Effects of Soft Contacts of Differing Thickness on Corneal Wound Healing in Rabbits

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This study was undertaken to determine the effects of thin (60 μm) and thick (240 μm) soft contact lenses of equal water content (70%) and power on nonlesioned and lesioned rabbit corneas. In one group of animals, corneas were not lesioned. Thin lenses were placed on left corneas and thick lenses on right corneas. In a second group, lesions were made in both corneas. Left corneas were covered with thin lenses and right corneas with thick lenses. Post-treatment times were 8 hr and 24 hr. At sacrifice, one-half of the cornea was fixed in 4% buffered glutaraldehyde for SEM study. The other half was cut into segments, fixed in a buffered glutaraldehyde–ruthenium red (RR) solution post-osmicated in osmium containing RR and prepared for TEM. At both 8 hr and 24 hr SEM showed cell migration in lesioned corneas covered with thin lenses but not in lesioned corneas covered with thick lenses. At 8 hr, TEM of nonlesioned and lesioned corneas showed no changes in the thickness of the corneal epithelium or the RR staining of the surface. At 24 hr, in nonlesioned corneas covered with thick lenses, the RR staining of microvilli and the height of the corneal epithelium were less than in nonlesioned corneas covered with thin lenses. In lesioned corneas covered with thick lenses, the thickness of the cornea was markedly reduced, the RR staining of microvilli was less and basal cells were more compressed than in lesioned corneas covered with thin lenses. The results of this study indicate that the thickness of a soft contact lens is important in treating corneal trauma. Invest Ophthalmol Vis Sci 30:2138-2147, 1989

A study of epithelial abrasion in rabbits using scanning electron microscopy showed that the immediate response to injury was separation and thickening of the basal and squamous epithelial cells at and near the margin of the wound. After 15 hr most of the epithelial cells at the wound margin were extensively flattened and showed a wide variety of surface ruffling and filopodia. Corneal reepithelialization proceeded with two or three cell layers moving over the basal lamina.1 Using the combined techniques of scanning and transmission electron microscopy, Kuwabara et al2 studied the healing process after superficial linear wounds of the rabbit cornea. The initial step of healing was the sliding of the epithelial cells into the tissue defect. The sliding cells extended fine processes into the tissue defect and eventually transformed into basal cells, with a thin basement membrane forming within a few days.

Past studies in our laboratory on corneal wound healing in rabbits have shown that cell migration is present at 16 to 24 hr.3 This migration was inhibited by placing soft contact lenses, containing 39% water with a low O₂ transmissibility (8.9 × 10⁻¹⁹ DK/L) over the lesioned corneas. In that same study soft contact lenses containing 58% water with an O₂ transmissibility (4.3 × 10⁻⁹ DK/L) did not inhibit movement.4 It is generally agreed that oxygen is the only metabolic requirement offered to the cornea from the tears, and other nutrients are supplied by the aqueous humor and limbal blood vessels.5,6

The precorneal tear film is an important functional unit in the physiology of the cornea. The mucous layer of the tear film, composed of hydrated mucopolysaccharides, rich in sialo-mucin, is intimately attached to the corneal epithelium.7 Studies suggest that the tear film is held in position against the epithelial surface of the cornea by a surface specialization known as microvilli and microplicate, which ultrastructurally exhibit a cell coat known as a glycocalyx8 that can be demonstrated with transmission electron microscopy by using the cationic dye ruthenium red (RR).9 This structural framework for tears is critical for the stability of the precorneal tear film which in turn is related to the health of the corneal and the healing process following injury.8

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The purpose of the present work was to study the effects of soft contact lenses of differing thickness, but of equal water content, on corneal wound healing in rabbits. Scanning electron microscopy was used to observe the margin of the lesion as related to epithelial cell migration and transmission electron microscopy was used to study the cellular responses of the epithelial cells and their surface glycocalyx.

