Nicholas H. Andrew, Sarmad Akkach, Robert J. Casson
A review of aqueous outflow resistance and its relevance to microinvasive glaucoma surgery
Survey of ophthalmology, 2020; 65(1):18-31

Published at: http://dx.doi.org/10.1016/j.survophthal.2019.08.002

Major review

A Review of Aqueous Outflow Resistance and its Relevance to Micro-invasive Glaucoma Surgery

Sarmad Akkach, MBBS,[1,2] Nicholas H. Andrew, MBBS, FRANZCO,[2,3] Robert J. Casson, DPhil, FRANZCO[2]

[1] Royal Victorian Eye and Ear Hospital, Melbourne, Victoria, Australia

[2] South Australian Institute of Ophthalmology, University of Adelaide, Adelaide, South Australia, Australia

[3] Department of Ophthalmology and Vision Sciences, University of Toronto, Toronto, Canada

*Authors Sarmad Akkach and Nicholas H. Andrew contributed equally to this manuscript

Corresponding author:
Dr Sarmad Akkach
Royal Victorian Eye and Ear Hospital
32 Gisborne St
East Melbourne
VIC 3002
Australia

Email: Akkach.sarmad@gmail.com
Tel: +61 421 661 417

Abstract

Primary open-angle glaucoma is the leading cause of irreversible blindness worldwide and intraocular pressure (IOP) reduction remains the only proven treatment strategy. Elevated IOP occurs due to impaired aqueous humour outflow. Both a passive model and a dynamic model have been used to explain trabecular outflow resistance. The passive model posits that the trabecular meshwork acts as a static filter that exerts stable and passive resistance to outflow. In contrast, the dynamic model involves a 'biomechanical pump.' In recent years, the range of surgical management options for glaucoma has dramatically expanded, particularly the class of procedures known as micro-invasive glaucoma surgery (MIGS). These procedures typically have narrow mechanisms of action and enhance specific outflow routes. Optimal patient outcomes with MIGS requires a clear understanding of aqueous outflow and a surgical approach that is targeted to overcome the site of abnormal resistance in the individual. We review the anatomy and physiology of trabecular and suprachoroidal outflow that is of relevance to MIGS surgeons.

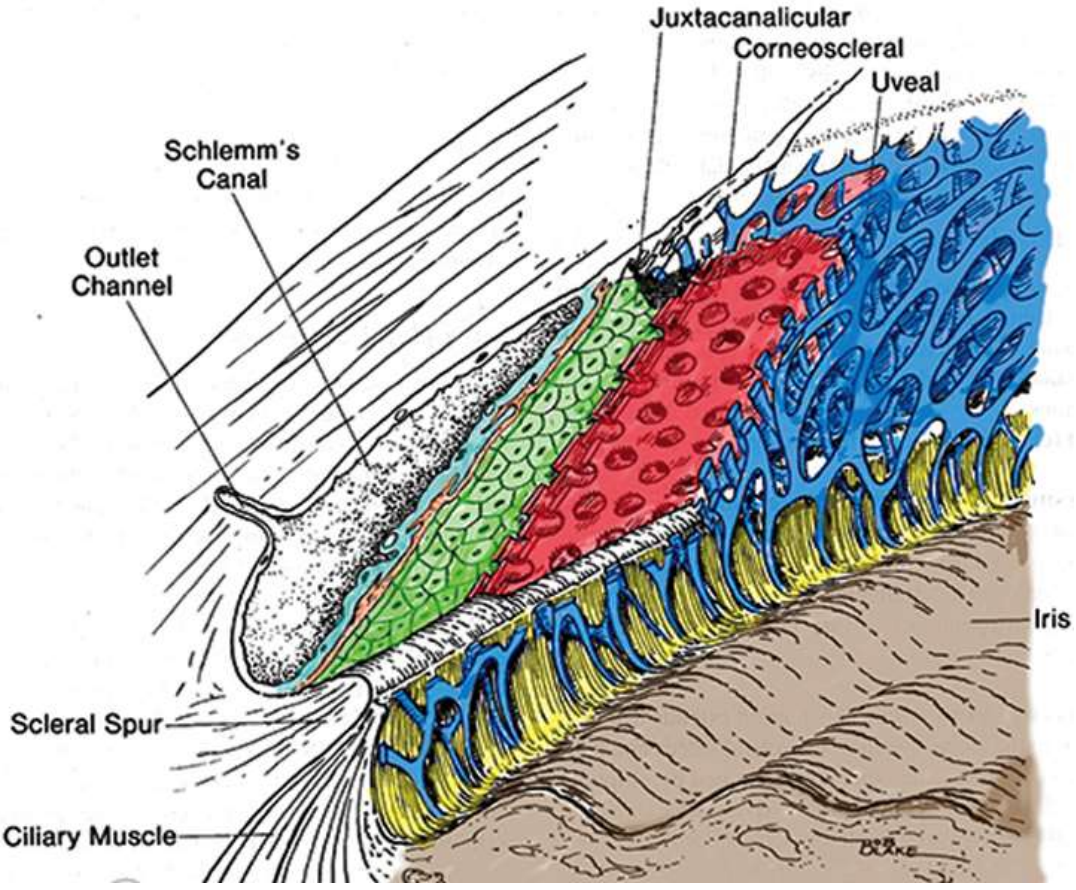
Key words: aqueous, outflow resistance, glaucoma, micro-invasive glaucoma surgery

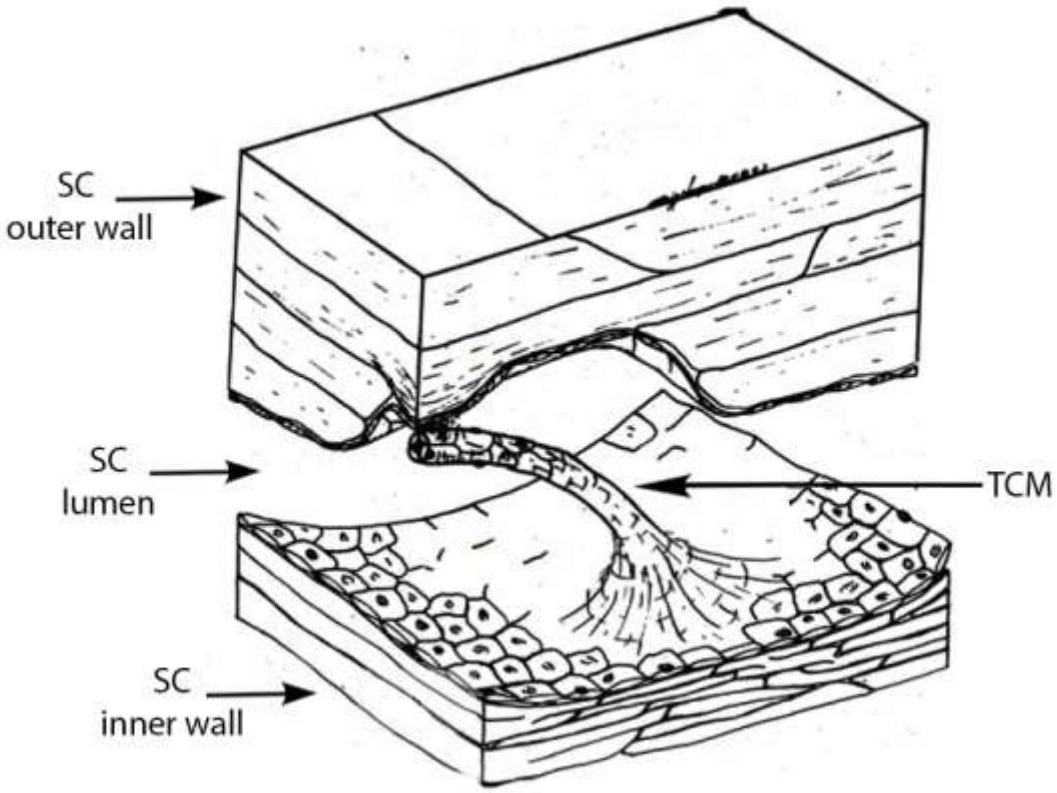
Introduction

Primary open-angle glaucoma (POAG) is the leading cause of irreversible blindness worldwide[74] and intraocular pressure (IOP) reduction remains the only proven treatment strategy.[14] Since elevated IOP occurs due to impaired aqueous humour outflow, surgical treatments either enhance drainage through existing physiologic outflow pathways or divert aqueous into new, non-physiologic pathways (such as subconjunctival drainage). The research effort to characterize outflow resistance is intensive and ongoing, and a clear understanding is increasingly important in this era of highly targeted procedures known as micro-invasive glaucoma surgery (MIGS). Here we provide a comprehensive review of the anatomy and physiology of trabecular and suprachoroidal outflow with particular emphasis on aspects relevant to MIGS.

Aqueous outflow pathways

Aqueous humour exits the eye via two routes: the trabecular pathway (Fig. 1a), and the non-trabecular pathway (Fig. 2). In the trabecular, or conventional outflow pathway, aqueous flows through the trabecular meshwork (TM) into Schlemm's canal (SC) and then into a network of downstream vessels. The TM comprises three regions: uveal meshwork, corneoscleral meshwork and juxtacanalicular tissue (JCT) (Fig. 1a).[84] The JCT is the outermost layer and abuts SC inner wall endothelium. Aqueous enters SC either by passing through pores in SC inner wall endothelium or by passing through transcanalicular microtubules (TCMs) (Fig. 1b).[13] Cell processes of trabecular lamellae attach to the inner wall of the lumen and limit its outward movement by exerting restraining tension (Fig. 1c). From SC, aqueous flows into collector channel entrances (CCEs) and then into aqueous, episcleral and conjunctival veins.[57] The trabecular outflow system can be subclassified into proximal and distal systems. The former refers to the TM (including the JCT), while the latter refers to the rest of the structures outlined above.[13]




