Original Article

Correlation between Morphologic Appearance and Function of Filtering Bleb *in vivo*

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Abstract

Purpose: Efficacy and long-term success of trabeculectomy largely depends on the postoperative development of a functioning filtering bleb. This study was designed to observe histological changes in filtering blebs after trabeculectomy using *in vivo* confocal microscopy (IVCM), and to investigate the correlation between morphologic appearance and function of filtering blebs.

Methods: A total of 46 glaucoma patients who had received a trabeculectomy unilaterally in the past 1 to 60 months underwent slit-lamp examination, applanation tonometry, and in vivo confocal microscopy. Eyes were classified into 4 groups according to the morphologic appearance of the filtering bleb based on the Kronfled system: type I blebs (8 eyes), type II blebs (14 eyes), type III blebs (16 eyes), and type IV blebs (8 eyes). The IVCM images were analyzed for the number of intraepithelial microcysts, the density of subepithelial connective tissue and the presence of blood vessels.

Results: Type II blebs presented with numerous intraepithelial vacuolar microcysts, while several large intraepithelial microcysts were found in type I blebs. Subepithelial connective tissue was widely spaced in type I and II blebs. In contrast, type Ⅲ and Ⅳ blebs showed few or no intraepithelial microcysts, and subepithelial connective tissue was densely distributed. Neovascularization was seen in 83.3% of failed blebs, whereas neovascularization was found in only 16.6% of successful blebs.

Conclusion: Different types of blebs reveal various histological characteristics at the cellular level, which appear to be correlated with postoperative filtering function. (***Eye Science 2011;26:201–207***)

Keywords: filtering blebs, trabeculectomy, histology, *in vivo*

Trabeculectomy is the most common surgical technique for glaucoma treatment[1,2]. The efficiency and long-term success of trabeculectomy largely depends on the postoperative development of a functioning filtering bleb overlying the scleral flap, which is determined by the postoperative wound healing process. Previous research reported bleb failure is caused by scarring within the tissue. According to previous studies, the success of filtering surgery is defined by postoperative intraocular pressure (IOP). Blebs with good function are categorized as type Ⅰ, blebs with an IOP <21 mmHg and not requiring antiglaucoma treatment are categorized as type Ⅱ, and nonfunctioning blebs with an IOP >21 mmHg and requiring antiglaucoma medication are categorized as type Ⅲ. However, there is no correlation between bleb appearance and IOP. The functional evaluation of blebs is difficult in clinical practice[3,4].

In vivo confocal microscopy (IVCM) is a relatively new, powerful optical technique used in previous studies. IVCM can provide details of ocular structures at the cellular level and additional morphologic information concerning intraepithelial cystic space, blood vessels, the density of connective tissue, and encapsulation. Previous investigations reveal that IVCM has already been used in numerous medical studies[5–7].

Through this study, we aim to better understand how morphologic changes affect the filtering function. Further studies focusing upon the correlation between the presence of these visible structures according to the Kornfeld classification criteria and the function of the filtering blebs should be performed. To evaluate the reliability of IVCM, we compared our *in vivo* morphological study findings to previous *in vitro* histopathologic results reported in the literatures[9–10].

Materials and methods

In this study, we retrospectively evaluated 46 fil-

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tering blebs of 46 patients who underwent trabeculectomy and were followed up at the Shenzhen Eye Hospital, Guangdong Province, China between May and September 2010. Informed written consent was obtained from all participants.

Twenty seven patients were female (58.7%) and 19 were male (41.3%); their age ranged from 30–72 years (mean±standard deviation; 51.3±16.4 years). There were 21 right eyes (45.7%) and 25 left eyes (54.3%). Of the 46 eyes, 30 (65.2%) had primary angle-closure glaucoma and 16 (34.8%) had open-angle glaucoma.

Most of the trabeculectomies were limbus-based (38 eyes) and were performed by three surgeons. In all cases, mitomycin C (MMC) was applied during the operation for 2–3 min using a small piece of surgical sponge soaked in 0.2 or 0.33 mg/ml MMC. After exposure, the conjunctival flap area was immediately irrigated with 250 ml balanced salt solution. The period between surgery and IVCM evaluation ranged from 1–60 months (mean±standard deviation; 17.1±25.2 months).

