Comparison of Laser Epithelial Keratomileusis With and Without Mitomycin C for Wavefront Customized Surface Ablations

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Mr Bains is a clinical consultant to NIDEK Co Ltd. All remaining authors have no proprietary or financial interest in the materials presented herein.

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ABSTRACT

PURPOSE: To investigate the efficacy of mitomycin C (MMC) in preventing haze formation in surface wavefront customized ablations with successful refractive treatment (laser epithelial keratomileusis [LASEK]) and to evaluate the safety of this technique on corneal stroma and endothelium.

METHODS: This study was a prospective, double-masked, randomized clinical trial involving 52 eyes (30 placebo and 22 MMC) of 26 patients. The manifest refractive spherical equivalent (MRSE), best spectacle-corrected visual acuity, uncorrected visual acuity, corneal pachymetry, topography, aberrometry, endothelial specular microscopy, contrast sensitivity, corneal confocal microscopy, and complaints of pain via a subjective questionnaire were recorded preoperatively and 90 days postoperatively.

RESULTS: The mean MRSE at 90 days postoperatively was −0.56 diopters (D) (−4.95±1.85 D, range: −8.00 to −1.62 D) for the MMC group and −0.49 D (−4.51±1.81 D, range: −7.75 to −2.25 D) for the placebo group. Higher order aberrations were similar between the placebo and MMC groups 90 days postoperatively (0.538±0.228 µm and 0.478±0.134 µm, respectively). Analysis of the endothelial cell count indicated a statistically significant decrease in endothelial cell density (P=.017) after LASEK, independent of MMC use.

CONCLUSIONS: The predictability of the final target refraction, induction of high order aberrations, and improvement in contrast sensitivity proved that the use of MMC was equally safe when compared to procedures that did not use MMC. In addition, the procedure was efficient in the prevention of corneal haze. [J Refract Surg. 2007;23:S1021-S1028.]

Myopia and astigmatism have been treated with reasonable success with radial keratotomy in the past.1 However, the precision of the excimer laser technology has iteratively been upgraded such that the results of the correction of myopia, as well as hyperopia and astigmatism, are reproducible and predictable.2

Initially, photorefractive keratectomy (PRK) accounted for 100% of excimer laser refractive surgeries. At the time, the limitations of PRK in treating cases of high myopia and astigmatism were already known. Regression of hyperopic treatments, postoperative pain, delay in visual recovery, and late corneal haze encouraged surgeons to investigate other refractive surgery options.3

In 1964, Barraquer3 produced a corneal disc procedure known as keratomileusis, and from this concept, Pallikaris et al4 developed laser in situ keratomileusis (LASIK) in which an excimer laser beam was delivered to the corneal stromal bed. Modern microkeratomes have produced corneal flaps with varying diameters and thicknesses with a high degree of predictability and a relatively low rate of complications.5

Thus, LASIK has gradually replaced PRK due to faster visual recovery, possibility of greater success in treating high ametropia, and higher levels of satisfaction among patients, including relatively little discomfort during the postoperative period.6

However, cutting the corneal stroma might interfere with collagen fiber within the cornea, and, as a result, the corneal structure may become unstable. Recent studies and empirical observations have indicated the necessity of a broader comprehension of corneal properties, especially related to the healing process and biomechanical qualities.7-10 Reports

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of corneal ectasia after LASIK have been widely published and trials to establish a safety profile for LASIK surgery candidates have been proposed. Despite all efforts to identify and avoid corneal procedures in patients with risk factors for corneal ectasia such as irregular corneal topography, thin preoperative corneal thickness, elevated posterior curvature, difference between superior and inferior corneal dioptic power, and posterior over anterior best fit sphere, reports of unexpected cases of ectasia show that more research is necessary to address issues regarding the corneal structure, integrity, and corneal response to LASIK.

As a result, surface ablation procedures that might preserve the corneal architecture, such as PRK, laser epithelial keratomileusis (LASEK), or epi-LASIK, are gaining popularity. However, corneal haze postoperatively remains a concern in surface ablation because it compromises corneal transparency and causes surface irregularity. Topical steroids have been the main prophylactic tool against haze, but recently, anti-proliferative drugs such as mitomycin C (MMC) have been used to modulate such an abnormal and unexpected corneal response. To date, no prospective, double-masked randomized comparative studies report the safety, efficacy, visual quality, corneal integrity, and wavefront aberration in the use of MMC for corneal surface ablations in primary cases versus a cohort of patients who did not receive MMC.

The purpose of this study was to investigate the efficacy of MMC in preventing haze formation in surface wavefront customized ablations and to evaluate the safety of this procedure on corneal stroma, the endothelium, and visual quality.