SC
outer wall
SC
lumen
TCM
SC
inner wall

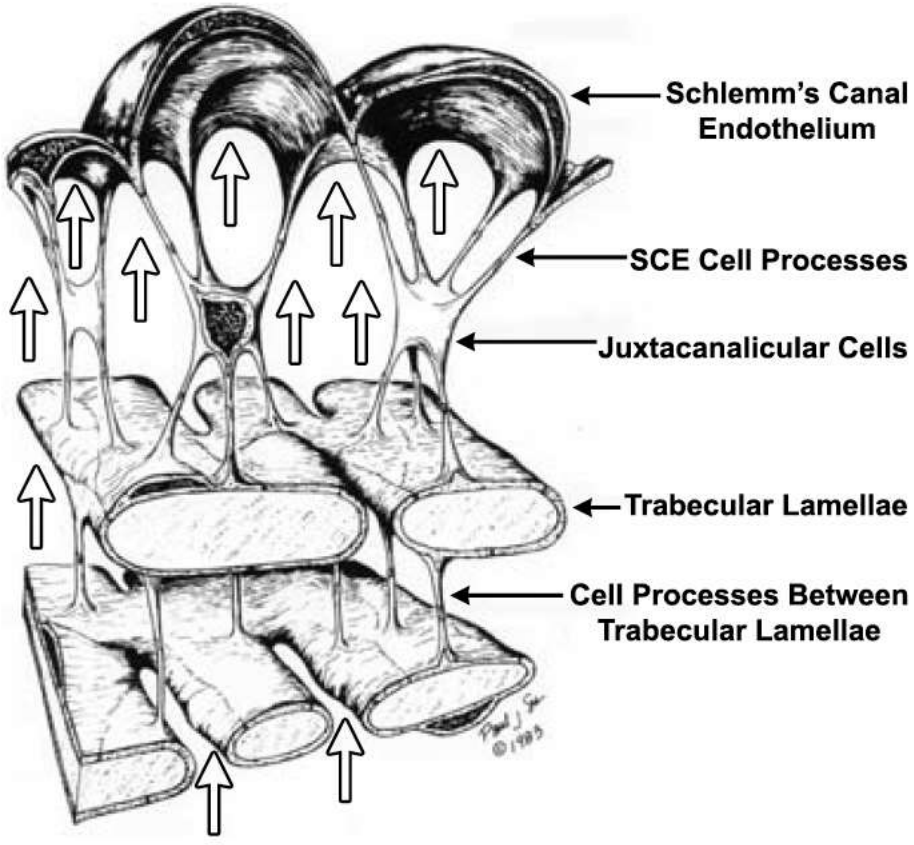

Schlemm's Canal
Endothelium
SCE Cell Processes
Juxtacanalicular Cells
Trabecular Lamellae
Cell Processes Between
Trabecular Lamellae

Fig. 1. (A) Trabecular outflow pathway. Cross-section showing layers of the TM that aqueous must traverse before entering SC. Aqueous exits SC through CCEs (outlet channels) located along the outer wall. Blue: uveal meshwork; red: corneoscleral meshwork; green: juxtacanalicular tissue.[2] (B) TCMs arise from SC inner wall and extend across the lumen towards the outer wall.[59] They are hollow and deliver aqueous into SC. Aqueous also enters SC by passing through pores in the SC inner wall endothelium. (C) Appearance of the trabecular outflow system at physiologic IOP. Arrows depict direction of aqueous flow. Cell processes attach to Schlemm's canal endothelium (SCE) and exert restraining tension to limit its outward movement.[51] (A) (Reprinted with permission. ©2010. Wolters Kluwer Health. All rights reserved), (B) (Reprinted with permission. ©1974. American Journal of Ophthalmology. All rights reserved), (C) (Reprinted with permission. ©2006. Springer Nature. All rights reserved).

Aqueous also exits the eye via the unconventional pathway, which includes the uveoscleral, uveovortex and uveolymphatic routes (Fig. 2).[29,50] The term "uveoscleral outflow" is often used in reference to all of these pathways but this is misleading. The preferred and more appropriate name is "non-trabecular outflow" and this term shall be used herein. It should be noted that a small amount of fluid also exits the eye via the corneal, iridial and retinal routes, but this flow is considered insignificant under physiological conditions.[11,75] Non-trabecular outflow begins with seepage of aqueous through the anterior face of the ciliary muscle to reach the supraciliary and suprachoroidal spaces. From here, fluid can drain via three possible routes: (1) through connective tissue of the sclera (uveoscleral flow); (2) into choroidal vessels and then vortex veins (uveovortex flow);[10,50] (3) into lymphatic vessels within the ciliary body (the uveolymphatic pathway) (Fig. 2). However, the existence of ciliary body lymphatic vessels is controversial.[50]

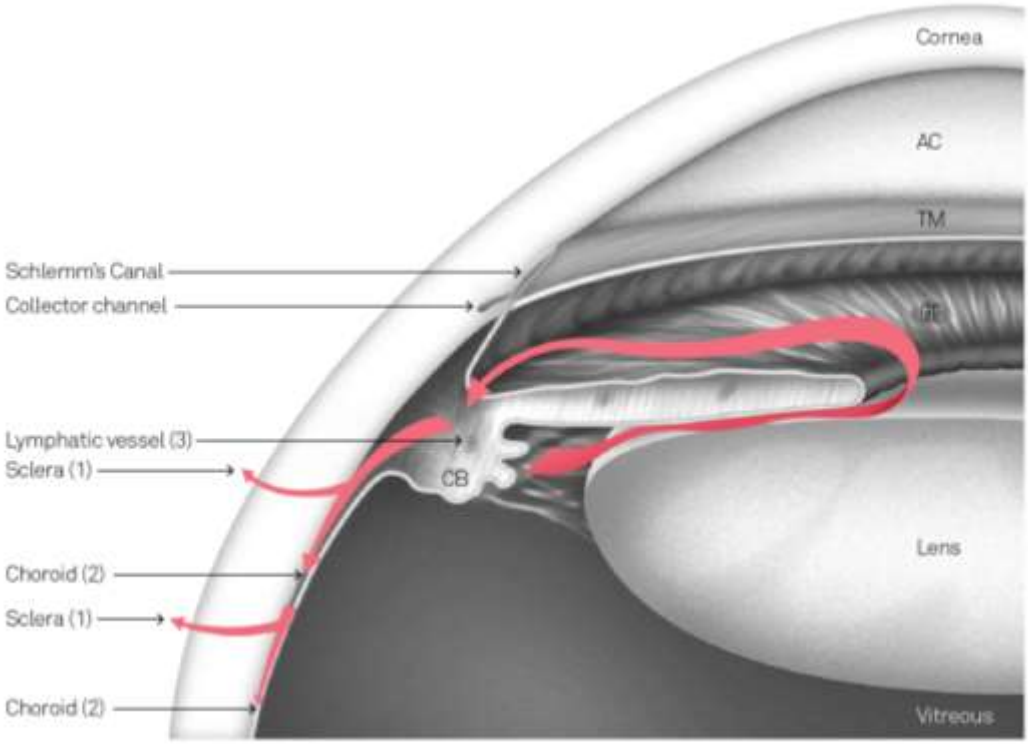


Fig. 2. Non-trabecular outflow pathway. Red arrow indicates direction of aqueous outflow. Aqueous can be seen to seep through the anterior face of the ciliary muscle to reach the supraciliary and suprachoroidal spaces. It then drains via three possible routes: (1) connective tissue of the sclera (uveoscleral flow); (2) choroid exiting via the vortex veins (uveovortex flow); (3) the lymphatic vessels within the ciliary body (the uveolymphatic pathway).

The flow-limiting step of the non-trabecular pathway is the resistance imparted by the muscle bundles and connective tissue of the ciliary body. This resistance is increased by drugs that increase ciliary muscle tone such as pilocarpine,[16] and is decreased by drugs that relax ciliary muscle tone, such as atropine and prostaglandin analogues. The latter have a slower, more significant hypotensive effect by reducing the amount of extracellular matrix between ciliary muscle bundles.[50] Non-trabecular outflow decreases by approximately 3.5% per decade,[87] which is attributable to an age-related increase in ciliary muscle connective tissue and a decrease in hydraulic conductivity of the sclera.[3,29,85]

The percentage of aqueous outflow draining via the non-trabecular pathway in humans is uncertain due to inherent difficulties in measuring this flow. Estimates vary

widely from less than 10% to more than 70% of total aqueous outflow, with measurement technique errors thought to account for most of the discrepancy.[50]
It is commonly quoted that non-trabecular outflow comprises approximately 50% of outflow in young healthy individuals but this flow declines with age and in glaucoma.[29,50,87,88]

Physiology of non-trabecular outflow

Evidence suggests that non-trabecular flow is driven by both hydrostatic and osmotic forces operating synergistically. Aqueous moves from the anterior chamber to the supraciliary space and then to the suprachoroidal space by travelling down a small hydrostatic pressure gradient. Emi and colleagues measured this gradient in a pivotal study of anaesthetized monkeys.[24] At an IOP of 15mmHg, pressure was 1mmHg lower in the supraciliary space and 4mmHg lower in the suprachoroidal space. Brisk drainage of fluid from the suprachoroidal space is thought to account for the lower pressures in this compartment. It has also been suggested that a "compact zone" of more densely packed collagenous tissue at the level of the ora serrata imparts resistance to flow between the supraciliary and suprachoroidal spaces, however the existence of this tissue is controversial.[64]

Aqueous reaching the suprachoroidal space predominately enters the choroidal vasculature by osmosis to drain via the uveovortex pathway. The uveoscleral pathway is driven by hydrostatic pressure and is likely much less important at physiologic IOPs.[24] In the monkey study by Emi and colleagues, rapidly lowering the IOP from 60mmHg to 5mmHg caused the pressure in the suprachoroidal space to become negative relative to atmospheric pressure. The authors reasoned that hydrostatically-driven uveoscleral flow could never produce negative pressures.[24] The predominate mechanism for drainage must therefore be uveovortex flow, driven by a large colloidal osmotic gradient. Further evidence for this comes from cyclodialysis studies, which have recorded profound hypotony despite relatively normal aqueous production rates on fluorphotometric testing.[83] When IOP is below episcleral venous pressure (EVP) non-trabecular flow must be the sole route of aqueous drainage, however, at low IOP there is inadequate hydrostatic pressure to drive the uveoscleral pathway.

Therefore, uveovortex outflow must underpin hypotony after cyclodialysis and must also be capable of draining large volumes.

Hydrostatic pressure in the suprachoroidal space is also important to uveovortex outflow. Positive pressure inhibits serum proteins diffusing out of the choroidal vasculature, which is freely permeable. Consequently, a large colloidal osmotic gradient can be maintained.[24,29] During hypotony, proteins exit the choroidal vessels but are too large to pass through the sclera. They therefore accumulate in the suprachoroidal space and contribute to the development of choroidal effusions.[29] It is important to point out a misunderstanding that commonly appears in physiology texts. That is, at IOPs below 7mmHg, fluid in the suprachoroidal space is unable to drain because hydrostatic pressure is lower than orbital pressure, resulting in choroidal effusions. This mechanism is incorrect because it falsely assumes that uveoscleral flow, driven by hydrostatic pressure, is the main route of non-trabecular outflow.[62]

A defining feature of non-trabecular outflow is that it is relatively unaffected by IOP.[50] In the monkey studies by Emi and colleagues,[24] increasing the IOP from 15mmHg to 60mmHg only increased the pressure difference between the AC and the suprachoroidal space from 4mmHg to 10mmHg. Thus, huge IOP spikes cause only a small increase in the pressure gradient driving non-trabecular flow, which remains fairly constant between an IOP of 4mmHg to 35mmHg.[9] In addition, increased IOP does not affect the colloid osmotic gradient pulling aqueous into the choroidal circulation, which is the main determinant of uveovortex flow.[50] Prior to the work of Emi and colleagues,[24] Bill[11] had proposed the "elastic sponge model" to explain how non-trabecular flow is IOP insensitive. This postulated that elevated IOP compressed the interstitial spaces between ciliary muscle bundles, resulting in increased resistance. However, it is inconsistent with several observations of outflow physiology and has now been superceded.[50] Non-trabecular flow can be rendered more IOP dependent by bypassing ciliary body resistance with cyclodialysis or a supraciliary stent. This eradicates most of the resistance to non-trabecular outflow and results in a four-fold increase in aqueous drainage, and may also lower IOP by focally interrupting ciliary body perfusion.[8,83] It has been hypothesized that in the initial stages of glaucoma there is increasing levels of trabecular resistance, with redirection of flow into the non-trabecular pathway, which is IOP insensitive.[86]

Trabecular outflow

Outflow resistance of the trabecular pathway: a brief history

In 1958 and 1963, Grant[39,40] published two studies that are considered seminal, because they demonstrated that IOP control and loss of IOP regulation in glaucoma can be localized to the outflow system. In these experiments normal enucleated eyes were cannulated to a constant-pressure outflow apparatus and the TM was progressively dissected ab interno to correlate the depth of TM dissection with the increase in outflow facility. Grant[39,40] demonstrated that a 360 degree ab interno trabeculotomy eliminated 75% of outflow resistance in normal eyes. This finding was later misinterpreted to mean that 75% of normal outflow resistance, and all of the increased resistance in open-angle glaucoma, is localised to the TM.

