# Noninvasive Evaluation of Phase Retardation in Blebs After Glaucoma Surgery Using Anterior Segment Polarization-Sensitive Optical Coherence Tomography

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**Purpose.** Evaluation of bleb morphology using anterior segment optical coherence tomography (OCT) can offer important information regarding bleb function after glaucoma surgery. However, analysis of tissue properties, such as scar fibrosis of blebs, is difficult with conventional OCT. The birefringence of the blebs as susceptible measure of fibrosis scar was evaluated using polarization-sensitive OCT (PS-OCT) and its relation with bleb function was assessed.

**Methods.** One hundred and fifty-three blebs of 122 patients that had undergone trabeculectomy or an Ex-Press tube shunt were examined. Also, in 14 blebs of 12 patients, consecutive measurements were performed for 2 months after surgery. The birefringence of blebs was evaluated by measuring alteration of phase retardation using PS-OCT. Functionality of the bleb was classified according to IOP and medication. The bleb morphology in terms of size and characteristics was evaluated using three-dimensional (3D) cornea and anterior segment OCT.

**Results.** The alteration of phase retardation of blebs had the largest impact on bleb functionality than bleb morphology as shown by multiple regression analysis. In consecutive measurements, no blebs showed abnormal phase retardation until 1 week after surgery. Some blebs showed partial increase of phase retardation at 1 month after surgery.

**Conclusions.** Intrableb fibrosis can be noninvasively evaluated with PS-OCT. Evaluation of birefringence by measuring phase retardation alterations using PS-OCT suggests new approaches for the postoperative management of glaucoma blebs regarding antifibrotic treatment for preventing IOP increases.

Keywords: polarization-sensitive optical coherence tomography, birefringence, fibrosis scar of blebs



Trabeculectomy is currently the most common glaucoma-filtering surgery. In this procedure, introduced in 1968 by Cairns,[1] the IOP is lowered by allowing drainage of aqueous humor from the anterior chamber of the eye to a bleb created between the conjunctiva and the sclera, from which the fluid is absorbed by the vasculature. In this way, IOP might be decreased to levels that can prevent further progression of visual field deterioration.[2] The Ex-Press glaucoma filtration device (Alcon, Fort Worth, TX, USA) is a nonvalved, implantable drainage device made of medical-grade stainless steel. The device is implanted below the scleral flap to drain aqueous humor from the anterior chamber to the subconjunctival space, creating a conjunctival filtration bleb, similar to trabeculectomy.[3] Both trabeculectomy and Ex-Press treatment require the exposure of a large area of sclera, and the induced damage to the subconjunctival tissues in the filtering area might cause scarring from fibrosis. Scarring of the conjunctiva can evolve with bleb encapsulation that prevents aqueous outflow. Eventually, this scarring, fibrosis, and encapsulation of the bleb results in poor IOP control,[4] which may need needling or revision to restore the filtering function of the bleb. The fibrosis has a central role in the scarring process and failure of the filtration bleb.[5] Many adjunctive antifibrotic agents that attempt to counteract this conjunctival scarring have been reported. Intraoperative use of two common antifibrotic agents, mitomycin C and 5-fluorouracil, have been shown to improve the outcomes of trabeculectomy.[6–8] If bleb fibrosis was recognized before IOP increase, additional antifibrotic treatment could be useful postoperatively. Recently, some instruments, such as ultrasound biomicroscopy, confocal microscopy, and anterior segment optical coherence tomography (OCT), evaluated the morphology of the filtration bleb.[9–13] However, they could not directly evaluate bleb scarring and fibrosis.

Polarization-sensitive optical coherence tomography (PS-OCT), which is a functional extension of OCT, can improve the contrast of the OCT image and can evaluate birefringence by imaging phase retardation of biological fibrous tissues.[14] Changes in the organization of fibrous tissues alter their birefringence and, hence, PS-OCT is useful for studying


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ultrastructural microscopic structural changes in fibrous tissues.

Although a previous study reported that PS-OCT detected the phase retardation of the filtrating bleb in an animal model and in a small number of patients in a clinical pilot study,[15] a clinical investigation evaluating the relationship between bleb function and alteration of phase retardation in the filtration bleb has not yet been conducted. In this study, we first investigated the alteration of phase retardation in filtering blebs, and then we assessed the relationship between phase retardation and bleb function using PS-OCT.

