Chapter 1

Schlemm's Canal: The Outflow "Vessel"

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Abstract

The aim of this chapter is to review the knowledge about the aqueous outflow through Schlemm's canal. Morphology of this canal and aqueous humor pathways from the anterior chamber through the trabeculum into suprascleral and conjunctival veins via connector channels are described. Additionally, the role of Schlemm's canal in the development of glaucoma and outflow resistance is discussed. Canalography as a more precise method of assessing the conventional drainage pathway and facilitating localization of an uncollapsed collector and aqueous veins is shown. Attention is also drawn to the relationship between aqueous and suprascleral veins and heartbeat.

Keywords: Schlemm's canal, aqueous humor, conventional drainage pathway, outflow resistance, canalography, glaucoma

1. Introduction

Aqueous humor (AH) is drained from the eye via two physiological pathways. The conventional path begins at the level of the irido-corneal trabecular meshwork (TM) and is responsible for approximately 83–96% of drainage. From the anterior chamber, the aqueous humor moves through the trabecular meshwork to Schlemm's canal (SC) and then to intrascleral collector channels (CCs), which lead to the intrascleral venous plexus, aqueous vessels, and venous vessels of the suprascleral space. Aqueous vessels begin as collector channels in the exterior wall of Schlemm's canal and can be seen on the surface of the eye in the corneal limbus.

Aqueous humor flows out of the anterior chamber as a mass stream regulated by a pressure gradient. In healthy human eyes, outflow facility has a value of 0.40 at 10 mmHg and is reduced with age. From a physiological perspective, the trabeculum, particularly the interior wall of

Schlemm's canal, and the trabecular meshwork near collector channels are the main sources of resistance to aqueous outflow, and the remaining part of resistance is located in the exterior wall and surrounding tissues. Elevated IOP in glaucoma is caused by an increase in aqueous outflow resistance on its drainage pathways, not by an increase in aqueous production. Many authors believe that the source of outflow resistance in correct eyes is found close to or in the area of the interior wall of Schlemm's canal. Outflow resistance is not constant but a function of IOP and rises as IOP rises.

The aqueous humor flows out of Schlemm's canal through one of 30 collector channels and aqueous veins (AVs) and then to the system of suprascleral veins, ophthalmic veins, and general circulation. According to Poiseuille's law, the resistance of aqueous veins should be insignificant if they are not collapsed or compressed. Provocative gonioscopy, during which blood reflux into Schlemm's canal is observed, is the simplest method of assessing the conventional drainage pathway and facilitating localization of an uncollapsed collector and aqueous vein. Assessment of the distribution of aqueous veins in canalography is a more precise method. Studies by Grieschaber et al. showed a relationship between postoperative intraocular pressure (IOP) level and the presence of reflux in Schlemm's canal before surgery and between the degree to which water veins were filled. Zou introduced the trabeculum bypass theory, which reduces resistance in this part of the drainage route. He observed increased flow through Schlemm's canal only in the quadrant where the implant was applied, and intraocular pressure reduction was dependent on initial pressure.

Aqueous and suprascleral veins oscillate according to heartbeat. These oscillations enable continuous lamellar flow. Pressure in aqueous veins is sufficiently high and enables reverse lamellar flow from suprascleral veins at cardiac diastole. At cardiac systole, pressure in aqueous veins increases and reverses the direction of aqueous flow with simultaneous blood reflux.

2. Schlemm's canal

Schlemm's canal (SC) was named in honor of the German anatomist, Friedrich Schlemm, who, in 1830, discovered the canal in the anterior chamber angle, draining aqueous humor (AH) into the bloodstream [1, 2]. It is a ring-like canal with a length of 36–40 mm encircling the cornea [3, 4] and directly adjacent to the juxtacanalicular trabecular meshwork (JCT) [5], and together with the trabecular meshwork (TM), it forms the conventional outflow pathway, which accounts for 50–90% of AH flow [6, 7]. Its cross-section has the shape of an elongated ellipse, with its longer axis measuring 150–350 μm. Three-dimensional visualizations have made it possible to take precise measurements of the canal, the cross-sectional area of which ranges from 4064 to 7164 μm^2 [8–12]. Rarely, the canal may be bi- or tripartite [13], and it may sometimes contain septa [14]. One of the primary functions of SC is to drain aqueous humor from the trabeculum to collector channels (CCs).

Due to its direct adjacency to the trabeculum, not all SC cells are identical [5, 15]. Owing to the canal's microanatomy, we can distinguish between the inner and outer wall, each built of a

continuous, single-cell layer of endothelium. The cells of both walls differ in terms of morphology [16], the presence of different marker expressions, cell organelles, and function [17]. The inner wall is more frequently analyzed because it presents the greatest resistance to drainage of AH [18–20]. Endothelial cells of the inner wall are shaped like paver stones, while the cells of the outer wall are smooth and flat [15]. Tight junctions of VE-cadherin as well as characteristic giant vacuoles and pores are the markers for cells of the inner wall. Desmin, reactivity to Factor VIII-related antigen, and the presence of Weibel-Palade bodies are the markers for cells of the outer wall [6, 21–26].

2.1. Embryogenesis

Schlemm's canal is a highly specialized vessel. Despite many similarities to vascular endothelium, the canal's embryonic origin and progression of its development have still not been precisely determined [5]. Earlier research suggested a vascular origin of cells [24, 27–29], but recent publications have classified them as unique endothelial cells with phenotypical traits of the endothelial cells of both blood and lymphatic vessels [30–33]. In humans, the prenatal development of SC begins with development of the trabeculum in the 17th week [15]; in the 24th week, the canal is already defined and encircles the limbus over 360°; and in the 36th week, the canal and collector channels are fully developed [34].

The organogenesis of SC was described by Kizhatil as a combination of the vascular developmental factors of angiogenesis and lymphangiogenesis. He termed this process "*canalogenesis*," which begins from the limbal vascular plexus [30]. The development of SC can be divided into four stages, starting from differentiation of the canal's precursor cells, proliferation and migration of frontal cells, formation of the canal's lumen, and separation from the venous vascular system [5]. PROX1 and VEGFR-3 expression is required for division of frontal cells and shaping them into the canal.

