Outflow enhancement by three different ab interno trabeculectomy procedures in a porcine anterior segment model

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Abstract

Purpose: To evaluate three different microincisional ab interno trabeculectomy procedures in a porcine eye perfusion model.

Methods: In perfused porcine anterior segments, 90 degrees of trabecular meshwork (TM) were ablated using the Trabectome (T; n = 8), Goniotome (G; n = 8), or Kahook device (K; n = 8). After 24 hours, additional 90 degrees of TM were removed. Intraocular pressure (IOP) and outflow facility were measured at 5 µl/min and 10 µl/min perfusion to simulate an elevated IOP. Structure and function were assessed with canalograms and histology.

Results: At 5 µl/min infusion rate, T resulted in a greater IOP reduction than G or K from baseline (76.12% decrease versus 48.19% and 47.96%, P = 0.013). IOP reduction between G and K was similar (P = 0.420). Removing another 90 degrees of TM caused an additional IOP reduction only in T and G but not in K. Similarly, T resulted in the largest increase in outflow facility at 5 µl/min compared with G and K (first ablation: 3.41 times increase versus 1.95 and 1.87; second ablation: 4.60 versus 2.50 and 1.74) with similar results at 10 µl/min (first ablation: 3.28 versus 2.29 and 1.90 (P = 0.001); second ablation: 4.10 versus 3.01 and 2.01 (P = 0.001)). Canalograms indicated circumferential flow beyond the ablation endpoints.

Conclusions: T, G and K significantly increased the outflow facility. In this model, T had a larger effect than G and K.

Keywords: ab interno trabeculectomy; intraocular pressure; outflow facility; canalogram; trabecular meshwork.

Introduction

Two common surgical procedures that are employed to treat patients with glaucoma involve trabeculectomy and glaucoma drainage devices. However, these invasive procedures are typically reserved for patients who are suffering from moderate or advanced glaucoma due to their risk profile and the associated complications, which include blebitis, corneal endothelial damage, diplopia, endophthalmitis, hardware erosions, and hypotony [1]. In an attempt to address the issues that impede trabeculectomy and glaucoma drainage devices, microincisional glaucoma surgeries (MIGS) have emerged [2]. MIGS involve an ab interno microincisional approach that spares the conjunctiva [3]. Research to date indicates these offer an improved risk profile, reduce IOP, and improve the patient's visual function. As such, they represent a viable surgical alternative to trabeculectomy and glaucoma drainage devices for treating patients who exhibit mild-to-moderate glaucoma [4]. A further advantage of MIGS is that they can be carried out together with cataract surgery. This is advantageous because glaucoma and cataracts frequently coexist and cataract surgery has been proven to contribute to a further reduction in intraocular pressure (IOP) [5].

MIGS improve pressure-dependent outflow via various approaches (1). These include shunting past or eradicating the trabecular meshwork (TM), (2) enhancing uveoscleral outflow by shunting into the suprachoroidal space, (3) reducing the ciliary body's production of aqueous humor, or (4) generating a subconjunctival drainage pathway [6]. The Trabectome (T), Goniotome (G), and Kahook (K) devices have all been employed effectively to remove the TM, the main site of outflow resistance in open-angle glaucoma [7]. T (Neomedix, Tustin, CA, USA) consists of a 19.5 gauge handpiece with an insulated footplate that contains aspiration, electrocautery, and irrigation. It was designed for movement along the TM while ablating a strip of TM and the inner wall of Schlemm's canal. This allows the aqueous humor to subsequently flow directly from the anterior chamber into the Schlemm's canal [6]. As opposed to ablating a strip of the TM, the Kahook dual blade (New World Medical, CA, USA) incorporates a special blade designed to fit the drainage angle anatomy of the eye to efficiently cut the TM tissue [8]. Unlike T, the K maintains the anterior chamber during the surgical procedure through viscoelastic material. However, previous research studies into use of K during cataract surgeries have found that retained viscoelastic material in patients can result in postoperative IOP spikes [9, 10]. The Goniotome (Neomedix, Tustin, CA, USA) is a new device that combines elements of both K and T. Like K, G consists of dual blades that cut into the TM tissue but they are serrated to initiate a double cut more reliably. However, like T, G involves an active irrigation and aspiration to control the stability of the anterior chamber without the need for viscoelastic material [11, 12]. Although separate studies have assessed and confirmed the efficacy of T and K, no study to date has evaluated G or performed a comprehensive comparison of the three techniques. In the past, outflow tracers were the primary method of examining flow [12–16] and the impact on the

outflow facility was not assessed. The purpose of this study was to compare the increase of outflow facility in a porcine perfusion model by each.