Grant[39,40] reported that outflow resistance was largely unaffected by incisions into the uveal and proximal corneoscleral meshwork. However, deeper incisions traversing full-thickness TM and inner wall of SC caused a profound reduction in outflow resistance. This finding lead to the erroneous conclusion that trabecular outflow resistance is localized to the juxtacanalicular portion of the TM and the inner wall of SC. This belief quickly gained traction in the literature and continues to be propagated by review articles in recent times.[12,28,34,49,72]

Over the following years, Grant and colleagues[22,23] published studies that superseded their earlier experiments and indicated alternate mechanisms for outflow resistance. These studies proposed that outflow resistance was not simply a product of TM permeability but was caused by collapse of SC and higher downstream resistance than previously thought. Their findings are particularly relevant today as they help to explain why trabecular microbypass stents do not lower IOP to EVP.[56] They also measured the change in outflow resistance of internalizing SC with ab interno trabeculotomy, and compared this to externalizing SC by removing the outer wall of SC and overlying sclera.[23] They demonstrated that 360 degree ab interno trabeculotomy eliminated only 27% of the total outflow resistance at an IOP of 10mmHg but 62% at an IOP of 20mmHg.[23] Paradoxically, removing the external wall of SC and overlying sclera (leaving the TM and inner wall of SC intact) also

eliminated 75% of the outflow resistance, leaving only 25% of the resistance to be accounted for by the TM.[23] In order to reconcile these seemingly contradictory findings Ellingsen and Grant[23] concluded that "resistance to aqueous outflow may normally depend in part upon an intact and unyielding outer wall of SC against which an intact inner wall is pressed by the IOP." Removing either the SC inner wall or the SC outer wall eliminates approximately 75% of outflow resistance by preventing apposition of these walls.

Despite Grant's valuable contribution to aqueous outflow physiology there are some important limitations to consider when interpreting his findings. An often-overlooked detail of Grant's[26] experiments is the elevated perfusion pressure used to measure outflow facility. The *ex vivo* eyes in Grant's studies were perfused at an IOP of 25mmHg. This is equivalent to an intracameral pressure of approximately 33mmHg *in vivo*, given the absence of EVP in cadaver eyes (assuming EVP is 8mmHg).[57] In light of the abnormally high perfusion pressures used, Rosenquist *et al*.[77] repeated Ellingsen and Grant's[23] earlier trabeculotomy experiments. In their study, they compared a lower IOP (7mmHg) and higher IOP (25mmHg), and reported reductions in outflow resistance up to 49% and 75%, respectively with 12 clock hour trabeculotomy (Fig. 3).[77] They concluded that at low IOPs, a relatively high portion of aqueous outflow resistance is situated downstream from SC but at higher IOPs this distal resistance is less important.[77]

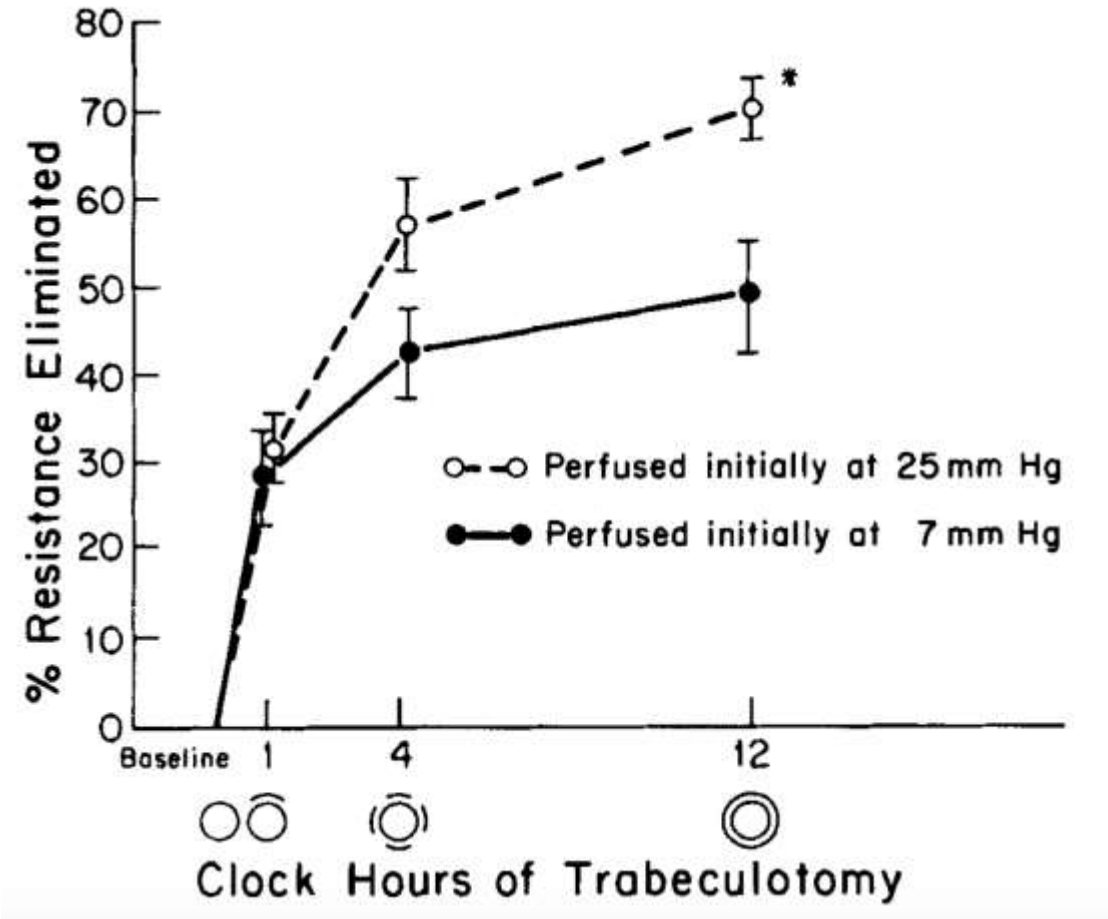


Fig. 3. Percentage of baseline resistance eliminated by sequential 1, 4 and 12 clock hour internal trabeculotomy in enucleated human eyes at 7mmHg vs. 25mmHg perfusion pressure.[77] (Reprinted with permission. ©1989. Taylor & Francis. All rights reserved.)

Further research in Grant's[22] lab demonstrated that outflow facility decreases as IOP increases, being more pronounced in glaucomatous eyes than normal eyes. Outflow facility declines gradually during prolonged periods of raised IOP, indicating that progressive changes to the outflow pathways occur.[22] To explain this finding, the authors consulted advisors in fluid mechanics. They postulated that the outflow system must not be geometrically fixed, but rather is physically altered by increasing flow and pressure.[22] They concluded that contact between the TM and SC outer wall was responsible for the increased resistance at higher IOPs.[22] The rise in outflow resistance becomes most evident at pressure levels where the canal begins to collapse.[89,90] IOP-dependent apposition of the TM against the SC outer wall has since been confirmed by numerous studies, including recent investigations using phase-

sensitive optical coherence tomography (PhS-OCT).[41-43,48,58,67,68] In the human eye, SC closure occurs even at relatively low pressures.[58]

Tensioning the TM to inhibit its excursion to the SC outer wall approximately halves trabecular outflow resistance.[40] This can be demonstrated experimentally by manipulating ciliary body tension or by depressing the lens posteriorly.[22,89,90] Lens depression eliminates nearly all of the IOP-related increase in outflow resistance up until an IOP of 40mmHg.[89,90] Tension on the ciliary body places tension on the scleral spur, which in turn pulls the TM away from the SC outer wall.[57] The increase in outflow facility is reversed by returning the lens to the neutral position or by disinserting the ciliary body from the scleral spur.[40] Conversely, outflow resistance is increased by rotating the ciliary body anteriorly (independent of the presence or absence of iris tissue).[40] At the time of performing these experiments it was hypothesized that tensioning the TM was increasing the permeability of the meshwork. While this may be true, the significance of SC collapse was only appreciated later.

Certain surgical procedures provide insight into the relative contributions that TM permeability and dynamic TM movement each have on outflow resistance. Nd-YAG laser trabeculopuncture aims to bypass proximal resistance by cutting holes through the TM into SC.[25,70] In a study conducted in monkeys, trabeculopuncture lowered IOP by up to 12mmHg.[70] However, IOP measurements returned to baseline by day eight due to healing of the puncture sites.[70] Excimer laser trabeculostomy (ELT), a procedure based upon the same outflow principles as Nd-YAG trabeculopuncture, minimizes the healing response by using photoablation without inducing thermal damage.[7] The procedure involves the creation of 10 laser ablations (trabeculostomies) distributed across 1 quadrant of the angle.[7] Similar to Nd-YAG trabeculopuncture, it theoretically allows aqueous to bypass the permeability resistance of the TM, yet the procedure would not be expected to prevent collapse of SC. Pache *et al*.[73] reported on 135 eyes with open-angle glaucoma or ocular hypertension treated with ELT as a standalone procedure. In the subgroup of individuals with baseline IOP >22mmHg, a mean IOP reduction of 31% was achieved (baseline mean IOP 27.9 ± 3.9mmHg, 1 year mean IOP 19.3 ± 5.5mmHg). Individuals with baseline IOP ≤21mmHg achieved

a mean IOP reduction of 13% (baseline mean IOP 20.2 ± 1.1mmHg, 1 year mean IOP 17.6 ± 3.3mmHg).[73] As illustrated in Fig. 3, these pressure reductions in the latter group are approximately half of what the studies of Rosenquist,[77] Grant and Ellingsen[22,23] would predict for a trabeculotomy involving 1 quadrant. The trabeculotomy, which addresses both TM permeability and SC collapse, appears to achieve greater IOP reductions than ELT, which only bypasses TM permeability resistance. This suggests that both TM permeability and SC collapse are important mechanisms in trabecular outflow resistance.

