Precise Identification of Filtration Openings on the Scleral Flap by Three-Dimensional Anterior Segment Optical Coherence Tomography

Toshihiro Inoue, Riyo Matsumura, Utako Kuroda, Kei-Ichi Nakashima, Takabiro Kawaji, and Hidenobu Tanibara

PURPOSE. To elucidate the potential of three-dimensional anterior segment optical coherence tomography (3D AS-OCT) for identifying filtration openings where aqueous humor flows from the sclera into the subconjunctival space.

METHODS. We used 3D AS-OCT and custom software to identify filtration openings, which were defined by pits and/or troughs in fluid-filled cavities in both horizontal and vertical rasters and corresponding C-scan images of scleral flap margin in the blebs. We measured bleb parameters, and at least three different reviewers surveyed the complete 3D images of the internal structure of the filtration blebs and associated findings.

RESULTS. We identified filtration openings in 118 (95%) of 124 eyes. Among these 118 eyes, we found only a single filtration opening in 90 eyes (76%) and two or more filtration openings in the remaining 28 eyes (24%). Filtration openings were located in the middle third of the scleral flap margins in 57 eyes (63%) of the 90 eyes. The pattern of incision and sutures used for closure of the conjunctival flap affected the location of the openings, but additional laser suture lysis did not. In 6 (5%) of the 124 eyes, filtration openings could not be identified because of high reflectivity and/or elevated bleb wall (2 eyes), and no fluid-filled cavities because a sponge-like structure masked the filtration openings just above the scleral flap (4 eyes).


Trabeculectomy has been regarded as the standard surgical modality for treatment of glaucoma refractory to medical and laser treatments. Many clinical investigations have shown that the existence of filtration blebs is correlated with intraocular pressure (IOP) values after trabeculectomy. Maintenance of filtration openings from anterior chamber to subconjunctival space, in particular, is the key for a favorable outcome of trabeculectomy. Thus, filtration blebs were assessed by using slit-lamp examinations and color photography,1–3 ultrasound biomicroscopy (UBM),4 conventional optical coherence tomography (OCT), and two-dimensional (2D) anterior segment optical coherence tomography (AS-OCT).5,6

The recent development of AS-OCT has allowed us to obtain information on internal bleb morphology in a noninvasive and safe manner. Clinical investigations using AS-OCT seem to be in good agreement with previous studies using the slit-lamp technique and those using UBM to assess anterior ocular segments. However, although a previous study using 2D AS-OCT described the presence of an internal ostia, the filtration openings could not be identified because of the limited number of sections (a radial section perpendicular to the limbus and/or a section tangential to the limbus through the site of maximal bleb elevation). In contrast, with the aid of three-dimensional (3D) AS-OCT, aqueous outflow channels at the margins of scleral flaps can be more easily identified via en face OCT images. In addition, in our previous case series of bleb revision, we found that filtration openings can be identified, by using preoperative 3D AS-OCT imaging, as pits and/or troughs in fluid-filled cavities located just above the scleral flap, which agrees with intraoperative confirmation of aqueous leakage from the sclera. From our experience with bleb revision, we hypothesized that the 3D technique, consisting of 512 scans, allows investigations of filtration openings. In addition, rotation of the scans by newly developed software and matching such scans with reconstructed C-scan images (tomographic image of the slice plane perpendicular to the depth direction) of the scleral flap margin can support precise identification of filtration openings. We therefore believed that 3D AS-OCT would be useful for determining a strategy for postoperative treatment of trabeculectomy such as needling and laser suture lysis.

In this study, we demonstrate that, with the aid of 3D AS-OCT and newly developed software, we could precisely identify filtration openings on the margins of the scleral flap created by trabeculectomy.

