Efficacy and Safety of Biodegradable Collagen–Glycosaminoglycan Polymer as a Material for Scleral Buckling

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PURPOSE. To test the efficacy and safety of a biodegradable collagen-glycosaminoglycan (CG) polymer as the material for scleral buckling in rabbit eyes.

METHODS. Segmental scleral buckling was performed by using a silicone sponge in one eye and a biodegradable CG polymer in the other eye of 20 rabbits. Wound and conjunctival reactions were evaluated by external photographs 1 day and then every week after surgery. Echography was used to evaluate the extent of the buckling effect. Electretinograms were used to evaluate the retinal function after scleral buckling. Histology and immunohistochemistry were used to check the tissue reaction and distribution of myofibroblasts during wound healing. Scanning electronic microscopy of buckling materials was used to analyze structural changes after episcleral implantation.

RESULTS. Biodegradable collagen initially achieved a buckling effect comparable to a silicone sponge; the buckling effect decreased after 1 month. Within 8 to 12 weeks, the collagen was gradually absorbed. After implantation, the collagen matrix degraded, and the pore size decreased as a result of compression and degradation. In contrast, no major structural changes were observed in silicone sponges, except some cell debris, fibrin, and blood cells were detected inside the porous structure of the sponge. The inflammatory responses were comparable between sponge and collagen in most areas of peribuckling histology. In areas of degraded collagen, a foreign body reaction was observable. Electretinography revealed no detectable difference in retinal function between control and peribuckling histology. In areas of degraded collagen, the inflammatory responses were comparable between sponge and collagen in most areas of peribuckling histology. In areas of degraded collagen, a foreign body reaction was observable. Electretinography revealed no detectable difference in retinal function between control and experimental eyes.

CONCLUSIONS. Biodegradable collagen was used effectively and safely as a material for scleral buckling. (Invest Ophthalmol Vis Sci. 2008;49:2673–2678) DOI:10.1167/iovs.07-1594

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3 mm long. The episcleral implants were approximately 10 mm long, and the sutures were tightened enough to produce a visible scleral invagination. The conjunctiva was closed with 7-0 polyglactin sutures (Vicryl; Ethicon, Somerville, NJ). At the end of the surgery and for the following 28 days, ophthalmic antibiotic ointment (tobramycin) was applied to the surgical eyes. Absorbable collagen explants were implanted in the right eyes, and silicone sponges (506 sponge; Mira Inc., Waltham, MA) were implanted in the left eyes as the control (Fig. 1).

**External Photos**

Each week, animals were anesthetized and external photographs were taken by a digital camera. The degree of conjunctival congestion and buckle absorption were recorded. Conjunctival congestion was evaluated according to the Cornea and Contact Lens Research Unit (CCLRU) grading scale.\(^\text{20}\) The severity of conjunctival redness ranged from very slight (grade 1), to slight (grade 2), to moderate (grade 3), to severe (grade 4). Signs of wound infection, buckle migration, and buckle exposure were also recorded.

**Echographic Examinations**

The effect of scleral indentation was recorded by echographic examinations each week according to previously published methods.\(^\text{21,22}\) After systemic anesthetization of animals, topical anesthetic agent (Novesin; Novartis, Hettlingen, Switzerland) was applied to the eye. An echographic machine (B-5500; Sonomed Inc., Lake Success, NY) was used to examine the effect of explant indentation on the sclera. The echographic probe (frequency: 10 MHz \(\pm 10\%\)) was directed toward the area of the scleral explants. B-scan mode was used to document the effect of scleral indentation while, A-scan mode was used to confirm the site of scleral indentation. A high spike was expected in the A-scan if the proper area of explants was recorded. The distance of the greatest indentation was measured as the buckling effect.

**Histology and Immunohistochemical Staining**

From 4 to 8 weeks after surgery, one rabbit was killed each week for histology. After fixation and dehydration of the eye cups, the samples were embedded in paraffin, serially sectioned (6 mm thickness), and stained for light microscopy with hematoxylin and eosin. The sections were examined in areas around the scleral explants. Additional tissue sections were used for immunohistochemistry to identify the distribution of myofibroblasts.\(^\text{17}\) Myofibroblasts synthesize collagen fibers in a direction parallel to their orientation and are actively involved during wound healing.\(^\text{25}\) Myofibroblasts contain the contractile apparatus of smooth muscle cells, \(\alpha\)-smooth actin (SMA), which was the species targeted by immunohistochemistry.

