Filtering Blebs Using 3-Dimensional Anterior-Segment Optical Coherence Tomography
A Prospective Investigation

Sachi Kojima, MD; Toshihiro Inoue, MD; Kei-Ichi Nakashima, MD; Ayako Fukushima, MD; Hidenobu Tanihara, MD

**IMPORTANCE** Post trabeculectomy changes in bleb parameters measured using 3-dimensional (3-D) anterior-segment optical coherence tomography (OCT) remain uncharacterized and might be related to postsurgical intraocular-pressure (IOP) control.

**OBJECTIVE** To evaluate time-dependent post trabeculectomy changes in filtering bleb parameters using 3-D anterior-segment OCT.

**DESIGN, SETTING, AND PARTICIPANTS** This prospective observational study was conducted at Kumamoto University Hospital, Kumamoto, Japan. Patients with open-angle glaucoma who underwent uncombined fornix-based trabeculectomy at Kumamoto University Hospital between January 1, 2012, and October 31, 2012, were included. Twenty-nine eyes were enrolled, 23 of which were followed up for 1 year without additional glaucoma surgical procedures; 3 required additional glaucoma surgery.

**INTERVENTIONS** Imaging filtering blebs using 3-D anterior-segment OCT.

**MAIN OUTCOMES AND MEASURES** The primary end points were changes in bleb parameters including the position and width of the filtration openings on the scleral flap, the total bleb height, fluid-filled cavity height, bleb wall thickness, and bleb wall intensity, which were measured using 3-D anterior-segment OCT. The secondary end points were postsurgical IOP measured 0.5, 3, 6, and 12 months after trabeculectomy, and the effects of aqueous cytokine levels on the bleb parameters.

**RESULTS** We observed increased total bleb height (0.82 to 1.25 mm; difference: 95% CI, 0.10 to 0.75; \( P = .01 \)), bleb wall thickness (0.46 to 0.61 mm; difference: 95% CI, 0.02 to 0.28; \( P = .03 \)), and distance from the top of the scleral flap to the filtration opening (1.69 to 2.16 mm; difference: 95% CI, 0.28 to 0.70; \( P < .001 \)), as well as decreased width of the filtration opening (2.08 to 1.12 mm; difference: 95% CI, −1.75 to −0.49; \( P = .002 \)) between 0.5 and 12 months post trabeculectomy. The filtration openings tended to close from the fornix side of the scleral flap during the wound healing process. Moreover, the width of the filtration opening at 0.5 months post trabeculectomy correlated with the IOP at 12 months (\( P = .02 \)). The aqueous humor level of monocyte chemoattractant protein-1 was correlated with the width of the filtration opening at 3 and 6 months post trabeculectomy.

**CONCLUSIONS AND RELEVANCE** The width of the filtration opening at 0.5 months post trabeculectomy correlated with the IOP at 12 months. The width of the filtration opening at the early stage may be a prognostic factor for long-term IOP control. Large-scale studies with longer follow-up periods are required.

**Author Affiliations:** Department of Ophthalmology, Kumamoto University, Kumamoto, Japan. 
**Corresponding Author:** Toshihiro Inoue, MD, Faculty of Life Sciences, Department of Ophthalmology, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan (noel@da2.so-net.ne.jp).
Intraocular-pressure (IOP) reduction has been the key to glaucoma management, and trabeculectomy has been regarded as the standard surgical modality. Reduction of IOP after trabeculectomy is related to the formation and morphology of filtering blebs; therefore, the structure of the filtering blebs has been assessed clinically using many imaging methods such as slitlamp examination, anterior-segment photography, ultrasound biomicroscopy, and advanced imaging technologies including optical coherence tomography (OCT). In particular, 3-dimensional (3-D) anterior-segment OCT is useful to identify the aqueous outflow route after trabeculectomy and to explore the morphology of the entire bleb.

