

Long-term Effects of Mitomycin C in Pterygium Surgery on Scleral Thickness and the Conjunctival Epithelium

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Purpose: To evaluate the long-term effects of intraoperative application of mitomycin C on the scleral thickness and the conjunctival epithelium at the surgical site of pterygium excision.

Design: Prospective observational case series.

Participants: Twenty-four patients who underwent excision of primary pterygium with intraoperative mitomycin C in our department during the year 1996.

Methods: Patients were evaluated by slit-lamp biomicroscopy, impression cytology, and high-frequency ultrasonography. Impression cytology was performed by applying a small nitrocellulose filter paper for a few seconds at the excision area and for a few seconds at the opposite perilimbal area, and subjecting the specimens to the periodic acid-Schiff-Gill modified Papanicolaou staining protocol. The morphology of the conjunctival epithelium and goblet cell density (GCD) were recorded. High-frequency ultrasound was performed at the same sites, and the scleral thickness was measured at a distance of 1 mm from the limbus.

Main Outcome Measures: Goblet cell density, conjunctival epithelial morphology, and the scleral thickness at the operated and nonoperated sites.

Results: All patients had successful pterygium removal with no corneal recurrence after a mean follow-up of 77.2 ± 3.9 months (range, 72–84). Impression cytology revealed normal nongoblet conjunctival epithelial cells at the excision area, with a 4-fold decrease in the GCD at the excision area when compared with the contralateral nonoperated site (296 ± 120 cells/mm² and 1183 ± 310 cells/mm², respectively; $P = 0.0036$). No differences were noted between the scleral thicknesses at the operated site (750 ± 70 μ m) and the opposite site (740 ± 80 μ m) ($P = 0.84$).

Conclusions: A single application of mitomycin C after pterygium excision is not associated with reduction in scleral thickness more than 6 years postoperatively. The conjunctival epithelium retains its normal phenotype, with a marked reduction of the GCD. *Ophthalmology* 2004;111:1522–1527 © 2004 by the American Academy of Ophthalmology.

Mitomycin C is an alkylating agent that inhibits DNA synthesis, resulting in long-term inhibition of Tenon's fibroblast proliferation. Its use in pterygium surgery was first reported in Japan in 1963¹ and in the United States in 1988,² and since then it has been described in numerous studies as an effective adjunct in reducing the recurrence rate of pterygium after excision. In addition to pterygium surgery, it has been successfully applied in other indications in ocular surface surgery, including ocular surface neoplasia,³ and symblepharon repair for ocular-cicatricial pemphigoid.⁴

One of the major concerns with the use of mitomycin C remains its safety. Although the complication rate associ-

ated with a single intraoperative application is low, various anecdotal case reports have created an unfavorable impact regarding the safety of mitomycin C among many ophthalmic surgeons.⁵ The complications of mitomycin C described to date are punctate keratitis, chemosis, delayed conjunctival wound healing,^{6–9} conjunctival granuloma, scleral melting, and corneal melting.^{5,10–13} In many cases, scleral melting is preceded by a nonhealing conjunctival epithelial defect. Therefore, the 2 main target tissues that can be affected by mitomycin C are the conjunctival epithelium and the sclera. Consequently, the main feared long-term effects are scleral thinning and eventual melting, and the possible damage to the ocular surface epithelium. To address these concerns, we have conducted a retrospective study evaluating the possible long-term effects of mitomycin C on scleral thickness and the conjunctival epithelium.

Patients and Methods

Patients and Surgical Technique

Informed consent was obtained from all patients. Institutional ethics committee approval was not required for this study. Thirty-

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eight patients underwent excision of a primary pterygium with intraoperative application of mitomycin C by one surgeon (JF-P) in our department from January 1996 through December 1996. Of these, 24 patients (13 male and 11 female) were available for examination for the purpose of this study. Patients underwent slit-lamp biomicroscopy, impression cytology of the conjunctival epithelium, and high-frequency ultrasound evaluation of the sclera.

