Effect of a Collagen Shield on Cat Corneal Epithelial Wound Healing

George J. Shaker, Shunsuke Ueda, Joseph A. LoCascio, and James V. Aquavella

We have previously demonstrated that a corneal bandage lens made from porcine scleral collagen may be useful in treating various ocular surface problems. In order to determine whether the collagen shield would accelerate epithelial healing, a 7 mm diameter circular area in the center of the left cornea of ten domestic cats was mechanically deepithelialized. In five of the cats, a 14.5 mm non-cross-linked collagen shield was then placed on the cornea covering the wound. Another shield was applied 24 hr after surgery. The wound size was determined immediately after surgery and at 8-hr intervals until wound closure. Using analysis of variance for experiments with repeated observations, there was a significantly greater healing response in the treated group than in the control group. There was, however, no significant difference in slope between the two groups, suggesting that the shield did not increase the speed of epithelial cell migration. Rather, the effect of the shield was most pronounced during the first 8 hr after wounding. In contrast to that of the treated group, the mean defect radius of the control group was larger at t = 8 hr than at t = 0 hr. The earlier wound closure exhibited by the treated group, which may be due to protection and lubrication of the epithelial cells at the margins of the fresh wound, suggests that the collagen shield may be useful in treating corneal surface conditions of which deepithelialization is a component. Invest Ophthalmol Vis Sci 30:1565-1568, 1989

Materials and Methods

Ten 2.5 to 3.5 kg domestic, female cats were divided into two groups with five in each group. Twenty minutes after administration of atropine (0.04 mg/kg subcutaneously) and acepromazine (0.1 mg/kg IM), general anesthesia was induced with ketaset (ketamine HCI 2.5 mg/kg IM, Veterinary Products, Bristol Laboratories, Syracuse, NY). Two drops of 0.5% proparacaine hydrochloride were then instilled into the surgical eye. The cats were subjected to mechanical debridement of a 7 mm diameter circular area of epithelium over the central cornea of the left eye. A 7 mm trephine was used to demarcate the area to be debrided. The ocular surface was stained with fluorescein to ensure the completeness of the debridement. In five of the ten cats, a 14.5 mm non-cross-linked collagen shield with a 9 mm base curve (Bausch and Lomb Pharmaceuticals, Inc., Clearwater, FL) was fit-
Fig. 1. Photograph of cat eye immediately after epithelial debridement and placement of collagen shield. White arrowhead indicates edge of collagen shield and black arrowhead points to margin of underlying epithelial wound.

ted over the wounded cornea (Fig. 1). The shields were made from porcine scleral tissue and as such consisted primarily of type I collagen with a small amount of type III collagen and were the only type of shield available at the time of this study. They were sterilized by exposure to gamma radiation. The non-cross-linked shields used were designated 12-hr shields based on their expected dissolution time. Their thickness ranged from 12.7 μm peripherally to 71.0 μm centrally.

All subjects were observed every hour for 4 hr. The status of the shields was carefully monitored at these observation periods. Shields were replaced every 24 hr postoperatively until wound closure.

In order to prevent mechanical injury of the ocular surface, an Elizabethan collar (#412 Saf-T Shield 12", EJAY International, Inc., Glendora, CA) was placed on all cats immediately after surgery and left in place until wound closure.

Clinical observations were recorded and photographs of the fluorescein-stained defect were taken every 8 hr from the time of surgery until complete wound closure. The photographs to measure wound size were along the visual axis. Particular attention was paid to the status of the cornea, conjunctival injection and edema, and to the presence or absence of exudate. Two drops of Gentamicin sulfate solution were applied to all surgical eyes immediately after surgery and then every 6 hr. All experiments were carried out in accordance with the ARVO Resolution on the Use of Animals in Research.

Wound size was determined by projecting a photograph of the fluorescein stained defect. The image of the defect was traced and the area determined using a Zeiss MOP-3 image analyzer (Carl Zeiss, Inc., Thornwood, NY). In order to standardize the magnification of the defect for all subjects at all observation periods, the focusing position was fixed for all photographs.