Methods and Materials

Study design

The aim of this study was to evaluate differences in how three microincisional procedures, T, K and G **(Fig. 1)**, efficiently reduced IOP. Two separate studies were performed. The first of these consisted of an eye perfusion study in which eight freshly dissected porcine anterior segments for each group were perfusion cultured at an infusion rate of 5 μl/min. After the baseline IOPs were obtained, each of the three devices, T, K, and Goniotome, were used to remove 90 degrees of TM from the sample. After 24 hours, a further 90 degrees of the TM were removed using the same procedure. Since it may not be possible to use glaucoma surgery to achieve a detectable IOP reduction in an eye that has a low baseline IOP [17], we also examined the IOP reduction at a higher infusion rate of 10 μl/min. The main outcomes of this study were IOP reduction and increase in outflow facility, the latter of which was calculated using the Goldmann equation. H&E staining was used for the histology. To access a direct view of the outflow pattern changes, two fresh pig eyes in each group underwent the corresponding procedures. The specimens then underwent a two-color canalogram [13, 16]. An upright fluorescence dissecting microscope was employed to visualize the outflow pattern.

Porcine eye perfusion culture, IOP, and outflow facility

The anterior segment perfusion culture followed our previous protocols [12, 18, 19]. Briefly, within two hours of sacrifice, fresh pig eyes from a local slaughterhouse were sterilized with 5% Betadine and subsequently bisected. The choroid, iris, lens, retina, and vitreous were gently removed. After undergoing a washing process with PBS three times, the anterior chambers were mounted onto an ex vivo perfusion system, and IOP measurements were taken in real time. The perfusion medium was composed of DMEM with 1% FBS and antibiotics. The baseline IOPs were obtained at 48 hours at a constant infusion rate of 5 μl/min. Following that, the uppermost 90 degrees of the TM was removed using either T, G or K. To ascertain how further TM removal resulted in an additional IOP reduction, a further 90 degrees of the TM was removed using the same procedure. Since a low baseline IOP may lead to an insignificant IOP reduction, we also examined the IOP responses at a higher perfusion rate of 10 μl/min at the baseline and after the first and second TM removal processes. If any clear signs of leakage (IOP< 5 mmHg at the baseline), contamination or obstruction (IOP>50 mmHg at any point) were observed at any time during the process, the samples were removed from the experiment [20].

Transducers were connected to each perfusion dish to measure the IOP in real time. The model incorporated the assumption that the episcleral venous pressure was zero. Thus, the individual outflow facility values were calculated from the infusion rates divided by the corresponding IOPs, according to Goldmann's equation [17, 21].

Canalograms

Outflow canalograms were performed in accordance with our previous protocol with some minor modifications [13, 16]. In brief, two fresh pig eyes were assigned to each group. After the retrobulbar adipose tissue and muscles were removed, the eyeballs with conjunctiva were mounted onto a foam head stand. A 3 mm corneal incision was made at the temporal side, the nasal TM was removed using either T, G or K. Two eyes with sham procedures served as the control. After the cornea incision was sealed with super glue, all the eyes were subjected to a two-color canalogram: A 20 gauge needle was intracamerally inserted through the nasal cornea, then the perfusate mixed with Texas Red (0.028 mg/ml, J60581, Alfa Aesar, Ward Hill, MA) and fluospheres® carboxylate-modified microspheres (1:100 dilution, F8813; Thermo Fisher, Eugene, OR) were gravity perfused into the chambers at a hydrostatic pressure equivalent to 15 mmHg. The differential outflow pattern was visualized with an epifluorescence dissecting microscope (Olympus SZX16 and DP80 Monochrome/Color Camera; Olympus Corp., Center Valley, PA). Pictures were taken at baseline and every 3 min up to 12 min at a 680 × 510-pixel resolution with 16-bit depth and 2 × 2 binning at a 200 ms exposure.

Histology

After perfusion culture, the anterior segments were dismounted and fixed with 4% PFA for 48 hours, embedded in paraffin, and then sectioned into 6 micron-thick slides. Hematoxylin and eosin (H&E) staining was subsequently performed for the histology using a previous protocol [18].

