In Vivo Confocal Microscopic Evaluation of Corneal Wound Healing after Epi-LASIK

Wei-Li Chen, Huai-Wen Chang, and Fung-Rong Hu

Purpose. To study the healing of corneal wounds after epikeratome laser-assisted in situ keratomileusis (epi-LASIK).

Methods. Twenty-seven patients who had undergone epi-LASIK for the treatment of myopia or myopic astigmatism in 46 eyes were enrolled. A single intraoperative application of 0.02% mitomycin C (MMC) for 20 to 30 seconds was used in 24 eyes with a refractive error of less than −6.0 D (MMC group). MMC was not given to eyes with myopia less than −6.0 D (non-MMC group). The eyes were examined by in vivo confocal microscopy at 1, 3, and 7 days after surgery and then weekly during the first month and once each at 3 and 6 months. Selected images of the corneal basal-apical surface epithelia and stromal reactions quantified by z-scan profile were evaluated.

Results. In vivo confocal microscopy showed that cells in most of the epithelial flaps were damaged during the first few days after surgery and were rapidly replaced by new growing cells. In the MMC and non-MMC groups, the corneal basal epithelial cells returned to their preoperative morphology in 0% and 13.6% of the eyes after 1 week, 37.5% and 36.4% after 2 weeks, and 87.5% and 86.3% after 1 month, respectively. The corneal apical surface epithelial cells in the MMC and non-MMC groups recovered their squamous morphology in 12.5% and 13.6% of the eyes after 1 week, 37.5% and 54.5% after 1 month, and 52.4% and 57.9% after 6 months, respectively. There was no difference in the stromal reaction between the groups at 1, 3, and 6 months after surgery.

Conclusions. Damage of the epithelial flaps after epi-LASIK was observed by in vivo confocal microscopy. MMC usage may cause more damage to the epithelial flaps. There was no difference in stromal reaction between the groups with and without MMC.

AAlthough laser-assisted in situ keratomileusis (LASIK) is the most popular surgical procedure for treating refractive errors, flap-related problems may still lead to undesirable consequences.1-4 Surface-ablation techniques, such as photorefractive kerectomy (PRK), laser-assisted subepithelial keratectomy (LASEK), and epikeratome laser-assisted in situ keratomileusis (epi-LASIK) have certain advantages over LASIK.

The reduced depth of treatment lessens the amount of adequate corneal tectonic strength. In addition, the lack of incision into the corneal nerves limits the severity of dry eye. Flap-related complications, such as epithelial ingrowth, flap striae, dislocation, or loss can also be avoided.5-8 Epi-LASIK is reported to preserve a viable epithelial sheet after replacement within at least 24 hours after treatment.9-10 It also provides lower levels of transforming growth factor-β1 in tears compared with LASEK and reduces the incidence of haze formation.11 Many patients have reported shortened durations of pain and satisfactory surgical outcomes.12-14 Although contradictory results have also been reported.15,16

To date, only a few investigators have studied the histopathology of the corneal epithelial flaps created with an epikeratome, and most specimens were obtained within 24 hours after surgery.15,16 To our knowledge, no researchers have sequentially observed the wound-healing process (namely, epithelial flaps and stromal reactions) microscopically after epi-LASIK. The purpose of this study was to assess by in vivo confocal microscopy the healing of corneal wounds during the 6 months after epi-LASIK.

Methods

Patients

The study procedure was approved by the Institutional Review Board for Human Studies of the National Taiwan University Hospital and adhered to the guidelines in the Declaration of Helsinki for research in human subjects. Twenty-seven patients (mean age ± SD, 29.2 ± 4.4 years; age range, 21–47; 46 eyes) who underwent epi-LASIK surgery between March 2005 and May 2006 for the treatment of myopia or myopic astigmatism were enrolled. All patients provided informed consent for the surgery and the in vivo confocal microscopic study. Inclusion criteria were >18 years of age, stable refraction, no previous refractive surgery, no ocular or systemic disease that could affect epithelial healing, tear break-up time of no less than 10 seconds, and Schirmer test with anesthesia of no less than 5 mm before surgery. Slit-lamp biomicroscopy and in vivo confocal microscopy were performed before surgery to rule out corneal disease.

Two physicians performed all the epi-LASIK procedures with an epikeratome (Centurion Epi Edge; Norwood Abbey, Melbourne, VIC, Australia) and an excimer laser (Technolas 217: Bausch & Lomb, Rochester, NY) and attempted to achieve eutmetria. Patients with incomplete flaps, free flaps, or poor adhesion of the epithelial flaps after surgery were excluded from the analysis.

During surgery, a 0.02% mitomycin C (MMC) solution was applied for 20 or 30 seconds to eyes with a refractive error of −6.0 to −8.0 D or more than −8.0 D, respectively. MMC was not given to eyes with myopia less than −6.0 D. Chilled physiologic saline (BSS Plus; Alcon Laboratories Inc, Ft. Worth, TX) was used to wash out any retained MMC.