**PATIENTS AND METHODS**

**Patients and Measurements**

A prospective, double-masked, randomized clinical trial was conducted on 52 eyes of 26 patients. A randomization schedule generated by a biostatistician was used to divide patients into two groups; one group underwent LASEK with MMC (MMC group) and the other group underwent LASEK with balanced salt solution (BSS) (placebo group). Patients were masked as to whether they received MMC until all study data were collected.

This study followed the tenets of the Declaration of Helsinki and informed consent was obtained from all participants after the nature and possible consequences of the study were explained. Pre- and postoperative assessment included manifest refractive spherical equivalent (MRSE), best spectacle-corrected visual acuity (BSCVA), uncorrected visual acuity (UCVA), ultrasound corneal pachymetry, endothelium specular microscopy, and confocal microscopy using the Confoscan II (NIDEK Co Ltd, Gamagori, Japan). Stromal keratocyte counts were performed with the automatic cell count device of the Confoscan II in an area of 300 µm², 10 to 20 µm beneath the basal cell layer. Mesopic contrast sensitivity was measured using the Pelli-Robson chart (Haag-Streit, Koniz, Switzerland) with distance correction in place. Wavefront aberrations and corneal topography were measured using the OPD-Scan wavefront aberrometer and corneal topographer (NIDEK Co Ltd). All wavefront measurements were performed using a pupil diameter of at least 6 mm and out to the 6th Zernike order. Patients were evaluated 1, 4, 7, 30, and 90 days postoperatively. Examinations performed during each follow-up are shown in Table 1.

**TABLE 1**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Preop</th>
<th>1 Day</th>
<th>4 Days</th>
<th>7 Days</th>
<th>30 Days</th>
<th>90 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refraction</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSCVA</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCVA</td>
<td>√</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specular microscopy</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contrast sensitivity test</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confocal microscopy</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aberrometry</td>
<td>√</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

BSCVA = best spectacle-corrected visual acuity, UCVA = uncorrected visual acuity
Postoperatively, all patients answered a subjective questionnaire that recorded pain after the surgical procedure. The pain scale classification was: grade 1, no complaint of pain; grade 2, foreign body sensation; grade 3, slight pain; grade 4, moderate pain; and grade 5, extreme pain.

Observations regarding corneal haze, stromal keratocyte count, and other aspects of confocal microscopy were recorded by a single examiner who was masked as to the use of MMC during LASEK.

**Surgical Procedure**

All surgeries were performed by three surgeons (E.M.N., W.P., K.N.) using the same surgical technique. Laser epithelial keratomileusis was performed under topical anesthesia. Twenty percent ethyl alcohol was delivered into a corneal marker placed on the cornea to form a well and held in place for 20 seconds. Subsequently, an epithelial flap was reflected towards the corneoscleral junction using a spatula. All eyes underwent optical path difference customization aspheric treatment (OPDCAT) using the NIDEK Advanced Vision Excimer Laser platform (NAVEX). The OPDCAT ablation algorithm uses aspheric optical and transition zones coupled with the treatment of ocular wavefront higher order aberrations. All data generated from the NIDEK OPD-Scan were imported into Final Fit ablation planning software (NIDEK Co Ltd), which allows the simulation of treatments based on the selection of various parameters such as treatment zones and ablation profile to customize the treatment to the eye. Once an adequate simulation was achieved the data were transferred via a universal serial bus to the excimer laser. The NIDEK EC-5000 CX II excimer laser equipped with a 200-Hz infrared eye tracker was used to deliver the ablation onto the cornea. All treatments were centered on the center of the pupil. After the laser ablation, 0.02% MMC or BSS (placebo) was delivered (per the randomization schedule) into a 7.0-mm diameter circular corneal marker to achieve a homogeneous exposure of the stroma to the drug or placebo. The eyes were exposed to either MMC or BSS for 30 seconds and rinsed with 50 mL of BSS before repositioning the epithelial flap and placement of a therapeutic contact lens. One drop of 1% ofloxacin and 1 drop of 1% prednisolone acetate were instilled into the eye prior to discharging the patient.

Upon discharge, the patient was instructed to instill 1% ofloxacin four times daily for 7 days, 1% prednisolone acetate four times daily for 10 days, and eye lubricant as needed for discomfort. Contact lenses were removed at the 4-day follow-up examination.

