Visualization of the Trabeculo-Descemet Membrane in Deep Sclerectomy After Nd:YAG Goniopuncture

An In Vivo Confocal Microscopy Study

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Objectives: To evaluate the morphologic characteristics of the trabeculo-Descemet membrane (TDM) by in vivo confocal microscopy (IVCM) after deep sclerectomy with collagen implant and to correlate the findings with the intraocular pressure (IOP)–lowering effect of goniopuncture.

Methods: Twenty eyes of 19 patients were evaluated in a prospective, observational case series. Examination using IVCM and measurement of IOP were performed 15 minutes before and 15 minutes after Nd:YAG goniopuncture.

Results: Two groups could be distinguished on the basis of morphologic characteristics of the TDM before goniopuncture. In group 1 (13 eyes), the TDM was characterized by the presence of an area of epithelial cells in the deep stromal level. After goniopuncture, an opening at the TDM with dispersed epithelial cells was visible. In group 2 (7 eyes), fibrotic tissue overlying the TDM was observed in all cases, and no openings were visible after goniopuncture. Group 1 had a statistically significant decrease in mean (SD) IOP after goniopuncture (21.6 [4.8] mm Hg before and 13.5 [4.6] mm Hg after, P = .008); there was no significant change in group 2 (19.2 [4.3] mm Hg before and 20.8 [7.5] mm Hg after, P = .30). There was a strong correlation between the presence of fibrous tissue and percentage of IOP lowering after goniopuncture (r = −0.89, P < .001).

Conclusions: The presence of fibrotic tissue covering the TDM is associated with failure of goniopuncture. Use of IVCM may be valuable in predicting the efficacy of goniopuncture in patients with elevated IOP after deep sclerectomy with collagen implant.

Deep sclerectomy with collagen implant (DSCI) has been introduced as a nonpenetrating surgical alternative to standard trabeculectomy. The success of this procedure depends largely on the surgical removal of the inner wall of the Schlemm canal and the creation of filtration through a thin trabeculo-Descemet membrane (TDM) to enable sufficient outflow of aqueous humor at the site of maximum outflow resistance. This lamella, however, can be too thick as a result of insufficient surgical dissection or can develop fibrosis at a later time (>9 months after the initial surgical procedure). Laser goniopuncture is performed after DSCI if there is insufficient percolation of aqueous humor at the TDM. This procedure consists of puncturing the TDM with an Nd:YAG laser through a goni-lens. Clinical trials have clearly shown the importance of goniopuncture as an adjunct to nonpenetrating glaucoma surgery (NPGS). With 10 years of follow-up after NPGS, 60% of patients required goniopuncture to achieve their target pressure, making this procedure an important part of postoperative follow-up. Success rates of NPGS complemented by goniopuncture are high and are similar to those with trabeculectomy. However, some cases do not respond to goniopuncture, and the mechanisms by which goniopuncture reduces intraocular pressure (IOP) are not fully understood.

Until recently, the clinical aspect of the TDM could only be grossly evaluated via gonioscopy or impression cytology. Laser scanning in vivo confocal microscopy (IVCM) is a noninvasive diagnostic tool for the microscopic analysis of the living human cornea. The newly introduced IVCM module for corneal analysis (Rostock Corneal Module/Heidelberg Retinal Tomograph II [HRT II]; Heidelberg Engineering Inc, Heidelberg, Germany) allows microscopic visualization of corneal and conjunctival tissues. This device has the advantages of short image acqui-
sition time, high resolution, and accurate depth assessment. Its use in the field of glaucoma has, so far, been limited to evaluation of postsurgical filtering blebs after trabeculectomy and conjunctival modifications in patients with glaucoma.9-11 The aim of our study was to describe the morphologic characteristics of the TDM after DSCI in eyes with open-angle glaucoma using IVCM and to evaluate whether morphologic aspects correlate with the IOP-lowering effect of Nd:YAG goniopuncture in an attempt to determine why some patients respond better than others to this intervention. To our knowledge, no attempt to determine why some patients respond better than others is described elsewhere.12 The study was performed in compliance with the tenets of the Declaration of Helsinki and with the Swiss federal regulations. All patients gave informed consent before their opera-

