Mitomycin C in Corneal Refractive Surgery

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Abstract. Mitomycin C has played a deciding role in the current revival of excimer laser surface ablation techniques. We review the literature regarding mechanism of action of mitomycin C, histological effects on the cornea, and indications, dose, exposure time, and toxicity of mitomycin C in corneal refractive surgery. Mitomycin C is an alkylating agent with cytotoxic and antiproliferative effects that reduces the myofibroblast repopulation after laser surface ablation and, therefore, reduces the risk of postoperative corneal haze. It is used prophylactically to avoid haze after primary surface ablation and therapeutically to treat pre-existing haze. There is no definite evidence that establishes an exact diopter limit or ablation depth at which to apply prophylactic mitomycin C. It is usually applied at a concentration of 0.2 mg/ml (0.02%) for 12 to 120 seconds over the ablated stroma, although some studies suggest that lower concentrations (0.01%, 0.002%) could also be effective in preventing haze when treating low to moderate myopia. This dose of mitomycin C has not been associated with any clinically relevant epithelial corneal toxicity. Its effect on the endothelium is more controversial: two studies report a decrease in endothelial cell density, but the majority of reports suggest that the endothelium is not altered. Regarding mitomycin C’s effect on keratocyte population, although animal studies report keratocyte depletion after its use, longer follow-up suggested that the initial keratocyte depletion does not persist over time. (Surv Ophthalmol 54:487–502, 2009. © 2009 Elsevier Inc. All rights reserved.)

Key words. advanced surface ablation • excimer laser surface ablation • haze • laser-assisted subepithelial keratectomy mitomycin • LASEK • laser subepithelial keratomileusis • mitomycin C • MMC • photorefractive keratectomy

Laser in situ keratomileusis (LASIK) has become the most popular corneal refractive surgery procedure because it provides a rapid postoperative recovery and less discomfort when compared to photorefractive keratectomy (PRK). Nevertheless, the possibility of sight-threatening complications associated with the stromal flap in LASIK, and the development of new surface ablation procedures, has led to a renewed interest in these techniques. Surface ablation has become the technique of choice in patients with thin corneal pachymetry, those at risk for trauma, and those with corneal surface problems such as dry eye, recurrent erosion syndrome, or basement membrane disease,108,153
and it has shown to be safe, effective, and predictable for treating low, moderate, and high myopia. Mitomycin C (MMC) has played a decisive role in the current revival of surface ablation techniques. The main complication associated with laser surface ablation was the loss of corneal transparency—corneal haze—that appeared most frequently associated with deep ablations (Fig. 1). The efficacy of the MMC in reducing the incidence of this complication has led to its widespread use in most refractive surgery practices. Nevertheless, the mechanism of action of this drug, the optimal dose and exposure time, the visual and refractive results of surface ablation techniques using MMC, and the possible toxicity of this drug remain subjects of intense debate.

MECHANISM OF ACTION

Mitomycin C was first isolated from cultures of Streptomyces caespitosus by Hata in 1956. It acts as a genotoxic antibiotic because of its alkylating action: once it becomes activated by enzymes such as the cytochrome p450 reductase, it produces cross-linking of the DNA molecules between adenine and guanine, thereby blocking DNA synthesis and secondarily inhibiting cell mitosis, causing cell cycle arrest. Although it acts primarily during the late G1 and S phases, it is non-cell cycle specific. As a result of its antimitotic effect, cells with a higher mitotic rate are more sensitive to its action, and it is widely used systemically as a chemotherapeutic agent. Nevertheless, some of the effects of MMC cannot be explained simply by this antiproliferative mechanism. MMC shows a cytotoxic effect that is not completely justified by its capacity to bind DNA.

Another point of debate is the possible long-term cellular effects of this drug. It is not clear whether cells repair the DNA damage caused by the MMC or whether its effects are permanent. Some studies with fibroblasts cultures suggest that these cells do not suffer a permanent inhibition after a single exposure to MMC and that the adjacent non-exposed cells can replace them.

DRUG EFFECTS ON THE CORNEA

Corneal haze is caused by the mechanisms of corneal wound healing that become activated after surface ablation and that are different from the wound healing process observed after LASIK. Epithelial damage and the secondary epithelial-stromal interaction seem to cause these differences. During surface ablation, both the deepithelialization and the laser ablation incite keratocyte apoptosis, which is followed by proliferation and migration of the surrounding keratocytes to repopulate the denuded stroma. In response to epithelial derived cytokines, especially TGFβ, some keratocytes differentiate into myofibroblasts. These cells are the base of corneal haze, as they scatter more light than quiescent keratocytes, not only from their nuclei, but also from their cell bodies and dendritic processes. In addition, they participate in extracellular matrix remodelling, resulting in a denser and more disorganized extracellular matrix, with abundant collagen type III, which contributes to the loss of corneal transparency (Figs. 2 and 3). These stromal healing mechanisms lead to the formation of a fibrotic and hypercellular scar in the anterior stroma.