2.2. Genetics

PROX1 (*prospero homeobox protein 1*) is the main regulator of lymphangiogenesis, and its expression is critical in transforming cells of the vascular endothelium into cells of the lymphatic endothelium [5, 30, 32]. Truong was the first to demonstrate a high level of expression of the PROX1 lymphatic transcription factor in the canal's endothelial cells, thus showing the similarity to lymphatic endothelial cells [7]. VEGFR-3 (*vascular endothelial growth factor receptor 3*), or FLT4, is a receptor belonging to the RTKs-KDR (*kinase insert domain-containing receptor*) family; it binds the VEGF-C and VEGF-D vascular endothelial growth factors, and its expression is typical of endothelium in lymphatic vessels [35]. Aspelund et al. [31] and Park et al. [32] presented the properties of the precursor cells of Schlemm's canal as well as key molecular mechanisms required for differentiation of these cells into the mature cells of the canal [31, 32]. Aspelund demonstrated that the VEGF-C vascular endothelial growth factor is necessary for activating migration of vascular endothelial cells and their further formation from transscleral venous vessels. He also demonstrated that precursor cells are, in essence, vascular endothelial cells with VEGFR-2 and TIE-2 (*tunica interna endothelial cell kinase*) expression. Precursor cells then gain PROX1 expression in order to create and form

the canal's lumen and also VEGFR-3 for later maturing of the canal's cells [31, 32]. Both aqueous humor and VEGF-C are required for proper SC development. A reduction in AH in mice resulted in the loss of elements of canal cells' lymphatic identity [32]. The direct relationship of the SC endothelium with JCT and the fact that the development of TM precedes the development of SC allow for the hypothesis that soluble factors from JCT cells may be of critical significance for obtaining phenotypical traits of SC cells. Because the inner wall of SC is in direct contact with the TM over a 360° circumference, modern canal surgery provides access to the entire inner wall of SC and the juxtacanalicular region without affecting the cornea, iris, and ciliary body. Canaloplasty may be used to deliver transgenic SC/TM vectors in glaucoma gene therapy [36].

2.3. Role of NO

Several studies have also documented the influence of cytokines (tumor necrosis factor (TNF)-α, interleukin (IL)1-α, IL-β, IL-8) released by TM cells on SC cells, as well as their influence on regulation of aqueous drainage [37, 38]. Nitrogen oxide (NO) was widely studied from the perspective of its role in modulating the behavior of SC cells and regulating aqueous flow [39, 40]. Stresses in the SC endothelium trigger no production in SC cells, similarly as in other vascular endothelial cells [39]. Nitrogen oxide also mediates volume reduction in SC cells, which is linked to facilitation of AH drainage [40].

2.4. Biomechanics

The hydraulic conductivity of the conventional aqueous drainage pathway amounts to approximately 10^{-7} cm^2 $s^{-1}g^{-1}$, and this value also sets the lower limit for hydraulic conductivity of the SC endothelium, which is 2–5 times greater than the hydraulic conductivity of brain endothelium and the greatest in the entire human body [41]. In SC, the biomechanical conditions acting on endothelial cells resemble the microenvironment in a lymphatic vessel [30]. In SC endothelial cells, the pressure gradient is distributed from the base to the apex of a cell similarly as in lymphatic vessels, but inversely to the distribution in the case of vascular endothelium [42]. In a typical blood vessel, the basement membrane and surrounding tissue provide additional support for endothelial cells, reducing circumferential, and radial stresses acting on cells. In the case of SC cells, the inverted pressure gradient caused by AH flowing into the canal's lumen generates a force that pushes cells away from the basement membrane [43]. However, in contrast to a lymphatic vessel, SC cells are bound by tight junctions, so they maintain the pressure difference between the eyeball and episcleral veins (EPV). Forces related to the pressure drop from the base to the apex of a cell result in cell deformation and the formation of large, dome-shaped diverticula into the canal's lumen, called giant vacuoles [41, 42, 44–47]. Besides tight junctions between endothelial cells, there are extensive links between endothelial cells and cells in the JCM area. These junctions are present when SC cells form protrusions to join with JCM cells, forming parachute-like structures. These junctions, described by Johnstone, play an important role in anchoring the canal's endothelial cells in response to increases in pressure [15, 48]. The size of SC's lumen changes in response to IOP fluctuations [48]. When IOP increases, the TM widens while the canal narrows, and this is

caused by an increase in the number of vacuoles and of the area of the extracellular matrix (ECM), as well as by the fact that both walls of the canal are closer to one another. At high IOP, the probability that the canal's walls will collapse and resistance on drainage outflow pathways will grow increases significantly [48]. When IOP increases to approximately 40 mmHg, the canal collapses, with the exception of segments containing septa [20, 49], which support the walls of SC and prevent occlusion of CCs [20, 48, 49]. In eyes with glaucoma, the lumen of SC is smaller than in healthy eyes [50].

2.5. Microanatomy-giant vacuoles and pores

Giant vacuoles are potential spaces between extracellular matrix (ECM) and the SC's inner wall cells [15]. Giant vacuoles form dynamically and respond to changes in intraocular pressure (IOP) instantaneously [5, 51]. Their quantity and size increase as IOP increases. After enucleation, the IOP drops to zero, and vacuoles disappear within a time of <3 min [52]. The majority of giant vacuoles are found near CCs outlets [52], which suggests that a greater pressure gradient is present at CCs outlets due to the greater aqueous flow [15]. Most probably due to the specific biomechanical microenvironment, endothelial cells are characterized by contractile properties and by an elastic modulus of 1–3 kPa [41], which is slightly greater than in the case of other endothelial cells [42, 47]. SC cells owe their capability of adapting to deformations to the cytoskeleton system fortified with actin microfilaments. Cells of the outer wall have star-shaped F-actin systems that pass through most cells, in contrast to the circumferential F-actin bands observed in endothelial cells of the inner wall [53]. The position of SC cells relative to ECM allows for reception of biomechanical signals from the ECM [37], which affect the expression of cells' genes and adapt them to changes in the rigidity. The rigidity and contractility of SC cells exhibited a strong response to pharmacological stimulation. Medications increasing resistance to drainage increased the rigidity of SC cells, and inversely, medications reducing resistance to drainage reduced the rigidity of these cells [41]. In eyes with glaucoma, endothelial cells are more sensitive and exhibit an amplified response to the increase in the substrate's rigidity that occurs in glaucoma [42]. Stress caused by a rise in IOP can increase cell surfaces by up to 50% and even cause them to thin out [22]. Tight junctions between endothelial cells of the inner wall are very sensitive to increases in IOP and become less complex when IOP is elevated [37]. Endothelial deformation may initiate the formation of pores mediating aqueous transport by loosening intercellular junctions [54–56].

Pores are structures in the inner wall with sizes ranging from 0.6 to 3 μm [25, 43, 57], and they are responsible for 10% of the resistance to aqueous drainage [37]. They may be found in the walls of giant vacuoles, but they may also be unrelated to them [21]. They form the main pathway of aqueous flow through the inner wall of SC. Two pore types have been identified and characterized: type I pores (transcellular) and type B pores (paracellular) [25]. They differ in their locations, filtration ability, and formation mechanisms [43]. B pores are larger, but they are outnumbered 3–4 to 1 by I pores. Type B pores form as a result of local loosening and widening of intercellular junctions [43]. Braakman et al. [26] presented a segmentation of the aqueous drainage stream, and type B pores account for the majority of aqueous flow. Type I pores may form as a result of a combination of deformations of the cellular membrane at the

base and apex of an endothelial cell, which may occur under the influence of the aqueous filtration stream, and caveolae, vesicles, and minipores [43, 58]. Pores in Schlemm's canal are most frequently formed from minipores 60 nm in size, covered by a diaphragm containing PLVAP (plasmalemma vesicle-associated protein) [43, 58]. Molecular pore formation processes are not well known, but PLVAP is most probably involved in them, considering that pore formation is significantly impaired in mice with PLVAP deficiency [58, 59]. Pore density in the interior wall fluctuates between 1000 and 2000/mm^2 [55, 60]. When IOP is elevated, the number of pores in the inner wall increases [25, 51, 55]. Giant vacuoles and pores are unique features of the endothelium of SC's inner wall and of the endothelium of the arachnoid villi in the central nervous system [42, 61, 62]. Scanning electron micrograph of the inner wall of Schlemm's canal can visualize the giant vacuoles and pores at the base of a bulging structure (**Figure 1**) [56].

