Control of Filtering Bleb Structure through Tissue Bioengineering: An Animal Model

Henry Shen-Lih Chen,[1,2] *Robert Ritch,*[3,4] *Theodore Krupin,*[5] *and Wei-Cherng Hsu*[6,7]

Purpose. To devise a means of providing controlled resistance between the anterior chamber and the subconjunctival space after trabeculectomy by implantation of a biodegradable, porous collagen matrix.

Methods. Matrices were implanted in the right eyes of 17 rabbits after trabeculectomy, while left eyes served as surgical controls. The scleral flap was sutured loosely, and the implant provided pressure on the scleral flap to reduce overfiltration. Trabeculectomy in the control eyes was performed with tight sutures using standard methodology. Intraocular pressure (IOP) was measured before surgery and on days 3, 7, 14, 21, and 28 after surgery. Masson trichrome and α-smooth muscle actin stains were used for histologic study of the filtering blebs.

Results. The initial postoperative IOP reduction was approximately equal, at 14% to 16%, for both groups. In the implanted group, the IOP continued to decrease to 55% below baseline at day 28 as the implant gradually degraded. In the control group, IOP had returned to the preoperative level by day 21. Histologic examination with Masson trichrome and α-smooth muscle actin stains showed a prominent bleb in the implanted group compared with scar formation and limited bleb formation in the control group.

Conclusions. Implantation of a biodegradable, porous collagen matrix in the subconjunctival space offers the potential for a new means of avoiding early scar formation and maintaining long-term IOP control by creating a loosely structured filtering bleb. (*Invest Ophthalmol Vis Sci.* 2006;47:5310–5314) DOI: 10.1167/iovs.06-0378

Trabeculectomy remains the criterion among surgical procedures for the reduction of intraocular pressure (IOP) in patients with glaucoma after medical and laser therapy have failed.[1,2] However, wound healing and scar formation result in fibrosis of the bleb or obstruction of the drainage fistula, eventually leading to bleb failure.[3] Hence, the inhibition of scar formation during the wound healing process should promote greater success.[4]

The two most important locations related to the success of trabeculectomy are the subconjunctival and the subscleral flap spaces. Too loose suturing of the scleral flap predisposes to a shallow or flat anterior chamber early after surgery. To minimize early hypotony, adjustable sutures and laser suture lysis may be used to titrate aqueous flow into the bleb and bleb size in the early postoperative period.

Clinically, mitomycin-C (MMC), 5-fluorouracil, and corticosteroids have all been used to inhibit fibroblast proliferation to prevent scar development after glaucoma surgery.[5,6] Antifibrosis agents, particularly MMC, increase the risk for late postoperative complications,[7] including wound leakage, hypotony, and maculopathy.[8–10] Resultant avascular thin conjunctiva decreases tissue defense mechanisms and increases the risk for bleb infection and endophthalmitis.[11]

The use of tissue bioengineering to prevent scar prevention is progressing rapidly.[12,13] The application of three-dimensional, collagen-glycosaminoglycan copolymers can lead to random and relatively loose reorganization of regenerating myofibroblasts, fibroblasts, and the secreted extracellular matrix (i.e., collagen), resulting in reduced scar formation.[14–16] These biodegradable implants may simultaneously provide physical resistance to overfiltration in the early stages and progressively may reduce this resistance as the implant degrades and a random connective tissue matrix permeates the spaces within the implant to create a loosely organized bleb structure.

We tested a three-dimensional collagen matrix placed in the subconjunctival space and on top of the scleral flap to achieve better postoperative IOP control and to reduce the risk for early shallow anterior chamber.