Materials and Methods

Adult New Zealand white rabbits, averaging 2.75 kg in weight, were anesthetized with ketamine, 0.5% ophthalmic drops (proparacaine hydrochloride ophthalmic solution) placed in the eyes, the nictating membranes removed and soft contact lenses were immediately placed over each eye. The lenses were of equal water content (70%) but differing thicknesses classified as thin (60 μm in thickness) and thick (240 μm in thickness). The lenses have a radius of 7.15, a DK at room temperature of 311011 and are approximately −3.50 diopters in power. These data are comparable to those reported for 2.75 kg animals in a study designing hydrophilic contact lenses for eye research using rabbits.10

In one group of six animals corneas were not lesioned. Thin lenses were placed on left corneas and thick lenses on right corneas. In a second group, trephine lesions 7.5 mm in diameter were marked centrally with an ocular trephine in both corneas and the epithelium was then denuded, leaving the basement membrane intact. The left cornea was covered with a thin lens and the right cornea with a thick lens. Post-treatment time periods were 8 and 24 hr.

At sacrifice the cornea was flooded with 4% glutaraldehyde, buffered with cacodylate, pH 7.4, and the same fixative was injected posterior to the cornea at the limbus. The cornea was then removed and halved. One half was prepared for study with the scanning electron microscope (SEM) and the other half was sectioned radially into segments and prepared for transmission electron microscopy (TEM). Since the contact lenses covered the entire corneal surface, all corneal samples studied, both SEM and TEM, were from covered areas. Half the cornea showing the lesion, central and peripheral areas was studied with SEM. The area immediately peripheral to the edge of the lesion was sectioned and studied with TEM.

Samples for SEM study were fixed in 4% glutaraldehyde, buffered with cacodylate, pH 7.4, post-osmicated in 2% osmium tetroxide, buffered with cacodylate, pH 7.4, dehydrated in graded acetones, critical point-dried and coated with gold–palladium alloy. Samples for TEM study were fixed in 1.2% glutaraldehyde buffered with 0.067 M sodium cacodylate, pH 7.3, containing ruthenium red in a final concentration of 0.001 g/ml with 0.1% CPC. They were rinsed in 0.15 M sodium cacodylate buffer and post-fixed in 1.67% osmium tetroxide buffered with 0.067 M sodium cacodylate, pH 7.3, containing ruthenium red in a final concentration of 0.001 g/ml. The samples were dehydrated in graded alcohols and embedded in Spurr low-viscosity resin. Thick plastic sections were stained with toluidine blue and viewed with the light microscope for orientation. When the entire epithelial layer was seen, ultrathin sections were obtained and mounted on uncoated grids. Some grids were stained with uranyl acetate and lead citrate and others were left unstained so as to assess the ruthenium red reaction for glycosaminoglycans (GAGs).

The investigations using animals in this study conform to the ARVO Resolution on the Use of Animals in Research.

Results

Scanning Electron Microscopy

For purposes of comparison, all scanning electron micrographs are shown at the same magnification. A lesioned cornea covered with a thin lens at 8 hr is shown in Figure 1. Signs of some cell migration are demonstrated by the presence of a few filopodia and the fact that the edges of the cells show a decrease in microvilli. A lesioned cornea covered with a thick lens at 8 hr is shown in Figure 2. The margins of the cells are rounded and there are no signs of filopodia. Cell migration is not apparent. A lesioned cornea covered with a thick lens at 24 hr is shown in Figure 3. Cell margins show ruffling, decreased microvilli and numerous filopodia indicating cell migration. A corneal lesion covered with a thick lens at 24 hr is shown in Figure 4. Rounded-up cells are shown along the margin of the lesion. Cell migration is not apparent.

Transmission Electron Microscopy

For purposes of establishing the staining reaction of ruthenium red, transmission microscopic observations were made of cornea stained only with ruthenium red (Fig. 5a). The presence of glycosaminoglycans (GAGs) is demonstrated by the staining reaction of the surface glycocalyx. In Figure 5b the cornea has been stained with ruthenium red followed by staining with uranyl acetate and lead citrate. When comparing these two figures, it is seen that the ruthenium red is responsible for the staining reaction at the surface of the cornea that represents glycosaminoglycans.

