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Article

Intraocular Pressure Reduction by Femtosecond Laser Created Trabecular Channels in Perfused Human Anterior Segments

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Purpose: This study investigated the initial feasibility of using femtosecond laser trabeculotomy (FLT) to create open channels through the trabecular meshwork into Schlemm's canal to lower intraocular pressure (IOP) in a perfused anterior segment model.

Methods: Human anterior segments (12 eyes) were assigned to either treatment ($n = 6$) or sham treatment ($n = 6$) groups. Both groups were perfused until a baseline IOP was recorded upon which a direct FLT treatment or a sham treatment was administered. IOP was recorded before and after the treatment. Spectral domain optical coherence tomography and second harmonic generation imaging we used to investigate the FLT channels.

Results: In the FLT group, there was a significant mean decrease in the IOP of 22% compared with the pre-FLT IOP (7.13 ± 2.95 mm Hg to 5.34 ± 1.62 mm Hg; $P < 0.05$). In the control group, the post-sham IOP remained relatively unchanged compared with the pre-sham IOP (6.39 ± 3.69 mm Hg to 6.67 ± 4.12 mm Hg).

Conclusions: The results of this study indicate that FLT treatment can significantly decrease the IOP in a perfusion model and may provide a potential noninvasive treatment option for primary open angle glaucoma.

Translational Relevance: Investigating the use of femtosecond lasers for photodisrupting the trabecular meshwork can lead to a clinically relevant alternative to current glaucoma procedures.

Introduction

Glaucoma is the leading cause of irreversible blindness worldwide, affecting approximately 64.3 million people in 2013. Estimates project that the incidences of glaucoma will increase from 76.0 million in 2020 to 111.8 million people in 2040.[1] Patients with primary open angle glaucoma present with elevated intraocular pressure (IOP), which is believed to be caused by an increased resistance to the normal outflow of aqueous humor from the eye.[2] The majority of aqueous humor outflow from the eye occurs through the traditional drainage pathway, which consists of specialized structures in the iridocorneal angle of the eye, or the junction of the cornea and iris. The structures consist primarily of the trabecular meshwork, juxtacanalicular tissue, and Schlemm's canal. Schlemm's canal is a vessel-like structure that runs circumferentially around the iridocorneal angle and drains aqueous humor into the episcleral venous system via collector channels.[3] The major sources of proximal outflow resistance that increase the IOP are thought to be the juxtacanalicular tissue and the inner wall of Schlemm's

canal.[3–7] An elevated IOP eventually leads to an acquired optic neuropathy in which the neuroretinal rim is thinned owing to the loss of retinal ganglion cells. When the loss of optic nerve tissue is significant, patients develop optic nerve–related visual field loss.

The management of glaucoma typically requires lifelong medication,[8,9] often in conjunction with surgical interventions including laser treatment[10–13] or, more recently, minimally invasive glaucoma surgeries (MIGS).[14–20] The common goal among the various therapies is to lower IOP to prevent loss of visual field from excessive pressure on the optic nerve. Although some MIGS procedures target the suprachoroidal space, most target the trabecular meshwork, juxtacanalicular tissue, and inner wall of Schlemm's canal to bypass the significant aqueous humor outflow resistance found in those tissues. Among the treatments targeting the aqueous humor outflow resistance in the juxtacanalicular region are the iStent (Glaukos, CA),[15,16] Hydrus microstent (Ivantis, Irvine CA),[14] and excimer laser trabeculostomy.[13] These three procedures create a direct conduit between the anterior chamber and Schlemm's canal, although all three require penetration into the anterior chamber with surgical tools through a corneal incision. Additionally, selective laser trabeculoplasty is a widely used, noninvasive laser procedure that targets the pigmented cells of the trabecular meshwork while causing minimal collateral damage to the meshwork itself. No direct connection between the anterior chamber and Schlemm's canal is created, although outflow through the trabecular meshwork is thought to be increased via biological changes in the tissue.[11,21]