**METHODS**

**PATIENTS**

This was a prospective, observational case series. Twenty eyes of 19 patients with primary and secondary open-angle glaucoma were included consecutively. All patients had undergone deep sclerectomy with a collagen implant and had an IOP higher than the individual target pressure. All operations were performed by the same experienced surgeon (T.S.). The DSCI procedure is described in greater detail elsewhere.1 The same examiner (A.D.), masked to the patients’ history, performed all IVCM examinations. The examination was performed after instillation of topical anesthesia and with the patient seated in front of the microscope. The objective was optically coupled with the ocular surface by a polymethyl methacrylate contact cap, using a highly viscous gel (Viscotears; Novartis AG, Basel, Switzerland). Images of the surgical site (at the 12-o’clock position) were acquired during downgaze. Images were recorded throughout all corneal layers. The presence of a rectangular area of epithelial-like cells in the deep stromal layer was determined and was used as a landmark to identify the site of the TDM. On IVCM, the morphologic aspect of the conjunctival epithelium is different from that of the corneal epithelium, especially for the superficial and intermediate cells.1 The criteria for intraoperative application of mitomycin were high risk for postoperative fibrosis (ie, age <60 years, African origin, previous conjunctival surgery, long-standing history of glaucoma treatment, previous uveitis, or trauma). After dissection of the superficial scleral flap, a sponge soaked with mitomycin, 0.02%, was applied for 30 seconds, and the area was then washed out with balanced salt solution. No patient had undergone a previous glaucoma laser procedure (eg, laser trabeculectomy or laser iridotomy) or filtering surgery.

Each patient was enrolled in the study after a complete ophthalmic workup, including applanation tonometry, gonioscopic evaluation of the surgically created trabeculo-Descemet window, and fundoscopy. The IVCM examinations were performed 15 minutes before and 15 minutes after goniopuncture. Goniopuncture with the Nd:YAG laser (Lasag AG; Thun, Switzerland) was performed when multiple holes became visible in the TDM. The IOP was measured 15 minutes after goniopuncture. Laser goniopuncture techniques and results have been reported elsewhere in greater detail.2

**IN VIVO CONFOCAL MICROSCOPY**

The IVCM images were obtained using a retina tomograph (Rostock Corneal Module) with a 63× objective (Carl Zeiss, Heidelberg Engineering GmbH, Heidelberg, Germany) adapted to the HRT II, covering an area of 400 × 400 μm with a resolution of 2 μm longitudinally and 4 μm transversely. The technical characteristics of the device have been described elsewhere.1 The technical configuration consisted of an acrylate contact cap, using a highly viscous gel (Viscotears; Novartis AG, Basel, Switzerland). Images of the surgical site (at the 12-o’clock position) were acquired during downgaze. Images were recorded throughout all corneal layers. The presence of a rectangular area of epithelial-like cells in the deep stromal layer was determined and was used as a landmark to identify the site of the TDM. On IVCM, the morphologic aspect of the conjunctival epithelium is different from that of the corneal epithelium, especially for the superficial and intermediate cells.1 Moreover, goblet cells are present, crowded in groups or mainly dispersed in the layer of conjunctival epithelial cells along with microcysts. Because neither goblet cells nor microcysts were observed in our patients and because the morphologic aspect was more characteristic of corneal epithelial than conjunctival cells, we assumed that cells observed on the TDM were corneal epithelial cells. Hyperreflective structures were observed around the islet of epithelial cells, which likely represented activated keratocytes (Figure 1). The following variables were identified and later correlated with IOP lowering after goniopuncture: visibility of the surgically exposed TDM, presence of epithelial cells, and presence of fibrous tissue.

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**Figure 1.** Epithelial cells (arrows) on the trabeculo-Descemet membrane (visualized by in vivo confocal microscopy).
tion. Because IVCM is part of routine clinical care in our department and no additional procedure was conducted solely for research purposes, no specific consent was required by the ethics committee of the University of Geneva. However, all patients were informed about the nature of the examination, and their oral consent was obtained before undergoing ICVM.

STATISTICAL ANALYSIS

The data were recorded prospectively. The Mann-Whitney and Fisher exact tests were used for comparing means of the 2 groups, as appropriate. Mean (SD) values were calculated for demographic and clinical variables. Spearman correlation was used to estimate the relationship between the presence of fibrous tissue and epithelial cells on the TDM and IOP lowering after goniopuncture. All P values reported are 2-tailed, and significance was defined as P < .05. All IVCM examinations were performed by a single experienced individual who was masked to the patient’s history. Statistical analysis was performed using commercially available software (Stata 10.0; StataCorp LP, College Station, Texas).