Materials and Methods

Implantation of the Biodegradable Porous Matrix

The drug-free, biodegradable, porous collagen matrix of 1% collagen/C-6-S copolymer was cut into small disks measuring 8 mm in diameter and 2 to 3 mm in thickness. The disks were immersed in 0.1 M phosphate-buffered saline (PBS) before implantation. Seventeen female New Zealand albino rabbits, each weighing between 2.5 to 3.5 kg, were anesthetized by intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg). Matrices were implanted in the right eyes, and the left eyes served as the surgical controls. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Trabeculectomies were performed using Watson's modification of the Cairns technique, described in detail elsewhere.[1,2] After a limbus-based conjunctival flap was fashioned, the sclera was exposed, and a rectangular 3 × 4-mm scleral flap was prepared. A 1 × 2-mm sclerostomy was followed by peripheral iridectomy. The scleral flap was closed with two relatively loose 10–0 nylon sutures. A porous collagen–glycosaminoglycan matrix was placed over the scleral flap, and the conjunctiva was closed with continuous 10–0 nylon sutures. Tenonectomy was not performed, and antifibrosis agents were not used. The same surgical procedure was performed on the left eyes without matrix implantation using normal suture tension for the scleral flap. Immediate postoperative IOP was similar for both eyes.

From the [1]Glaucoma Service, Department of Ophthalmology, Chang Gung Memorial Hospital, Taipei, Taiwan; [2]Chang Gung University, College of Medicine, Tao-Yuan, Taiwan; [3]New York Eye and Ear Infirmary, New York, New York; [4]The New York Medical College, Valhalla, New York; [5]Department of Ophthalmology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois; [6]Taipei Medical University, Taipei, Taiwan; and the [7]Department of Ophthalmology, Taipei City Hospital, Taipei, Taiwan.

Presented in part at the 18th Congress of the Asia-Pacific Academy of Ophthalmology, Taipei, Taiwan, Republic of China, March 10–14, 2001 and at the 2006 World Ophthalmology Congress, São Paulo, Brazil.

Supported by Life Spring Biotech Co. Ltd.

Submitted for publication April 5, 2006; revised June 11, 2006; accepted October 17, 2006.

Disclosure: **H.S.-L. Chen**, Life Spring Biotech Co. Ltd. (F); **R. Ritch**, (P); **T. Krupin**, (P); **W.-C. Hsu**, (P)

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Wei-Cherng Hsu, Taipei Medical University, 7F, No. 3, Alley 18, Lane 170, Sec 4, Chungsiau East Road, Taipei, Taiwan; cyao@seed.net.tw.

IOP was measured (Tono-Pen; Medtronic, FL) after the rabbits were anesthetized with half-dosage intramuscular ketamine (35 mg/kg) and xylazine (5 mg/kg) on days 3, 7, 14, 21, and day 28. The percentage postoperative IOP reduction was calculated as: [IOP change (%) = (postoperative IOP – preoperative IOP) × 100%]/Preoperative IOP.

Student *t* test ($P < 0.05$) was used to compare IOP between the control and the collagen implant eyes.

Histologic Evaluation after Collagen Matrix Implantation

Rabbits were killed by excess ketamine (35 mg/kg) and xylazine (5 mg/kg) on days 3, 7, 14, 21, and 28 after implantation. Eyes, including the eyelids, were quickly removed and fixed overnight in 4% formaldehyde. The conjunctiva and implant with the underlying scleral bed was dissected, dehydrated, and embedded in paraffin. Sections were cut by a microtome at 7 μm and stained with hematoxylin and eosin (H&E) for general histologic observation and with Masson trichrome stain to assess collagen deposition and remodeling. Additional tissue sections were used for α-smooth muscle actin (SMA) immunocytochemistry to identify the distribution of myofibroblasts.

Implant Hydraulic Pressure after Saturation with Physiological Buffer

The collagen matrix containing 0.25%, 0.5%, and 1% collagen/C-6-S copolymers were cut into disks of 7, 7.5, 8, 8.5, and 9 mm in diameter and 2 to 3 mm in thickness. The dry disks ($n = 10$ for each diameter) were weighed and placed in 0.1 M PBS and were weighed again after saturation. The saturated static pressure of the matrix per unit area was calculated on the basis of the following equation:

Saturated static pressure of the implant

= (weight of saturated implant

− weight of dry implant)/Area of implant.