PATIENTS AND METHODS

This cross-sectional study included eyes that had undergone trabeculectomy as described in the following text: A fornix-based or limbal-based conjunctival flap was created and a 4-mm-wide half-layer triangle scleral flap was also created. Mitomycin C (0.4 mg/mL) was applied on and under the scleral flap and under the conjunctiva for 4 minutes, followed by irrigation with 200 mL physiologic saline. A deep limbal block was excised to create a fistula in the anterior chamber, and
peripheral iridectomy was then performed. The scleral flap and the conjunctival flap were sutured with 10-0 nylon. Cases without visible blebs, which are considered as unfunctioning blebs, were excluded.

We used slit-lamp examinations and color photography of the anterior ocular segment to evaluate the appearance of blebs. Then the IOP was measured and internal bleb structures were assessed with 3D AS-OCT (CASIA; Tomey Corp., Nagoya, Japan). For the 3D AS-OCT assessment, a patient was asked to look down, and the examiner's finger gently elevated the upper lid to expose the filtration bleb. The examined area was chosen to include the entire scleral flap and bleb. For each bleb, at least two ways of scans were obtained as horizontal and vertical rasters, and each raster consisted of 512 scans.

At least three different reviewers evaluated the complete 3D images of the internal structure of the filtration blebs and associated findings, and independently assessed filtration openings in the 3D AS-OCT images. When the opinions differed among the reviewers, a majority decision was chosen. Filtration openings were identified by the presence of pits and/or troughs in fluid-filled cavities in both horizontal and vertical rasters and by a corresponding C-scan image of scleral flap margins in the bleb (Fig. 1). These blebs in which filtration openings could be identified by 3D AS-OCT were called type F. The numeral indicates the number of filtration openings, so F1 means a bleb with a single filtration opening, as identified by 3D AS-OCT (Fig. 1A), and F ≥ 2 means a bleb in which two or more filtration openings were noted (Fig. 1B). Blebs in which filtration openings could not be identified were subdivided into two groups: high reflectivity and/or elevated bleb wall (type H, Fig. 2A), and masked by a sponge-like structure with low reflectivity (type S, Fig. 2B).

In this study, to identify filtration openings, we used criteria defined on the basis of scleral flap margins and fluid-filled cavities as described earlier. However, especially in cases of scleral flaps created for trabeculectomy at the temporal (or nasal) upper quadrants, the margins of the scleral flap are often difficult to see because 3D AS-OCT has only vertical and horizontal rasters and because the C-scan image plane does not always agree with the scleral plane. Therefore, we developed and installed a new software (CASIA bleb assessment software, version 4.0L; Tomey Corp.) into 3D AS-OCT for rotation of the data that we obtained (Fig. 3). The software allowed us to approve arbitrary cross-section images from the restructured 3D data, and thereby we could identify filtration openings on the plane along the scleral flap (see Supplementary Material and Supplementary Video S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10941/-/DCSupplemental).

**Statistical Analysis**

Characteristics of patients and type F blebs were compared by using Student's t-test or the χ² test. Values of P < 0.05 were considered statistically significant.

**RESULTS**

Included were 124 glaucomatous eyes (106 patients; 70 males and 36 females). The mean (±SD) was 66.3 ± 12.7 years of age. The diagnoses included primary open-angle glaucoma in 29 eyes, exfoliation glaucoma in 39 eyes, primary angle closure glaucoma in 7 eyes, and secondary glaucoma in 49 eyes. For each case, the IOP was determined as the average of three measurements at the time of the 3D AS-OCT examination. The mean (±SD) IOP value was 14.1 ± 5.6 mm Hg with an average of 0.3 ± 0.8 antiglaucoma medications. In addition, certain patients had undergone previous surgery: phacoemulsification (and intraocular lens implantation) in 60 eyes; vitrectomy in 15 eyes; and failed trabeculectomy in 20 eyes.

