A Novel Schlemm's Canal Scaffold Increases Outflow Facility in a Human Anterior Segment Perfusion Model

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PURPOSE. An intracanalicular scaffold (Hydrus microstent) designed to reduce intraocular pressure as a glaucoma treatment was tested in human anterior segments to determine changes in outflow facility (C).

METHODS. Human eyes with no history of ocular disease or surgeries were perfused within 49 hours of death. The anterior segments were isolated and connected to a perfusion system. Flow rates were measured at pressures of 10, 20, 30, and 40 mm Hg. The scaffold was inserted into Schlemm's canal of the experimental eye, while a control eye underwent a sham procedure. Flow rate measurements were repeated at the four pressure levels. Individual C values were computed by dividing the flow rate by its corresponding pressure, and by averaging the four individual C measurements. The change in C between control and experimental eyes was assessed by the ratio of the baseline and second C measurement. In two eyes, the placement of the scaffold was evaluated histologically.

RESULTS. After scaffold implantation in the experimental eyes, the average C increased significantly from baseline ($n = 9$, $P < 0.05$). Ratios of C at all pressure levels, except for 10 mm Hg, were significantly higher in experimental eyes ($n = 9$) than control eyes ($P < 0.05$, $n = 7$). Histologically, the scaffold dilated Schlemm's canal with no visible damage to the trabecular meshwork.

CONCLUSIONS. The Hydrus Microstent provided an effective way to increase outflow facility in human eyes ex vivo. (*Invest Ophthalmol Vis Sci.* 2012;53:6115–6121) DOI:10.1167/iovs.12-9570

A major risk for glaucoma is elevated intraocular pressure (IOP). Clinically available methods to reduce IOP are by pharmacologic means, laser therapy, or surgical intervention.[1]

Current surgical options are invasive, and can result in unstable IOP and high complication rates.[2] The standard surgical filtration procedure for glaucoma is trabeculectomy, the success of which depends on the formation of a filtering bleb in the subconjunctival space. Glaucoma drainage devices have been used as another surgical means to reduce IOP. Similar to trabeculectomy, existing drainage devices provide a path for aqueous humor to flow into the subconjunctival space, and success depends on the formation of a fibrous capsule in this space.[3,4] Malformed blebs and capsules are the main reason for the failure of these procedures. Therefore, new glaucoma surgeries are being investigated to avoid the problems associated with traditional filtration procedures.

Several glaucoma implants have been designed to provide a pathway for fluid flow across the trabecular meshwork (TM) and into Schlemm's canal (SC). The improvement in outflow facility (C) has shown promise.[5-10] There are three advantages to this approach. First, restoring conventional outflow into SC avoids the complications and adverse effects associated with drainage into the subconjunctival space. Second, bypassing the TM, specifically the juxtacanalicular tissue (JCT), and inner wall of SC, the region of greatest outflow resistance,[11-17] should increase C significantly. Lastly, the downstream resistance provided by the episcleral venous pressure reduces the incidence of hypotony without the use of an additional resistance mechanism.

Methods, such as goniotomy and the Trabectome procedure,[18-23] have been explored to create an opening into SC by cutting through the TM. Whether the TM remains open after wound healing is unclear. Alternatively, rather than bypassing the TM, some glaucoma surgeries, such as canaloplasty and viscocanalostomy, dilate SC to increase circumferential flow and access to collector channels. There is some evidence that these nonpenetrating procedures may cause ruptures in the inner wall of SC and JCT resulting in a reduction in the outflow resistance.[24] Additionally, dilation of the SC may be a factor in the IOP reduction. In canaloplasty, increased tension of the suture is associated with greater SC dilation and IOP reduction.[9] It was reported decades ago that SC collapses with elevated IOP as a consequence of bowing of the trabecular meshwork and inner wall of SC toward the outer wall.[25] In a recent study, histologic evidence has demonstrated that the collapse of SC, and herniation of the TM into collector channel ostia under increased pressure levels in enucleated bovine eyes, led to changes in preferential flow patterns.[26] Taken together, these studies provide substantial evidence supporting the premise that an opening in the TM combined with prevention of collapse of SC may provide a means to reduce IOP.

