The Effect of Collagen Shields on Rabbit Corneal Reepithelialization After Chemical Debridement

Jeffrey D. Robin, Cheryl L. Keys, Loree A. Kaminski, and Marios A. G. Viana*

We evaluated the effect of precarved collagen lenses on the kinetics of epithelial wound healing in an experimental model of corneal erosions. After induction of anesthesia, central corneal erosions of 5-mm diameter were created in New Zealand white rabbits using n-heptanol. Animals were randomly assigned either to the treatment group or to one of three control groups. Each animal in the treatment group received a precarved collagen shield made from porcine sclera. Immediately after creation of the corneal epithelial defects, topical fluorescein sodium was applied, and the corneas were photographed. Similar follow-up examinations were conducted at 5, 24, 30, 48, 72, and 96 hr after defect creation. Epithelial defect areas were calculated by projecting the photographic slides onto a computerized digitizing pad. Reepithelialization kinetics were compared for the four treatment groups. When initial wound size was taken into account, no significant difference between mean reepithelialization rates was noted. These results indicate that collagen lenses do not adversely affect the speed of corneal reepithelialization, and may, because of their documented biodegradability and drug delivery capability, be useful in the clinical management of corneal epithelial erosions. Invest Ophthalmol Vis Sci 31:1294-1300, 1990

Defects of the corneal epithelium are encountered frequently in clinical ophthalmology. They may occur after trauma or ocular surgery, as well with corneal exposure, keratoconjunctivitis sicca, or other ocular surface diseases. Because the corneal epithelium forms a physical barrier to the penetration of most microbial organisms, epithelial defects may allow for the initiation of corneal infections, stromal melting, and perforation. Therefore, the goal of treatment for these defects is to reestablish an intact epithelial layer as rapidly as possible.

Recently, precarved corneal collagen shields made from porcine sclera or bovine tendon have been introduced to the ophthalmic market. These biodegradable contact lenses have been used primarily as ocular lubricants and to provide postsurgical comfort. The results of clinical studies have suggested that collagen shields may have a positive effect on the rate of corneal epithelial wound healing, as well as on the morphology of healing epithelial cells, in a variety of medical and surgical conditions. Aquavella and colleagues have demonstrated that the use of collagen shields in an experimental model of corneal epithelial and stromal wounds provided for increased epithelial cell differentiation. Additionally, Shaker et al recently demonstrated that, in an experimental animal model, the use of collagen shields after mechanical debridement did not significantly increase corneal reepithelialization rates. In an attempt to characterize further the relationship between collagen shields and corneal epithelial healing, we investigated the effects of these lenses upon healing kinetics in an experimental model of chemically debrided corneal epithelial wounds.

Materials and Methods

Experimental Design

For this study, 64 young adult New Zealand White rabbits (2–3 kg) of either sex were used. All animals were housed at the Biological Resources Laboratories of the University of Illinois College of Medicine at Chicago. Animal care and housing were in compliance with the ARVO Resolution on the Use of Animals in Research.

Each of the 128 eyes was randomly assigned to one of the four treatment groups. The treatment groups...
used in the study were as follows: 1) balanced salt solution (BSS; Alcon, Ft. Worth, TX); 2) topical combination antibiotic ointment, consisting of polymyxin B sulfate, neomycin sulfate, and bacitracin zinc (Ocutricin®; Pharmacia, Hauppauge, NY); 3) excision of the nictitating membrane (NM) plus Ocutricin® ointment; and 4) precarved porcine collagen shield plus NM excision and Ocutricin® ointment. The rationale for the use of antibiotic ointment and NM excision involved the threat of infectious keratitis in this experimental model and the possible mechanical effects of the NM on both the shield and the epithelial defect. We elected not to include an occlusion group, namely tarsorrhaphy, since in preliminary experiments we consistently noted corneal epithelial edema and breakdown 48–96 hr after central tarsorrhaphy (unpublished data). We believe that this is related to the marked corneal edema that results from the severe hypoxic stress placed on the cornea by occlusion in this animal model.7

**NM Excision**

For animals in groups 3 and 4, the NMs were excised immediately after the biomicroscopic examination. Upon the induction of general and application of topical anesthesia, the apex of the membrane was grasped with toothed forceps and pulled laterally. The base of the triangular membrane was clamped with a curved hemostat and the membrane then was sharply dissected above the level of the clamp. Hemostasis was achieved with cauterization of the excised surface. The extent of the excision was such that the remaining base of the membrane could not slide over the cornea, and thus was not able to dislodge the collagen shield.