The stiffness of the matrix can prevent the reduction of the wound defect by a purse string effect and can maintain the size of the bleb without collapse. Strain is the change in length/original length. When the matrix is compressed by external force, it becomes more rigid and more difficult to deform. To assess the relationship between strain and the pressure of the collagen matrix, we gradually compressed the matrix by external force on top of it and measured the pressure required to reduce it from the initial height (100%) to half that (50%).

Measurements were compared using a one-tailed Student *t*-test.

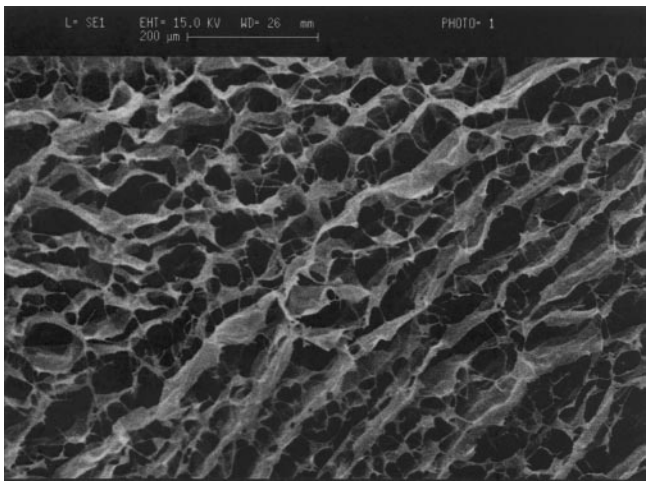

FIGURE 1. Scanning electron microscopic image of collagen matrix.

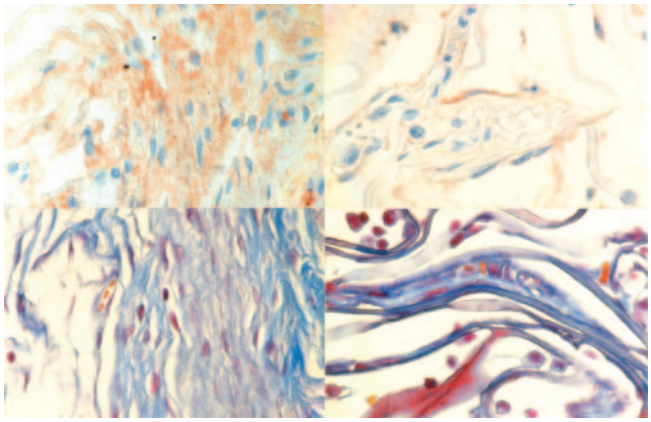

FIGURE 2. Day 14. *Left*: (*upper*) α-SMA and (*lower*) Masson trichrome stains show high cell density with close cell-to-cell contact with less extracellular space in the control group. *Right*: (*upper*) α-SMA and (*lower*) Masson trichrome stains show less cell-to-cell contact with prominent extracellular space in the implanted group.

RESULTS

Collagen Matrix

Scanning electron microscopy revealed that the collagen matrix consisted of a diffusely porous material. The pore size of the collagen matrix ranged from 20 μm to 200 μm (Fig. 1).

Hematoxylin-Eosin Staining

Wound areas of implanted and control eyes showed a typical acute inflammatory response at days 3 and 7 after surgery. Elongated fibroblasts, macrophages, and different types of lymphocytes aggregated on the surface of the implant. The implanted matrix began to degrade after 7 days following surgery.

Ingrowing cells were distributed throughout the porous pattern of the implant but less densely than on the surface. Inflammatory responses decreased gradually from day 14 and subsided completely by day 21 after surgery in the control group and by day 28 as the implant degraded completely.

Masson Trichrome and α-SMA Staining

In the control eyes, immunostaining of α-SMA showed numerous myofibroblasts aligned parallel to the sclera surface until day 14 after surgery and compactly aggregated collagen fibers secreted by myofibroblasts. In contrast, the implanted eyes showed fewer myofibroblasts, which adhered randomly to the remaining matrix (Fig. 2) and the surrounding wound area (Fig. 3). During the period of degradation of the implant, the bleb space remained prominent (Fig. 4).