**Electron Microscopy (EM)**

Before and 6 weeks after implantation, the scleral explants were scanned via electron microscopy. After an intravenous overdose of pentobarbital sodium (100 mg/ml; Sigma-Aldrich, St. Louis, MO), the eyeballs were enucleated for morphology. The scleral explants were fixed in 3% glutaraldehyde in sodium phosphate buffer for 1 hour at 4°C, postfixed in 1% osmium tetroxide, dried to the critical point, sputter-coated in platinum, and photographed by microscope (model S-5000; Hitachi, Tokyo, Japan).

**Electroretinograms**

Some absorbable buckling materials, such as gelatin, may cause a low-grade inflammatory reaction with vitreous haze and even macular edema.\(^\text{1}\) Therefore, the ERG was used to evaluate retinal functional changes after implantation of buckling materials in the rabbits. One week after the scleral implants were placed and immediately before death, flash ERGs were recorded (UTAS-E 300; LKC Technology, Gaithersburg, MD) to assess the retinal function of collagen-implanted and control eyes. The rabbits were dark-adapted for 1 hour before the ERG. After they were anesthetized, the rabbits were placed on a heating pad. The recording Ag:AgCl electrode was placed on the cornea with 0.5% methyl cellulose as a conductive medium. A reference electrode was attached to the shaven skin of the head, and a ground electrode was clipped to the ear. A single-flash light (duration, 100 ms) 30 cm from the eye was used as the light stimulus. The responses were amplified with a gain setting \(\pm 500 \mu V\) and filtered with an amplifier (low, 0.3 Hz; high, 500 Hz). Data were acquired, digitized, and analyzed (EM) for Windows, ver. 2.6; LKC Technology) running on an IBM-compatible computer. The amplitudes and the implicit times of the a- and b-waves were measured and averaged.

**Statistical Analysis**

The Wilcoxon signed-ranks test was used to test for significant differences in the buckle effects and ERG results between collagen-implanted and control eyes. The data were obtained and analyzed with statistical software (SPSS ver. 11.0; SPSS Inc., Chicago, IL) and are expressed as the mean \(\pm SD\), with \(P < 0.05\) considered to be significant.

**RESULTS**

**External Photos**

The degree of conjunctival congestion was similar between eyes implanted with silicone sponges and those implanted with collagen polymers (Fig. 2A). After surgery, moderate to severe conjunctival congestion lasted for \(\sim 4\) to 5 weeks. After that, the redness decreased gradually. There was no significant difference between the two groups in the degree of conjunctival redness assessed by the CCLRU grading scale up to 10 weeks after surgery (Fig. 2B). None of the eyes developed buckle extrusion, intrusion, or infection.

**Buckle Height Determination**

After surgery, the silicone sponges maintained a very stable buckle height. In contrast, the buckling effect of the CG matrix degreased gradually (Fig. 3). The buckle created by each material was noted immediately after surgery; the buckling effect was similar between the two materials. One week after implantation, there was still no significant difference in buckle height between the silicone sponge and the collagen matrix (3.79 \(\pm 0.25\) mm vs. 3.64 \(\pm 0.31\) mm, \(n = 20\) each; \(P = 0.07\)). One month after implantation, 72% of the buckle height remained in the collagen-implanted eyes (3.68 \(\pm 0.24\) mm vs. 2.42 \(\pm 0.55\) mm, \(n = 7\) each; \(P = 0.017\)). Six weeks after implantation, 43% of the buckle height remained in the collagen-implanted eyes (3.59 \(\pm 0.23\) mm vs. 1.59 \(\pm 0.87\) mm, \(n = 8\); \(P = 0.17\)). Eight weeks after implantation, only 18% of the buckle height remained in the collagen-implanted eyes (3.7 \(\pm 0.11\) mm vs. 0.60 \(\pm 0.58\) mm, \(n = 5\); \(P = 0.043\); Fig. 3). Most of the CG matrices were reabsorbed 8 to 12 weeks after episcleral implantation.