In our previous study, we showed that the potential filtration opening identified by 3-D anterior-segment OCT images corresponded to the intraoperative findings of the filtration point. Moreover, with the aid of 3-D anterior-segment OCT and CASIA bleb-assessment software, we identified the exact filtration opening on the margins of the scleral flap created by trabeculectomy in most cases. These results indicated that 3-D anterior-segment OCT might be a useful tool for assessing the internal morphology of filtering blebs, thereby allowing us to potentially predict surgical results.

Methods

Patients
All investigations adhered to the tenets of the Declaration of Helsinki. This prospective observational study was approved by the institutional review board and ethics committee of Kumamoto University and was registered with the University Hospital Medical Information Network Clinical Trials Registry of Japan (ID UMIN000006008; date of access and registration, July 21, 2011). Each patient provided written informed consent for participation in the study. This study included patients with open-angle glaucoma who underwent uncomplicated fornix-based trabeculectomy with mitomycin C at the Kumamoto University Hospital between January 1, 2012, and October 31, 2012, and provided analyzable 3-D anterior-segment OCT images of filtering bleb 0.5 months after trabeculectomy (Figure 1). Patients who had undergone ocular surgical procedures previously were excluded from this analysis, except for those who underwent phacoemulsification before trabeculectomy. When both eyes in a patient met the inclusion criteria, only the eye that was treated first was included in this analysis.

Surgical Procedures
Two experienced surgeons (T.I. and H.T.) conducted all surgical procedures included in the present study in an identical manner. Aqueous humor samples were collected at the beginning of surgery before the incisional procedures, and all trabeculectomies followed the same surgical procedure, as described previously. Postoperatively, all patients received a similar topical medical regimen: 1% topical atropine sulfate for 1 week as well as 0.1% topical betamethasone and 1.5% levofloxacin for approximately 3 months. Laser suture lysis was not conducted during the 0.5- to 12-month observational period after surgery. The surgeons who conducted the primary trabeculectomy judged the necessity for postoperative glaucoma eye drops based on the IOP values, the levels of visual field disturbance, and bleb appearances by slitlamp without knowing the bleb parameters in the 3-D anterior-segment OCT images. When the postoperative IOP could not be controlled by the maximum dose of eye drops, additional glaucoma surgical procedures were conducted.

Data Collection
Baseline IOP was determined as the average of 3 measurements taken during 3 consecutive visits before trabeculectomy. At 0.5, 3, 6, and 12 months after trabeculectomy, filtering bleb images were acquired using 3-D anterior-segment OCT (CASIA; Tomey Corp, as described previously. At each visit, eyes were examined by slitlamp, and IOP values were measured using a Goldmann tonometer between the hours of 1 PM and 4 PM. When additional glaucoma surgical procedures (including needling) were conducted, the eye was excluded from the analysis for time-dependent changes in the bleb parameters.

3-Dimensional anterior-segment OCT images of filtering blebs were evaluated using CASIA Bleb Assessment Software version 4.0L (Tomey), as described previously. Briefly, we rotated the 3-D anterior-segment OCT images to make the C-scan image plane agree with the scleral plane. Next, we identified the filtration opening, which was defined by the presence of pits and/or troughs in the fluid-filled cavities in both the horizontal and vertical rasters and by a corresponding C-scan image of scleral flap margins in the bleb (Figure 2; Video). In our previous study, we categorized the 3-D anterior-segment OCT images of filtering blebs based on the visibility of the filtration opening. Blebs in which filtration openings could be identified by 3-D anterior-segment OCT were termed type F. The numeral indicated the number of filtration openings (eg, F1 represents a bleb with a single filtration opening as identified by 3-D anterior-segment OCT and F2 describes a bleb in which ≥2 filtration openings were noted). Blebs in which the filtration openings could not be identified were subdivided into 2 groups: those masked by a high-reflectivity and/or
elevated bleb wall (type H) or by a spongelike structure with low reflectivity (type S). Flat nonfunctioning blebs were defined as type N. For type F blebs, we examined consecutive filtration openings on the scleral flap using custom software and determined the distance over which they occurred (filtration opening width). Moreover, the center of the opening was defined as the top filtration distance (TFD) (Figure 3A). When the eye had 2 or more filtration openings, the widths were summed and the TFDs were averaged. Three reviewers, who were blinded to the clinical background factors, evaluated the complete 3-D anterior-segment OCT images of the internal structure of the filtering blebs and associated findings and independently assessed the images. When opinions differed among the reviewers, a majority decision was used. After identification of the filtration openings, the reviewers measured other bleb parameters including the total bleb height, fluid-filled cavity height, bleb wall thickness, and bleb wall intensity (Figure 3B) in the horizontal image at the center of the filtration opening or at the center of the scleral flap when the filtration opening was unidentifiable. The mean values of the bleb parameters from 3 independent reviewers were used for further analysis.