## Methods

### Patients

We examined 153 blebs of 122 patients with glaucoma at Tsukuba University Hospital (Ibaraki, Japan) between April 2013 and October 2013. Moreover, consecutive measurements were performed in 14 blebs of 12 patients (eight men, four women) for 2 months after surgery. Table 1 summarizes the demographic and clinical characteristics of patients. To evaluate normal conjunctiva by PS-OCT, we also examined 10 eyes of 10 healthy subjects (68.6 ± 7.7 years, mean ± SD) without ocular abnormalities other than cataract and mild refractive error. The research followed the tenets of the Declaration of Helsinki and written informed consent was obtained from each participant. The study was approved by institutional review board of the Tsukuba University.

Trabeculectomies were performed as follows: a fornix based conjunctival flap and a 4-mm wide half-layer square scleral flap were created. Two to four small pieces of cellulose sponges soaked in topical 0.04% mitomycin C were applied to exposed tissues, between the Tenon capsule and the posterior surface of the conjunctiva, which are adjacent to episcleral tissue, and the scleral flap for 3 minutes. After 3 minutes all sponges were removed and the entire surgical field was irrigated thoroughly with 200 mL of physiologic saline solution. A trabecular block was excised, and a peripheral iridectomy was then performed. The scleral flap was closed with four interrupted 10-0 nylon sutures (MANI, Tochigi, Japan), and the conjunctiva was closed tightly with interrupted 10-0 nylon sutures. For the Ex-Press tube shunt procedure, a wound was created with a 27-G needle and the shunt device was then placed below the scleral flapthrough the ostium opened with the needle. Topical 0.04% mitomycin C was applied in the same way as for the trabeculectomy. The scleral flap and the conjunctival flap were sutured with 10-0 nylon.

To check the appearance of the blebs, patients were evaluated with slit-lamp examination and color photographs of the anterior ocular segment were analyzed. All IOP measurements were performed using Goldmann applanation tonometry. Three-dimensional (3D) cornea and anterior segment OCT (3D CAS-OCT; CASIA, Tomey Corp, Aichi, Japan) based on swept-source OCT technology was also used.

A custom-made 3D anterior segment PS-OCT built by the Computational Optics Group (University of Tsukuba) was used in this study.[15] The system was also based on swept-source OCT technology. Its light source sweeps over 110-nm across a center wavelength of 1.3 μm with a sweeping frequency of 30,000 Hz. In addition to conventional OCT images, PS-OCT provides the microstructural properties of the tissue by revealing its birefringent property. Phase retardation is one of the parameters that define birefringence. A birefringent material including birefringent tissue is known to have two special polarization states so called characteristic polarizations. If the polarization state of a light is one of the characteristic polarizations, the polarization state cannot be altered by the tissue birefringence, only the phase and the intensity are altered. Assuming two beams with the two characteristic polarizations propagate through a birefringent tissue, the phase difference between the two beams increases as the propagation distance increases. The phase retardation is defined as the phase difference induced by the tissue birefringence. Since stronger birefringence creates larger phase retardation, the phase retardation can be used as an indirect measure of birefringence. It should be noticed that the phase retardation is a cumulative quantity, which is affected by the birefringence of the tissue along the entire depth of the light propagation. In the case of PS-OCT, the phase retardation measured at a certain point is affected by the birefringence of all tissues located superiorly to the point of measurement. According to these properties of phase retardation, in the phase retardation image, the local alteration of color (phase retardation) along the depth indicates the existence of birefringence.

The PS-OCT system used in this study was based on tomographic Jones matrix measurement and is referred to as Jones matrix OCT.[16,17] The Jones matrix OCT requires use of two incident polarization states of light for full recovery of the Jones matrix of the sample. In our PS-OCT, it was achieved using an electro-optic modulator.[15,17] The effect of fiber birefringence was cancelled out by multiplying the tissue Jones matrix by the inverse of the Jones matrix from a reference surface,[18] which, in our case, was the air tissue interface. Upon matrix diagonalization, the Jones matrix gave the round-trip phase retardation, which was represented over the range 0 to π.[16,17] The details of this PS-OCT are described elsewhere.[15]