Statistics

A sample size calculator was used to determine the minimum number of eyes for an adequate study power. With a baseline IOP of 11.64+/- 1.45 mmHg [18], a minimum sample size of 7 is required to detect an 18% IOP reduction [22] with an alpha of 0.05 and a power of 0.80.

The quantitative results of this study are presented as the mean +/- standard deviation. The data were processed by PASW 18.0. A one-way ANOVA was used to compare the data for different groups at individual time points. Paired t-tests were used for an in-group comparison of IOP and outflow facility before and after treatment.

Results

IOP reduction

A total of 21 eyes were included in the perfusion study, of which, 8 were in K, 7 in G, and 6 in T. Two eyes in T exhibited clear signs of leakage and one eye in G showed signs of infection; as such, these three samples were excluded from further analysis.

At a 5 µl/min infusion rate, the baseline IOPs were comparable across the three groups (16.50+/-4.34 mmHg in T, 14.20+/- 1.00 mmHg in G, and 15.93+/-2.08 mmHg in K, P=0.795). The removal of the first 90 degrees of the TM using T resulted in greater IOP reduction than the reduction achieved by removing 90 degrees of the TM using G and K (76.12% versus 48.19% and 47.96% in IOP reduction from each respective baseline, P=0.013); however, the level of IOP reduction with G was similar to K (P=0.420). An additional 90 degrees of TM removal caused a further minor IOP reduction in T (4.45% decrease) and Gs only (9.32% decrease) but not in K (6.98% increase). The largest IOP reduction (13.30+/-4.08) and lowest postoperative IOP (3.21+/-0.70 mmHg) was achieved using T.

When the infusion was increased to 10 µl/min, the baseline IOPs increased to 22.53+/-8.23 mmHg in T, 22.60 mmHg +/- 0.98 mmHg in G, and 24.73+/- 3.72 mmHg in K. No significant difference in the baseline was observed (P=0.910). Like the IOP response at 5 µl/min infusion, T resulted in the greatest IOP reduction after both the first and second processes of removing 90 degrees of the TM (75.03% versus 54.20% and 53.48% after the first 90 degree TM removal; 80.02% versus 63.97% and 54.32% after the second 90 degrees of TM removal), while G and K did not further reduce the IOP (54.20% versus 53.48%, P=0.445 and 63.97% versus 54.32%, P=0.090, respectively).

Increase of outflow facility

According to the Goldmann equation, there is an inverse relationship between outflow facility and IOP when the episcleral vein pressure is zero. At a 5 µl/min infusion, the baseline outflow facility was comparable across the three groups (0.39+/-0.11 µl/min/mmHg in T, 0.36+/-0.03 µl/min/mmHg in G, and 0.36+/-0.05 µl/min/mmHg in K). Consistent with the IOP responses, the outflow facility in T increased by 3.41 times from the baseline after the first 90 degrees of TM ablation. This increase was significantly higher than the 1.95 times observed in G and 1.87 times that observed in K. An additional 90 degrees of TM was removed by either T (from 3.41 to 4.60) or Goniotome (from 1.95 times to 2.50 times), resulting to a further increase in the outflow facility. However, the removal of an additional 90 degrees of the TM using K did not significantly increase the outflow facility (1.87 times increase after the first TM removal versus 1.74 times after the second TM removal).

The outflow facility increased when the infusion rate was increased to 10 μl/min; however, there was no statistical difference among the groups at the baseline (P= 0.590). The first 90 degrees of TM ablation with T resulted in a 3.28 times increase in the outflow facility from the baseline, significantly higher than that of G (2.29 times) and K (1.90 times, P=0.001). The removal of an additional 90 degrees of the TM resulted in increased trabecular outflow in T and G only but not in K. Overall, T achieved a 4.1 fold increase in outflow facility compared to 3.01 fold in G and 2.01 times in K (P=0.001).

Outflow pattern

To better visualize the differential outflow pattern, we performed a two-color canalogram on the fresh pig eyes after surgery. Consistent with our previous studies, the TM permeability of the control eye with intact TM to 0.5 μm microspheres was low (Fig. 3 A, the middle band), while all three surgeries promoted the microspheres passing through the TM removal site into downstream outflow tracts (Fig. 3B, 3C, and 3D, the middle bands). In contrast to the 0.5 μm microspheres, Texas Red with a smaller size in diameter can easily access the downstream outflow tracts. The fluorescence intensity increased as the time lapsed. The post-surgery specimens exhibited an earlier Texas Red filling and a higher fluorescence intensity at the TM removal sites (Fig. 3B, 3C, and 3D, upper band), than the control (Fig. 3A, upper band).