On postoperative day 21, diminished bleb size in the control eyes was even more pronounced, combined with the aggregation of collagen fibers in the subconjunctival space (Fig. 5). Control eyes 28 days after surgery showed a diminished bleb space with dense linear collagen filling in the subconjunctival space. The implanted group on postoperative day 21 had a prominent bleb with some collagen dispersed inside the subconjunctival space, and the structured implant was no longer visible (Fig. 6). Comparison of the depths of the blebs revealed that the blebs of the implanted group were five to six times deeper than those of the control group (the diameter of a 10–0 nylon is used here as a unit).

Mechanical Pressure

As a reservoir, the implant can hold water and simultaneously provide pressure on top of the scleral flap. The pressure of

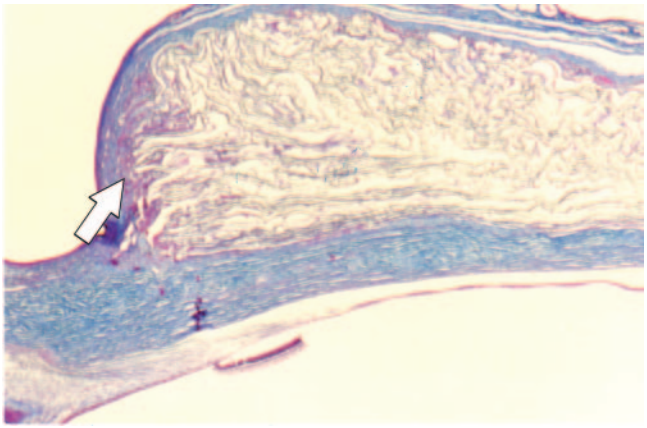

FIGURE 3. Masson trichrome stain, day 7. The intact implant occupies the subconjunctival space, with cells (*white arrow*) migrating on its surface. Nuclei are stained *brown*. Original magnification, ×40.

different collagen concentrations varies: the higher the concentration, the higher the mechanical pressure (Fig. 7).

IOP

On the first postoperative day, the anterior chambers were formed in both groups, implying adequate tension over the scleral flap in both groups. Three days after surgery, IOP reduction was similar in the control (–16%) and implanted (–14%) eyes. The control group had an approximately 20% decrease in IOP for the first 2 weeks, with IOP reverting to the preoperative IOP on day 21. In contrast, IOP decreased steadily in the implanted eyes, reaching an approximately 55% reduction on postoperative day 28 (Fig. 8). IOP was significantly lower in the implanted eyes than in control eyes on days 7, 14, 21, and 28 ($P < 0.05$).

Relationship between Pressure Created from the Collagen Matrix and Strain Induced by External Compressive Force

When the compressive force (or weight) was applied on top of the collagen matrix, the pressure the matrix created against the compressive force increased when the percentage of strain became higher (Fig. 9).

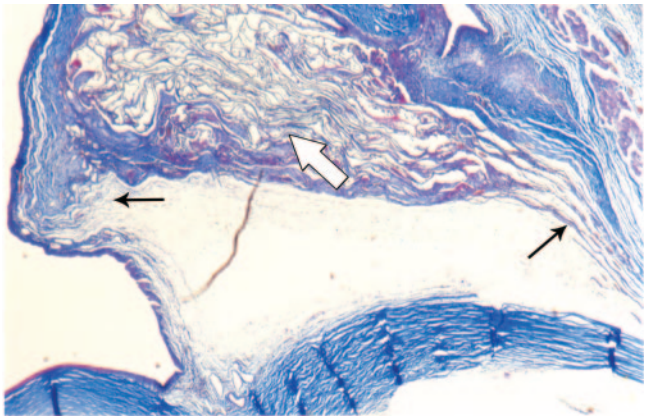

FIGURE 4. Partially degraded implant (*white arrow*) inside a prominent bleb. Cells are present inside the implant, as opposed to the region in which the implant has degraded, where only collagen remains (*black arrow*). Masson trichrome stain, day 14. Original magnification, ×20.