Despite the emergence of MIGS and various minimally invasive laser therapies such as selective laser trabeculoplasty, there is still an unmet medical need for a noninvasive method that avoids penetrating corneal incisions while causing minimal collateral damage to create channels connecting the anterior chamber to Schlemm's canal. To this end, this study aimed to investigate the feasibility of using a femtosecond laser trabeculotomy (FLT) to create clear drainage channels through the trabecular meshwork and into Schlemm's canal to lower the IOP. Femtosecond lasers for ophthalmic surgery have been commercially available since 2000, and their safety profile, lack of collateral damage to surrounding ocular tissue, and noninvasive capabilities are well-documented.[22–25] In this proof-of-concept study, we report on the efficacy of using FTL to lower the IOP in a perfused human cadaver tissue model.

Methods

Laser System

A commercially available OneFive Origami XP laser (NKT Photonics, Birkerød, Denmark) generating amplified femtosecond laser pulses at a 1030-nm wavelength was used in the experiments. The laser beam was directed through a pair of x–y scanning galvo mirrors into an objective lens focusing the laser beam to a spot size of approximately 5 mm. The objective lens was mounted to a *z*-axis controller located above a sample holder. Custom LabView software was written to control the galvo mirrors and z-motor so that the laser focus could be scanned in a three-dimensional rectangular raster pattern. A red HeNe laser was coaligned with the femtosecond laser to assist in aiming the femtosecond beam onto the trabecular meshwork. An operating microscope eyepiece was mounted above the objective to give a direct view of the aiming beam and trabecular meshwork.

Cadaver Tissue, Perfusion, and IOP Measurements

Twelve dissected corneoscleral shells with no history of glaucoma, ocular hypertension, or trabecular meshwork procedures were received within 24 hours of death from the San Diego Eye Bank and preserved in Optisol GS corneal transplant media (Chiron Intraoptics, Irvine, CA). Six eyes received FLT (FLT group) and six eyes received a sham treatment (control group). This study was performed in compliance with the principles of the Declaration of Helsinki.

The constant flow, variable pressure anterior segment perfusion model used in this study is based on established methods frequently described in the literature.[26–28] This method has also been used to evaluated MIGS implants (iStent) in the trabecular meshwork.[29] IOP (mm Hg), as opposed to outflow facility (μL/min/mm Hg), was measured because of the clinical relevance of IOP in glaucoma management. The sample tissues were mounted to a custom anterior segment perfusion device before being placed into a tissue culture incubator at 37 °C, 5% CO_2 and 95% humidity (Sheldon Manufacturing Inc, Cornelius, OR). The inlet of the anterior segment perfusion device was connected to a syringe pump (PHD 2000, Harvard Apparatus, Inc., Holliston, MA) which provided an inflow of cell perfusion media (Dulbecco's modified eagle media supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 5 μg/mL amphotericin B)
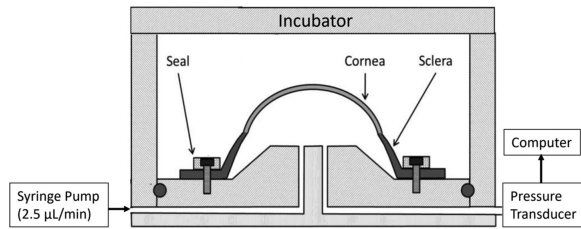
Figure 1. Perfusion system. The anterior segments were perfused with a syringe pump in an incubator at a 2.5 μL/min flowrate. The pressure was monitored by pressure transducers and recorded by computer.
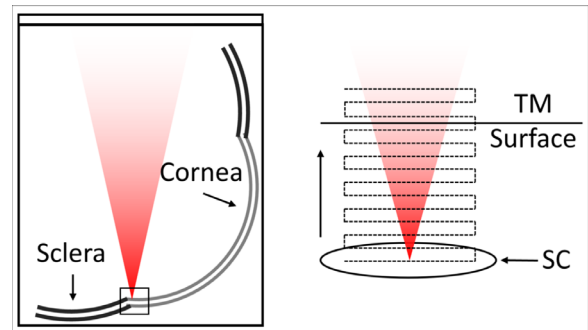

