In Vivo Identification of the Posttrabecular Aqueous Outflow Pathway Using Swept-Source Optical Coherence Tomography

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PURPOSE. The purpose of this study was to investigate a novel imaging technique to identify the continuous post trabecular aqueous outflow pathway from a single B-scan, using swept-source optical coherence tomography (SS-OCT).

METHODS. Three-dimensional volume scans of the temporal or nasal side of the anterior segment area at the limbus were acquired from 11 eyes of 11 healthy subjects, using SS-OCT. The aqueous outflow pathway was identified using an en face OCT image and reconstructed images of the vasculature (vasculature map). Delineation of the whole aqueous outflow pathway in a single B-scan was accomplished by reslicing the volume scan.

RESULTS. The post trabecular aqueous outflow pathway was successfully identified in 10 eyes (90.9%). Combined with a flattening technique, the en face video and vasculature map showed a clear blood stream that could not be observed on a sequential stack of B-scans. In the en face images, the vessels were widely branched in the episcleral venous plexus, perpendicularly penetrating the scleral stroma. Vessels running parallel to Schlemm’s canal and the collector channels were observed in the deeper region of the sclera. The average longitudinal diameter of the vessel was 29.7 ± 6.6 μm at the episcleral venous plexus, and it was significantly larger than that in the deep scleral aqueous plexus (22.0 ± 4.8 μm; P = 0.0002).

CONCLUSIONS. The continuous post trabecular aqueous outflow pathway could be identified from a single B-scan and quantitatively analyzed using SS-OCT with en face imaging and volume scan reslicing.

Keywords: aqueous flow, glaucoma, optical coherence tomography

The drainage system of the aqueous humor is composed of two types of pathways: the conventional trabecular meshwork outflow pathway and the uveoscleral outflow pathway.1,2 Most of the total aqueous humor outflow is considered removed through the conventional outflow pathway, and generally, ocular hypertension is induced by impairment of this pathway, causing abnormal outflow resistance in glaucoma.3,4 To date, filtration surgery, such as trabeculotomy, which provides an artificial nonphysiological resistance in glaucoma.3,4 To date, filtration surgery has been used mainly to address ocular hypertension. However, it is well known that filtration surgery is associated with risks including blebitis, aqueous humor leakage from the bleb, ocular hypotension, and others.

Recently, treatments targeting the conventional outflow pathway to increase aqueous humor outflow with milder complications than filtration surgery have gained popularity.5 Although trabeculotomy has conventionally been performed, the recent development of microinvasive canal-based surgery, including procedures based on use of the iStent (Glaukos, Laguna Hills, CA, USA) and Trabectome (Neomedix, Tustin, CA, USA), has attracted increasing attention.5–9 However, the drawback to these procedures is that the outcome of the surgery is difficult to predict.10 Compared to filtration surgery, these newer procedures are unreliable, and there is still room for improvement. One of the reasons for this difficulty is the absence of technology to visualize the pathway preoperatively and postoperatively, and another is the difficulty that may arise from the clinician’s lack of understanding of the anatomical structure of the pathway. Because microinvasive canal-based surgery is expected to become increasingly popular, development of the technology for visualizing the conventional pathway and improvement of our understanding of the drainage system of the aqueous humor are needed.9

In this study, we describe a novel method to identify the continuous post trabecular aqueous outflow pathway, from Schlemm’s canal to the episcleral venous plexus, from a single B-scan, and we investigated the pathway’s morphologic features in normal subjects.

METHODS

This was a prospective, observational, cross-sectional case series study. This study was approved by the Institutional Review Board and Ethics Committee at Kyoto University Graduate School of Medicine. All aspects of the study adhered to the tenets of the Declaration of Helsinki. After the study design and risks and benefits of participation were thoroughly explained to them, written informed consent was obtained from each participant.
Subjects

Eleven normal volunteers with no history of ocular or systemic disease were recruited to the study. The right eye from each subject was included.