In 118 (95%) of the 124 eyes with filtration blebs, filtration openings were identified by the presence of pits and/or troughs in fluid-filled cavities in both horizontal and vertical rasters, and these findings were supported by the matching locations on the scleral flap margins in the C-scan images. Among the 118 eyes with filtration openings, 90 (76%) eyes showed only a single filtration opening (type F1), and the remaining 28 (24%) eyes showed two or more filtration openings (type F ≥ 2). Patients with type F1 blebs and those with type F ≥ 2 blebs demonstrated no statistically significant differences in IOP values, age, and postoperative follow-up periods (respectively, P = 0.101, 0.844, and 0.093). Next, we measured the distance between the top of the scleral flap and the filtration opening and called it the top filtration distance (TFD). By using the TFD, we plotted the filtration openings (Fig. 4). In 90 eyes with a single filtration opening (type F1), the openings were identified on the scleral flap margins and the TFD values (in mm) were 0 to 0.9, 1.0 to 1.9, 2.0 to 2.9, and ≥ 3.0, respectively, in 10 (11%), 36 (40%), 38 (42%), and 6 (7%) of the plots. Thus, in the middle (1.0 ≤ TFD < 3.0) of the scleral flap margins, 74 (82%) of the 90 eyes had a single...
filtration opening (Fig. 4A). Similarly, in 28 eyes with multiple filtration openings, in >80% of the cases, TFD values were in the middle of the scleral flap margins (Fig. 4B).

In the patients’ medical records, the pattern of incision and sutures used for closure of the conjunctival flap, limbal-based or fornix-based, was analyzed in relation to the locations of filtration openings (Figs. 4C, 4D). Interestingly, only 2 eyes (7%) among 72 eyes with fornix-based conjunctival flap had filtration opening with TFD value of ≥3.0 mm, whereas 4 eyes (22.2%) among 18 eyes with limbal-based conjunctival flap had a filtration opening at the site, and the ratios were significantly different between fornix-based and limbal-based eyes (P < 0.0001). Furthermore, the use of laser suture lysis was also analyzed in relation to the locations of filtration openings. Our analysis showed that the side without residual suture was not the dominant side for filtration openings. In addition, the pattern of TFD in the side without suture is not different from that with suture (P = 0.2348 and 0.2330: residual sutures were on the left and the right, respectively), suggesting that the side of laser suture lysis did not affect the side of the filtration openings (Figs. 4E, 4F).

Among 28 eyes categorized as F ≥ 2, 25 eyes (89%) showed filtration openings on both sides of the scleral flap margins. In the remaining 3 eyes, multiple openings were observed on the same side of the scleral flap margins. To elucidate whether the locations of filtration openings were similar, we analyzed the locations in the 25 F ≥ 2 eyes. As Figure 5 shows, 20 (80%) of the 25 eyes had differences in TFD values for both filtration openings of within 0.8 mm.

In the remaining 6 (5%) of the 124 eyes with filtration blebs, filtration openings could not be identified. The reasons for this failure to detect filtration openings include high reflectivity and/or elevated bleb wall (type H) in 2 eyes, and no fluid-filled cavities, because a sponge-like structure masked the filtration openings along the scleral flap margin.11 On the basis of our experience, in the present study, we used 3D AS-OCT and custom software to define filtration openings on the basis of pits and/or troughs in fluid-filled cavities in both horizontal and vertical rasters and corresponding C-scan images of scleral flap margins. However, to do this, we had to clearly see the scleral flap margins in the 3D AS-OCT images. It was relatively easy for us to identify the scleral flap margins when the flap was created at the uppermost region, but not in the temporal (or nasal) scleral flaps. This difficulty was caused by problems in

**DISCUSSION**

Our results showed that, in most eyes with functional filtration blebs, we could use 3D AS-OCT to identify the exact filtration openings from the scleral flap margin into the bleb space. The 3D technique allowed us to evaluate the surgical wounds and internal structures of the filtration blebs.9,10 In cases of bleb revision, we could confirm that aqueous humor could flow out from the patent internal ostium through a filtration opening during surgery. Also, preoperative and postoperative 3D AS-OCT images clearly showed the presence of filtration openings because of the pits and/or troughs in the fluid-filled cavities. C-scan images provided precise identification of the filtration openings along the scleral flap margin.11 On the basis of our experience, in the present study, we used 3D AS-OCT and custom software to define filtration openings on the basis of pits and/or troughs in fluid-filled cavities in both horizontal and vertical rasters and corresponding C-scan images of scleral flap margins. However, to do this, we had to clearly see the scleral flap margins in the 3D AS-OCT images. It was relatively easy for us to identify the scleral flap margins when the flap was created at the uppermost region, but not in the temporal (or nasal) scleral flaps. This difficulty was caused by problems in
identifying a complete scleral flap in the C-scan images. By using our novel custom software, however, we could easily rotate and tilt our 3D AS-OCT images on the screen display. Thus, in this study, our clinical data for glaucomatous eyes after trabeculectomy clearly showed the results of the filtration operations.