Slit-lamp examinations were performed by the same investigator who was masked for the IOP and clinical history of the patients. A set of reference photographs for all filtering blebs was also taken and analyzed. Based on the Kornfeld classification criteria, the patients were classified into four groups according to the morphologic appearance of the filtering bleb. Type I blebs are thin-walled, polycystic, and have transconjunctival fluid flow. Type Ⅱ blebs are characterized as being shallow, thicker, more diffuse, perilimbally extended, and relatively avascular. Type Ⅲ blebs are flattened with little or no function; in these blebs, the scarred conjunctiva firmly adheres to the underlying sclera. Type Ⅳ blebs are encapsulated (Tenon cyst) and characterized by a firm, localized, dome-shaped cavity with engorged surface blood vessels[8].

The patients were examined using a new generation in vivo confocal microscope (Rostock Cornea Module (RCM)/Heidelberg Retina Tomograph Ⅲ (HRT Ⅲ); Heidelberg Engineering, Inc., Heidelberg, Germany) after undergoing a slit-lamp examination and Goldmann applanation tonometry. Blebs were observed using the following criteria: the extent of vascularization, cork screw vessels, microcysts and encapsulation. The blebs were photographically documented. An immersion lens covered by a polymethyl metacrylate cap was used as the objective lens of the microscope; magnification was ±60. A diode laser with a wavelength of 670 nm was the light source for the HRT Ⅲ/Rostock Cornea Module. The detecting area was 400 μm × 400 μm, with 384±384 pixel images and 1 μm optical resolution.

Epithelium and subepithelial connective tissue of the conjunctiva located over the trabeculectomy site were analyzed. Each eye was examined for less than 5 min; no side effects were noted during IVCM observation.

The IVCM images were evaluated for conjunctival epithelium changes, the presence and number of intraepithelial microcysts ranging from 0 (none) to 3 (numerous), the density of subepithelial connective tissue ranging from 0 (loose) to 3 (dense), the presence of blood vessels, and encapsulation of the bleb according to a previous IVCM study[5]. Statistical values for the number of microcysts, the density of subepithelial connective tissue, and the relative risk of the appearance of new vessels were calculated for each group and compared using the nonparametric Mann-Whitney U test. Probability values less than 0.05 were considered significant different.

Results

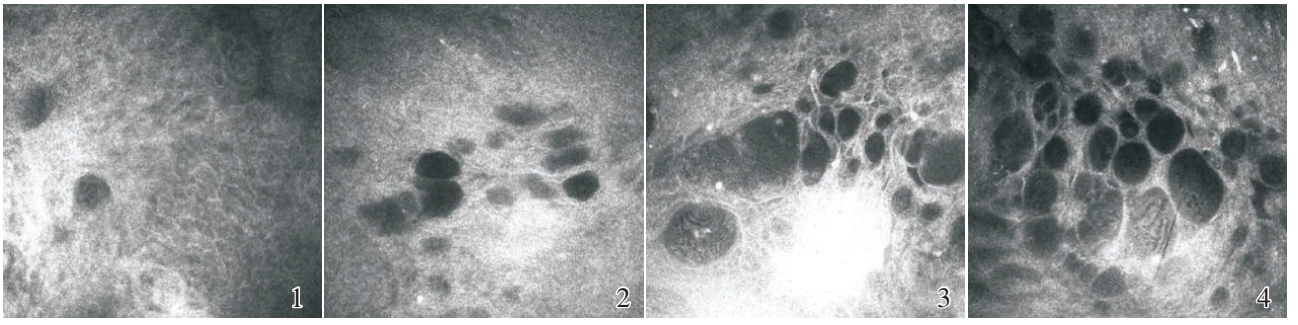
In group 1 (8 eyes; type I blebs), the IVCM showed the blebs' conjunctiva were extremely thin and epithelial cells were hardly seen. Several large optically clear space filled with fluid was seen, corresponding to microcysts. The number of microcysts was 0 or 1(mean±SEM, 0.623±0.665). Subepithelial connective tissue was loosely arranged, having a density of 0 or 1(mean±SEM, 0.109±0.467).