We evaluated a novel SC scaffold (Hydrus Microstent; Ivantis, Inc., Irvine, CA) in human anterior segments. The scaffold is comprised of Nitinol (nickel-titanium alloy), and has a non-luminal open design to improve flow of aqueous humor into SC and gain better access to collector channels within the canal. The Microstent (Fig. 1A) is designed to increase C by
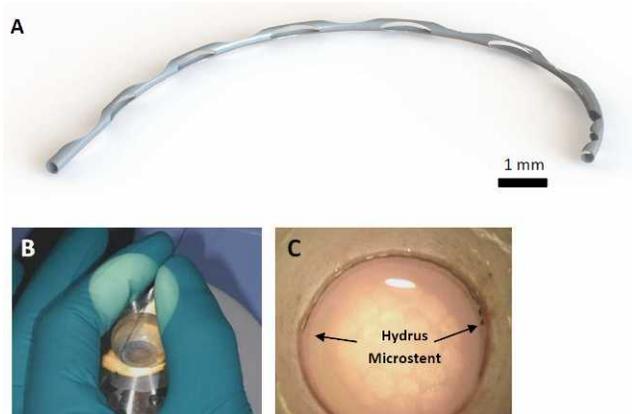
FIGURE 1. Hydrus Microstent. (**A**) The Microstent design consists of a flexible Nitinol scaffold with windows along its walls to dilate SC and allow aqueous humor to flow through. (**B**) Insertion of the Microstent into SC of an ocular anterior segment using a custom designed delivery system. (**C**) Final placement of Microstent in SC.

bypassing the TM and dilating 5 clock hours (approximately 15 mm) of SC to increase circumferential flow. It creates a maximum SC dilation of 241 μm or approximately 4 to 5 times the natural cross-sectional area of SC. The Microstent effect on C was evaluated and compared to baseline and to control eyes. Additionally, the effects on C during SC collapse were investigated at different pressure levels before and after Microstent insertion. Lastly, the tissue manipulation during Hydrus implantation was evaluated in some eyes to determine its effect on C.

METHODS

Eye Preparation

Human whole globes were obtained from National Disease Research Interchange (Philadelphia, PA) or Lions Eye Institute (Tampa, FL). All experiments were approved by the University of Nebraska Medical Center Institutional Review Board before study initiation and are in compliance with the Declaration of Helsinki. The 15 pairs of eyes included in our study had no reported history of previous ocular diseases or surgeries. Enucleated eyes were wrapped in saline-wetted gauze, placed in moist chambers, and shipped on ice. All eyes were perfused within 49 hours postmortem.

Anterior segments consisting of cornea, anterior sclera, and trabecular meshwork were prepared by cutting along the equator of the globe to separate the two hemispheres. The choroid, ciliary body, iris, and lens were removed and discarded along with the posterior segment. Effects of perfusion pressure on C were compared in anterior segments versus whole globes.

Perfusion System

An isotonic solution was prepared with 5.5 mM of glucose in Dulbecco's phosphate buffer saline (276 ± 12 mOsm; Sigma Chemical Co., St. Louis, MO) as a substitute for aqueous humor.[27] A perfusion setup similar to that described previously[28] was used to perform the measurements (Fig. 2). The height of fluid columns with a fixed diameter (4 mm) was used to set the pressure in the perfusion system. Calibration of the transducers was done before each experiment by comparing the transducer reading at specific fluid column heights. For 10 pairs of anterior segments, the fluid column was linked to a custom-made fixture that secured the eye. For whole globes, a 25-gauge needle connected to the fluid column was inserted into the posterior chamber of the eye. Paired eyes were set in a water bath maintained at 34°C. Before each experiment, the system
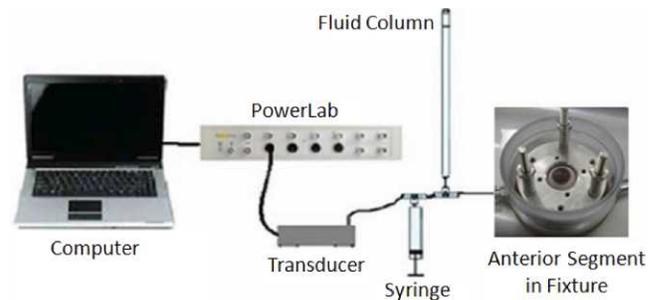