Figure 2. (A) General orientation of the tissue sample in the cuvette relative to the laser. (B) Rectangular raster laser scanning pattern. The pattern traverses the full depth of the trabecular meshwork (TM), thus connecting the artificial anterior chamber to the Schlemm's canal (SC).

at a constant, physiological rate of 2.5 mL/min. The outlet of the anterior segment perfusion device was connected to a computer-controlled pressure gauge that recorded the pressure within the eye at 1-minute intervals. Figure 1 shows the schematics of the perfusion system.

The eye was perfused for at least 12 hours before treatment; steady-state IOP (baseline) was maintained for 4 hours before the FLT surgery or sham treatment was performed. Likewise, the eye was perfused for at least 12 hours after treatment, after which a steady-state IOP (final IOP) was maintained for 4 hours before the experiment was terminated.

FLT and Sham Treatments

Once an anterior segment reached the steady-state (baseline) IOP, the tissue was removed from the incubator and unmounted from the perfusion device. The tissue was then secured in a custom cuvette so that the corneal endothelium and trabecular meshwork were directed upward along the axis of the laser beam. The sample was oriented so that the incoming femtosecond laser beam was nearly orthogonal to the trabecular meshwork. The tissue was submerged in perfusion media and the top of the cuvette was covered with a microscope slide so that no air gap remained between the media and microscope slide. Laser scan parameters were a 15 μJ pulse energies, a 1 kHz repetition rate, and 5 × 5 × 5 μm spot, line, and layer separations. The surface of the trabecular meshwork was located using the red HeNe aiming laser. The raster scan was then programmed so that the scan began 200 microns beneath the surface of the trabecular meshwork and advanced outward toward the media filled cuvette. Given the angle of incidence and the reported average thickness of the trabecular meshwork (174.16 ± 28.14 μm),[30] a standard 200 μm depth was chosen to ensure the scan penetrated through the inner

wall of Schlemm's canal. The pattern was scanned outward past the surface of the trabecular meshwork to ensure the ablation of the entire tissue region of interest and the creation of a contiguous channel from the anterior chamber into Schlemm's canal. During the FLT treatment, three channels were created in the trabecular meshwork measuring 1000 mm wide × 250 mm high, and through the full depth of the trabecular meshwork. Figure 2 illustrates the general orientation of the tissue and the laser pattern location in relation to Schlemm's canal while also showing the pattern traversing the full depth of the trabecular meshwork. The three FLT channels were separated by at least 1 mm. The tissue samples were outside of the incubator for about 20 minutes for a typical FLT treatment cycle. The control eyes underwent a sham treatment in which they were handled identically to the FLT eyes with the exception that the femtosecond laser was not applied.

Imaging

A custom-built spectral domain optical coherence tomography (OCT) was used to verify the patency of the channels. The spectral domain OCT had a central wavelength of 850 nm and theoretical axial and lateral resolutions of 2.7 μm and 8.2 μm in air, respectively. Second harmonic generation (SHG) imaging and autofluorescence imaging were also used to further investigate the appearance of the FLT channel. A commercially available multiphoton microscope (Leica SP8 Falcon, Wetzlar, Germany) was used to perform the nonlinear optical imaging. The wavelength of the femtosecond excitation laser was set to 820 nm and the collection band for SHG and autofluorescence were set to 390 to 420 nm and 450 to 550 nm, respectively.