A unifying model to explain all of the observations on trabecular resistance must incorporate both SC closure (dynamic) and TM permeability resistance (passive). Three key points should be emphasized in regards to the trabecular outflow pathway. Firstly, SC closure is important and accounts for the dramatic increase in outflow resistance as IOP rises. It also explains why this increase in resistance is eliminated by tensioning the scleral spur posteriorly or by removing either the inner wall or outer wall of SC.[22,23,77] Secondly, the TM imparts a passive resistance to permeability, as demonstrated by the immediate IOP-lowering effect of Nd-YAG trabeculopuncture or ELT.[70] Further evidence is found in the dissection studies of Ellingsen and Grant.[23] In 1972 they reported that if they accidentally pierced the TM while removing the SC outer wall the outflow resistance was dramatically lower than if the TM remained intact.[23] In addition, it is self-evident that TM permeability and TM motion must be coexistent phenomena, as only tissues resisting a force undergo deformation. Thirdly, resistance downstream from SC accounts for a significant proportion of outflow resistance at low-normal IOPs.[77]

Passive model of trabecular resistance

The traditional model posits that the TM acts as a filter that exerts stable and passive resistance to outflow. The resistance is entirely dependent on its permeability, which is regulated by changes in the extracellular matrix of the JCT.[32,71] The model relies on the assumption that the JCT has a geometrically stable structure that is not significantly altered by short-term IOP fluctuations.[57] Several pieces of evidence have already been highlighted that argue against this model as being solely responsible for trabecular outflow resistance.[52,55]

Modern histologic studies indicate that the JCT has insufficient extracellular matrix to solely account for the resistance of the trabecular pathway.[31,35,57 27,81] The JCT became the favoured candidate for trabecular resistance based upon studies of enucleated eyes that were typically fixed under conditions of hypotony.[53] Such conditions made the JCT appear more compact than it is in vivo.[53] In contrast, the spaces in the uveal and corneoscleral meshwork are too large to provide meaningful levels of resistance.[13,49,84] A more general argument against the passive model is that it implies that most structural elements of the TM serve no purpose, which seems counter-evolutionary.[52] For example, in the passive model it would appear that the trabecular beams (lamellae) and surrounding cells, which comprise the majority of the TM, serve no function.[52]

Dynamic model of trabecular resistance

The dynamic model proposes that trabecular outflow involves a 'biomechanical pump' that is powered by the ocular pulse pressure, blinking, and saccadic eye movements.[22,23,58,89,90] The flexible TM distends and recoils in sync with the cardiac cycle, actively moving aqueous into SC. Unlike the passive model, the dynamic model links trabecular structure and function. All components of the TM have functional significance including the trabecular lamellae, JCT, SC inner wall endothelium and TCMs.[52] Our understanding of this model is largely derived from the work of Murray Johnstone.[52,53,61]

Ex vivo histologic[58,61] and PhS-OCT[67,68] research indicates that the TM moves outward during pulse-synchronous increases in IOP. During systole, the outward movement of the TM increases pressure in SC, compressing its lumen and forcing aqueous into collector channels and aqueous veins. During diastole, the IOP drops and the TM recoils towards the AC, which reduces the pressure in SC and allows entrance of aqueous from the AC.[54] The ocular pulse amplitude in normal eyes is approximately 3mmHg[63] and blinking and eye movements generate forces of 10mmHg,[51] providing ample energy to power this system.

When IOP is raised, the TM is stretched towards the SC outer wall.[60] This stretching moves the trabecular tissues up their length-tension curve, resulting in an increased

force of recoil with the minor fluctuations in IOP occurring during the cardiac cycle.[51,61] In normal eyes this causes the stroke volume of the trabecular pump to increase, which helps to return IOP back towards its homeostatic set point.[51,61] This can be seen as increased pulsatile flow within aqueous veins.[60] As IOP rises, stoke volume will continue to increase up until the point that SC closure occurs.[51,61] The power of the trabecular pump is dependent upon the recoil force of the TM, which is dependent upon its elasticity and the tension exerted by the scleral spur.[60] In this way, short term IOP regulation is mediated by changes in stroke volume, and long term IOP regulation is mediated by changes in the intrinsic distension and recoil characteristics of the extracellular matrix of the trabecular lamellae.[51] The trabecular lamellae and SC endothelium must maintain distension-recoil responses within a narrow range to maintain normal IOP homeostasis. In this way, the entire TM and SC complex becomes a tensionally-integrated system able to sense strain and alter its biomechanical characteristics accordingly.[51,60]

In glaucoma, the TM becomes stiffer and loses its elasticity and recoil.[92] The result is a progressive decline in trabecular movement, which in turn decreases the efficiency of the trabecular pump. As IOP climbs, the SC inner wall is pushed into appositional closure against the SC outer wall and herniations of TM into CCE are seen (Fig. 4).[36] In normal eyes, transient SC closure is a normal phenomenon that can occur at relatively low IOPs and is entirely reversible. However, in glaucomatous eyes, SC closures and TM herniations are less reversible,[36] possibly as a result of a less pliable TM and more prolonged and frequent episodes of SC closure. Aqueous cannot enter a collapsed SC, and neither can it travel circumferentially within a collapsed SC to reach collector channels. Consequently, the area of angle available for aqueous drainage decreases, resulting in higher IOPs. This creates a vicious cycle culminating in the trabecular apparatus losing regulatory control of IOP.[51]

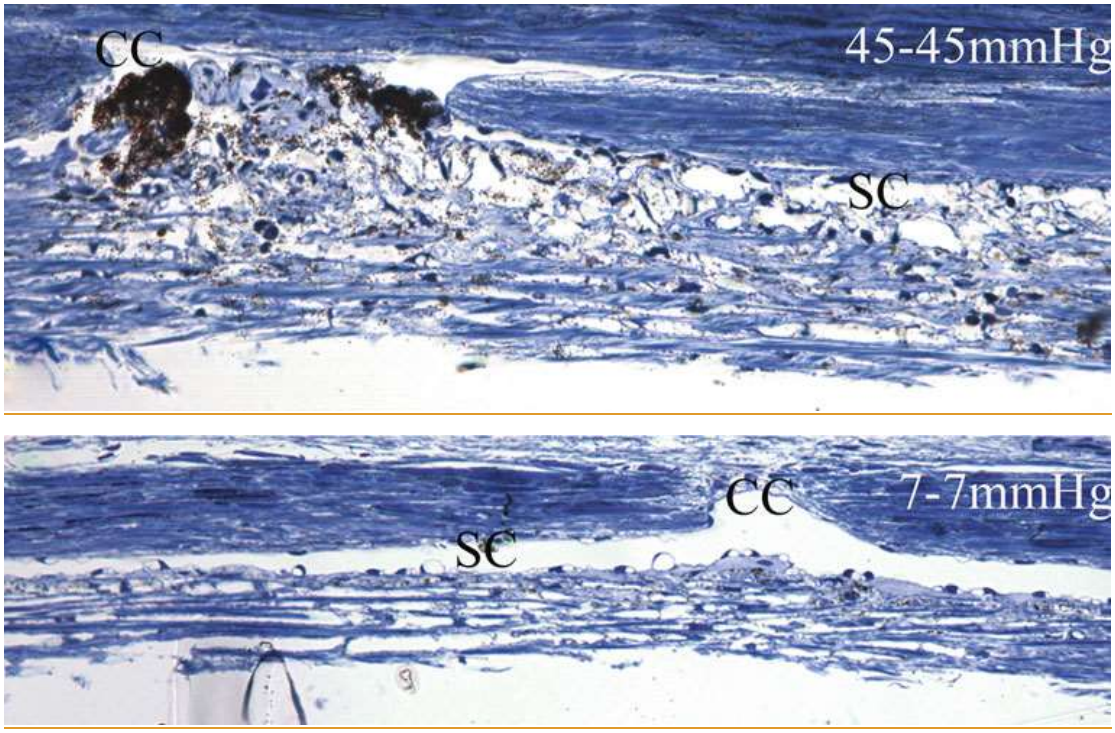


Fig. 4. Light microscopy images of CCE of normal eyes. (A) At a perfusion pressure of 45mmHg, SC is collapsed and TM is herniating into CCE. (B) At a perfusion pressure of 7mmHg, SC is open and no herniations are visible.[38] SC, Schelmm's canal; CC, collector channel. (Reprinted with permission. ©2014. Springer. All rights reserved).

Clinical manifestations of a failing trabecular biomechanical pump

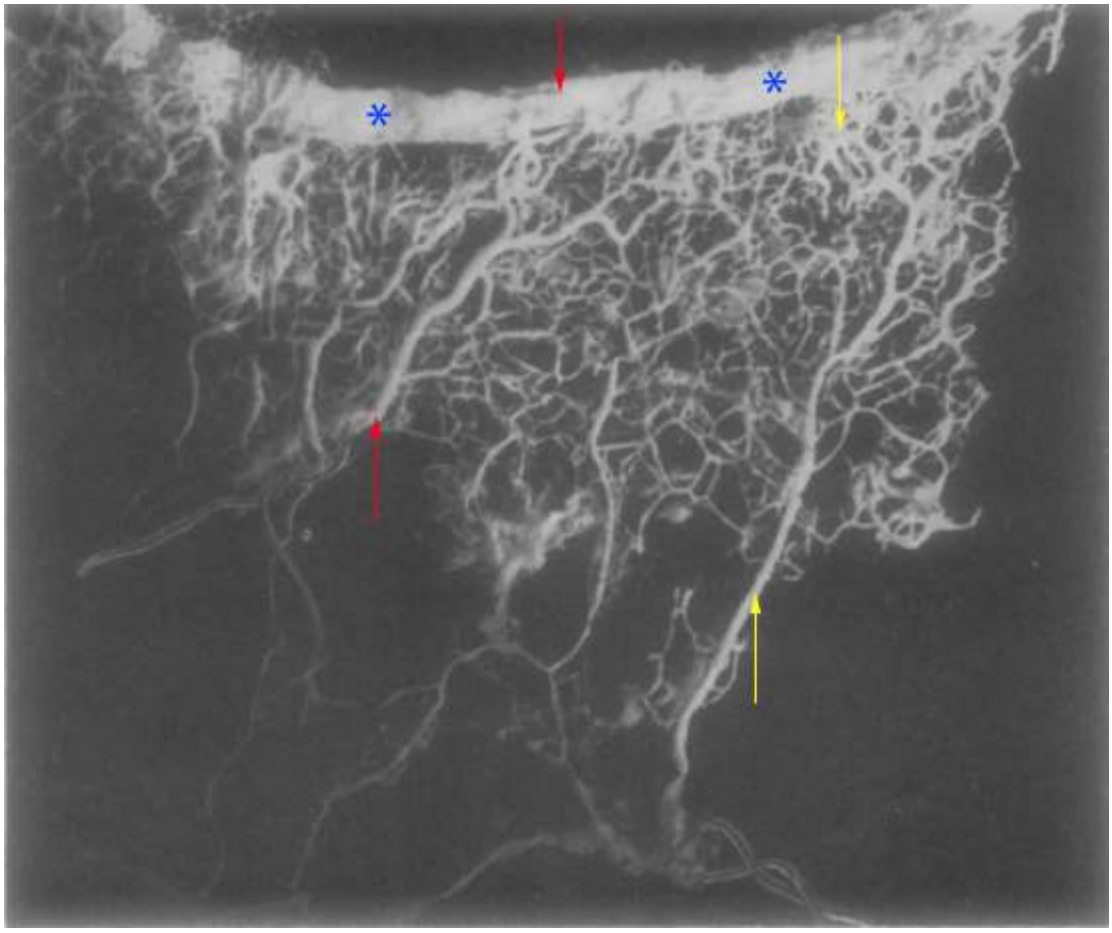
Several clinical manifestations of pump failure can be used to assess the health of the trabecular pathway and estimate the success of trabecular MIGS procedures. In a significant proportion of normal eyes there is pulse-synchronous flow within aqueous veins, which becomes more vigorous as IOP is elevated.[5,65] Johnstone[51,61] proposes that this is evidence of a healthy trabecular pump that can change its stroke volume with IOP. In contrast, pulsatile flow is sluggish or absent in glaucomatous eyes. When it is present it tends to disappear with only small increases in IOP (as measured with ophthalmodynamometry), and the aqueous veins then undergo retrograde filling with blood.[5,65 51] The explanation for this is that SC closure occurs when IOP is raised even a small amount, and this causes the pressure in aqueous veins to fall below EVP, which causes retrograde blood filling.[51] These observations suggest impaired trabecular pump function in glaucoma.