FIGURE 2. Experimental setup for C measurement of human anterior segments. A 10-mL syringe filled with a glucose-saline solution was connected to a three-way stopcock that was linked with pressure tubing to a pressure transducer and a fluid column. Transducers were connected using an analogue cable to a computer running PowerLab software and calibrated using the fluid column. For the anterior segment perfusion experiments, the fluid column was linked to a custom-made fixture that secured the eye.

pressure was referenced to zero pressure corresponding to zero flow. PowerLab software (ADInstruments, Bella Vista, Australia) recorded the pressure for the duration of the experiment.

Tissue Exclusion Criteria

Eyes were excluded from the study based on signs of leaks or obstructions, baseline nonphysiologic C, and difficulties with Hydrus placement. Rapid pressure loss and appearance of infused fluorescein tracer around the sealing ring were indications of leaks. Eyes with baseline C of higher than 0.7 μL/min/mm Hg were excluded because a nonphysiologically high value may indicate damaged tissue. C values above 0.7 μL/min/mm Hg are greater than 4 standard deviations from the mean and are considered statistical outliers. Implantation was considered poor when the Hydrus punctured through the TM or SC outer wall, or hit an obstruction within SC during implantation.

C Value Calculations

C values were calculated from the stabilized flow rates measured at set perfusion pressures of 10, 20, 30, and 40 mm Hg. As described previously,[28] the fluid exiting the system was proportional to the decline in height of the fluid column or decline in pressure over time. The flow rate at a set pressure was calculated from the volume displacement in the fluid column in the selected timeframe. Transducers measured a steady linear reduction in pressure over the time interval that each pressure level was assessed. Less than 1 mm Hg of total drop in pressure occurred during this time. Average pressures and flow rates were determined from a minimum of 5 minutes of data in which pressure decline was stabilized.

Using Goldmann's equation, individual C values at the four perfusion pressures (10, 20, 30, and 40 mm Hg) were calculated from the stabilized flow rates divided by the corresponding average pressures. It was assumed that episcleral venous pressure was zero and the stabilized flow measurements were equivalent to trabecular outflow.[13,14,29] The average C for each eye was the mean of C values calculated from the 4 pressure levels.

Implant Study Design

Ten pairs of human eyes were perfused at IOPs of 10, 20, 30, and 40 mm Hg to obtain an overall baseline C measurement and C values that corresponded to each pressure level. After baseline assessment, the anterior segments were removed from the fixtures and placed onto a surgical platform. The experimental eye was implanted with the Hydrus Microstent (Figs. 1A, 1C). With a 27-gauge needle, a small incision was

TABLE 1. Information on Donors Contributing Anterior Segments Receiving Hydrus Microstents ($n = 9$ Eyes)

Donor	Sex	Cause of Death	Age, y	Post Mortem Time, h
1	M	Lymphoma	67	44
2	F	Breast cancer	61	41
3	M	CHF	77	46
4	M	Perforated bowel	62	36
5	M	Heart failure	70	33
6	M	Stroke/septic shock	69	42
7	F	CHF	74	49
8	M	Carcinogenic shock	65	34
9	M	Myocardial infarction, CHF	80	31
Average ± SEM			69.4 ± 2.2	39.4 ± 2.1

CHF, congestive heart failure; SEM, standard error of the mean.

made through the TM and inner wall of SC. Next the Microstent was advanced through the incision and along SC (Fig. 1B) until only 1 to 2 mm of the Microstent remained in the anterior chamber. The contralateral control eye was left undisturbed. Eyes then were remounted into the fixtures and C was re-assessed. A third C measurement was made after making an incision into the TM with a 27-gauge needle (four control eyes), or after removing the Hydrus (four experimental eyes).

Whole Globe-Anterior Segment Study Design

For five pairs of eyes, the right eyes were dissected into anterior segments and the left eyes remained as intact whole globes. C was assessed at 10, 20, 30, and 40 mm Hg. The C values at each pressure level were averaged over duplicate assessments and plotted to determine the change in C with increased pressure.