In the following pairs of transmission electron mi-
Fig. 1. SEM showing the margin (arrows) of a corneal lesion covered with a thin lens at 8 hr. The cell margins show signs of migration such as decreased microvilli and a few filopodia. ×1200.

crographs, for purposes of comparison, all low-power micrographs are shown at the same magnification and all high-power micrographs are shown at the same magnification. All corneas in all groups were measured with a metric rule, allowing relative increases and decreases in width to be noted. Since all samples were processed for transmission electron microscopy in the same manner, changes in width are not related to fixation. There was very little difference, when comparing the nonlesioned corneas covered with thin (Fig. 6a, b) and thick lenses (Fig. 7a, b) at 8 hr.

Fig. 2. SEM showing the margin of a corneal lesion covered with a thick lens at 8 hr. The cell margins are round and blunt exhibiting no signs of cell migration. ×1200.
In a nonlesioned cornea covered with a thin lens at 24 hr (Fig. 8a, b) the superficial, middle and basal cells of the cornea are distinguishable and the surface glycocalyx is heavily stained. When the nonlesioned cornea covered with a thick lens at 24 hr (Fig. 9a) is compared with that covered with a thin lens (Fig. 8a), it is seen that there is a slight decrease in the width of the corneal epithelium. In addition, the staining reaction of the surface glycocalyx shown in Figure 9b appears less than that shown in the nonlesioned cornea with the thin lens in Figure 8b.

Transmission electron micrographs of a lesioned cornea covered with a thin lens at 8 hr are shown in Figure 10a, b. The superficial and basal cells are dis-
Fig. 5. (a) TEM of cornea stained only with ruthenium red showing the affinity of the surface glycocalyx (arrow) for glycosaminoglycans. ×30,000. (b) TEM of cornea stained with ruthenium red and counterstained with uranyl acetate and lead citrate showing the staining reaction of the surface (arrow). ×30,000.

Fig. 6. (a) TEM of a nonlesioned cornea covered with a thin lens at 8 hr. The superficial (S), middle (M), and basal (B) cells are distinguishable. ×4575. (b) Area outlined in (a) shown at a higher magnification to demonstrate the staining reaction of the surface glycocalyx (arrows). ×33,625.
Fig. 7. (a) TEM of a nonlesioned cornea covered with a thick lens at 8 hr. The superficial (S), middle (M), and basal (B) cells are distinguishable. X4575. (b) Area outlined in (a) shown at higher magnification to demonstrate the staining reaction of the surface glycocalyx (arrow). X33,625.

tinguishable and the surface glycocalyx is well stained. When the lesioned cornea covered with a thick lens at 8 hr (Fig. 11a) is compared with that covered with a thin lens at eight hours (Fig. 10a), it is seen that the widths of the corneal epithelium are approximately the same. The staining reaction of the surface glycocalyx shown in Figure 11b, however, appears greater than that shown in Figure 10b.

A lesioned cornea covered with a thin lens at 24 hr is shown in Figure 12a and b. Superficial and basal cells are distinguishable and the surface glycocalyx is well stained. When the lesioned cornea covered with a thick lens at 24 hr (Fig. 13a) is compared with that covered with a thin lens (Fig. 12a), it is seen that the width of the corneal epithelium is slightly less. The staining reaction of the surface glycocalyx shown in Figure 13b also appears less than that shown in the lesioned cornea with the thin lens in Figure 12b.

**Discussion**

Past studies in our laboratory on corneal wound healing in rabbits have shown that cell migration is present at 16 to 24 hr. Our observations agreed essentially with the work reported by Pfister. He, too, used an epithelial lesion centrally placed with a trephine. At 15 hr following injury, he described the characteristics of cell migration. More recent studies in our laboratory showed this migration was inhibited by placing soft contact lenses containing 39% water with a low O₂ transmissibility (8.9 × 10⁻¹0 DK/L) over the lesioned cornea. In that same study soft contact lenses containing 58% water with an O₂ transmissibility (4.3 × 10⁻⁹ DK/L) did not inhibit movement. A study of the effects of soft contacts on long-term corneal wound healing, 72 and 120 hr, in rabbits showed that by 120 hr reepithelialization of 7.5 mm centrally placed trephine lesions was complete. In the present study it is apparent that the thickness of a soft contact lens is a factor influencing corneal healing following trauma.