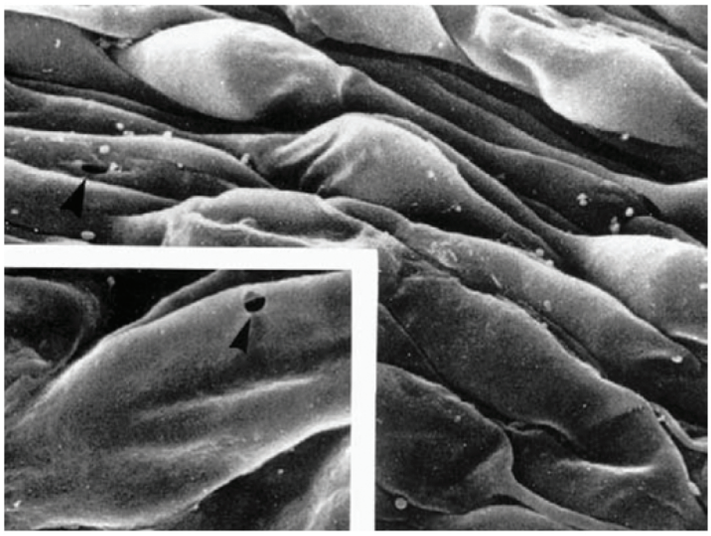

Figure 1. Scanning electron micrograph of the giant vacuoles and pores of the inner wall of Schlemm's canal. A pore (arrow) is observed at the base of a bulging structure (modified from Allingham et al. [56]).

The formation of giant vacuoles is directed in one direction, providing a preferential aqueous drainage pathway through the endothelium by means of a one-way valve mechanism. In the case of a pressure increase in episcleral veins and in SC that exceeds IOP, the number of vacuoles and pores decreases, preventing blood reflux from SC into the anterior chamber [42, 63, 64]. Certain medications, such as glycocorticosteroids or sphingosine-1-phosphate (S1P), which induce polymerization of the cytoskeleton's proteins [65, 66], may inhibit the formation and reduce the density of vacuoles, increasing resistance to drainage [67, 68]. Eyes with glaucoma exhibit reduced pore density, which emphasizes the critical role of the inner wall in maintaining homeostasis of AH. The aqueous flow resistance is considerably increased by the hydrodynamic interaction between pores and their basal substrate- subendothelial (basement membrane of SC cells and extracellular matrix of JCT) [69]. In particular, flow is concentrated near every pore, forming funnels that flow through the region of extracellular matrix closest to a given pore, which significantly reduces the effective area available for flow through these

regions [42]. The goal of glaucoma therapy oriented toward SC may be to increase pore density, and thus drainage, leading to reduction in IOP [55].

2.6. Distribution of aqueous humor

AH in Schlemm's canal is not distributed uniformly through the canal's inner wall, but rather appears preferentially at certain locations. Drainage of AH most frequently occurs near CCs [70]. Twice as many giant vacuoles are present near collectors, which suggests that aqueous flow through the inner wall is dependent on the value of pressure [52]. Studies involving the application of fluorescent markers have also demonstrated an elevated level of markers in the pigmented part of TM adhering to CCs, suggesting that the preferred drainage outflow pathways are present near collectors [70]. Histological research on human eyes has proven that, between the 25th and 30th year of life, CCs are randomly distributed around the eye, with preferential dislocation in the inferior nasal quadrant [1, 71]. This has been confirmed by three-dimensional micro-CT tests [72]. There is high diversity in the size of CCs outlets, with values ranging between 5–50 μm and up to 70 μm depending on the type of test [1, 71, 72]. From the CCs, AH flows through a winding system of venous plexuses, from the deep scleral plexus, through the limbal plexus, to the intrascleral plexus, which ultimately leads to the episcleral veins [6].

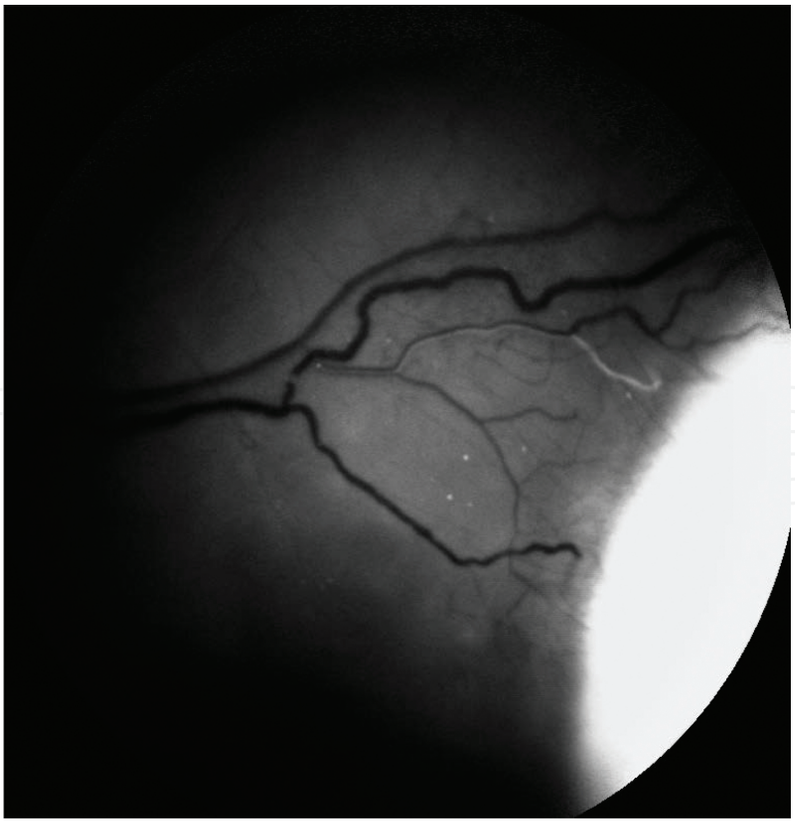

Figure 2. Aqueous veins.