MMC is useful in laser surface ablation because of its capacity to interfere with this stromal wound healing process. MMC is usually applied over the de-epithelialized stroma after the laser ablation has been performed. Animal studies have shown, within the first hours after its application, a higher rate of keratocyte apoptosis, followed 24 hours after...
wards by a reduced keratocyte repopulation. Four weeks later, there is a lower keratocyte and myofibroblast density and less deposit of collagen and extracellular matrix, compared to untreated eyes. These effects in the corneal stroma result in increased corneal transparency after surface ablation in animal models. In human corneas maintained in vitro, Rajan et al. also detected a lower keratocyte proliferation after MMC application, although they did not observe the initial increase in keratocyte apoptosis.

The initial increase in keratocyte apoptosis is related to the cytotoxic effect of the MMC, whereas the antimitotic effect of this drug is responsible for blocking keratocyte activation and differentiation into myofibroblasts. This latter mechanism seems more effective than cytotoxic elimination of the already differentiated myofibroblasts. As Netto et al. demonstrated in rabbit corneas, Sadeghi et al. had already shown that the concentration of MMC needed in vitro to achieve its antiproliferative effect was lower than that to cause cytotoxicity. In fact, MMC seems more effective as a prophylactic agent, to prevent haze, than as a therapeutic agent to eliminate pre-existing haze. Nevertheless, no consensus has been reached, and some authors consider the antimitotic activity the more important mechanism of action, while others support that the cytotoxic effect.

**FIRST USES OF MMC IN CORNEAL REFRACTIVE SURGERY**

The first studies of MMC in surface ablation were performed in animals. Talamo et al. suggested in 1991 the use of postoperative MMC to modulate the corneal wound-healing response to surface ablation. They showed that the application of topical MMC in rabbit corneas during the 2 weeks after surgery resulted in less subepithelial collagen deposition. Schipper et al. observed that intraoperative 0.04% MMC for 5 minutes after surface ablation in rabbits resulted in less scar tissue and lower keratocyte density.

Majmudar et al. pioneered its use to treat corneal scars secondary to refractive procedures. They applied 0.02% MMC intraoperatively for 2 minutes and found a clinically significant improvement in corneal transparency. It was subsequently proposed as a prophylactic agent to prevent haze formation after primary surface ablation. Carones et al. applied MMC after surface ablation to correct myopia from -6.00 to -10.00 D. They reported less haze and better predictability of visual results in the group that received intraoperative MMC compared to the control group, with no side effects.

**Uses of MMC in Corneal Refractive Surgery**

**USE OF MMC TO TREAT CORNEAL HAZE**

Intraoperative MMC, along with scraping of the corneal surface, has shown to be effective in increasing the transparency of corneas with haze following previous refractive surgery procedures.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Number of Eyes</th>
<th>Manuscript Type</th>
<th>Preop Spherical Equivalent (diopters)</th>
<th>MMC Dose (%)</th>
<th>MMC Application Time</th>
<th>Follow-up (months)</th>
<th>Haze</th>
<th>Visual Results</th>
<th>Refractive Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argento et al4</td>
<td>30 MMC 28 controls</td>
<td>Retrospective, comparative study</td>
<td>MMC: $-5.72 \pm 2.82$ controls: $-5.81 \pm 2.74$</td>
<td>0.02</td>
<td>75 sec</td>
<td>6</td>
<td>MMC-group: 0% controls: trace 17.9%, grade 1: 3.6% (p &lt; 0.001)</td>
<td>Efficacy index: MMC: 0.954 controls: 0.909 (p &lt; 0.01)</td>
<td>No significant difference</td>
</tr>
<tr>
<td>Bedei et al8</td>
<td>62 MMC 62 controls</td>
<td>Prospective, randomized, comparative study</td>
<td>$&gt;-5.00$</td>
<td>0.02</td>
<td>12</td>
<td></td>
<td>Less haze in MMC-group (p = 0.005)</td>
<td>Better BCVA in MMC-group (p = 0.013)</td>
<td>eyes ±0.50 D: MMC: 69.3% controls: 50%</td>
</tr>
<tr>
<td>Carones et al15</td>
<td>30 MMC 30 controls</td>
<td>Prospective, randomized, comparative study</td>
<td>MMC: $-7.75 \pm 0.86$ controls: $-7.79 \pm 0.87$ (p = 0.82)</td>
<td>0.02</td>
<td>2 min</td>
<td>6</td>
<td>Haze grade ≥1: MMC: 0% controls: 63% (p = 0.01)</td>
<td>Better UCVA (p ≤ 0.01) and BCVA (p ≤ 0.001) in MMC-group. Loss of 1-3 lines of BCVA: MMC: 0% controls: 23.3% (p = 0.0006)</td>
<td>eyes ±0.50D: MMC: 87% controls: 47%</td>
</tr>
<tr>
<td>Gambato et al12</td>
<td>36 MMC 36 controls</td>
<td>Prospective, randomized, comparative study</td>
<td>MMC: $-9.40 \pm 1.74$ controls: $-8.71 \pm 1.67$ (p = 0.03)</td>
<td>0.02</td>
<td>2 min Mean:18 (range 12–36)</td>
<td></td>
<td>Haze grade ≥1: MMC: 0% controls: 20%</td>
<td>UCVA (logMAR): MMC: 0.4 ± 0.48 controls: 0.5 ± 0.53 (p = 0.03)</td>
<td>No significant difference</td>
</tr>
</tbody>
</table>

BCVA = best-corrected visual acuity; MMC = mitomycin C; UCVA = uncorrected visual acuity.
In contrast with the trend to shorten exposure time and lower dosage when MMC is used prophylactically in primary surface ablation, therapeutic MMC is generally still applied at the same concentration (0.02%) and for the same duration (2 minutes) as in those first studies. The cytotoxic effect to produce apoptosis of pre-existing myofibroblasts seems lower than MMC’s capacity to prevent their appearance when applied prophylactically. Netto et al showed that 4 weeks after application of 0.02% MMC for 2 minutes to treat pre-existing haze in rabbit corneas, myofibroblasts could still be detected. These cells tended to disappear progressively during the first 6 months after surgery.

