Inhibition of Conjunctival Scarring and Contraction by a Porous Collagen-Glycosaminoglycan Implant

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PURPOSE. To study the healing processes of full-thickness wounds in the adult rabbit conjunctiva after grafting with a porous collagen-glycosaminoglycan (CG) copolymer matrix.

METHODS. A 7-mm trephine was used to produce lesions of the bulbar conjunctiva down to the level of the bare sclera. Full-thickness removal of the conjunctiva and Tenon’s capsule created a reproducible wound bed. Wounds either remained ungrafted (control) or were grafted with CG matrix. In previous studies, this CG matrix has induced partial regeneration of the dermis in the human, the swine, and the guinea pig. Healing of the conjunctival epithelium and underlying stroma was evaluated by histology, immunohistochemistry, and measurement of wound contraction kinetics.

RESULTS. By 28 days, ungrafted wounds had closed by contraction (26.4% ± 5.0% fornix shortening) and the formation of scar-like tissue comprising an aligned array of dense collagen populated with occasional fibroblasts. Grafting of identical defects with CG copolymer matrix resulted in inhibition of wound contraction (6.8% ± 3.2% fornix shortening) and the formation of a tissue that resembled normal conjunctival stroma, being composed of a loose network of collagen fibers and fibroblasts. Contractile fibroblasts (myofibroblasts) were identified at the edge of both ungrafted and grafted wounds during the period of active contraction. Both ungrafted and grafted wounds were completely re-epithelialized by 28 days.

CONCLUSIONS. Implantation of CG copolymer matrix drastically reduced contraction and promoted the formation of a nearly normal subconjunctival stroma. (Invest Ophthalmol Vis Sci. 2000;41:2404–2411)

Spontaneous healing of wounds of the adult conjunctiva consists of inflammation followed by re-epithelialization, wound contraction, and the formation of subconjunctival fibrous scar.1 Epithelialization is a regenerative process by which the wound is covered by migration, proliferation, and differentiation of the cells from the wound edge. Wound contraction serves to reduce the size of the wound by inward movement of the wound edges. Although conjunctival wounds are capable of spontaneous re-epithelialization, the result of pathologic healing of the subepithelial stroma is scar formation. Fibrous scar is the result of migration and proliferation of fibroblasts followed by synthesis and remodeling of collagen in the wound bed. During scar synthesis, aligned bundles of collagen are deposited in the wound, providing tensile strength to the scar tissue.2

Conjunctival scarring is a final common pathway for a myriad of ophthalmic disorders, representing a broad range of causes including infectious (e.g., trachoma), immunologic (e.g., Stevens-Johnson syndrome, ocular cicatricial pemphigoid), traumatic (e.g., chemical burns), and postsurgical (pterygium, glaucoma filtering procedures, anophthalmic socket). Prevention and modulation of conjunctival scarring could favorably impact each of these disorders.

Substantial evidence indicates that wound contraction in connective tissues is cell mediated3 and that the active cell type is a modified contractile fibroblast, the myofibroblast.4,5 Myofibroblasts contain the contractile apparatus of smooth muscle cells, α-smooth muscle actin (SMA), and can be identified by immunohistochemical staining with antibodies to α-SMA.6 Contractile fibroblasts have been identified in contracting wounds in the skin,6 cornea,7 and conjunctiva.8,9 Myofibroblasts form intercellular and cell-to-stroma connections, which serve to transmit the contractile force and impart some degree of organization to surrounding collagen fibers.10

The formation of scar occurs by the deposition, maturation, and organization of newly synthesized collagen. Fibroblasts, the major source of collagen, become oriented in the wound bed and synthesize collagen fibers in a direction parallel to their orientation.11 The uniaxial orientation of collagen in scar tissue is different from the random alignment of collagen fibers in normal connective tissues.12

Previous studies in this laboratory have demonstrated that a highly porous chemical analogue of extracellular matrix, composed of type I collagen and chondroitin 6-sulfate (CG copolymer matrix), induces partial regeneration of the injured dermis in the human,13,14 the swine,15 and the guinea pig.17,18 Implantation of the CG copolymer in these models resulted in
the inhibition of wound contraction and the regeneration of a nearly physiological dermis. Inhibition of dermal wound contraction by CG matrices may be simply interpreted as the interruption of alignment of contractile myofibroblasts and collagen fibers in the wound bed by the randomly oriented pore walls of the matrix.19

In the present study, we attempted to modulate the healing process after wounding of the rabbit conjunctiva by implanting a porous CG copolymer graft. We hypothesized that the porous CG copolymer would reduce scarring and inhibit wound contraction and shortening of the fornix. We established an anatomically constant wound model for examining the healing of full-thickness conjunctival wounds. Healing was evaluated by histology (hematoxylin and eosin [H&E], Masson’s trichrome, polarized light, and α-SMA immunohistochemistry) and by the kinetics of wound contraction as measured by shortening of the conjunctival fornix at the location of the wound.

