Glaucoma

Foreign Body Reaction in Glaucoma Drainage Implant Surgery

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Submitted: November 15, 2012
Accepted: May 2, 2013

Purpose. To investigate the histopathology of the foreign body reaction (FBR) and the effect of aqueous humor on it in glaucoma drainage implant surgery.

Methods. A glaucoma drainage device was implanted into 20 New Zealand white rabbits. We monitored the histopathology of blebs at microscopic levels from 3 days to 8 weeks postoperatively. Hematoxylin and eosin staining, Masson’s trichrome staining, anti-actin and α-smooth muscle immunofluorescence staining, and antiproliferating cell nuclear antigen immunohistochemistry were performed. To observe effects of aqueous humor on FBR, we designed two implant models. One group received a plate with a tube placed in the anterior chamber (experimental group), whereas the other received the plate cut from the tube (control group).

Results. Foreign body giant cells were found along the inner border of blebs, and the innermost layer of blebs demonstrated a densely packed collagenous stratum in both groups. The number of foreign body giant cells was suppressed in the experimental group compared with the control group (P < 0.001). Fibroblast division was more active in the experimental group than that in the control group. Masson’s trichrome staining demonstrated that the innermost avascular collagenous layer was much thicker in the experimental group than that in the control group (P = 0.021). The extent of α-SMA staining was greater in the experimental group than that in the control group.

Conclusions. In the aqueous humor environment, wound healing around a glaucoma drainage implant revealed a unique FBR with the relatively small number of foreign body giant cells and reinforced fibrotic encapsulation.

Keywords: foreign body reaction, glaucoma drainage implant surgery, aqueous humor, wound healing, myofibroblast

Glaucoma drainage devices are being used with increased frequency for the treatment of glaucoma cases that are complicated or unresponsive to medications.1,2 Implantation of an aqueous drainage device is affected by aqueous humor because glaucoma implants are designed to allow the flow of aqueous humor, which bathes the surgical wound. Aqueous humor contains various cytokines and chemical mediators that significantly influence the local cellular response.3 Additionally, aqueous humor exerts effects in vitro that suggest a potential role in wound healing in vivo. Dilute aqueous humor stimulates proliferation of cultured fibroblasts.4–6

Biocompatibility is generally defined as the ability of a biomaterial, prosthesis, or medical device to induce an appropriate host response in a specific application.7 Practically, the biological response to a medical device influences its ability to perform as intended. Unlike other biomaterial devices, an aqueous drainage valve uniquely requires sufficient diffusion of an aqueous solution out of the surrounding fibrous capsule for proper function. Understanding the biological effect of aqueous humor on the foreign-body response is necessary for development of a thin permeable capsule around the biomaterial device and better aqueous passage through the bleb wall. Foreign body reaction (FBR), comprising macrophages and foreign body giant cells (FBGCs), is the response of inflammatory and wound healing responses following implantation of a medical device or prosthesis. Retention of foreign bodies leads to formation of a dense, hypocellular, collagen-rich capsule. Despite the increasing use of glaucoma drainage devices, the FBR remains incompletely understood and the effect of aqueous humor remains to be elucidated.

In the present study, we investigated morphologic characteristics of the FBR in glaucoma drainage implant surgery, and the effect of aqueous humor on it at both gross and microscopic levels by implanting a standard and modified silicone glaucoma device that does or does not allow aqueous humor flow, respectively, in the rabbit.

Methods

Animals

Twenty adult New Zealand White rabbits (2.0–3.0 kg; 20 weeks old) were used. All rabbits were confirmed to be free of ocular diseases. All animals were handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The Animal Care Committee of the College of
Medicine at the Catholic University of Korea approved the protocols and monitored animal use.

**Implant Model**

All operations were performed by a single surgeon (KJJ) using a glaucoma drainage device (Model FP7 Ahmed Glaucoma Valve [AGV]; New World Medical, Inc., Rancho Cucamonga, CA), which consisted of a silicone tube connected to a silicone sheet valve held in a silicone body. Both eyes of the animals except four rabbits, which assigned to nonoperated group underwent AGV implantation, and their right or left eyes were randomly assigned to the experimental group with anterior chamber tube placement or the control group without tube placement.