Histology

At the completion of the C assessments, one pair of eyes was processed for histologic examination of the Microstent placement and the appearance of the TM after insertion. These eyes were perfused with a fixative of 4% glutaraldehyde and 10% neutral buffered formalin (NBF) for one hour at a pressure of 10 mm Hg in the right eye and 30 mm Hg in the left eye. The eyes were immersed in the fixative for approximately 24 hours, and then were placed in 10% NBF for shipment to Wasatch Histo Consultants (Winnemucca, NV) for processing. Each eye was embedded in polymethyl methacrylate and cut into 1 mm sections. Sections containing the Microstent were mounted on plastic slides, then polished and stained with hematoxylin and eosin.

Statistical Analysis

Using two-tailed Student's *t*-tests, paired comparisons were made within the same eye, pre- and post-insertion or sham procedure, and between the paired anterior segments and whole globes. Hydrus-implanted eyes were compared to control eyes using unpaired Student *t*-tests. A *P* value of less than 0.05 was considered statistically significant. The logarithmic

converted ratios of C after manipulation (implantation, sham procedure, or removal of implant) to baseline were compared between the control and experimental eyes. The slope of C with IOP level after implantation or sham procedure was compared to its baseline measurement to determine if pressure-induced changes in C were affected by the Hydrus.

RESULTS

The donor information is summarized for the experimental group ($n = 9$) in Table 1 and the control group ($n = 7$) in Table 2. Eyes were excluded from the study for leaks or clogs in the system or fixture ($n = 5$), nonphysiologically high C values at baseline measurement ($n = 2$), and poor Hydrus insertion ($n = 1$). In experimental eyes, the Microstent significantly ($P < 0.01$) increased the average C from a baseline of 0.19 ± 0.02 to 0.39 ± 0.07 μL/min/mm Hg (mean ± SEM, $n = 9$). There was no significant difference between the average C after the sham procedure in control eyes (0.20 ± 0.03 vs. 0.23 ± 0.03 μL/min/mm Hg, $n = 7$), or between baseline measurements in the control and experimental eyes (Fig. 3A). The log-converted ratios of average C were significantly higher ($P < 0.05$) in the experimental eyes (2.11 ± 0.312, $n = 9$) than the control eyes (1.27 ± 0.16, $n = 7$). Likewise, the log-converted ratios of the individual C values measured at 20, 30, and 40 mm Hg were significantly higher in the experimental eyes than the control eyes ($P < 0.05$, Fig. 3B). C values measured at 10 mm Hg had too much variability to show significance between the control and experimental eyes.

The removal of the Hydrus significantly reduced ($P < 0.05$) the ratio of C values (Hydrus removed/baseline 1.81 ± 0.43, $n = 4$) when compared to the implanted eye (Hydrus/baseline 2.75 ± 0.44, $n = 4$; Fig. 4B). The ratio of baseline C to C values after an incision (1.26 ± 0.13, $n = 4$) was not significantly different from baseline compared to the sham procedure (1.12 ± 0.11, $n = 4$).

C values in anterior segments were found to increase with perfusion pressure increases (Figs. 5A, 5B). The slope of the

TABLE 2. Information on Donors Contributing Control Anterior Segments ($n = 7$ Eyes)