2.7. Aqueous veins

Aqueous humor moves through the TM into SC, issuing forth from its lumen into CCs, aqueous veins (AVs) (**Figure 2**) and the system of episcleral veins (EPV) (**Figure 3**), ocular veins, and into the general circulation [21, 73]. AVs have lumens that are directly connected to CCs, and because of this, they are directly connected to the episcleral veins draining blood into the general circulation, bypassing the deep scleral and intrascleral venous plexuses [74, 75]. AVs containing initially clean AH are joined to episcleral veins filled with blood, which is why transition zones can be identified on the surface of the conjunctiva as large vessels with a transparent, central lumen bounded by dark blood from all sides. Linear stratification into AH and blood occurs due to the differences in these fluids' viscosity and density [76]. The composition of blood and aqueous in transition zones changes as IOP changes. Direct observation of these changes is a reliable indicator for assessment of the effectiveness of topical and surgical therapy oriented toward IOP reduction in glaucoma [77]. AVs differ in their position, size, and anatomical configuration. In a slit lamp test, 2–3 AVs are usually visible, and sporadically, up to 6 AVs may be seen [78, 79]. AVs are nonuniformly distributed and are present in the greatest number in the inferior nasal quadrants [78, 80]. Their size varies from 20 to 100 μm, 50 μm on average [78, 81, 82]. Histologically, AVs cannot be distinguished from conjunctival and EPV [80].

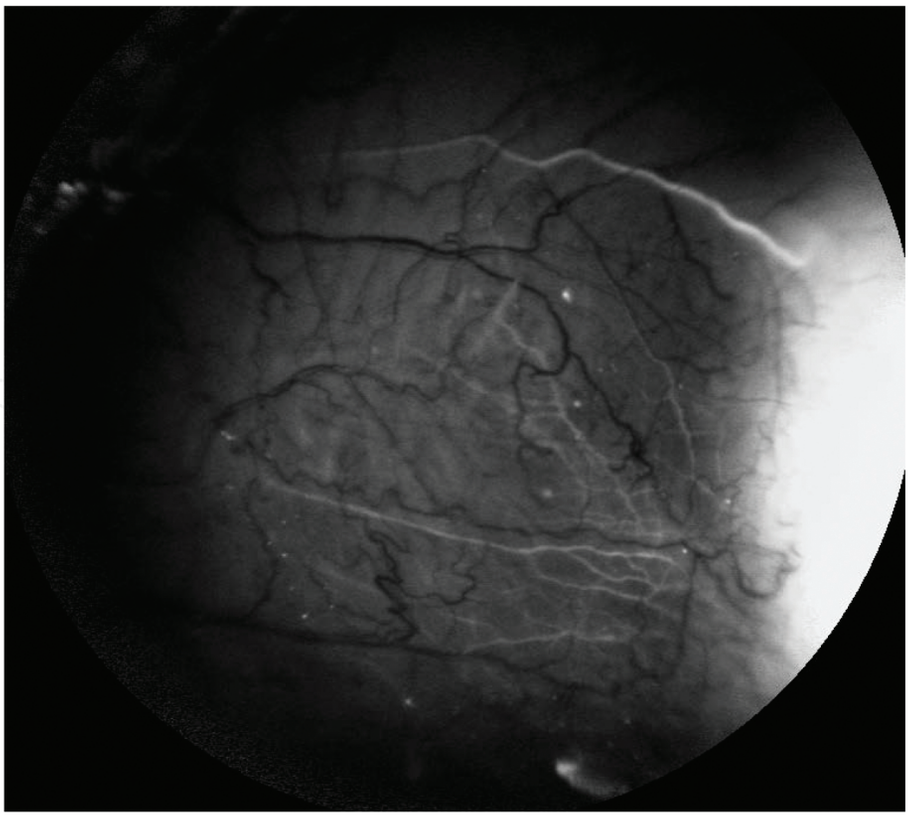

Figure 3. Episcleral veins.

2.8. Aqueous humor drainage

IOP is the primary factor affecting AH drainage. Drainage through the conventional outflow pathway is directly proportional to the IOP value within the range of physiological values [83, 84]. Drainage facility is the measure of how easily AH leaves the eye, and it is the inverse of resistance to drainage [20, 85]. In healthy human eyes, drainage facility has a value of 0.40 μl/min/mmHg at an IOP of 10 mmHg [86]. The main point of resistance to AH drainage is located at the JCM level in juxtacanalicular connective tissues, in the inner wall of SC, and its basement membrane [21, 69]. Elevated IOP in glaucoma is caused by an increase in aqueous outflow resistance on its drainage outflow pathways, not by an increase in aqueous production [87]. AH flow, defined as the movement of AH from the posterior chamber of the eye through the pupil into the anterior chamber, is lower than aqueous production because it does not include the AH that leaves the posterior chamber via other pathways [85]. The value of AH flow through the anterior chamber is not dependent on sex [88]. AH flow amounts to 2.4 ± 0.6 μl/min and decreases with age by 2% per decade [88], which may result in a reduction of up to 30% [89]. It has also been observed that flow is halved during the night (1.13–1.6 μl/min) as compared to the day (3.0–3.1 μl/min) [89, 90]. In studies with fluorescein, it was observed that flow value is also significantly lower in eyes with pseudoexfoliation syndrome than in physiologically correct eyes [88].

2.9. Effective filtration areas

Based on observations of the distribution of pigment and perfusion markers, it was determined that, circumferentially, drainage of AH in healthy eyes is nonuniform and segmented [91–94]. At any given time, only some AH drainage pathways are actively involved in aqueous percolation. These active areas are called effective filtration area (EFA) [50, 80, 95]. EFA is a valuable method for measuring resistance to flow and the effects of IOP changes. Segmented drainage has been described in mice [96], pigs [97], cows [91, 93], monkeys [94], and humans [70, 97, 98]. Higher marker concentration was present in the TM neighboring the outlets of CCs, and in humans, more pigment was also observed at this location, suggesting that EFA locations can be determined by using pigment distribution as a marker [80]. A sudden increase in IOP in cow eyes caused a significant reduction in EFA [91, 93]. When IOP increased suddenly, the marker was present in a greater concentration near CC outlets. When IOP was correct, the drainage patterns were more uniform, and when IOP was elevated, drainage became more segmented [91]. EFA reduction is linked to reduction in drainage facility and is reversed when pressure is reduced from high to normal level [99]. EFA reduction was also observed in an animal model, in eyes with glaucoma and chronic IOP elevation that had undergone laser therapy [100]. In this study, reduction in the marker level was determined in regions of the TM that had undergone laser therapy. It was stated that active drainage shifted from areas that had undergone laser therapy to areas not affected by therapy. In a study, where a marker was applied, significant EFA reduction was observed in eyes with glaucoma in comparison with healthy eyes [101]. In addition, the inversely proportional dependence between EFA and IOP has been documented on an animal model in the eyes of a mouse with ocular hypertension [96].