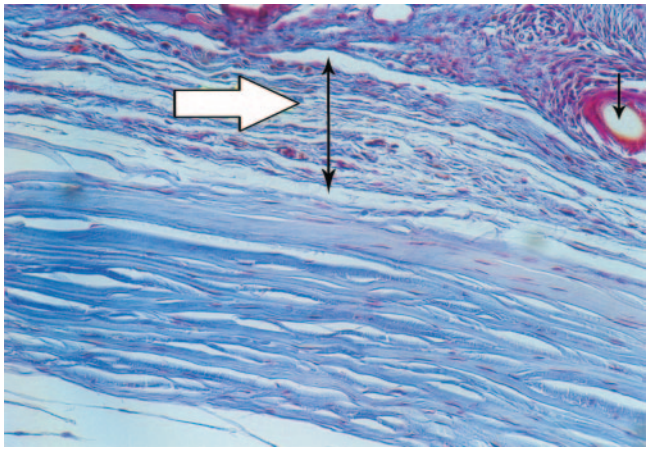

FIGURE 5. Linear collagen (*white arrow*) deposited inside the collapsed bleb (*long black arrow*) in control group. Sclera was seen in this view. 10–0 nylon (20- to 30-μm diameter; *short black arrow*). Masson trichrome stain, day 21. Original magnification, ×200.

DISCUSSION

The most critical factor about the final IOP after filtering surgery is the wound-healing process.[3] If healing can be con-

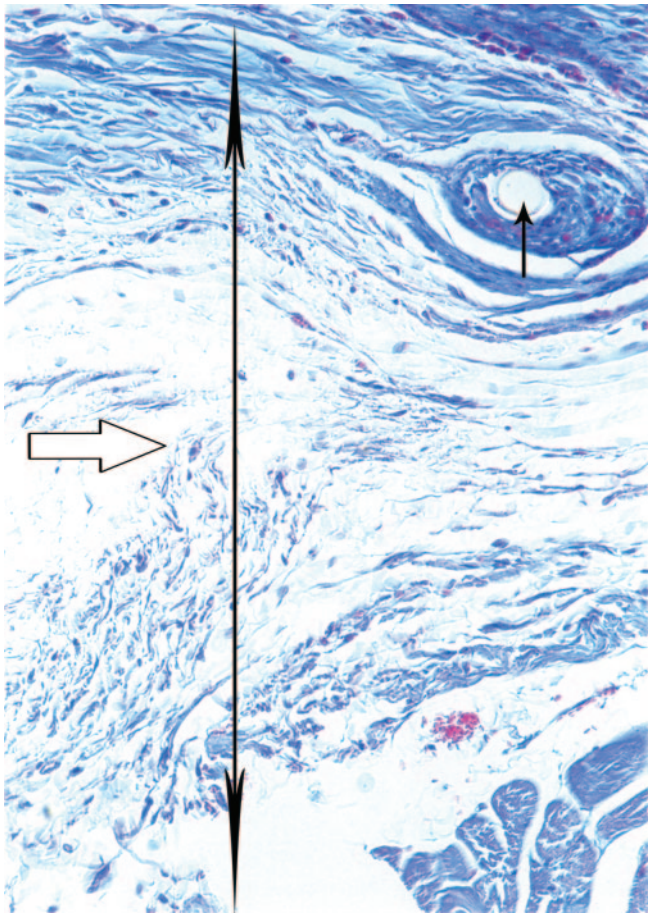

FIGURE 6. Randomized collagen (*white arrow*) deposited in prominent bleb (*long black arrow*) in implanted group (depth indicated by *long black arrow*, 16× the diameter of a 10–0 nylon, indicated by *short black arrow*). No sclera was seen in this view. 10–0 nylon (20- to 30-μm diameter, *black arrow*) was located at right upper corner. Masson trichrome stain, day 21. Original magnification, ×200.