Optical Coherence Tomography Imaging

A swept-source optical coherence tomography (SS-OCT) (deep range imaging [DRI] OCT-1 model; Topcon, Tokyo, Japan) system, which uses a tunable laser as the light source, was operated at 100,000 Hz, and an A-scan repetition rate at a wavelength of 1050 nm was used to obtain the study images. Because DRI OCT-1 was originally developed for use in the posterior pole, its use for anterior segment OCT requires some ingenuity. First, subjects were instructed to gaze to the right or left side, using an external fixation lamp, in order to scan the temporal or nasal side of the anterior segment area covering the corneal limbus and farther than approximately 6 mm from the corneal limbus to the conjunctiva (Fig. 1). Then, the OCT probe was moved closer to the eye from the farthest position, but it did not exceed the switching point (11 mm from the farthest position), which enabled rapid changing of focus from the anterior segments to the posterior pole. For image acquisition, a three-dimensional (3D) volume scan mode containing $512 \times 256$ axial scans with a scan length of $3 \times 3$ mm was used. The image acquisition time was approximately 3 seconds for 1 volume scan. Both the nasal and the temporal sides of the corneal limbus images were acquired for each subject, and the image with better quality was included in the analyses. Note that the lateral resolution of the image was unknown because of the inaccuracy of the scan length, whereas the axial resolution could be defined as 2.6 μm/pixel.\textsuperscript{11,12}

Visualization of the Scleral Venous Plexus

En Face OCT Imaging. En face images were generated by using built-in software (Enview; Topcon). To obtain a stack of frames parallel to the scleral surface for better visualization of the vessel, the flattening function, which was originally developed for automatic correction of the concavity of the posterior pole in Enview, was used. In this study, flattening was performed at the level of the conjunctival epithelium, which was misidentified as the inner limiting membrane by the software (Fig. 1). Nine consecutive frames from each en face video were averaged for the purpose of noise reduction (moving average).

Motion Contrast Enhancement. In the en face images, cross-sections of the vessels in the sclera were delineated as dark dots or line segments, indicating that the blood cells and flow could not be detected with this SS-OCT system. Meanwhile superficial conjunctival vessels were detected as low-intensity tubes with a high-intensity line corresponding to the reflection from blood cells at the center of the tube. However, in the video of the en face images played from the level of the episclera to the interface between the sclera and the ciliary body, dark cross-sections of the vessels were detected as dark, moving objects against the bright sclera, although the blood flow itself could not be seen (Supplementary Video S1). Therefore, the map of the scleral vessels (vasculature map) was constructed as a trajectory of dark, moving objects by using the motion contrast enhancement technique, which was originally developed for capillary visualization, assisted by the use of adaptive optics scanning laser ophthalmoscopy without a contrast dye (Fig. 2).\textsuperscript{13–15}

First, 202 frames spanning from the episclera to the interface between the sclera and ciliary body were extracted, and after application of a Gaussian blur filter, 201 division images were
calculated by dividing the pixels between sequential frames as
\[
D j(x, y) = I_j(x, y)/I_{j+1}(x, y),
\]
where \(I_j(x, y)\) represents the intensity of frame \(j\). Then, the division images were divided into three equal stacks of frames, and for each stack, the variance of the pixels among all of the division images at each \(x\)-\(y\) position was calculated to visualize the contrast-enhanced vessel images. As a consequence, three vascular images of different depths were obtained, and the images were merged in different colors (Fig. 3E, superficial layer network, blue; intermediate layer network, green; deep layer, red) in order to visualize the connection between the layers. Digital image processing was performed by using ImageJ software (National Institutes of Health, Bethesda, MD; in the public domain: http://rsb.info.nih.gov/ij/index.html), and a series of ImageJ commands was performed automatically by using a macro (Supplementary Fig. S1).

