Original Article

# Effect of mitomycin-C on corneal endothelial cell parameters after refractive surface ablation procedures

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## ABSTRACT

**Background:** The effect of mitomycin-C (MMC) on the reduction of endothelial cell count in the cornea remains controversial. We aimed to evaluate the effect of MMC on corneal endothelial cell parameters after refractive surface ablation procedures, including photorefractive keratectomy (PRK) and laser epithelial keratomileusis (LASEK).

**Methods:** In this interventional, comparative, follow-up study, 342 eyes of 171 patients were followed up for 6 months. Patients undergoing PRK or LASEK were included and were divided into two groups: group one (188 eyes of 94 patients) with an ablation depth of ≥ 65 µm and who received intraoperative 0.02% MMC for 30 s, and group two (154 eyes of 77 patients) with an ablation depth of < 65 µm and who received balanced salt solution for 30 s. Changes in endothelial cell density (ECD), central corneal thickness (CCT), coefficient of variation (CV), and hexagonality values were compared between the groups at 3 and 6 months after surgery.

**Results:** The mean ± standard deviaiton (SD) age of the patients was 28.11 ± 6.56 years. The mean ± SD ECD did not change significantly in either group between the baseline and at 3 and 6 months postoperatively. The baseline mean ECD was significantly higher in group one than that in group two ($P < 0.001$) and remained so at 3 ($P = 0.002$) and 6 months ($P = 0.022$) postoperatively. The baseline hexagonality value was lower in group one ($P = 0.173$), with a gradual decrease during the postoperative follow-up as compared with that in group two ($P = 0.016$ and 0.001 at 3 and 6 months postoperatively, respectively). Group one had a significantly lower CCT at 3 and 6 months postoperatively (both $P < 0.001$) and a higher mean CV (3 months: $P = 0.028$; 6 months: $P = 0.328$).

**Conclusions:** A single intraoperative application of MMC for 30 s as prophylaxis for corneal haze development during refractive surface ablation procedures had no significant effect on ECD up to 6 months postoperatively. Future studies with a contralateral-eye design (to neutralize factors specific to the individual patient), a larger sample size, and longer follow-up are necessary to confirm or disprove our observations.

**KEY WORDS**
mitomycin-C, corneal endothelium, surface ablation, corneal haze, specular microscopy, corneal opacity, endothelial cell density, central corneal thickness, coefficient of variation, hexagonality

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## INTRODUCTION

Corneal haze is a known complication of certain types of refractive surgeries, mostly occurring after photorefractive keratectomy (PRK); however, it has also been reported after other surface ablation procedures, such as laser epithelial keratomileusis (LASEK) or epipolis LASIK (epi-LASIK) [1]. Immediately after PRK, keratocytes, which exist underlying the excimer laser-ablated tissue, undergo apoptosis in proportion to the amount of laser ablation [2], as seen at 3 months postoperatively [3]. Haze formation, i.e., decreased subepithelial corneal transparency, which is related to aberrant epithelial and stromal wound healing processes, remains a major concern in laser ablation procedures [4]. Clinically significant haze occurs in only 0.5-3% of cases in the post-refractive surgery period; however, PRK can specifically cause reticular or dense haze. In addition, the higher the refractive correction, the higher the risk of stromal haze, with the refractive outcome being less predictable [5].

Mitomycin-C (MMC) is an alkylating agent that acts as an antifibrotic and antiproliferative agent [6]. The immediate post-ablation application of MMC can prevent the recurrence of subepithelial fibrosis and scarring after refractive surgery [6]. Many surgeons use MMC prophylactically or as an adjunct for the prevention of corneal haze, particularly in cases of high-myopia [7-10], even though MMC remains "off-label" for haze prevention after corneal refractive surgery [11]. The application of MMC remains controversial in patients with low myopia (< 5 diopters [D] to 6 D myopia) because the incidence of haze is considerably lower in these patients [12]. However, the positive effect of MMC in preventing haze, even in patients with low myopia, has been documented [13]. There is no consensus on the "best" dose and application duration for MMC, but it is routinely administered using a disk soaked in 0.02% (0.2 mg/mL) of MMC from 12 s up to 2 min [12].

In this study, we assessed the possible effects of a single intraoperative application of topical 0.02% MMC for 30 s on the corneal endothelium and haze formation after refractive surface ablation procedures, including PRK or LASEK. We assessed its effects on specular microscopy findings, including endothelial cell density (ECD), central corneal thickness (CCT), coefficient of variation (CV), and hexagonality.

## METHODS

In this interventional, non-randomized, comparative follow-up study, patients who were scheduled for refractive surface ablation procedures (PRK or LASEK) for myopia were recruited from July 2014 to September 2017 at Basir Eye Clinic, Private Ophthalmology Center, Tehran, Iran. The study adhered to the tenets of the Declaration of Helsinki and was approved by the ethics committee at the institute level. Informed consent was obtained from the patients before surgery. They were informed that their participation in the study was voluntary and that they could withdraw from the study at any point in time without negative consequences to themselves.

In total, 180 eyes would require in each group to obtain 80% power to detect a 3.0% difference in the mean ECD between the groups at a two-sided 5.0% significance level. The population proportion and alpha level (margin of error) were calculated as 3% and 2.96%, respectively.