General anesthesia was achieved by intramuscular injection of 15 mg/kg ketamine plus zolazepam (Zoletil; Virbac, Carros, France) and 5 mg/kg xylazine hydrochloride (Rompun; Bayer, Leverkusen, Germany). Local anesthesia was provided by proparacaine hydrochloride (Alcaine 0.5%; Alcon Laboratories, Inc., Fort Worth, TX).

A fornix-based incision was made through the conjunctiva and Tenon’s capsule (TC) in the superior temporal quadrant; radial-relaxing incisions on one side of the conjunctival flap were made to improve surgical exposure. The plate was inserted under the conjunctiva and TC and secured to the sclera with nonabsorbable sutures 2 to 3 mm posterior to the limbus. Priming of the valve was performed by irrigation with balanced salt solution through the tube, ensuring patency prior to insertion. In the experimental group, the tube was trimmed to the appropriate length with a beveled edge, so that it extended approximately 2 to 3 mm into the anterior chamber. In the control group, we designed the nonaqueous plate, extending approximately 2 to 3 mm into the anterior chamber.

For fluorescein staining, sections were incubated in 10% sodium hyaluronate 1.65%–sodium chondroitin sulfate 4% (DisCoVisc; Alcon Laboratories, Inc.) was injected to reform the aqueous humor. Next, a paracentesis was created with a 23-gauge needle from 1 to 2 mm posterior to the limbus. The conjunctiva was reapproximated with 8-0 vicryl sutures. After insertion, the whole tube was loosely secured to the sclera with a 10-0 nylon suture. The anterior chamber through a 23-gauge needle, and the tube was inserted under the conjunctiva and TC and secured to the sclera with nonabsorbable sutures 2 to 3 mm posterior to the limbus. Priming of the valve was performed by irrigation with balanced salt solution through the tube, ensuring patency prior to insertion. In the experimental group, the plate was designed with a beveled edge, so that it extended approximately 2 to 3 mm into the anterior chamber. In the control group, we designed the nonaqueous plate, extending approximately 2 to 3 mm into the anterior chamber.

**Validation of Aqueous Humor Flow Into Subtenon’s Space**

At first, to confirm the presence of aqueous humor to the blebs in the experimental group, we observed whether aqueous humor was detected when the blebs walls punctured just before enucleation. Second, intraocular pressure (IOP) was measured after general anesthesia at baseline (prior to operation) and at 3 days, and 2, 4, and 8 weeks postoperatively. IOP was measured in both eyes (Tono-Pen XL; Medtronic Solan, Jacksonville, FL). Three readings were taken and averaged.

**Gross Examination of Blebs After Tube Implantation**

At various time points until 8 weeks after the operation, the animals were randomly euthanized by intravenous administration of potassium chloride, and four animals were assigned to each time point (3 days, and 2, 4, and 8 weeks postoperatively). Before enucleations, all eyes underwent slit-lamp examinations for confirmation of a patent tube tip and evaluation of bleb appearance, including vascularity, on the designated days.

All eyes were enucleated and conjunctival incisions were made along the limbus, except the valve-implanted area, and four rectus muscles were dissected. Extracted eyeballs were immersed overnight in 4% paraformaldehyde in phosphate-buffered saline (PBS) at 4°C.

After fixation of eyeballs, the fibrous capsules around the valve were excised slightly and blebs were turned inside out to examine their inner surface by slit-lamp bimicroscopy.

Photographs of the external and inner surfaces of blebs were taken using a camera (EOS 50D; Canon, Tokyo, Japan) built into the slit-lamp bimicroscopy instrument (BM 900; Haag-Streit, Koeniz, Switzerland).

**Histological Staining**

Blebs surrounding the device were excised and tissues were embedded in paraffin. Four sections per each eye were obtained and one of the sequential 3-μm sections was stained with hematoxylin and cosin (H&E) to calculate the number of FBGCs, except those that abut the suture material. Sections were stained using a trichrome stain kit (Masson’s, HT15; Sigma-Aldrich, St. Louis, MO) to identify collagen quantitatively and qualitatively based on blue staining.

Slides were scanned and investigated using a commercial scanner (Panoramic MIDI Scanner; 3DHISTECH Ltd., Budapest, Hungary). The height of the collagen-stained area was calculated using the distance-measuring tool in the scanner. The mean of the three height measurements along the bleb wall was calculated and compared between the two groups.