Multiple Immunoassay Analyses
An xMAP (Luminex) multiplex bead-based immunoassay was used, as described previously,15 to determine the levels of 4 cytokines and 4 growth factors in the aqueous humor: interleukin (IL)-6, IL-8, monocyte chemoattractant protein–1 (MCP-1), tumor necrosis factor–α, epidermal growth factor, vascular endothelial growth factor, platelet-derived growth factor (PDGF)-AA, and PDGF-AB/BB. Finally, we analyzed the correlations of bleb parameters with aqueous humor cytokine levels.

Statistical Analysis
Data were analyzed using JMP version 8 (SAS Institute). The patients' characteristics and bleb parameters were compared using the Wilcoxon signed-rank test or the χ² test between the groups. Time-dependent changes in the bleb parameters were calculated using paired 2-tailed t tests. Correlations of the bleb parameters with the IOP value or aqueous humor cytokine levels were assessed by calculating Spearman correlation coefficients. Multiple stepwise-regression analyses were performed to determine the bleb parameters affecting the IOP control at 12 months after trabeculectomy. After the first calculation, factors with $t^2$ values less than 2 were excluded, and recalculation proceeded with the remaining factors. $P < .05$ was considered to indicate statistical significance.

Results

Patients
Twenty-nine eyes (29 patients) were enrolled in this study between January and October 2012; 23 eyes (79.3%) that completed 1-year investigations without additional ocular surgical procedures were placed in group A, and the time-dependent changes in the bleb parameters were analyzed. Three eyes (10.3%) that required additional glaucoma surgical proce-
dures within 1 year after trabeculectomy were placed in group B and the early bleb parameters were compared with those in group A. Three eyes (10.3%) were excluded from the analysis for the following reasons: loss to follow-up because of admission to another hospital for general disease before 3 months posttrabeculectomy (1 eye) and protocol deviations (failure to acquire reliable anterior-segment OCT images at 6 months after trabeculectomy) during the observational period (2 eyes) (Figure 1). eTable 1 in the Supplement lists the baseline characteristics of the patients. Needlings for 2 eyes and a repeat trabeculectomy for 1 eye were conducted in cases in group B. The average IOP value in group B before repeat glaucoma surgical procedures was 28 mm Hg with glaucoma eye drops. No severe complications causing permanent visual loss were observed.