All of the patients who were scheduled for either PRK or LASEK were enrolled unless they met one or more of the following exclusion criteria: unstable refraction, previous ocular surgery, lenticular changes, inadequate wound-healing process (e.g., connective tissue disease or diabetes), comorbid disease that would cause severe dry eyes, corneal dystrophy, corneal ectasia and keratoconus (suspected or definite), uveitis, glaucoma, moderate or severe meibomian gland dysfunction, history of herpetic keratitis, and retinal disease. Cases with mesopic pupil diameter > 6 mm or patients whose eyes could not belong to the same treatment group because of differing ablation depths were also excluded. Patients using contact lenses were asked to stop wearing their lenses for 1–2 and 3–4 weeks preoperatively for soft and hard lenses, respectively [14]. Masking was followed appropriately; the surgeon, person performing specular microscopy, and the person who read and interpreted the printout reports of specular microscopy were masked.

Preoperative assessment included complete ophthalmological examination, mesopic pupil diameter measurement, determining ocular dominance, measurement of uncorrected (UCDVA), and best-corrected distance visual acuity (BCDVA) using a Snellen chart (auto chart projector CP 670; Nidek Co., Ltd , Gamagori, Japan), ultrasonic corneal pachymetry (UP-800; Nidek Co., Ltd.), applanation tonometry (Perkins; Clement Clarke, Haag-Streit, Harlow, United Kingdom), keratometry (Javal Schiotz keratometer; Haag-Streit, Bern, Switzerland), corneal topography (EyeSys Vista topographer/VFA Tracey; EyeSys Vision Inc., Houston, TX, USA), dilated fundoscopy examination, dilated and undilated slit-lamp biomicroscopy examination (Photo-Slit Lamp BX 900; Haag-Streit, Koeniz, Switzerland), autorefractometry (Topcon KR-8800 refractometer; Topcon Corporation, Tokyo, Japan), manifest and cycloplegic refraction, calculation of spherical equivalent (SE; sphere + 1/2 cylinder), tomography (Pentacam ® HR Premium; Oculus Optikgerate GmbH, Wetzlar, Germany), and

wavefront analysis (OPD-Scan II Wavefront Aberrometer; Nidek Co., Ltd.). Slit-scanning confocal microscopy (Confoscan 4; Nidek Co., Ltd.) was also performed immediately before surgery.

The central corneal ECD, percentage of hexagonal cells, CV, and CCT were evaluated using specular microscopy (SM; Topcon SP-3000P; Topcon Corporation). After the initial examinations and determination of the required laser ablation depth, based on the magnitude of myopia, the patients were assigned to two groups: patients in group one needed higher myopic correction and an ablation depth of ≥ 65 μm, and those in group two needed an ablation depth of < 65 μm.

The refractive surface ablation procedures (PRK or LASEK) were performed by a single surgeon (H.M.R) using an excimer laser (Technolas C-LASIK 217 excimer laser; Bausch and Lomb, Chiron Technolas GmbH, Dornach, Germany). Preoperatively, 5% tetracaine (Sina Daru, Tehran, Iran) eye drops was instilled to anesthetize the cornea. A 5% povidone-iodine solution was utilized for sterilization, and an eyelid speculum was used to keep the eye open during the procedure. The epithelium was marked with an 8-mm-diameter corneal marker centered over the pupil. The epithelium was removed using a spatula. After contact with 20% alcohol solution for 20 s, a cellulose sponge was used to remove the alcohol, and the undersurface was gently washed with balanced salt solution (BSS). The epithelium was removed with a hockey knife, and the flap edges were dried with a sponge. Stromal ablation was performed using an excimer laser. The ablation zone (optical zone) was always larger than the mesopic pupil diameter and was kept between 6 and 6.5 mm. The laser ablation was centered on the visual axis.

When the ablation depth was ≥ 65 μm, a 7-mm round cellulose sponge soaked in 0.02% MMC was applied for 30 s. The corneal surface and entire conjunctiva were subsequently irrigated with 50 mL of cold BSS to remove the residual MMC. In patients with ablation depths < 65 μm, no MMC was used, and the eye was irrigated with cold BSS after ablation. Finally, a bandage contact lens (PureVision™; Bausch & Lomb, Rochester, NY, USA) was applied over the cornea of the operated eye.

Postoperatively, for both groups, 0.3% ciprofloxacin (Sina Darou; every 6 h until complete re-epithelialization), ketorolac trometamine eye drops (Sinarolac®; Sina Darou; every 6 h for the first 24 h), and 1% betamethasone drops (Sina Darou; every 6 h for 2 weeks) were administered, after which the third was changed to fluoromethalone (Sina Darou; every 6 h for the first month, every 8 h for the second month, and every 12 h for the third month, after which it was discontinued). By 3–5 days after the surgery (after complete re-epithelialization), the contact lenses were removed, and non-preservative-containing artificial tears (Valean Darou, Tehran, Iran) were applied every 3–4 h in the first month.