In group 2 (14 eyes;type II blebs),the IVCM images revealed numerous optically clear space filled with fluid between normal epithelial cells. These micro cysts were particularly numerous and even largely confluent in type II blebs; the number of microcysts ranged from 2–3(mean±SEM,2.864±0.473). Subepithelial connective tissue was equally loose to that in group 1,with a density of 0 or 1(mean±SEM,0.475±0.539).

In group 3 (16 eyes; type Ⅲ blebs), conjunctival and corneal cells were perfectly visible with a normal appearance. Very few and relatively non-transparent microcysts were observed between superficial conjunctival cells. Type Ⅲ blebs had no microcysts. The subepithelial connective tissue was densly filled with few or no clear spaces, having a density of 2 or 3 (mean±SEM, 2.364±0.661).

In group 4 (8 eyes; type Ⅳ blebs), within the clinically encapsulated nonfunctioning blebs, dense fibrotic tissue evocative of encapsulation was observed. One or two relatively large microcysts were

A. Rating of microscysts in the conjunctival epthelium: 1, 2, 3 or 4.



B. Rating of density of subepithelial connective tissue: 1, 2, 3, or 4.

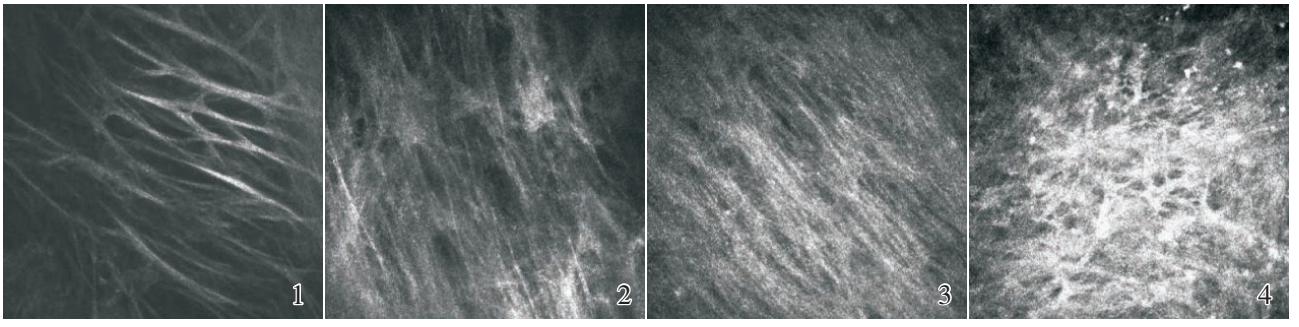


Figure 1 *In vivo* confocal microscopy images of blebs (400 μm × 400 μm).