Schlemm's canal

Schlemm's canal was first described by Friedrich S. Schlemm[80] in 1830 after observing this structure in the eye of a man who had been hung. Since it was filled with blood at the time, he considered it to be a venous sinus.[80] Its role in aqueous humour drainage and outflow resistance was subsequently investigated by numerous authors.

In 1934, ocular pathologist Georgiana Dvorak-Theobald[20] undertook a detailed anatomical study of SC, believing that "anatomic variations have an important bearing upon clinical pathology." She dissected a human eye into 810 serial sections, each 15μm thick, cut horizontally, the first and last section being through the outer margins of SC. For each section she outlined the lumen of the vessels on a different sheet of paper to eventually reproduce wax models of SC with its anatomic relations to veins, arteries and nerves.[20] Using the models she was able to demonstrate that SC does not consist of a sharp inner margin which one is accustomed to seeing in textbook illustrations, but rather its border with the trabeculae is highly irregular.[20]

In 1951, Norman Ashton[6] produced even more detailed models of SC and aqueous veins using neoprene casting. This technique, which was first used by Lieb[69] in 1940 for renal vascular studies, is well suited to demonstrating the complex relationship of SC to the downstream collectors (Fig. 5). From his model, Ashton[6] concluded that aqueous veins either arise directly from SC or communicate with it indirectly via anastomotic branches between the superficial and deep scleral plexuses.



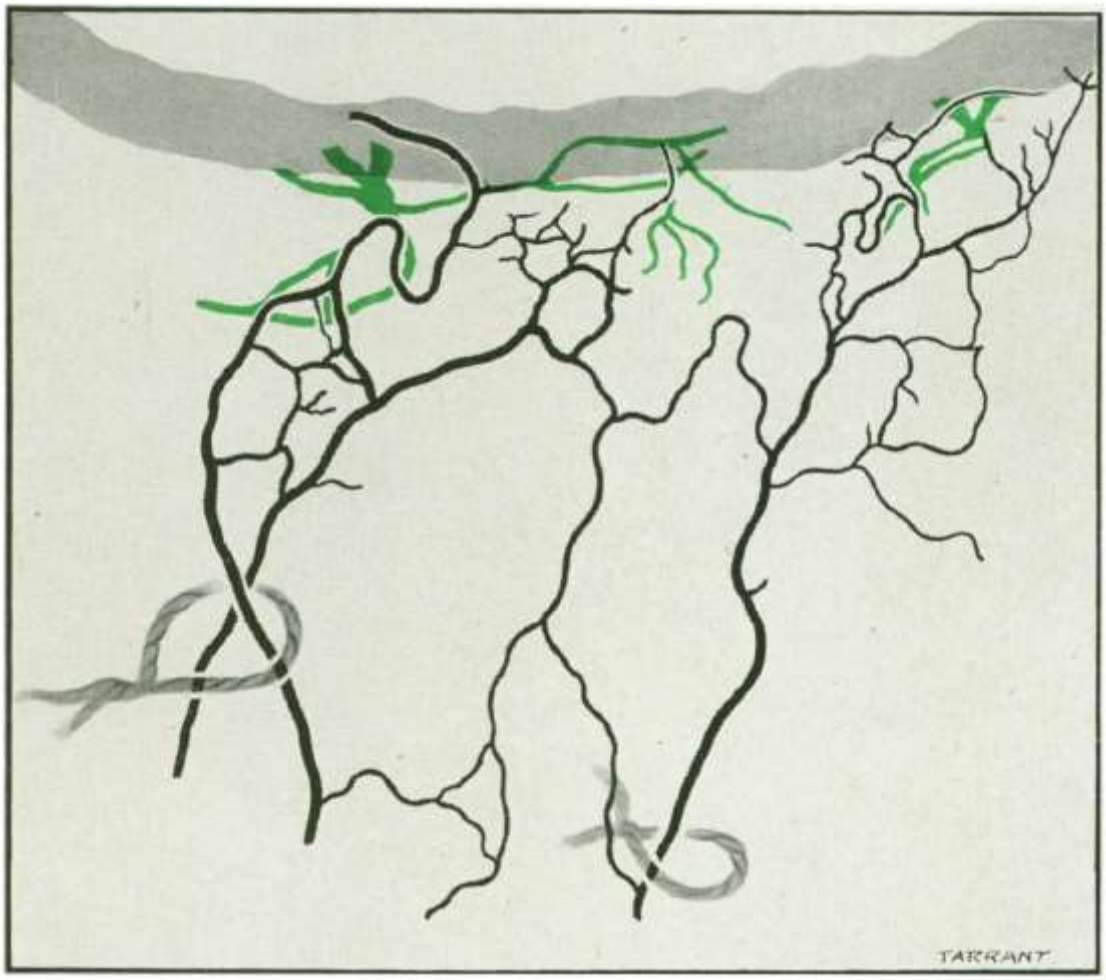


Fig. 5. (A) Neoprene cast of SC demonstrating two aqueous veins. Blue asterix denotes SC. Red arrows denote aqueous vein arising directly from SC. Yellow arrows denote aqueous vein indirectly arising from SC via anastamotic branches between the superficial and deep scleral plexuses. (B) Drawing from neoprene cast shown in Fig. 3a. Deep scleral plexus is shown in dark green. Superficial scleral plexus is depicted in light green. The aqueous vein on the left arises directly from SC by a hook-shaped origin. The aqueous vein on the right does not directly connect with the canal.[6] (Reprinted with permission. © 1951. BMJ. All rights reserved).

These findings have since been adapted and simplified into anatomy textbook descriptions, such as 'Clinical Anatomy of the Eye' by Snell and Lemp.[82] Here SC is described as "a sinus, which is oval or triangular in cross section." The illustrations depict a well-demarcated circular structure. The problem with this common description is that it neglects the functional aspect of the tissue including its ability to dynamically change shape in response to IOP fluctuations. Therefore, this

conceptualization of the anatomy can inadvertently lead to a misunderstanding of the physiology.

Collector channel entrances

CCEs or ostia refer to the entrance point of collector channels that exit from the external wall of SC. They typically turn abruptly to join a deep scleral plexus of collector vessels that are typically orientated parallel to SC.[57] Histological studies of human eyes indicate that CCEs vary greatly in size (from 5μm to 70μm) and are more numerous in the infero-nasal quadrant.[21,46,76] Their location is of particular relevance to trabecular microbypass stents because there is preferential drainage through TM adjacent to CCEs and minimal circumferential flow within SC.[39,45]

There is emerging evidence that CCEs open and close and thus play a role in regulating outflow resistance.[47,48] Scanning electron microscopy reveals hinged septate at the CCEs – a configuration that permits rapid movement in response to pressure changes (Fig. 6).[57] This was first reported by Rohen and Rentsch[76] in 1968, who identified collagen flaps at CCEs as an important source of resistance, claiming that the flaps are held open by attachments to the TM. The more recent mechanism proposed is that highly elastic TCMs connect the TM to the hinged collaged flaps, thereby allowing CCEs to open and close in synchrony with pressure-dependent movement of the TM.[57] This is supported by recent studies using high-resolution OCT.[48] Cells containing smooth muscle myosin have been identified near CCEs but a contractile mechanism for outflow regulation has not been confirmed.[17]

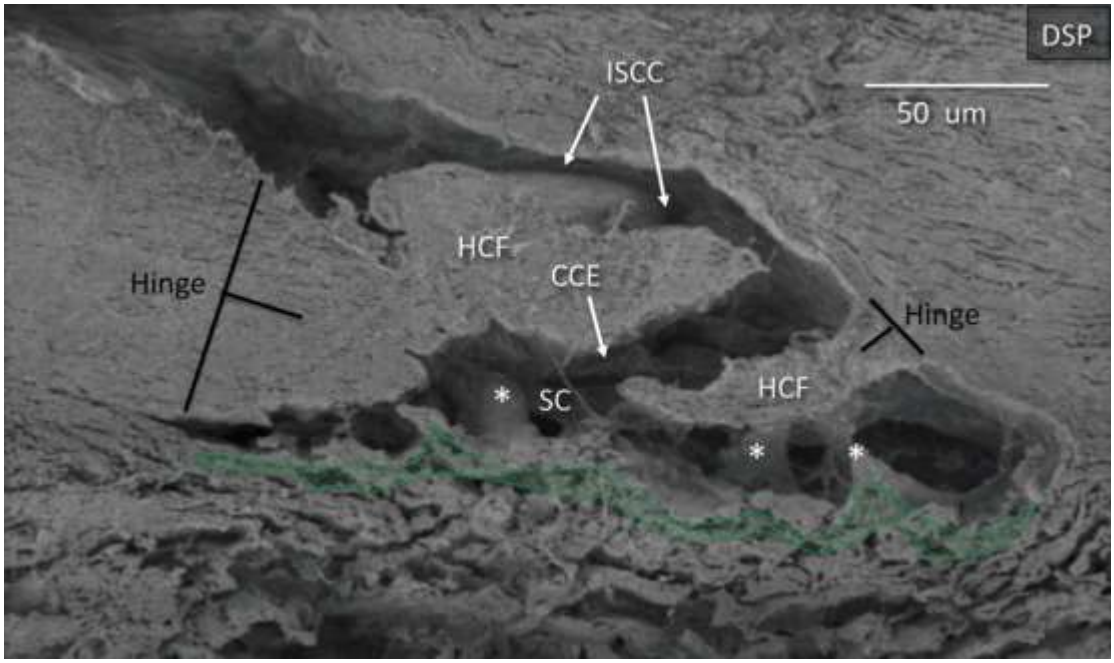


Fig. 6. Collector channel entrance (CCE) and hinged collagen flaps (HCF) in deep scleral plexus. SC can be seen opening into a CCE. Two hinged collagen flaps can be seen surrounding the convoluted pathway into the intrasclaeral collector channels (ISCC). Black T denotes hinge locations. Green outline denotes JCT space. White asterisk denotes TCMs, which connect SC inner wall to the hinged collagen flaps. If the TM moves, the hinged collagen flaps must also move because of their attachment to the TCMs.[57] (Reprinted with permission. ©2016. Kugler Publications. All rights reserved.)