Internal bleb structures were assessed with 3D CAS-OCT and PS-OCT. For the 3D CAS-OCT and PS-OCT assessments, the patient was asked to look down, and the examiner gently elevated the upper lid to expose the filtration bleb. We took care not to exert any pressure on the globe and the bleb. Measurements were performed by experienced examiners SF and SB for both CAS-OCT and PS-OCT. Each filtering bleb was scanned at least twice using 3-D CAS-OCT and PS-OCT and the best image was selected for subsequent analyses. Blebs were scanned with a horizontal, fast raster pattern on scanning ranges of 12 × 12 mm$^2$, including 512 horizontal × 128 vertical A-lines in both instruments. Tomographic volumes of a

**Table 1.** Demographic and Clinical Characteristics of Patients

| | Observational Case Series | Consecutive Measurements |
|---|---|---|
| *N* eyes (patients) | 153 (122) | 14 (12) |
| Age, y (range) | 68.0 ± 12.7 (26–83) | 67.4 ± 10.4 (47–80) |
| Sex (male/female), *n* | 75/47 | 8/4 |
| Glaucoma category, *n* (%) | | |
| Primary open-angle glaucoma | 73 (47.7) | 4 (28.6) |
| Primary angle-closure glaucoma | 12 (7.9) | 0 (0) |
| Pseudoexfoliation glaucoma | 36 (23.5) | 6 (42.9) |
| Secondary glaucoma | 32 (20.9) | 2 (14.3) |
| Neovascular glaucoma | 0 (0) | 2 (14.3) |
| Surgery | | |
| Trabeculectomy, *n* | 114 | 9 |
| Ex-press tube shunt surgery, *n* | 39 | 5 |

Values are presented as the mean ± SD.

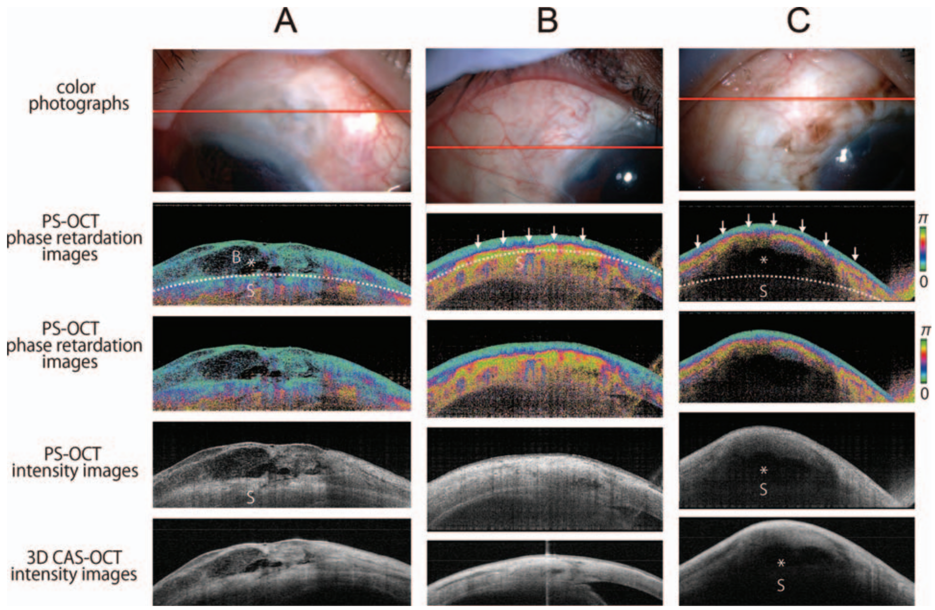


**Figure 1.** Three cases of representative photographs, phase retardation, and intensity images with PS-OCT, and intensity images with 3D CAS OCT. Polarization-sensitive OCT phase retardation grade 1 bleb (**A**) and two cases of PS-OCT phase retardation grade 3 blebs (**B**, **C**). *Dotted line* indicates boundary between conjunctiva and sclera. No abnormal phase retardation was found in grade 1 bleb (**A**) and irregular and abnormal phase retardation was found in grade 3 blebs (*arrow* [**B**, **C**]).

structural OCT and PS-OCT (cumulative phase–retardation) were obtained by a single scan.

The obtained OCT images were analyzed both qualitatively and quantitatively as described previously[12] (Supplementary Material). Horizontal and vertical length of the internal fluid-filled cavity, height of internal fluid-filled cavity, maximum and minimum bleb wall thickness, total bleb height, volume of internal fluid-filled cavity, volume of hyporeflective area, number of microcysts, rate of lake under the sclera flap (LUSF), and rate of filtration opening on the sclera flap were measured.