All patients underwent excision of the pterygium and received a single intraoperative application of mitomycin C as previously described.¹⁴ Briefly, the pterygium head was excised from the cornea with a disposable crescent knife (Alcon Laboratories, Fort Worth, TX). The conjunctival epithelium covering the body of the pterygium was separated from the pterygium body with spring-action scissors. Then, the pterygium body was separated from the episclera and the medial rectus fascia and excised, leaving the overlying conjunctiva intact. A sponge soaked in a solution of 0.2-mg/ml mitomycin C was applied for a period of 5 minutes. This was followed with copious irrigation with 5 to 10 ml of balanced salt solution of the space between the conjunctiva and the sclera. The conjunctiva was then sutured to the sclera with approximately 5 separated 10-0 nylon sutures, leaving an area of 3 mm of bare sclera between the conjunctival edge and the limbus.

All patients were treated with topical 0.1% dexamethasone 6 times daily and topical 0.3% gentamicin 3 times daily, for the first postoperative week. Topical dexamethasone was applied 4 times daily for the first 3 weeks, and then 3 times daily, and discontinued 3 months postoperatively. Topical gentamicin was discontinued between 1 and 2 weeks postoperatively, when epithelialization was complete.

Slit-Lamp Biomicroscopy

Slit-lamp biomicroscopy was performed to evaluate the final clinical outcome after pterygium surgery. The recurrence rate was graded on a scale of 1 to 4, as previously described.¹⁵ Briefly, grade 1 indicates a normal appearance of the operated site; grade 2 indicates the presence of fine episcleral vessels in the excised area, extending to the limbus, but without any fibrous tissue; grade 3 demonstrates fibrovascular tissue in the excised area, reaching to the limbus but not invading the cornea; and grade 4 represents a true corneal recurrence, with fibrovascular tissue invading the cornea.

High-Frequency Ultrasound Evaluation of Scleral Thickness

Twenty-megahertz anterior segment B-scan echography (I³ SYSTEM-ABD, Innovative Imaging, Sacramento, CA) was used to evaluate scleral thickness. This system has an axial resolution of 0.075 mm and a lateral resolution of 0.090 mm while scanning a sector angle of 30°. The scanning probe was submerged in methylcellulose solution, which was applied in a scleral shell inserted between the lids. The thickness of the sclera was measured in the treated eye and the nontreated eye (which served as a control) at the 3-o'clock and 9-o'clock positions at a constant distance from the limbus (1–2 mm). The measurement did not include the conjunctiva. The average of 3 repeated consecutive measurements was used for the purpose of this study.

Impression Cytology of the Conjunctival Epithelium

Impression cytology was performed at least 1 week after the high-frequency ultrasound examination, as previously described by Tseng and Prabhasawat.^{16,17} Briefly, nitrocellulose filter paper

(Millipore, Bedford, MA) with a pore size of 0.22 mm was cut into asymmetric rectangular pieces. A piece of this filter paper was applied over the conjunctival surface after application of a drop of topical anesthetic. Two pieces of filter paper were applied to each patient—one over the area of the excised pterygium (usually, the nasal perilimbal bulbar conjunctiva) and one over the contralateral conjunctiva (usually, the temporal bulbar conjunctiva). The filter paper pieces were gently pressed for 5 seconds against the conjunctiva, and then peeled off and immediately placed in a fixative containing 70% alcohol and 37% ethyl alcohol in galcial acetic acid.

Specimens were stained with periodic acid–Schiff–Gill's modified Papanicolaou stain. Specimens were rehydrated in consecutive decreasing concentrations of ethanol, then oxidized for 5 minutes in 0.5% periodic acid, rinsed in distilled water, immersed for 5 minutes in pure Schiff reagent, soaked for 2 minutes in 0.5% sodium bisulfite, and soaked in running tap water for an additional 5 minutes. Specimens were then counterstained with Gill's hematoxylin for 2 minutes, followed by 3-minute immersion in 0.5% hydrochloride solution, and afterwards rinsed in tap water. After dehydration in 95% ethanol, specimens were stained with orange G6 for 2 minutes, and EA 50 dye, followed by destaining in 95% ethanol after each dye. Specimens were then dehydrated in absolute alcohol, immersed in xylene, and mounted on cover-slipped slides.

The mean goblet cell density (GCD) was determined for each specimen by averaging the number of goblet cells in five 1-mm² areas defined by a calibrated grid under light microscopy at a magnification of $\times 100$.

Statistics

Paired Student's *t* test and power analysis were used to analyze the scleral thickness and impression cytology results. Data are expressed as means \pm standard deviations, and the differences were considered statistically significant at $P < 0.05$.