The aqueous veins

Karl Ascher first described aqueous veins in the January issue of the *American Academy of Ophthalmology*, 1942.[4] Ascher's[5] work is considered ground breaking because it demonstrated that aqueous is not stagnant but instead flows. Although many ophthalmologists today would regard this concept as self-evident, this was not appreciated prior to Ascher. His work prompted research interest into aqueous physiology, outflow routes, and the mechanisms regulating IOP.

In 1961, Ascher[5] published *The Aqueous Veins*, an extensive analysis of the anatomy and physiology of these structures. Aqueous veins are epithelial-lined vessels in the conjunctival and subconjunctival tissue that return aqueous humour to the systemic circulation.[5] They arise either directly from SC by a hook-shaped origin or indirectly

via the deep scleral plexus.[6] They are visible on the surface of the eye and are distinguished from blood vessels by their pale colour and transparency.[5] Usually 2-3 aqueous veins are visible but up to 6 may be seen in some eyes.[18] Their distribution around the limbus is asymmetric, with the majority of aqueous veins located in the infero-nasal quadrant, followed by the infero-temporal quadrant.[18] Few are seen superiorly.[18] Ascher[5] noted pulsatile flow within the veins, but was unable to explain the significance of this and the observation unfortunately went largely ignored for over 50 years. Today, trabecular MIGS has revitalised interest in aqueous veins as these vessels permit direct visualisation of how canal-based procedures enhance aqueous outflow. It should also be noted that surgery on the conjunctiva, episclera and sclera can damage these vessels, as well as other downstream collectors, and thereby increase the resistance of the distal system.

Micro-invasive glaucoma surgery (MIGS): theory into practice

MIGS and 'MIGS-like' procedures aim to lower IOP by targeting three different outflow pathways: trabecular drainage, suprachoroidal drainage, and subconjunctival drainage.[15] The scientific rationale of MIGS procedures is founded upon the outflow resistance studies that have been discussed in this paper. For instance, the rationale for many of the trabecular devices is based on the supposition that the majority of trabecular outflow resistance is located in the TM and inner wall of SC. Only if this traditional model is accepted can many of the devices be considered to have a firm evidence base for their design. The scientific rationale for and theoretical limitations of trabecular and suprachoroidal MIGS procedures are summarized in Table 1.

Trabecular MIGS devices

It is self-evident that trabecular MIGS procedures require a healthy downstream collector system and normal EVP to work well. However, it is suspected that various stressors may cause the downstream collectors to become dysfunctional, or even undergo atrophy. Such stressors include advanced age, surgery (i.e. cautery of the sclera), and long-standing glaucoma causing SC closure, collector system stasis and collapse. It is biologically plausible that there is an optimal window of time for trabecular MIGS in the natural history of open-angle glaucoma. Even individuals with

advanced glaucoma may once have been good candidates for trabecular MIGS while their downstream collector system was still healthy.

Before proceeding with trabecular MIGS it is important to assess the health of the downstream collector system. Certain clinical signs can help in deciding whether the collector system is functional (or at least amenable to rejuvenation), or whether the system should be abandoned in favour of the suprachoroidal or subconjunctival route. On history, a functioning trabecular pathway is suggested by a favourable response to selective laser trabeculoplasty (SLT) or pilocarpine. On examination, one can observe for pulsatile flow within aqueous veins or blood reflux into SC on gonioscopy. Blood reflux can be triggered by applying pressure to the episcleral vessels with a flanged gonioscope, by "pumping" a gonioscope on the cornea to generate a suction effect on the eye, or by lowering IOP below EVP. Robert Stegmann's[37] team correlated blood reflux into SC with fluorescein egress into episcleral veins in individuals with POAG undergoing canaloplasty. At the start of the operation, the IOP was lowered with a paracentesis and gonioscopy was performed to grade blood reflux into SC. Fluorescein chanellography and canaloplasty was then performed using a microcatheter. Individuals with good circumferential blood reflux into SC had excellent fluorescein egress into episcleral veins, whereas those with patchy or no blood reflux into SC had much poorer fluorescein egress.[37] The results suggest that eyes that do not have blood reflux into SC do not have a patent distal collector system, at least in that region of the angle. When SC blood reflux is observed, the speed at which SC fills with blood, and then empties of blood during dynamic manoeuvres, is thought to reflect the health of the downstream collectors.[78,79]

Trabecular meshwork pigment can be used to locate CCEs and areas of functioning angle. Drainage through the TM occurs preferentially near high-flow collector channels, indicating that aqueous flow across the TM is sensitive to downstream pressure.[45] This has been demonstrated experimentally using enucleated healthy human eyes perfused with fluorescent beads. Beads are found to accumulate in pigmented TM near CCEs,[45] and by the same process, pigment floating in the aqueous tends to deposit in TM adjacent to functioning CCEs.

If a trabecular microbypass procedure is performed to a focal area of angle selected at random then Rosenquist's[77] study of 1989 would predict only a modest IOP reduction. In enucleated human eyes perfused at an IOP of 25mmHg, a one-clock hour trabeculotomy produced 41% of the effect of a twelve-hour trabeculotomy (Fig. 3). Given that the effective filtration area comprises only a fraction of the total angle, and that there is minimal circumferential flow within SC, placing a microbypass stent in meshwork remote from a functioning CCE may have minimal effect. Greater IOP reductions can be expected if microbypass is targeted to sites of high flow collector channels. Fortunately, these are most numerous in the inferonasal and superonasal quadrants, which is surgically convenient. In keeping with this, in 1971, Ellingsen and Grant[22] reported that trabeculotomies made in the nasal hemisphere increased outflow more than trabeculotomies made in the temporal hemisphere. Functioning collectors can be further targeted by looking for segments of angle with increased TM pigment or increased (and brisk) blood reflux into SC.

As discussed earlier, Rosenquist[77] also found that circumferential trabeculotomy reduced outflow resistance by up to 49% at low IOPs (7mmHg) but up to 75% at higher IOPs (25mmHg). This suggests that the resistance of the downstream collectors is relatively stable and unchanged by IOP, whereas the resistance of the TM varies with IOP. At higher pressures, TM resistance increases (due to SC collapse) and accounts for a higher proportion of the total resistance. The clinical significance of this is that individuals with high IOP may get significant benefit from procedures that address both TM resistance and SC collapse (such as Hydrus or goniotomy). In contrast, individuals with low IOPs would be expected to have less response to this intervention and may need a treatment that addresses downstream resistance (such as viscocanaloplasty) to achieve IOP reduction.

Given the mounting evidence suggesting that SC closure and TM herniations into CCEs play an important role in glaucoma, the ideal trabecular MIGS procedure would address trabecular permeability, SC occlusion, TM herniations into CCEs, and downstream resistance. In theory, microbypass devices may reduce the pressure gradient across the TM and therefore reduce TM excursion, however they don't directly prevent SC closure. In contrast, an intracanalicular scaffold would be expected to prevent SC closure in the quadrant of the device.

Suprachoroidal MIGS devices

Suprachoroidal MIGS devices bypass the ciliary body resistance to form a direct communication between the anterior chamber and the suprachoroidal space. The huge absorptive capacity of the choroidal vasculature makes hypotony possible with this route. However, hypotony can potentially be limited by the device itself. Long-term, the main factor limiting IOP control is encapsulation of the device by scar tissue.[1] The healing response is less pronounced with ab interno devices that spare the need for conjunctival and scleral dissection.[29] In the future it may be possible to modulate this healing response with the use of antifibrotic agents, drug-eluting stents, and expansion of the suprachoroidal reservoir with ophthalmic viscosurgical devices.[91]

Anatomic target	Scientific rationale	Theoretical limitations
Trabecular devices		
General comments	Increases trabecular outflow by one or more of: 1. Increasing TM permeability 2. Preventing SC collapse 3. Reversing TM herniations into CCEs 4. Viscodilating downstream collectors Outcome is dependent upon the extent of angle treated and the health of the downstream collector system. Increased TM pigment and brisk blood reflux into SC suggest a functioning downstream system. IOPs in the very low teens can be achieved with healthy collectors,[44] whereas higher IOPs are expected with collector dysfunction.	Cannot lower IOP below EVP (but therefore low risk of hypotony). May damage endothelium of SC and CCEs, leading to scarring/stenosis. Hyphaemas (including late hyphaemas) if IOP falls below EVP, such as may occur if filtration surgery is performed later.[30] It is unknown how these procedures affect hinged collaged flaps at CCEs. Hinged collagen flaps may also have independent contractile mechanisms that would likely be unaffected.[17]
Procedures localized to segments of angle (e.g. iStent trabecular microbypass, Hydrus	Provides a portion of the increase in outflow achieved with a circumferential trabeculotomy (see Fig 3).	Aqueous outflow is segmental. Devices that treat only small segments of angle are likely to

intracanalicular scaffold, ELT, Kahook Dual Blade, Trabectome)	Magnitude of IOP reduction maximised by targeting functioning CCEs and aqueous veins. These are most numerous in the infero-nasal quadrant.[5,21,46,76] Supported by experimental evidence that trabeculotomies made in nasal hemisphere increase outflow more than trabeculotomies made in temporal hemisphere.[22]	be less effective, unless placed near large CCEs or aqueous veins arising directly from SC. Do not address dynamic influences on outflow resistance, such as appositional closure of SC and TM herniations into CCEs (partially addressed by Hydrus, trabectome, and Kahook Dual Blade).
	iStent Focally bypasses TM permeability resistance. Implanting multiple devices increases the success,[19] given increased likelihood of positioning at least one of the devices near a functioning CCE.[45] May possibly preserve the trabecular pumping mechanism.	
	Hydrus intracanalicular scaffold Prevents SC collapse and stretches the TM over the windows, which may increase TM permeability.	
	ELT Makes full thickness ostia in TM and inner wall of SC to bypasss TM permeability resistance.	

	Kahook Dual Blade or Trabectome Physically removes the TM and inner wall of SC to overcome permeability resistance and prevent SC collapse and TM herniations into CCEs.	
Procedures addressing entire angle (e.g. GATT)	The perfusion studies of Grant and colleagues would predict that 360 degree trabeculotomy should remove approximately 50% of outflow resistance for eyes with an IOP in the low teens, and approximately 75% of outflow resistance for eyes with an IOP of approximately 25mmHg – 33mmHg.[77] By disinserting TM, GATT should remove TM herniations into CCEs and prevent SC collapse.	Complete disruption of the trabecular pumping mechanism.
Ab interno canaloplasty	Viscodilation attempts to restore patency of SC and the downstream collector channels, and unplug herniations of TM into CCEs. Causes "microperforations" in TM and SC inner wall, which may increase TM permeability.	In advanced glaucoma, downstream collector system may be atrophic and not amenable to rejuvenation with viscodilation.[37] The duration of action of ab interno viscodilation is unknown and would be

		expected to be transient, especially without placement of a tensioning suture in the canal. However, there is some evidence that the therapeutic response is sustained at least in the medium term.[33,66] Does not prevent SC collapse.
Suprachoroidal devices		
Ab interno suprachoroidal devices (e.g. CyPass, iStent Supra)	Provides direct communication between AC and the suprachoroidal space. Bypasses the ciliary muscle, which is thought to be the main site of resistance in non-trabecular flow.[29,50] Increases the IOP sensitivity of the non-trabecular pathway. Does not affect structural integrity and dynamic motion of TM, therefore not expected to disrupt trabecular pump.[52]	Does not address trabecular outflow resistance. Unique risk profile, including risk of hypotony.