The bleb area was defined by the intensity images of 3D CAS OCT and PS-OCT. The intensity image and phase retardation images of PS-OCT were created from the same spectral data set and, hence, were perfectly registered to each other. Based on the PS-OCT phase retardation images obtained, the filtering blebs were classified into three grades. Each PS-OCT image was examined and graded by three experienced ophthalmologists (SH, GK, KI). All of them were blinded to the results provided by the other graders and also to the classification of the bleb function. According to those results, an average grade was calculated for each bleb and the resulting numbers were rounded off. The bleb birefringence grade was defined as "Grade 1" if the abnormal phase retardation evaluated in the bleb was one-third or less, as "Grade 2" if abnormal phase retardation was more than one-third but two-thirds or less, and as "Grade 3" if abnormal phase retardation was more than two-thirds. The phase retardation was identified as depth-oriented alteration in a PS-OCT image.

The functionality of the bleb was classified based on IOP and medications according to a previous study.[13] Bleb function was defined as "good" if the IOP was equal or lower than 14 mm Hg without glaucoma medication, as "moderate" if the IOP was higher than 14 mm Hg but equal or lower than 18 mm Hg or without glaucoma medication, and as "poor" if the IOP was higher than 18 mm Hg or if glaucoma medication had been prescribed to treat IOP measuring higher than 18 mm Hg on two consecutive postoperative visits.

For all 153 blebs, the mean and SD values for patient characteristics and also for each parameter of intrableb structures evaluated by 3D CAS-OCT were calculated and compared. A one-way ANOVA was performed to compare age, period after surgery, IOP, and each OCT parameter among each bleb function group. The rate of PS-OCT phase retardation grade, the LUSF, and the filtrationopening on the sclera flap were examined by a $\chi^2$ test for independence among good, moderate, and poor bleb function groups. Multiple regression analyses were performed in 153 blebs to investigate the relationship between these parameters and bleb function. Explanatory variables were age, period after operation, PS-OCT phase retardation grade, and 3D CAS-OCT parameters. Multiple regression analyses were also performed including 114 eyes in the trabeculectomy group and 39 eyes in the Ex-Press tube shunt group. All tests of associations were considered statistically significant if $P$ was less than 0.05.The analyses were carried out using a commercial software package (StatView software, version 5.0; SAS, Inc., Cary, NC, USA).

## Results

### Phase Retardation of Blebs After Glaucoma Surgery

Representative photographs, phase retardation and intensity images with PS-OCT and intensity images with 3D cornea and anterior segment OCT (3D CAS-OCT) are shown in Figure 1. A case of PS-OCT phase retardation Grade 1 blebs is shown in Figure 1A. A 72-year-old female patient had a good bleb in her left eye. Seven years after trabeculectomy, the IOP was 7 mm Hg without glaucoma medication (Fig. 1A). In a PS-OCT phase retardation Grade 1 bleb, the vast green region above the sclera and no abnormal birefringence was found in the phase retardation image, whereas the sclera showed strong phase retardation. Diffusely thickened blebs with a large hyporeflective area and multiple microcysts were observed in the intensity images with PS-OCT and 3D CAS-OCT. Two cases of a PS-OCT phase retardation Grade 3 bleb are shown in Figures

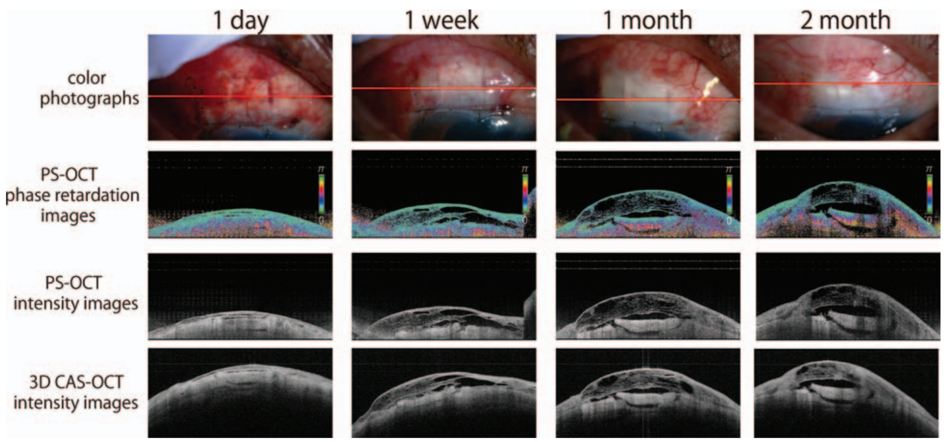


FIGURE 2. Photographs, phase retardation and intensity images with PS-OCT, and intensity images with 3D CAS OCT at 1 day, 1 week, and 1 and 2 months after surgery. Cases of no increase of phase retardation after surgery.