**Statistical Analysis**

Continuous and categorical data were analyzed using the *t* test and Chi-square test, respectively. To analyze changes in means across time, repeated measures analysis of variance (ANOVA) was used. The analyses

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo Group</th>
<th>MMC Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male (%)</td>
<td>26.7</td>
<td>36.4</td>
<td>.45</td>
</tr>
<tr>
<td>Age (y)</td>
<td>32.7±7.2</td>
<td>38.8±8.7</td>
<td>.01</td>
</tr>
<tr>
<td>MRSE (D)</td>
<td>−4.51±1.81</td>
<td>−4.95±1.85</td>
<td>.41</td>
</tr>
<tr>
<td>Visual acuity (logMAR)</td>
<td>0.008±0.205</td>
<td>−0.184±0.338</td>
<td>.03</td>
</tr>
<tr>
<td>RMS (µm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.679±2.188</td>
<td>5.874±2.443</td>
<td>.76</td>
</tr>
<tr>
<td>Higher order</td>
<td>0.343±0.074</td>
<td>0.343±0.114</td>
<td>.98</td>
</tr>
<tr>
<td>Coma</td>
<td>0.148±0.091</td>
<td>0.161±0.066</td>
<td>.55</td>
</tr>
<tr>
<td>Trefoil</td>
<td>0.234±0.084</td>
<td>0.220±0.119</td>
<td>.63</td>
</tr>
<tr>
<td>Tetrafoil</td>
<td>0.074±0.041</td>
<td>0.089±0.047</td>
<td>.24</td>
</tr>
<tr>
<td>Spherical aberration</td>
<td>0.098±0.052</td>
<td>0.097±0.047</td>
<td>.95</td>
</tr>
<tr>
<td>Higher order astigmatism</td>
<td>0.066±0.041</td>
<td>0.081±0.046</td>
<td>.22</td>
</tr>
<tr>
<td>Endothelial cell count (cells/mm²)</td>
<td>2989.3±452.6</td>
<td>3167.0±471.1</td>
<td>.20</td>
</tr>
</tbody>
</table>

MRSE = manifest refractive spherical equivalent, RMS = root-mean-square

P < .05 was considered statistically significant.
were performed using SAS version 9.1 (SAS Institute Inc, Cary, NC). A P value <.05 was considered statistically significant.

**RESULTS**

A statistically significant difference was noted in mean patient age between the two groups (Table 2) (P=.01). Preoperatively, no other statistically significant differences were observed between the placebo (n=30) and MMC (n=22) groups (Tables 2 and 3).

Postoperative refractive stability is plotted in Figure 1. No significant differences in MRSE were observed between the groups at 7-day (P=.82), 30-day (P=.97), or 90-day (P=.80) follow-up (Table 3).

Higher order aberrations were similar between the placebo and MMC groups at 30 days (0.533±0.230 μm and 0.577±0.231 μm, respectively, P=.54) and 90 days postoperatively (0.538±0.228 μm and 0.478±0.134 μm, respectively, P=.33) (Fig 2). After 30 days postoperatively, statistically significant increases were noted in mean contrast sensitivity in the MMC group at all spatial frequencies (Table 4). Clinically significant haze was not observed in either group during the entire postoperative period. However, an increase of the
reflectivity of the anterior stroma extracellular matrix was commonly observed during confocal microscopy in the placebo group (Fig 3).

Patients reported overall pain as a “foreign body sensation.” No statistically significant difference was noted in the subjective pain score between groups on postoperative days 1 ($P_{=}.40$), 4 ($P_{=}.09$), or 7 ($P_{=}.92$).

A statistically significant recovery in keratocyte population was noted between 30- and 90-day follow-up in both groups ($P_{=}.005$). No differences occurred between the placebo and MMC groups at 30 days postoperatively (194.9±60.2 and 176.4±55.2, respectively, $P_{=}.35$) or at 90 days postoperatively (251.9±60.7 and 211.8±50.7 respectively, $P_{=}.05$). Analysis of the endothelial cell count indicated a statistically significant decrease in endothelial cell density ($P_{=}.017$) after LASEK, independent of whether MMC was used (Fig 4). There were no statistically significant differences in endothelial cell density between groups at postoperative days 7 ($P_{=}.23$), 30 ($P_{=}.02$) or 90 ($P_{=}.83$).

Figure 5 shows the predictability of both groups.