Results

The mean age of the 24 patients in this study was 48.3 ± 11.2 years (range, 28–64). The mean age of the 14 patients (9 male and 5 female) who were not available for the study was 45.4 ± 8.4 years (range, 32–61). There was no significant difference between the mean ages of the study group and of those who were absent. All 38 patients had undergone the same surgical procedure and the same postoperative protocol. Review of the medical records of the 14 patients who did not show for the study showed that none had experienced any adverse events during their last follow-up period (mean, 14.2 ± 2.1 months after surgery).

Impression cytology and high-frequency ultrasonography were performed after a mean follow-up period of 77.2 ± 3.9 months (range, 72–84 months after surgery).

Twenty-two of the 24 patients studied had no recurrence (grade 1), 1 patient had a grade 2 result (fine conjunctival vessels), and 1 patient had a conjunctival recurrence (grade 3). None of the patients had any postoperative complications.

Mean scleral thicknesses were 750 ± 70 μm at the surgical site and 740 ± 80 μm at the nonoperated eye (no significant difference) (Fig 1). None of the patients had any clinical evidence of scleral thinning at the operated site. Power analysis was performed to evaluate the strength of the study sample size to detect a significant decrease in scleral thickness. To detect a 75- μm decrease in scleral thickness (which is the axial resolution of our high-frequency ultrasound system, and equals 10% of scleral thickness), and assuming a standard deviation of 80 μm of scleral thickness (as