Histology

Normal porcine TM exhibited a multilayer structure that was pigmented in a scattered way (Fig. 5). Unlike the human eye, the size of pig Schlemm's canal was smaller and not continuous (black arrows). No obvious damage to the adjacent sclera and corneal endothelium was observed following any of the three procedures. In the samples in T, a larger proportion and the full thickness of the TM was centrally ablated in comparison to that observed in the samples in K and G. Occasional contracted collagen was noted along the residual TM in T suggesting a thermal effect. All devices removed the large porcine TM incompletely.

Discussion

This is the first ex vivo study to systematically assess the change of outflow facility after a different extent of TM ablation using three different techniques. The juxtacanalicular meshwork and the inner wall of Schlemm's canal are the major aqueous outflow resistant sites, accounting for approximately 75% of the conventional outflow resistance [23]. Either removing [24, 25] or bypassing [21, 26] these tissues can lead to a reduction in IOP. In our previous study, we characterized an ab interno goniotomy device with an active irrigation and aspiration and found that the anterior chamber depth and the nasal angle remained steady throughout the entire procedure, in contrast to a lower anterior chamber stability in a passive dual blade goniotomy [12]. In the current study, we investigated the IOP-lowering efficacy of the same newly

developed device in a porcine eye perfusion model and compared its performance with a plasma-mediated ablation and a passive dual blade. In the current study, T resulted in the highest IOP reduction but these results have to be interpreted with caution because porcine eyes have an angular aqueous plexus different from human eyes [27]: the TM is thicker and a single lumen Schlemm's canal is absent. Consequently, the TM ablation is deeper allowing the adjacent TM to prolapse into the plasma ablation area which may lead to thermal effects that are absent in human eyes.

In the current study, we utilized an ex vivo eye perfusion system that allowed us to continuously perfuse the tissue-culturing medium at different infusion rates [28]. IOP can be measured at 1 min intervals using a pressure transducer. We found that T was a slightly more effective method of reducing IOP. With a similar baseline, T reduced IOP by 76.12% in comparison to 48.19% in G and 47.96% in K after the first 90 degrees of TM ablation.

The greatest IOP reduction and increase in outflow facility was observed after the first 90 degrees of TM removal in all three procedures. Additional TM removal achieved only a minor further reduction in IOP. This observation was aligned with the findings of a clinical study by Khaja and colleagues, that there was no significant correlation between the ablation arc and IOP reduction or final IOP after Trabectome surgery [29]. Similar results have been observed in studies of alternative devices for MIGS. Under a human eye perfusion culture, Hunter et al. found that a single trabecular bypass stent decreased IOP by 6 mmHg from the baseline while the addition of a second one reduced the IOP by another 2.9 mmHg [30]. One possible explanation for this observation was that, once the minimum TM removal required to maintain the circumferential aqueous outflow in the Schlemm's canal was established, the additional arc of TM removal became less important. Despite no continuous Schlemm's canal in the pig, there is circumferential flow as our prior studies demonstrated [13, 14, 16], presumably because the Schlemm's canal-like segments are connected. Limited access to angle structures, highlighted by the microsphere canalograms are sufficient to provide outflow beyond the ablated TM. We have observed the preferential entry of a tracer into the supranasal and infranasal quadrants before [15].

Previous studies suggested that the episcleral venous pressure (EVP), which is part of the IOP Goldmann equation, is higher in glaucomatous eyes [31, 32]. The EVP in our model is likely very low in absence of a venous circulation. This can explain why the postoperative IOPs in the current study reached a level that is lower than typically reported in clinical studies and it may provide indirect evidence of the role of elevated EVP in the etiology of glaucoma [33].