**Epithelial Defect Creation**

Animals were anesthetized with intramuscular injections of a 1:1 mixture of ketamine hydrochloride 25 mg/kg (Ketaset®; Aveco, Fort Dodge, IA) and xylazine hydrochloride 5 mg/kg (Gemini®; Rugby Laboratories, Rockville Centre, NY), as well as with topically applied proparacaine hydrochloride 0.5% (Ak-Taine®; Akorn, Abita Springs, LA). Baseline biomicroscopic examinations were then performed on each animal, in order to rule out preexisting corneal abnormalities.

For this study, circular epithelial wounds were created in each eye using a method previously described by Cintron et al. Briefly, a no. 50 filter paper disc (Whatman, Maidstone, England), 5 mm in diameter, was soaked in n-heptanol (Sigma, St. Louis, MO), blotted, and then applied to the central cornea of one eye for 30 sec. The disc then was removed and the eye was thoroughly irrigated with 10 ml of BSS applied dropwise.

**Documentation of Epithelial Defect Size**

Immediately after creation of the epithelial defects, topical fluorescein sodium was applied in order to visualize the defect area. This staining method was preferred because topically applied fluorescein has been shown not to adversely affect corneal epithelial healing. The tip of a fluorescein-impregnated strip (Fluoret®; Smith & Nephew, Romford, England) was moistened with BSS and the stain was applied directly onto the inferior bulbar conjunctiva. The eyelids were manually closed, in order to spread the stain, and the ocular surface was rinsed with sterile saline.

The corneas were photographed on Ektachrome 100 ASA color slide film (Eastman Kodak, Rochester, NY) with a 35-mm camera (Nikon, Tokyo, Japan) equipped with a 1:1 macrofocusing lens (Kiron, Tokyo, Japan). A Kodak Wrattan No. 47A (cobalt blue) excitation filter (Eastman Kodak) was attached over the strobe in order better to define the stained region of the defect. The animal's head was held stationary during photography. The camera was positioned directly in front the animal's eye and was stabilized using a tripod. Each photograph included a label with the animal's number for slide identification and a millimeter ruler for slide calibration.

**Collagen Shield Hydration and Insertion**

Precarved porcine collagen shields (Bio-Cor® 72 hr; Bausch & Lomb, Clearwater, FL) were custom manufactured to fit the corneas of this animal model (base curve 9.0 mm, diameter 10.0 mm). The collagen in these shields is cross-linked to the degree that dissolution in vitro occurs in approximately 72 hr. Each shield was hydrated by filling the bowl of its case with BSS for approximately 1 min. The shield was placed on the ocular surface of each animal in group 4 with blunt-tipped forceps. After placement, the shield and the eye both were hydrated with BSS.

**Follow-Up Examinations**

For follow-up examinations, the animals were sedated and topically anesthetized as described above. Each animal's corneas were stained and photographed at 5, 24, 48, 72, and 96 hr after defect creation. After photography, antibiotic ointment was reapplied to each eye in groups 2, 3, and 4. Additionally, for each group 4 eye, a cotton swab was used to displace the collagen shield from its position on the central cornea, in order to permit biomicroscopic ex-
amination and photographic documentation of the defect area; the undissolved portion of the shield then was repositioned over the defect.