The UCDVA and haze formations were assessed 1 week postoperatively. Additionally, UCDVA, BCDVA, spherical and cylinder refractive errors, SE, and haze were evaluated 1 month postoperatively. The UCDVA, BCDVA, CCT, corneal curvature, intraocular pressure (IOP), spherical and cylindrical refractive errors, SE, haze formation, and specular microscopy indices, including ECD, CV, CCT, and percentage of hexagonal cells, were assessed 3 and 6 months postoperatively. Haze formation was assessed based on the corneal haze grading scale [15], as shown in Table 1.

Data were collected, and statistical analyses were performed using IBM SPSS Statistics for Windows (version 24.0; IBM Corp., Armonk, NY, USA). Statistical comparisons were performed using the paired *t*-test for intragroup comparisons and the unpaired *t*-test for comparisons between the two groups. The safety index was defined as the mean postoperative BCDVA/mean preoperative BCDVA. The efficacy index was defined as the mean postoperative UCDVA/mean preoperative BCDVA. Data are presented as mean ± standard deviation (SD), median (range), or frequency (percentage). The chi-square test was used to assess haze formation postoperatively. Statistical significance was set at $P < 0.05$.

## RESULTS

**Table 1. Corneal haze grading scale [15]**

| Grade | Definition |
|---|---|
| **0** | Clear, no opacity noticed during microscopic slit-lamp examination |
| **0.5** | Trace of or faint haze noticed only using indirect, broad tangential illumination |
| **1** | Haze of minimal density noticed with difficulty using direct or diffuse examination |
| **2** | Mild haze easily evident using direct focal slit lamp illumination |
| **3** | Moderate opacity partially obscuring details of iris |
| **4** | Severe opacity completely obscuring the details of intraocular structures |

Initially, 190 patients were enrolled in the study. After the exclusion of 19 cases due to missing data, 171 patients were finally included. The mean ± SD age of the patients was 28.11 ± 6.56 years. The patients were divided into two groups based on the depth of ablation: a group of 94 patients (188 eyes) with an ablation depth of ≥ 65 μm who received MMC during surgery, and a group of 77 patients (154 eyes) with an ablation depth of < 65 μm who did not receive MMC during surgery (Figure 1).

Table 2 shows demographic characteristics, baseline and postoperative parameters, and postoperative haze statuses of the two groups. Both groups were comparable in terms of age, sex, ocular dominance, re-epithelialization time, UCDVA, BCDVA, and corneal haze (Table 2). The MMC-treated group had a significantly higher degree of preoperative myopia ($P < 0.001$ for preoperative SE) and had a significantly higher depth of ablation ($P < 0.001$). In the BSS-treated group, majority of the patients had low myopia (54.55%), followed by moderate myopia (45.45%), but none had high myopia. In the MMC group, most patients had moderate myopia (62.77%), followed by low (23.4%) and high myopia (13.83%), which was expected because only patients with deeper ablation depths (≥ 65 μm) were included in this group. However, postoperative SE was comparable between the two groups except at the 3-month follow-up visit, during which the MMC-treated group had slightly less myopia ($P = 0.03$). This difference was resolved 6 months postoperatively ($P = 0.080$; Table 2).

The specular microscopy findings of the two groups are shown in Table 3. The mean ± SD of the preoperative and 6-month postoperative ECD in group one were 3003 ± 283 cells/mm$^2$ and 2970 ± 256 cells/mm$^2$, respectively, and in group two were 2827 ± 287 and 2860 ± 261 cells/mm$^2$, respectively. Although the baseline CCT was higher in group one ($P = 0.116$), it declined significantly more in group one than in group two, with a thinner CCT at the 3- and 6-month follow-up visits (both $P < 0.001$), which was expected because patients who received MMC received deeper ablations.

The preoperative mean ECD at the baseline was slightly higher in group one and remained significantly higher at 3 and 6 months postoperatively ($P < 0.001$, $P = 0.002$, and $P = 0.022$, respectively). The mean ECD