This study had several limitations. First, our results were based on an ex vivo perfusion model. Immune response and fibrosis, both of which could significantly impact postsurgical IOP, were not taken into consideration [34, 35]. Second, pig eyes have a much thicker TM layer than human eyes without a

continuous Schlemm's canal [36]. These anatomic differences between species may result in a different IOP response. Third, EVP was likely close to zero in the perfusion model; however, it is higher in glaucoma patients [31, 32]. However, the widespread use of porcine eyes in glaucoma research by our group [18, 37–44] and others [45–53] and in glaucoma surgery models [16, 41, 54–56] validates and justifies its use in this study. In particular, outflow enhancement by trabecular ablation [14, 16] or trabecular bypass surgery [16] demonstrated a behavior highly similar to human eyes.

In conclusion, in this porcine ex vivo model, three MIGS all yielded a significant enhancement of outflow. Plasma-mediated trabecular meshwork ablation (Trabectome) yielded slightly more IOP reduction and a higher increase of outflow facility than a new dual blade device that has active irrigation and aspiration ports (Goniotome) or than a passive dual blade device (Kahook dual blade).

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Figure Captions
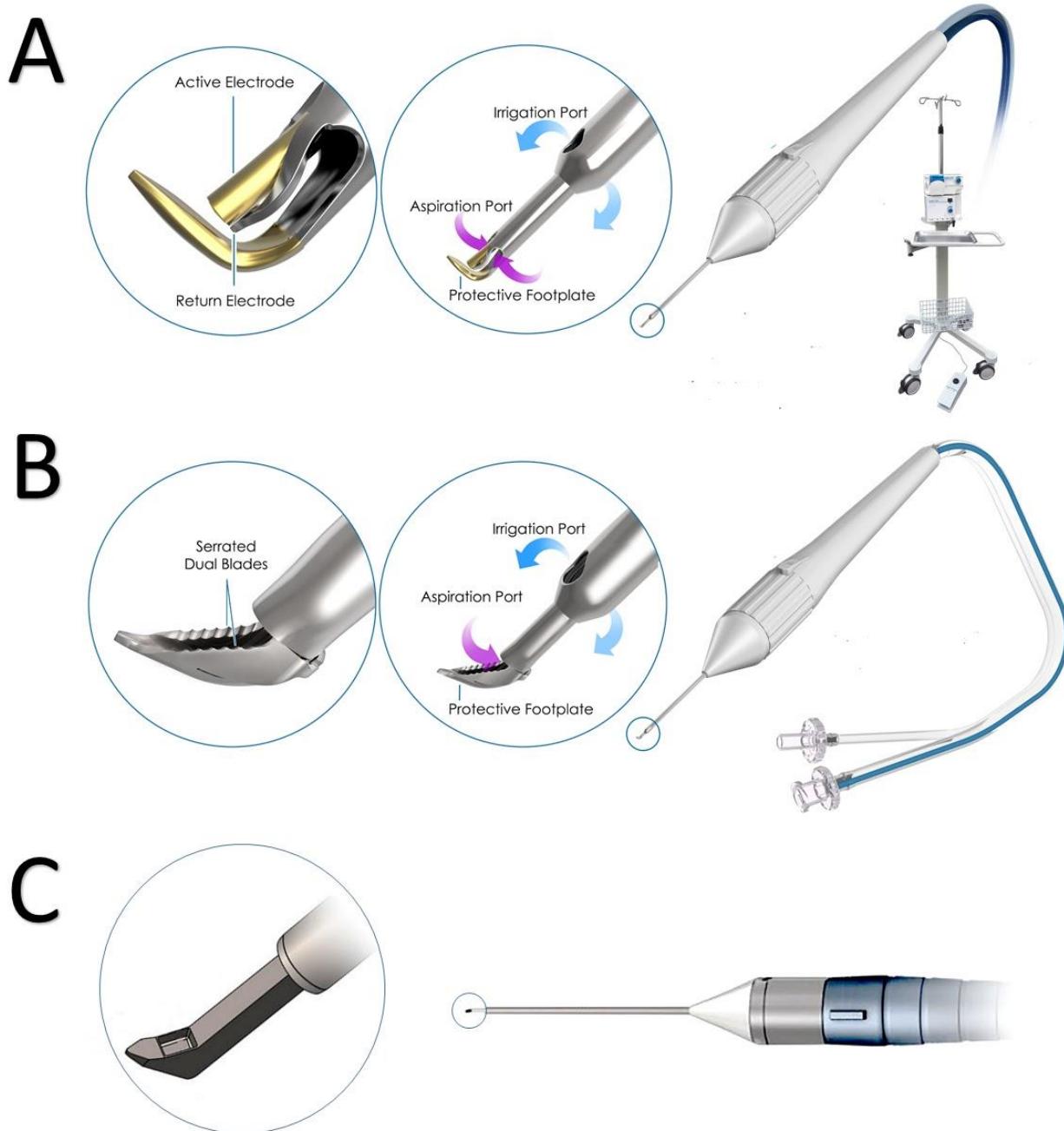
Fig. 1 Three microincisional glaucoma devices. T has a bipolar electrode that molecularizes and ablates the TM. It incorporates active irrigation and aspiration (A) ports. G has serrated dual blades that stretch and cut the TM. An active irrigation and aspiration direct the TM into the opening between the blades (B). K