METHODS

Synthesis of CG Copolymer Matrix

CG copolymer matrices were produced as previously described.18,20 Briefly, a coprecipitate of type I bovine tendon collagen (Integra Lifesciences, Plainsboro, NJ) and chondroitin 6-sulfate (Sigma, St. Louis, MO) in 0.05 M acetic acid was freeze-dried to yield a highly porous sheet 2 mm in thickness. The freeze-drying process yields a network of CG copolymer with approximately 95% pore volume fraction and average pore diameter of 138 ± 9 μm.18 The sheets of CG copolymer were cross-linked by dehydrothermal treatment consisting of exposure to a vacuum at a temperature of 105°C for 24 hours. Disks of CG copolymer, 8-mm diameter with 2-mm thickness, were cut by trephination and further cross-linked by immersion in 0.25% aqueous glutaraldehyde for 24 hours. Residual glutaraldehyde was removed by exhaustive rinsing in multiple changes of phosphate-buffered saline (PBS) over 48 hours.

Surgical Procedure

All investigations conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Massachusetts Institute of Technology Committee on Animal Care. Twenty-one female New Zealand albino rabbits weighing between 2.5 and 3.5 kg were anesthetized by intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg). The eyelids were held open during surgery by a speculum, and a surgical microscope was used during all procedures. Two circular lesions were formed on the right eye of each animal, located at the 10 o’clock (superior lateral) and 8 o’clock (inferior lateral) positions at a distance of 2 mm away from the corneal–scleral limbus (Fig. 1). A 7.0-mm diameter vacuum trephine (Katena, Denville, NJ) was used to completely remove the conjunctival epithelium, substantia propria, and the Tenon’s capsule down to the level of the bare sclera, taking care to avoid damage to the underlying sclera. Occasionally, the trephine did not completely sever the Tenon’s capsule. In these cases, Vannas scissors were used to remove the remaining tissue. Slight retraction of the wound edges resulted in a final wound diameter of approximately 8 mm. One of the two conjunctival wounds remained ungrafted without further manipulation and served as a control. The remaining wound on the same eye was grafted with a disc of CG copolymer 8.0 mm in diameter and 2.0 mm thick, by using eight interrupted 8-0 polygactin sutures (Fig. 1). The location of the grafted wound and ungrafted control was alternated among animals between the superior lateral and inferior lateral positions. Topical 0.5% erythromycin ophthalmic ointment (Bausch & Lomb, Tampa, FL) was applied immediately after surgery and once daily for 1 week after the operation.

Gross examination of grafted and ungrafted wounds included observation for clinical signs of fibrosis and scarring, including thinning of the conjunctiva, subepithelial whitening and opacity, and the presence of symblepharon. Conjunctival contraction was qualitatively observed as gross shortening of the conjunctival fornix and deformation of the adjacent conjunctiva.

The depth of the conjunctival fornix above each wound was measured immediately after surgery and at postoperative days 1, 7, 14, 21, and 28. Each animal was anesthetized, and a blunt probe was inserted into the fornix at the 8 o’clock and 10 o’clock positions directly above each wound. The probe depth in the fornix was determined by reading scale markings on the surface of the probe. The percentage of fornix shortening was calculated at any given time as the percentage decrease in fornix depth from the fornix depth measured immediately after surgery (day 0).

