Determination of Bleb Capsule Porosity With an Experimental Glaucoma Drainage Device and Measurement System

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IMPORTANCE Control of intraocular pressure after implantation of a glaucoma drainage device (GDD) depends on the porosity of the capsule that forms around the plate of the GDD.

OBJECTIVE To compare capsular porosity after insertion of 2 different GDDs using a novel implant and measurement system.

DESIGN, SETTING, AND SUBJECTS We performed an experimental interventional study at an eye research facility in a tertiary eye care center. Testing was performed on 22 adult New Zealand white rabbits that received the experimental GDD or an existing GDD.

INTERVENTIONS A new experimental GDD, the Center for Eye Research Australia (CERA) implant, was created using computer-aided design and a 3-dimensional printer. The CERA GDDs were implanted in the eyes of rabbits randomized into 1 of the following 3 groups: with no connection to the anterior chamber (n = 7), with connection to the anterior chamber for 1 week (n = 5), and with connection to the anterior chamber for 4 weeks (n = 5). In a control group (n = 5), a pediatric GDD was implanted without connection to the anterior chamber. We measured the capsular porosity using a pressure-gated picoliter pump at a driving pressure of 12 mm Hg. The animals were killed humanely for histologic study.

MAIN OUTCOMES AND MEASURES Porosity of the fibrous capsule around the implant.

RESULTS We found no difference in mean (SEM) capsular porosity between the CERA (3.39 [0.76; 95% CI, 1.43-5.48] μL/min) and pediatric (4.52 [0.52; 95% CI, 3.19-5.95] μL/min) GDDs (P = .28, unpaired t test) at 4 weeks without aqueous exposure. Mean (SEM) capsular porosity of CERA GDDs connected to the anterior chamber at 1 week was 2.46 (0.36; 95% CI, 1.55-3.44) μL/min but decreased to 0.67 (0.07; 95% CI, 0.49-0.86) μL/min at 4 weeks (P = .001, unpaired t test).

CONCLUSIONS AND RELEVANCE Our experimental method permits direct measurement of capsular porosity of an in situ GDD. In a comparison between an experimental (CERA) and an existing GDD, no differences were identified in capsular porosity or histologic reaction between the implants. These results suggest that the CERA GDD model can be used to test key components of glaucoma surgery and implant design.
The aim of glaucoma surgery is to lower the intraocular pressure (IOP) by creation of a supplementary filtering channel for aqueous humor outflow. In this postoperative state, the total outflow facility of the eye is the sum of existing outflow (trabecular plus uveoscleral outflow) plus the surgically induced outflow. In clinical practice, lowering of the IOP after surgery is taken as evidence of increased surgical outflow. Contributions from reduced aqueous production and changes to nonsurgical outflow (ie, altered trabecular meshwork flow) cannot be excluded. Outflow and IOP have a nonlinear relationship, and inferring outflow facility from IOP may not be accurate. For example, outflow facility after an attack of angle-closure glaucoma has been shown to remain reduced despite return of the IOP to a reference value. In glaucoma surgical research, an exact measurement of surgical outflow is preferred.

A previously reported model of surgical outflow identified the following key components that determined success: (1) egress of aqueous from the anterior chamber, (2) the porosity of the subconjunctival tissue through which the aqueous passes, and (3) the absorption capacity of the subconjunctival tissue. This model further predicts that capsular porosity is the key measurable variable of glaucoma filtration surgery. This report describes the effect of aqueous flow on the capsular porosity of the CERA GDD.

To address these issues, we developed a novel (to the best of our knowledge) 2-tubed implant created with a 3-dimensional printer (Center for Eye Research Australia glaucoma drainage device [CERA GDD]) and used a highly accurate pressure-gated syringe pump to test the capsular porosity of the system. This report describes the effect of aqueous flow on the porosity of the fibrous capsule around the CERA GDD.

Methods

GDD Design and Development

This research was performed under institutional ethics guidelines specified by the Institutional Animal Research Ethics Committee of The Royal Victorian Eye and Ear Hospital. To overcome difficulties in cannulation, we designed a GDD with 2 tubes attaching to the plate. To compare outcomes with previous data derived from a pediatric GDD (Molteno GDD; Molteno Ophthalmic Ltd), we modeled the plate to have a similar surface area and shape. The CERA GDD has a diameter of 9.2 mm and can be implanted easily in a rabbit eye. The CERA GDDs were designed using computer-aided design tools (Solidworks; http://www.solidworks.com/) (eFigure in the Supplement). The designed components were subsequently batch produced by means of additive fabrication. The implant body was created using a proprietary biocompatible material (MED610; Stratasys) via a 3-dimensional printer (Objet Connex350; Stratasys). Implants were created with a flange for gluing of the silicone tubing, and the base curve was increased to accommodate the rabbit eye. The flanges included holes to improve the ease of suture placement, which is more difficult in the rabbit owing to access and scleral dimensions.

