Long-term Effects on Corneal Keratocytes of Mitomycin C During Photorefractive Keratectomy: A Randomized Contralateral Eye Confocal Microscopy Study

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THE AUTHORS HAVE NO FINANCIAL OR PROPRIETARY INTEREST IN THE MATERIALS PRESENTED HEREIN.

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PURPOSE: To evaluate the long-term side effects of mitomycin C (MMC) assisted photorefractive keratectomy (PRK) on corneal keratocytes of highly myopic eyes.

METHODS: Twenty-eight patients with bilateral myopia from −7.00 to −14.25 diopters (D) underwent PRK on both eyes, one eye of each patient received topical application of 0.02% MMC for 2 minutes immediately after the PRK procedure. Corneal keratocyte density was quantified by corneal confocal microscopy at baseline and 5 years postoperatively.

RESULTS: Photorefractive keratectomy reduced keratocyte density in the most anterior stromal layer, without a statistically significant difference between MMC and standard treated eyes. Posterior stromal layers showed no signs of keratocyte loss with either techniques.

CONCLUSIONS: Phototherapeutic keratectomy with 0.02% topical MMC has no significant side effects on corneal keratocytes compared to standard PRK, as documented by in vivo corneal confocal microscopy.

Excimer laser photorefractive keratectomy (PRK) continues to have a relevant role in the surgical correction of refractive errors, particularly in eyes more prone to detrimental side effects after LASIK (eg, lower corneal thickness and high myopia).1,2 Highly myopic eyes, which need significant volumes of tissue removed to correct the refractive error, seem the best candidates for PRK. Unfortunately, correction of high myopia with PRK leads to haze development, compromising final visual function. To overcome the development of stromal haze, the prophylactic use of topical mitomycin C (MMC) during PRK has been successfully advocated.3-5 Gambato et al4 reported that topical MMC significantly lowers the incidence of haze in highly myopic eyes compared to standard corticosteroid treatment. Recently, the use of topical MMC for corneal haze prevention has been cautioned because of presumed long-term side effects of this topically applied drug.6

The aim of this study is to report the in vivo long-term effects, quantified by corneal confocal microscopy, on corneal keratocytes of eyes that underwent PRK with topical MMC for the correction of high myopia compared to fellow eyes that underwent standard PRK treatment without MMC.

PATIENTS AND METHODS

This prospective, randomized, double-masked study comprised 28 patients (56 eyes) with bilateral high myopia (defined as ≥7.00 diopters [D]) who underwent PRK treatment with MMC in one eye and PRK treatment without MMC in the fellow eye. All eyes underwent corneal confocal microscopy at baseline and 5 years after bilateral PRK. To circum-
vent any significant differences in the amount of tissue removed between eyes, only patients with a baseline difference of <0.75 D bilaterally were included. At baseline, one eye was randomly assigned, per a randomization schedule, to receive topical MMC treatment, and the fellow eye was treated without MMC. This contralateral eye study approach was chosen to limit the influence of wound-healing characteristics on the study.

This study was approved by the institutional review board of Padova University Hospital. Prior to participating in the study, each patient signed a detailed informed consent that outlined the risks and benefits of the study. This study was double masked during surgery and follow-up. Inclusion criteria were preoperative consent that outlined the risks and benefits of participating in the study, each patient signed a detailed in-board of Padova University Hospital. Prior to participation the study.

Surgery and follow-up. Inclusion criteria were preoperative consent that outlined the risks and benefits of participating in the study, each patient signed a detailed in-board of Padova University Hospital. Prior to participation the study.

Corneal topography, and stable manifest refraction as acuity (BSCVA) of 20/25 or better in both eyes, normal wound healing, immunodeficiency, previous intraocular or corneal surgery, glaucoma or glaucoma suspect, active ocular disease or corneal abnormality, hard contact lenses.