1B and 1C. Case 1: A 66-year-old male patient had a flat bleb in his right eye, then two years after trabeculectomy, the IOP was 16 mm Hg with full glaucoma medication (Fig. 1B). Case 2: Four years after trabeculectomy, a 56-year-old male with a collapsed flat bleb (Fig. 1B) had an IOP of 21 mm Hg with full glaucoma medication (Fig. 1C). In this bleb, a fluid pool was almost invisible. The whole region of the conjunctiva presented with irregular and abnormal phase retardation as shown by the corresponding phase retardation images. No hyporeflective area and microcysts were observed in the intensity images with PS-OCT and 3D CAS-OCT (Fig. 1B). In case 2, a highly elevated localized bleb and a large fluid pool surrounded by hyperreflective tissue were observed (Fig. 1C). Despite a highly elevated appearance, it was not possible to determine the normality of this tissue property from OCT intensity images. In contrast, the corresponding phase retardation image presented strong-phase retardation that was encircling the fluid pool (Fig. 1C). According to the general anatomy of the eye, conjunctival structures do not possess phase retardation properties.[19,20] In healthy subjects, there is no abnormal phase retardation in conjunctiva (Supplementary Fig. S1). Hence, this appearance in Figures 1B and 1C indicates abnormal phase retardation.

## Early Change of Birefringence in Blebs After Surgery

Consecutive measurements were performed in 14 patients for 2 months after surgery. Nine blebs were functionally classified as "good," three were "moderate," and two were "poor" at 2 months after surgery. All blebs showed no abnormal phase retardation until 1 week after surgery; however, seven blebs showed partially increased phase retardation at 1 month after surgery. One bleb was encapsulated at 1 month after surgery. A case of no increase in phase retardation after surgery is shown in Figure 2. Another case of partial increase in phase retardation after surgery is shown in Figure 3. Blebs were separated into two groups, "no increase of phase retardation" and "increase of phase retardation." No increase of phase retardation group showed PS-OCT phase retardation Grade 1 at 1 month after surgery. On the other hand, increase of phase retardation group showed PS-OCT phase retardation Grades 2 or 3 at 1 month after surgery. Though both groups showed no increase of IOP at 1 month after surgery, increase of phase retardation group showed higher IOP than no increase of phase retardation group at 4 months after surgery (Fig. 4).

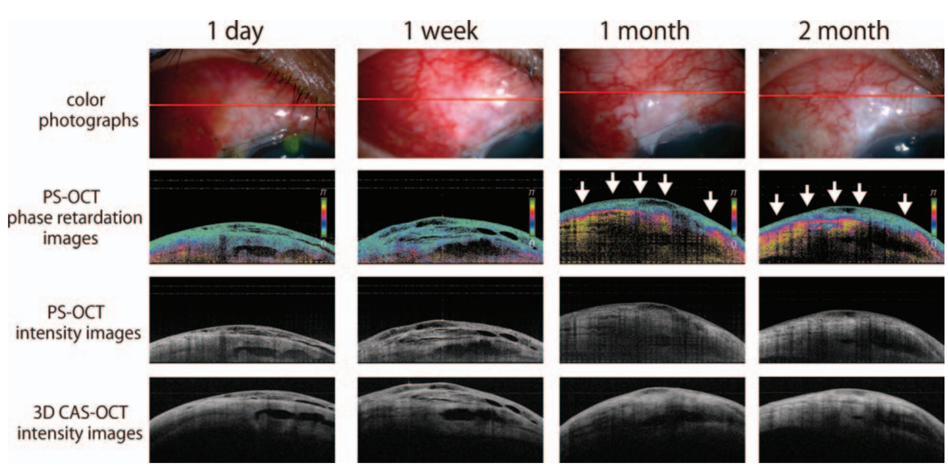


FIGURE 3. Photographs, phase retardation and intensity images with PS-OCT, and intensity images with 3D CAS OCT at 1 day, 1 week, and 1 and 2 months after surgery. Cases of partial increase of phase retardation after surgery. *Arrows* indicate irregular and abnormal phase retardation.