Statistics

Paired *t*-tests were performed to determine whether significant differences existed between pretreatment and post-treatment perfusion pressures in each study arm (FLT and sham) and an unpaired *t*-test was used to determine if a difference existed between the baseline perfusion pressures in both arms (FLT vs. sham).
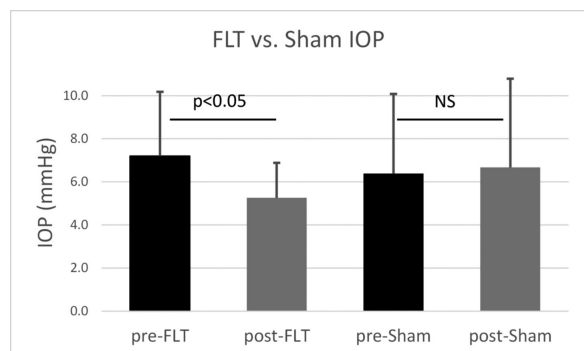

Figure 3. There was an average reduction in IOP of 22% in the FLT-treated eyes compared with baseline ($n = 6$). There was no significant change in IOP before and after the sham procedure in the control group ($n = 6$).

Results

Overall, the perfusion pressure was decreased in the perfused anterior segments that underwent FLT with a mean decrease in the perfusion pressure of 22% from baseline. In the FLT group, the mean pre-FLT perfusion pressure was 7.13 ± 2.95 mm Hg and the post-FLT pressure was significantly reduced to 5.34 ± 1.62 mm Hg ($P < 0.05$). In the control group, the presham pressure was 6.39 ± 3.69 mm Hg, and the postsham pressure remained statistically unchanged at 6.67 ± 4.12 mm Hg ($P = 0.28$). These results are summarized in Figure 3. There was no statistically significant difference between the pre-FLT perfusion pressure and the presham perfusion pressure ($P = 0.73$). The data for all eyes are presented in Table. A sample graph showing the pressure over time in a perfused anterior segment treated with FLT is presented in Figure 4; the hashmarks represent 4 hours of elapsed time during which the pressure of the perfused anterior segment was stabilizing.

Figure 5 shows a representative image of the three FLT channels and where they are located relative to the cornea, sclera, and trabecular meshwork. The darkest band of trabecular meshwork just above the scleral

Table. IOP Values Before and After and Percent Change in IOP for the FLT Treatment Group and the Sham Control Groups

|	IOP Data		
FLT #	Pre-FLT IOP (mm Hg)	Post-FLT (mm Hg)	Percent of IOP Change
1	6.20 ± 0.20	5.08 ± 0.42	18.06
2	8.33 ± 0.12	5.39 ± 0.10	35.29
3	7.04 ± 0.43	5.31 ± 0.16	24.57
4	3.57 ± 0.18	3.13 ± 0.90	12.32
5	4.88 ± 0.20	4.60 ± 0.29	5.74
6	12.80 ± 0.74	8.53 ± 1.10	33.36
Mean	7.13	5.34	21.55
Standard deviation	2.95	1.62	10.68
Standard deviation	0.0364		
Sham #	Pre-Sham (mm Hg)	Post-Sham (mm Hg)	Percent of IOP Change
1	2.81 ± 0.08	2.79 ± 0.24	0.71
2	2.34 ± 0.45	2.07 ± 0.06	11.54
3	5.94 ± 0.31	5.67 ± 0.27	4.55
4	4.61 ± 0.34	5.02 ± 0.33	−8.89
5	11.90 ± 0.20	12.55 ± 0.51	−5.46
6	10.71 ± 0.64	11.92 ± 0.36	−11.30
Mean	6.39	6.67	−1.48
Standard deviation	3.69	4.12	7.94
P value	0.2866		
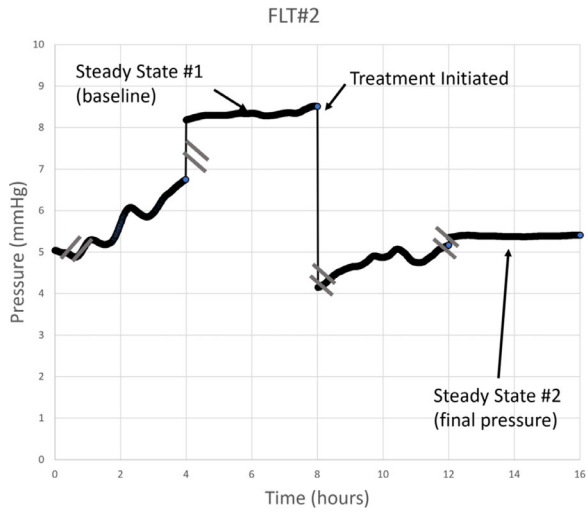
Figure 4. Pressure data from FLT eye #2 is presented. The gray hashmarks indicate four hours of lapsed time (not shown). The time between treatment and reperfusion was roughly 20 minutes.
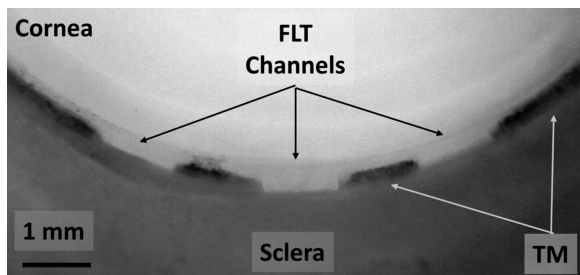