Printed GDDs were repeatedly washed and inspected for consistency and integrity. Silicone tubing was glued to the plates using medical-grade silicone adhesive (Silastic; Dow Corning). The silicone adhesive was allowed to cure thoroughly, after which the CERA GDDs were washed and sterilized with a hospital-grade hydrogen peroxide system before use.

Experimental design required occlusion of the unused tube, which was achieved with a novel tube plug. The plug was developed using the same process and manufactured from the same material as the plate. Prototype plugs were tested for capacity to withstand hydraulic resistance by increasing the intraluminal hydraulic pressure of the tube (>50 mm Hg). The plug remained in place and did not leak at high pressure.

In Vivo Testing of the CERA GDD

The study was conducted on the right eyes of 22 rabbits divided into 4 groups. Animals were housed under conditions consistent with the Association for Research in Vision and Ophthalmology statement on the use of animals in research.

Initially, we implanted the CERA GDD and the pediatric GDD for 4 weeks with no aqueous exposure (no flow) and examined the histologic reaction and the porosity of the capsule. We then tested the CERA GDDs with unimpeded aqueous flow for 1 and 4 weeks. Eyes then underwent histologic examination.

The CERA GDDs were inserted in 1 eye of New Zealand white rabbits in a technique described elsewhere. In brief, GDDs were checked for patency and inserted into the superolateral subconjunctival space. Some GDDs in the no-flow groups were inserted in the inferior fornix to save animals and time because we did not expect the tissue response to be different in the superior or inferior quadrants. When one tube was placed within the anterior chamber, the other was plugged and placed under the nasal conjunctiva. When neither tube was introduced into the anterior chamber, both were plugged and tucked under the conjunctiva. Tubes and plate were sutured into place with 10-0 nylon sutures, and the conjunctiva was closed. Topical corticosteroids were given for 24 hours and antibiotics for 72 hours.

When taking porosity measurements, the tube in the anterior chamber was completely blocked with an external polyglyactin ligature (6-0 Vicryl; Ethicon), and the second (plugged) tube was exposed and attached to a pressure-gated picoliter syringe driver pump (PHD ULTRA CP 4400 remote syringe pump; Harvard Apparatus [http://www.harvardapparatus.com/webapp/wcs/stores/servlet/haisku2_10001_11051_68273_-1_HAI_ProductDetail_N_37295_44353] (Figure 1)). The system was primed with Hank balanced saline solution with fluorescein dye added to expose leak-
age if present. Flow was measured from the pump apparatus after setting the pressure to 12 mm Hg. Flow rates were initially high, and measurements were obtained once the system had reached stability (usually <3 minutes). The system was considered stable when the pump was able to maintain a preset pressure of 12 mm Hg for at least 5 minutes.

For data analysis, the animals were divided into 4 groups. Group 1 (n = 7) had a CERA GDD with both tubes left tucked into the subconjunctival space, providing no access to the anterior chamber. Group 1 animals underwent testing and were killed at 4 weeks after implantation. Group 2 (n = 5) had a standard commercially available pediatric GDD with the tube tucked under the conjunctiva and not connected to the anterior chamber. Porosity measurements were taken and animals were killed at 4 weeks. Group 3 (n = 5) had a CERA GDD with one tube in the anterior chamber and the second tube plugged. Capsular porosity was measured and animals were killed at 1 week after implantation. Group 4 (n = 5) had the same procedure as group 3 except that the measurements were taken 4 weeks after implantation (resulting in 4 weeks of aqueous exposure).

Animals were killed using a standard protocol of intracardiac barbiturate. The eyes were taken and immediately placed in fixative. After embedding the eyes in wax, the GDDs and capsules were identified and multiple sections were taken through the implant. Sections were initially stained with hematoxylin-eosin.

**Results**

Twenty-two CERA GDDs were implanted. Implantation was relatively straightforward with the design changes. The extra tube remained buried without obvious reaction of the overlying conjunctiva. By the time of testing, the plug remained in place in all experiments. Cannulation was straightforward, and closure of the tube into the anterior chamber was achieved in all experiments. Stable flow rates at preset IOPs were achieved in all eyes within 5 minutes. Results are shown in the Table.

Results for groups 1 and 2 showed that the tissue reaction to the plate was similar despite different manufacturing techniques, surface characteristics, and material. In the absence of aqueous, no difference was detected in the capsular poros-
ity between the 2 groups (mean [SEM], 3.39 [0.76; 95% CI, 1.43-5.48] vs 4.52 [0.52; 95% CI, 3.19-5.95] μL/min; \( P = .28 \), unpaired \( t \) test) (Figure 2). Histologic evaluation of the capsule showed a similar appearance and dimensions of the capsular condensation (Figure 3). One CERA GDD in group 1 had to be excluded owing to blockage in the system because no flow could be measured even at high pressures. This eye was excluded from further analysis.