Exclusion criteria were keratoconus, keratoconus suspect, active ocular disease or corneal abnormality, systemic disease likely to affect wound healing, unstable keratometry readings with irregularly shaped mires, use of systemic medications likely to affect wound healing, immunodeficiency, previous intraocular or corneal surgery, glaucoma or glaucoma suspect, and corneal thickness that would require ablation within 300 µm of the endothelium.

Surgery and Postoperative Follow-up

The operative and postoperative protocols have been described in detail previously. Briefly, after topical anesthesia (oxybuprocaaine 0.4%, Novartis; Novartis Pharma, Milan, Italy) and chemical epithelial removal by a 20% ethanol-balanced salt solution instilled in a 7-mm optical zone maker, all eyes were treated using with the COMPeX 300 laser (InPro, Norderstedt, Germany) using a Gaussian profile laser beam. The mean depth of ablation was 96±18 µm (range: 65 to 120 µm). Immediately after treatment, MMC-treated eyes received a single topical application of MMC 0.02% (diluted with balanced salt solution) administered by placing a soaked sponge (7 mm in diameter) over the ablated stroma for 2 minutes. The corneal surface and entire conjunctival fornix were thoroughly irrigated with 20 cc of balanced salt solution. Fellow eyes did not receive MMC and underwent standard PRK and received a sponge soaked in balanced salt solution in place of MMC, with the same post-treatment procedure. After full corneal reepithelialization, all eyes were treated with artificial tears (hyaluronic acid 0.2%, Hylalistil; Sifi, Catania, Italy) three times daily for 3 months. Moreover, MMC-treated eyes received a placebo solution (hyaluronic acid 0.2%) three times daily for 1 month, whereas corticosteroid-treated fellow eyes received fluoromethalone sodium 2% (Flumetol S; Farmila, Milan, Italy) three times daily for 1 month. The dosage of corticosteroids and placebo was reduced to twice daily for the second month postoperatively and once daily for the third month postoperatively.

Confocal Microscopy

Corneal confocal microscopy was used to evaluate corneal layers in vivo. Corneal confocal microscopy was performed using a Confoscan 3.0 corneal confocal microscope (NIDEK Co Ltd, Gamagori, Japan) with a 40× surface-contact objective, using a standardized technique. To analyze corneal keratocyte density before and at 5 years postoperatively, five stromal layers were considered, as described by Erie et al: 1) 0% to 10% (anterior), 2) 11% to 33%, 3) 34% to 66%, 4) 67% to 90%, and 5) 91% to 100% (posterior) depth. One image was selected from each stromal layer for assessment of cell density. Keratocyte nuclei (cells) were identified as bright objects within an area of the same size for each selected image to calculate cell density by using a custom computer program. The mean cell density in each layer after PRK was compared with the mean cell density in the corresponding layer before PRK, and the two treatment groups were also compared.

Statistical Analysis

Statistical analysis of variance for repeated measures was used. The F statistic is a value resulting from a standard statistical test used in analysis of variance and regression analysis to determine whether the variances between the means of two populations are significantly different. For practical purposes, this value determines the P value. A P value <.01 was considered statistically significant.

Results

Fifty-six highly myopic eyes of 28 patients (16 women and 12 men) were included in this study. Mean patient age was 39.7±7 years. Preoperative refractive error was 9.50±1.84 D (range: −7.25 to −14.25 D) in MMC-treated eyes versus 9.00±1.79 D (range: −7.00 to −14.25 D) in corticosteroid-treated eyes (P=.02). No eyes showed signs of delayed reepithelialization or had any adverse side effects during the duration of follow-up.

Keratocyte density in the four posterior stromal...
sections remained unchanged when compared to the preoperative evaluation (from sections 2 to 5: \( F=0.02, P=.90; F=1.12, P=.29; F=0.83, P=.37; \) and \( F=0, P=.97 \)). The most anterior section showed a statistically significant reduction in keratocyte density at 5-year follow-up compared to the preoperative quantification (\( P<.0001 \) in MMC-treated eyes, \( P<.0001 \) in corticosteroid-treated eyes) (Figs 1 and 2; Table). However, no statistically significant difference was noted between groups (\( F=0.38, P=.54 \)).