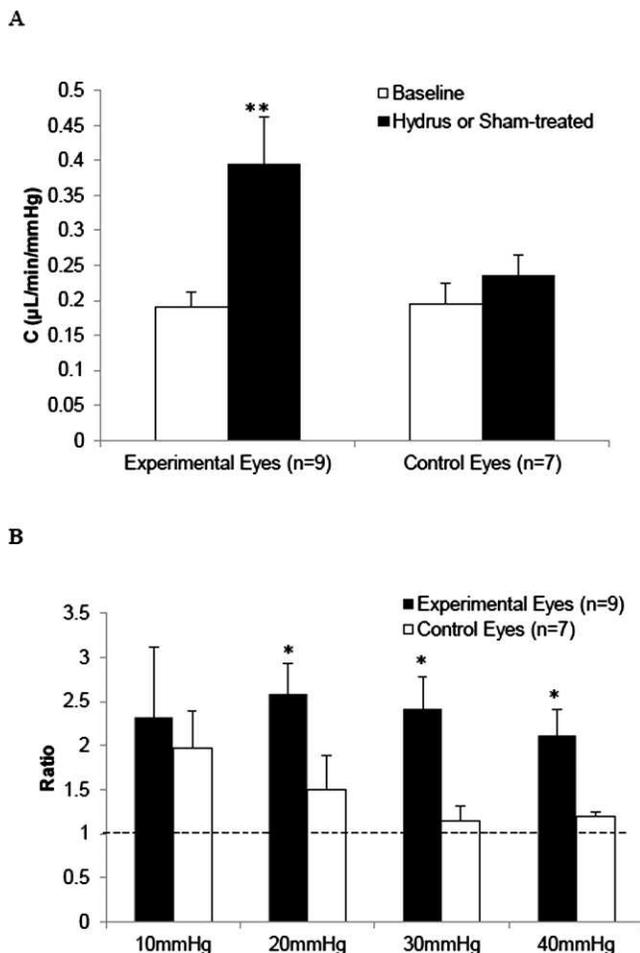| Donor	Sex	Cause of Death	Age, y	Post Mortem Time, h
1	M	Lymphoma	67	44
2	F	Breast cancer	61	41
3	M	CHF	77	46
6	M	Stroke/septic shock	69	42
7	F	CHF	74	49
8	M	Carcinogenic shock	65	34
10	F	Breast cancer	47	38
Average ± SEM			65.7 ± 3.7	41.8 ± 2.0

A

B

FIGURE 3. Average C is the mean of the individual C measured at perfusion pressures of 10, 20, 30, and 40 mm Hg. (**A**) Experimental eyes ($n = 9$) had increased C (arithmetic mean ± SEM) after Microstent insertion compared to the sham-treated control eyes (**$P < 0.01$, $n = 7$). (**B**) The log-converted ratio (manipulation/baseline) of the individual C values (arithmetic mean ± SEM) measured at 10, 20, 30, and 40 mm Hg for the experimental eyes (Hydrus/baseline; *black bars*, $n = 9$) was significantly higher than the control eyes (sham-treated/baseline; *white bars*, $n = 7$) at 20, 30, and 40 mm Hg (*$P < 0.05$) but not 10 mm Hg pressure.

increase in C with pressure (C/pressure) was significantly higher after Hydrus implantation than at baseline ($P < 0.05$, $n = 9$, Fig. 5A). Sham control eyes ($n = 7$) showed no significant increase in the slope (Fig. 5B). In the whole globe verses anterior segment study, two of the anterior segments had leaks, leaving five paired donor eyes (Table 3) in the analysis. The anterior segments showed increases in C with increases in IOP (slope of C versus IOP $0.0034 ± 0.0037$ µL/min/mm Hg^2, mean ± SE, $n = 5$, $P < 0.02$), while the contralateral whole globes showed no significant change in C with increases in IOP (slope of C versus IOP $-0.0042 ± 0.0024$ µL/min/mm Hg^2, Fig. 5C).

Histologic examination of cross-sections of regions of the eyes with the Microstent showed widely dilated SCs in eyes fixed at either 10 or 30 mm Hg fixation pressures compared to the SC regions without the Microstent (Fig. 6). At fixation pressures, the TM appeared to be intact and stretched similarly by the Microstent.
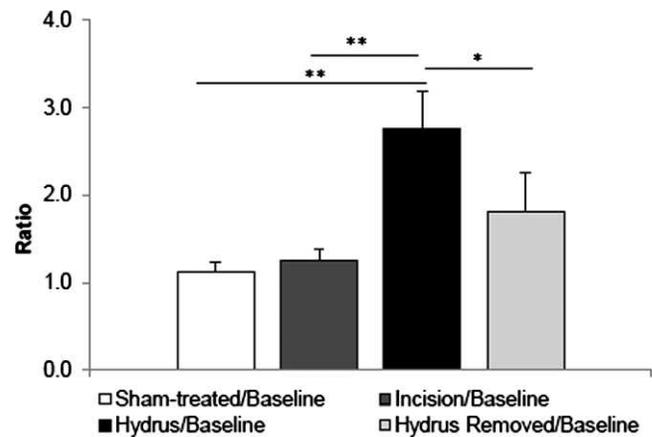