**Immunohistochemistry and Immunofluorescence Staining**

Immunohistochemical staining was performed using standard protocols with dextran polymer reagents (Envision + DAB system; Dako, Glostrup, Denmark). For antigen retrieval, sections were heated for 20 minutes at 95°C in antigen retrieval solution (Target Retrieval Solution [citrate pH 6] PTLink; Dako). Pretreated slides were incubated with peroxidase blocking solution (Dako) for 10 minutes, and then with antiproliferating cell nuclear antigen (PCNA; 1:500; Sigma-Aldrich). Antibodies were detected using the dextran polymer reagent for mouse antibody for 30 minutes. PCNA-positive cells were stained with 3,3′-diaminobenzidine (DAB) for 10 minutes, and nuclei were then stained with Mayer’s hematoxylin for 2 minutes. Sections were mounted (Permount; Fischer Scientific, Pittsburgh, PA) onto slides. Slides were examined using a microscope (Axioimager A1; Carl Zeiss, Oberkochen, Germany).

For fluorescence staining, sections were incubated in 10% normal donkey serum in PBS for 1 hour at room temperature, followed by incubation with monoclonal antiaactin, α-smooth muscle (1:4500; Sigma-Aldrich) at 4°C overnight. After several washes with PBS, sections were incubated with a goat anti-mouse Alexa 546 secondary antibody (Molecular Probes, Eugene, OR). The nuclei were stained with 4,6-diamidino-2-phenylindole (DAP), mounted using antifade medium (Vectashield; Vector Laboratories, Burlingame, CA). The slides were observed and photographed under a confocal laser scanning microscope (LSM 510 Meta; Carl Zeiss).

**Ultrastructural Examination**

Tissues from blebs 4 weeks postoperatively were fixed in 2.5% glutaraldehyde at room temperature to identify the cells...


**TABLE. Intraocular Pressure Assessed at Different Time Points**

<table>
<thead>
<tr>
<th>Time of Enucleation</th>
<th>Group</th>
<th>Baseline IOP, mm Hg</th>
<th>Postoperative IOP, mm Hg</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No operation</td>
<td>Control</td>
<td>10.58 ± 0.32</td>
<td>11.43 ± 1.06</td>
<td>0.638</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>10.75 ± 0.50</td>
<td>5.25 ± 0.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3 days</td>
<td>Control</td>
<td>10.65 ± 0.60</td>
<td>10.35 ± 0.47</td>
<td>0.262</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>10.50 ± 0.64</td>
<td>5.00 ± 0.38</td>
<td>0.001</td>
</tr>
<tr>
<td>2 weeks</td>
<td>Control</td>
<td>10.93 ± 0.90</td>
<td>10.92 ± 0.88</td>
<td>0.988</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>10.92 ± 0.88</td>
<td>4.50 ± 0.43</td>
<td>0.002</td>
</tr>
<tr>
<td>4 weeks</td>
<td>Control</td>
<td>10.65 ± 0.60</td>
<td>10.40 ± 0.20</td>
<td>0.529</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>9.82 ± 0.57</td>
<td>6.58 ± 1.37</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Data are expressed as the means ± SD. Significant decreases in IOP preoperatively to postoperatively in the experimental group.

* Mann-Whitney U test.

covering the inner surface of blebs. After washing with PBS, samples were in 1% fixed osmium tetroxide, dehydrated in alcohol, and embedded in Epon resin. Samples were sectioned and observed under a transmission electron microscope (TEM) (JEM1010; JEOL, Tokyo, Japan).

**Statistical Analysis**

Values are presented as the mean ± SD. Statistical analyses were performed using commercial software (SPSS software, version 17.0; SPSS, Inc., Chicago, IL). The difference in the number of FBGCs and thickness of fibrous capsules between the control and experimental groups was analyzed using the Mann-Whitney U test. Values of P < 0.05 were deemed to indicate statistical significance.

**Results**

During the follow-up period, all rabbits appeared to be healthy and ate normally. No severe systemic complication was observed in any of the animals.

**Confirmation of Aqueous Humor Flow Into Subtenon’s Space**

Once punctured in all experimental groups, the blebs spilled out a gush of aqueous humor. We could confirm that the aqueous humor occupied the space between the aqueous-drainage valve and Tenon’s tissue. The Table summarizes the mean IOP at baseline and after aqueous humor drainage-valve implantation. Significant IOP decreases were observed in the experimental group with tube placement into the anterior chamber, from baseline to 3 days, and 2, 4, and 8 weeks postoperatively.