Figure 5. A dissecting microscope image of three FLT channels in a human eye. The corneoscleral shell was positioned so that the endothelium faced upward; the tissue was imaged from above. The three channels were 1000 μm wide by 250 μm high, and spanned the full depth of the trabecular meshwork (TM).
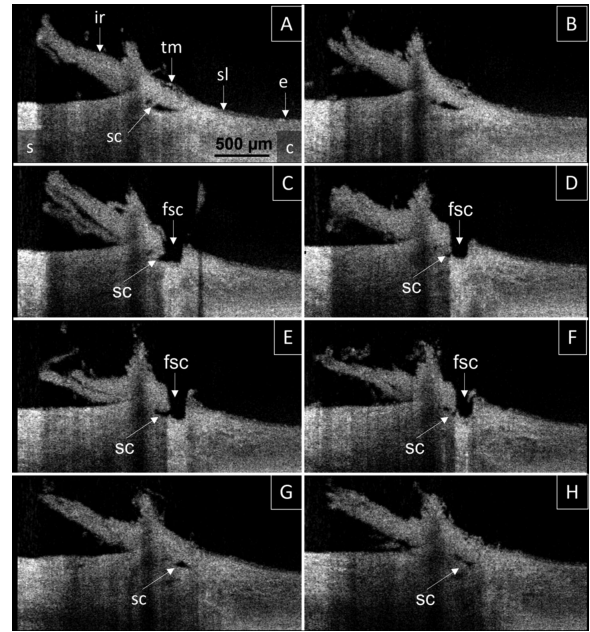

Figure 6. (A–H) The en face OCT images of 2 mm of corneoscleral/trabecular tissue along the circumferential axis of the eye. The distance between each panel is 250 μm. Sclera (s) is on the left; cornea (c) is on the right. The endothelium (e), Shwalbe's line (sl), and iris root (ir) are also visible. (A, B) Normal tissue with trabecular meshwork (tm) and Schlemm's canal (sc) intact. (C–F) The femtosecond laser drainage channel (fsc) penetrating the SC through the TM. (G–H) Regular tissue adjacent to the femtosecond surgical channel.
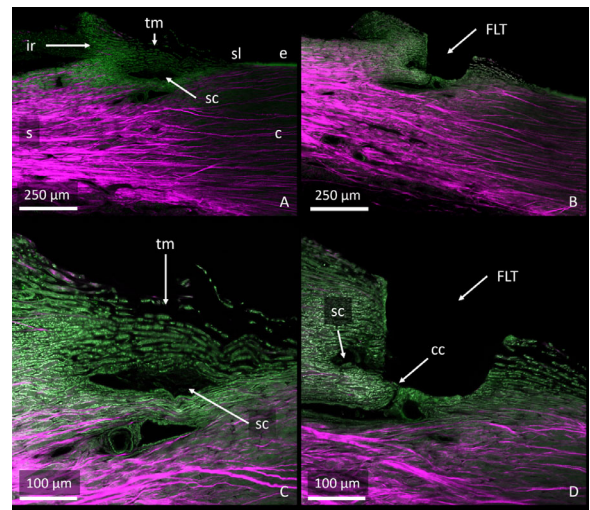