**Electroretinograms**

The ERG amplitude and the implicit time of the b-wave of the collagen-implanted eyes were not significantly different from those of the sponge-implanted eyes eyes, either at 1 week after surgery or immediately before death (Fig. 4). There was no noticeable change in ERG a-wave amplitude or implicit time between the two groups (data not shown).

**Histology and Immunohistochemistry**

The histology and immunohistochemistry results are shown in Figure 5. In eyes implanted with silicone sponges, the tissues around the sponge formed a pseudocapsule (a capsule with endothelial lining). The pseudocapsule was mainly composed of fibroblast-like cells. There were a few neutrophils and lymphocytes scattered around the buckel material and in areas
with stitches. In some areas, plasma cells were also detected. No foreign body giant cells were found. In eyes implanted with CG matrix, more inflammatory reactions were noted in areas inside the collagen material. No capsule formed around the CG matrix. In CG-degraded areas, there was less inflammation than in areas inside the CG matrix undergoing active degradation. The degree of inflammation was compatible with that in the eyes implanted with silicone sponges. Some foreign body giant cells were clustered around the collagen and around the area of the stitches. There was more neutrophil and lymphocyte infiltration in the pericollagen areas, indicating a mixed acute and chronic inflammatory reaction. Some plasma cells were also detected. The choroid and retina adjacent to the implant appeared to be intact, except for signs of compression.

In CG-implanted eyes, immunostaining for α1-SMA showed randomly distributed myofibroblasts adhering to the remaining CG matrix. In contrast, SMA-positive cells were found only in the structure of vessel walls inside the capsule in the sponge-implanted eyes. The randomly distributed myofibroblasts that produced collagen in the random alignment were different from the uniaxial orientation of the myofibroblasts and collagen in scar tissue. No retinal edema or retinal inflammation was noted. Only retinal compression was noted in the area directly underneath the scleral buckling.

Scanning Electronic Microscopy (SEM)

SEM of the silicone sponge revealed numerous porous structures on the surface of the silicone sponge. The size of these porous structures ranged from 20 to 400 μm. After the sponge was implanted as an episcleral buckling agent, no major structural changes were observed, except for some cell debris, fibrin, and blood cells detected inside these porous structures.

**FIGURE 1.** Gross appearance of collagen matrix (top) and 506 silicone sponge (bottom).

**FIGURE 2.** External photographs and conjunctival congestion scores of eyes after implantation of CG matrix and silicone sponge. (A) Collagen matrix was implanted in the right eye and a silicone sponge in the left eye. The degree of conjunctival congestion was comparable between these two groups. Buckling in eyes implanted with silicone sponges remained stable after surgery, whereas the collagen matrix was reabsorbed gradually. (B) There was no difference in conjunctival congestion scores between these two treatment groups up to 10 weeks after surgery.
SEM of the CG matrix revealed that the collagen matrix consisted of collagen plates loosely packed together. Randomly oriented pore walls were observed on the collagen matrix surface. The pore size of the collagen matrix ranged from 20 to 200 μm. After the sponges were implanted episclerally for 6 weeks, the morphology of the collagen matrix changed significantly. The collagen matrix degraded partially and the pore size decreased as a result of compression and degradation (Fig. 6).

**DISCUSSION**

Buckling implants close retinal tears and provide mechanical support until the retina and choroid have reattached with chorioretinal scar formation. Permanent implants have been used for decades for scleral buckling in retinal detachment surgery. In cases with a fixed retinal fold, multiple breaks, or advanced proliferative vitreoretinopathy (PVR), permanent support to reattach the retina is usually needed. Permanent implants can cause long-term complications such as intrusion, extrusion through the conjunctiva, infection, pain, or diplopia.2–13 In retinopathy of prematurity–induced retinal detachment treated with scleral buckling, additional procedures must be performed early in infancy to sever or remove the permanent buckling materials, to prevent the inhibition of normal eye growth.6 In simple uncomplicated cases of retinal detachment, the need for a buckling effect is temporary.24