**Calculation of Epithelial Defect Area**

The resultant 35-mm slides were projected from a fixed distance onto a digitizing pat (Summagraphics Bit-Pad II Digitizer; ADTech, Park Ridge, IL) linked to a personal computer. After calibrating for the magnification of each projected slide, a hand-held cursor was used to trace the outlines of the epithelial wounds onto the bit-pad. Each slide area was traced by a masked observer a minimum of three times. Using a personal computer, epithelial defect areas were calculated and recorded.

**Statistical Analysis**

Because epithelial healing appears to be a nonlinear function, and the initial size of the epithelial defect may directly affect the rate of subsequent healing, we considered the differences in initial mean defect size among the respective treatment groups to be a potentially significant experimental factor when comparing the healing rates. The statistical analysis required the estimation of a suitable model of epithelial healing; the identification of the healing rate parameters; and the statistical comparison of such parameters. In addition, the analysis considered the adequacy of modeling the results according to the following parameters: the area of the defect (S); the radius of the defect (R); the total period of time needed to close the defect; and the "healing phase" of the reepithelialization process.

The initial levels of defect area among the four groups were compared using an analysis of variance (ANOVA) procedure. We then estimated an exponential model of reepithelialization, including the latent phase. This was accomplished by estimating the usual linear regression of the natural log of the defect area (ln S) with time (t).

There were two reasons for adopting the ln S transformation. First, if the derivatives dS/dt were proportional to S at the start of the healing process, then it would suggest an exponential decay of wound areas. Second, if the linear relationship between ln S and t were examined the decay of both the wound area and wound radius with time. Reepithelialization rates during the healing phase were estimated by linear regression analysis of both of these functions. When divided by the square root of $\pi$, the parameter $a_1$ in the model

$$S^{1/2} = a_0 + a_1t$$

represents the rate of wound radius closure with time. The statistical comparisons of these estimated slopes were performed after the m-group analysis for comparing linear regression coefficients.

**Results**

Biomicroscopic examinations, prior to creation of the defects, revealed no corneal abnormalities. The initial wounds were quite irregular in shape and had areas ranging from 17.50 to 41.36 mm², with mean wound areas (Table 1) among the four treat-

### Table 1. Comparison of defect areas and healing rates at $t = 0$

<table>
<thead>
<tr>
<th>Group*</th>
<th>n</th>
<th>Mean ± SE</th>
<th>Median</th>
<th>ds/dt†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>26.80 ± 0.60</td>
<td>26.30</td>
<td>-0.20</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>28.04 ± 0.75</td>
<td>27.90</td>
<td>-0.28</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>26.47 ± 0.78</td>
<td>25.70</td>
<td>-0.12</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>31.32 ± 0.88</td>
<td>31.60</td>
<td>-0.94</td>
</tr>
</tbody>
</table>

* Group 1, BSS only; group 2, antibiotic ointment; group 3, antibiotic ointment plus NM excision; group 4, collagen shield plus NM excision.
† ds/dt refers to the first derivative of defect area with respect to time.
Table 2. Defect areas (mm², mean ± SE) at each time point

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Group*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>0</td>
<td>26.80 ± 0.60</td>
</tr>
<tr>
<td>5</td>
<td>25.09 ± 0.51</td>
</tr>
<tr>
<td>24</td>
<td>9.58 ± 0.45</td>
</tr>
<tr>
<td>30</td>
<td>7.96 ± 0.45</td>
</tr>
<tr>
<td>48</td>
<td>4.42 ± 0.62</td>
</tr>
<tr>
<td>72</td>
<td>0.53 ± 0.34</td>
</tr>
<tr>
<td>96</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* Group 1, BSS only; group 2, antibiotic ointment; Group 3, antibiotic ointment plus NM excision; group 4, collagen shield plus NM excision.

ment groups varying from 26.47 to 31.32 mm². This range, we believe, was the result of diffusion of the n-heptanol away from the filter paper disc during its application on the corneal surface. Statistical comparison, by ANOVA, revealed a significant difference ($P < 0.01$) among the initial defect sizes.