Histologic and Immunohistochemical Staining

Groups of rabbits were killed on day 1 (n = 4), day 7 (n = 4), day 14 (n = 5), and day 28 (n = 6) for histologic examination. Intact eyes including eyelids were removed en bloc by orbital exenteration and fixed by immersion in 4% formaldehyde overnight. The portion of each eye that included either the grafted or ungrafted wound site, including the underlying scleral bed, was dissected and embedded in paraffin. Sections were cut on a microtome at 7 μm and stained with H&E for general cell morphology and Masson’s trichrome to assess collagen deposition and remodeling (reorganizing). Identification of specific cell types was based on cell morphology observed in sections stained with H&E. Polymorphonuclear neutrophils (PMNs) were identified by characteristic multilobed nuclei and fine cytoplasmic granules. Eosinophils were characterized by bi-lobed or multilobed nuclei and larger eosinophilic granules. Basophils possessed less lobulated, pale nuclei with cytoplasmic granules that were less numerous and variable in size. Fibroblasts were characterized by elongated morphology and oval nuclei. Slides processed for histology were also viewed under cross-polarizing filters to visualize the organization and alignment of collagen fibers.

Additional tissue sections were stained with an antibody for α-SMA to identify cells with contractile potential (myofibroblasts). Briefly, sections were deparaffinized, rehydrated, washed three times (5 minutes each) with PBS, and blocked with 10% normal goat serum (Gibco, Gaithersburg, MD). A monoclonal antibody for α-SMA (Sigma, St. Louis, MO) was used at a dilution of 1:400. To verify the specificity of the primary antibody, adjacent tissue sections were similarly processed except for the replacement of the primary antibody with nonimmune mouse serum. Slides were then incubated with biotinylated goat anti-mouse IgG (Sigma) diluted 1:400, reacted with avidin-biotin complex (Vector, Burlingame, CA), and developed with AEC chromogen (Sigma), which yields a red-brown color indicating positive stain.
RESULTS

Day 1

At day 1 after surgery, both grafted and ungrafted wounds showed infiltration of acute inflammatory cells (PMNs, basophils, and eosinophils). Grafted wounds were more strongly infiltrated by inflammatory cells than ungrafted wounds. Neither control nor grafted wounds showed any significant growth of epithelium at the wound edges. No decrease in fornix depth was observed in wounds grafted with CG copolymer, and only a slight change (decrease of 5.0% ± 5.0%) was observed for the ungrafted group. Immunostaining for α-SMA was negative in the wound bed of both control and grafted animals; however, the smooth muscle cells and/or pericytes of blood vessels and the ciliary muscle of the adjacent tissue stained positively. Few fibroblasts were present in the wound bed, and deposition of new collagen was not detected.

Day 7

A hypercellular condition was noted in ungrafted wounds at day 7, consisting predominantly of inflammatory cells with occasional elongated cells resembling fibroblasts. The inner one third to one half of the grafted CG copolymer adjacent to the sclera was infiltrated by inflammatory cells and fibroblasts from the wound bed (Fig. 2). A thin epithelial layer had almost completely covered ungrafted control wounds by 7 days. A few epithelial cells with prominent secretory vesicles, morphologically resembling goblet cells, were present. Only the edges of the grafted wounds were covered with a thin layer of epithelium. The CG copolymer graft was partially degraded at 7 days, and the remaining material (approximately 70% of initial area) was grossly visible in the wound bed. Although some degradation of the peripheral edges of CG copolymer grafts was observed at this time, the pore structure of the remaining graft material remained intact, as observed in histologic sections (Fig. 2).

Measurements of fornix depth indicated that the fornices directly adjacent to ungrafted control wounds had contracted 18.7% ± 2.1%, whereas the fornices above wounds grafted with CG copolymer had contracted 4.6% ± 2.1% (Table 1 and Fig. 3). The difference between groups was statistically significant (P < 0.001). Immunostaining for α-SMA revealed that elongated cells with fibroblastic morphology stained positive for α-SMA at the edge of ungrafted wounds. In grafted wounds, cells at the wound edge as well as cells that had infiltrated the matrix stained for α-SMA.

The deposition of a loose meshwork of new collagen adjacent to fibroblasts in the ungrafted wound was apparent at 7 days after surgery (Fig. 2). In histologic sections from grafted wounds stained with Masson’s trichrome, it was difficult to distinguish between newly synthesized collagen and partially degraded CG copolymer matrix.

FIGURE 1. Photographs of rabbit eyes showing both ungrafted (A) and matrix-grafted (B) lesions of the bulbar conjunctiva. In both cases, the margin of the wound was marked before surgery with surgical ink applied to the edge of the trephine.