An optimal implant for patients who need only temporary scleral buckling could be made of a slowly biodegradable material with good biocompatibility, such as donor tissue or specially prepared materials. Donor tissues, such as fascia lata25 and sclera,26-27 are derived from either autogenous or cadaveric sources. The drawbacks of using these tissues are the additional surgery necessary to obtain autologous tissues or the risk of infection or other unknown risks when using donor tissues. Specially prepared materials, such as gelatin28–31 and bovine fibrin,32 have been used as scleral implants. Gelatin is somewhat brittle and must be implanted under the scleral flap.1 Once implanted, it gradually liquefies and is replaced by a thin layer of granulation tissue. Because of this tissue reaction, gelatin may cause a low-grade inflammatory reaction with vitreous haze and even macular edema.1

Because temporary buckling materials are sometimes desirable and because of the shortcomings of available temporary buckling materials, biodegradable scleral implants comprised of a CG copolymer were developed and tested in this rabbit animal model. The CG copolymer is a biocompatible material that undergoes biodegradation during the wound healing process in vivo. The rate of degradation of the CG copolymer depends on the tissue reaction and the dosage of anti-inflammatory medication.33–35 Beyond passive degradation, the CG copolymer matrix can even induce partial regeneration of the injured derrmis in humans and animals.36–38 Implantation of the CG copolymer in these models inhibited wound contraction and stimulated regeneration of a nearly physiological derrmis. Implantation of CG copolymer matrix in a surgery-induced conjunctival defect drastically reduced wound contraction and guided the healing of subconjunctival stroma with a more physiological collagen deposition.39 Conjunctival scarring was inhibited and fornix shortening was prevented after implantation.18 In agreement with these other research reports, our study also shows that application of CG copolymer can lead to random and relatively loose reorganization of regenerating myofibroblasts, that might reduce scar formation.17 These findings suggest that the CG matrix provides a physiological structure for tissue repair, inducing a conjunctival wound to heal more in a physiological than a pathologic process.

Potential complications associated with CG copolymers are comparable with those of silicon sponges before they are degraded. These complications include infection, extrusion, anterior segment ischemia, changes in refractive error, choroidal detachment, motility disturbance, and failure to reattach. However, CG copolymers are reabsorbed within a couple of months after implantation, which might reduce the complications of chronic infection, motility disturbance, and induced
refractive error. If the underlying etiology that induces PVR and retinal redetachment after the CG copolymer has degraded, then permanent scleral buckling materials will be needed because they could provide a persistent vitreous base support to counter the tractional force.

CG polymer, implanted on top of the sclera like a sponge, is easier to handle, less brittle, and less antigenic than gelatin. Therefore, simple retinal detachment may be treated more safely and with more physiologic wound healing by using this material as a biodegradable implant. The stiffness of the CG material and the rate of its degradation can be adjusted according to each patient’s particular need by changing the level of collagen cross-linkage and the concentration of collagen. Thus, CG matrix could be a good biodegradable material for scleral buckling procedures in the future. These promising results justify controlled clinical trials with this biodegradable material, which might allow the combined advantages of precise localization similar to silicone sponge explants, reversibl-

**FIGURE 5.** Histology and immunohistochemistry results after implantation of sponges or CG copolymers. H&E staining showed numerous neutrophils and lymphocytes inside the degrading CG matrix. In sponge-implanted eyes, pseudocapsule formation around sponge was noted, with fewer neutrophils and lymphocytes. In CG-implanted eyes, immunostaining for α-SMA showed randomly distributed myofibroblasts (black arrows) adhering to the degrading CG matrix (white arrowheads). In contrast, SMA-positive cells were found only in the structure of vessel walls inside the capsule in the sponge-implanted eyes (black arrows). Scale bar, 20 μm.

**FIGURE 6.** SEM of silicone sponge and collagen matrix before and after episcleral implantation. Cut end of collagen-CG matrixes shows irregularly shaped cells (interstices) 20 to 400 μm in size before implantation. After implantation, the morphology of the collagen matrix changed significantly. The collagen matrix degraded, and the pore size decreased as a result of compression and degradation. SEM of the silicone sponge revealed numerous porous structures on the surface of the silicone sponge. After implantation of the sponge as an episcleral buckling agent, no major structural changes were observed, except for some cell debris, fibrin, and blood cells detected inside these porous structures.
ity similar to inflatable balloon treatment, and perhaps even enhanced wound healing.

References