RESULTS

Twenty eyes (13 right and 7 left) of 19 patients were included in this study. The mean age of the patients was 67.9 (9.0 [range, 51-88]) years. Diagnosis was primary open-angle glaucoma (14 cases) and pseudoexfoliative glaucoma (6 cases). All eyes had previously undergone deep sclerectomy with mitomycin. The mean preoperative IOP was 24.3 (5.2) mm Hg, with a mean (SD) of 2.7 (1.3) drugs used per eye. No significant intraoperative complications had occurred. After the operation, there were no signs of anterior chamber inflammation; also, there were no cases of hyphema, early or late hypotony, choroidal detachment, or shallow anterior chamber. The postoperative regimen in all patients consisted of topical therapy (an antibiotic/corticosteroid fixed-dose combination for 4 weeks followed by an antibiotic/nonsteroidal anti-inflammatory drug combination for 8 weeks). All patients developed IOP higher than the target level at 2 consecutive visits after the operation and were scheduled for goniopuncture. The mean (SD) time of Nd:YAG goniopuncture after DSCI was 17.3 (15.5 [range, 4-63]) months. One patient with advanced glaucoma had a pregoniopuncture IOP of 12 mm Hg with use of 3 glaucoma drops and was included because of drug intolerance.

Visualization of the TDM by IVCM was possible in all eyes. Two groups could be distinguished on the basis of morphologic characteristics of the TDM and IOP lowering after goniopuncture. The groups were similar in terms of age, sex, glaucoma diagnosis, mean time after surgical intervention, and preoperative medications (Table). In group 1 (13 eyes), IVCM examination showed an area of epithelial cells over the TDM (Figure 2A). Fifteen minutes after goniopuncture, an opening at the site of the TDM with dispersed epithelial cells clearly became visible (Figure 2B). In group 2 (7 eyes), hyperreflective

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P Value . . ./.16b/.16b .66b .92b .82c .19c .001c .35c .04c

Abbreviations: ellipses, not applicable; F, female; IOP, intraocular pressure; L, left; M, male; PEXG, pseudoexfoliative glaucoma; POAG, primary angle-open glaucoma; R, right.

a Same patient.
b Fisher exact test.
c Mann-Whitney test.
structures at the TDM site were observed in all cases (example shown in Figure 3). There was a strong correlation between the presence of fibrotic tissue and percentage of IOP lowering after goniopuncture ($p = -0.89, \ P < .001$).

Group 1 had a statistically significant IOP decrease 15 minutes after goniopuncture (mean [SD], 21.6 [4.8] mm Hg before and 13.5 [4.6] mm Hg after, $P = .008$); there was no significant change in group 2 (19.2 [4.3] mm Hg before and 20.8 [7.5] mm Hg after, $P = .30$) (Figure 3). One month after goniopuncture, no patient in group 1 required glaucoma medication to reach the target IOP. All group 2 patients required glaucoma medication to control IOP by the first month after goniopuncture (up to only 2 patients at 1 week after goniopuncture). There was no correlation between the observation of a fibrotic membrane at the TDM and any variation in surgical technique during DSCI or unique characteristics of the patients.

To our knowledge, this is the first report of noninvasive microscopic analysis of TDM after NPGS. This study assessed morphologic features of the TDM associated with IOP lowering after goniopuncture following DSCI, accessible only by IVCM. Little is known about the microscopic aspects of the TDM. The normal Descemet membrane is not visible in young people and becomes more visible with age.¹⁰ Until the advent of IVCM, a detailed examination of the TDM would have been possible only through invasive methods; hence, little research has been done.

Using IVCM, we observed that the TDM of eyes with significant IOP lowering after goniopuncture was characterized by the presence of an area of epithelial cells at the TDM. The presence of a fibrotic membrane covering the TDM, however, was associated with a significant probability for goniopuncture failure. This was well demonstrated in a woman (aged 88 years) who required bilateral goniopunctures 5 years after her first surgery. In one eye of this patient, IVCM showed dense fibrosis of the TDM that remained unchanged after goniopuncture. This was accompanied by an insignificantly reduced IOP (from 27 mm Hg to 22 mm Hg) after goniopuncture. In the contralateral eye, however, the TDM could be perforated easily by goniopuncture in the absence of fibrosis, and IOP dropped significantly (from 22 mm Hg to 6 mm Hg) after 1 day. Anterior segment optical coherence tomography of that eye showed a significant increase of the intrascleral volume and the presence of conjunctival microcysts after goniopuncture (data not shown). We have no explanation for the difference in the morphologic characteristics of the TDM in the eyes of the same patient. This woman had a similar duration of use of glaucoma medications in both eyes and, therefore, a similar medication-induced risk of conjunctival scarring. However, to date, the effect of this risk factor on the TDM has not been studied. Such a scenario is possible but, in the absence of scientific data, remains speculative; this is a topic for future research.