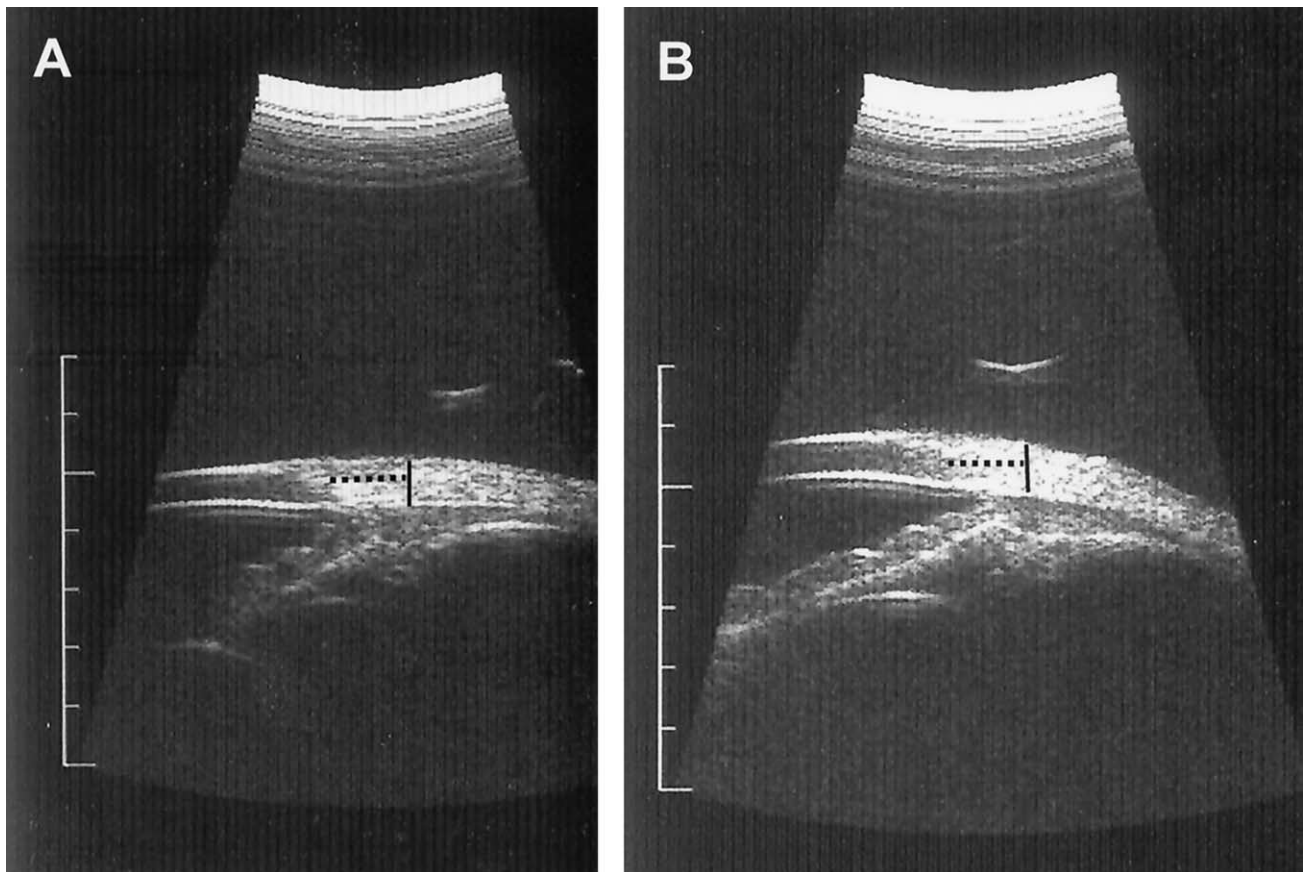


Figure 1. High-frequency ultrasound evaluation of scleral thickness in a patient who had pterygium excision and mitomycin C application 74 months previously. The scleral thickness was measured 1 mm from the limbus at the surgical site (A) and the corresponding contralateral nonoperated site (B). Dashed lines demonstrate the 1-mm distance from the limbus, and solid lines show the location of scleral thickness assessment. No differences were noted between the mean scleral thickness values of the 2 sites in the study group.

found in the normal nonoperated site), a study sample of 24 patients yields a power of 0.8886.

The impression cytology specimens showed an equally abundant nongoblet epithelial cell population at both the surgical and the corresponding nonoperated sites (Fig 2). No signs of squamous metaplasia were seen at either site. There was no difference in the cellular yield of specimens between the 2 sites. However, a significant difference was noted in the mean GCDs between the operated and the contralateral sites. In the nonoperated area the mean GCD was 1183 ± 310 cells/mm² (Fig 2B, D), whereas in the operated site it was 296 ± 120 cells/mm² (Fig 2A, C) ($P = 0.0036$).

Discussion

Mitomycin C is one of the modalities employed to reduce the recurrence rate in modern pterygium surgery. The intraoperative application of mitomycin C is very effective, and the drug is used widely by ophthalmologists. There are abundant data regarding the early postoperative adverse effects of mitomycin C. However, very little is known about the long-term effects of this drug, and controversies over its safety are not yet settled.¹⁸ Specifically, concern still exists regarding the long-term effects of mitomycin C on scleral

integrity. Over the years, anecdotal case reports of postsurgical necrotizing scleritis after pterygium surgery with mitomycin C have been reported.^{5,10-13} This information, combined with a lack of long-term data, deterred many ophthalmologists from using mitomycin C in pterygium surgery. Some have cautioned against the use of mitomycin C in routine pterygium surgery, restricting its application to severely complicated cases only.¹⁸ Therefore, long-term data on the scleral integrity and the conjunctival epithelial phenotype at the site of mitomycin C application are extremely important to the ophthalmic community.

The normal scleral thickness is maximal near the optic nerve ($1000 \mu\text{m}$), whereas the minimal scleral thickness is behind the rectus muscle tendinous insertions ($300 \mu\text{m}$).¹⁹ At the area of the pterygium, between the limbus and the muscle insertion, the normal scleral thickness varies between $800 \mu\text{m}$ adjacent to the limbus and $600 \mu\text{m}$ at the rectus muscle insertion. High-frequency ultrasonography performed at the site of mitomycin C application demonstrated a scleral wall thickness similar to that of the contralateral nonoperated site in the same patients ($750 \pm 70 \mu\text{m}$ and $740 \pm 80 \mu\text{m}$, respectively; both fall within the normal range). This demonstration, after a mean follow-up of >6