USE OF MMC IN PRIMARY SURFACE ABLATION PROCEDURES

Several comparative studies have reported less incidence of haze, and better visual and refractive outcomes, when using intraoperative MMC during surface ablation procedures in moderate and high myopia (Table 1). The corneal wound-healing response to laser surface ablation is directly related to ablation depth. This was initially the main limitation for PRK, since treating high refractive defects was associated with a stromal response that caused significant haze and refractive regression, resulting in poorer outcomes as compared to LASIK. The introduction of MMC has improved the outcomes of surface ablation in high myopia. A comparison between 114 eyes treated with laser-assisted subepithelial keratectomy (LASEK) and 0.02% MMC for 60 seconds and 114 refractive-matched eyes treated with LASIK to correct a myopic defect of −7.00 D or greater showed no difference between techniques regarding safety and efficacy and in UCVA or BSCVA 3 months after surgery. A trend toward overcorrection was detected in the LASEK with MMC group, despite the planned undercorrection of 10%.

The wound-healing response to the laser ablation seems to be one of the causes of refractive regression. Because the MMC reduces that response, it causes a tendency to overcorrection that needs to be compensated for with an appropriate adjustment of the laser nomogram. Although each surgeon should define their own adjustment based on his/her results, it is advisable to perform an undercorrection of about 10% of the preoperative spherical refraction, sometimes higher depending on patient age and preoperative refractive defect (Carones et al: 10%; Lacayo et al: 8–15%; Camellin: 20% in low myopia; our group: 10%). Usually, the cylinder programmed ablation does not need to be modified.

The use of MMC in thin corneas (thinner than 500 µm) has been the subject of only one study in which intraoperative MMC did not seem to increase the risk of postoperative corneal instability with a follow-up of 15 months. More studies with longer follow-up are needed to further elucidate the safety of MMC in thin corneas.

USE OF MMC IN ADVANCED SURFACE ABLATION PROCEDURES

The sight-threatening complications associated with LASIK led to a renewed interest in surface ablation techniques. New procedures appeared, aiming to improve outcomes and postoperative rehabilitation over those of conventional PRK. These techniques, together with the development of better excimer laser platforms, were embraced under the name of Advanced Surface Ablation (ASA).

Mitomycin C has been shown to be effective in LASEK. Currently, the criteria to apply MMC during LASEK are the same as in PRK. However, if this procedure demonstrates a lower incidence of haze as compared to conventional mechanical PRK, MMC may be less necessary. De-epithelialization with 20% ethanol produces less inflammatory response and delayed keratocyte apoptosis than manual mechanical deepithelialization in rabbits. Clinical studies comparing PRK with mechanical versus ethanol deepithelialization (in both cases discarding the epithelium) showed less haze after ethanol use. On the other hand, the effect of replacing the epithelial flap obtained with 20% ethanol has been studied in animals, showing less and delayed apoptosis when it was repositioned compared to no replacement of the flap. In clinical studies, repositioning of the ethanol-obtained epithelial flap has also been associated with less postoperative haze. Two animal studies have shown that MMC and ethanol applied together are synergistic, leading to increased keratocyte apoptosis in the anterior stroma and to a lower keratocyte density in the anterior stroma 4 weeks after the surgery. If ethanol use during LASEK produces similar effects as MMC, MMC will not be as necessary as in conventional PRK.

Regarding the other ASA technique—the epipolis-LASIK (epi-LASIK)—the only study comparing this technique with conventional PRK showed no significant difference in the incidence of haze. There are no specific recommendations yet for MMC use in epi-LASIK different from those in PRK.

USE OF MMC DURING SURFACE ABLATION AFTER OTHER CORNEAL SURGICAL PROCEDURES

Treating residual refractive error with LASIK after previous corneal surgeries, such as radial keratot-
Residual refractive errors after LASIK could benefit from treatment with surface ablation when an in-the-bed enhancement is not possible. Carones et al., however, reported the development of dense corneal haze several months after treatment of post-LASIK residual refraction with surface ablation. Even though other series report a much lower incidence, haze may still appear even with shallow ablations—Cagil et al. reported haze with corrections of −2.00 D or greater—or when treating residual hyperopia. It has been suggested that surface ablation with MMC in such cases could allow treatment of these cases while avoiding the risk of haze. Srinivasan et al. reported good visual and refractive results when treating residual refraction from +0.75 to −2.38 D after LASIK using PRK with intraoperative MMC, with a tendency to transitory overcorrection one month postoperatively. We have noted a tendency to overcorrection, and thus recommend caution when using surface ablation with MMC to treat post-LASIK residual refraction.