**Identification of the Post trabecular Aqueous Outflow Pathway**

The post trabecular aqueous outflow pathway was identified by applying the same image processing technique as the spatiotemporal image generation. Because the venous plexus in the sclera has a complex 3D structure and because the constructed vascular images were too faint to fully identify the aqueous outflow pathway, the post trabecular aqueous outflow pathway was identified by using both the constructed vascular images and the original en face videos. Moreover, an en face video emphasizing the vasculature was generated by calculating the differences between the division images, and this video was also used for identification as necessary (Supplementary Video S1). In fact, the video files were opened and viewed in ImageJ software by freely moving between video frames in forward and reverse directions so that the relationship between the cross-sections of the vessels and frames became evident. After identifying the pathway by making a segmented line selection on the vascular image, we copied the selection to an en face video, followed by reslicing the sequential frames to obtain the new B-scan image. This image contained the continuous cross-section of the vessels from Schlemm’s canal to the episcleral venous plexus. Successful identification of the post trabecular aqueous outflow pathway was defined as full delineation of the pathway, that is, from Schlemm’s canal to the episcleral venous plexus in the...
newly resliced B-scan, and eyes with partial delineation of the pathway were excluded from the study. Because many vasculature samples were depicted incompletely, especially in deeper locations as the OCT signal attenuated, processes of trial and error were performed to obtain a limited number of continuous pathways from Schlemm’s canal to the episcleral venous plexus, generating multiple unsuccessful pathways with blind ends on the reconstructed B-scans. Schlemm’s canal was identified as a dark, hyporeflective luminal structure that ran parallel to the limbus, using both the B-scans and the en face images (Figs. 3C1, 4F). The longitudinal diameter of the vessel was measured at a superficial location, and the deepest location was used for the analyses. The measurements were repeated three times and averaged. Moreover, to quantify the tortuosity of the pathway, the ratio of the straight line distance from Schlemm’s canal to that of the first bifurcation of the superficial layer network (Fig. 3E, merged in blue) in the x-y plane was calculated as the tortuosity index. All digital images were processed by a single operator (A.U.), using ImageJ software and a software plug-in.

**Statistics**

All values are means ± SD, where applicable. Differences between diameters of superficial vessels and those of vessels at the deepest location were analyzed using paired t-tests. The relationship between the tortuosity index and the average longitudinal diameter of the vessel was analyzed using Pearson’s correlation coefficient. A P value of <0.05 was considered statistically significant. All analyses were performed using StatView statistical software (version 5.0; SAS Institute, Cary, NC, USA).

**RESULTS**

Subjects’ average age was 29.9 ± 5.7 years old (range, 23–41 years of age). Successful identification of the post trabecular aqueous outflow pathway was achieved in 10 eyes (90.9%), and 1 eye with partial delineation of the pathway was excluded from the study (Supplementary Fig. S2). Eleven eyes (11 subjects) were examined and one eye was excluded from the study. The OCT data sets from the remaining 10 eyes were analyzed. The remaining 10 eyes (29.1 ± 4.8 years; range, 23–41 years) were included in the current study. In all 10 eyes, the en face videos with flattening showed clear cross-sections of the vessels distributed over a larger area than the en face videos without flattening (Supplementary Video S2). The en face videos showed widely branched vessels in the episclera, which joined to several vessels that perpendicularly penetrated the scleral stroma (Supplementary Video S1; Fig. 4). In the episclera, small interconnecting vessels forming a vascular hexagonal meshwork were observed between the larger vessel

**Figure 3.** Identification of the post trabecular aqueous outflow pathway. (A1-3, B1-3, C1-3). Images from different depths: episcleral venous plexus, intermediate scleral aqueous plexus, and deep scleral aqueous plexus. (A1, B1, C1) En face OCT images. Vessels were detected as dark lines and dots against the bright scleral stroma. Schlemm’s canal was seen as a dark curved line parallel to the corneal limbus (red arrowheads). (A2, B2, C2) Constructed vasculature maps. Bright vessels (yellow arrowheads) are shown against a dark background. (C1) Multiple vessels approaching Schlemm’s canal, which may correspond to collector channels, were observed (yellow arrowheads). (A3) Vasculature maps superimposed on the en face images (A1, B1, C1). (D) Identified aqueous outflow delineated on the en face image (yellow line). (E) Merged vessel image shown in different colors (superficial layer network, blue; intermediate layer network, green; deep layer, red) for better visualization of the connection between layers (white arrowheads). The tortuosity index was defined as the ratio of the actual distance to the linear distance from Schlemm’s canal (yellow arrowhead) to the first bifurcation of the superficial layer network merged in blue (red arrowhead) in the x-y plane to quantify the tortuosity of the pathway. (F) A reconstructed B-scan obtained by reslicing the volume scan data along the identified aqueous outflow pathway in the x-y plane ((D) yellow line and (E) white arrowheads). The upper region of the image corresponds to the outer side, and the lower region of the image corresponds to the inner side. Post trabecular aqueous outflow pathways are shown as a dark line against the bright high-intensity scleral stroma with a stair-like shape (red arrowheads). The stair-like shape appeared to be composed of three steps, the episcleral venous plexus, the intermediate scleral aqueous plexus, and the deep scleral aqueous plexus, which was connected to Schlemm’s canal (yellow arrowhead).
streams. In the deeper location of the sclera, vessels parallel to Schlemm’s canal and multiple vessels approaching Schlemm’s canal that might correspond to collector channels were observed (Figs. 4, 5). In some cases, vessels parallel to Schlemm’s canal were shown in sequential frames, suggesting the presence of a rich and dense venous plexus in the circumferential direction.