2.10. Pulsating flow

Drainage of AH is a complex process. Besides the traditional approach, according to which the AH moves passively in a combined stream through TM into SC, downward along the pressure gradient determined by the heart [102], a significant effect of the active process driven by means of a mechanical pump is also assumed [77]. Pulsating flow occurs as a result of oscillating compressive force caused by transitional IOP increases occurring during the cardiac cycle, blinking, and eye movements. These transitional IOP spikes cause microscopic deformations of the flexible structural elements of drainage outflow pathways. During contraction, the canal's endothelial cells move to the outside, forcing AH flow to the outlets of CCs and AVs. When the value of IOP drops, flexible elements move back to their original configuration, which leads to a relative reduction in pressure in SC inducing AH flow into the SC's lumen [11, 102, 103]. The theory that pulsating flow drives AH drainage is reflected in the dynamic equilibrium between AH and blood in AVs [104]. During contraction, the pulse wave causes flow of AH through AVs, resulting in visible widening of the aqueous layer in their lumens [78]. Eyes with glaucoma exhibit reduced pulsating flow in comparison with healthy eyes [105, 106]. In healthy eyes, the TM is susceptible to deformation under the influence of naturally occurring, dynamic changes in pressure and volume of AH flow from the anterior chamber to SC. Reduction in pulsating flow in glaucoma may be caused by changes in the TM's elasticity [75].

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References

[1] Dvorak-Theobald G. Schlemm's canal: its anastomoses and anatomic relations. Trans Am Ophthalmol Soc. 1934;32:574–95. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1315493/

[2] Mansouri K, Shaarawy T. Update on Schlemm's canal based procedures. Middle East Afr J Ophthalmol. 2015;22:38–44. doi:10.4103/0974-9233.148347

[3] Freddo TF, Johnson M. The eye's aqueous humor. Aqueous humor outflow resistance. Curr Topics Membr. Elsevier; 2008;62:161–192 doi:org/10.1016/S1063-5823(08)00406-7

[4] Seiler T, Wollensak J. The resistance of the trabecular meshwork to aqueous humor outflow. Graefe's Arch Clin Exp Ophthalmol = Albr von Graefes Arch für Klin und Exp Ophthalmol. 1985;223:88–91. doi:10.1007/BF02150951

[5] Dautriche C, Tian Y, Xie Y, Sharfstein S. A closer look at Schlemm's canal cell physiology: implications for biomimetics. J Funct Biomater. 2015;6:963–85. doi:10.3390/jfb6030963

[6] Goel M, Picciani RG, Lee RK, Bhattacharya SK. Aqueous humor dynamics: a review. Open Ophthalmol J. 2010;4:52–9. doi:10.2174/1874364101004010052

[7] Truong TN, Li H, Hong Y, Chen L. Novel characterization and live imaging of Schlemm's canal expressing Prox-1. PLoS One. 2014;9:1–8. doi:10.1371/journal.pone.0098245

[8] Kagemann L, Wang B, Wollstein G, Ishikawa H, Nevins JE, Nadler Z, et al. IOP elevation reduces Schlemm's canal cross-sectional area. Invest Ophthalmol Vis Sci. 2014;55:1805–9. doi:10.1167/iovs.13-13264

[9] Kagemann L, Wollstein G, Ishikawa H, Bilonick R a, Brennen PM, Folio LS, et al. Identification and assessment of Schlemm's canal by spectral-domain optical coherence tomography. Invest Ophthalmol Vis Sci. 2010;51:4054–9. doi:10.1167/iovs.09-4559

[10] Kagemann L, Nevins JE, Jan N-J, Wollstein G, Ishikawa H, Kagemann J, et al. Characterisation of Schlemm's canal cross-sectional area. Br J Ophthalmol. 2014;98:10–4. doi:10.1136/bjophthalmol-2013-304629

[11] Kagemann L,Wollstein G, Ishikawa H, et al. 3D visualization of aqueous humor outflow structures in-situ in humans. Exp Eye Res. 2011;141(4):520–9. doi:10.1016/j.exer.2011.03.019

[12] Sambasivarao S V. Visualization of the conventional outflow pathway in the living human eye. Ophthalmology. 2013;18(9):1199–216. doi:10.1016/j.ophtha.2012.02.032

[13] Ten Hulzen RD, Johnson DH. Effect of fixation pressure on juxtacanalicular tissue and Schlemm's canal. Investig Ophthalmol Vis Sci. 1996;37:114–24. http://iovs.arvojournals.org/article.aspx?articleid=2180115

[14] Dietlein TS, Jacobi PC, Lüke C, Krieglstein GK. Morphological variability of the trabecular meshwork in glaucoma patients: implications for non-perforating glaucoma surgery. Br J Ophthalmol. 2000;84:1354–9. doi:10.1136/bjo.84.12.1354

[15] Ethier CR. The inner wall of Schlemm's canal. Exp Eye Res. 2002;74:161–72. doi:10.1006/exer.2002.1144

[16] Lütjen-Drecoll E, Rohen JW. Endothelial studies of the Schlemm's canal using silver-impregnation technic. Albr von Graefe's Arch Clin Exp Ophthalmol. 1970;180:249–66. http://www.ncbi.nlm.nih.gov/pubmed/4938983

[17] Karl MO, Fleischhauer JC, Stamer WD, Peterson-Yantorno K, Mitchell CH, Stone RA, et al. Differential P1-purinergic modulation of human Schlemm's canal inner-wall cells. Am J Physiol Cell Physiol. 2005;288:784–94. doi:10.1152/ajpcell.00333.2004

[18] Vranka JA, Kelley MJ, Acott TS, Keller KE. Extracellular matrix in the trabecular meshwork: intraocular pressure regulation and dysregulation in glaucoma. Exp Eye Res. 2015;133:112–25. doi:10.1016/j.exer.2014.07.014

[19] Acott TS, Kelley MJ. Extracellular matrix in the trabecular meshwork. Exp Eye Res. 2008;86:543–61. doi:10.1016/j.exer.2008.01.013

[20] Johnson MC, Kamm RD. The role of Schlemm's canal in aqueous outflow from the human eye. Investig Ophthalmol Vis Sci. 1983;24:320–5. http://www.ncbi.nlm.nih.gov/pubmed/6832907

[21] Tamm ER. The trabecular meshwork outflow pathways: structural and functional aspects. Exp Eye Res. 2009;88:648–55. doi:10.1016/j.exer.2009.02.007

[22] Hamanaka T, Bill A, Ichinohasama R, Ishida T. Aspects of the development of Schlemm's canal. Exp Eye Res. 1992;55:479–88. doi:10.1016/0014-4835(92)90121-8

[23] Heimark RL, Kaochar S, Stamer WD. Human Schlemm's canal cells express the endothelial adherens proteins, VE-cadherin and PECAM-1. Curr Eye Res. 2002;25:299–308. http://www.ncbi.nlm.nih.gov/pubmed/12658549

[24] Hamanaka T, Bill A. Platelet aggregation on the endothelium of Schlemm's canal. Exp Eye Res. 1994;59:249–56. doi:10.1006/exer.1994.1106

[25] Ethier CR, Coloma FM, Sit AJ, Johnson M. Two pore types in the inner-wall endothelium of Schlemm's canal. Invest Ophthalmol Vis Sci. 1998;39:2041–8. http://www.ncbi.nlm.nih.gov/pubmed/9761282