For retreatment over a previous surface ablation, there is no consensus on the need for MMC. Some authors have performed surface ablation enhancements without MMC and found no increased incidence of postoperative haze when treating low residual myopia. As a result of the presence of activated keratocytes at the site of ablation, haze may appear and cause a decrease in best-corrected visual acuity. Therefore, it has been suggested that MMC would be useful in surface ablation enhancements. There are, however, no studies addressing the safety of re-applying the drug, and caution is recommended when using it during re-treatments.

**DOSE AND EXPOSURE TIME IN PRIMARY SURFACE ABLATION**

MMC was initially applied at a concentration of 0.02% for 2 minutes over the ablated stroma. Subsequently, when applied prophylactically during primary surface ablation, the tendency has been to use a lower dose and a shorter exposure time. Sadeghi et al. applied MMC on cultured human keratocytes and reported that its antiproliferative effect was achieved with much lower doses than the cytotoxic effect. After a 5-minute exposure the lowest concentration that significantly (>50%) inhibited keratocyte proliferation was 0.05 mg/ml. After that exposure time, the median inhibitory dose was 0.038 mg/ml (0.0038 %) and the median lethal dose was much higher than the greatest concentration tested in the study (0.5 mg/ml [0.05%]).

Netto et al. applied MMC prophylactically at two different concentrations (0.02% and 0.002%) using three different exposure times (12 seconds, 1 minute, 2 minutes) in rabbits. They observed that, even though 0.02% MMC applied for 2 minutes achieved the greatest reduction in the postoperative myofibroblast population, 0.002% MMC applied for 12 seconds was equally effective in preventing postoperative haze. The difference in the myofibroblast density did not seem to have clinical relevance, although a larger study might be required to find a significant difference.

In another study in rabbits, Song et al. compared the keratocyte apoptosis rate after deep-epithelialization and application of 0.02% MMC for 15, 30, 60, and 120 seconds and of MMC 0.005%, 0.01%, 0.02% and 0.04% for 2 minutes. They observed more apoptosis with both greater concentrations and longer exposure times, but the correlation was stronger and statistically significant with concentration than exposure time.

In human corneas maintained in vitro, Rajan et al. applied 0.02% MMC for 1 or 2 minutes after laser ablation. They observed an initial decrease in the number of keratocytes in the ablated stroma similar in the control group and in both MMC-treated groups. Afterwards, the keratocyte repopulation started first in the control group, then in the MMC-1 minute group, and then in the MMC-2 minutes group. Four weeks afterwards, the keratocyte density in the anterior stroma was significantly lower in the groups that received MMC, with lower density associated with longer exposure times.

There are only three clinical studies using MMC at a lower concentration than 0.02%. Camellin reported his results using just a “brushstroke” of 0.01% MMC after LASEK in 86 eyes compared to 100 control eyes. He detected less haze in the MMC group, although the incidence was low in both groups. Thornton et al. retrospectively reviewed the outcomes after LASEK of 83 eyes treated with 0.002% MMC (for 45 to 120 seconds) versus 92 control, non-MMC eyes. They found significantly less haze in the MMC-treated group. However, haze in 0.002% MMC-treated eyes that received ablation...
for more than ~9.00 D showed a bimodal response over time, peaking 1 month after the surgery and again 1 to 2 years afterwards. They also reported some cases of dense haze in high myopes treated with low-dose MMC. A retrospective review by the same authors157 analyzed 126 eyes treated with 0.002% MMC versus 95 eyes treated with 0.02% MMC. They found less haze in the 0.02% MMC group, especially in the subgroups of high myopia (more than ~6 D) and deep ablation (more than 75 μm) and reported some cases of moderate to severe haze in the group that received 0.002% MMC. As mentioned previously, Sadeghi et al showed that the median inhibitory dose of the MMC on cultured human keratocytes is 0.038 mg/ml (0.0038%), almost twice the concentration applied in the eyes that received 0.002% MMC.157

Therefore, 0.02% MMC still seems to be the most effective option for high myopia.157 More studies are needed to establish the efficacy of lower concentrations for moderate myopia.

Based on the study by Netto et al,107 and because there is a lack of other evidence to establish the optimal exposure time, the tendency has been to shorten exposure times in order to reduce the side effects of the MMC. The drug is usually applied for 12 seconds to 1 minute, depending on the ablation depth.4,75,80,156 Animal studies by Song et al suggest, however, that changes in the exposure time have less impact on the absorption of MMC by the cornea and aqueous humor than changes in concentration.

The factor most clearly related to development of haze is ablation depth.83,96 Individual and ethnic factors,149 however, may result in different corneal wound-healing responses in two patients receiving the same surgery, and other extrinsic factors, such as the exposure to ultraviolet radiation,101,148 can modulate that response. Therefore, there is no established ablation depth under which there is no risk for haze. When treating myopia, some authors suggest applying prophylactic MMC when ablation exceeds a certain number of diopters (such as ~6.00D107), a particular ablation depth (50 μm,32 75 μm,75 or 100 μm93), or when the ablation depth/corneal thickness ratio is equal to or higher than 0.18.83,95

Surface ablation is less popular for the treatment of hyperopia because LASIK obtains better results.145,160 Some reports, however, suggest that ASA procedures could be safe, effective, and predictable in these eyes.5,112 No study has defined the indications for using prophylactic MMC in hyperopic ASA. The incidence of haze after hyperopic surface ablation seems to be higher than after myopic ablation,111 and although haze after hyperopic ablation is located at the mid-periphery of the cornea, it leads to important refractive regression. Therefore, it is probably advisable to use MMC even with low hyperopic corrections12 until further comparative studies become available.