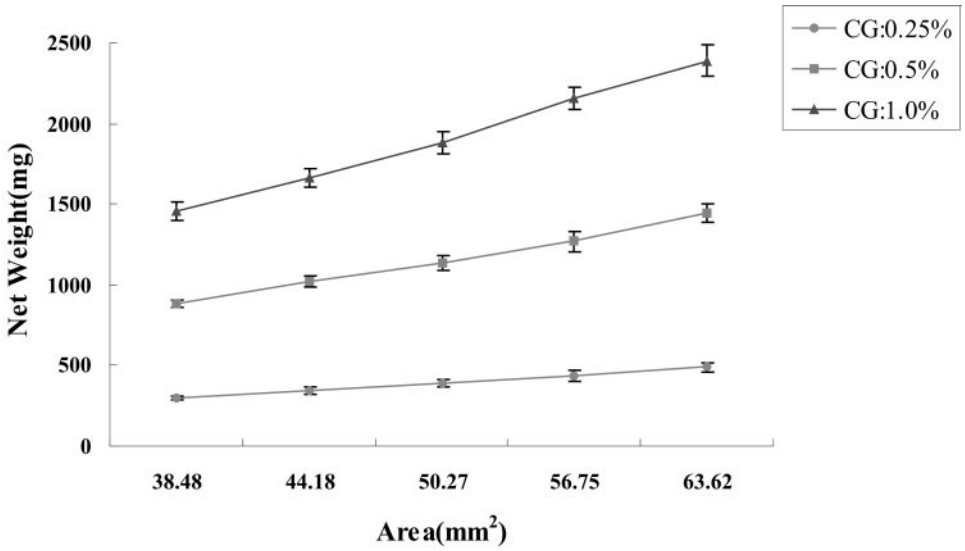

FIGURE 7. Resultant change of mechanical pressure caused by different concentrations of collagen/C-6-S in matrices. Course of IOP after implantation of the collagen matrix.

trolled, patients can better achieve low target IOP. However, scar formation is the most common cause of bleb failure.[17] To circumvent the wound-healing process, particularly in complicated cases of glaucoma, aqueous drainage implants are often used.[18] The complications of tube-shunt surgery include excessive aqueous outflow, tube obstruction, corneal damage, strabismus, tube migration, and long-term foreign body reaction usually resulting from the characteristics of nonbiodegradable devices.[19,20]

To improve the results of trabeculectomy, a new physiological environment of the bleb should be established with dynamic drainage of the aqueous from the anterior chamber to the conjunctival space. A major complication of filtration surgery scarring is the formation of Tenon capsule cysts consisting of compressed collagen lamellae with few cells and no epithelial lining, preventing reabsorption of aqueous humor.[21]

Antifibrosis agents have markedly improved trabeculectomy success rates by preventing scarring. However, these agents cause irregularities in the conjunctival epithelium, breaks in the basement membrane, and hypocellularity of the conjunctiva and subconjunctival tissue, each of which may predispose to bleb leaks[22] and bleb-related infections.[11] The ideal augmented surgical technique should control postoperative IOP while normalizing wound healing without inhibiting fibroblast proliferation.

Based on an animal model of conjunctiva wound healing,[16] wound contraction occurs during an acute inflammatory stage with the presentation of myofibroblasts. In this model, deposition of dense, linear collagen deposition results in scar formation and completion of the healing process. In contrast, the application of a three-dimensional collagen matrix leads to a random reorganization of regenerating myofibroblasts, fibroblasts, and the secreted extracellular matrix, resulting in reduced scar formation.[14–16]

The current three-dimensional collagen matrix has been designed to achieve three goals: physiological environment to control cell ingrowth, stiffness to maintain the inner porous structure and to preserve the function of the reservoir without collapse during the period of wound contraction, and physical weight on the scleral flap to prevent shallow anterior chambers. In this study, avoiding excessive pressure on top of the scleral flap was secondary to attaining IOP control, and different tensions of sutures were used. Based on the similar IOPs at day 3 in both groups, we believe the effects of the implant will be more focused on these three goals. Two parameters, concentration and temperature, were related to the inner structure of the matrix. To achieve the proper weight and pore size for the implant, we selected a 1% collagen concentration that underwent a freeze-drying process in this study.