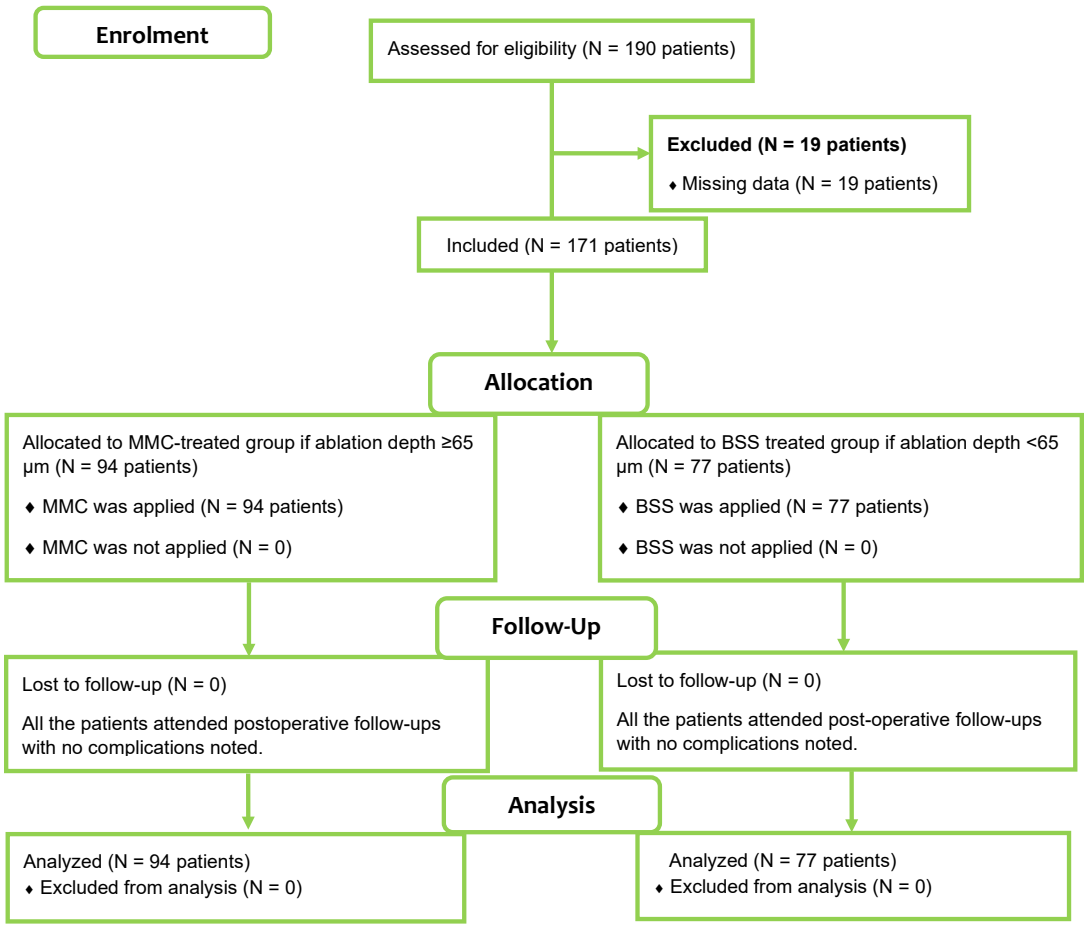


**Figure 1. Allocation of study participants to the MMC-treated or BSS-treated group following refractive surface ablation procedures. Abbreviations: N, number; MMC, mitomycin C; μm, micrometer; BSS, balanced salt solution.**

in group one decreased at a steady rate during follow-up visits, though this was not statistically significant ($P$ = 0.365 and $P$ = 0.830 at 3 and 6 months postoperatively, respectively, as compared with that at baseline). The mean ECD increased slightly in group two, though this was also not significant ($P$ = 0.353 and $P$ = 0.690 at 3 and 6 months postoperatively, respectively, as compared with the baseline; Table 3).

CV was higher in group one than that in group two at the baseline, though this difference was not statistically significant ($P$ = 0.658) and remained significantly higher at the 3-month follow-up as compared with that of group two ($P$ = 0.028). At the 6-month visit, it remained higher, but the difference was no longer statistically significant ($P$ = 0.328; Table 3).

The hexagonality index of cells was higher in group two at baseline, though this was not statistically significant ($P$ = 0.173), and remained significantly higher throughout the postoperative follow-ups as compared with that of group one ($P$ = 0.016 and $P$ = 0.001 at 3 and 6 months postoperatively, respectively; Table 3). No intraoperative or postoperative complications were noted in the study participants throughout the 6-month follow-up period.

## DISCUSSION

**Table 2. Demographic characteristics and baseline and postoperative visual parameters in the study groups**

| Variables | | | Group 1 | Group 2 | *P*-value |
|---|---|---|---|---|---|
| **Age (y), Mean ± SD** | | | 28 ± 6.0 | 27 ± 6.0 | 0.933 |
| **Sex (Male/Female), n (%)** | | | 21 (22.3) / 73(77.7) | 16 (20.8) / 61(79.2) | 0.577 |
| **Ocular dominance, n (%)** | **Cases with OD dominance** | | 48 (51.1) | 38 (49.4) | 0.158 |
| | **Cases with OS dominance** | | 46 (48.9) | 39 (50.6) | |
| **Efficacy index*** | **1-week post-op** | | 4.22 | 179.48 | N.A. |
| | **1-month post-op** | | 1.79 | 37.33 | |
| | **3-month post-op** | | 0.64 | 13.70 | |
| | **6-month post-op** | | 0.40 | 3.09 | |
| **Safety index**** | **1-month post-op** | | 0.13 | 0.96 | N.A. |
| | **3-month post-op** | | 0.10 | 0.32 | |
| | **6-month post-op** | | 0.03 | 8.10 | |
| **UCDVA > 20/40 at 6-month post-op, n (%)** | | | 72 (76.59) | 46 (59.74) | 0.091 |
| **UCDVA > 20/25 at 6-month post-op, n (%)** | | | 70 (74.46) | 46 (59.74) | 0.259 |
| **BCDVA (logMAR) at 6-month post-op, Mean ± SD** | | | 0.04 ± 0.17 | 0.0 ± 0.01 | 0.185 |
| **UCDVA (logMAR) at 6-month post-op, Mean ± SD** | | | 0.99 ± 0.85 | 0.83 ± 0.74 | 0.584 |
| **Mean ablation depth (µm), Mean ± SD** | | | 86.39 ± 17.05 | 46.8 ± 11.8 | **< 0.001** |
| **Re-epithelialization (day), Mean ± SD** | | | 3.87 ± 0.66 | 3.62 ± 0.86 | 0.130 |
| **Cases with Myopia, n (%)** | **low (< -3 D)** | | 22 (23.40) | 42 (54.55) | **< 0.001** |
| | **moderate (-3 D to -6 D)** | | 59 (62.77) | 35 (45.45) | |
| | **high (-6 D to -9 D)** | | 13 (13.83) | 0 (0.0) | |
| **SE (D), Mean ± SD** | **pre-op** | | - 4.43 ± 2.29 | - 2.91 ± 0.96 | **< 0.001** |
| | **1-week post-op** | | - 0.77 ± 1.36 | - 0.37 ± 0.82 | 0.160 |
| | **1-month post-op** | | - 0.15 ± 0.49 | - 0.17 ± 0.33 | 0.880 |
| | **3-month post-op** | | - 0.03 ± 0.46 | - 0.16 ± 0.30 | **0.030** |
| | **6-montn post-op** | | - 0.01 ± 0.76 | - 0.09 ± 0.25 | 0.080 |
| **Haze, n (%)** | **Day 1 postop** | **Clear** | 174 (92.55) | 136 (88.31) | 0.340 |
| | | **Trace** | 14 (7.45) | 18 (11.69) | |
| | **6-month post-op** | **Clear** | 180 (95.74) | 140 (90.91) | 0.080 |
| | | **Trace** | 8 (4.26) | 14 (9.09) | |