A therapeutic bandage contact lens was inserted immediately after surgery and was removed within 7 days after surgery. Artificial tears and 0.1% fluorometholone were applied 4 times daily for 1 month and were gradually tapered over 4 months. Gentamicin (0.3%) was applied four times daily for 1 week.

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In Vivo Confocal Microscopy

In vivo confocal microscopy was performed before surgery and on days 1, 3, 5, and 7 after surgery. Evaluations were repeated weekly in the first month and once each at 3 and 6 months. Before the therapeutic bandage contact lenses were removed, the patients were examined with the lenses in place. After the contact lenses were discontinued, examinations were performed without contact lenses. Before in vivo confocal microscopic examination, one drop of 0.5% proparacaine solution and artificial tears was instilled into the lower conjunctival sac. The patient was then seated at an examination table with the head in a headrest. The center of the cornea was examined with a confocal microscope (Confoscan 3.4.1; Nidek Technologies, Padova, Italy) equipped with a standard 40× water-immersion front lens. The microscope was set to the automatic mode. A scan of the full thickness of the cornea was automatically performed in each participant; the examinations lasted 1 to 3 minutes. Each scan recorded 350 images at a distance of 4 µm, on a z-axis.

Image Analysis of Corneal Basal Epithelial Cells

In vivo confocal microscopy was used to classify the corneal basal epithelium as follows: (1) intact cellular border without a visible nucleus (Fig. 1A); (2) elongated shape with intact cellular borders but no visible nucleus (Fig. 1B); (3) amorphous and poorly identified appearance (Fig. 1C); (4) patchy whitening of the cellular sheet in which some areas showed intact borders without visible nuclei, whereas some areas demonstrated unidentified cellular morphology (Fig. 1D); (5) basal cells with prominent nuclei and high nucleus/cytoplasm (N/C) ratios but no identifiable cellular borders (Fig. 1E); or (6) cellular borders and low N/C ratios (Fig. 1F).

Image Analysis of Corneal Apical Surface Cells

Cells above the basal cells were defined as corneal apical surface cells. In the early postoperative period, the severe epithelial cellular damage makes it difficult to identify the corneal epithelial cells precisely at different depths. However, it is easy to identify basal epithelial cells because of their typical appearance, as shown in Figure 1, and their location immediately superficial to the corneal stroma. Thus, those cells that were located above the basal cells were defined as corneal apical surface cells in this study. Apical cells cannot exist if no basal layer is visualized under this definition. It is also impossible to identify the origin of the different layers of cells in this study by in vivo confocal microscopy.

Results were classified as cells showing large and flat superficial epithelia with small nuclei (Fig. 2A); elongated superficial epithelial cells (Fig. 2B); cells with high N/C ratios but without the normal large, squamous appearance (Fig. 2C); large, flat epithelial cells with multidirectional elongation (Fig. 2D); exfoliating superficial epithelial cells resembling sloughing or necrotic cells (Fig. 2E); or amorphous and poorly identified cells (Fig. 2F).

Image Analysis of the Stromal Reaction

The stromal reaction was evaluated by stromal scatter based on the reflectivity (Confoscan 3; Nidek Technologies) tied to the light intensity. The z-scan system (a graphic showing the depth coordinate on the z-axis and the level of reflectivity on the y-axis) with the profile of scattered light assessment was used. Three consecutive measurements were performed on the same examination, to quantify the light intensity. Three parameters were used to represent the stromal reaction: the peak of the light intensity in the whole stroma, the average light intensity of the anterior stroma within the anterior 50 µm of depth, and the average light intensity of the whole stromal layer.

Measurement of Corneal Epithelial Thickness

All areas of the z-scan curve where the basal epithelium and the apical surface epithelial cells could be clearly recognized were recorded. The depth values on the z-axis indicated by the software were used to determine corneal epithelial thickness, which was the distance between the innermost basal epithelium and the most superficial apical surface epithelial cells.

Statistical Analysis

Data regarding corneal epithelial thickness and stromal reactions were calculated as means ± standard deviations. Statistical analyses of the results were performed by Student’s t-test for postoperative stromal reaction. P < 0.05 was considered statistically significant.

Results

Patients who completed the 1-, 3-, and 6-month follow-up examinations numbered 27 (24 and 22 eyes in the groups with and without MMC treatment, respectively), 25 (22 and 20 eyes
with and without MMC), and 22 (21 and 19 eyes with and without MMC).

Healing of Basal Cells Observed by In Vivo Confocal Microscopy

Table 1 shows the distribution of basal epithelial morphologies in the treatment groups. Before surgery, all eyes had intact cellular borders without visible nuclei (Fig. 1A). This pattern was also observed in cells thought to be in a late differentiating phase during wound healing. In the MMC and non-MMC treatment groups, the corneal basal epithelial cells returned to this pattern (Fig. 1A) in 0% and 13.6% of the eyes at 1 week, 37.5% and 36.4% after 2 weeks, and 87.5% and 86.3% after 1 month, 52.4% and 57.9% at 6 months, respectively.