The number of pathways identified was 1 in 8 eyes and 2 in 2 eyes. Of 10 eyes, the aqueous outflow pathways were identified in 4 eyes (40%) with 5 pathways by using only the constructed vasculature map. For 3 eyes (30%) with 4 pathways, both the constructed vasculature map and the original en face video were required for identification. In the remaining 3 eyes (30%) with 3 pathways, the aqueous outflow pathways were identified mainly by using the en face video because of the low image quality of the vasculature map. In all of the resliced B-scans, the identified posttrabecular aqueous outflow pathways showed a dark line against a bright, high-intensity stroma of the sclera and had a stair-like shape (Fig.3). The stair-like shape appeared to be composed of 3 steps, that is, the episcleral venous plexus, the intermediate scleral aqueous plexus, and the deep scleral aqueous plexus, which connected to Schlemm’s canal. Note that these steps do not necessarily correspond to the vasculature map, which is conveniently divided into three different depths. Three steps were connected to each other by the steeper vessel. Intriguingly, there were several variations of the stair-like shape (Fig. 3). The tilt and length of the steps, as well as the connections between the steps, had individual differences, and some had almost no steps.

The average longitudinal diameter of the vessel was $29.7 \pm 6.6 \mu m$ at the episcleral venous plexus, and it was significantly larger than that of the deep scleral aqueous plexus ($22.0 \pm 4.8 \mu m; P = 0.0002$). In the $x$-$y$ plane, the pathways meandered, and the average tortuosity index was $1.80 \pm 0.45$, suggesting that it is impossible to obtain the whole posttrabecular aqueous outflow pathway in one rectilinear B-scan without identifying the meandering of the vessels. The average tortuosity index showed a significantly negative correlation with the average longitudinal diameter of the vessel at the deep scleral aqueous plexus ($R = -0.745; P = 0.0314$). The average tortuosity index also showed a negative correlation with the average longitudinal diameter of the vessel at the episcleral venous plexus but without statistical significance ($R = -0.643; P = 0.0881$).

**DISCUSSION**

The aim of this study was to identify the continuous posttrabecular aqueous outflow pathway in a single B-scan, using simple volume scans from SS-OCT. To the best of our knowledge, this is the first report describing the method for characterizing the morphologic features of the outflow pathway in a single B-scan. The results showed that the identified posttrabecular aqueous outflow pathways had a stair-like shape with several variations. Moreover, with this method, the longitudinal diameter and the tortuosity of the vessels could be measured. Because the method can be performed with a commercially available OCT machine and does not require hardware reconstruction, it is readily available and has the potential to be used for the basic processing necessary for morphologic analyses of the aqueous outflow pathway.