**Abbreviations: BSS, balanced salt solution; MMC, mitomycin C; y, years; SD, standard deviation; n, number; %, percentage; OD, right eye; OS, left eye; post-op, postoperatively; pre-op, preoperatively; N.A., not available; UCDVA, uncorrected distance visual acuity; BCDVA, best-corrected distance visual acuity; logMAR, the minimum angle of resolution; µm, micrometer; D, diopter; µm, micrometer; *the Efficacy index, the mean postoperative UCDVA/ mean preoperative BCDVA [16];**the safety index, the mean postoperative BCDVA/ mean preoperative BCDVA [16]. *P*-value < 0.05 is shown in bold. Note: re-epithelialization is equal with day of contact lens removal; Group 1 with an ablation depth of ≥ 65 µm, who received intraoperative MMC 0.02% for 30 seconds; Group 2 with an ablation depth of < 65 µm, who received BSS for 30 seconds.**

**Table 3. Baseline and post-operative specular microscopy findings in the study groups**

| Time Point | Variable | Group 1 | Group 2 | *P*-value |
|---|---|---|---|---|
| **Baseline, Mean ± SD** | **CCT (μm)** | 542 ± 34 | 534 ± 30 | 0.116 |
| | **CV** | 30.40 ± 6.68 | 29.95 ±7.16 | 0.658 |
| | **ECD (cells/mm²)** | 3003 ± 283 | 2827 ± 287 | **< 0.001** |
| | **Hexa (%)** | 56 ± 11 | 59 ± 9 | 0.173 |
| **3-month post-op, Mean ± SD** | **CCT (μm)** | 447 ± 35 | 482 ± 33 | **< 0.001** |
| | **Intragroup *P*-value** | **< 0.001** | **< 0.001** | |
| | **CV** | 32.98 ± 5.14 | 31.43 ± 4.09 | **0.028** |
| | **Intragroup *P*-value** | **< 0.001** | **0.077** | |
| | **ECD (cells/mm²)** | 2978 ± 281 | 2849 ± 255 | **0.002** |
| | **Intragroup *P*-value** | 0.365 | 0.353 | |
| | **Hexa (%)** | 55 ± 12 | 59 ± 8 | **0.016** |
| | **Intragroup *P*-value** | 0.570 | 0.999 | |
| **6-month post-op, Mean ± SD** | **CCT (μm)** | 447 ± 33 | 473 ± 27 | **< 0.001** |
| | **Intragroup *P*-value** | **< 0.001** | **< 0.001** | |
| | **CV** | 33.11 ± 4.49 | 32.43 ± 4.3 | 0.328 |
| | **Intragroup *P*-value** | **< 0.001** | **< 0.001** | |
| | **ECD (cells/mm²)** | 2970 ± 256 | 2860 ± 261 | **0.022** |
| | **Intragroup *P*-value** | 0.830 | 0.690 | |
| | **Hexa (%)** | 53 ± 10 | 59 ± 8 | **0.001** |
| | **Intragroup *P*-value** | 0.120 | 0.999 | |

**Abbreviations: BSS, balanced salt solution; MMC, mitomycin-C; SD, standard deviation; CCT, central corneal thickness; μm, micrometers; CV, coefficient of variation; ECD, endothelial cell density; cells/mm², cells per square millimeter; Hexa, hexagonality value in percentage; post-op, postoperatively. *P*-value < 0.05 is shown in bold. Note: Group 1 with an ablation depth of ≥ 65 μm, who received intraoperative MMC 0.02% for 30 seconds; Group 2 with an ablation depth of < 65 μm, who received BSS for 30 seconds.**

In this study, we found no ECD reduction at 6 months post-refractive surgery in MMC-treated eyes as compared with the control group. However, the postoperative CCT and hexagonality were significantly lower in the MMC-treated group. CV was higher in group one than in group two at baseline, though the difference was not statistically significant, and remained significantly higher at the 3-month follow-up as compared with that of group two. Furthermore, the postoperative corneal haze rate was comparable between the study groups at the 6-month postoperative visit.