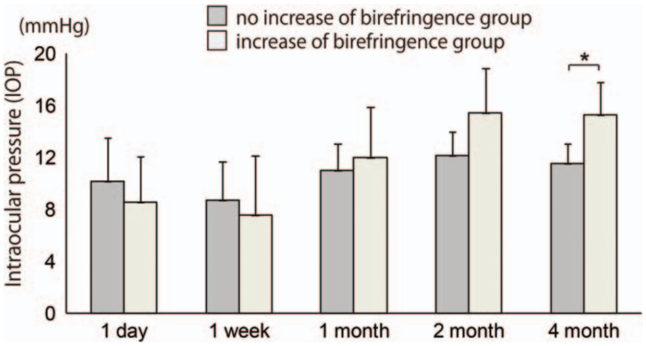


**FIGURE 4.** The change of IOP after glaucoma surgery in the group with no increase of phase retardation and in the group with increase of phase retardation. ($n = 7$ in each group). *Bar graph* shows the mean ± SD (*$P = 0.017$).

### Relation Between Bleb Function and Bleb Characteristics Measured by 3D CAS-OCT and PS-OCT

The characteristics of the blebs with good, moderate, and poor function are shown in Table 2. Significant differences were observed among the three groups except for height of the internal fluid-filled cavity.

Table 3 summarizes the results of multiple regression analysis in relation to bleb function. Explanatory variables were age, period after operation, PS-OCT phase retardation grade, and 3D CAS-OCT parameters. The PS-OCT phase retardation grade showed the highest correlation to bleb function ($\beta = 0.429$, $P < 0.001$). Moreover, we analyzed the data in each surgery type group. The PS-OCT phase retardation grade also showed the highest correlation to bleb function in the trabeculectomy group ($\beta = 0.386$, $P < 0.001$) and in the Ex-press tube shunt group ($\beta = 0.435$, $P < 0.01$).

## DISCUSSION

In the present study, the internal phase retardation of the blebs was examined using PS-OCT. As shown in Figures 1 and 2, the phase retardation images obtained were dramatically different between blebs with good and poor functions. We also found that bleb function classified by IOP and medication was affected by multiple factors, of which alteration of phase retardation in blebs had the largest impact on bleb function as shown by the multiple regression analysis. We have previously reported a rabbit model of trabeculectomy and investigated the phase retardation of blebs using PS-OCT.[15] No abnormal phase retardation was found in the bleb with no evident scarring by histologic analysis. Conversely, abnormal strong phase retardation was found in the collapsed blebs, and the histologic cross-sections indicated that the strong phase retardation was due to scarring.

The most important factor in the success of trabeculectomy is whether the filtering bleb becomes and remains functional. Because bleb function is believed to depend on the degree of bleb fibrosis and obstruction of intrascleral aqueous humor flow, previous studies evaluated fibrosis or blockage of fluid flow by analyzing the bleb morphology. Large and tall blebs with internal hyporeflexivity and an increased number of microcysts were all features that correlated with the functionality of the blebs.[10,12,13] In the current study, PS-OCT could evaluate bleb fibrosis and the phase retardation of blebs had the largest impact on bleb functionality. Polarization-sensitive OCT also provided the possibility of estimating a prognostic value for the filtering bleb function, as the group with increase of phase retardation showed higher IOP than the group with no increase of phase retardation at 4 months after surgery. However, the sample size for consecutive measurements was small and the observation period was short. In a further study aiming to evaluate prognostic ability of PS-OCT, we are planning to follow a larger number of patients subjected to glaucoma filtering bleb surgery and obtain consecutive measurements using PS-OCT for a longer period.