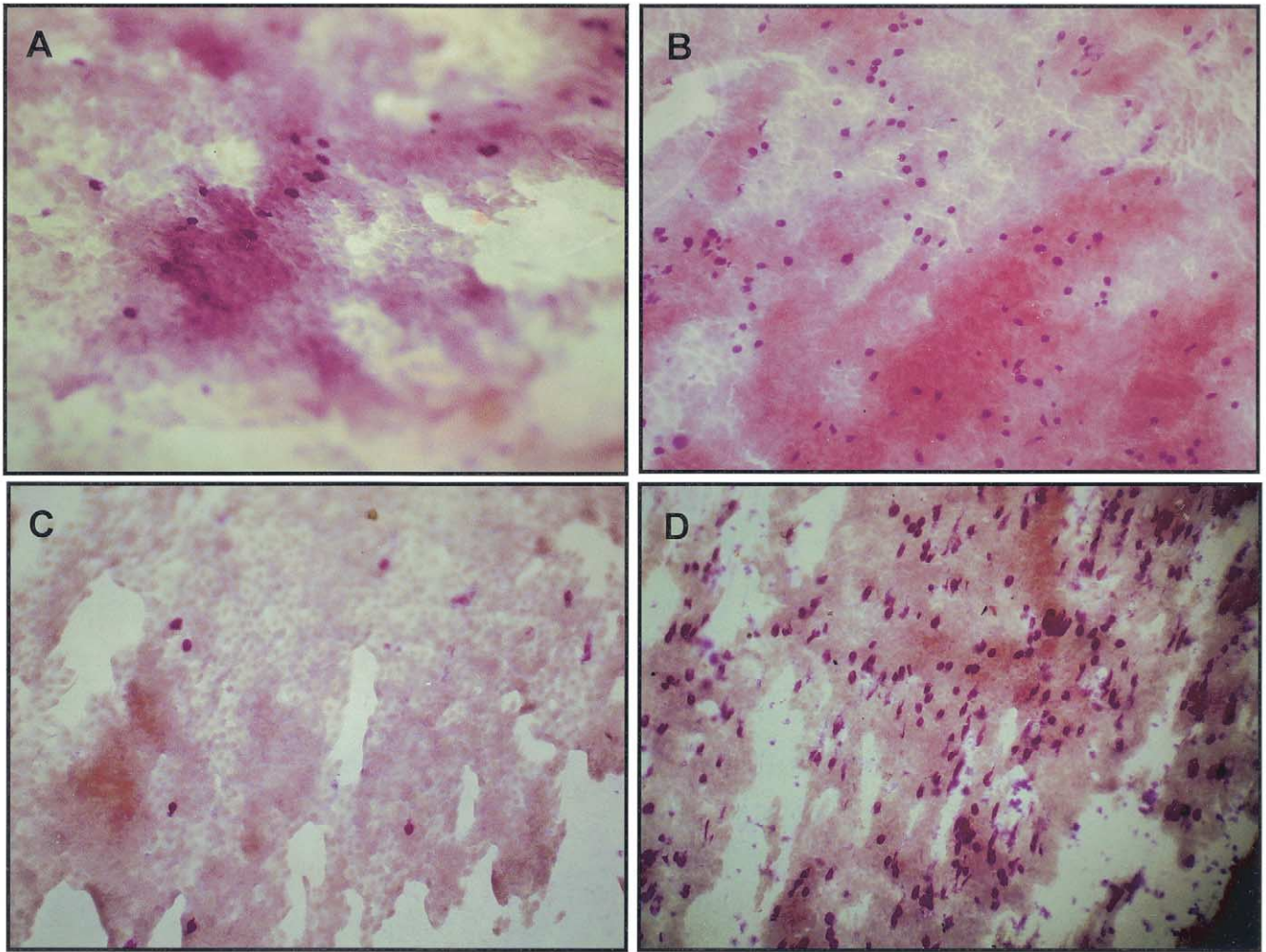


Figure 2. Impression cytology specimens from 2 patients 74 and 77 months, respectively, after pterygium excision and mitomycin C application. Specimens were taken from the surgical site (A and C, respectively) and from the corresponding contralateral nonoperated site (B and D, respectively). No differences were noted between the nongoblet conjunctival epithelial phenotypes. However, a marked reduction of the goblet cell density was demonstrated in the operated site (A, C) compared with the nonoperated site (B, D).

years, indicates a lack of any long-term damage to the scleral wall by the single intraoperative 5-minute application of mitomycin C.