**Gross Appearance of Blebs**

In the slit-lamp examination, the extent of vascularity at the superficial surface of the conjunctiva was prominent and the vessel diameter was relatively larger 3 days and 2 weeks postoperatively and decreased over the period to 8 weeks postoperatively in the control and experimental groups. We identified no distinct difference between the two groups in bleb vascularity.

Regarding the inner side of blebs, both groups revealed a relatively avascular and smooth surface throughout the postoperative period. Compared with the control group, the inner bleb in the experimental group seemed to be thicker and much more robust. In the control group without aqueous humor flow, but not in the experimental group, there was invasion of fibrous tissue into the opening of the valve from 4 weeks postoperatively.

**Histochemical Staining: Formation of FBGCs and Collagen Synthesis**

Histologic examination of H&E-stained sections revealed inflammatory cell infiltration throughout the blebs in both groups during the early postoperative period, which peaked 3 days postoperatively and decreased 2 weeks postoperatively.

In both groups, FBGCs were found and most of them were located along the inner border of blebs, and many of them were present until 8 weeks postoperatively. However, the mean number of FBGCs was 12.58 ± 2.02 per slide in the control group and 0.58 ± 1.00 per slide in the experimental group (P ¼ 0.001; Fig. 1). Additionally, FBGCs around the suture material were detected at similar levels in both groups but were not included in the comparison of the number of FBGCs. In the control group, some macrophages were in contact with their surface, which seemed to be about to fuse into a giant cell. The nuclei of giant cells were arranged in a disorganized manner, and the cytoplasm was elongated along the lining of the inner bleb surface.

In Masson’s trichrome stain, the quantity of collagen deposition (fibrosis) is associated with the staining density and extent (blue) (Fig. 2). In nonoperated eyes, the subepithelial connective tissue of the conjunctiva was loosely arranged and displayed weak blue staining. Additionally, the sclera showed a densely packed collagen layer with intense blue staining.

Four weeks postoperatively, Masson’s trichrome stain showed modest blue staining of subepithelial Tenon’s capsule, and dense blue (for collagen) and dense pink (for the cytoplasm) staining of the innermost layer. The staining density of subepithelial Tenon’s collagen did not differ markedly between the groups, but the thickness of the innermost layer was higher in the experimental group than that in the control group (P ¼ 0.021).

Eight weeks postoperatively, subepithelial Tenon’s tissue clearly demonstrated three layers in all capsules of both groups: (1) an outer dense fibrous layer that stained more densely blue than that at 4 weeks postoperatively; (2) an inner loose fibrous layer with mild blue collagen deposition; and (3) the innermost layers that demonstrated an avascular and densely packed collagenous stratum intensively stained blue. The intensity of blue collagen deposition in the innermost layer was the strongest among the three layers and was similar to that of sclera. In the experimental group, the innermost avascular collagenous layer was much thicker than that in the control group (P ¼ 0.021).
Immunohistochemistry and Immunofluorescence Staining

Proliferation of Fibroblasts. From 2 to 4 weeks postoperatively, the distinct hypercellular innermost layer was conspicuous in the H&E staining of blebs (Fig. 3). This hypercellular layer was more prominent and thicker in the experimental group than in the control group. PCNA immunohistochemistry staining results are shown later in the text (see Fig. 5). Numerous PCNA-expressing cells were observed 2 weeks postoperatively, and the number decreased from 4 to 8 weeks postoperatively in both groups. Cell division was prominent in the inner hypercellular layer of both groups throughout the postoperative period. The proportion of PCNA (+) cells was greater in the experimental group than that in the control group from 2 to 8 weeks postoperatively.

Transformation of Fibroblasts to Myofibroblasts. α-SMA immunostaining is shown in Figure 4. Virgin conjunctiva and sclera were not stained with α-SMA. In eyes with an implanted device, α-SMA stained mostly in the inner border of blebs. The number of FBGCs was significantly higher in the control group compared with that in the experimental group (P < 0.005). CE, conjunctival epithelium; S, sclera; AGV, Ahmed glaucoma valve; TC, Tenon’s capsule. Scale bars: 1000 μm (A, C, D); 200 μm within the box (A); 100 μm (E-J); 50 μm within the box (E, G).