FIGURE 2. Micrographs illustrating histologic differences between ungrafted and matrix-grafted wounds after 7 days (A, B) and 28 days (C, D). Paraffin-embedded sections stained with Masson’s trichrome. Collagen stained blue, cellular material red, and cell nuclei purple. (A) The ungrafted control wound 7 days after surgery contained a loose network of faintly staining collagen (FC) with an abundant cell population, predominantly composed of fibroblasts and acute inflammatory cells. Filled arrows: blood vessels. (B) A matrix-grafted wound at 7 days showing darkly stained matrix pore walls and traces of faintly stained collagen within the pores. Arrowheads: typical pore wall. The matrix pores also contained a population of fibroblasts and acute inflammatory cells. In both (A) and (B), the sclera (S) is visible at the bottom of the wound. (C) Ungrafted control wound 28 days after surgery contained a densely packed array of darkly stained collagen fibers (DC) with occasional elongated fibroblasts (open arrows). Filled arrow: blood vessel. (D) Matrix-grafted wound at 28 days showing a more loosely organized network of collagen (LC) and fibroblasts (open arrows). Scale bar, (A, B) 50 μm; (C, D) 10 μm.
Day 14
The epithelial layer above ungrafted wounds was slightly thicker than that observed at day 7. Re-epithelialization over the CG copolymer was complete by 14 days, and a few goblet-like cells were observed near the wound margin. Degradation of the CG copolymer grafts was more complete at day 14 (approximately 45% of initial area by gross observation) than at day 7.

At day 14, ungrafted wounds continued to be populated by inflammatory cells and fibroblasts, with the fibroblast becoming the more abundant cell type. Grafted wounds exhibited a hypercellular condition, with PMNs persisting in the regions of undegraded matrix. At postoperative day 14, closure of ungrafted wounds by contraction continued, evidenced by fornical shortening (23.3% of ungrafted wounds by contraction continued, evidenced by regions of undegraded matrix. At postoperative day 14, closure

Day 21

| Table 1. Comparison of Wound Characteristics for Ungrafted and Grafted Wounds |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| **Fornix Shortening (%)**       | **α-SMA–Positive Cells** | **Re-epithelialization** | **Collagen Deposition** | **Collagen Organization** |
| Day 1                           | Day 7            | Day 14           | Day 21           | Day 28           |
| Ungrafted                       | Grafted          | Ungrafted        | Grafted          | Ungrafted        | Grafted          |
| 5.0 ± 0.0                       | 4.7 ± 2.2        | 23.5 ± 7.0       | 27.2 ± 5.9       | 6.8 ± 3.3        |
| 0.0 ± 0.0                       | 5.1             | 7.0 ± 2.4        | NA              | +               |
| 18.7 ± 2.2                      | +               | +               | NA              | +               |
| 27.2 ± 5.9                      | NA              | NA              | NA              | NA              |
| 10.2 ± 5.0                      | +               | +               | NA              | NA              |
| 26.5 ± 5.1                      | +               | +               | +               | Loose, no alignment |
| 6.8 ± 3.3                       | +               | +               | +               | Loose, no alignment |

NA, not applicable.

* α-SMA staining was positive at wound edge and within undegraded CG copolymer but was negative in regions of degraded CG copolymer.
† Distinction between degradation of CG copolymer and deposition of new collagen was not possible by histology at these stages.

decreased fornix shortening (6.9% ± 2.4%, Table 1 and Fig. 3). Staining for α-SMA was positive at the wound edge and in the middle of ungrafted wounds (Table 1 and Fig. 3). Positive-stained cells in ungrafted wounds were predominantly aligned parallel to the sclera surface. Staining for α-SMA in grafted wounds was positive at the wound edge and within undegraded CG copolymer (Fig. 4). α-SMA-positive cells in grafted wounds displayed a more random orientation, after the irregular contour of the matrix pore walls. Staining was negative in regions where degradation of the CG copolymer was complete (Fig. 5).

Subepithelial tissue of ungrafted wounds contained a loose meshwork of collagen fibers predominantly oriented parallel to the sclera surface and the plane of the conjunctival epithelium (Fig. 6). In grafted wounds, loosely organized collagen fibers and fibroblasts had replaced regions of degraded CG copolymer. In the regions of undegraded CG copolymer, it was difficult to distinguish newly synthesized collagen from the partially degraded CG copolymer, when using Masson’s trichrome stain.

