus connects two goblet cells at different stages of discharge. Note the stoma in the apical membrane of the lower goblet cell. ×11,500.

Fig. 12. The surface remains of an exhausted goblet cell. The ragged surface aperture lies in a depression. Stereo, ×11,000.
ous openings scattered over the upper and lower tarsal conjunctival surface and the nictitating membrane (Fig. 13). These gland openings were formed by surface epithelium lining the entry into the duct. Wolfring's glands open to the surface near the attachment of the tarsi but the orifices could not be differentiated from the glands of intra-epithelial origin. Meibomian gland orifices showed irregular epithelial edges.

When upper and outer cul-de-sac specimens were fixed as stretched preparations, the orifices of two other glands became visible. The opening of a lacrimal tubule was evident showing a small tab at the surface. Saccular gland orifices typically showed a mucinous discharge to the surface (Fig. 14).

Discussion
When superficial conjunctival mucin was partially removed, individual cells of the conjunctival surface, like the cornea, were found to be covered by fine cytoplasmic protrusions (microvilli) in intimate relation to their chemically unremovable "fuzzy coat." These findings are consistent with TEM findings in the human conjunctiva which, in addition, showed occasional small crypts with secretory granules standing in rows close to the plasma membrane of the free surface. These secretory granules may be identical to "membrane coating granules" of a glycoprotein nature (fuzzy coat), found on other free surfaces and between superficial cells of the human oral epithelium.

The sequential development of the holes
in hypermature superficial cells of corneal epithelium has led to a new concept of cellular exfoliation in the rabbit. This controlled resurfacing of new cells with mucin was thought to increase corneal wetting/ability hence lending considerable stability to the precorneal tear film. Since corneal and conjunctival epithelia are embryologically similar tissues, it is reasonable to expect similar exfoliation patterns. Differences from the cornea are apparent. There were frequent multiple mounds and full-thickness holes in individual dark conjunctival cells as opposed to usually a single depression or hole in dark cells of the central cornea. Most full-thickness holes in the surface conjunctival epithelium probably developed initially from intracellular cysts with rounded surfaces. Unroofing of the cyst exposed the interior of the cyst with a subsequent breakdown of cell structure posterior to the cyst. Alternately, a simultaneous breakdown of both anterior and posterior cyst walls could expose the succeeding second layer cell directly. Surface changes and cellular holes were usually found in dark cells while the succeeding cell always appeared as a light cell. It appears that as the cell advances to the surface from the second layer it increases its number of surface microvilli, attains a larger surface area, and becomes darker under the SEM by emitting fewer secondary electrons.

Subsurface vesicles similar to those described in this study have been noted in TEM of the rabbit surface corneal epithelium. "Craters" of surface corneal epithelium examined by SEM were thought to possibly result from collapse of the intracellular vesicle during vacuum dessication. It is probable that the findings of that study correspond to either unroofed vesicles or full-thickness holes described in this paper.

In the present study peripheral cornea and conjunctiva showed unroofing of the anterior cyst wall with some cells showing breakdown of the posterior cyst wall to form full-thickness holes. Unroofing of the
Cyst unroofed or simultaneous anterior and posterior cyst rupture

Full thickness cellular hole with partial exposure of second layer cell

Elevation and rolling of edges of hole

Progressive destruction of surface cell by expanding hole

Second layer light cell surfaced

Fig. 15. Projected exfoliation patterns of the external rabbit eye based on SEM.

cyst did give a craterous appearance to the posterior cyst wall but it was generally smooth with little appearance of any microvilli. It is impossible to know from this study if any of these cysts have functions other than exfoliation in normal, healthy surface cells. A diagram of this concept of exfoliation is summarized in Fig. 15.

The presence of filopodia in the transitional zone of conjunctival epithelium, from tarsus to cul-de-sac, appears to represent a localized adaptation of cellular interconnections not previously described. The possibility exists that long filopodia in that area may expand and contract by their contained filaments. In an area where lid movement causes extensive expansion and compression of conjunctival tissues, filopodia would appear to be a delicate expression of tissue form fitted to function.

The specific surface pattern of the goblet cell life cycle is presented in this study. The time lapse from the secretion of one cell to its next secretion is unknown. However, the time period may be similar to the 82-hour period required by intestinal goblet cells in their maturative migration from the base to the tip of the villus. Individual goblet cells of conjunctiva are thought to have indefinite life-spans. This is based on the fact that goblet cells are apical secretors which show continuous integrity of the main cell body and firm anchoring within the epithelium even in the postsecretory phase (resting stage). The spreading of this continuously produced mucinous goblet cell secretion over the cornea by blinking forms the basis of the current concept of corneal wettability.

The preocular tear film is made up of a continuous composite tear layer over cornea and conjunctiva. A special pattern of exfoliation would be necessary to both tissues to ensure a wettable surface. Those differences which do exist could be explained on the basis of different metabolic environments. However, the particular manner of cell desquamation in the cornea would be expected to be optically superior to its counterpart process in the conjunctiva.
REFERENCES