Ultrastructural Findings

At the ultrastructural level, most of the lining cells along the inner bleb wall were elongated and spindly in the experimental group (Fig. 5). Further examination of the cells revealed that the cytoplasm was extensive, and rough endoplasmic reticula (rERs) were remarkably numerous and had inflated cisterns. The cells also contained longitudinally and peripherally disposed myofilaments, but they were devoid of a surrounding basal lamina, which is observed in smooth muscle cells. Thus, we suggest that lining cells along the inner bleb were myofibroblasts. Moreover, that finding was consistent with the staining of lining cells with α-SMA.

In the control group, numerous mononuclear macrophages and multinucleated giant cells were present on the inner lining.
and in the bleb area. FBGCs as well as macrophages exhibited several finger-like projections. The cytoplasm contained numerous mitochondria, lysosomes, and cytoplasmic vacuoles. A variable number of cell nuclei with a typical margin of peripherally condensed chromatin was observed.

**DISCUSSION**

In the unique aqueous humor environment, we focused on morphologic characteristics of the FBR around a glaucoma drainage implant. In both groups, FBGCs were found along the inner border of blebs, and the innermost layer of bleb revealed a densely packed collagenous stratum in both groups. However, FBR was definitely altered by aqueous humor. First, FBGCs, a hallmark of FBR, were small in number around the implant with continuous aqueous humor flow. Second, aqueous humor promoted fibrosis of blebs surrounding the implant device. Third, transformation of fibroblasts to myofibroblasts, as well as proliferation of fibroblasts, was stimulated by the aqueous humor.

Several growth factors have been identified in aqueous humor, such as basic fibroblast growth factor (FGF), epidermal growth factor, transforming growth factor-β (TGF-β), insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), transferrin, and interleukin-6. Growth factors such as PDGE FGE, and TGF-β are important for the growth of fibroblasts and blood vessels.

When macrophages encounter large foreign bodies, they may fuse to form a large multinucleated cell called the FBGC, the primary cellular mediators of the foreign body response. Although these macrophages and FBGCs tend to remain at the surface of an implant for the duration of its residence, whether they remain activated, releasing their lysosomal constituents, or become quiescent is unknown. In the ultrastructural evaluation of the current study, FBGCs were found to contain lysozyme, at a level similar to that of adjacent macrophages. Additionally, whether these FBGCs are “more or less inflammatory” than a collection of macrophages is difficult to predict. Formation of foreign body giant cells surrounding the implant device was suppressed by the aqueous humor. Giant cell formation is stimulated by activated T cells and macrophages through CC chemokine ligand 2 or interleukin-1 and interleukin-13. It is not clear whether these cytokines are suppressed in the anterior chamber. We cannot determine the exact cause and effect of lower degree of FBGC formation throughout the wound healing response in a glaucoma drainage implant surgery. Further study will be required to elucidate the meaning of the result.

The end-stage healing response to biomaterials is generally fibrosis or fibrous encapsulation. The encapsulation process is a very general phenomenon that occurs around materials implanted within tissues. This encapsulation results from

**Figure 2.** Collagen synthesis by fibroblasts is stimulated by aqueous humor flow. Four weeks postoperatively, Masson’s trichrome stained subepithelial Tenon’s capsule modestly blue, the innermost layer intensely both blue for collagen and pink for cytoplasm (A, B). Eight weeks postoperatively, subepithelial Tenon’s tissue clearly displayed three layers in all capsules of both groups (C, D): (1) an outer dense fibrous layer (asterisk), (2) an inner loose fibrous layer; and (3) the innermost layers that demonstrated an avascular and densely packed collagenous stratum (arrowhead). In the experimental group (B, D), the innermost avascular collagenous layer was much thicker than that in the control group (A, C) (E, P = 0.021, all). Scale bar: 500 μm.

**Figure 3.** Cell division after glaucoma drainage device implantation. Numerous PCNA-expressing cells were found 2 weeks postoperatively, and decreased from 4 to 8 weeks postoperatively in the control group (A, C, E) compared with the experimental group (B, D, F). Cell division was prominent in the innermost hypercellular layer of both groups throughout the postoperative period. The proportion of PCNA (+) cells was greater in the experimental group than that in the control group from 2 to 8 weeks postoperatively. Scale bar: 100 μm.
collagen neogenesis by fibroblasts. In the current study, encapsulation occurred regardless of aqueous humor and FBGC formation. However, the thickness of the fibrous capsule was much greater in the experimental group due to soaking of aqueous humor around the implanted device.