Because the vessels are intricately buried in the sclera, it is almost impossible to delineate the continuous pathway in a single B-scan, and a simple B-scan will provide discontinuous cross-sections of the vessels. The method proposed herein makes it possible to depict a continuous cross-sectional image of the pathway, from Schlemm’s canal to the episcleral venous plexus, in one slice by reslicing the 3D volume data along the 2D pathway, identified using a vasculature map and en face video. This method was originally developed in the field of computed tomography (CT) as “multiplanar reconstruction” (MPR), which includes such well-known basic scan images as...
the axial, sagittal, and coronal planes. Multiplanar reconstruction is not a new technology and has been widely used in CT imaging. Multiplanar reconstruction is a method for reconstructing new cross-sections by reslicing the 3D CT volume. Because CT scans initially obtain scan images perpendicular to the bed on which patients lie, scans used for diagnosis are always reconstructed into axial, sagittal, and coronal planes and any other unique plane by reslicing the original volume data sets. Other than these basic scans, MRI images that are resliced along regions of interest also have been used to obtain new scans and are called curved MPR, such as a scan along the coronary arteries on the surface of the heart or a scan along a row of teeth.17–20 The method proposed in this study is identical to that used in curved MPR in CT, and the introduction of the concept of curved MPR in OCT imaging has not been described in previous reports. We obtained an OCT volume scan and then resliced it to reconstruct a single B-scan that depicts a continuous cross-sectional image of the outflow pathway from Schlemm’s canal to the episcleral venous plexus. We believe that these newly reconstructed B-scans, which are easily understood visually, may be able to provide more useful information than the original B-scans.

Due to recent advancements in hardware and software, technologies including the SS system and multiple scan averaging that were developed in posterior segment OCT have been incorporated in anterior segment OCT systems, achieving high-speed, high-quality, and high-penetration imaging.21–27 In this study, en face OCT imaging, which has also been used in retinal and choroidal diseases, was applied for visualization of the vasculature,28,29 whereas previous studies reported B-scan-based vessel image construction.30,31 Combined with the flattening technique, the use of the en face video and its projection (vasculature map) revealed a clear blood stream that was not depicted by a sequential stack of B-scans. The motion contrast enhancement technique was originally developed using adaptive optics scanning laser ophthalmoscopy (AOSLO) imaging, which allows the researcher to correct for ocular aberrations and provides high-resolution retinal images. With AOSLO, a projection of the blood cells onto a cone mosaic can be recorded on sequential frames, and the trajectory of the blood cells can be delineated as vasculature images by using the motion contrast enhancement technique.15–15 Due to the flattening of the video, cross-sections of the vessels in the sclera in this study, which move in sequential frames, could be

Figure 5. Variations in the morphologic characteristic of the identified post trabecular aqueous outflow pathways. (A–F) Temporal limbus of a 26-year-old woman. (G–I) Nasal limbus of 23-year-old woman. (M–R) Nasal limbus of 37-year-old woman. (S–X) Nasal limbus of 27-year-old man. (A, G, M, S) Vasculature maps of the episcleral venous plexus. Interconnecting small vessels forming a vascular hexagonal meshwork could be observed between the larger vessel streams. (B, H, N, T) Vasculature maps from the intermediate area of the sclera. The vessels (yellow arrowheads) were less delineated than those in the episcleral area. (C, I, O, U) Vasculature maps from the deep area of the sclera. The vessels parallel to Schlemm’s canal (red arrowheads) and vessels connecting to Schlemm’s canal (yellow arrowheads) were observed. (D, J, P, V) Merged vessel images in different colors (superficial layer network, blue; intermediate layer network, green; deep layer, red) for better visualization of the connection between the layers. (E, K, Q, W) The identified aqueous outflow delineated on the en face image (yellow line). Red arrowheads indicate the location of Schlemm’s canal. (F, L, R, X) Reconstructed B-scan obtained by reslicing the volume scan data along the identified aqueous outflow pathway in the x–y plane (yellow line in E, K, Q, W). Post trabecular aqueous outflow pathways appeared as a dark line against the bright high-intensity scleral stroma and had a stair-like shape that was connected to Schlemm’s canal (yellow arrowhead). Note that there are several variations of the stair-like shape. The tilt and length of the steps, as well as the connections between the steps, had individual differences, and some had almost no steps (yellow arrow).
similar to cross-section of moving blood cells in the AOSLO video. Therefore, the trajectory of the moving cross-sections can be obtained as an enhanced vessel image in the sclera analogous to the parafoveal capillary network visualization in the AOSLO video. AOSLO data are in good agreement with previous OCT studies and casting studies using neoprene, as we were able to observe vessels running posteriorly to form anastomoses and drain into the episcleral plexus, which contains small vessels that form a vascular hexagonal meshwork. However, our images also presented different characteristics from the images described in those studies. First, the images in this study had fewer vessels than previously reported images, particularly in the deeper locations of the sclera. Compared to the rich venous plexus and vascular connections with Schlemm’s canal in casting studies and OCT studies, our results showed only several vessels parallel to Schlemm’s canal and the collector channels. In humans, the collector channels arise at irregular intervals from the outer wall of Schlemm’s canal, and there are thought to be 25 to 35 collector channels. Therefore, our results may delineate only one quarter to one third of the channels present. One of the reasons for this difference may be the lower lateral resolution due to the small number of raster scans in DRI OCT-1, which was originally designed for fundus imaging. Another reason is the noise caused by the corneal stroma and palisades of Vogt, which differ in pattern from frame to frame. Through the motion contrast enhancement technique, these structures were unintentionally shown as moving objects and were not canceled out by the background on vasculature maps. Therefore, capillaries may not be shown, and only the larger vessels, including aqueous veins, might be imaged. Another difference is the stair-like formation of the vascular network, which was visualized using the above-mentioned curved MPR technique to reslice the volume scan. The en face videos and vasculature maps in this study revealed that the running directions of the blood vessels vary depending on the depth of the en face slice; that is, we observed widely branched vessels in the episclera venous plexus, several vessels perpendicularly penetrating the scleral stroma, and the vessels running parallel to and connecting to Schlemm’s canal in the deeper location of the sclera. These differences in the pattern of the vasculature depending on the depth are considered to contribute to the stair-like formation seen in the curved MPR, and they suggest that the 3D structure of the aqueous humor outflow is composed of three venous plexuses (the deep scleral aqueous plexus, the intermediate scleral aqueous plexus, and the episcleral venous plexus), which are vertically interconnected.