During the course of the follow-up examinations, the collagen shields were noted to dissolve incompletely, into a somewhat gelike consistency. In no case, however, were we not able to identify the shield and manipulate it, even at the 96-hr time point.

Examination of the mean defect areas for each time point (Table 2) revealed that all defects were completely healed by 96 hr; the vast majority in fact, were healed by 72 hr. The variation in mean epithelial defect area with respect to time (epithelial healing rate) for each treatment group was determined to be nonlinear (Fig. 1). For example, the mean healing rate for all treatment groups between the 0- and 24-hr time points was 0.63 mm²/hr (range 0.57–0.71 mm²/hr), whereas the mean rate between the 24- and 48-hr time points was 0.32 mm²/hr (range 0.21–0.41 mm²/hr).

The numeric first derivatives ($dS/dt$) of each treatment group’s median healing rates with respect to time at $t_0$ (Table 1)—the initiation of the healing process—indicated that the healing rate was directly related to the initial wound area, with larger wounds demonstrating a faster initial decrease in wound areas. The overall relationship between $dS/dt$ and $S$ suggested an exponential model to the data.

Using the exponential model, the slope of the linear regression of $\ln S$ on time is represented by $a_t$, the rate of healing per unit of defect area. Comparison of the values for $a_t$ (Table 3), by m-group analysis, demonstrated no significant difference ($P = 0.10$). This exponential model applied to the reepithelialization process reasonably well, with $R^2$ ranging from 0.88 to 0.97, and was considered adequate for the purpose of statistically comparing the unitary closure rates.

Examination of reepithelialization rates during the healing phase (Table 4), either by decay of wound area or wound radius, revealed no significant differences among the treatment groups ($P > 0.5$).

**Discussion**

The modern use of extraneous substances to protect the ocular surface dates back to Ridley who, in the 1930s, applied prefit, polymethyl methacrylate scleral shells to protect diseased corneas. Gassett and Kaufman, in 1970, introduced the therapeutic use of hydrophilic contact lenses. These “bandage” lenses have received wide clinical acceptance for use in such disorders as recurrent erosions, exposure keratopathy, keratoconjunctivitis sicca, bullous keratopathy, chemical and other toxic keratopathies, filamentary keratitis, and persistent epithelial defects. However, the chronic use of hydrophilic lenses, particularly in eyes with diseased ocular surfaces, has been complicated by corneal edema, stromal vascularization, sterile infiltrates, and infectious keratitis.

The problems associated with therapeutic hydrophilic lenses have prompted the search for other materials for ocular surface bandages. Collagen, the most abundant protein in humans, is the major protein component of healing wounds and is metabolized by...
demonstrate that, when used in patients with corneal epithelial defects, collagen shields significantly increase corneal reepithelialization rates following chemical wounding. It has been recognized widely that the healing of corneal epithelial defects is a complex and only incompletely understood process. In each of our treatment groups, the process of reepithelialization appeared to be nonlinear, consistent with the observations of Crosson and coworkers. Reported differences between the conclusions drawn from studies of the kinetics of epithelial healing may depend on several factors, including the method used to create the wound, the method used to determine the wound area, the initial size of the wound, and the statistical method selected to analyze the rate of reepithelialization.

We fully recognize that the choice of method of epithelial wound creation is a somewhat controversial issue. We chose to use n-heptanol to create the epithelial wounds, primarily because this long-chain fatty acid is known to disrupt the plasma membranes of epithelial cells without damaging the underlying basal lamina, a concern in mechanical debridement. As has been noted previously, a problem with n-heptanol epithelial debridement is the tendency for diffusion of the chemical, and subsequent deepithelialization, away from the filter paper disc. In our study, this diffusion resulted in a wider range of initial wound areas than probably would have occurred with mechanical debridement. However, in view of the fact that there are no published reports directly comparing the effects of chemical and mechanical debridement upon corneal reepithelialization, we believed that chemical deepithelialization was preferable because of our desire to retain an intact basal lamina. Because this issue remains controversial, our laboratory is currently involved in a direct comparison of the effects of chemical and mechanical debridement upon the kinetics and morphology of corneal reepithelialization.