has dual blades that cut the TM tissue. It has no irrigation or aspiration ©
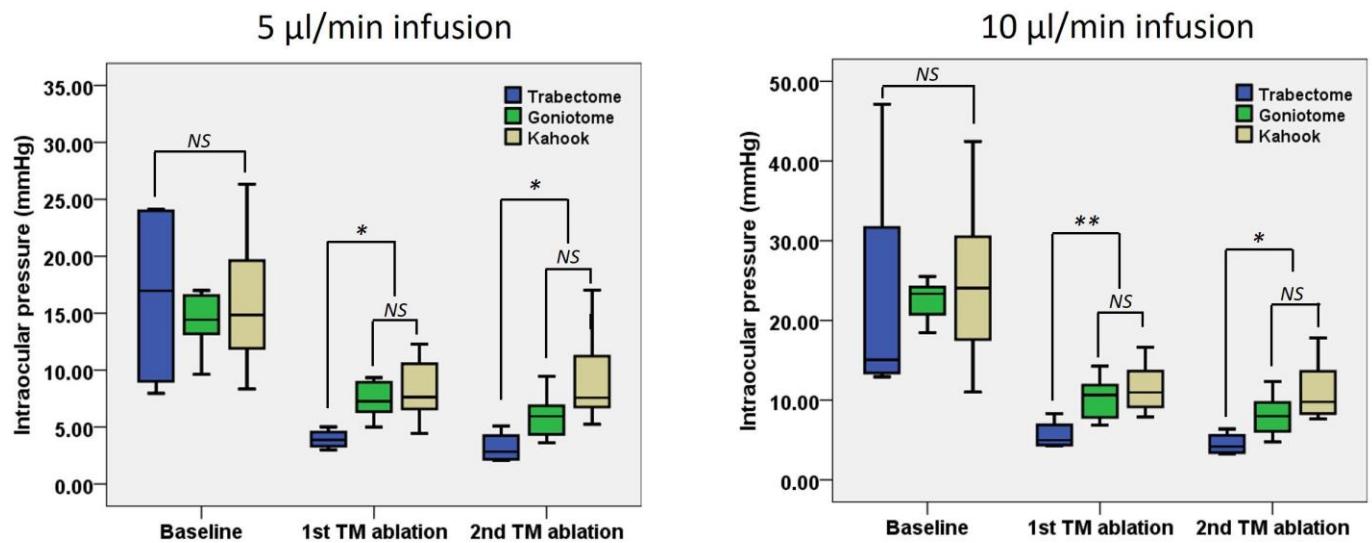
Figure 1. IOP reduction by TM ablation with three minimally invasive approaches