Groups 3 and 4 showed a marked decline in capsular porosity of the CERA GDD after 4 weeks. One-week capsular porosity was moderate (mean [SEM], 2.46 [0.36; 95% CI, 1.55-3.44] μL/min) but declined by 4 weeks after insertion (mean [SEM], 0.67 [0.07] [95% CI, 0.49-0.86] μL/min; \( P = .001 \), unpaired \( t \) test) (Figure 2). In aqueous-exposed CERA GDDs, marked thickening of the capsule was seen from weeks 1 to 4 (Figure 4).

**Discussion**

Measurement of IOP or morphologic/histologic analysis of blebs as the end point for glaucoma surgical research is unreliable. Intraocular pressure has a nonlinear relationship to outflow and outflow facility, and surgery for glaucoma may affect all aspects of the inflow/outflow dynamics of the eye. \(^4\) We believe that, to advance measurement of healing responses after GDD implantation, we need surgical models that have clear, unambiguous, and functionally significant end points. To this end, we have developed methods to

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**Table. Surgical Outflow at 1 and 4 Weeks in CERA and Pediatric GDDs**

<table>
<thead>
<tr>
<th>Implant</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Difference, Groups 2 − 1 (^b)</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Difference, Groups 4 − 3 (^c)</th>
</tr>
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<tbody>
<tr>
<td>Fluid</td>
<td>None</td>
<td>None</td>
<td>NA</td>
<td>Aqueous</td>
<td>Aqueous</td>
<td>NA</td>
</tr>
<tr>
<td>Duration, wk</td>
<td>4 of 52</td>
<td>4 of 52</td>
<td>NA</td>
<td>1 of 52</td>
<td>4 of 52</td>
<td>NA</td>
</tr>
<tr>
<td>Porosity, μL/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 1</td>
<td>1.36</td>
<td>3.22</td>
<td>1.86</td>
<td>1.53</td>
<td>0.49</td>
<td>−1.04</td>
</tr>
<tr>
<td>Rabbit 2</td>
<td>1.71</td>
<td>3.38</td>
<td>1.67</td>
<td>2.10</td>
<td>0.55</td>
<td>−1.55</td>
</tr>
<tr>
<td>Rabbit 3</td>
<td>1.89</td>
<td>5.08</td>
<td>3.19</td>
<td>2.44</td>
<td>0.69</td>
<td>−1.75</td>
</tr>
<tr>
<td>Rabbit 4</td>
<td>3.95</td>
<td>5.10</td>
<td>1.15</td>
<td>2.55</td>
<td>0.72</td>
<td>−1.83</td>
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<tr>
<td>Rabbit 5</td>
<td>5.50</td>
<td>5.84</td>
<td>0.34</td>
<td>3.70</td>
<td>0.89</td>
<td>−2.81</td>
</tr>
<tr>
<td>Rabbit 6</td>
<td>5.91</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>3.39 (2.02)</td>
<td>4.52 (1.16)</td>
<td>1.64 (1.05)</td>
<td>2.46 (0.80)</td>
<td>0.67 (0.16)</td>
<td>−1.80 (0.64)</td>
</tr>
<tr>
<td>SEM (95% CI)</td>
<td>0.76 (1.43 to 5.48)</td>
<td>0.52 (3.19 to 5.95)</td>
<td>0.47 (0.44 to 2.93)</td>
<td>0.36 (1.55 to 3.44)</td>
<td>0.07 (0.49 to 0.86)</td>
<td>0.29 (−2.54 to 1.00)</td>
</tr>
</tbody>
</table>

Abbreviations: CERA, Center for Eye Research Australia; GDD, glaucoma drainage device; NA, not applicable.

\(^a\) Groups are described in the In Vivo Testing of the CERA GDD subsection of the Methods section.

\(^b\) \( P = .28 \), unpaired \( t \) test.

\(^c\) \( P = .001 \), unpaired \( t \) test.