Table 3. Estimation of healing rates per unit defect area over entire study

<table>
<thead>
<tr>
<th>Group*</th>
<th>( a_1 \pm SE ) (mm/hr)</th>
<th>Correlation</th>
<th>Regression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.058 ± 0.0044</td>
<td>-0.98</td>
<td>97.23</td>
</tr>
<tr>
<td>2</td>
<td>-0.057 ± 0.0059</td>
<td>-0.97</td>
<td>94.93</td>
</tr>
<tr>
<td>3</td>
<td>-0.038 ± 0.0028</td>
<td>-0.98</td>
<td>97.53</td>
</tr>
<tr>
<td>4</td>
<td>-0.054 ± 0.0087</td>
<td>-0.94</td>
<td>88.58</td>
</tr>
</tbody>
</table>

* Group 1, BSS only; group 2, antibiotic ointment; group 3, antibiotic ointment plus NM excision; group 4, collagen shield plus NM excision.
† A represents the estimated healing rate per unit defect area in the exponential model (1).

naturally occurring proteases. Purified heterologous collagen has been used recently for a variety of medical indications, including the treatment of burns. In ophthalmology, collagen has been used for temporary punctal plugs and in external eye patches. Furthermore, Gegel et al. recently demonstrated that collagen gel is an excellent substrate for the in vitro growth of corneal epithelial cells.

The biocompatibility and biodegradability of collagen, plus its ready availability, provided the impetus for the concept of a therapeutic collagen lens. Weissman and Lee, additionally, have recently demonstrated that biodegradable collagen lenses have oxygen transmissibility levels adequate for corneal epithelial metabolism. The therapeutic use of collagen lenses was first described by Fyodorov and colleagues, who used a preliminary version of the collagen shield to provide comfort in cases of bullous keratopathy and to enhance epithelial healing after radial keratotomy. Recent clinical studies evaluated the use of commercially available collagen shields for corneal reepithelialization after a variety of surgical and medical conditions. Although lacking rigid controls and statistical analyses, these series did demonstrate that, when used in patients with corneal epithelial defects, collagen shields were generally well tolerated and did not appear to interfere with the reepithelialization process. To the best of our knowledge, however, no controlled analyses to date have demonstrated that collagen shields significantly increase corneal reepithelialization rates following chemical wounding.

Table 4. Estimation of mean healing rates during “healing” phase using wound area or wound radius

<table>
<thead>
<tr>
<th>Using wound area*</th>
<th>Using wound radius†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group‡</td>
<td>( a_2 \pm SE ) (mm/hr)</td>
</tr>
<tr>
<td>1</td>
<td>-0.237 ± 0.0698</td>
</tr>
<tr>
<td>2</td>
<td>-0.279 ± 0.0576</td>
</tr>
<tr>
<td>3</td>
<td>-0.252 ± 0.0650</td>
</tr>
<tr>
<td>4</td>
<td>-0.289 ± 0.0495</td>
</tr>
</tbody>
</table>

* By m-group analysis, \( P > 0.5 \).
† By m-group analysis, \( P > 0.5 \).
‡ Group 1, BSS; group 2, antibiotic ointment; group 3, antibiotic ointment plus NM excision; group 4, collagen shield plus NM excision.
§ A represents the estimated healing rate in the corresponding linear model.

It has been recognized widely that the healing of corneal epithelial defects is a complex and only incompletely understood process. In each of our treatment groups, the process of reepithelialization appeared to be nonlinear, consistent with the observations of Crosson and coworkers. Reported differences between the conclusions drawn from studies of the kinetics of epithelial healing may depend on several factors, including the method used to create the wound, the method used to determine the wound area, the initial size of the wound, and the statistical method selected to analyze the rate of reepithelialization.