Fig. 2 Reduction of intraocular pressure. (*Left)* IOP reduction is shown for T, G and K at 5 µl/min. All three groups had a comparable baseline IOP (T: n=6; G: n=7; K: n=8; NS = no significance). T resulted in the greatest reduction in IOP in comparison to the other two groups (*P<.05) with no significance between G and K. (*Right*) IOP reduction at 10 µl/min. The baseline was higher for all groups. T resulted in the greatest reduction in IOP compared to G and K (**P<.01)
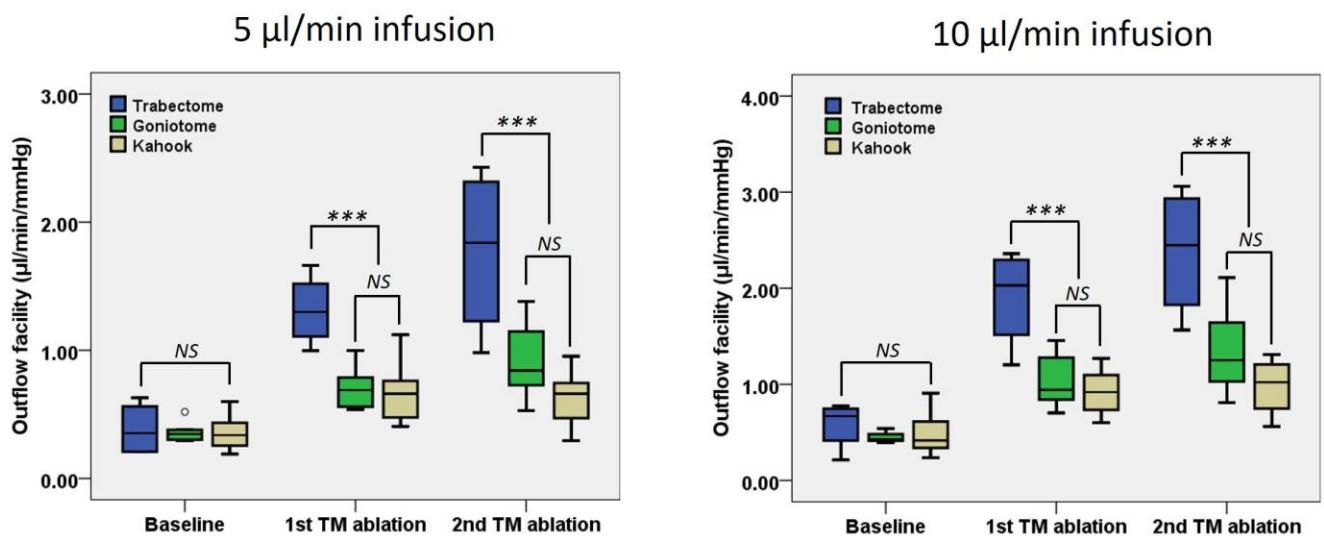
Fig. 3 Increase in outflow facility. (*Left*) The outflow facilities are shown for T, G and K at 5 µl/min. All three

groups had a comparable baseline IOP (T: n=6; G: n=7; K: n=8; NS = no significance). T resulted in the greatest increase in outflow facility compared to the other two groups (***P<0.001). (*Right*) Outflow facility at 10 µl/min. T led to the greatest increase in outflow facility compared to the other two groups (***P<0.001)
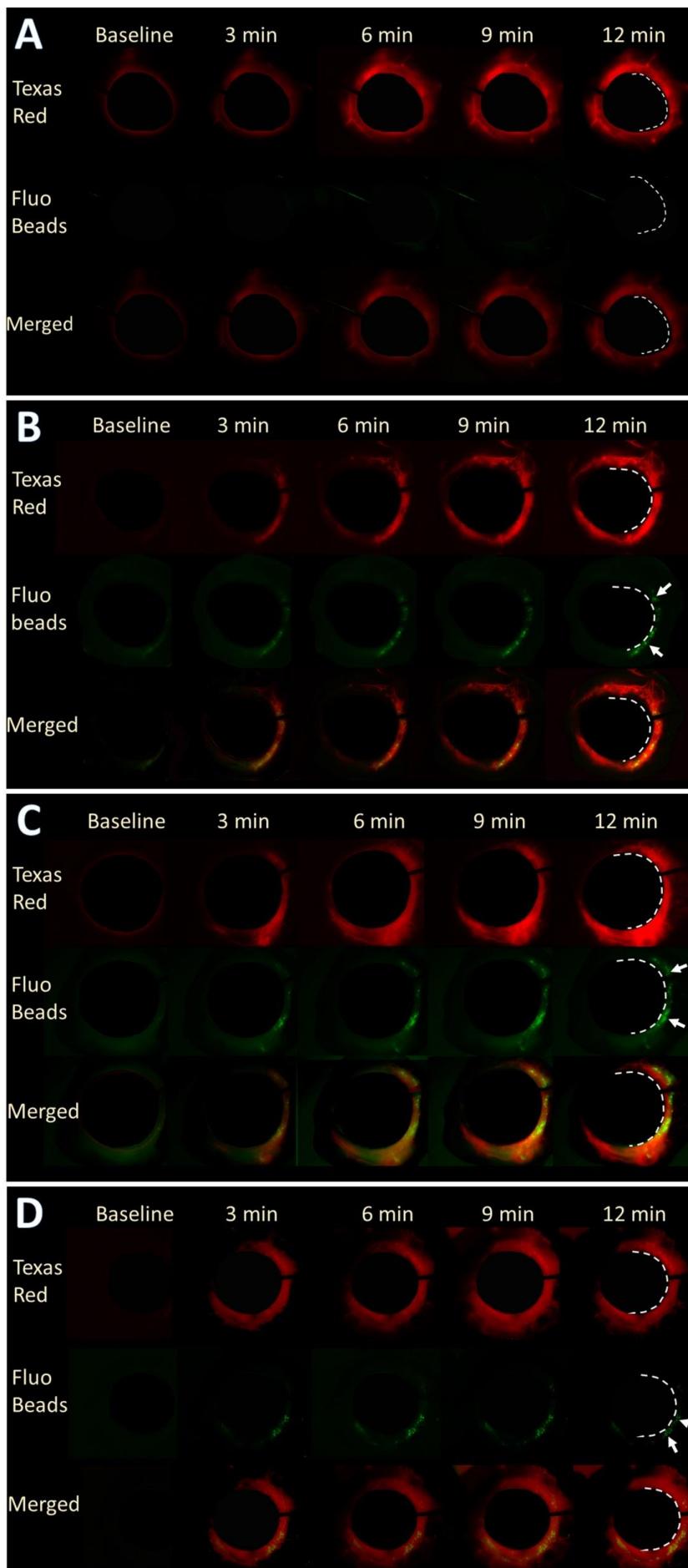
Fig. 4 Outflow pattern in canalograms. Canalograms are shown at different time points using Texas Red to