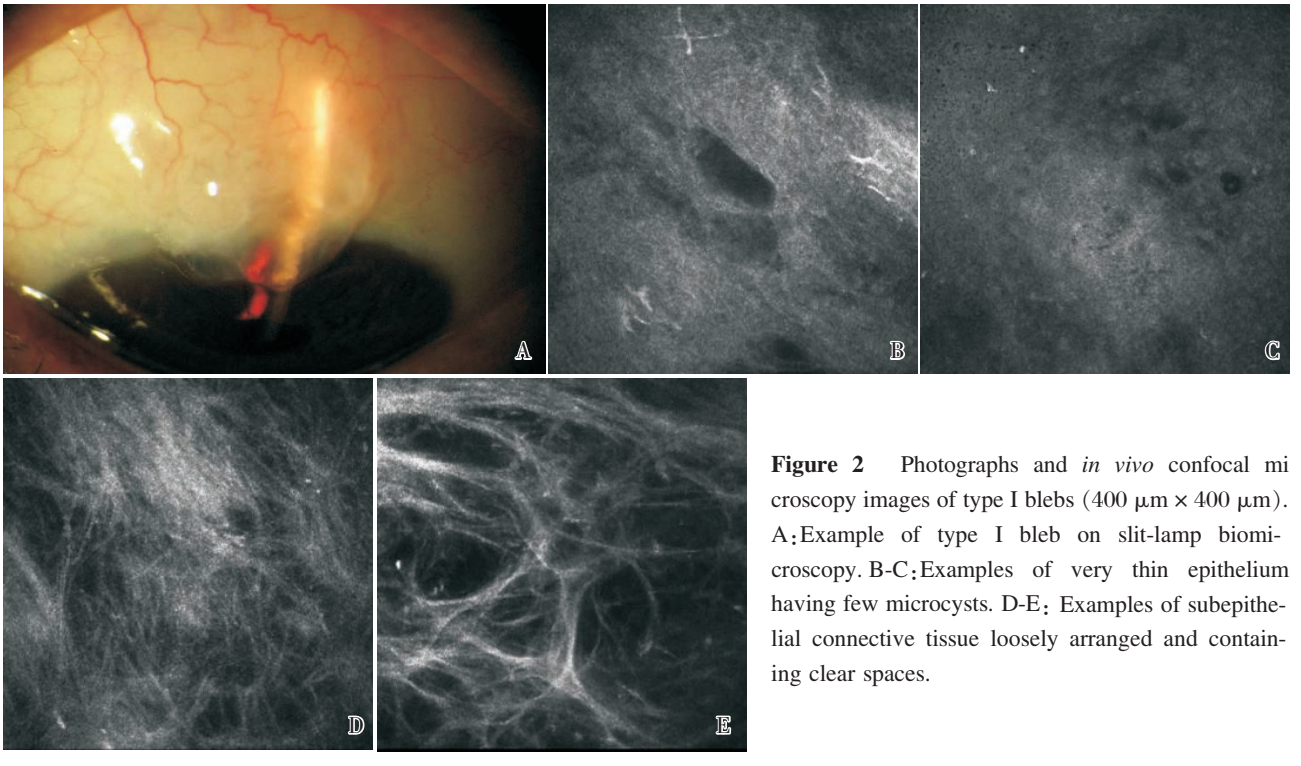


Figure 2 Photographs and *in vivo* confocal mi croscopy images of type I blebs (400 μm × 400 μm). A: Example of type I bleb on slit-lamp biomicroscopy. B-C: Examples of very thin epithelium having few microcysts. D-E: Examples of subepithelial connective tissue loosely arranged and containing clear spaces.