The role of the MMC during refractive surface ablation procedures as a corneal healing modulator is supported by a report published by the American Academy of Ophthalmology [17]. A single intraoperative application of 0.02% MMC for 12–60 s, depending on the ablation depth, is commonly implemented in refractive surface ablation [11]. Currently, there is controversy about the effect of MMC on the reduction of corneal endothelial cell counts. The existing literature is inconsistent as to whether and under what circumstances MMC causes endothelial cell loss [18]. The effectiveness of MMC has been stated to be both concentration- and time-dependent [19]. However, the efficacy of haze prophylaxis was conserved after a 12-s application of 0.02% MMC following PRK, versus that after a 60- or 120-s application, with no significant changes in visual outcome [20]. Sixty-, 30-, and 15-s exposures of MMC 0.01% following wavefront-guided PRK for higher myopia (a manifest SE -4.50 to -9.00 D and astigmatic ≤ 3.00 D) revealed no clinically significant difference in haze formation as compared with a 4-month steroid taper [21]. Low-dose MMC of 0.01% had the same efficacy as that of a standard dose of 0.02% in haze prophylaxis after PRK, along with reduced side effects and reduced future complications at the 6-month follow-up [22]. Hence, the optimal contact time of 0.02% MMC should be determined.

A review of more than a decade of studies on the effect of different exposure times of 0.02% MMC during refractive surface ablation procedures revealed that there were two approaches to these studies. Most of these clinical studies, including the present study, determined the effectiveness and safety of an exposure time of 0.02% MMC, and some compared different exposure times of 0.02% MMC. Furthermore, the majority reported no difference in preoperative versus postoperative corneal ECD when using 0.02% MMC with an exposure time of ≤ 2 min during refractive surgery [23].

Gambato et al. [24] examined 28 patients with high-myopia who underwent PRK with an application

of 0.02% MMC for 120 s. Confocal microscopic parameters were evaluated at the baseline and at 5 years postoperatively. The contact time and follow-up duration were longer than those in our study, and they only examined ECD, epithelial thickness, and corneal nerve status. They found no significant reduction in ECD at 5 years postoperatively [24]. In the present study, the preoperative mean ECD at the baseline was slightly higher in the MMC-treated eyes and remained significantly higher at 3 and 6 months postoperatively. Although the rate of change in ECD was different between the two groups, at the 3- and 6-month follow-ups, the mean ECD did not differ significantly as compared with the baseline in either group.

Goldsberry et al. [25] also applied 0.02% MMC for 12 s in 16 eyes with an ablation depth >75 μm and found no changes in the ECD and percentage of hexagonal cells at 1 year postoperatively [25]. Likewise, we did not observe a significant change in the percentage of hexagonal cells and ECD at 3 and 6 months postoperatively versus that at baseline in MMC-treated eyes. In contrast, MMC-treated eyes had a significantly lower percentage of hexagonal cells and higher ECD at the 3- and 6-month postoperative visits as compared with the control group. The latter could be justified by a significantly higher baseline mean ECD in MMC-treated eyes, yet the former was comparable between groups at the baseline. The longer follow-up period and smaller sample size in Goldsberry et al.'s study may explain the differences with our results.

Shojaei et al. [13] studied the effect of the shortest exposure time (5 s) to 0.02% MMC as compared to saline during the refractive surface ablation procedure in 152 eyes with low myopia during PRK on haze formation. Haze grades were significantly lower in the MMC-treated group, though ECD was comparable with that in the placebo group 6 months postoperatively [13]. Despite a similar follow-up period, the mean SE and exposure time were less than those in the current study. Their findings signify the safety and efficacy of a short-duration application of 0.02% MMC in eyes with low myopia, with an ablation depth < 65 μm. The current study confirms the safety and efficacy of 0.02% MMC with a longer exposure time at an ablation depth of ≥ 65 μm for a higher degree of myopia. However, the haze formation was comparable between both groups in the current study but was insignificantly lower in MMC-treated eyes. Considering the significantly higher degree of myopia and ablation depth in these eyes, this could be clinically significant.

Sy et al. [26] compared the manifest refraction SE after PRK between 90 patients with myopic astigmatism (30 patients receiving MMC for 30 s during PRK, 30 not receiving MMC during PRK, and 30 who underwent LASIK). Despite having a comparative UCDVA, the refractive outcomes were more variable in the MMC-treated group at the 12-month follow-up after surgery. They suggested that 0.02% MMC should be used with caution during PRK [26]. We found a comparable postoperative SE between the two groups except at the 3-month follow-up visit, in which the MMC-treated group had slightly but statistically significantly less myopia. However, the difference resolved at the 6-month visit. These changes might indicate a variable refractive outcome in MMC-treated eyes, but this was not the objective of our study. Since the severity of haze formation and corneal ECD were not evaluated by Sy et al. [26], it is not possible to comment on the safety of MMC based on their results, nor to compare the results of the two studies concerning endothelial cell parameters. Torricelli et al. [27] examined the effectiveness of 0.02% MMC during PRK in patients with myopia based on intraocular straylight values and found a similar result in MMC-treated versus non-treated eyes 4 months postoperatively [27]. Although the MMC dose and contact time were similar to those in the present study, they did not investigate the effect of MMC at the cellular level.