trace all outflow and fluorescent microspheres (Fluo Beads) to indicate the entry points into the downstream outflow system. Almost no microspheres passed through the TM in the control eye after 12 minutes (A) compared to the eyes that had been operated on using T (B, white arrows), G (C, white arrows), and K (D, white arrows). The post-surgery eyes exhibited a greater fluorescence intensity than the control
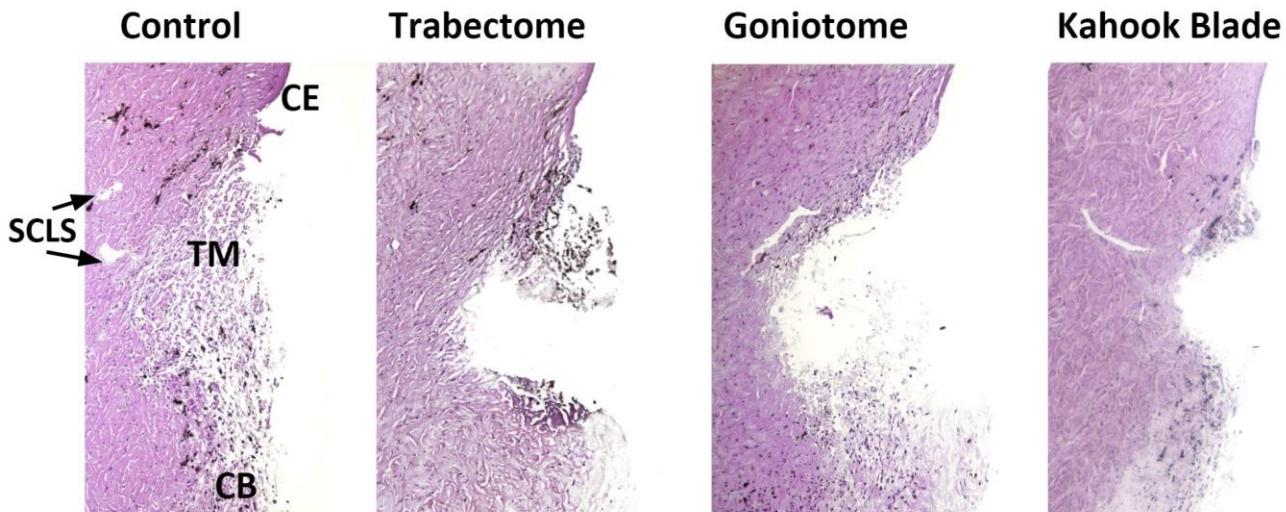
Fig. 5 Histology of the anterior chamber angle (H&E staining). TM: trabecular meshwork. SCLS: Schlemm's canal-like segments of the porcine angular aqueous plexus. CE: corneal endothelium. CB: ciliary body stump