In clinical practice with glaucoma valve implantation, aqueous humor pools in the space between the endplate of the implant and surrounding, nonadherent fibrous capsule when flow occurs through the tube placed in the anterior chamber. Aqueous humor then proceeds through the capsule via passive diffusion and is absorbed by periocular capillaries. It is the fibrous capsule around the endplate that offers the major resistance to aqueous humor flow with drainage implants. Thus, the degree of IOP reduction observed following glaucoma drainage implant surgery might depend on capsular thickness and the total surface of encapsulation. A lower postoperative IOP might be expected with a thinner capsule. Failure to control IOP after glaucoma drainage implant surgery may occur secondary to heavy encapsulation of the bleb around the endplate. Fibrosis and fibrous capsule formation might be aggravated by the many growth factors in the aqueous humor. PDGF, IGF-1, and TGF-β recruits fibroblasts to the wound, and TGF-β causes wound fibroblasts to synthesize more collagen, and transform into myofibroblasts. Mechanically, application of heavy pressure or mechanical stretching of fibroblasts was reported to promote fibroblast activation and proliferation. Aqueous humor in the interface between the implant and fibrous capsule flows constantly and struggles to pass through the fibrous capsule, so fibroblasts in the capsule might be under greater pressure than would be the case in the absence of fluid flow.

The main characteristics of myofibroblasts include a spindle-shaped morphology, immunostaining for α-SMA, and ultrastructural features of prominent rER and peripheral myofilaments such as contractile filaments. Thus, our data suggest that lining cells under the effect of aqueous humor included myofibroblasts. Further studies are required to evaluate the reason why aqueous humor flow induced more conversion of fibroblasts, although lining myofibroblasts facing the implant device might be influenced by direct contact with the aqueous humor, and some constituents, such as TGF-β, might stimulate fibroblasts to transform into myofibroblasts. The extent of α-SMA staining was greater under aqueous humor flow. In the wound healing response, myofibroblasts play an important role by offering local contraction of the matrix, a process supported by collagen synthesis and secretion. The greater conversion of fibroblasts to myofibroblasts seems to be related to a thicker fibrous capsule around the device in FBR under aqueous humor flow.

**Figure 4.** Transformation of fibroblasts to myofibroblasts was enhanced by aqueous humor. (A, B) Four and 8 weeks postoperatively, the extent of α-SMA staining was greater in the experimental group (D, F) than that in the control group (C, E); however, the α-SMA–positive area was lower at 8 weeks compared with that at 4 weeks postoperatively in both groups. α-SMA stained just the inner border of blebs in the experimental group, both 4 and 8 weeks postoperatively. Scale bar: 100 μm.