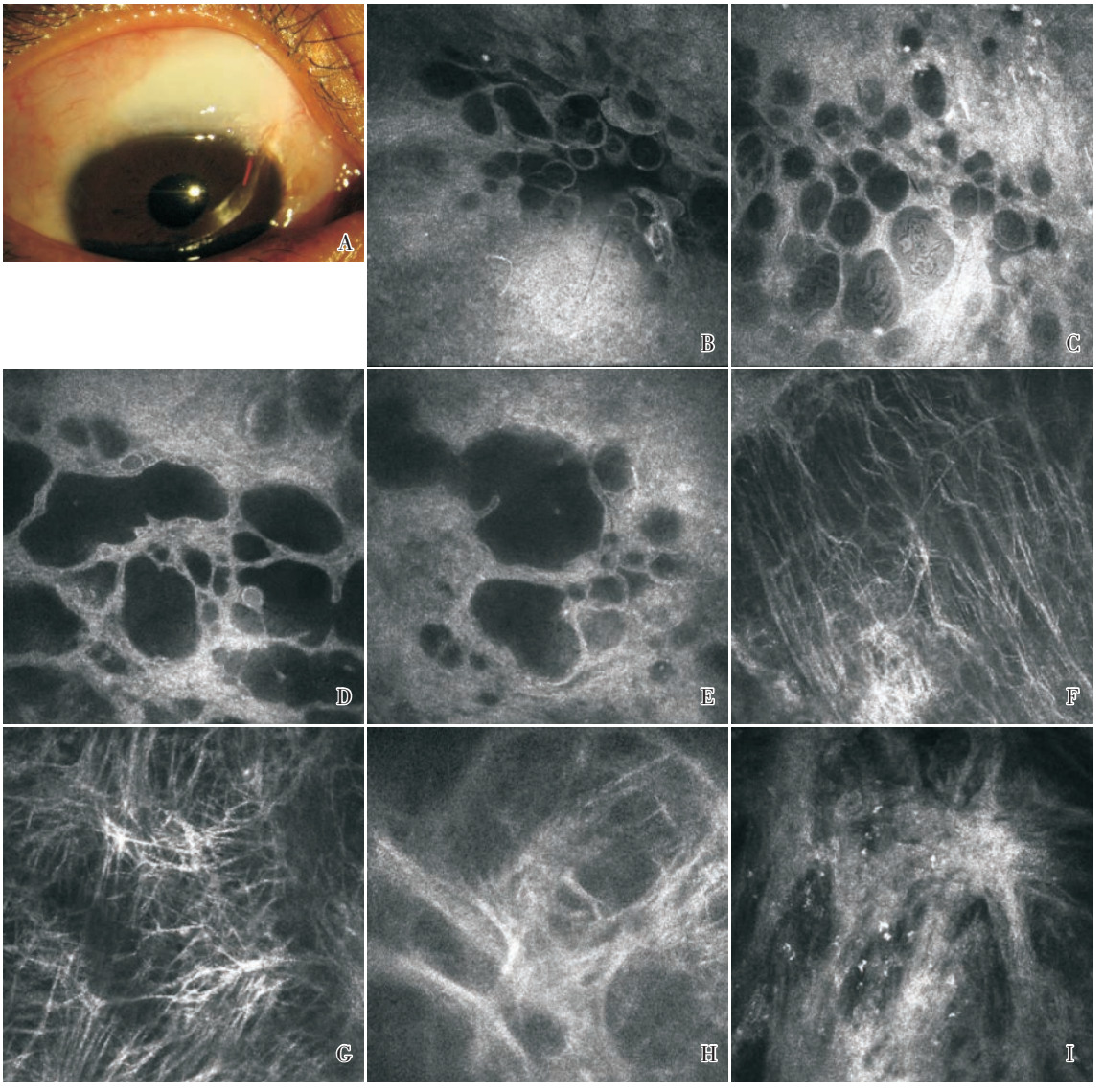


Figure 3 Photograph and *in vivo* confocal microscopy images of type II blebs (400 μm × 400 μm). A: Example of type II bleb on slit-lamp biomicroscopy. B-E: Examples of numerous microcysts in the conjunctival epithelium. F-I: Examples of subepithelial connective tissue widely spaced, containing clear spaces.

found, mostly in the central part of the bleb (mean ±SEM, 0.813 ±0.367). The subepithelial connective tissue was densely arranged with high reflectivity, few or no spaces, with a density of 2 or 3(mean±SEM, 0.813 ± 0.367).

Some of these microcysts showed hyper-reflective microdots in the superficial epithelium layer and might have been necrotic epithelial cells or inflammatory cells. New vessels were found in 13.6% (3/22)of the successful blebs (groups 1 and 2), whereas they were found in 83.3% (20/24) of the failed blebs (groups 3 and 4).

The quantity of microcysts and the density of the connective tissue were statistically significant between the functioning (groups 1 and 2) and nonfunctioning (groups 3 and 4) blebs. The IOP and the quantity of microcysts were highly negatively correlated (r=–0.837, P=0.000). The IOP and the density of microcysts were highly positively correlated (r=–0.926, P=0.000). The probability of finding blood vessels is higher in nonfunctioning blebs. The relative risk of the appearance of new vessels and nonfunctioning blebs was strongly related(RR=4.99, P=0.001).