**Figure 2. Mean Capsular Porosity of the Printed Center for Eye Research Australia (CERA) Glaucoma Drainage Devices by Aqueous Flow**

**Figure 3. Histologic Findings in the Rabbit Eyes Showing Fibrous Capsular Formation at 4 Weeks in the No-Flow State**

A. Eye with pediatric glaucoma drainage device (GDD). B. Eye with Center for Eye Research Australia (CERA) GDD (hematoxylin-eosin; original magnification \( \times 10 \)). Measurements indicate thickness of the capsule.
measure capsular porosity, which detailed engineering modeling previously identified as the most important characteristic for success.4,6

We have used GDDs as the preferred method of creating outflow and drainage in an animal model.9-11 Although we have an imperative to limit the use of experimental animals, no method exists to emulate the complex cellular and extracellular dynamics of the capsular development and changes over time in response to various stresses other than the live animal model. In the experimental setting, GDDs offer 2 advantages compared with nonimplant surgery for glaucoma. First, the size and location of the bleb can be predefined. Hence, the area or volume being tested can be standardized. Second, GDDs allow us to control flow of aqueous to the bleb in the early postoperative period (built-in flow restriction mechanism or external ligature), providing us an opportunity to study the effect of aqueous on the development of the fibrous capsule.

However, current GDDs are not designed for capsular porosity measurements in experimental animals. Previous work has shown that, although cannulation of the internal ostium of the tube is possible while in situ, thus keeping the anterior chamber formed, the process is technically challenging and cannot be repeated on the same eye.6,13 We concluded that, to interrogate the GDD capsule porosity and the factors involved in its final state, we needed a redesigned implant and testing system. We developed the CERA GDD in response to these demands. This implant system allows us to change features of the implant and determine their effect on capsular porosity, thus supplying a direct measure of surgical effectiveness.

Glaucoma drainage devices are, in effect, foreign bodies under the conjunctiva.14,15 We assume that the material, shape, and surface characteristics will all have some effect on the reaction of tissue around the implant. Because this tissue reaction will contribute to capsular porosity, we sought to isolate the effects of aqueous and test the pediatric GDD (polypropylene injection-molded plate) and the CERA GDD (proprietary biocompatible polymer from the 3-dimensional printer) tissue reaction in the absence of aqueous. Although we have not examined the histologic findings in great detail, the capsular appearance was similar between the 2 GDDs at 4 weeks with no exposure to aqueous. Among CERA GDDs, the capsules were thicker when exposed to aqueous for 4 weeks compared with 1 week of exposure. We also tested the capsular porosity in implants not exposed to the aqueous, and we identified no difference in the 2 GDD types. From these data, we can reasonably conclude that both materials were similar in the cellular and extracellular reaction that they incite in the absence of aqueous.

The key difference in the CERA GDD is the second tube, which allows the capsular porosity to be tested in situ without breaching the globe or disturbing the IOP. Because we are interested in the capsular porosity and not the volume of the capsule per se, control of the IOP is critical. Decompression of the eye produces inward bulging of the sclera, which may distort the plate and capsule and affect porosity measurements.

Figure 4. Histologic Findings in the Rabbit Eyes With Printed Center for Eye Research Australia (CERA) Glaucoma Drainage Devices (GDDs) Showing Fibrous Capsular Formation

A, Eye with 1 week of aqueous flow (hematoxylin-eosin; original magnification ×10). B, Eye with 4 weeks of aqueous flow (periodic acid-Schiff; original magnification ×10).

The 2-tube implant allows relatively easy access to the tube from the subconjunctival space away from the plate without the need to disturb the anterior chamber. We closed off the tube to the anterior chamber with a 6-0 suture through the limbal conjunctiva. We added fluorescein dye to the test fluid to monitor any backflow into the anterior chamber.

In our view, capsular porosity has the most validity when measured at physiologic pressures. Previous work has used higher testing pressures that may produce nonuniform and temporary changes in porosity due to compaction or tearing of the capsular tissue. With the use of highly accurate picoliter pressure-gated syringe pumps, the capsule porosity can be reproducibly tested at lower pressures. The pump we chose could maintain a user-defined pressure to within ±2% and can provide flow rates as low as 3.06 pL/min.

We chose the pressure of 12 mm Hg to test initially. We also tested at 16 mm Hg after testing at 12 mm Hg. Results were similar and did not vary between the GDDs, and they do not add to the discussion. Because the capsule was probably never subjected to a driving pressure of 16 mm Hg or more in a normal
New Zealand white rabbit, we did not include porosity measurements at higher IOPs.

Conclusions

We isolated the effects of aqueous and tested the capsular porosity and histologic reaction of the subconjunctival space to the GDD plate in the absence of aqueous. The effect of aqueous on the capsular porosity is marked with reduction of porosity of greater than 80% from 1 to 4 weeks. Flow of aqueous appears to have a much greater effect on capsular porosity than material changes when using these 2 plate materials in a direct comparison. Whether this effect would be seen in a wide range of materials is not known, but it is a challenging observation because material considerations have been dominant in GDD design for many years. The CERA GDD and measurement system accurately determine the tissue porosity and can be used to characterize surgical devices or techniques in terms of a functional end point. The system also has the potential to become a model for testing the influence of GDD materials, designs, and drugs on the porosity of the fibrous capsule.