Day 21
Histologic data were not available for day 21 wounds, because no animals were killed at that time. However, measurements of fornix depth provided data for wound contraction. At day 21, fornix shortening of ungrafted wounds (27.1% ± 5.8%) was significantly greater (P < 0.05) than that of grafted wounds (10.1% ± 3.4%, Table 1 and Fig. 3). Neither grafted nor ungrafted wounds showed any significant increase in contraction between days 14 and 21 (Table 1 and Fig. 3).

Day 28
Clinical observation of ungrafted animals at 28 days showed an apparent thinning and depression of the conjunctiva at the wound site. A slight increase in conjunctival opacity was also observed. In two of six ungrafted animals, there was notable distortion of the intact conjunctiva surrounding the ungrafted wounds. Symblepharon was not present in either ungrafted or grafted wounds. Matrix-grafted wounds did not exhibit thinning or distortion of the conjunctiva, but a slight increase in opacity at the wound site was observed at day 28.
In contrast to earlier time points, day 28 ungrafted wounds exhibited hypocellular appearance and no evidence of a persisting acute inflammatory response. The CG copolymer in grafted wounds was almost completely degraded (approximately 5% of initial area) and was barely distinguishable in the wound bed. Acute inflammatory cells were identified at day 28 in the small regions of undegraded CG copolymer. Both ungrafted and grafted wounds were completely covered by an epithelial layer 2 to 3 cells thick. A few gobletlike cells were present above both peripheral and central regions of the wounds in both groups.

Neither ungrafted nor grafted 28-day-old wounds showed significant fornical contraction in addition to that observed at day 14 ($P > 0.6$). At 28 days, ungrafted wounds showed 26.4% ± 5.0% fornix shortening, whereas grafted wounds showed 6.8% ± 3.2% fornix shortening (Table 1 and Fig. 3). The difference between groups was statistically significant ($P < 0.01$). At day 28, α-SMA staining was negative in the middle and edge of ungrafted wounds with the exception of pericytes and the smooth muscle cells of blood vessels. Staining for α-SMA was no longer observed at the edges of grafted wounds but remained positive within undegraded regions of CG copoly-

![Figure 4](http://boorjam.org/images/figure4.png)

**Figure 4.** Paraffin-embedded sections from ungrafted and grafted wounds at 14 days. (A) Section from an ungrafted control wound stained with an antibody for α-SMA. Red-brown stain indicates positive labeling of numerous fibroblasts as well as the vascular cells of small blood vessels (arrows). (B) Section adjacent to (A) stained with an identical protocol except the substitution of nonimmune serum for the primary antibody to verify antibody specificity. Neither the fibroblasts nor small blood vessels were labeled. (C) Section from a matrix-grafted wound showing α-SMA–positive cells within the matrix pores. Also staining was a thin layer of vascular cells surrounding a large, newly formed blood vessel (arrows) filled with erythrocytes. (D) Section adjacent section to (C) stained by replacing the primary antibody with nonimmune serum. Neither fibroblasts nor the blood vessel was labeled. Hematoxylin counterstain.

![Figure 5](http://boorjam.org/images/figure5.png)

**Figure 5.** Paraffin-embedded section showing the edge of the partially degraded CG matrix 14 days after implantation. The section was immunostained for α-SMA. Positive-staining cells (red-brown, filled arrows) were present in the undegraded matrix, but were not present in adjacent tissue in which the matrix had degraded. Blood vessels (open arrows) also stained positive. Inset: regions of undegraded matrix (UM), degraded matrix (DM), and underlying muscle (M). Hematoxylin counterstain.

![Figure 6](http://boorjam.org/images/figure6.png)

**Figure 6.** Paraffin sections from 14-day-old ungrafted (A) and matrix-grafted (B) wounds stained with Masson's trichrome. (A) The thin epithelial layer (red) above the ungrafted wound contained a few gobletlike cells (arrows). The subepithelial tissue consisted of a loosely packed network of collagen fibers (blue) and fibroblasts (red with purple nuclei). The collagen fibers are predominantly oriented in parallel between the epithelial layer and the underlying muscle (M). (B) The epithelial layer above the grafted wound also contained gobletlike cells at 14 days. The randomly oriented pore walls of undegraded matrix (blue-staining trabeculae) were present in the subepithelial space. The matrix pores contained cells (red with purple nuclei) and blood vessels (open arrows).
mer. Staining was negative in regions of degraded CG copolymer, which represented the majority of the grafted wound bed. Collagen fibers in day 28 ungrafted wounds retained their linear alignment, but were more densely packed than those in day 14 wounds (Fig. 2). The dense, aligned collagen displayed intense birefringence when viewed using polarized light (Fig. 7). Fibers tended to be highly aligned between the conjunctival epithelium and the sclera surface. In contrast, collagen fibers in grafted wounds were more randomly oriented (Fig. 2) and exhibited only sparse, discontinuous birefringence (Fig. 7). The density and organization of collagen in the grafted wounds was similar to that of the subepithelial connective tissue (stroma) of the normal conjunctiva (Fig. 7).