Table 1. Scientific rationale for and theoretical limitations of trabecular and suprachoroidal MIGS, based on aqueous drainage physiology**.**

Key: AC, anterior chamber; CCEs, collector channel entrances; EVP, episcleral venous pressure; ELT, excimer laser trabeculostomy; GATT, gonioscopy-assisted transluminal trabeculotomy; IOP, intraocular pressure; JCT, juxtacanalicular tissue; TM, trabecular meshwork; SC, Schlemm's canal

Summary

It is appropriate to move towards an integrated model whereby the TM, SC, collector channels, and distal outflow pathways function as a sophisticated organ system that works in unison to control trabecular outflow.[13] Therefore, abnormalities in both the proximal and distal portions of the system are important in understanding loss of IOP homeostasis in glaucoma.[13]

The perfect glaucoma procedure would rejuvenate the outflow systems of the eye to their premorbid state and direct aqueous down physiologic routes. This would require restoring normal flexibility and permeability of the TM and SC, rejuvenating the downstream collector system, and reducing ciliary muscle resistance. No current single procedure achieves all of these goals, but improvements in technology may allow us to bridge this gap. It is conceivable that a targeted procedure, likely incorporating sustained drug delivery, could consistently obtain a very low IOP with minimal complications. In addition, the development of imaging modalities to assess the health of the distal collector system pre-operatively would help with prognostication and patient selection for trabecular procedures.

For individuals with early glaucoma and a functional downstream collector system, goniotomy or multiple targeted trabecular bypass devices (that ideally prevent SC collapse) may be an appropriate strategy unless a very low IOP target is required. Goniotomy needs to be circumferential or near-circumferential to maximise trabecular outflow.[77] In more advanced glaucoma with dysfunctional collectors, or in individuals requiring lower IOPs, trabecular bypass could be combined with viscocanaloplasty to reduce the resistance of the downstream collectors. However, this approach may be ineffective in very late cases where the distal system is atrophic. Alternatively, one could choose a suprachoroidal device, after considering its unique risk profile. A key advantage of canal-based procedures is that there is theoretically no risk of hypotony.

A clear understanding of outflow function and dysfunction has become more important in this era of MIGS procedures with very specific mechanisms of action. We hope that by meticulously reviewing outflow anatomy and physiology, and bringing to light historical findings that may have been forgotten or overlooked, we

may help to optimize the surgical treatment of glaucoma and ultimately reduce glaucoma blindness worldwide.

Methods of literature search

In preparing this review, we conducted PubMed, EMBASE, and Google Scholar searches using the following key words in various combinations: aqueous, outflow resistance, micro-invasive glaucoma surgery, glaucoma, aqueous veins, Schlemm's canal, and collector channel entrances. In addition, reference lists from the selected articles were used to identify additional articles not included in the electronic databases. This included hard copy and electronic textbooks, which were accessed from various libraries. From the searches, all articles pertaining to the relevant topic were included in this review. No constraints were placed on publication date or publication language.

Disclosure

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

References

1. Agnifili L, Costagliola C, Figus M, Iezzi G, Piattelli A, Carpineto P, et al. Histological findings of failed gold micro shunts in primary open-angle glaucoma. *Graefes Arch Clin Exp Ophthalmol*. 2012; 250(1): 143-9
2. Allingham RR, Damji KF, Freedman SF, Moroi SE, Rhee DJ, Shields MB. *Shields Textbook of Glaucoma*: Wolters Kluwer Health, 2010.
3. Alm A, Nilsson SF. Uveoscleral outflow--a review. *Exp Eye Res*. 2009; 88(4): 760-8
4. Ascher KW. Aqueous veins. *Am J Ophthalmol*. 1942; 25: 31-8
5. Ascher KW. *The Aqueous Veins*. Illinois: Charles C Thomas, 1961.
6. Ashton N. Anatomical study of Schlemm's canal and aqueous veins by means of neoprene casts. Part I. Aqueous veins. *Br J Ophthalmol*. 1951; 35(5): 291-303
7. Berlin MS, Funk J, Pache M, Wilmsmeyer S, Giers U, Kleineberg L, et al. Excimer Laser Trabeculostomy 2004 [cited 2018 29 April]:[12-7 pp.]. Available from: http://glaucomatoday.com/pdfs/0504_07.pdf
8. Bill A. The routes for bulk drainage of aqueous humour in rabbits with and without cyclodialysis. *Doc Ophthalmol*. 1966; 20: 157-69
9. Bill A. Conventional and uveo-scleral drainage of aqueous humour in the cynomolgus monkey (Macaca irus) at normal and high intraocular pressures. *Exp Eye Res*. 1966; 5(1): 45-54
10. Bill A, Phillips CI. Uveoscleral drainage of aqueous humour in human eyes. *Exp Eye Res*. 1971; 12(3): 275-81
11. Bill A. Basic physiology of the drainage of aqueous humor. *Exp Eye Res*. 1977; 25(Supplement 1): 291-304
12. Braunger BM, Fuchshofer R, Tamm ER. The aqueous humor outflow pathways in glaucoma: A unifying concept of disease mechanisms and causative treatment. *Eur J Pharm Biopharm*. 2015; 95(Pt B): 173-81
13. Carreon T, van der Merwe E, Fellman RL, Johnstone M, Bhattacharya SK. Aqueous outflow - A continuum from trabecular meshwork to episcleral veins. *Prog Retin Eye Res*. 2017; 57: 108-33
14. Casson RJ, Chidlow G, Wood JP, Crowston JG, Goldberg I. Definition of glaucoma: clinical and experimental concepts. *Clin Exp Ophthalmol*. 2012; 40(4): 341-9
15. Conlon R, Saheb H, Ahmed, II. Glaucoma treatment trends: a review. *Can J Ophthalmol*. 2017; 52(1): 114-24
16. Crawford K, Kaufman PL. Pilocarpine antagonizes prostaglandin F2 alpha-induced ocular hypotension in monkeys. Evidence for enhancement of Uveoscleral outflow by prostaglandin F2 alpha. *Arch Ophthalmol*. 1987; 105(8): 1112-6
17. de Kater AW, Spurr-Michaud SJ, Gipson IK. Localization of smooth muscle myosin-containing cells in the aqueous outflow pathway. *Invest Ophthalmol Vis Sci*. 1990; 31(2): 347-53
18. de Vries S. *De zichtbare afvoer van het kamerwater*. 1st ed. Amsterdam: Drukkerij Kinsbergen, 1947.
19. Donnenfeld ED, Solomon KD, Voskanyan L, Chang DF, Samuelson TW, Ahmed, II, et al. A prospective 3-year follow-up trial of implantation of two trabecular microbypass stents in open-angle glaucoma. *Clin Ophthalmol*. 2015; 9: 2057-65
20. Dvorak-Theobald G. Schlemm's Canal: Its Anastomoses and Anatomic Relations. *Trans Am Ophthalmol Soc*. 1934; 32: 574-95
21. Dvorak-Theobald G. Further Studies on the Canal of Schlemm: Its Anastomoses and Anatomic Relations Georgiana. *Am J Ophthalmol*. 1955; 39(4, Part 2): 65-89
22. Ellingsen BA, Grant WM. The Relationship of Pressure and Aqueous Outflow in Enucleated Human Eyes. *Invest Ophthalmol*. 1971; 10(6): 430-7

23. Ellingsen BA, Grant WM. Trabeculotomy and sinusotomy in enucleated human eyes. *Invest Ophthalmol*. 1972; 11(1): 21-8
24. Emi K, Pederson JE, Toris CB. Hydrostatic pressure of the suprachoroidal space. *Invest Ophthalmol*. 1989; 30(2): 233-8
25. Epstein DL, Melamed S, Puliafto CA, Steinert RF. Neodymium:YAG Laser Trabeculopuncture in Open-angle Glaucoma. *Ophthalmol*. 1985; 92(7): 931-7
26. Epstein DL. Schlemm Canal Surgery 2013 [cited 2018 20 June]:[46-9 pp.]. Available from: http://glaucomatoday.com/pdfs/gt09113_F_epstein.pdf
27. Ethier CR, Kamm RD, Palaszewski BA, Johnson MC, Richardson TM. Calculations of flow resistance in the juxtacanalicular meshwork. *Invest Ophthalmol*. 1986; 27(12): 1741-50
28. Ethier CR. The inner wall of Schlemm's canal. *Exp Eye Res*. 2002; 74(2): 161-72
29. Figus M, Posarelli C, Passani A, Albert TG, Oddone F, Sframeli AT, et al. The supraciliary space as a suitable pathway for glaucoma surgery: Ho-hum or home run? *Surv Ophthalmol*. 2017; 62(6): 828-37
30. Francis BA, Singh K, Lin SC, Hodapp E, Jampel HD, Samples JR, et al. Novel glaucoma procedures: a report by the American Academy of Ophthalmology. *Ophthalmol*. 2011; 118(7): 1466-80
31. Freddo TF, Gong H. Anatomy of the Ciliary Body and Outflow Pathways. In: Tasman W, Jaeger EA, editors. *Duane's Ophthalmology*. 13th ed. Philadelphia: Lippincott Williams & Wilkins, 2013.
32. Gabelt BAT, Kaufman PL. Aqueous Humor Hydrodynamics. In: Kaufman PL, Alm A, editors. *Adler's Physiology of the Eye*. 10th ed. St. Louis: Mosby, 2003. p. 237-89.
33. Gallardo MJ, Supnet RA, Ahmed IIK. Circumferential viscodilation of Schlemm's canal for open-angle glaucoma: ab-interno vs ab-externo canaloplasty with tensioning suture. *Clin Ophthalmol*. 2018; 12: 2493-8
34. Goel M, Picciani RG, Lee RK, Bhattacharya SK. Aqueous humor dynamics: a review. *Open Ophthalmol J*. 2010; 4: 52-9
35. Gong H, Ruberti J, Overby D, Johnson M, Freddo TF. A new view of the human trabecular meshwork using quick-freeze, deep-etch electron microscopy. *Exp Eye Res*. 2002; 75(3): 347-58
36. Gong H, Freddo TF, Zhang Y. New Morphological Findings in Primary Open-Angle Glaucoma. *Invest Ophthalmol*. 2007; 48(13): 2079-
37. Gong H, Huang R, Zhu J, Stegmann R. Blockages of collector channel ostia exist in patients with Primary Open Angle Glaucoma (POAG). American Glaucoma Society 19th annual meeting; 5-8 March; San Diego, CA2009
38. Gong H, Francis A. Schlemm's Canal and Collector Channels as Theraputic Targets In: Samples J, Ahmed, IIK, editor. *Surgical Innovations in Glaucoma*. New York, NY: Springer, 2014. p. 3-25.
39. Grant W. Further studies on facility of flow through the trabecular meshwork. *AMA Arch Ophthalmol*. 1958; 60(4): 523-33
40. Grant WM. Experimental aqueous perfusion in enucleated human eyes. *Arch Ophthalmol*. 1963; 69: 783-801
41. Grierson I, Lee WR. Changes in the monkey outflow apparatus at graded levels of intraocular pressure: a qualitative analysis by light microscopy and scanning electron microscopy. *Exp Eye Res*. 1974; 19(1): 21-33
42. Grierson I, Lee WR. The fine structure of the trabecular meshwork at graded levels of intraocular pressure. (1) Pressure effects within the near-physiological range (8-30 mmHg). *Exp Eye Res*. 1975; 20(6): 505-21
43. Grierson I, Lee WR. The fine structure of the trabecular meshwork at graded levels of intraocular pressure. (2) Pressures outside the physiological range (0 and 50 mmHg). *Exp Eye Res*. 1975; 20(6): 523-30