**Time-Dependent Changes in Bleb Parameters After Trabeculectomy**

Lower IOP values were observed at 3, 6, and 12 months posttrabeculectomy than at 0.5 months posttrabeculectomy in 23 eyes in group A (12.6 mm Hg, difference: 95% CI, 1.75-6.42, P = .001; 13.2 mm Hg, difference: 95% CI, 2.75-6.56, P < .001; and 13.0 mm Hg, difference: 95% CI, 2.29-6.58, P < .001, respectively). Additionally, a significantly greater number of eye drops was used at 12 months after surgery than at 0.5 months postsurgery (8.5 mm Hg; difference: 95% CI, 0.02-1.12; P = .045). Significant changes in bleb type composition were observed at 6 and 12 months after trabeculectomy compared with that at 0.5 months postsurgery (P = .03 and P = .003, respectively) (Figure 4A). The rate of F1 tended to increase over time, while that of F2 decreased accordingly, suggesting that the filtration openings on the scleral flap exhibited continuous closing after trabeculectomy. To further explore time-dependent changes in the filtration openings, we compared the TFDs and the filtration opening widths (Figure 4B and C). Compared with the values at 0.5 months, larger TFDs were observed at 3, 6, and 12 months after surgery (2.07, difference: 95% CI, 0.14-0.65, P = .004; 2.13, difference: 95% CI, 0.21-0.72, P = .003; and 2.16, difference: 95% CI, 0.28-0.70, P < .001, respectively), and smaller filtration opening widths were observed at 6 and 12 months postsurgery (1.35, difference: 95% CI, −1.57 to −0.35, P = .005 and 1.12, difference: 95% CI, −1.75 to −0.49, P = .001, respectively). Interestingly, the fornix-side edge of the filtration opening shifted toward limbus (0.66 mm and 1.60 mm at 0.5 and 12 months, respectively; difference: 95% CI, −1.75 to −0.49, P = .002, respectively). As shown in the schematic representation of the filtration opening changes (Figure 4D), these results suggest that filtration openings tend to close from the fornix side of the scleral flap. Additionally, we compared the total bleb height, fluid cavity height, wall thickness, and wall intensity at each point after trabeculectomy (Figure 4E-H).

**Prognostic Factors of Postsurgical Intraocular Pressure Control**

To identify the prognostic factors of postsurgical IOP control among the bleb parameters, we evaluated the correlation of each bleb parameter at 0.5 months after trabeculectomy with the postoperative IOP at 12 months. The width of filtration openings at 0.5 months after trabeculectomy was negatively associated with the IOP value at 12 months (R² = 0.249; t = −2.64; P = .02) (Figure 5A). Intraocular pressure values at
Figure 4. Time-Dependent Changes in the Bleb Parameters Assessed by 3-Dimensional Anterior-Segment Optical Coherence Tomography

A, Bleb types classified along 3-dimensional anterior-segment optical coherence tomographic images. Time-dependent changes in bleb parameters (B-H).  
\( p < .05 \)
\( p < .01 \) compared with the value 0.5 months after trabeculectomy.
12 months posttrabeculectomy were obtained during treatment with glaucoma eye drops in 4 eyes; the mean (SD) IOP values were 18.0 (4.3) mm Hg in the eyes with glaucoma eye drops and 11.9 (4.4) mm Hg in the eyes without eyedrops. In contrast, we found no correlations in the other bleb parameters at 0.5 months after surgery with the IOP value at 12 months. Multiple regression analysis revealed that the width of the filtration openings was the only factor related to IOP at 12 months among the bleb parameters at 0.5 months posttrabeculectomy (proportion of variance, 6.958; $P = .02$) (eTable 2 in the Supplement).

**Correlation of Bleb Parameters With Aqueous Humor Cytokine Levels**

Of the 26 eyes, 19 provided sufficient volumes of aqueous humor to measure cytokine levels. The mean (SE) levels of IL-6,
IL-8, MCP-1, tumor necrosis factor-α, PDGF-AA, and vascular endothelial growth factor were 109.7 (102.4), 14.0 (2.1), 1469.4 (104.3), 1.0 (0.1), 18.7 (1.2), and 52.8 (6.4) pg/mL, respectively; PDGF-AB/BB was below detectable levels in all samples. The Table summarizes the cytokines that were correlated with the bleb parameters. Tumor necrosis factor-α levels were negatively correlated with the total bleb height and fluid-filled cavity height, whereas the bleb wall intensity was positively correlated. In contrast, MCP-1 levels were negatively correlated with the bleb wall thickness and the width of the filtration openings. Additionally, the wall thickness was negatively correlated with vascular endothelial growth factor and IL-8 levels.