First, with the aid of our novel software and 3D AS-OCT, we precisely identified filtration openings in 118 (95%) of 124 eyes after trabeculectomy. In 90 (76%) of these 118 eyes, we found only a single filtration opening. This observation seems to agree with our previous results in which only a single filtration point was present on the scleral flap margin. In eyes after trabeculectomy, aqueous humor can flow out from any point on the scleral flap margins, at least during the early postoperative period. As the postoperative period continues, wound-healing activity causes more sealing of the scleral flap margin. During this sealing phase, the scleral flap margin, with no or scant aqueous flow, is quite likely easily sealed by wound healing. In contrast, sustainable filtration opening forms at the leakiest point on the scleral flap margin. As shown in Figure 4, most filtration openings were localized on the middle of the scleral flap margins. This predominant localization on the middle may result from the complicated combination of various factors for postoperative wound-healing activities. Among them, the pattern of incision and sutures used for closure of the conjunctival flap affected the localization of filtration openings. The bleb with fornix-based conjunctival flap had fewer filtration openings around the limbus (TFD ≥ 3.0 mm) compared with blebs with a limbal-based conjunctival flap. This difference may be caused by conjunctival sutures along the limbus that might close the fistula under the scleral flap near the limbus. These findings agree with the characteristic shape of clinically observed blebs with a fornix-based conjunctival flap, which has a more diffuse width and lower height compared with blebs that are limbal-based.

Second, in 28 eyes (24%), multiple filtration openings were observed on the margins of the same scleral flap. The formation of two or more filtration openings may be caused

FIGURE 3. Typical examples of blebs in which filtration openings were identified by using new software for rotation of the 3D AS-OCT data that we obtained. En face (top left images), vertical (top right images), horizontal (bottom left images), and C-scan (bottom right images) 3D AS-OCT images of blebs were shown. Red and blue lines indicate horizontal and vertical axes of 3D AS-OCT, respectively. Yellow lines indicate the z-axis of the 3D AS-OCT data, corresponding to the C-scan image. The rotation angle of each axis was shown as a scheme with a red square and an orange arrow in the right of the image, and was indicated as an actual value in the rightmost of the all images: 3D rotation. (A) 3D AS-OCT images without rotation. The margin of the scleral flap was difficult to see because the C-scan image plane did not agree with the scleral plane. (B) 3D AS-OCT images of the same eye after rotation. A filtration opening was clearly identified as pit and trough (arrows). (See Supplementary Material and Supplementary Video S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10941/-/DCSupplemental.)
by low wound-healing activity and/or the existence of similar aqueous flow resistance at multiple points. As clinicians, we should pay attention to the fact that the presence of multiple filtration openings cannot always ensure potent IOP-reducing effects, because no significant differences in IOP values were found between F1 and F ² groups. Also, some F1 eyes had an elevated IOP (>20 mm Hg). Thus, the presence of filtration openings in the blebs cannot guarantee enough IOP reduction to inhibit progression of glaucomatous optic neuropathy. Further investigation will be needed for determination of the clinical significance for IOP management.