Gharaee et al. [28] used 0.02% MMC for 5 s for each diopter correction in patients with myopia ranging from -1.00 D to -7.00 D and astigmatic ≤ 3.00 D [28]. Similar to our results, postoperative ECD and polymegathism did not significantly change up to the sixth month postoperatively. However, the SD of cell size and pleomorphism increased significantly, though this was not related to MMC exposure time. Similar to our results, they found that 0.02% MMC was harmless to the endothelium at an exposure time of < 30 s [28]. In contrast to our results, CCT was increased significantly at the sixth month as compared to the first month postoperatively. Although they did not specify the exact ablation depth, a possible explanation for the steady and significant decrease in CCT in MMC-treated eyes is the greater ablation depth in the current study.

Our study has several limitations, including a short follow-up period and low study power; we used a power of 80%, although a power of 90% would have been more ideal. The long-term side-effects of MMC remain unclear because the surviving corneal cells could suffer DNA damage that would potentially augment ultraviolet-induced DNA injury that could present as corneal thinning or edema long after the operation [12]. We propose that a long-term follow-up of patients and assessment of the efficacy of a more diluted MMC dose, shorter exposure time, or both, on haze formation and corneal toxicity should be performed in the future.

## CONCLUSIONS

A single intraoperative application of MMC for 30 s as prophylaxis for corneal haze during refractive surface ablation procedures was effective and safe up to 6 months postoperatively. Future studies with a contralateral-eye design, a larger sample size, and longer follow-up are needed to confirm or refute our observations.

## ETHICAL DECLARATIONS

**Ethical approval:** The study was approved by the ethics committee at the institute level. Informed consent was obtained from all of the patients before surgery. They were informed that their participation in the study was voluntary, and that they could withdraw from the study at any point in time without negative consequences to themselves.

**Conflict of interest:** None.

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## REFERENCES

1. Margo JA, Munir WM. Corneal Haze Following Refractive Surgery: A Review of Pathophysiology, Incidence, Prevention, and Treatment. Int Ophthalmol Clin. 2016;56(2):111-25. doi: 10.1097/IIO.0000000000000112 pmid: 26938342
2. Ambrósio R Jr, Wilson S. LASIK vs LASEK vs PRK: advantages and indications. Semin Ophthalmol. 2003;18(1):2-10. doi: 10.1076/soph.18.1.2.14074 pmid: 12759854
3. Wilson SE. Analysis of the keratocyte apoptosis, keratocyte proliferation, and myofibroblast transformation responses after photorefractive keratectomy and laser in situ keratomileusis. Trans Am Ophthalmol Soc. 2002;100:411-33. pmid: 12545703
4. Torricelli AA, Santhanam A, Wu J, Singh V, Wilson SE. The corneal fibrosis response to epithelial-stromal injury. Exp Eye Res. 2016;142:110-8. doi: 10.1016/j.exer.2014.09.012 pmid: 26675407
5. Ghanem RC, Ghanem VC, Ghanem EA, Kara-José N. Corneal wavefront-guided photorefractive keratectomy with mitomycin-C for hyperopia after radial keratotomy: two-year follow-up. J Cataract Refract Surg. 2012;38(4):595-606. doi: 10.1016/j.jcrs.2011.11.032 pmid: 22440434
6. Mearza AA, Aslanides IM. Uses and complications of mitomycin C in ophthalmology. Expert Opin Drug Saf. 2007;6(1):27-32. doi: 10.1517/14740338.6.1.27 pmid: 17181449
7. Hashemi H, Taheri SM, Fotouhi A, Kheiltash A. Evaluation of the prophylactic use of mitomycin-C to inhibit haze formation after photorefractive keratectomy in high myopia: a prospective clinical study. BMC Ophthalmol. 2004;4:12. doi: 10.1186/1471-2415-4-12 pmid: 15363107
8. Gambato C, Ghirlando A, Moretto E, Busato F, Midena E. Mitomycin C modulation of corneal wound healing after photorefractive keratectomy in highly myopic eyes. Ophthalmology. 2005;112(2):208-18; discussion 219. doi: 10.1016/j.ophtha.2004.07.035 pmid: 15691552
9. Muller LT, Candal EM, Epstein RJ, Dennis RF, Majmudar PA. Transepithelial phototherapeutic keratectomy/photorefractive keratectomy with adjunctive mitomycin-C for complicated LASIK flaps. J Cataract Refract Surg. 2005;31(2):291-6. doi: 10.1016/j.jcrs.2004.04.044 pmid: 15767148
10. Argento C, Cosentino MJ, Ganly M. Comparison of laser epithelial keratomileusis with and without the use of mitomycin C. J Refract Surg. 2006;22(8):782-6. doi: 10.3928/1081-597X-20061001-08 pmid: 17061715
11. Arranz-Marquez E, Katsanos A, Kozobolis VP, Konstas AGP, Teus MA. A Critical Overview of the Biological Effects of Mitomycin C Application on the Cornea Following Refractive Surgery. Adv Ther. 2019;36(4):786-797. doi: 10.1007/s12325-019-00905-w pmid: 30859502
12. Carlos de Oliveira R, Wilson SE. Biological effects of mitomycin C on late corneal haze stromal fibrosis following PRK. Exp Eye Res. 2020;200:108218. doi: 10.1016/j.exer.2020.108218 pmid: 32905844
13. Shojaei A, Ramezanzadeh M, Soleyman-Jahi S, Almasi-Nasrabadi M, Rezazadeh P, Eslani M. Short-time mitomycin-C application during photorefractive keratectomy in patients with low myopia. J Cataract Refract Surg. 2013;39(2):197-203. doi: 10.1016/j.jcrs.2012.09.016 pmid: 23183351
14. Shehadeh-Mashor R, Mimouni M, Shapira Y, Sela T, Munzer G, Kaiserman I. Duration of contact lens removal before myopic refractive surgery. Eur J Ophthalmol. 2021;31(4):1695-1699. doi: 10.1177/1120672120949101 pmid: 32811175
15. Lim WK, Soh ZD, Choi HKY, Theng JTS. Epithelium-on photorefractive intrastromal cross-linking (PiXL) for reduction of low myopia. Clin Ophthalmol. 2017;11:1205-1211. doi: 10.2147/OPTH.S137712 pmid: 28721004
16. Chen X, Shen Y, Xu H, Wang X, Zhou X. One-year natural course of corneal densitometry in high myopic patients after implantation of an implantable collamer lens (model V4c). BMC Ophthalmol. 2020;20(1):50. doi: 10.1186/s12886-020-1320-x pmid: 32050942
17. Majmudar PA, Schallhorn SC, Cason JB, Donaldson KE, Kymionis GD, Shtein RM, et al. Mitomycin-C in corneal surface excimer laser ablation techniques: a report by the American Academy of Ophthalmology. Ophthalmology. 2015;122(6):1085-95. doi:

10.1016/j.ophtha.2015.01.019 pmid: 25795477
18. Karaarslan C. New Bubble Mitomycin C Application Technique for Haze Prevention following Customized Photorefractive Keratectomy. Indian Journal of Pharmaceutical Sciences. 2020:31-5. doi: 10.36468/pharmaceutical-sciences.spl.56
19. Rajan MS, O'Brart DP, Patmore A, Marshall J. Cellular effects of mitomycin-C on human corneas after photorefractive keratectomy. J Cataract Refract Surg. 2006;32(10):1741-7. doi: 10.1016/j.jcrs.2006.05.014 pmid: 17010877
20. Virasch VV, Majmudar PA, Epstein RJ, Vaidya NS, Dennis RF. Reduced application time for prophylactic mitomycin C in photorefractive keratectomy. Ophthalmology. 2010;117(5):885-9. doi: 10.1016/j.ophtha.2009.10.024 pmid: 20163867
21. Hofmeister EM, Bishop FM, Kaupp SE, Schallhorn SC. Randomized dose-response analysis of mitomycin-C to prevent haze after photorefractive keratectomy for high myopia. J Cataract Refract Surg. 2013;39(9):1358-65. doi: 10.1016/j.jcrs.2013.03.029 pmid: 23830559
22. Naderi M, Ahmadi M, Jadidi K, Alishiri A, Rafizadeh P. Comparison of standard and low dose mitomycin C in the prevention of corneal haze following photorefractive keratectomy. Iranian Journal of Ophthalmology. 2010;22(3):13-6. Link
23. Santhiago MR, Netto MV, Wilson SE. Mitomycin C: biological effects and use in refractive surgery. Cornea. 2012;31(3):311-21. doi: 10.1097/ICO.0b013e31821e429d pmid: 22157595
24. Gambato C, Miotto S, Cortese M, Ghirlando A, Lazzarini D, Midena E. Mitomycin C-assisted photorefractive keratectomy in high myopia: a long-term safety study. Cornea. 2011;30(6):641-5. doi: 10.1097/ICO.0b013e31820123c8 pmid: 21242784
25. Goldsberry DH, Epstein RJ, Majmudar PA, Epstein RH, Dennis RF, Holley G, et al. Effect of mitomycin C on the corneal endothelium when used for corneal subepithelial haze prophylaxis following photorefractive keratectomy. J Refract Surg. 2007;23(7):724-7. doi: 10.3928/1081-597X-20070901-14 pmid: 17912945
26. Sy ME, Zhang L, Yeroushalmi A, Huang D, Hamilton DR. Effect of mitomycin-C on the variance in refractive outcomes after photorefractive keratectomy. J Cataract Refract Surg. 2014;40(12):1980-4. doi: 10.1016/j.jcrs.2014.02.048 pmid: 25305150
27. Torricelli AA, Parede TR, Netto MV, Crestana FP, Bechara SJ. Intraocular straylight before and after low myopic photorefractive keratectomy with and without mitomycin C. Arq Bras Oftalmol. 2016;79(2):88-91. doi: 10.5935/0004-2749.20160027 pmid: 27224070
28. Gharaee H, Zarei-Ghanavati S, Alizadeh R, Abrishami M. Endothelial cell changes after photorefractive keratectomy with graded usage of mitomycin C. Int Ophthalmol. 2018;38(3):1211-1217. doi: 10.1007/s10792-017-0584-5 pmid: 28612330