### PREPARATION AND APPLICATION OF THE MITOMYCIN C

The MMC dilution may be prepared as follows: 5 ml of balanced salt solution (BSS) or distilled water are added to 2 mg of MMC, to obtain a 0.4 mg/ml dilution of MMC. Using an insulin syringe, we take 0.5 ml of this solution and we add 0.5 ml of BSS or distilled water, thus obtaining 1 ml of 0.02% MMC versus 95 eyes treated with 0.02% MMC. As mentioned previously, Sadeghi et al showed that the median inhibitory dose of the MMC on cultured human keratocytes is 0.038 mg/ml (0.0038%), almost twice the concentration applied in the eyes that received 0.002% MMC.157

There are several ways of applying the MMC over the ablated stroma.12,57,65 The easiest way to avoid leakage of the MMC to the peripheral cornea or the limbus is to use a round cellulose sponge approximately 7–9 mm in diameter. This is soaked in the MMC solution and placed carefully over the ablated stroma. This technique results in the release of a reproducible amount of MMC.55 Jain et al proposed the use of a ring instead of a complete disk, in order to diminish the exposure of the central cornea to the MMC,57 and reported good results.86 A small piece of a cellulose sponge soaked in the MMC solution may be used to apply a brushstroke of MMC over the ablated stroma.12

### Adverse Effects on the Cornea

The complications associated with MMC in pterygium and glaucoma surgeries3,35,40,92 have not been reported in refractive surgery. Mitomycin C may cause vascular endothelial injury140 and, secondarily, tissue necrosis.80 While the tissues in contact with MMC during pterygium and glaucoma surgeries are richly vascularized and may be damaged through that ischemic mechanism, the avascular cornea is not affected. When applied to the cornea, MMC could potentially damage all three main corneal cell types by direct citotoxicity: epithelial (differentiated epithelium and limbal cells), stromal (keratocytes), and endothelial cells. Only the first two have substantial mitotic activity. Because cells with a higher mitotic rate are more sensitive to the MMC, the epithelium and the keratocytes would be expected to be more susceptible to MMC toxicity than the endothelium.166

### EFFECT ON THE CORNEAL EPITHELIUM

Animal studies have shown variable results regarding the effect of MMC in the corneal epithe-
lium. Chang reported a dose-dependent delay in re-epithelialization after application of 0.01% and 0.02% MMC for 2 minutes, but another study in rabbits did not find any re-epithelialization delay associated with MMC.

Rajan et al applied 0.02% MMC for 1 and 2 minutes on human corneas in vitro. They found a delay in the latency until the re-epithelialization started that was dependant on the duration of the application of the MMC, but no difference in the epithelial migration rate (once the re-epithelialization began) between the group that received MMC for 1 minute compared to controls. They observed a statistically significant delay until the corneal epithelialization was complete in the group that received MMC for 2 minutes.

Despite these experimental observations, clinical studies suggest a lack of relevant epithelial toxicity (Table 2). In addition, studies of repeated topical application of MMC to treat ocular surface neoplasias, where the drug also contacts the limbus, do not show epithelial changes, thus suggesting the absence of limbal toxicity.

When used prophylactically, the MMC is currently applied for a shorter time. Given the results of Rajan et al, those short exposure times could explain why corneal epithelial complications are rarely seen after MMC use during surface ablation.

There is another aspect of the possible epithelial toxicity of MMC that has not been thoroughly studied: the effect on the development of epithelial hyperplasia. Epithelial hyperplasia has been described following surface ablation, especially with small optical zones (≤5 mm) and deeper ablations, where the change in dioptric power at the edge of the ablation zone is more abrupt. The epithelium reacts to stromal loss with hypertrophy of the cells of the basal layer and, if this hypertrophy does not result in a smooth corneal surface, epithelial hyperplasia develops.

As the epithelium plays an important role in determining the dioptric power of the total cornea, this epithelial hyperplasia is thought to be one cause of refractive regression after surface ablation. Another study using confocal microscopy on human corneas after surface ablation found no epithelial hyperplasia and no relation between postoperative epithelial thickness and refractive regression. They did detect an increment in stromal thickness related to postoperative regression.