FIGURE 4. C values were measured three times in the control and experimental eyes. The ratio of C after implantation to C at baseline (Hydrus/baseline, *black bar*, arithmetic mean ± SEM) and after Hydrus removal to baseline (Hydrus Removal/baseline, *dark gray bar*) in experimental eyes ($n = 4$) was compared to ratio after the sham procedure to baseline (sham-treated/baseline, *white bar*) and after an incision into the TM to baseline (Incision/baseline, *light gray bar*) in the contralateral control eyes ($n = 4$). The removal of the Hydrus significantly reduced the log-converted ratio from implantation ($P < 0.05$), while an incision into the TM was not significantly different from the sham treatment in the control eyes. Additionally, the log-converted ratio of the implanted eyes was significantly higher than that of the incision and sham-treated eyes ($P < 0.01$). There was no significant difference between the ratios of Hydrus removal (*dark gray bar*), sham-treated (*white bar*), and incision (*light gray bar*) to its baseline C.

DISCUSSION

We report four key findings about the Hydrus Microstent. First, the Hydrus increased C in a human anterior segment perfusion model. Second, the increase in C appeared to be independent of the implantation procedure. Third, C increased more as perfusion pressure increased in eyes implanted with the Hydrus when compared to baseline. Lastly, histologic evidence showed that SC dilated several-fold, yet the TM remained intact after the Hydrus implantation.

Hydrus Increases C

The Hydrus Microstent caused a significant increase in C at pressures of 20, 30, and 40 mm Hg compared to the controls and baselines. Due to high variability, C measured at 10 mm Hg after implantation was not significantly different from baseline or control measurements. It is possible that the eyes were not at equilibrium when evaluated. Each pressure level was assessed for 20 minutes starting with the 10 mm Hg pressure; therefore, the tissue may not have equilibrated fully before data collection. Another possibility is that the perfusion system was not sensitive enough to detect differences in flow at the low pressure.

The anterior segment perfusion model has been used previously to evaluate C after implantation of a trabecular micro-bypass (iStent and iStent inject; Glaukos Corp., Laguna Hills, CA).[6,10] Procedural differences between our study and the iStent studies make comparison of results problematic. Two versions of the iStent were evaluated in an organ culture perfusion model in which the eyes were perfused with culture media for days at a set flow rate of 2.5 µL/min.[6,10] Our study investigated the effect of the Hydrus Microstent on C in anterior segments perfused for 6 hours or less with saline-glucose solution at four different pressure levels. Although our study and the iStent studies used different
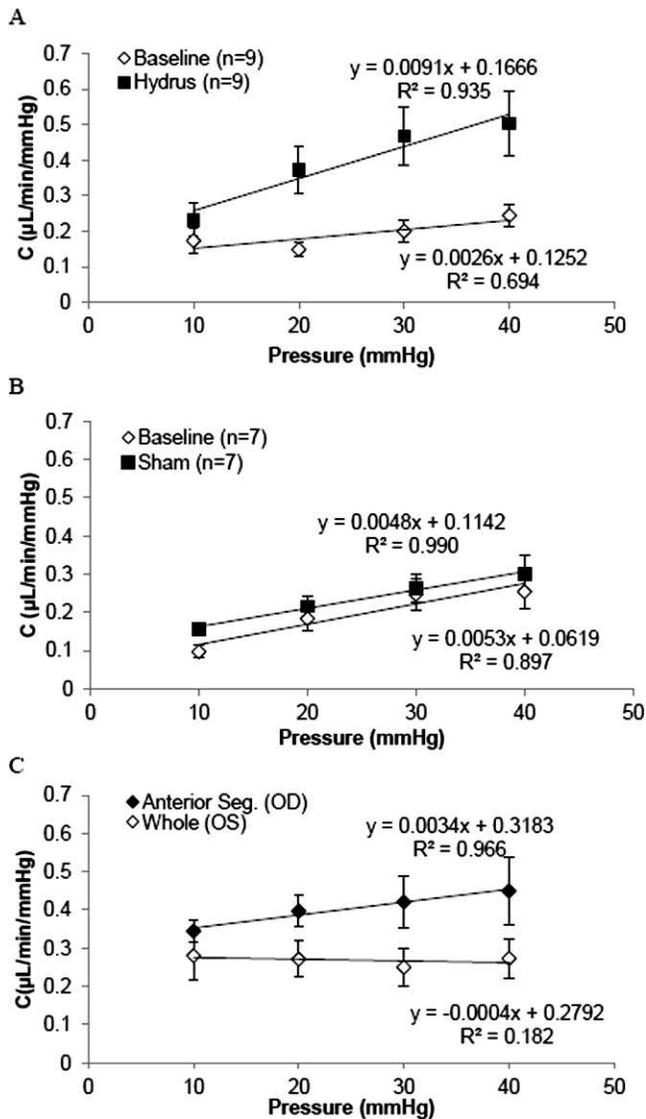
A