44. Grover DS, Smith O, Fellman RL, Godfrey DG, Gupta A, Montes de Oca I, et al. Gonioscopy-assisted Transluminal Trabeculotomy: An Ab Interno Circumferential Trabeculotomy: 24 Months Follow-up. *J Glaucoma*. 2018; 27(5): 393-401
45. Hann CR, Fautsch MP. Preferential Fluid Flow in the Human Trabecular Meshwork Near Collector Channels. *Invest Ophthalmol*. 2009; 50(4): 1692-7
46. Hann CR, Bentley MD, Vercnocke A, Ritman EL, Fautsch MP. Imaging the Human Aqueous Humor Outflow Pathway in Human Eyes by Three Dimensional Micro-Computed Tomography (3D micro-CT). *Exp Eye Res*. 2011; 92(2): 104-11
47. Hann CR, Vercnocke AJ, Bentley MD, Jorgensen SM, Fautsch MP. Anatomic changes in Schlemm's canal and collector channels in normal and primary open-angle glaucoma eyes using low and high perfusion pressures. *Invest Ophthalmol Vis Sci*. 2014; 55(9): 5834-41
48. Hariri S, Johnstone M, Jiang Y, Padilla S, Zhou Z, Reif R, et al. Platform to investigate aqueous outflow system structure and pressure-dependent motion using high-resolution spectral domain optical coherence tomography. *J Biomed Opt*. 2014; 19(10): 106013
49. Johnson M. 'What controls aqueous humour outflow resistance?'. *Exp Eye Res*. 2006; 82(4): 545-57
50. Johnson M, McLaren JW, Overby DR. Unconventional aqueous humor outflow: A review. *Exp Eye Res*. 2017; 158: 94-111
51. Johnstone M. A New Model Describes an Aqueous Outflow Pump and Explores Causes of Pump Failure in Glaucoma. In: Grehn F. SR, editor. *Glaucoma Essentials in Ophthalmology*. 1 ed. Berlin, Heirdelberg: Springer, 2006. p. 3-34.
52. Johnstone M. Aqueous outflow: the case for a new model. *Rev Ophthalmol*. 2007; (14): 79-84
53. Johnstone M, Jamil A, Martin E. Aqueous Veins and Open Angle Glaucoma. In: Schacknow PN, Samples JR, editors. *The Glaucoma Book: A Practical, Evidence-Based Approach to Patient Care*. New York, NY: Springer New York, 2010. p. 65-78.
54. Johnstone M, Martin E, Jamil A. Pulsatile flow into the aqueous veins: manifestations in normal and glaucomatous eyes. *Exp Eye Res*. 2011; 92(5): 318-27
55. Johnstone M. New Concepts in Our Understanding of Glaucoma 2013 [cited 2018 20 June]:[22-5 pp.]. Available from: http://glaucomatoday.com/pdfs/gt09113_F_johnstone.pdf
56. Johnstone M. Outflow Resistance: An analysis of early and recent research 2015 [cited 2018 15 June]:[13-5 pp.]. Available from: http://glaucomatoday.com/pdfs/gt0115_bench.pdf
57. Johnstone M. Intraocular pressure control through linked trabecular meshwork and collector channel motion In: Knepper PA, Samples JR, editors. *Glaucoma Research and Clinical Advances 2016 to 2018*: Kugler Publications, 2016.
58. Johnstone MA, Grant WG. Pressure-dependent changes in structures of the aqueous outflow system of human and monkey eyes. *Am J Ophthalmol*. 1973; 75(3): 365-83
59. Johnstone MA. Pressure-dependent changes in configuration of the endothelial tubules of Schlemm's canal. *Am J Ophthalmol*. 1974; 78(4): 630-8
60. Johnstone MA. The aqueous outflow system as a mechanical pump. *Int Glaucoma Rev*. 2003; (5): 14
61. Johnstone MA. The aqueous outflow system as a mechanical pump: evidence from examination of tissue and aqueous movement in human and non-human primates. *J Glaucoma*. 2004; 13(5): 421-38
62. Kaufman PL, Levin LA, Adler FH, Alm A. *Adler's Physiology of the Eye*: Saunders/Elsevier, 2011.
63. Kaufmann C, Bachmann LM, Robert YC, Thiel MA. Ocular pulse amplitude in healthy subjects as measured by dynamic contour tonometry. *Arch Ophthalmol*. 2006; 124(8): 1104-8

64. Kelly DE, Hageman GS, McGregor JA. Uveal compartmentalization in the hamster eye revealed by fine structural and tracer studies: implications for uveoscleral outflow. *Invest Ophthalmol Vis Sci*. 1983; 24(9): 1288-304
65. Kleinert HW. The compensation maximum; a new glaucoma sign in aqueous veins. *AMA Arch Ophthalmol*. 1951; 46(6): 618-24
66. Lewis RA, von Wolff K, Tetz M, Koerber N, Kearney JR, Shingleton BJ, et al. Canaloplasty: Three-year results of circumferential viscodilation and tensioning of Schlemm canal using a microcatheter to treat open-angle glaucoma. *J Cataract Refract Surg*. 2011; 37(4): 682-90
67. Li P, Reif R, Zhi Z, Martin E, Shen TT, Johnstone M, et al. Phase-sensitive optical coherence tomography characterization of pulse-induced trabecular meshwork displacement in ex vivo nonhuman primate eyes. *J Biomed Opt*. 2012; 17(7): 076026
68. Li P, Shen TT, Johnstone M, Wang RK. Pulsatile motion of the trabecular meshwork in healthy human subjects quantified by phase-sensitive optical coherence tomography. *Biomed Opt Express*. 2013; 4(10): 2051-65
69. Lieb E. Demonstration of vascular tree with neoprene. *J tech Methods*. 1940; 20: 48
70. Melamed S, Pei J, Puliafito CA, Epstein DL. Q-switched neodymium-yag laser trabeculopuncture in monkeys. *Arch Ophthalmol*. 1985; 103(1): 129-33
71. Millar JC, Gabelt BAT, Kaufman PL. Aqueous Humor Dynamics. In: Tasman W, Jaeger EA, editors. *Duane's Ophthalmology*. 13th ed. Philadelphia: Lippincott Williams & Wilkins, 2013.
72. Overby DR, Stamer WD, Johnson M. The changing paradigm of outflow resistance generation: towards synergistic models of the JCT and inner wall endothelium. *Exp Eye Res*. 2009; 88(4): 656-70
73. Pache M, Wilmsmeyer S, Funk J. Laser surgery for glaucoma: excimer-laser trabeculotomy [German]. *Klin Monbl Augenheilkd*. 2006; 223(4): 303-7
74. Pascolini D, Mariotti SP. Global estimates of visual impairment: 2010. *Br J Ophthalmol*. 2012; 96(5): 614-8
75. Pederson JE, Cantrill HL. Experimental retinal detachment. V. Fluid movement through the retinal hole. *Arch Ophthalmol*. 1984; 102(1): 136-9
76. Rohen JW, Rentsch FJ. Morphology of Schlemm's canal and related vessels in the human eye [German]. *Albrecht von Graefes Arch Klin Exp Ophthalmol*. 1968; 176(4): 309-29
77. Rosenquist R, Epstein D, Melamed S, Johnson M, Grant WM. Outflow resistance of enucleated human eyes at two different perfusion pressures and different extents of trabeculotomy. *Curr Eye Res*. 1989; 8(12): 1233-40
78. Schirmer KE. Reflux of blood in the canal of Schlemm, quantitated. *Can J Ophthalmol*. 1969; 4(1): 40-4
79. Schirmer KE. Gonioscopic assessment of blood in Schlemm's canal. Correlation with glaucoma tests. *Arch Ophthalmol*. 1971; 85(3): 263-7
80. Schlemm F. Ueber einen kreisförmigen dünnhäutigen Kanal in der Verbindungsstelle von Slerotica und Cornea im menschlichen Auge [German]. *Ztschr f Ophth*. 1830; 1(30): 543-4
81. Seiler T, Wollensak J. The resistance of the trabecular meshwork to aqueous humor outflow. *Graefes Arch Clin Exp Ophthalmol*. 1985; 223(2): 88-91
82. Snell RS, Lemp MA. *Clinical Anatomy of the Eye*. 2nd ed: Blackwell Science Ltd,. 1997.
83. Suguro K, Toris CB, Pederson JE. Uveoscleral outflow following cyclodialysis in the monkey eye using a fluorescent tracer. *Invest Ophthalmol Vis Sci*. 1985; 26(6): 810-3
84. Tamm ER. The trabecular meshwork outflow pathways: structural and functional aspects. *Exp Eye Res*. 2009; 88(4): 648-55
85. Tamm S, Tamm E, Rohen JW. Age-related changes of the human ciliary muscle. A quantitative morphometric study. *Mech Ageing Dev*. 1992; 62(2): 209-21

86. Toris C, Camras C. Aqueous humor dynamics II. Clinical studies. In: Civan M, editor. *The Eye's Aqueous Humor*. Waltham, MA: Elsevier Inc, 2008. p. 231-72.
87. Toris CB, Yablonski ME, Wang YL, Camras CB. Aqueous humor dynamics in the aging human eye. *Am J Ophthalmol*. 1999; 127(4): 407-12
88. Toris CB, Koepsell SA, Yablonski ME, Camras CB. Aqueous humor dynamics in ocular hypertensive patients. *J Glaucoma*. 2002; 11(3): 253-8
89. Van Buskirk EM. Changes in the facility of aqueous outflow induced by lens depression and intraocular pressure in excised human eyes. *Am J Ophthalmol*. 1976; 82(5): 736-40
90. Van Buskirk EM. Anatomic correlates of changing aqueous outflow facility in excised human eyes. *Invest Ophthalmol Vis Sci*. 1982; 22(5): 625-32
91. Vinod K. Suprachoroidal shunts. *Curr Opin Ophthalmol*. 2018; 29(2): 155-61
92. Wang K, Read AT, Sulchek T, Ethier CR. Trabecular meshwork stiffness in glaucoma. *Exp Eye Res*. 2017; 158: 3-12