A change in the pattern of epithelial hyperplasia associated with the use of MMC could be a subtle sign of its epithelial toxicity. Rajan et al using human corneas in vitro, found a normal epithelial thickness and morphology 1 month after the application of 0.02% MMC for 1 minute when compared to the control group, but the epithelial layer was less differentiated and significantly thinner in the group that received 0.02% MMC for 2 minutes. These differences might have disappeared with longer follow-up. Chen et al applied 0.02% MMC for 20–30 seconds after epi-LASIK and found that, 1 week postoperatively, the number of basal cells with normal morphology was greater in the control group that did not receive MMC, but they did not find any differences in subsequent examinations. The number of apical cells with normal morphology was only slightly higher in the control group in examinations 2 weeks, 1 month,

### Table 2

**Clinical Studies Reporting The Effect of Mitomycin C (MMC) on the Corneal Epithelium after Surface Ablation**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Number of Eyes</th>
<th>Manuscript Type</th>
<th>MMC Dose (%)</th>
<th>MMC Application Time</th>
<th>Effect on the Epithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argento et al</td>
<td>30 MMC-treated 28 controls</td>
<td>Retrospective, comparative study</td>
<td>0.02</td>
<td>75 sec</td>
<td>No difference in re-epithelialization</td>
</tr>
<tr>
<td></td>
<td>100 controls</td>
<td>Prospective, nonrandomized, comparative study</td>
<td>0.01</td>
<td>Brushstroke</td>
<td>No difference in re-epithelialization</td>
</tr>
<tr>
<td>Camellin</td>
<td>30 MMC-treated 30 controls</td>
<td>Prospective, randomized, comparative study</td>
<td>0.02</td>
<td>30 sec</td>
<td>No difference in re-epithelialization</td>
</tr>
<tr>
<td>Carones et al</td>
<td>36 MMC-treated 36 controls</td>
<td>Prospective, randomized, comparative study</td>
<td>0.02</td>
<td>2 min</td>
<td>No difference in re-epithelialization</td>
</tr>
<tr>
<td>Gambato et al</td>
<td>1 Case report</td>
<td>Case report</td>
<td>0.02</td>
<td>2 min</td>
<td>Persistent punctate keratitis</td>
</tr>
<tr>
<td>Kymionis et al</td>
<td>52 MMC-treated 52 controls</td>
<td>Prospective, randomized, comparative study</td>
<td>0.02</td>
<td>45 sec</td>
<td>No difference in re-epithelialization</td>
</tr>
<tr>
<td>Leccisotti</td>
<td>1,011</td>
<td>Retrospective case series</td>
<td>0.02</td>
<td>30 sec - 2 min</td>
<td>Delay in 2 eyes</td>
</tr>
<tr>
<td>Lee et al</td>
<td>35</td>
<td>Retrospective case series</td>
<td>0.02</td>
<td>2 min</td>
<td>No epithelial toxicity reported</td>
</tr>
</tbody>
</table>


and 6 months after the surgery. In a group of 64 eyes, we found a statistically significant increase in central corneal thickness from 1 to 3 months after surface ablation, with no significant difference in the corneal thickness increase between the group that received intraoperative MMC and the group that did not receive MMC (Teus MA et al. Effect of mitomycin C on epithelial hyperplasia after LASEK. EVER meeting, Portoroz, Slovenia, October 3–6, 2007). More studies with longer follow-up measuring epithelial and stromal thicknesses after surface ablation would help define whether the MMC interferes with the normal pattern of corneal regrowth seen after laser surface ablation.

**EFFECT ON THE CORNEAL STROMA**

Keratocytes constitute the second cell type exposed to the MMC. It is in fact the cytotoxic and antiproliferative effects of the MMC on the corneal stromal cellularity that produce its capacity to reduce haze, since it inhibits its activation, proliferation and differentiation into myofibroblasts. This antimitotic effect has led to concern over a possible long-term depletion of keratocyte population.

The long-term effect of the laser ablation itself on stromal population is itself controversial. Studies disagree: some find a similar or higher postoperative keratocyte density whereas others have reported a decreased stromal population after surface ablation with no adjunctive MMC.

The few studies using intraoperative MMC also report contradictory results (Table 3). All reporting keratocyte depletion are animal or laboratory studies and all have a short follow-up (1 to 3 months). Those animal and human studies with longer follow-up (6 months or more) demonstrate that, after the initial depletion, the keratocytes proliferate, and no significant decrease in their density is found 6 to 12 months after the use of MMC. Midena et al detected keratocyte depletion 5 years after surface ablation compared to preoperative levels, regardless of whether MMC was used. The keratocytes of the posterior cornea do not seem to be altered by the use of MMC.

Qazi et al reported a case of late dense haze that developed after uncomplicated primary surface ablation with prophylactic MMC performed 17 months previously. We have also observed late corneal scarring that developed following epithelial trauma 1 year after LASEK with intraoperative 0.02% MMC for 1 minute. These two cases and the observations made by Gambato et al suggest that stromal cellularity does not significantly suffer from permanent MMC effect and that the keratocytes keep their capacity to activate and proliferate in response to a corneal trauma.

MMC has been shown to reduce haze associated with ultraviolet B (UV-B) irradiation after surface ablation in rabbits. Irradiation alone causes cellular toxicity and reduces cell growth in cultures of porcine corneal fibroblasts, effects that are enhanced when MMC was applied before the UV-B irradiation. This observation has led to the recommendation that UV-B protection be used in eyes treated with surface ablation and intraoperative MMC until the synergistic effect of MMC and UV-B on long-term keratocyte population is clarified.

The keratocyte density that a cornea needs to maintain to keep its normal function is not known, nor whether a depletion of keratocytes would carry a higher risk of long-term corneal instability. Whether MMC effects on the stromal population could facilitate post-surface ablation ectasia or corneal melting is not known, although no case of ectasia or corneal melting after surface ablation with MMC has yet been reported.