B

C

Figure 5. C increased with increasing perfusion pressure in control and experimental eyes. (**A**) The slope of C was significantly higher (*P* < 0.05) in experimental eyes (*n* = 9) post-implantation (0.0091 C/pressure, *black boxes*) in comparison with baseline (0.0026 C/mm Hg, *white diamonds*). (**B**) The slope of C was not significantly different in the control eyes (*n* = 7) after the sham procedure (0.0053 C/pressure, *white diamond*) in comparison with baseline (0.0048 C/pressure, *black square*). (**C**) C in anterior segments versus whole globes. The C (mean ± SEM) increased with perfusion pressure in the anterior segment (*black diamond*), while it was unchanged with perfusion pressure in its paired whole globe (*white diamond*, *n* = 5 pairs).
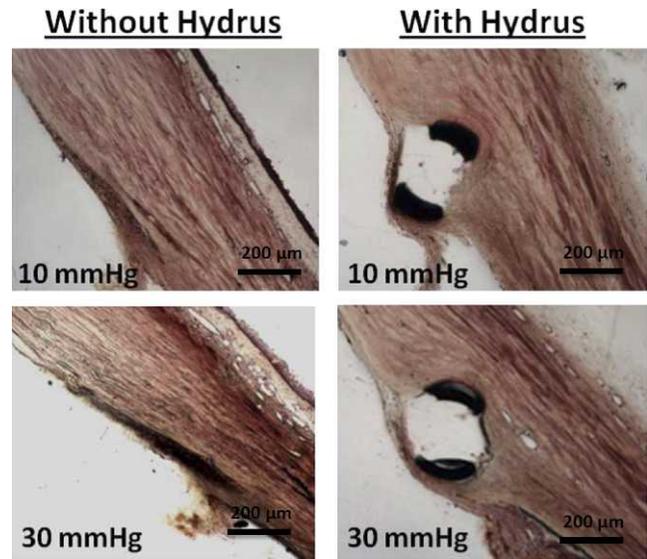
Without Hydrus   **With Hydrus**

Figure 6. The right eye of an 80-year-old male (donor 9, Table 1) was perfusion fixed for one hour at 10 mm Hg (*top*), while the left eye was perfusion fixed at 30 mm Hg (*bottom*) for a similar length of time. The images on the *left* show cross-sectional views of a region of the eye without the Microstent. The trabecular meshwork is compressed but visibly intact in both eyes. The images on the *right* show the Microstent in SC perfusion fixed at 10 (*top*) and 30 (*bottom*) mm Hg.

methods, all studies found an increase in C after implantation in comparison with controls.

Implantation Procedure Alone Has Insignificant Effects on C

It has been proposed that some procedures involving SC, like canaloplasty, may cause tears in the walls of SC resulting in an increase in C.[24] To determine whether the implant or the implantation procedure itself affected C, C values were measured one final time after the Hydrus was removed. The results showed that C of the implanted eyes returned toward baseline levels after the Hydrus was removed. These results provide strong evidence that the Hydrus Microstent itself rather than the implantation procedure alone, increases C. Also, an incision into SC resulted in no significant increase in C from its baseline measurement, demonstrating further evidence that the manipulation of the tissue is not contributing significantly to the C changes.