The underlying mechanism of successful IOP-lowering goniopuncture is not understood fully. After successful DSCI, low IOPs, typically approximately 5 mm Hg, are achieved on the first postoperative day before reaching a peak between the first and second month, followed by a stable plateau for the remaining follow-up period.¹⁷ We hypothesize that this IOP curve reflects the migration of epithelial cells to the TDM from the adjacent corneal epithelium of the remaining TDM. These migrating epithelial cells are undetectable by slitlamp biomicroscopy but are easily demonstrated by IVCM; IVCM is also able to image stromal keratocyte nuclei in the normal cornea.¹² However, keratocytes cannot be visualized in the TDM by IVCM because this depth is out of the device’s range. In stromal wound healing, keratocytes become activated, transform into fibroblast-like cells, and migrate to the site of injury. In fact, the formation of a stromal...
scar is a dynamic process that leads to formation of stromal fibrotic tissue. When documented with IVCM, this tissue appears as a hyperreflective area as noted after corneal trauma, radial keratotomy, and infectious keratitis, as we have observed in clinical settings. In our patients, the aspect of the hyperreflective structure observed at the site of the TDM, as well as around and sometimes over the area of epithelial cells, is highly suggestive of stromal fibrotic tissue. In this group, no openings were visible after gonipuncture. However, because we did not perform in vitro histologic testing to correlate with IVCM, we can only hypothesize about the cellular components of the structures observed on images obtained via IVCM.

Depending on the surgical technique, it is possible that some amount of residual stromal tissue is left behind at the TDM. We assume that the stimulation of these remaining keratocytes would lead to the development of fibrous tissue. To date, all published studies of keratocyte density have examined only the central cornea. This density appears to vary with stromal depth, but the exact gradient of keratocyte distribution is controversial. Some studies have shown a gradual decrease with increasing stromal depth; others found the lowest cell density in the midstroma. There seems to be some agreement that keratocyte density is significantly lower in the posterior stroma compared with the anterior stroma. The interplay of keratocytes and the depth of surgical excision are currently unknown factors in the outcome of NPGS.

The main advantage of IVCM is the fact that it allows close histologic examination of all cellular components of the cornea and the conjunctiva in a noninvasive manner. It is a technique that enables a prospective and repeated evaluation of the cornea and the conjunctiva at high magnification. This makes the IVCM an interesting tool for glaucoma care and research, such as the analysis of filtering blebs after glaucoma operations. Currently, postoperative bleb assessment is based on slitlamp microscopy, but the short image acquisition time and high resolution of IVCM allow a more detailed examination of conjunctival tissue. To our knowledge, only 1 IVCM study has examined the bleb morphologic characteristics after NPGS. This might be explained partly by the fact that, in contrast to trabeculectomy, the long-term success of NPGS is less dependent on the postoperative development of a functioning filtering bleb. In NPGS, the crucial site that determines the success of an operation is the exposed TDM. It is observed as an acellular layer between the posterior stroma and endothelium. Because the causes for surgical failure can remain unclear at slitlamp examination, some investigators have looked for new evaluation techniques, such as ultrasound biomicroscopy and anterior segment optical coherence tomography. The main advantage of IVCM compared with these techniques is the possible visualization at the cellular level of the TDM. Ultrasonographic biomicroscopy has been used extensively to study postoperative characteristics, such as the size and volume of the intra-scleral space after NPGS. These factors, however, are an indirect measure of filtration after NPGS. Moreover, ultrasonographic biomicroscopy is unable to visualize the TDM at the microscopic level, as was done in the present study. Recently, a prototype noncontact IVCM using a 50X lens (Nikon Inc, Tokyo, Japan) has become available. This approach has the advantage of providing information about bleb morphologic characteristics without the risk of iatrogenic bleb injury or infection. The main limitation of IVCM for the analysis of filtering blebs is that only a limited part of the conjunctiva covering the bleb can be examined at a time. This makes conclusions from such focal examination unreliable.

In conclusion, this article presents descriptive IVCM findings gained from examining patients with elevated IOP after NPGS procedures and Nd:YAG gonipuncture. The study is limited by its small sample size. Despite this weakness, the strong correlation observed between the morphologic characteristics of the TDM and the IOP response to gonipuncture gives credibility to our findings beyond the limited sample size and opens a new path of research into the mechanisms of nonpenetrating glaucoma operations. The present findings suggest that there is a correlation between morphologic characteristics, such as the presence of fibrotic tissue at the TDM, and the failure of gonipuncture. In these eyes, Nd:YAG gonipuncture may not produce any clinical benefit.

Submitted for Publication: May 21, 2010; final revision received December 11, 2010; accepted January 10, 2011.
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Financial Disclosure: None reported.
Previous Presentation: This study was presented in part at the Congress of the European Glaucoma Society; June 4, 2008, Berlin, Germany.
Additional Contributions: Mauro T. Leite, MD, assisted with statistical analysis.

REFERENCES