**EFFECT ON THE CORNEAL ENDOTHELIUM**

The third corneal cell type exposed to the MMC in corneal refractive surgery is the endothelium. Endothelium has the least mitotic activity in normal conditions as a result of contact inhibition and the presence in the aqueous humor of inhibitory factors. Torres et al and Song et al detected the presence of MMC in the aqueous humor after its application over the de-epithelialized cornea in animal models, suggesting that the drug contacts the posterior stromal layers and the endothelium. This raises the question of potential deep corneal toxicity. Direct exposure of the endothelium to the MMC at the concentrations used on the ocular surface would rapidly cause endothelial damage. Fortunately, apart from accidentally instillation of MMC into the anterior chamber during glaucoma filtering surgery, such concentrations do not reach the endothelium, as the concentration of MMC detected in the anterior chamber after its application over the de-epithelialized cornea is much lower. McDermott et al and Garweg et al showed that direct application of MMC (on human corneas maintained in vitro and on endothelial cell cultures) at concentrations of 100 μg/ml (0.01%) or lower did not result in endothelial toxicity, whereas application of 200 μg/ml (0.02%) MMC rapidly induced edema with marked ultrastructural changes. Torres et al and Song et al measured MMC concentration in the aqueous humor after application of 0.02% MMC for 2 minutes and found an
aqueous humor concentration much lower than 0.002%. Nevertheless, this small concentration has been shown to cause cross-linking and double-strand breaks of corneal endothelial DNA in goat corneas.\textsuperscript{128}

The possible long-term effects of these changes on human corneas in vivo are still controversial, since two recent studies suggest a decrease in corneal endothelial cell density, whereas the majority of them report no endothelial change at all (Table 4).\textsuperscript{115} In fact, there have been no reports of corneal edema after the usual dose and exposure time of intraoperative MMC in surface ablation. The only case of corneal edema after MMC application in refractive surgery occurred in a patient after repeated postoperative topical application of MMC.\textsuperscript{117} Garweg et al\textsuperscript{43} had shown that MMC cytotoxicity appeared if the exposure was maintained chronically for 7 days, even with low concentrations, which could explain the case of corneal edema.\textsuperscript{117}

Chang\textsuperscript{21} applied 0.01% and 0.02% MMC for 2 minutes in rabbit corneas and reported a dose-dependent transient edema and a decrease in endothelial cell density. The fact that the rabbit corneal endothelium has continuous mitotic activity,\textsuperscript{60} unlike human endothelium, may have led to a higher sensitivity of the rabbit endothelium to the antimitotic action of the MMC.

Among the human studies, Morales et al\textsuperscript{99} found significant cell loss in the MMC group (nine eyes), compared to the control group (nine eyes). However, the high standard deviation of endothelial cell counts make results in studies with few patients difficult to interpret, because the probability of having cases with extreme counts (too low or too high) in a given group is high. There are two main ways to decrease the uncertainty: decrease the standard deviation (i.e., increase the reproducibility of the measurement) or increase the number of cases studied. Most human studies that include a large number of patients do not detect that endothelial cell decrease (Table 4), and two of them\textsuperscript{30,80} actually found a statistically significant increase in the endothelial cell density 3 to 6 months after surgery. This increase may have resulted from the cessation of contact lens wear.\textsuperscript{116} Another explanation may be the change in corneal magnification after laser ablation. The decrease in the keratometric values after myopic laser ablation profiles would produce a decrease in the magnification of the image of the endothelial cells obtained by specular microscopy.\textsuperscript{55} The cells would consequently appear smaller than in the preoperative picture and would thus be counted erroneously as being more numerous.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Number of Eyes (patients)</th>
<th>Manuscript Type</th>
<th>MMC Dose (%)</th>
<th>MMC Application Time</th>
<th>Follow-up (months)</th>
<th>Endothelial Cell Density</th>
<th>Endothelial Cell Morphology</th>
<th>Clinically Evident Corneal Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Benito-Llopis et al</td>
<td>48 (24)</td>
<td>Prospective, nonrandomized, comparative study</td>
<td>0.02</td>
<td>30 sec</td>
<td>3</td>
<td>Non-decreased</td>
<td>n.a.</td>
<td>No</td>
</tr>
<tr>
<td>Diakonis et al</td>
<td>15 (15)</td>
<td>Prospective, randomized, comparative study</td>
<td>0.02</td>
<td>15 sec</td>
<td>12</td>
<td>Non-decreased</td>
<td>Unchanged</td>
<td>No</td>
</tr>
<tr>
<td>Gambato et al</td>
<td>36 (36)</td>
<td>Prospective, randomized, comparative study</td>
<td>0.02</td>
<td>2 min</td>
<td>Mean: 12</td>
<td>n.a.</td>
<td>Unchanged</td>
<td>No</td>
</tr>
<tr>
<td>Goldsberry et al</td>
<td>16 (8)</td>
<td>Prospective case series</td>
<td>0.02</td>
<td>12 sec</td>
<td>Mean: 18</td>
<td>Non-decreased</td>
<td>Unchanged</td>
<td>No</td>
</tr>
<tr>
<td>Leccisotti</td>
<td>52 (52)</td>
<td>Prospective, randomized, comparative study</td>
<td>0.02</td>
<td>45 sec</td>
<td>12</td>
<td>Non-decreased</td>
<td>n.a.</td>
<td>No</td>
</tr>
<tr>
<td>Lee et al</td>
<td>359</td>
<td>Retrospective case series</td>
<td>0.02</td>
<td>30 sec–2 min</td>
<td>3 (96 eyes followed up to 6 months)</td>
<td>Non-decreased</td>
<td>Unchanged</td>
<td>No</td>
</tr>
<tr>
<td>Morales et al</td>
<td>9 (9)</td>
<td>Prospective, randomized, comparative study</td>
<td>0.02</td>
<td>30 sec</td>
<td>3</td>
<td>Decreased</td>
<td>n.a.</td>
<td>No</td>
</tr>
<tr>
<td>Nassaralla et al</td>
<td>22 (14)</td>
<td>Prospective case series</td>
<td>0.02</td>
<td>2 min</td>
<td>6</td>
<td>Non-decreased</td>
<td>n.a.</td>
<td>No</td>
</tr>
<tr>
<td>Nassiri et al</td>
<td>76 (48)</td>
<td>Prospective, nonrandomized, comparative study</td>
<td>0.02</td>
<td>10–50 sec</td>
<td>6</td>
<td>Decreased</td>
<td>n.a.</td>
<td>No</td>
</tr>
<tr>
<td>Wallau et al</td>
<td>44 (44)</td>
<td>Prospective, randomized, comparative study</td>
<td>0.002</td>
<td>1 min</td>
<td>6</td>
<td>Non-decreased</td>
<td>Unchanged</td>
<td>No</td>
</tr>
<tr>
<td>Zhao et al</td>
<td>174 (89)</td>
<td>Prospective case series</td>
<td>0.02</td>
<td>15 sec</td>
<td>6</td>
<td>Non-decreased</td>
<td>Unchanged</td>
<td>No</td>
</tr>
</tbody>
</table>
The lack of clinically evident endothelial toxicity and the mentioned studies (Table 4) suggest that one application of MMC at the low concentration used in refractive surgery is probably insufficient to produce a significant cytotoxic effect in the endothelium. Long-term studies are still needed to further establish its safety.