**DISCUSSION**

The surgical procedure chosen for the present study was LASEK. Advantages of using the epithelial layer as a biological bandage after ablation are decreased discomfort and late haze formation.$^{11}$ Decreased migration of inflammatory cytokines and lower quantities of TGF-β dissolved in tears of patients who underwent LASEK have been observed.$^{15}$ Therefore, LASEK itself might be responsible for the inhibition of haze in the patients enrolled in this study. Further studies are re-
Mitomycin C is extracted from a bacteria (Streptomyces caespitosus) and its mechanism of action focuses on the inhibition of keratocyte activity, decrease of collagen formation, and stimulation of cellular apoptosis. Mitomycin induces apoptosis of keratocytes and myofibroblasts, but the predominant effect in inhibiting or treating haze appears to be at the level of blocking replication of keratocytes or other progenitor cells of myofibroblasts. The corneal healing process is induced by the activation and proliferation of keratocytes. The collagen tissue produced by these cells is significantly unorganized compared to normal stromal tissue and shows matrix-free areas and fibers with an irregular stereospatial relationship. Therefore, any healing process on the cornea, theoretically, induces a loss of corneal transparency.

In ophthalmology, MMC has been widely used for glaucoma filtering surgeries, superficial keratectomies during pterygium excision, and ocular surface reconstruction in pemphigoid, superficial corneal degenera-

Figure 4. Endothelial cell count over time of eyes that underwent LASEK with or without MMC using the NAVEX platform.

Figure 5. Refractive outcome over time of eyes that underwent LASEK A) with or B) without MMC using the NAVEX platform. SE = manifest refractive spherical equivalent.
tions, and neoplasias. In refractive surgery, the use of MMC was originally proposed by Talamo et al\textsuperscript{19} to inhibit the corneal wound healing response that might produce opacities (such as haze) and scars. Initially, it was exclusively used in retreatments after radial keratotomy, previous LASIK, and unsuccessful or complicated keratomileusis to avoid complications.\textsuperscript{14} Corneal complications after MMC use include corneal edema, recurrent erosion, melting, and perforation.\textsuperscript{20}

The presence of MMC in the anterior chamber after topical application (during PRK) has been reported.\textsuperscript{21} Cytotoxicity of MMC in the anterior chamber can cause lasting damage to the endothelial cell layer.\textsuperscript{22} Recent studies of endothelial cell counts have reported increases, decreases, and no significant alteration in the cell count after topical application of MMC during refractive surgery.\textsuperscript{22-24} In the current study, a statistically significant decrease in endothelial cell count was found regardless of MMC use. In a similar study using PRK and epi-LASIK, Diakonis et al\textsuperscript{22} reported a decrease in endothelial cell. However, once the repeatability of the instrument was included in the analyses, no change in endothelial cell density was found.\textsuperscript{22} Although not investigated, we believe this may explain the decrease observed in the current study. Longer term follow-up studies involving the use of MMC to establish its effects on the different aspects of the corneal integrity are warranted for definitive conclusions.

The lack of difference in refractive outcomes between groups is similar to that reported in other studies. In a retrospective analysis of a smaller sample size (n=30) and a slightly higher preoperative MRSE, Argento et al\textsuperscript{25} found no difference in MRSE or UCVA 6 months postoperatively. In a prospective, nonrandomized study, de Benito-Llopis et al\textsuperscript{23} found no differences in outcomes despite treating a higher MRSE in the MMC group.

A clinically significant increase in contrast sensitivity was observed in the MMC group only. Increases in contrast sensitivity after MMC use for PRK have been reported.\textsuperscript{26} The increase in contrast sensitivity was observed 6 months after PRK\textsuperscript{26} whereas we found an increase after 1 month postoperatively. This relatively quick rehabilitation of visual quality may be an indicator of the faster wound healing seen in LASEK. The increase in contrast sensitivity may portend better visual quality in eyes that undergo LASEK with topical MMC. Age and preoperative contrast sensitivity cannot account for the differences in visual quality between groups, as the MMC group was older and had a lower preoperative contrast sensitivity (Table 4). Perhaps the lower reflectivity of the extracellular matrix of the anterior stroma in the MMC group indicates lower corneal light scatter in this group, which may explain the difference in contrast sensitivity.\textsuperscript{27} However, further studies are required to verify these subjective observations.

A drawback of this study is the difference in the number of eyes in each group; however, some patients dropped out of the study and could not be contacted. Additionally, longer term follow-up, especially when studying an anti-proliferative agent, is warranted.

The use of MMC in customized surface treatments seems to be comparable to the same surgical procedure without the use of this anti-proliferative drug. The predictability of the final target refraction, high order aberration induction, contrast sensitivity improvement, and evaluation of stromal and endothelial cells suggests that the use of MMC is equally safe when compared to procedures without its use. The lack of difference in haze formation may be due to the low myopia treated in this study; however, one study has reported a decrease in haze due to MMC use regardless of the amount of refractive error.\textsuperscript{25} The results of our investigation indicate that LASEK with MMC is not required for the magnitude of refractive error treated in this trial. In addition, LASEK itself proved to be efficient in the prevention of corneal haze.

REFERENCES