[26] Braakman ST, Read AT, Chan DW-H, Ethier CR OD. Colocalization of outflow segmentation and pores along the inner wall of Schlemm's canal. Exp Eye Res. 2015;130:87–96. doi:10.1016/j.exer.2014.11.008

[27] Smelser GK, Ozanics V. The development of the trabecular meshwork in primate eyes. Am J Ophthalmol. 1971;71:366–85. http://www.ncbi.nlm.nih.gov/pubmed/5542135

[28] Wulle KG. Electron microscopic observations of the development of Schlemm's canal in the human eye. Trans Am Acad Ophthalmol Otolaryngol. 1968;72:765–73. http://www.ncbi.nlm.nih.gov/pubmed/5692862

[29] Foets B, van den Oord J, Engelmann K, Missotten L. A comparative immunohistochemical study of human corneotrabecular tissue. Graefe's Arch Clin Exp Ophthalmol = Albr von Graefes Arch für Klin und Exp Ophthalmol. 1992;230:269–74. doi:10.1007/BF00176303

[30] Kizhatil K, Ryan M, Marchant JK, Henrich S, John SWM. Schlemm's canal is a unique vessel with a combination of blood vascular and lymphatic phenotypes that forms by a novel developmental process. PLoS Biol. 2014;12:e1001912. doi:org/10.1371/journal.pbio.1001912

[31] Aspelund A, Tammela T, Antila S, Nurmi H, Leppänen V-M, Zarkada G, et al. The Schlemm's canal is a VEGF-C/VEGFR-3-responsive lymphatic-like vessel. J Clin Invest. 2014;124:3975–86. doi:10.1172/JCI75395

[32] Park D-Y, Lee J, Park I, Choi D, Lee S, Song S, et al. Lymphatic regulator PROX1 determines Schlemm's canal integrity and identity. J Clin Invest. 2014;124:3960–74. doi:10.1172/JCI75392

[33] Karpinich NO, Caron KM. Schlemm's canal: More than meets the eye, lymphatics in disguise. J Clin Invest. 2014;124:3701–3. doi:10.1172/JCI77507

[34] Ramírez JM, Ramírez AI, Salazar JJ, Rojas B, De Hoz R, Triviño A. Schlemm's canal and the collector channels at different developmental stages in the human eye. Cells Tissues Organs. 2004;178:180–5. doi:10.1159/000082248

[35] Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. FASEB J. 1999;13:9–22. http://www.ncbi.nlm.nih.gov/pubmed/9872925

[36] Tian B, Kaufman PL. A potential application of canaloplasty in glaucoma gene therapy. Transl Vis Sci Technol. 2013;2(1):2. doi:10.1167/tvst.2.1.2

[37] Alvarado JA, Yeh R-F, Franse-Carman L, Marcellino G, Brownstein MJ. Interactions between endothelia of the trabecular meshwork and of Schlemm's canal: a new insight into the regulation of aqueous outflow in the eye. Trans Am Ophthalmol Soc. 2005;103:148–63. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1447570/

[38] Alexander JP, Acott TS. Involvement of the Erk-MAP kinase pathway in TNFalpha regulation of trabecular matrix metalloproteinases and TIMPs. Invest Ophthalmol Vis Sci. 2003;44:164–9. http://www.ncbi.nlm.nih.gov/pubmed/12506070

[39] Ashpole NE, Overby DR, Ethier CR, Stamer WD. Shear stress-triggered nitric oxide release from Schlemm's canal cells. Invest Ophthalmol Vis Sci. 2014;55:8067–76. doi:10.1167/iovs.14-14722

[40] Ellis DZ, Sharif NA, Dismuke WM. Endogenous regulation of human Schlemm's canal cell volume by nitric oxide signaling. Invest Ophthalmol Vis Sci. 2010;51:5817–24. doi:10.1167/iovs.09-5072

[41] Zhou EH, Krishnan R, Stamer WD, Perkumas KM, Rajendran K, Nabhan JF, et al. Mechanical responsiveness of the endothelial cell of Schlemm's canal: scope, variability

and its potential role in controlling aqueous humour outflow. J R Soc Interface. 2012;9:1144–55. doi:10.1098/rsif.2011.0733

[42] Stamer WD, Braakman ST, Zhou EH, Ethier CR, Fredberg JJ, Overby DR, et al. Biomechanics of Schlemm's canal endothelium and intraocular pressure reduction. Prog Retin Eye Res. 2014;44:1–13. doi:10.1016/j.preteyeres.2014.08.002

[43] Braakman ST, Pedrigi RM, Read AT, Smith JAE, Stamer WD, Ethier CR, et al. Biomechanical strain as a trigger for pore formation in Schlemm's canal endothelial cells. Exp Eye Res. 2014;127:224–35. doi:10.1016/j.exer.2014.08.003

[44] Miteva DO, Rutkowski JM, Dixon JB, Kilarski W, Shields JD, Swartz MA. Transmural flow modulates cell and fluid transport functions of lymphatic endothelium. Circ Res. 2010;106:920–31. doi:10.1161/CIRCRESAHA.109.207274

[45] Raviola G, Raviola E. Paracellular route of aqueous outflow in the trabecular meshwork and canal of Schlemm. A freeze-fracture study of the endothelial junctions in the sclerocorneal angle of the macaque monkey eye. Investig Ophthalmol Vis Sci. 1981;21(1 II):52–72.http://www.ncbi.nlm.nih.gov/pubmed/7251302

[46] Overby DR, Zhou EH, Vargas-Pinto R, Pedrigi RM, Fuchshofer R, Braakman ST, et al. Altered mechanobiology of Schlemm's canal endothelial cells in glaucoma. Proc Natl Acad Sci USA. 2014;111:13876–81. doi:10.1073/pnas.1410602111

[47] Zeng D, Juzkiw T, Read AT, Chan DWH, Glucksberg MR, Ethier CR, et al. Young's modulus of elasticity of Schlemm's canal endothelial cells. Biomech Model Mechanobiol. 2010;9:19–33. doi:10.1007/s10237-009-0156-3

[48] Johnstone MA. Pressure-dependent changes in nuclei and the process origins of the endothelial cells lining Schlemm's canal. Invest Ophthalmol Vis Sci. 1979;18:44–51. http://www.ncbi.nlm.nih.gov/pubmed/103860

[49] Van Buskirk EM. Anatomic correlates of changing aqueous outflow facility in excised human eyes. Invest Ophthalmol Vis Sci. 1982;22:625–32. http://www.ncbi.nlm.nih.gov/pubmed/7076408

[50] Allingham RR, de Kater AW, Ethier RC. Schlemm's canal and primary open angle glaucoma: correlation between Schlemm's canal dimensions and outflow facility. Exp Eye Res. 1996;62:101–10. doi:10.1006/exer.1996.0012

[51] Epstein DL, Rohen JW. Morphology of the trabecular meshwork and inner-wall endothelium after cationized ferritin perfusion in the monkey eye. Investig Ophthalmol Vis Sci. 1991;32:160–71. http://www.ncbi.nlm.nih.gov/pubmed/1987099