Figure 7. *Magenta* represents the SHG signal which primarily shows the collagen structure of the cornea and sclera. *Green* represents the autofluorescence signal that primarily shows the TM and iris structures. A The intact trabecular meshwork (tm), Schlemm's canal (sc), iris root (ir), Schwalbe's line (sl), corneal endothelium (e), sclera (s), and cornea (c) are visible. (B) Sn FLT channel approximately 1 mm away from the intact TM region in (A). A collector channel is visible within the FLT region. (C, D) Higher resolution images taken from the trabecular regions in (A) and (B), respectively.

spur was targeted during the FLT. OCT and SHG were used to determine if the channels had penetrated the Schlemm's canal through the trabecular meshwork and to give finer detail regarding the appearance of the channels. Figure 6 displays en face OCT images of the channels and the in-tact tissues adjacent to the channels. Figures 6A, B, G, and H show intact trabecular meshwork and Schlemm's canal tissues on either side of the FLT channel, whereas Figures 6C to F show the FLT channel. The eight images were taken at 250-μm increments and clearly demonstrate that the FLT channel penetrated the Schlemm's canal. Figure 7 shows SHG/autofluorescence images of an FLT channel (Figs. 7B and D) as well as intact tissues adjacent to the FLT channel (Figs. 7A and C). The images clearly show the channels fully penetrating the Schlemm's canal through the trabecular meshwork.

Discussion

The purpose of this study was to determine if FLT channels through the trabecular meshwork would significantly decrease the anterior chamber perfusion pressure in an anterior segment perfusion model. Overall, the data support the statement that the femtosecond laser-created channels through the trabecular meshwork resulted in a significant decrease in the perfusion pressure. Anterior segment perfusion is a generally accepted technique that has been used for decades in the study of the aqueous humor outflow pathway.[26–28,31,32] It is important to recognize that the baseline IOP values reported in this study were generally lower than the average physiological IOP of 15 mm Hg.[33] This is due in part to the lack of episcleral venous back pressure in the perfusion model which, in the living eye, is 7 to 11 mm Hg.[34–36] Given the range of episcleral venous pressure, the reported IOP values are well within the realm of reason. Furthermore, the pressure values reported in this study can be converted to outflow facility for comparison with values in the literature via the standard Goldmann equation, $P_0 = (F/C) + P_v$, where P_0 is the measured pressure (mm Hg), F is the inflow (μL/min), C is the outflow facility (μL/min/mm Hg), and P_v is the episcleral venous pressure, which is absent in this study. The results are in line with previously reported values of outflow facility ranging from 0.14 to 0.47 μL/min/mm Hg in the perfused human anterior segment when compared with the average 0.37 μL/min/mm Hg measured in this study.[31,37–39]