We fully recognize that the choice of method of epithelial wound creation is a somewhat controversial issue. We chose to use n-heptanol to create the epithelial wounds, primarily because this long-chain fatty acid is known to disrupt the plasma membranes of epithelial cells without damaging the underlying basal lamina, a concern in mechanical debridement. As has been noted previously, a problem with n-heptanol epithelial debridement is the tendency for diffusion of the chemical, and subsequent deepithelialization, away from the filter paper disc. In our study, this diffusion resulted in a wider range of initial wound areas than probably would have occurred with mechanical debridement. However, in view of the fact that there are no published reports directly comparing the effects of chemical and mechanical debridement upon corneal reepithelialization, we believed that chemical deepithelialization was preferable because of our desire to retain an intact basal lamina. Because this issue remains controversial, our laboratory is currently involved in a direct comparison of the effects of chemical and mechanical debridement upon the kinetics and morphology of corneal reepithelialization.
The degree of effect, if any, of initial defect size upon epithelial healing rate is another issue upon which not all investigators agree. Jumblatt and Neufeld33 concluded that larger epithelial wounds close at a faster rate than do smaller ones. They noted that, using n-heptanol in a similar experimental model, the wound closure rate for the first 24 hr after the creation of a 7-mm-diameter wound was 0.88 mm²/hr, whereas for a 6-mm-diameter wound it was 0.65 mm²/hr. Matsuda and coworkers,11 using a mechanical debridement model, concluded likewise that defect size was related to reepithelialization rate. They observed mean healing rates of 0.91 mm²/hr for 8-mm-diameter wounds and 0.37 mm²/hr for 4-mm-diameter wounds. However, Crosson et al,10 using a mechanical debridement model, noted no effect of initial wound size on reepithelialization rates.

Because of the wide variation that we noted in initial defect areas among the treatment groups, our statistical analysis took into account the possibility that initial wound size can affect epithelial healing rates. Because of the irregular, nonspherical initial wounds, we believed that the recently described linear analysis model of Crosson et al10 would not have been best suited for our data.30 The statistical model that we developed, namely the exponential analysis of healing rates per unit defect area, did enable us to evaluate the entire reepithelialization process. In the current study, analyses of reepithelialization rates per unit of defect area revealed no statistically significant differences among the treatment groups. These results are similar to those of a recently published report by Shaker et al,4 which demonstrated, in a feline model of mechanical debridement, that corneal reepithelialization rates were not significantly accelerated by the use of corneal shields. These investigators also likewise noted an increased rate of reepithelialization in the first few hours after defect creation. Whether this phenomenon is the result of protection of the migrating cells, lubrication, or early deposition of shield collagen onto the deepithelialized cornea, is still unclear.6

Therapeutic goals in the treatment of epithelial erosions primarily involve the reduction of discomfort and the prevention of secondary infection. None of the currently available treatment modalities, including patching and therapeutic hydrophilic lenses, has been proven to affect the speed of reepithelialization.34,36 Therefore, in the current study, we did not expect the use of collagen shields to accelerate epithelial wound healing rates. Extrapolating to the clinical management of corneal epithelial erosions, therefore, it may be expected that collagen shields will not interfere with normal corneal reepithelialization. If this proves to be the case, the use of these lenses may offer distinct advantages over current therapies. Compared to patching, shields provide the patient with some degree of sight, and as recent experimental evidence indicates,35–39 may allow for the delivery of prophylactic or therapeutic levels of antibiotics. In addition, in certain clinical situations, a corneal erosion may actually represent the initial manifestation of an infectious keratitis, in which case placement of a patch actually may encourage intracorneal microbial growth, or at the minimum, delay diagnosis of the infectious process.40 Compared to therapeutic hydrophilic lenses, collagen shields, because of their biodegradability, do not require fitting. Furthermore, according to O'Brien et al,39 collagen shields can deliver greater concentrations of antibiotics to the cornea than can hydrophilic lenses. Therefore, we believe that collagen shields will play an active role in the future management of corneal epithelial defects, and currently we are conducting clinical evaluations of their efficacy.

Key words: collagen shields, epithelium, epithelial wound healing, rabbit, cornea

References