C Increases with IOP Elevation in the Anterior Segment Perfusion Model

With the Hydrus Microstent implanted into SC, the slope of C versus perfusion pressure increased in comparison with baseline. One explanation is that the Hydrus causes a further

TABLE 3. Information on Donors Contributing Pairs of Eyes for Anterior Segment versus Whole Eye Perfusion Study (*n* = 5 Pairs)

Donor	Sex	Cause of Death	Age, y	Post Mortem Time, h
11	F	Subarachnoid hemorrhage	53	45
12	M	Cardiac arrest, sepsis	53	41.5
13	M	Respiratory failure, sepsis	56	44
14	M	Lymphoma	72	24.5
15	F	Bladder cancer	55	33.5
Average ± SEM			57.8 ± 3.6	37.7 ± 3.9

reduction in downstream resistance with increasing IOP by increasing circumferential flow and pressure in SC. Increased circumferential flow allows for greater access to collector channels, while increased pressure may cause dilation of collector channels. A previous study showed a small C increase with a pressure elevation in the range of 7 to 25 mm Hg after a 4-clock trabeculotomy.[30] In both of these experiments, the Hydrus implantation or trabeculotomy causes a reduction in transmural pressure across the anterior chamber and SC that would cause increased pressure in SC at elevated IOP during perfusion. Increased pressure in SC causes a further dilation of SC and collector channels, and a decrease in flow resistance in these channels.

It has been shown previously that C decreases with IOP increases in whole globes[29,31,32] and sometimes increases with IOP increases in anterior segments.[17,33] In our study, whole globes showed no significant change in C with increasing pressure, whereas the contralateral anterior segments showed an increase in C with increasing pressures. Although not confirmed experimentally, one theory for the discrepancies found between anterior segments and whole globes is based on the differences in the streamlines or direction of fluid flow in each model. Whole globes have an angle that is intact and fluid enters from the posterior chamber, through the pupil, and then drains into the angle and TM. On the other hand, in anterior segments, fluid enters the TM directly, since the iris, ciliary body, and lens all are removed. These differences in the fluid dynamics and supportive tissue around the scleral spur induce variations in the direction and magnitude of drag forces on the TM, potentially affecting its deformation and outflow resistance.

Schlemm's Canal Dilates in Areas of Implantation

Histologic examination showed that the Hydrus Microstent dilated SC when perfusion fixed at 10 mm Hg in one eye and 30 mm Hg in the paired eye. The areas of the trabecular meshwork with the Microstent appeared stretched but intact in both eyes. Breaks in the inner and outer wall of SC have been proposed as a mechanism for increased outflow in canaloplasty. Examination to identify microscopic breaks in SC was not performed in our study. However, removal of the Hydrus reduced C; if breaks had occurred, they appear to have had a minimal effect on C.

Study Limitations

A few limitations of our study warrant discussion. First, cell viability was not assessed in the donated globes. It is reasonable to assume that postmortem cell viability decreases with time, which could affect C. However, linear regression analysis showed that during the time of this experiment there was no correlation of postmortem time and C. Since our study focused on mechanical manipulation of the SC rather than pharmacologic response of the TM, C changes from the Hydrus implant are expected to be less affected by postmortem cellular changes. On the other hand, the cell viability may confound the pressure-induced C changes observed in our study, since cellular response or contraction to pressure changes may affect the outflow resistance. Another limitation of the study was that, because this was an acute ex vivo experiment, cellular responses to the dilation of SC and tissue remodeling could not be evaluated.

Conclusions

The Hydrus Microstent significantly increased C in human anterior segments. It is designed to direct aqueous humor across the trabecular meshwork and into a dilated SC, thus bypassing the main site of outflow resistance. Future studies evaluating different lengths of intracanalicular scaffolds may provide information on the amount of SC dilation needed to optimize circumferential flow and C. Controlled, randomized, multicenter studies are needed to prove long-term safety and efficacy of the Hydrus Microstent in reducing IOP in glaucomatous eyes.

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