It is important to notice that in the current study, several encapsulated blebs were included in the poor bleb function group. Encapsulation of the filtering bleb occurs in approximately 10% of eyes following trabeculectomy, partly due to conjunctival and episcleral fibrosis.[21–23] Encapsulation occurs in the early postoperative period approximately 2 to 4 weeks when adhesions form between the episcleral tissues and

**TABLE 2.** Comparisons Among Good, Moderate, and Poor Bleb Function Groups

| Bleb Function | Good | Moderate | Poor | *P* Value |
|---|---|---|---|---|
| Number of eyes | 73 | 16 | 64 | |
| Age | 70.7 ± 10.6 | 67.6 ± 10.5 | 64.9 ± 14.7 | 0.027 |
| Period after surgery, d | 242.5 ± 766.9 | 317 ± 802.5 | 1162.0 ± 1232.5 | <0.001 |
| IOP | 9.7 ± 3.0 | 15.8 ± 1.3 | 16.0 ± 4.4 | <0.001 |
| PS-OCT phase retardation grade | | | | |
| Grade 1 | 60.8% | 37.5% | 15.6% | |
| Grade 2 | 32.4% | 56.3% | 31.3% | <0.001 |
| Grade 3 | 6.8% | 6.2% | 53.1% | |
| 3D CAS-OCT parameters | | | | |
| Horizontal length of the internal fluid-filled cavity, mm | 3.03 ± 1.68 | 3.08 ± 1.65 | 2.18 ± 1.89 | 0.014 |
| Vertical length of the internal fluid-filled cavity, mm | 2.39 ± 1.52 | 2.22 ± 1.39 | 1.77 ± 1.44 | 0.046 |
| Height of internal fluid-filled cavity, mm | 0.46 ± 0.24 | 0.46 ± 0.20 | 0.47 ± 0.39 | 0.999 |
| Maximum bleb wall thickness, mm | 0.81 ± 0.34 | 0.83 ± 0.35 | 0.55 ± 0.35 | <0.001 |
| Minimum bleb wall thickness, mm | 0.47 ± 0.30 | 0.61 ± 0.29 | 0.36 ± 0.26 | 0.002 |
| Total bleb height, mm | 1.26 ± 0.46 | 1.30 ± 0.37 | 1.03 ± 0.59 | 0.021 |
| Volume of internal fluid-filled cavity, $mm^3$ | 8.44 ± 10.12 | 4.84 ± 4.41 | 4.04 ± 6.99 | 0.009 |
| Volume of hyporeflective area, $mm^3$ | 14.43 ± 11.23 | 12.01 ± 7.92 | 6.29 ± 7.67 | <0.001 |
| Number of microcysts | 25.80 ± 19.23 | 21.63 ± 13.87 | 7.59 ± 10.26 | <0.001 |
| Rate of LUSF | 35.6% | 56.3% | 51.6% | 0.103 |
| Rate of filtration opening on the sclera flap | 94.5% | 87.5% | 73.4% | 0.002 |

**Table 3.** Multiple Regression Analyses Between Bleb Function and Patient Characteristics PS-OCT and 3D CAS-OCT Parameters

| | $\beta$ | SE | *P* Value |
|---|---|---|---|
| Age | −0.184 | 0.005 | 0.004 |
| Period after surgery, d | 0.186 | 0.001 | 0.006 |
| PS-OCT phase retardation grade | 0.429 | 0.076 | <0.001 |
| 3D CAS-OCT parameters | | | |
| Horizontal length of the internal fluid-filled cavity, mm | −0.133 | 0.045 | 0.128 |
| Height of internal fluid-filled cavity, mm | 0.257 | 0.271 | 0.004 |
| Maximum bleb wall thickness, mm | −0.176 | 0.257 | 0.078 |
| Minimum bleb wall thickness, mm | 0.170 | 0.265 | 0.039 |
| Volume of hyporeflective area, $mm^3$ | −0.235 | 0.008 | 0.009 |

Tenon's capsule, creating a fibrous, vascular cyst that entraps the aqueous and creates a localized and dome-shaped bleb. The associated IOP increases in the first 6 weeks postoperatively and may lead to additional early needle manipulation of the encapsulated bleb. As shown Figure 1C, the encapsulated bleb was highly elevated and showed strong abnormal phase retardation, which was encircling the fluid pool. Inclusion of several encapsulated blebs might have affected the results in the current and previous studies, with small differences in the morphologic comparisons associated with each bleb function group.