[52] Parc CE, Johnson DH, Brilakis HS. Giant vacuoles are found preferentially near collector channels. Invest Ophthalmol Vis Sci. 2000;41:2984–90. http://www.ncbi.nlm.nih.gov/pubmed/10967055

[53] Ethier CR, Read AT, Chan D. Biomechanics of Schlemm's canal endothelial cells: influence on F-actin architecture. Biophys J. 2004;87(4):2828–37. doi:10.1529/biophysj.103.038133 [cited 2016 Mar 12]

[54] Johnson M, Shapiro A, Ethier CR, Kamm RD. Modulation of outflow resistance by the pores of the inner wall endothelium. Investig Ophthalmol Vis Sci. 1992;33(5):1670–5. http://www.ncbi.nlm.nih.gov/pubmed/1559767

[55] Johnson M, Chan D, Read AT, Christensen C, Sit A, Ethier CR. The pore density in the inner wall endothelium of Schlemm's canal of glaucomatous eyes. Investig Ophthalmol Vis Sci. 2002;43(9):2950–5. http://www.ncbi.nlm.nih.gov/pubmed/12202514

[56] Allingham RR, De Kater AW, Ethier CR, Anderson PJ, Hertzmark E, Epstein DL. The relationship between pore density and outflow facility in human eyes. Investig Ophthalmol Vis Sci. 1992;33(5):1661–9. http://www.ncbi.nlm.nih.gov/pubmed/1559766

[57] Grierson I, Lee WR. Pressure-induced changes in the ultrastructure of the endothelium lining Schlemm's canal. Am J Ophthalmol. 1975;80:863–84. http://www.ncbi.nlm.nih.gov/pubmed/811121

[58] Herrnberger L, Ebner K, Junglas B, Tamm ER. The role of plasmalemma vesicle-associated protein (PLVAP) in endothelial cells of Schlemm's canal and ocular capillaries. Exp Eye Res. 2012;105:27–33. doi:10.1016/j.exer.2012.09.011

[59] Herrnberger L, Hennig R, Kremer W, Hellerbrand C, Goepferich A, Kalbitzer HR, et al. Formation of fenestrae in murine liver sinusoids depends on plasmalemma vesicle-associated protein and is required for lipoprotein passage. PLoS One. 2014;9(12):e115005. doi:10.1371/journal.pone.0115005 [cited 2016 Mar 26]

[60] Sit AJ, Coloma FM, Ethier CR, Johnson M. Factors affecting the pores of the inner wall endothelium of Schlemm's canal. Invest Ophthalmol Vis Sci. 1997;38:1517–25. http://www.ncbi.nlm.nih.gov/pubmed/9224279

[61] Bill A, Svedbergh B. Scanning electron microscopic studies of the trabecular meshwork and the canal of Schlemm–an attempt to localize the main resistance to outflow of aqueous humor in man. Acta Ophthalmol. 1972;50:295–320. doi:10.1111/j.1755-3768.1972.tb05954.x

[62] Tripathi BJ, Tripathi RC. Vacuolar transcellular channels as a drainage pathway for cerebrospinal fluid. J Physiol. 1974;239:195–206. http://www.ncbi.nlm.nih.gov/pubmed/4369428

[63] Pedrigi RM, Simon D, Reed A, Stamer WD, Overby DR. A model of giant vacuole dynamics in human Schlemm's canal endothelial cells. Exp Eye Res. 2011;92:57–66. doi:10.1016/j.exer.2010.11.003

[64] Alvarado JA, Betanzos A, Franse-Carman L, Chen J, González-Mariscal L. Endothelia of Schlemm's canal and trabecular meshwork: distinct molecular, functional, and

anatomic features. Am J Physiol Cell Physiol. 2004;286:621–34. doi:10.1152/ajpcell.00108.2003

[65] Filla MS, Schwinn MK, Nosie AK, Clark RW, Peters DM. Dexamethasone-associated cross-linked actin network formation in human trabecular meshwork cells involves β3 integrin signaling. Investig Opthalmol Vis Sci. 2011;52:2952. doi:10.1167/iovs.10-6618

[66] Clark AF, Wilson K, McCartney MD, Miggans ST, Kunkle M, Howe W. Glucocorticoid-induced formation of cross-linked actin networks in cultured human trabecular meshwork cells. Invest Ophthalmol Vis Sci. 1994;35:281–94. http://www.ncbi.nlm.nih.gov/pubmed/8300356

[67] Underwood JL, Murphy CG, Chen J, Franse-Carman L, Wood I, Epstein DL, et al. Glucocorticoids regulate transendothelial fluid flow resistance and formation of intercellular junctions. Am J Physiol. 1999;277:330–42. http://www.ncbi.nlm.nih.gov/pubmed/10444410

[68] Sumida GM, Stamer WD. Sphingosine-1-phosphate enhancement of cortical actomyosin organization in cultured human Schlemm's canal endothelial cell monolayers. Invest Ophthalmol Vis Sci. 2010;51:6633–8. doi:10.1167/iovs.10-5391

[69] Johnson M. What controls aqueous humour outflow resistance? Exp Eye Res. 2006;82:545–57. doi:10.1016/j.exer.2005.10.011

[70] Hann CR, Fautsch MP. Preferential fluid flow in the human trabecular meshwork near collector channels. Invest Ophthalmol Vis Sci. 2009;50:1692–7. doi:10.1167/iovs.08-2375

[71] Rohen JW, Rentsch FJ. Morphology of Schlemm's canal and related vessels in the human eye. Albr von Graefes Arch für Klin und Exp Ophthalmol Albr von Graefe's Arch Clin Exp Ophthalmol. 1968;176:309–29. http://www.ncbi.nlm.nih.gov/pubmed/5305008

[72] Hann CR, Bentley MD, Vercnocke A, Ritman EL, Fautsch MP. Imaging the aqueous humor outflow pathway in human eyes by three-dimensional micro-computed tomography (3D micro-CT). Exp Eye Res. 2011;92:104–11. doi:10.1016/j.exer.2010.12.010

[73] Toris CB. Aqueous humor dynamics and intraocular pressure elevation. Second Ed. Glaucoma. Elsevier Ltd; 2015. 47–56. http://linkinghub.elsevier.com/retrieve/pii/B9780702051937000066

[74] Ascher KW. The aqueous veins: I. Physiologic importance of the visible elimination of intraocular fluid. Am J Ophthalmol. 1942;25:1174–209. doi:10.1016/S0002-9394(42)90325-8

[75] Schacknow PN SJ. The glaucoma book: a practical, evidence-based approach to patient care. 1st ed. Vol. 53, J Chem Inf Model. New York: Springer-Verlag; 2010. 1689–1699

[76] Meighan SS. Blood vessels of the bulbar conjunctiva in man. Br J Opthalmol. 1956;40:513–26. http://www.ncbi.nlm.nih.gov/pubmed/13364178

[77] 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;13:421–38. http://www.ncbi.nlm.nih.gov/pubmed/15354083

[78] De Vries S. De zichtbare afvoer van het kamerwater. 1st ed. Drukkerij Kinsbergen. Amsterdam; 1947