Discussion

A filtering bleb assessment based on slit-lamp

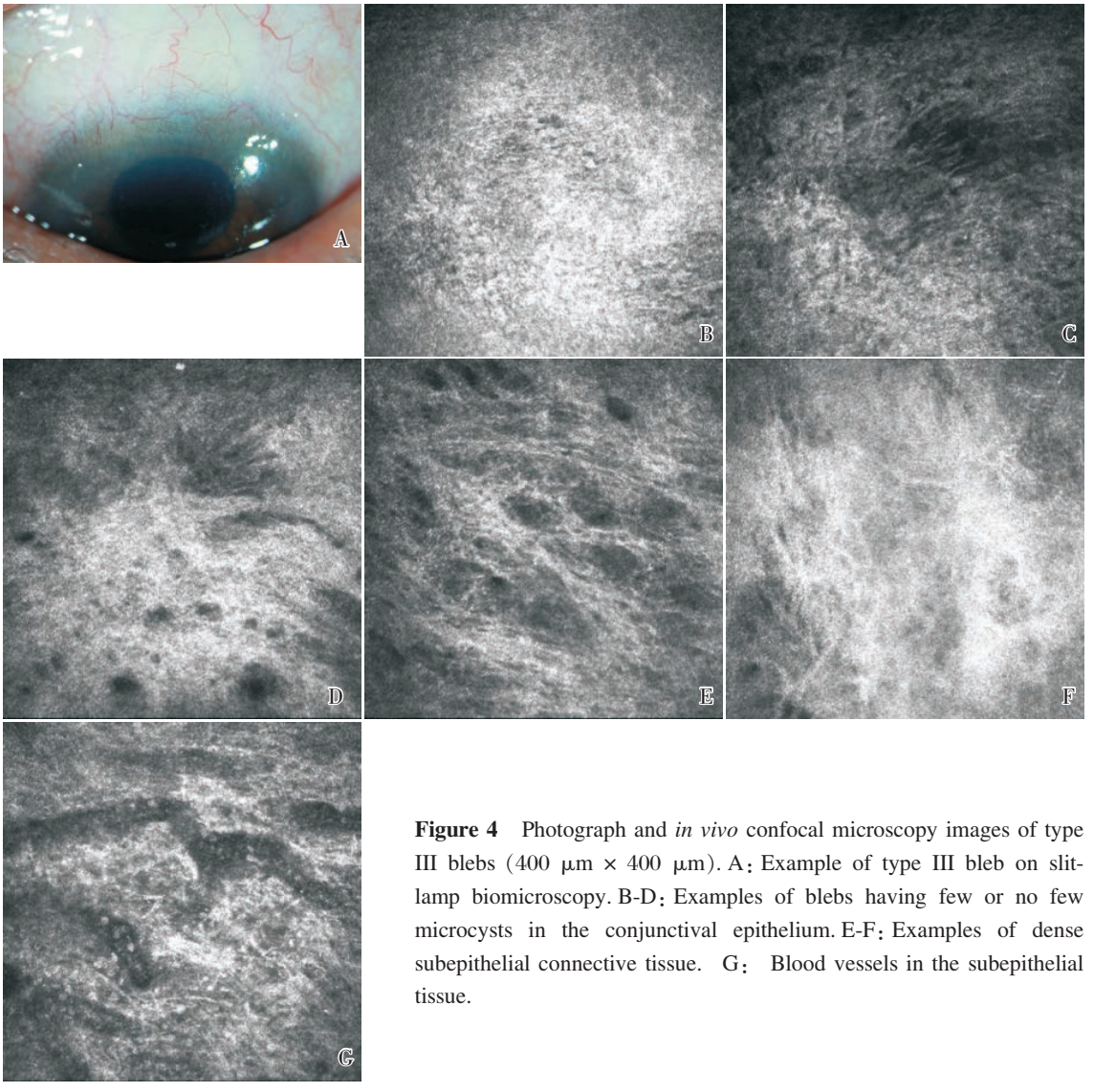


Figure 4 Photograph and *in vivo* confocal microscopy images of type III blebs (400 μm × 400 μm). A: Example of type III bleb on slit-lamp biomicroscopy. B-D: Examples of blebs having few or no few microcysts in the conjunctival epithelium. E-F: Examples of dense subepithelial connective tissue. G: Blood vessels in the subepithelial tissue.

biomicroscopy is a common method used in clinics[11-12]. The Kornfeld classification criteria is the most common assessment method used in China, so we analyzed the IVCM images based on the four types of blebs and described their characteristics.