**DISCUSSION**

Numerous investigators have attempted to modulate corneal wound healing responses after PRK to preserve corneal clarity and the induced refractive correction. Intraoperative topical application of MMC after PRK has been introduced recently as an approach to minimize haze development.\(^3\)\(^-\)\(^5\)\(^-\)\(^8\) Different studies have recorded successful clinical results following PRK with MMC in treating and preventing corneal haze formation. Gambato et al\(^4\) reported a statistically significant decrease in haze formation after the prophylactic use of 0.02% MMC during PRK in highly myopic eyes. High myopes are likely the best candidates for this surgical procedure, compared to standard postoperative treatment, without any midterm significant side effects. Furthermore, Gambato et al\(^4\) confirmed their observations using corneal confocal microscopy, which documented, for the first time, the in vivo apoptotic action of MMC on stromal keratocytes.

Recently, Wilson\(^6\) advised caution when using MMC,

![Figure 1](image1.png)

**Figure 1.** A) Preoperative and B) 5-year postoperative corneal confocal microscopy of the posterior stromal layer of a highly myopic eye that underwent MMC-assisted PRK. No significant difference in corneal keratocytes density was found. Corneal keratocyte nuclei appear as highly reflective, irregularly bordered spots on a dark gray background.

![Figure 2](image2.png)

**Figure 2.** A) Preoperative and B) 5-year postoperative corneal confocal microscopy of the anterior stromal layer of a highly myopic eye that underwent MMC-assisted PRK. The corneal keratocyte cell density is significantly different. This can be appreciated by comparing the two images, where the number of brilliant corneal keratocyte nuclei is lower in the right versus the left image.
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particularly during PRK for low to moderate myopia, because of unknown long-term effects. Our data objectively confirm that the density of anterior corneal keratocytes is reduced in PRK MMC-treated human eyes, but this reduction is equivalent to that induced by standard PRK. Posterior corneal stroma layers did not show signs of keratocyte loss, as previously reported for standard PRK and LASIK by Erie et al. Therefore, at 5-year follow-up highly myopic eyes undergoing PRK with intraoperative prophylactic topical MMC do not show signs of side effects induced by the use of 0.02% MMC. This study confirms the relevant role of corneal confocal microscopy in the in vivo evaluation of the cornea after any refractive surgery procedure, with or without corneal wound healing modulation.

REFERENCES


### TABLE

Density of Corneal Keratocytes at Baseline and 5-year Follow-up for the MMC and Corticosteroid Treatment Groups

<table>
<thead>
<tr>
<th>Corneal Keratocyte Density (keratocytes/0.13 sq.mm)</th>
<th>MMC-treated Eyes</th>
<th>Corticosteroid-treated Eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mean ± standard deviation)</td>
<td>Baseline 5-year</td>
<td>Baseline 5-year</td>
</tr>
<tr>
<td>Corneal Stromal Layers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (Anterior)</td>
<td>449.8±58.5</td>
<td>305.3±59.9*</td>
</tr>
<tr>
<td></td>
<td>386.0±53.9</td>
<td>371.8±56.1</td>
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<tr>
<td>2</td>
<td>376.7±53.4</td>
<td>387.1±53.3</td>
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<tr>
<td>3</td>
<td>366.9±55.5</td>
<td>384.9±46.8</td>
</tr>
<tr>
<td>4</td>
<td>363.5±53.9</td>
<td>392.2±53.2</td>
</tr>
<tr>
<td>5 (Posterior)</td>
<td>363.5±53.9</td>
<td>392.2±53.2</td>
</tr>
</tbody>
</table>

*Statistically significant difference (P<.0001).