**EFFECT ON THE CILIARY BODY AND THE INTRAOCULAR PRESSURE**

Several studies have suggested a cytotoxic effect of topical MMC on the ciliary body when applied transsclerally, and such cytotoxicity might be involved in the postoperative hypotony that may appear after its use in glaucoma surgery. Only one recent study analyzes this effect after applying MMC over the ablated cornea. Kymionis et al. applied 0.02% MMC during 2 minutes in 20 rabbit corneas previously treated with a 7-μm deep surface ablation. The contralateral eyes served as controls. They did not find differences in the intraocular pressure between eyes treated with MMC and the fellow eyes up to 3 months postoperatively. They also did not find any difference between preoperative and postoperative intraocular pressure. Optic and electronic microscopy did not show morphological changes in the ciliary body. These results suggest that the amount of MMC that enters the anterior chamber and gets in contact with the ciliary body is insufficient to cause toxicity.

**Conclusions**

Evidence 1 clinical trials are clearly needed to definitely establish the efficacy and safety of the use of MMC in corneal refractive surgery. Nevertheless, the current available evidence supports the use of this drug in surface ablation procedures.

Mitomycin C has shown to decrease the incidence of haze after surface ablation refractive surgery. Its use allows treatment with surface ablation not only of low and moderate myopia, but also of high myopia with similar visual and refractive results as LASIK. There is a trend toward reduced dosage and exposure time of MMC; several studies suggest that even low concentrations over short times are effective in reducing the risk of haze. Some studies, however, suggest that lower concentrations (0.002%) may not be adequate to prevent haze after surface ablation for high myopia. More long-term clinical studies are needed to establish the efficacy of lower concentrations to avoid haze in moderate myopia. There is not enough evidence to establish the criteria for the use of prophylactic MMC in hyperopic ablations. The usual intraoperative dose of 0.02% MMC has not been associated with any clinically relevant corneal toxicity. More studies are needed to evaluate the effect of this drug on postoperative corneal epithelial hyperplasia and stromal regrowth. Long-term depletion of keratocyte population after MMC application is controversial, and its consequences to corneal stability still needs to be analyzed. Human studies are needed to confirm the lack of toxicity to the ciliary body and of effect on the postoperative intraocular pressure, as suggested by animal studies.

**Method of Literature Search**

Articles regarding mitomycin C were identified through a multistage systematic approach. First, we conducted a computerized search of the Medline database using PubMed (www.pubmed.com). Last search was performed in September 2008. A comprehensive search was made using the terms: haze, excimer laser surface ablation, advanced surface ablation, photorefractive keratectomy, PRK, laser-assisted subepithelial keratectomy, laser subepithelial keratomileusis, LASEK, epipolis LASIK, epi-LASIK, LASIK, laser in situ keratomileusis and all of those terms followed by “AND” and the following: mitomycin, mitomycin-C, MMC, alkylating agent, MMC toxicity. Second, all entries were critically reviewed and those considered to be of significance were used, including those written in English, Spanish, and French, and also those from the non-English literature if an English abstract was available. Next, we reviewed the reference section of each article, to detect other studies not captured by the Medline search. Once these articles were critically reviewed, they were included if they were considered to add additional data or to refute previous information.

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