### Table. Correlations of Bleb Parameters With Aqueous Humor Cytokine Levels

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time After Surgery, mo</th>
<th>0.5</th>
<th>3</th>
<th>6</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bleb height</td>
<td>Cytokine</td>
<td>VEGF</td>
<td>TNF-α</td>
<td>TNF-α</td>
<td>TNF-α</td>
</tr>
<tr>
<td>t value</td>
<td>−2.46</td>
<td>−4.35</td>
<td>−3.48</td>
<td>−2.63</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>.03</td>
<td>&lt;.001</td>
<td>.003</td>
<td>.02</td>
<td></td>
</tr>
<tr>
<td>Fluid-filled cavity height</td>
<td>Cytokine</td>
<td>TNF-α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleb wall thickness</td>
<td>Cytokine</td>
<td>MCP-1, VEGF</td>
<td>IL-8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>−2.38, −2.34</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>Both .03</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleb wall intensity</td>
<td>Cytokine</td>
<td>TNF-α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>NS</td>
<td>2.68</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Width of filtration openings</td>
<td>Cytokine</td>
<td>MCP-1</td>
<td>MCP-1</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>−2.45</td>
<td>−2.47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>.03</td>
<td>.03</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** IL, interleukin; MCP-1, monocyte chemoattractant protein-1; NS, no significant cytokine levels; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

Comparison of Bleb Parameters Between Groups A and B

The bleb parameters at 0.5 months posttrabeculectomy in group B, which resulted in early surgical failure, were compared with those in group A (Figure 5B). Among the parameters, only the width of the filtration opening differed significantly between the groups (P = .003), indicating that the smaller filtration opening width at 0.5 months after trabeculectomy may result in subsequent early surgical failure in terms of IOP control.

Discussion

A filtering bleb can become nonfunctional in a time-dependent manner owing to reduced aqueous outflow volume caused by excess wound healing of the subconjunctival tissue and scleral flap.18,19 In this study, both the IOP and the number of eye drops increased significantly within 12 months after trabeculectomy. This result is probably related to the subconjunctival wound healing process, as just described. Our results suggest that the filtration opening tends to close from the fornix side of the scleral flap (Figure 4D). The earlier closure of the fornix-side opening might be due to the distance from the window through the anterior chamber because the fornix-side opening is more distant from the window compared with the limbal-side opening. Therefore, the current of aqueous humor might be slower and more prone to closure at the fornix side.

A limitation of the present study was the relatively small sample size; in particular, group B included only 3 eyes. Another limitation was the exclusion of 10.3% of enrolled patients within 1 year. Considering the chronic pathological progression of glaucoma, the 1-year follow-up results are not always consistent with the long-term results. In addition, as described in the Methods section, at least 3 different reviewers evaluated the complete 3-D images of the internal structure of the filtration blebs and associated findings and independently assessed the filtration openings in the 3-D anterior-segment OCT images to avoid interobserver variability in identifying filtration openings. When the opinions differed among the reviewers, the majority decision was chosen. Because these assessments tended to be qualitative, reproducibility was not statistically assessed. Considering these issues, we must interpret the data carefully because they were not truly conclusive in terms of long-term surgical success. Therefore, larger-scale studies with longer follow-up times are required.