Because our criteria are based on the presence of a fluid-filled cavity and a visible scleral flap margin, we could not identify filtration openings in some eyes with an elevated and/or high-reflectivity bleb (type H) or with a sponge-like structure masking the fluid-filled cavity (type S bleb). Neither type possessed fluid-filled cavities, but the significance of this finding differs for each type. Because AS-OCT has limited penetration,⑦⑧⑩⑫ bleb walls with high reflectivity and large fluid-filled cavities cause attenuation of light, which results in shadows. Although, as discussed by Kawana et al.,⑩ 3D AS-OCT using a 1.3-μm light source has greater penetration than does conventional OCT with an 830-nm light source, our present study suggested that, in some cases of elevated blebs (type H), this limited penetration of 3D AS-OCT still results in a failure to identify filtration openings. Also, because thick bleb walls with low reflectivity are thought to be associated with good IOP control, high reflectivity in thick bleb walls may suggest the occurrence of a scarring response and induce increased IOP because of inhibited filtration.⑧⑩⑫⑭ Also, one group of investigators reported that the internal reflectivity of filtration blebs is significantly correlated with IOP values.⑮ This result is supported by findings that, in encapsulated blebs, a thick bleb wall with high reflectivity was revealed by AS-OCT⑮ and that high reflectivity in AS-OCT images corresponds to the dense scar tissue that we observed in our previous
The existence of active filtration. However, in our previous reports, the description of a multiloculated structure suggested a multiloculated structure with low reflectivity. In previous between laser suture lysis and filtration openings.

To conduct a prospective study to elucidate the relationship near a filtration opening is cut by using a laser. We are planning correct, laser suture lysis may be more effective when a suture enlarge the already formed filtration route. If this hypothesis is cannot usually change the aqueous outflow route, but it can

In view of these data, we hypothesized that laser suture lysis
despite the pattern of remaining sutures and cut nylon sutures.

Formation of filtration openings occurs predominantly
during early postoperative periods, and one possible application of our knowledge about filtration openings to clinical practice may be in laser suture lysis. Previously, a group in Singapore reported an increased bleb height and decreased reflectivity on an AS-OCT image after laser suture lysis. In this present study, with the aid of 3D AS-OCT, we could compare the location of filtration openings. As an interesting finding, our preliminary prospective observation of a small case series of laser suture lysis showed that filtration openings after laser application were the same as those before laser treatment. Also, a scatterplot analysis of filtration openings in type F blebs demonstrated that opening locations were similar despite the pattern of remaining sutures and cut nylon sutures. In view of these data, we hypothesized that laser suture lysis cannot usually change the aqueous outflow route, but it can enlarge the already formed filtration route. If this hypothesis is correct, laser suture lysis may be more effective when a suture near a filtration opening is cut by using a laser. We are planning to conduct a prospective study to elucidate the relationship between laser suture lysis and filtration openings.

With regard to multiloculated blebs, type S blebs evidenced a multiloculated structure with low reflectivity. In previous reports, the description of a multiloculated structure suggested the existence of active filtration. However, in our previous experience of bleb revision, during the surgery we confirmed that a multiloculated bleb structure did not always mean active influx into the loculated part of the bleb. However, in general, thick and low-reflectivity bleb walls in 3D AS-OCT images are thought to be associated with good IOP control. Although the sample number was small in the present study, the IOP of four eyes with type S blebs was well controlled, which agrees with the above-mentioned data. This finding also suggests the presence of an active filtration opening, but it is quite likely concealed by the sponge-like structure, through which aqueous humor can easily flow. Also, because the multiloculated structure is often associated with increased bleb height, shadows as mentioned earlier may cause a vague image of the scleral flap margin. More resolving power may be required to establish new criteria for diagnosis of type S blebs.

Since the present study is cross-sectional, the indications for fornix-based or limbal-based were not defined by a unified way. This could affect the identification of the filtration opening, and the issue should be addressed in prospective studies in the future.

In conclusion, our present study showed that, in most eyes with functional filtration blebs, we could identify the precise filtration openings from the scleral flap margin into the bleb space with the use of 3D AS-OCT and our new custom software. The 3D technique allows complete evaluation of surgical wounds and internal structures of the filtration blebs, which suggests the clinical usefulness of 3D AS-OCT in treatment decision making after trabeculectomy.

**References**