In order to make the healing curve more linear, the wound area was converted to the spherical radius using the equation described by Crosson et al.10

Statistical Analysis

Linear regression analysis was performed for each subject so that an estimate of slope, which reflects the speed of cell migration, and expected healing time could be determined. Analysis of variance for experiments with repeated observations was performed to assess the overall response to treatment.11

Results

The collagen shields were observed to have lost their structural integrity by 4 hr after application. Only collagenous film over the ocular surface was evident in two eyes at 2 hr, in one eye at 3 hr, and in two eyes at 4 hr. There was no evidence that any of the shields had fallen out.

There was mild to moderate conjunctival edema and injection in all of the eyes in both groups during the first 24 to 48 hr after surgery with no apparent differences between groups. There was no evidence of infection in any of the eyes.

Wound closure occurred sooner in the treated than in the control group, as is reflected in the graph of defect radius vs. time (Fig. 2). During the first 8 hr, the size of the defect increased in four of the five control eyes while it decreased in four of the five treated eyes.

Simple linear regression of each subject's healing curve using the spherical radius data beginning at 8 hr after surgery yielded a mean coefficient of determination of 0.93 ± 0.09 for the control eyes and of 0.98 ± 0.01 for the treated group, in both cases suggesting strong linearity. Regression analysis yielded a mean slope of -0.10 ± 0.03 mm per hour for the control group and -0.11 ± 0.01 for the treated group. There was no significant difference in slope between the two groups. The mean expected healing time for the control group was 49.32 ± 10.22 hr and for the treated group was 37.97 ± 4.07 hr using individual regression analysis.

The time factor in analysis of variance for experiments with repeated observations evaluates all of the
data together with respect to the trend over time. As expected, starting at 8 hr, there was a statistically significant trend toward decreasing wound size \( (P < 0.0002) \). With respect to each group's trend over time (slope), the two groups did not differ significantly (time:group interaction: \( P = 0.67 \)). The group analysis evaluates differences among the means of the two groups. The \( P \) value of 0.02 indicates, overall, a significantly greater healing response in the treated group than in the control group.

**Discussion**

As a major connective tissue protein in animals, collagen accounts for approximately 25% of the total protein in vertebrates.\(^{12}\) Collagen has gained much popularity as a biomaterial due to various mechanical, chemical and biological properties. Most applicable to its use as an ocular surface bandage are the ability to control collagen cross-linking, collagen's low antigenicity and its potential effect on wound healing.\(^{13}\) In ophthalmology, collagen has been used to make scleral buckling devices,\(^{14}\) lacrimal drainage plugs\(^{15}\) and an external eye patch.\(^{16}\)

The shields used in this experiment consist of non-cross-linked tropocollagen molecules. Exposure to UV irradiation causes cross-linking between tropocollagen molecules and is being used to produce shields that are more resistant to dissolution and therefore retain their structural integrity for a longer period of time. Shields lasting up to 72 hr are currently being produced. Shield dissolution appears to involve both enzymatic and mechanical action. Agitation in phosphate-buffered saline for up to 2 weeks resulted in no visible changes to the shields. The rate of shield dissolution probably depends on several host factors, including tear volume, tear enzyme concentration, degree of inflammation and blink rate. Differences in these and possibly other parameters may explain the variability in dissolution rate observed in this study.

All eyes exhibited mild to moderate conjunctival injection and edema postoperatively. Since there was no apparent difference between the control and treated groups in this respect, these inflammatory changes were attributed to the wounding itself and not to the collagen shields. In a clinical study in which a collagen shield was applied one time either postoperatively or for corneal epitheliopathy, all of 55 patients were observed to have tolerated the shield well.\(^{8}\) Although additional studies will be necessary to evaluate longer-term tolerance to the shields, previous experience with collagen preparations in ophthalmology are encouraging in this regard.