DISCUSSION

Scarring and contraction of the conjunctiva are common complications of many ocular diseases (autoimmune disorders, trauma, and infection) and surgical procedures (glaucoma filtering surgery). Clinical implications include fornix foreshortening, symblepharon, cicatricial entropion, limited extraocular movement associated with diplopia, and glaucoma filter bleb failure. Investigators have attempted to prevent scarring with pharmacological approaches, such as controlling levels of transforming growth factor (TGF)-β,21,22 using 5-fluorouracil,23 mitomycin C,24 or D-penicillamine25 with varying degrees of success. However, because of complications of toxicity and limited clinical efficacy, there are no widely accepted effective pharmaceutical therapies to prevent conjunctival scarring. Surgical interventions have included various reconstructive techniques,26-27 the use of autogenous conjunctiva28 or mucous membrane grafts,29-31 allograft tissue such as amniotic membrane,32 and implanted synthetic materials.33 Morbidity at the harvest site is an inherent disadvantage of autogenous tissues.34 Allograft tissues are subject to donor availability and host immune response. Synthetic materials often do not become well integrated with the host tissue. A therapy is needed that prevents contraction and scarring after damage to the conjunctiva.

The purpose of this study was to examine spontaneous healing of the damaged conjunctiva and to attempt to modify the wound healing response by implantation of a porous CG copolymer matrix. Previous studies have demonstrated that a similar implant inhibited wound contraction and prevented scar formation in animal models of dermal wound healing,16-18 as well as in the human.14,15 In the present study, we focused on four aspects of wound healing: inflammation, re-epithelialization, wound contraction, and the deposition and organization of collagen in the wound bed.

Inflammatory and Immune Responses

Cells that are characteristic of an acute inflammatory response were present in both ungrafted and grafted wounds at days 1 and 7. Acute inflammatory cells subsided by day 14 in ungrafted wounds but persisted until day 28 in regions of undegraded CG copolymer in grafted wounds. Because these cells were not present in regions where the matrix had degraded, it is expected that the inflammatory response would have eventually subsided when the CG material had been completely resorbed.

No excessive accumulation of plasma cells or lymphocytes was identified in the vicinity of the CG copolymer at any time point (data not shown), indicating that there was no immune rejection of the bovine collagen or the chondroitin 6-sulfate components of the CG copolymer. The CG copolymer has not elicited persistent acute inflammatory response or immune rejection when implanted into a number of tissues, including skin,16-18 peripheral nerve,35 and spinal cord.56

FIGURE 7. Polarized light micrographs illustrating differences in subepithelial collagen organization in a 28-day ungrafted wound (A), a 28-day matrix-grafted wound (B), and the normal conjunctiva (C). Collagen fibers in the ungrafted wound show a high degree of alignment indicated by parallel bands of birefringence (arrows). In contrast, collagen fibers of the matrix-grafted wound display scattered birefringence with random orientation (arrows). The birefringence pattern of the matrix-grafted wound is similar to that of the normal conjunctiva. Paraffin-embedded sections stained with Masson’s trichrome and viewed using cross-polarizing filters.

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Re-epithelialization
At 7 days after surgery, re-epithelialization of ungrafted wounds was nearly complete, whereas only the edges of grafted wounds were covered with epithelium. The more prominent contraction of ungrafted wounds had decreased the wound size and may have allowed re-epithelialization to occur in a shorter time. In addition, the irregular surface of the porous CG copolymer may not have provided a favorable substrate for the migration of epithelial cells over the graft. By 28 days after surgery, a complete epithelial layer that contained gobletlike cells covered both ungrafted and grafted wounds. Our findings concerning the time scale of re-epithelialization of ungrafted control wounds are consistent with other studies in which smaller, 4-mm diameter, full-thickness conjunctival wound were completely re-epithelialized after 7 days and contained goblet cells after 14 days.57