Type I blebs had a thin epithelium and loosely ar ranged connective tissue. The histological changes[13-14] of the leaking blebs were similar to the IVCM results. The conjunctiva epithelium was thin or defective and there was a denaturation of the collagen beneath the conjunctiva. The thinning or absence of conjunctival epithelium, degeneration of subepithelial fibrous connective tissue, and lack of blood vessels may have been caused by bleb leakage and low IOP. Type Ⅱ blebs had numerous microcysts, widely spaced connective tissue, and were relatively avascular. A histological study of a diffuse functioning bleb showed uniform three-layered non-keratinizing stratified conjunctiva and loose subepithelial connective tissue[15]. The small cystic spaces interspersed within the loose conjunctival stroma presumably provided numerous conduits for aqueous drainage and lower IOP. Type Ⅲ blebs had dense connective tissue and vascularization, unassociated with the microcysts. In contrast, type Ⅳ blebs had relatively large microcysts in the central area and were surrounded by dense fibrotic tissue and vessels. Both kinds of nonfunctioning blebs had dense connective tissues and abundant vessels. Although relatively large microcysts were found in the central part of type IV blebs, the surrounding dense connective tissues impeded aqueous humor outflow, leading to increased IOP.

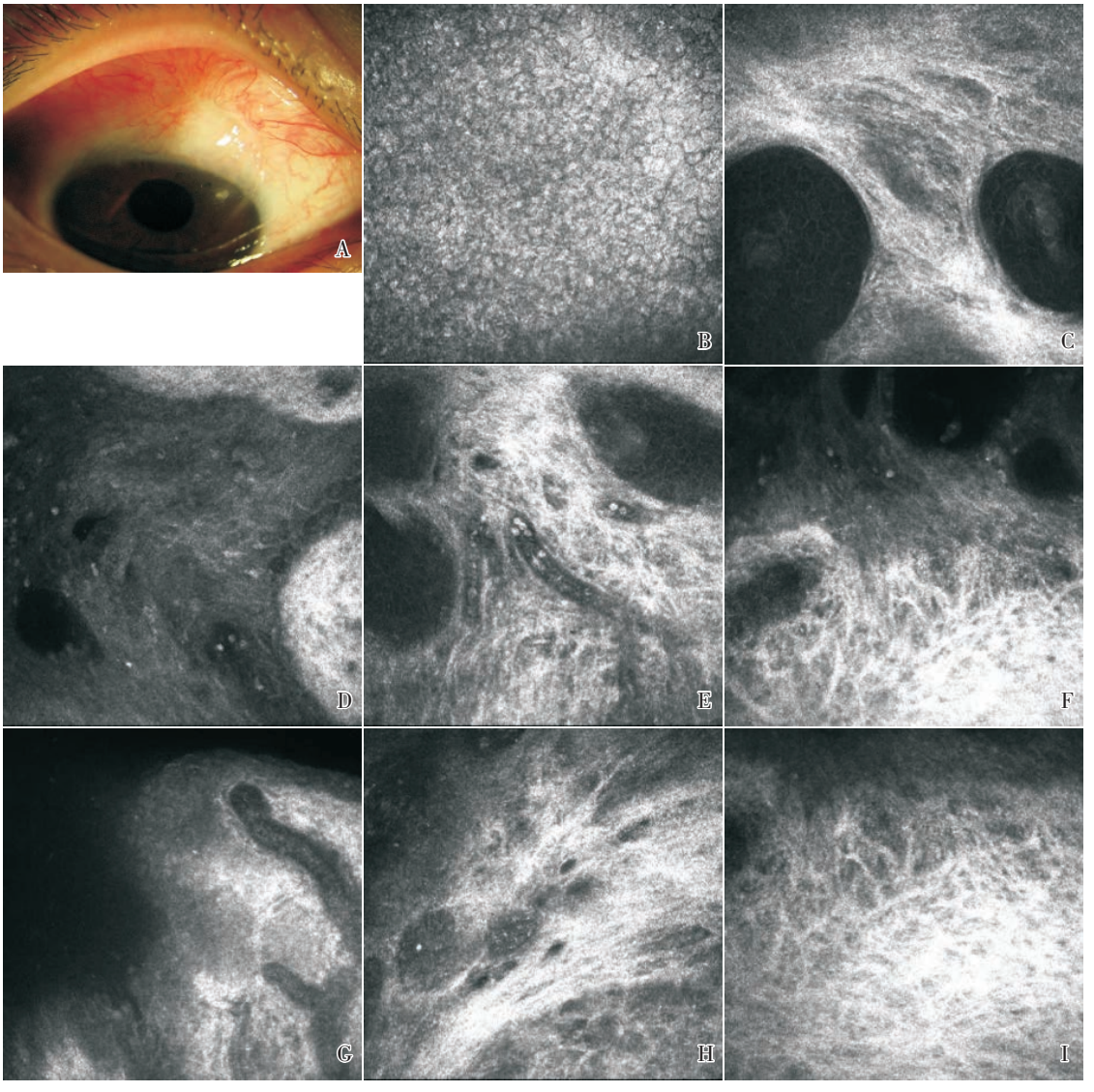


Figure 5 Photograph and *in vivo* confocal microscopy images of type IV blebs(400 μm × 400 μm). A: Example of type IV bleb on slit-lamp biomicroscopy. B: Example of conjunctival epithelium with a normal appearance. C-G: Examples of large microcysts in the central area of a bleb, surrounded by dense connective tissue. H-I: Examples of dense subepithelial connective tissue with few or no clear spaces. E-G: Blood vessels in the subepithelial tissue.

Our study revealed the functioning blebs had in traepithelial optically-empty microcysts and/or loosely arranged connective tissue. Conversely, the nonfunctioning blebs had few microcysts and/or dense connective tissue. Both the functioning and nonfunctioning blebs showed similar features to those in previous studies[5-7].

Based on previous light and electron microscopy studies, the functioning blebs had numerous intraepithelial optically-empty microcysts and the nonfunctioning blebs had few or none. The subepithelial connective tissue of functioning blebs was loosely arranged with widely spaced collagen. Nonfunctioning blebs had dense collagenous connective tissue[9-10]. Our results are in good agreement with previous *in vitro* histological study describing functioning and nonfunctioning blebs suggesting the reliability of this *in vivo* morphological technique.