We observed more rapid wound closure in the treated corneas. The fact that there was no significant difference between the slopes of the two groups suggests that the actual rate of migration of epithelial cells was not accelerated. Rather, the effect of the shield was most pronounced during the first 8 hr after debridement. Damage to cells outside the trephination mark was present at 8 hr in four of the control subjects compared to only one experimental subject. The stimulus in both instances was the same, suggesting that the collagen shield may act as a bandage lens and as a lubricant, in both ways protecting the margins of the freshly debrided wound from further damage. It must also be considered that the shields used in this study dissolved very rapidly after surgery and that new shields were not applied until 24 hr after surgery. It will be interesting to see whether longer-acting shields will affect the healing curve differently.

It is of interest that the healing curves observed in this study appeared biphasic, a phenomenon which was reported by Crosson et al\(^{10}\) to occur after mechanical epithelial debridement of the rabbit cornea. Although we did not attempt to determine its duration due to insufficient observation points, there appeared to be an initial latent phase followed by a linear healing phase. The high coefficients of determination obtained applying simple linear regression to the data beginning at the 8 hr observation period support this concept.

The principle of treating ocular surface disease with a bandage lens is not new. One important therapeutic benefit appears to be the reduction of mechanical trauma to the epithelium, secondary either to lid abnormalities or to normal blinking, which may reduce pain and potentiate the process of epithelial adhesion to the underlying tissue.\(^{2}\) Under normal
conditions, regenerating epithelium may require 1 week or more to form strong adhesions to underlying basement membrane and up to 3 weeks to adhere to underlying tissue if the basement membrane is not intact.\(^2\)\(^3\) During the last 20 years, hydrophilic bandage lenses have been used with variable success depending on the condition being treated.\(^2\)\(^3\)

In the development of hydrophilic lenses, great attention has been paid to oxygen diffusibility through the lens. By reducing the thickness and increasing the water content of hydrophilic lenses, oxygen diffusion has improved significantly. Still, reduced oxygen tension at the corneal surface remains a concern with the use of therapeutic lenses, since they are usually worn 24 hr/day, which requires wear with lid closure. While the partial pressure of oxygen in the tear film of an eye with open lids is approximately 155 mm Hg, lid closure reduces this pressure to 40 to 50 mm Hg.\(^2\) Epithelial hypoxia may contribute to the corneal neovascularization which can complicate hydrophilic lens wear.\(^4\)\(^1\)\(^8\) We expect that unlike that through standard hydrophilic bandage lenses, the oxygen diffusibility through the collagen bandage lens may increase over time as the collagen biodegrades.

Contrary to our results with the collagen shield, hydrophilic bandage lens wear was found to reduce significantly the rate of corneal epithelial wound healing in rabbits,\(^1\)\(^9\) although the effect of a wide range of therapeutic bandage lenses on epithelial healing needs to be more fully investigated.

Infection is an infrequent, but potentially serious, complication of hydrophilic lens wear, particularly in the presence of epithelial or stromal defects.\(^2\)\(^4\)\(^5\) As a soluble lens, the collagen shield is designed for one-time application and to be replaced after dissolution. It is conceivable that reduced handling and the decrease in the duration of wear of each individual shield may result in a decreased rate of infection, although this question will require further study.

The natural biodegradability and high absorptive capacity\(^2\)\(^0\) of collagen suggest that the collagen shield may be useful as a drug delivery agent. Collagen shields presoaked in tobramycin solution have been found to result in higher drug levels both in corneal tissues and aqueous humor than subconjunctival injection of tobramycin both 1 and 4 hr after administration.\(^2\)\(^1\)

In summary, we observed that the collagen shield accelerated corneal epithelial wound closure in cats. This effect, which may be due to lubrication and protection of the corneal surface, and the possible role as a drug delivery device, suggest that the collagen shield may be useful in treating various ocular surface conditions.

Key words: epithelial wound healing, cat cornea, corneal bandage lens, collagen, epithelial wound size analysis

Acknowledgment

The authors gratefully acknowledge the assistance of Manuel del Cerro, MD in reviewing the manuscript.

References