Aqueous humor drainage across the scleral flap was controlled not only by sutures but also by pressure created by the collagen matrix implant placed on top of the flap. According to observations of static pressure in this study, we can expect gradual reduction of the implant-induced pressure as the collagen gradually degrades. During the degradation process, the shape and porosity of the implant are maintained despite any

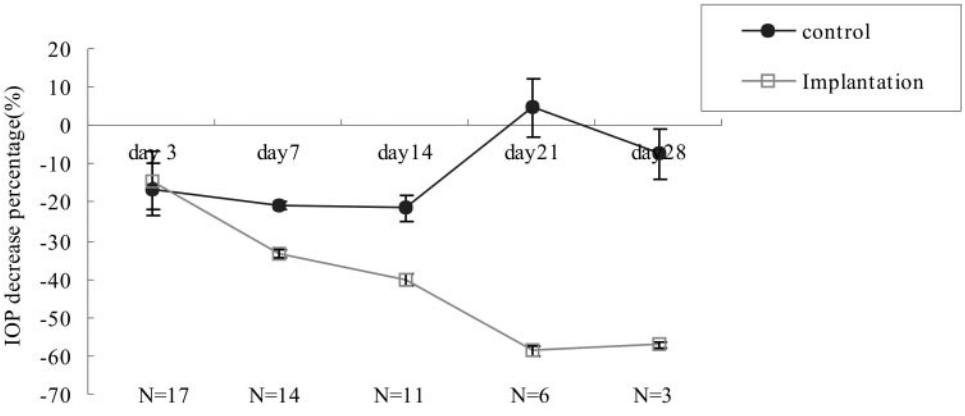

FIGURE 8. The pressure of the implant against compression increased as the strain increased.

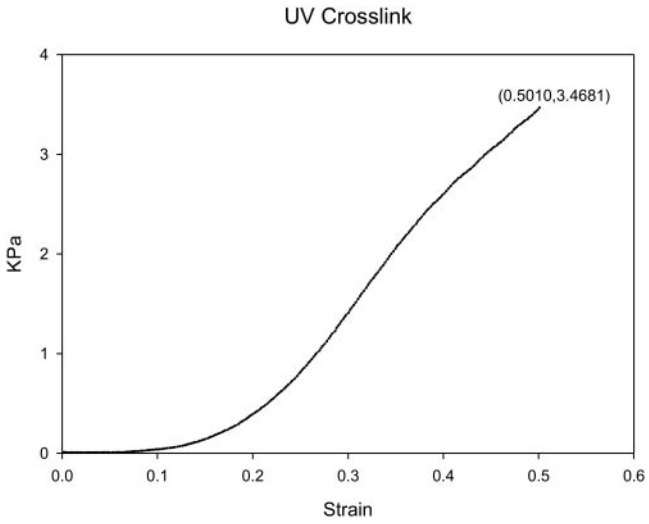

FIGURE 9. The pressure the matrix created against the compressive force increased when the percentage of strain became higher.

force exerted by wound contraction. This creates an equilibrium; its function is maintained until it is completely degraded.

Based on previous findings,[16] the collagen matrix provides a physiological structure for tissue regrowth (epithelium, stroma, and vessels), inducing a conjunctival wound to heal in a more physiological than pathologic process. In the case of filtering surgery in this rabbit model, a 1-month degradation period was sufficient to create a prominent bleb compared with that in the control group. The healing process of the conjunctival stroma in the present study is different from that described in Hsu et al.[18] after a purely conjunctival wound. The conjunctival stroma in this study became one part of the aqueous system with a prominent bleb, and the collagen dispersed inside the aqueous humor was indistinguishable from the surrounding collagen with no scar formation.

The collagen implant offers a potential alternative to antifibrosis agents because it produces more loosely organized bleb tissue than a bleb created without antifibrosis agents and yet more abundant tissue than one created with antifibrosis agents. This new approach, making use of a degradable collagen implant to normalize filtering surgical wound healing, offers potential benefits regarding physics and physiology.