According to our findings, the microscopic evidence of loose connective tissue is indicative of the way in which aqueous outflows. The intraepithelial microcysts are presumably related to transconjunctival aqueous filtration. This supports the argument that the microcysts are channels for the passage of aqueous humor, which is in agreement with the literature[16].

Compared with the failed blebs, successful filtering blebs had a greater number of microcysts. We suggest the quantity of microcysts in the conjunctival epithelium is highly related to the filtering function of blebs. Based on correlation analysis, the densities of the microcysts were negatively correlated with IOP. The denser the microcysts were, the lower the IOP was. Our data also showed the IOP of nonfunctioning blebs exceeded the normal upper limit. Therefore, it can be concluded that the quantity of microcysts affects bleb function. In nonfunctioning blebs, the subepithelium connective tissue was relatively dense, whereas it was loosely arranged in functioning blebs. Correlation analysis showed that IOP and the density of connective tissue were highly positively correlated; the denser the connective tissue was, the higher the IOP was. Similarly, we can conclude that the density of connective tissue affected the formation of blebs. These results are in agreement with those reported in a previous study[7].

In this study, we observed the appearance of new vessels in all groups. New vessels were found only in 16.6% of favorable blebs, compared to 83.3% of unfavorable blebs. These results are similar to those of Labbes[5]. The emergence of blood vessels is a strong relative risk factor of nonfunctioning blebs; their emergence greatly increased the possibility of nonfunctioning filtration blebs forming. Abundant vessels prompted proliferation of blebs and increased the scarring tendency. Consequently, the emergence of blood vessels was proved to be one of the important factors affecting the formation of functioning blebs. This supports the clinical experience of considering the hyperemia in impending bleb failure.

In conclusion, The IVCM study proved to be valuable in the morphologic evaluation of filtering blebs. It presents a microscopic architecture consistent with in vitro histological examination and correlates well with the postoperative filtering function. Observation of functioning and failing blebs using *in vivo* confocal microscopy has increased our understanding of tissue changes after filtration surgery.

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