[79] Johnstone M, Jamil A, Martin E. Aqueous veins and open angle glaucoma. In: The Glaucoma Book. New York, NY: Springer New York; 2010:65–78. DOI: 10.1007/978-0-387-76700-0_7

[80] Surgical Innovations in Glaucoma. Springer Science & Business Media; 2014;307. https://books.google.com/books?id=7uS5BAAAQBAJ&pgis=1 http://www.springer.com/us/book/9781461483472

[81] Stepanik J. Measuring velocity of flow in aqueous veins. Am J Ophthalmol. 1954;37:918–22. doi:10.1016/0002-9394(54)91933-9

[82] Goldmann H. Weitere Mitteilung über den Abfluss des Kammerwassers beim Menschen. Ophthalmologica. 1946;112:344–9. http://www.ncbi.nlm.nih.gov/pubmed/20293720

[83] Morrison JC, Pollack IP. Glaucoma: science and practice. Thieme; New York Stuttgart 2003. https://books.google.pl/books?isbn=1588905918

[84] Glaucoma Research and Clinical Advances 2016 to 2018. Kugler Publications; 2016:336. https://www.kuglerpublications.com/index.php?p=316&page=publication

[85] Morrison JC, Freddo TF TC. Anatomy and physiology of aqueous humor formation. Glaucoma Science and Practice. Stuttgart: Thieme New York; 2003:4–41. http://s2.bitdownload.ir/Ebook/Biology/Medicine/Glaucoma%20-%20Science%20and%20Practice%20-%20J.%20Morrison,%20I.%20Pollack%20(Thieme,%202003)%20WW.pdf

[86] Brubaker RF. The effect of intraocular pressure on conventional outflow resistance in the enucleated human eye. Invest Ophthalmol. 1975;14:286–92. http://www.ncbi.nlm.nih.gov/pubmed/1123284

[87] Grant WM. Clinical measurements of aqueous outflow. AMA Arch Ophthalmol. 1951;46:113–31. http://www.ncbi.nlm.nih.gov/pubmed/14856471

[88] Brubaker RF. The flow of aqueous humor in the human eye. Trans Am Ophthalmol Soc. 1982;80:391–474. http://www.ncbi.nlm.nih.gov/pubmed/1748546

[89] Reiss GR, Lee DA, Topper JE, Brubaker RF. Aqueous humor flow during sleep. Invest Ophthalmol Vis Sci. 1984;1:776–8. http://www.ncbi.nlm.nih.gov/pubmed/6724850

[90] Viggiano SR, Koskela TK, Klee GG, Samples JR, Arnce R, Brubaker RF. The effect of melatonin on aqueous humor flow in humans during the day. Ophthalmology. 1994;101:326–31. doi:10.1016/S0161-6420(94)31332-7

[91] Battista SA, Lu Z, Hofmann S, Freddo T, Overby DR, Gong H. Reduction of the available area for aqueous humor outflow and increase in meshwork herniations into collector channels following acute IOP elevation in bovine eyes. Investig Ophthalmol Vis Sci. 2008;49:5346–52. doi:10.1167/iovs.08-1707

[92] Camp JJ, Hann CR, Johnson DH, Tarara JE, Robb RA. Three-dimensional reconstruction of aqueous channels in human trabecular meshwork using light microscopy and confocal microscopy. Scanning. 2006;19:258–63. doi:10.1002/sca.4950190402

[93] Lu Z, Overby DR, Scott PA, Freddo TF, Gong H. The mechanism of increasing outflow facility by rho-kinase inhibition with Y-27632 in bovine eyes. Exp Eye Res. 2008;86:271–81. doi:10.1016/j.exer.2007.10.018

[94] Lu Z, Zhang Y, Freddo TF, Gong H. Similar hydrodynamic and morphological changes in the aqueous humor outflow pathway after washout and Y27632 treatment in monkey eyes. Exp Eye Res. 2011;93:397–404. doi:10.1016/j.exer.2011.05.012

[95] Cha EDK, Xu J, Gong L, Gong H. Variations in active outflow along the trabecular outflow pathway. Exp Eye Res. 2016;146:354–60; doi:10.1016/j.exer.2016.01.008

[96] Swaminathan SS, Oh D-J, Kang MH, Ren R, Jin R, Gong H, et al. Secreted protein acidic and rich in cysteine (SPARC)-null mice exhibit more uniform outflow. Invest Ophthalmol Vis Sci. 2013;54:2035–47. doi:10.1167/iovs.12-10950

[97] Keller KE, Bradley JM, Vranka JA, Acott TS. Segmental versican expression in the trabecular meshwork and involvement in outflow facility. Invest Ophthalmol Vis Sci. 2011;52:5049–57. doi:10.1167/iovs.10-6948

[98] Yang CYC, Liu Y, Lu Z, Ren R, Gong H. Effects of Y27632 on aqueous humor outflow facility with changes in hydrodynamic pattern and morphology in human eyes. Investig Ophthalmol Vis Sci. 2013;54:5859–70. doi:10.1167/iovs.12-10930

[99] Zhu JY, Ye W, Wang T, Gong HY. Reversible changes in aqueous outflow facility, hydrodynamics, and morphology following acute intraocular pressure variation in bovine eyes. Chin Med J (Engl). 2013;126:1451–7. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3771502/

[100] Zhang Y, Toris CB, Liu Y, Ye W, Gong H. Morphological and hydrodynamic correlates in monkey eyes with laser induced glaucoma. Exp Eye Res. 2009;89:748–56. doi:10.1016/j.exer.2009.06.015

[101] Cha E, Jin R, Gong H. The relationship between morphological changes and reduction of active areas of aqueous outflow in eyes with primary open angle glaucoma. Invest Ophthalmol Vis Sci. 2013;54:2291–2291. http://iovs.arvojournals.org/article.aspx?articleid=2146968

[102] Bill A. Blood circulation and fluid dynamics in the eye. Physiol Rev. 1975;55:383–417. http://www.ncbi.nlm.nih.gov/pubmed/1153528

[103] Johnstone M, Martin E, Jamil A. Pulsatile flow into the aqueous veins: manifestations in normal and glaucomatous eyes. Exp Eye Res. 2011;92:318–27. doi:10.1016/j.exer.2011.03.011

[104] Zamir M, Budwig R. Physics of pulsatile flow. Appl Mech Rev. 2002;55:35. doi:10.1007/978-1-4612-1282-9

[105] Kleinert H. The visible flow of aqueous humor in the epibulbar veins. II. The pulsating blood vessels of the aqueous humor. Albr von Graefe's Arch für Ophthalmol. 1952;152:587–608. http://www.ncbi.nlm.nih.gov/pubmed/12976314

[106] Kleinert HW, Ascher KW, Goldmann H, Duke-Elder WS, Goldmann H, Ascher KW, et al. The compensation maximum. AMA Arch Ophthalmol. 1951;46:618. http://www.ncbi.nlm.nih.gov/pubmed/14868091

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