Wound Contraction
In the present study, partial closure of ungrafted and grafted conjunctival wounds occurred by contraction during the period when myofibroblasts were present in the middle of the wound and at the wound edge. Our data indicate that contraction (as measured by fornical shortening) of both ungrafted and grafted wounds was most active during the period up to 14 days after surgery (Fig. 3), reaching an apparent plateau thereafter. We observed the presence of myofibroblasts at the edges of ungrafted and grafted wounds at days 7 and 14. Myofibroblasts were no longer present at the wound edge in both groups by day 28, with the exception of a few labeled cells in the undegraded matrix in grafted wounds. The correlation between the active period of wound contraction (between days 1 and 14) and the presence of α-SMA-positive fibroblasts in both ungrafted and grafted wounds lends evidence to the hypothesis that myofibroblasts actively participate in conjunctival wound contraction. The appearance and disappearance of myofibroblasts have been shown by other investigators to coincide with the active phase and cessation of contraction during healing of skin wounds.5,58–59 Similar results were observed in a model of contraction of glaucoma filtering blebs, where myofibroblasts were identified in the bleb peripheral margin 10 days after surgery.10 There is also substantial evidence that fibroblasts that do not express the α-SMA-positive phenotype may be involved in wound contraction.40–41 These findings provide a possible explanation for the day 1 fornical shortening of ungrafted wounds (Fig. 3) before α-SMA staining was observed.

Compared with the ungrafted group, the presence of the CG copolymer in grafted wounds significantly reduced wound contraction, evidenced by statistically significant differences in fornix shortening at days 7, 14, 21, and 28 (P < 0.05 for all time points). The presence of the CG copolymer may have inhibited contraction of grafted wounds during the early stages when a significant amount of undegraded CG copolymer matrix was present in the wound bed. Previous studies of the inhibition of wound contraction by CG copolymer matrix in a dermal wound model have led to the hypothesis that the random porous structure CG copolymer interrupts the continuity of myofibroblasts in the wound, thus preventing contraction.19

Collagen Deposition and Organization
Numerous investigators have studied the role of myofibroblasts in contraction and alignment of collagen during healing of various tissue types including skin,42–43 cardiac muscle,44 tendon,45 and conjunctiva.10 The pattern of collagen deposition as well as the subsequent cross linking and remodeling of collagen are factors that may determine the final collagen configuration and the functional characteristics of repair tissue or scar.46 In this study, initial deposition of new collagen in the ungrafted wound was found at day 7. At day 28, mature subepithelial fibrous scar was identified in ungrafted wounds by its dense linear alignment of collagen57 and its hypocellular condition.48–49 It is interesting to speculate that over the time course of this study myofibroblasts may have been involved in imparting such a high degree of organization to the collagen fibers. Dermal scar, which is composed of highly aligned collagen fibers, has mechanical properties distinctly different from those of normal dermis.50 We would therefore expect that the randomly oriented collagen fibers of the matrix-grafted wounds would exhibit mechanical behavior dissimilar to that of aligned collagen fibers of scar tissue.

We observed that fibroblasts within the CG copolymer matrix tended to adhere to the randomly oriented pore walls and thus adopt a random orientation (Figs. 2, 4). In other models of wound healing, fibroblasts deposit collagen in a direction parallel to their alignment.12 It is not surprising that the random orientation of collagen fibers in day 28 matrix-grafted wounds reflects the random orientation of cells in the matrix pores at earlier time points. The open pore structure of the CG copolymer provided space for fibroblast ingrowth, and the irregular geometry of the pore walls may have disrupted the linear pattern of collagen deposition by fibroblasts.

CONCLUSION
In this investigation, we demonstrated that implantation of a CG copolymer matrix inhibited wound contraction in a full-thickness defect of the rabbit conjunctiva. Wounds that were not grafted with CG copolymer closed by a combination of wound contraction and formation of subconjunctival scar. Subconjunctival scar was characterized by a linear array of densely packed collagen fibers with a sparse population of fibroblasts. In the presence of the CG copolymer graft, the wound healing response was modified toward regeneration of nearly physiological subepithelial stroma rather than contraction and scar formation. Subepithelial tissue in the grafted group comprised a loosely organized network of randomly oriented collagen that resembled that of the normal bulbar conjunctiva. In both ungrafted and grafted wounds, the presence of myofibroblasts at the wound margin coincided with the active phase of wound contraction.

References
Collagen-Glycosaminoglycan Conjunctival Implant

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