References

1. Cairns JE. Trabeculectomy: preliminary report of a new method. *Am J Ophthalmol*. 1968;66:831–845.
2. Watson PG, Barnett F. Effectiveness of trabeculectomy in glaucoma. *Am J Ophthalmol*. 1975;79:831–845.
3. Arici MK, Demircan S, Topalkara A. Effect of conjunctival structure and inflammatory cell counts on intraocular pressure after trabeculectomy. *Ophthalmologica*. 1999;213:371–375.
4. Skuta GL, Parrish RK II. Wound healing in glaucoma filtering surgery. *Surv Ophthalmol*. 1987;32:149–170.
5. Palmer SS. Mitomycin as adjunct chemotherapy with trabeculectomy. *Ophthalmology*. 1991;98:317–321.
6. Rockwood EJ, Parrish RK II, Heuer DK. Glaucoma filtering surgery with 5-fluorouracil. *Ophthalmology*. 1987;94:1071–1078.
7. Katz GJ, Higginbotham EJ, Lichter PR. Mitomycin C versus 5-fluorouracil in high-risk glaucoma filtering surgery. *Ophthalmology***.** 1995;102:1263–1269.
8. Sinnreich Z, Barishak R, Stein R. Leaking filtering blebs. *Am J Ophthalmol*. 1978;86:345–349.
9. Stamper RL, McMenemy MG, Lieberman MF. Hypotonous maculopathy after trabeculectomy with subconjunctival 5-fluorouracil. *Am J Ophthalmol*. 1992;114:544–553.
10. Sinnreich Z, Barishak R, Stein R. Leaking filtering blebs. *Am J Ophthalmol.* 1978;86:345–349.
11. Greenfield DS, Suner IJ, Miller MP. Endophthalmitis after filtering surgery with mitomycin. *Arch Ophthalmol*. 1996;114:943–949.
12. Yannas IV, Lee E, Orgill DP, Skrabut EM, Murphy GF. Synthesis and characterization of a model extracellular matrix that induces partial regeneration of adult mammalian skin. *Proc Nat Acad Sci USA*. 1989;86:933–937.
13. Yannas IV. Studies on the biological activity of the dermal regeneration template. *Wound Repair Region*. 1998;6:518–524.
14. Orgill DP, Butler CE, Regan JF. Behavior of collagen-GAG matrices as dermal replacement in rodent and porcine models. *Wounds*. 1996;8:151–157.
15. Yannas IV, Burke JF, Orgill DP, Skrabut EM. Wound tissue can use a polymeric template to synthesize a functional extension of skin. *Science*. 1982;215:174–176.
16. Hsu WC, Spilker MH, Yannas IV, Rubin PA. Inhibition of conjunctival scarring and contraction by a porous collagen-glycosaminoglycan implant. *Invest Ophthalmol Vis Sci*. 2000;41:2404–2411.
17. Broadway DC, Chang LP. Trabeculectomy, risk factors for failure and the preoperative state of the conjunctiva. *J Glaucoma*. 2001; 10:237–249.
18. Coleman AL, Hill R, Wilson MR. Initial clinical experience with the Ahmed glaucoma valve implant. *Am J Ophthalmol*. 1995;120:23–31.
19. Lieberman M, Ewing R. Drainage implant surgery for refractory glaucoma. *Int Ophthalmol Clin*. 1990;19:802–810.
20. Gedde SJ, Foster RE, Rockwood EJ. Late endophthalmitis associated with glaucoma drainage implants. *Ophthalmology*. 2001;108: 1323–1327.
21. Buskirk EM van. Cysts of Tenon's capsule following filtration surgery. *Am J Ophthalmol*. 1982;94:522–527.
22. Holger M, Georg A, Bernd K, Michael D. Histopathology of episcleral fibrosis after trabeculectomy with and without mitomycin C. *Graefe's Arch Clin Exp Ophthalmol***.** 1996;234:364–368.