The total bleb height increased significantly until 6 months, as did the wall thickness until 3 months after surgery (Figure 4E and G). These bleb parameter values were almost the same be-
between 6 and 12 months after surgery, suggesting that the bleb formation was under construction during the first 6 months along the inflow of the aqueous humor and had matured at 6 months after surgery. Meanwhile, we observed a decreased width of filtration opening (Figure 4C) between 6 and 12 months after surgery. Additionally, a shift in bleb type from F2 to F1 suggested that the number of filtration openings decreased during that period (Figure 4A). Therefore, chronic-wound healing may proceed with unchaged bleb appearance, resulting in decreased aqueous outflow from the anterior chamber into the bleb. If this is the case, it might not be possible to predict future bleb failure by slitlamp examination, and the width of the filtration opening on the scleral flap may be a more-sensitive prospective factor of the surgical failure than bleb appearance. Thus, information about internal structures of blebs may be valuable for postsurgical follow-up, and 3-D anterior-segment OCT could be a useful tool with which to improve surgical results. For instance, we may determine the optimal timing of laser suture lysis or needling bleb revision based on the internal bleb parameters measured by 3-D anterior-segment OCT at an early postsurgical stage. It may also be possible to suture the scleral flap less tightly to achieve wide filtration openings after surgery. However, a loosely sutured scleral flap may lead to severe hypotony in the early postsurgical stage; therefore, the most suitable procedure for wider filtration openings would be difficult to establish.

The univariate correlation analysis (Figure 5A) and the multiple regression analysis indicated that longer width in filtration openings on the scleral flap at 0.5 months were correlated with lower IOPs at 12 months post trabeculectomy (eTable 2 in the Supplement). Moreover, the eyes that required repeat glaucoma surgical procedures had smaller widths of filtration openings at 0.5 months (group B) compared with those that did not require repeat surgery (group A) (Figure 5C), whereas the other bleb parameters were not different between the groups (Figure 5B and D-G). Considering these results, maintaining large filtration openings during the early stage may be important to ensure identical surgical results at later stages. From this perspective, a larger filtration opening could maintain the volume of aqueous outflow, and the aqueous flow itself may inhibit closure of the opening.

In this study, MCP-1 levels in the aqueous humor were negatively correlated with the width of the filtration openings at 3 and 6 months after surgery (Table). Monocyte chemoattractant protein-1 is known to recruit and activate inflammatory cells; thus, it can induce inflammatory responses and fibrosis in the filtering bleb and may enhance the closure of the filtration openings on the scleral flap. Our previous study revealed that the MCP-1 level in the aqueous humor was a prognostic factor for the outcome of trabeculectomy using the Cox proportional hazards model.20 Furthermore, IL-6, IL-8, and MCP-1 levels were closely correlated in the analysis of samples from 73 eyes with open-angle glaucoma.15 Considering that the filtration opening widths at 0.5 months were correlated with the IOP values at 12 months post trabeculectomy, aqueous MCP-1 in consort with other cytokines may play a significant role in the wound healing process and subsequent IOP control after trabeculectomy.

The manner of creating the conjunctival flap affects the bleb morphology and its internal structure. Hamanaka et al21 analyzed both limbal- and fornix-based trabeculectomy and found that IOP control in limbal-based trabeculectomy seemed to be more dependent on large, thinned-wall blebs than in fornix-based trabeculectomy. Furthermore, the distribution patterns of the filtration openings were different between fornix- and limbal-based blebs. Thus, it is intriguing to compare the time-dependent changes in bleb parameters measured by 3-D anterior-segment OCT between the bleb types.

Conclusions

To our knowledge, this is the first study to evaluate time-dependent changes in filtration openings on the scleral flap after trabeculectomy using 3-D anterior-segment OCT in the peer-reviewed literature. The filtration opening width at the early stage after trabeculectomy may be a prognostic factor for long-term IOP control.

REFERENCES


**OPHTHALMIC IMAGES**

**Bead Block Embolization of a Retinal Arteriole**

Yasser M. Elshatory, MD, PhD; Vinay A. Shah, MD

An 11-year-old child experienced blurred vision of the left eye after a Bead Block (size range, 100-300 μm) embolization procedure to debulk a maxillary tumor. A, Fundus photograph. B, Early-phase fluorescein angiography showed an arteriolar occlusion with a visible round gray embolus. Visual acuity stabilized to 20/100. C, Optical coherence tomography of the optic nerve allowed measurement of the bead (238 μm).

**Correction:** This article was corrected for a typographical error on December 22, 2014.