Mitomycin C has been used widely in pterygium surgery at our institute since 1989. In our previous and current reports,^{3,14,20} we have not encountered even a single case of scleral thinning, scleral melting, or scleral perforation as a result of mitomycin C used in pterygium surgery. We attribute these results to a careful selection of the patients, the close postoperative follow-up, and our method of surgery. We preserved the conjunctiva overlying the pterygium body by dissecting it from the pterygium body and suturing the conjunctiva back to the sclera at the end of the procedure, thus allowing the epithelium to slide over the areas of bare sclera and cover the wound faster. We speculate that the reported cases of scleral melting are a result of leaving a large area of bare sclera after mitomycin C application, without careful follow-up. When the wound fails to epithelialize, this will result in a vascular compromise to the

surgical site, which, when coupled with the permanent inhibition of fibroblast proliferation, exposes the sclera to avascular necrosis. In addition, we avoid thermal cautery of any bleeding vessels during surgery. These methods keep the conjunctival and episcleral vasculature intact and prevent long-term damage to the scleral wall. In addition, some of the mitomycin C-associated complications reported in the literature were a result of poor selection of patients, who had ocular surface or immune disorders, as well as poor follow-up and postoperative management.⁵ After pterygium excision with mitomycin C, patients should be closely followed up until epithelialization is complete. Any delay in epithelialization should be promptly treated, as it may result in scleral melt.

Impression cytology was used in this study to assess the effect of mitomycin C on the conjunctival GCD and on the overall conjunctival epithelial phenotype. The temporal bulbar conjunctiva of the same eye was used as a control for impression cytology. Although the ideal control would have

been the nasal bulbar conjunctiva of the contralateral eye, we could not use it because the nasal conjunctival area of the contralateral eye either had a pterygium or had already undergone pterygium surgery in the majority of our patients.

We found that the conjunctival epithelium retained its normal phenotype and did not transform into squamous metaplasia, a pattern usually associated with keratoconjunctivitis sicca and different cicatrizing diseases and ocular irritation disorders.^{16,21} These findings show, as expected, that mitomycin C did not have a long-term effect on the morphology of the nongoblet conjunctival epithelium population. Morphologic changes in the conjunctival epithelium during topical mitomycin C treatment for primary acquired melanosis with atypia were reported during the 2- to 3-week treatment period.²² These features included nuclear enlargement and chromatin hyperchromasia in the superficial epithelial layers in conjunctival biopsies. However, these alterations in epithelial morphology were described during the period of intensive topical treatment with mitomycin C drops,²² whereas our study was performed 6 years after a single intraoperative application of the drug.

Conversely, an almost 4-fold decrease in GCD was noted in the surgical site relative to the nonoperated site. Reduced GCD was previously reported in a group of patients 12 months after pterygium surgery with mitomycin C.²³ In that study, an overall decrease in GCD was found also in patients who had pterygium excision using a bare sclera technique as well as in pterygium patients with a conjunctival autograft. The GCD gradually returned to its normal level in the latter 2 groups over a 12-month period, but in the mitomycin C group it remained significantly lower than the normal level.²³ In our study we found a markedly low GCD after a mean period of 6 years, whereas our comparison includes corresponding conjunctival sites in the same patient. These data are augmented by a recent report of a possible increased goblet cell population that exists in primary pterygia before surgery.²⁴ If the GCD preoperatively is higher over the pterygium area than over the normal conjunctiva, then the absolute loss of goblet cells is much higher than what is demonstrated in our study. Taken together, these findings imply that there is a true sustained impairment of the goblet cell population over time as a result of mitomycin C application. However, this decrease of GCD is limited to the surgical site, and its clinical significance is not yet understood. We feel that a localized decrease of goblet cells in a restricted area does not affect the otherwise healthy ocular surface, and has a limited effect on the total mucin production. Further studies are needed to evaluate the causes of this increased susceptibility of the goblet cells to antiproliferative agents.

The long-term safety of mitomycin C in pterygium surgery should be assessed in association with the surgical method used and with regard to the inclusion criteria of patients. We have shown previously that careful patient selection (e.g., a healthy ocular surface, a normal tear film, lack of signs and symptoms of ocular irritation or dry eye syndrome) and the use of a single intraoperative application of 0.2-mg/ml mitomycin C, though preserving the overlying conjunctiva, minimize the risks of the severe complications

associated with mitomycin C. Our present data demonstrate a lack of long-term damage to the sclera at the operated site, with a possible localized deficiency in the number of conjunctival goblet cells. Nowadays, we are avoiding our previous technique of leaving 3 mm of bare sclera and instead suture the conjunctiva close to the limbus to increase the safety of the procedure. Future modifications of our technique, such as mitomycin C application combined with a conjunctival autograft or with amniotic membrane transplantation, may further augment the safety of the procedure and may improve the long-term health of the ocular surface as manifested by the conjunctival epithelium phenotype.

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