Corneal epithelium has a high oxygen demand to support the aerobic metabolism indispensable for the cornea to maintain its transparency. Atmospheric oxygen is necessary to prevent an accumulation of lactate in the cornea. A shift of aerobic to anaerobic
metabolism results in an accumulation of lactate. This is accompanied by disappearance of epithelial glycogen, swelling of corneal epithelium and impairment of corneal transparency.\textsuperscript{14} Glycogen depletion of epithelium is concomitant with ATP depletion with anoxia and epithelial trauma related to wearing contact lenses.\textsuperscript{15}

The atmospheric oxygen required by the corneal epithelium is dissolved in the tear film, when the eye is open.\textsuperscript{16} The tear film, in fact, is the major source of oxygen for the cornea.\textsuperscript{17} The microvilli and micropli- cacae of the superficial corneal cells greatly augment the free surface area, therefore increasing tear film retention. The glycoproteins absorbed to the surface of these cell surface specializations are essential contributors to tear film stability.

The results of the present study show that the thin soft contact lens (60 \(\mu\)m in thickness) is more beneficial to the healing of corneal wounds than the thick lens (240 \(\mu\)m in thickness). At 8 hr there was little observable difference between lesions covered with thick or thin contact lenses. This may be related to epithelial closure in the rabbit cornea being described as a biphasic process consisting of an initial latent phase with no epithelial movement followed by a linear healing phase.\textsuperscript{17} At 24 hr the lesions covered with the thin lenses were healthier when compared with those covered with the thick lenses. With the thin lenses the epithelial cells appear less vacuolated; thus, less edema is present. The cellular structure and thickness of the cornea is more comparable to that of the unlesioned cornea. With a thick lens over the lesion the basal cells are flattened or compressed. Instead of the long axis of the cell being vertical to the corneal surface, it is parallel. Changes in the basal cell layer primarily account for the decrease in corneal thickness at this time period with the thick lens.

Cellular migration of surface cells about the lesion margin is evidenced by filopodia and ruffled cell margins. With a thick lens over the lesion rounded
Fig. 9. (a) TEM of a nonlesioned cornea covered with a thick lens at 24 hr. The superficial (S), middle (M), and basal (B) cells are distinguishable. ×4575. (b) TEM of corneal surface in (a) shown at higher magnification to demonstrate the staining reaction of the surface glycocalyx (arrow). ×33,625.

Fig. 10. (a) TEM of a lesioned cornea covered with a thin lens at 8 hr. The superficial (S) and basal (B) cells are distinguishable. ×4575. (b) Area outlined in (a) shown at higher magnification to demonstrate the staining reaction of the surface glycocalyx (arrow). ×33,625.
Fig. 11. (a) TEM of a lesioned cornea covered with a thick lens at 8 hr. The superficial (S) and basal (B) cells are distinguishable. ×4575. (b) Area outlined in (a) shown at higher magnification to demonstrate the staining reaction of the surface glycocalyx (arrow). ×33,625.

cells are apparent at its margin. Apparently the level of anoxia is such that there is an insufficient level of ATP to metabolically drive the contractile proteins involved in cell motility. Another important observation was that of the greater staining reaction of the external corneal surface cells with ruthenium red.

Fig. 12. (a) TEM of a lesioned cornea covered with a thin lens at 24 hr. The superficial (S) and basal (B) cells are distinguishable. ×4575. (b) Area outlined in (a) shown at higher magnification to demonstrate the staining reaction of the surface glycocalyx (arrow). ×33,625.
This indicates the presence of glycoproteins providing stability to the tear film and providing oxygen to the cornea to promote the healing process.

**Key words:** cornea, scanning electron microscopy, wound healing, transmission electron microscopy, ruthenium red

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**References**