Interestingly, in the consecutive measurements taken for 2 months after surgery, all blebs showed no abnormal phase retardation until 1 week after surgery. However, seven blebs showed partially increased phase retardation at 1 month after surgery. One bleb was encapsulated at 1 month after surgery. These periods might be related to the intrableb fibrosis of the encapsulated bleb. A previous study reported that blebs with uniform reflectivity were significantly more likely to have worse function than multiform blebs in the early postoperative stage.[13] Multiform bleb walls had hyporeflective areas that seemed to represent loosely-arranged connective tissue, subconjunctival separation and microcysts. Blebs with multiple-layer structures at 2 weeks were associated with better bleb function at 6 months. In the current study, multiform blebs showed no abnormal birefringence until 2 months after surgery (Fig. 2). In contrast to a previous report using 3D CAS-OCT, seven cystic blebs were included in the current study.[24] Strong, abnormal phase retardation tissue encircled the cystic bleb, however, no abnormal phase retardation was found inside (Supplementary Fig. S2). According to an evaluation using anterior segment OCT and histologic observation, Kojima, et al.[25] described the thin bleb walls of a cystic bleb, which were formed by the conjunctival epithelium without connective tissue. We intend to assess the relationship between cystic bleb formation and intrableb phase retardation in further study.

Similar with current study, Inoue et al.[26] reported the importance of evaluation of filtration openings. In the current study, the proportion of filtration openings of the scleral flap was significantly different among the three bleb function groups (Table 2). In contrast to previous study, LUSF showed no significant differences among all three bleb function groups.[27]

Currently, several morphologic diagnostic methods, such as laser scanning in vivo confocal microscopy and anterior segment OCT, allowed a precise analysis of the filtering bleb structure and function after glaucoma surgery by analyzing the conjunctival and subconjunctival tissues features.[24,28] Laser scanning in vivo confocal microscopy can assess local bleb morphology and evaluate the presence of epithelial microcysts, the density of subepithelial connective tissue and the presence of blood vessels. In comparison with OCT, high-resolution laser scanning in vivo confocal microscopy provides a smaller measurement area ($300 \times 300$ μm) and requires contact with the bleb surface. Moreover, confocal microscopy possesses only short-image penetration from 10 to 20 μm and, hence, is only capable of visualizing the outermost surface of the bleb (i.e., the epithelium). Thus, OCT might identify other types of microcysts that are different from the ones evaluated by confocal microscopy. Polarization-sensitive OCT provides not only the structural tomography of the bleb but also visualizes its polarization property that is associated with the microstructure of the sample.

Mitomycin C is important in inhibiting intrableb fibrosis. The application of mitomycin C may have increased the frequency and/or degree of formation of hyporeflective areas in the bleb walls by inhibiting intrableb fibrosis.[13] Recently, another antifibroticagent, the TGF-β, was also investigated.[29] Although it is difficult to evaluate intrableb fibrosis directly with standard modalities, PS-OCT can evaluate intrableb fibrosis by measuring its phase retardation. In the future, PS-OCT might be a useful instrument to evaluate the effects of antifibrotic agents to prevent the increase of IOP after surgery.

Note that the intensity image and phase retardation images of PS-OCT were created from the same spectral data set and, hence, were perfectly registered to each other. Because PS-OCT is also able to detect bleb morphology, as with conventional 3D CAS-OCT, it is possible for PS-OCT to acquire information on internal tissue properties and bleb morphology simultaneously. The alteration of phase retardation in blebs had the largest impact on bleb function as shown by multiple regression analysis. Thus, if the data of internal tissue properties, such as birefringence and bleb morphology were combined, we could obtain even more precise information regarding bleb function.

Our study has some limitations. The study carried out to date was based on the phase retardation obtained from the PS-OCT system. It should be noted that the phase retardation images do not represent the localized birefringence property but rather is a cumulative effect of the depth-resolved property. For a better understanding of bleb function and its related-phase retardation, analysis of the localized birefringence property of the tissue should be carried out.[30] This analysis would determine the future direction of research on imaging bleb function using PS-OCT. Additionally, further signal processing has enabled a more accurate quantitative measurement of phase retardation.[31] This provides higher sensitivity and specificity in the investigation.

In conclusion, we confirmed that PS-OCT could noninvasively measure birefringence in filtrating blebs after glaucoma surgery. The bleb function was most associated with PS-OCT phase retardation grades followed by patient age, height of the internal fluid-filled cavity, volume of the hyporeflective area, and minimum bleb wall thickness. No bleb showed abnormal phase retardation until 1 week after surgery, however some blebs showed partial increase of phase retardation at 1 month after the procedure.

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