Our study has the following limitations: (1) As stated above, the vessels might not have been perfectly delineated, although the larger vessels that are considered to represent the main outflow pathways were successfully identified with the proposed method, except in one subject. Full delineation of the capillaries in the deep sclera may be achieved by improving the system software to spatially control the scan protocol. (2) Because OCT was originally developed for use in the posterior pole but was used for anterior segment OCT in this study, the lateral resolution of the image was unknown. Therefore, length measurements should be performed in the axial direction. Meanwhile, the use of DRI OCT-1 enabled us to process the en face images with flattening in the built-in software. (3) Discrimination between veins (draining aqueous humor) and arteries can be difficult. The continuity with Schlemm’s canal was used to identify the veins in this study. (4) Vessel images of the vasculature map were slightly faint, although the main purpose of this study was to identify the aqueous humor outflow pathway in the resliced B-scans. Because the method used in this study was based on intensity images and was entirely different from OCT angiography technology, the blood flow signal was not used, and the contrast of the vessel image was relatively low. Moreover, projection images from stacks might create ambiguity and confusion from the mixing of structures from different levels. The application of high-penetration OCT angiography technology in future studies may improve the accuracy of detection of the aqueous humor outflow pathway and may enable us to differentiate among blood, aqueous humor, aqueous-blood admixture, and lymph. (5) Our findings with the proposed method have not been validated in this study. The main reason for the lack of validation in this study is due to the unique nature of this approach, and there is no direct method to validate the approach in a living human. However, the motion contrast enhancement technique in retinal vessels using AOSLO has been validated by the gold standard technique of fluorescein angiography. Instead, we have validated the fact that the vasculature-like map that we constructed through our method is identical to the actual vasculature by reslicing the volumetric data along with the vasculature-like map and showing reconstructed continuous hyporeflective lines as vessels from Schlemm’s canal to the episcleral venous plexus.

In conclusion, continuous post trabecular aqueous outflow pathway could be identified from a single B-scan, using SS-OCT with en face imaging and reslicing 3D volume data (curved MPR technique). This technique effectively reconstructs the complex 3D structure into visually apparent 2D images and promises to provide an opportunity for research in unexplored fields of the post trabecular aqueous outflow pathway and the changes related to glaucoma surgery.

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