There are numerous anterior segment perfusion studies in the literature that have also investigated trabecular bypass, specifically by MIGS devices, and demonstrated IOP reductions or increases in outflow facility. Bahler et al.[29] (2012) showed nearly a 50% decrease in the IOP from baseline using an iStent (Glaukos, San Clemente, CA) in the perfused anterior segment. Another study evaluating the iStent, iStent Inject, and Hydrus microstent reported a 32% IOP decrease for one iStent, a 47% IOP decrease for two iStents, an 11% IOP decrease for two iStent Injects, and a 79% IOP decrease for the Hydrus microstent.[40] The reported IOP decreases varied widely between the studies, although all showed a general decrease in the IOP. The FLT procedure in the current study demonstrates the same IOP decrease trend in an anterior segment perfusion model as the established trabecular bypass MIGS procedures. FLT is similar to microtrabecular bypass implants in that it also provides a direct route for aqueous humor through the trabecular meshwork into Schlemm's canal. It should be noted that, in this study, no attempt was made to optimize the size, number, or locations of the FLT channels, and further research and refinement are needed to establish optimal surgical parameters to decrease the IOP.

SHG and OCT confirmed that the FLT channels penetrated the Schlemm's canal through the full thickness of the trabecular meshwork. The depth of the laser raster pattern beyond the surface of the trabecular meshwork (200 μm) was chosen based on the upper end of trabecular meshwork thickness ranges reported in the literature.[30,41] For this reason, the outer wall of Schlemm's canal was not entirely unperturbed, especially in the case of a shallow trabecular meshwork. In fact, Figure 7 shows that the outer wall of Schlemm's canal was photodisrupted to some degree, although the depth of the damage was approximately 10 μm. In future studies, it may be possible to image or measure the trabecular meshwork geometry beforehand to fine tune the scan depth to avoid the outer wall. Otherwise, the well-defined edges of the channel indicate that collateral damage to the surrounding tissue was minimal. The trabecular sheets in Figure 7 seem to be fully intact adjacent to the channel. Additionally, the presence of a collector channel in Figure 7B is noteworthy.

A major shortcoming of this study was that FLT was performed directly on a corneoscleral anterior segment artificially mounted such that the trabecular meshwork was directly facing the axis of the femtosecond laser beam. Such an orientation is obviously not realistic for any clinical setting. To achieve clinical relevance, the FLT procedure would need to be performed through the intact cornea via a goniotype lens. A previous study sought to do this in the primate trabecular meshwork using a femtosecond laser through a gonio lens, although the investigators were unable to penetrate the Schlemm's canal.[42] There are significant technical barriers related to focusing a femtosecond laser into the iridocorneal angle via a gonio lens while maintaining a sufficiently tight spot size for successful trabecular meshwork photodisruption. The investigators in the above study used a 0.15 NA focusing lens along with a standard trabeculoplasty goniolens (Magna View Gonio Laser Lens, OMVGL; Ocular Instruments Inc., Bellevue, WA) that was designed to deliver spot sizes on the order of hundreds of microns to the trabecular meshwork. This delivery system did not compensate for optical aberrations introduced by the gonio delivery and likely resulted in a large spot size not adequate for photodisrupting the trabecular meshwork. Previously, the same group demonstrated that ablation of the trabecular meshwork directly, that is, not through the cornea and similar to the present study, was possible with a femtosecond laser, although they did not investigate IOP effects with a perfusion model.[43] Before this study,

our group used a 1.7-μm amplified femtosecond pulses to photodisrupt the iridocorneal angle, creating partial thickness and subsurface drainage channels via an ab externo approach through the sclera.[44,45] Although the approach did decrease the IOP in rabbit eyes, the procedure did not specifically target the trabecular meshwork or Schlemm's canal, but instead created a very thin scleral region through which the aqueous humor could filter from the eye. Although there were limitations in the current study, the data are encouraging and provide sufficient evidence that FLT has the potential to decrease the IOP in a clinical setting. The technical engineering problems of maintaining tight focus of the femtosecond laser pulses through the peripheral cornea must be addressed.

The creation of femtosecond laser drainage channels through the trabecular meshwork of perfused human anterior segments resulted in a marked reduction of perfusion pressure compared with baseline and a control group. The results of this study support the further investigation and development of FLT as a noninvasive treatment for glaucoma.

Acknowledgments

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