**Figure 5.** Ultrastructural features of cells occupying the inner bleb. (A) In the control group, numerous mononuclear macrophages (black arrowhead) and multinucleated giant cells (black arrow) occupied the inner lining and bleb areas. The cytoplasm contained numerous mitochondria (white arrow) and lysosomes (white arrowhead). (B) In the experimental group, lining cells along the inner bleb wall were elongated and spindly, and contained synthetic machinery, including rough endoplasmic reticulum (rER; asterisk). Further examination of the cells revealed that the cytoplasm was extensive, and rERs were remarkably numerous and had inflated cisterns. These cells also contained longitudinally and peripherally disposed myofilaments (arrow). (C) Higher-magnification view of the boxed areas in (B) showing an enlarged rER with ribosomes (arrow). (D) Higher-magnification view of longitudinally arranged myofilaments (arrowhead). Scale bars: 2 μm (A, B); 200 nm (C, D).
The rabbit has been shown to be an aggressive wound healing response, such that glaucoma filtration surgeries in rabbits fail within 2 to 3 weeks postoperatively in rabbits due to aggressive wound healing at the surgical site.20,21 Regarding glaucoma drainage device implantation, Jacob et al.22,23 documented that rabbits developed fibrous encapsulation and maintained functioning blebs for more than 3 to 6 weeks after Baerveldt drainage device implantation. They found that the fibrous capsule thickness was thinner, and the amount of type III collagen was less in the modified implants with porous cellular ingrowth material to the drainage plate or 5-fluorouracil treatment.22,23 However, they did not observe proliferation of fibroblasts or the degree of conversion of fibroblasts to myofibroblasts. In our study, we investigated those factors associated with fibroblast activities, and observed FBGCs in detail. Molteno et al.24,25 investigated histologic features of capsules between 4 days and 23 years after insertion of Molteno implants in human. They showed that the capsule was thicker with immediate drainage of aqueous (one-stage technique) compared with delayed drainage of aqueous (the second stage of a two-stage insertion). Quantitative results of their study, regarding the effects of aqueous humor on bleb, correspond to our qualitative findings that aqueous humor stimulated collagen synthesis and transformation of fibroblasts to myofibroblasts, and proliferation of fibroblasts after Ahmed glaucoma valve implantation in rabbit models. However, their researches are not case-controlled studies, and aqueous humor was not allowed to drain into the bleb cavity at a certain fixed time in cases with delayed drainage of aqueous. There is a possibility that the differences between the experimental and control group become less remarkable in human eyes because the wound healing response in rabbits is known for its vigor and aggressiveness as compared with that in humans.26 However, it is definitely helpful to investigate how aqueous affects each step during encapsulation of blebs in rabbits because it is challenging to observe it in a standardized manner for humans. The rabbit has been considered as a useful model for evaluating histopathology or clinical outcome in glaucoma surgery.20-23,27,28 In addition, it has been known that there are little difference in protein distribution between human and rabbit aqueous humor.29 Therefore, we believe that our findings may apply to humans to a certain extent.

Following the insertion of a glaucoma drainage device in human with glaucoma, an initial hypotensive phase lasting 1 to 4 weeks, is followed by the hypertensive phase that begins 3 to 6 weeks after the surgery and can last for 4 to 6 months.2 Therefore, rabbits did not show definitive hypertensive phase in our study, regarding the effects of aqueous humor on bleb, correspond to our qualitative findings that aqueous humor stimulates collagen synthesis and transformation of fibroblasts to myofibroblasts, and proliferation of fibroblasts after Ahmed glaucoma valve implantation in rabbit models. However, their researches are not case-controlled studies, and aqueous humor was not allowed to drain into the bleb cavity at a certain fixed time in cases with delayed drainage of aqueous. There is a possibility that the differences between the experimental and control group become less remarkable in human eyes because the wound healing response in rabbits is known for its vigor and aggressiveness as compared with that in humans.26 However, it is definitely helpful to investigate how aqueous affects each step during encapsulation of blebs in rabbits because it is challenging to observe it in a standardized manner for humans. The rabbit has been considered as a useful model for evaluating histopathology or clinical outcome in glaucoma surgery.20-23,27,28 In addition, it has been known that there are little difference in protein distribution between human and rabbit aqueous humor.29 Therefore, we believe that our findings may apply to humans to a certain extent.

Following the insertion of a glaucoma drainage device in human with glaucoma, an initial hypotensive phase lasting 1 to 4 weeks, is followed by the hypertensive phase that begins 3 to 6 weeks after the surgery and can last for 4 to 6 months.2 However, rabbits did not show definitive hypertensive phase for postoperative 8 weeks, corresponding to results of previous studies using a Baerveldt glaucoma drainage device.22,23 In the current study, IOP increased modestly from postoperative 4 to 8 weeks, but which was within normal range. One of the reasons might be that we did not induce a glaucoma model in rabbits before implantation of glaucoma drainage device. Some of the aqueous humor might flow through trabecular meshwork. There is also a possibility that different wound healing response in rabbits might influence on postoperative course of IOP.

We found that the aqueous humor reinforced the fibrotic encapsulation surrounding an implant device in a rabbit model. The ultimate aim of glaucoma valve implantation is proper IOP control with sufficient drainage of the aqueous humor, which may be possible using a more a permeable capsule with a less fibrotic reaction. Our findings may give a picture of the appropriate foreign body reaction around the device in the unique aqueous humor environment, suggesting that modulation of the aqueous humor may be an important target for the permeable bleb formation surrounding an implant.

Acknowledgments
The authors alone are responsible for the content and writing of the paper.

Disclosure: K.I. Jung, None; S.-B. Lee, None; J.H. Kim, None; C.K. Park, None

References