During the first few days after surgery, elongated superficial epithelial cells (Fig. 2B) and large flat epithelial cells with multidirectional elongation (Fig. 2D) were observed in the MMC and non-MMC groups. The cause of these patterns (Figs. 2B and 2D) was thought to be mechanical stretching of epithelial flaps during surgery. Exfoliating superficial epithelial cells (Fig. 2E) and amorphous cells (Fig. 2F) were believed to be caused by severe pathologic changes (most likely cell death) and were found only in the first few weeks after surgery in the groups treated with and without MMC. Cells with a high N/C ratio but without normal large, squamous appearance were considered active regenerative cells without full differentiation (Fig. 2C). Such cells could be found after postoperative day 3, and were seen in most eyes between 3 weeks and 3 months after surgery.

Stromal Reaction Observed by In Vivo Confocal Microscopy

The peak of the light intensity in the whole stroma was found within the anterior 12 μm of depth in all eyes. Table 3 presents the comparison of the stromal reactions between the MMC and non-MMC treatment groups. In all three parameters which represent stromal reaction, there were no differences observed between the groups at 1, 3, and 6 months after surgery (P > 0.05).

Corneal Epithelial Thickness

The total corneal epithelial thicknesses in the MMC and non-MMC groups were, respectively, 54.2 ± 4.5 and 56.1 ± 5.1 μm at 1 month (P > 0.05), 56.1 ± 6.7 and 55.2 ± 4.9 at 3 months (P > 0.05), and 58.1 ± 5.1 and 57.3 ± 7.2 at 6 months (P > 0.05).

Representative Cases

Patient 1 (case 1) was a 24-year-old man who had a preoperative refractive error of −4.0 D. Figure 3 shows the sequential confocal microscopic findings in the basal epithelial cells after epi-LASIK. The cells looked normal on day 1 but demonstrated notable pathologic changes (amorphous and poorly identified basal cells) on days 3 and 5. On day 7, small cells with prominent nuclei but no identifiable cellular borders were found. On day 14, the basal cells had cellular borders and a lower N/C ratio. Normal basal cells with intact cellular borders but no visible nuclei appeared at 1 month after surgery.

Patient 2 (case 2) was a 37-year-old woman who underwent epi-LASIK in her left eye, which had a preoperative refractive error of −5.5 D. Figure 4 shows the sequential results. The basal epithelial cells appeared healthy throughout the observational period. However, pathologic changes were seen in the apical surface cells on days 1 and 7. After 2 weeks, these cells

![Figure 2](image-url)
had high N/C ratios but not the normal, large, squamous appearance. A normal, large, flat epithelium with small nuclei was observed after 1 month. Among all the eyes, only two in the group with no MMC treatment were found to have a morphologically healthy basal epithelium, as seen in case 2 throughout the observational period.

**DISCUSSION**

Normal wound healing after epithelial debridement involves three distinct but continuous phases. In the first phase, hemidesmosomes are lost and provisional attachment complexes, called focal contacts, form. The epithelial cells flatten, migrate, and slide as a single-layered intact sheet to cover the denuded surface. During the second phase, cells distal to the original wound proliferate to repopulate the wound area, and cell stratification and differentiation occur. In the third phase, hemidesmosomes reform, and extracellular matrix is synthesized and reassembled.

After epi-LASIK, healing of the corneal epithelium may differ from normal epithelial healing. The replaced epithelial flap, whether dead or alive, stays in front of the leading edge, and a

<table>
<thead>
<tr>
<th>Time after Surgery</th>
<th>Border (+)</th>
<th>Nucleus (-)</th>
<th>Elongated</th>
<th>Amorphous</th>
<th>Patchy Whitening of Cell Sheet</th>
<th>Border (-) Nucleus (+)</th>
<th>Border (+) Nucleus (+)</th>
<th>Total Number</th>
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<tr>
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<td>1 d</td>
<td>8.3 (2)</td>
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<td>4.2 (1)</td>
<td>50 (12)</td>
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<td>25 (6)</td>
<td>0 (0)</td>
<td>41.6 (10)</td>
<td>33.8 (8)</td>
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<td>2 wk</td>
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<td>3 wk</td>
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<td>Eyes without MMC treatment</td>
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<tr>
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<td>45.5 (10)</td>
<td>18.2 (4)</td>
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<td>3 d</td>
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<td>50 (11)</td>
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<td>36.4 (8)</td>
<td>4.6 (1)</td>
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<td>22.7 (5)</td>
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<td>31.8 (7)</td>
<td>31.8 (7)</td>
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<td>2 wk</td>
<td>36.4 (8)</td>
<td>0 (0)</td>
<td>4.5 (1)</td>
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<td>9.1 (2)</td>
<td>50 (11)</td>
<td>22</td>
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<td>13.7 (3)</td>
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<td>3 mo</td>
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Data are the percentage (number) of eyes. MMC: MMC treatment during operation; border (+) nucleus (-), intact cellular borders without visible nuclei; elongated: elongated basal epithelial cells with intact cellular borders, but no visible nuclei; amorphous: amorphous and poorly identified basal epithelial cells; patchy whitening of cell sheet: cells in some areas showed normal appearance, whereas cells in other areas demonstrated patchy whitening, and cellular morphology was not identifiable; border (-) nucleus (+): basal cells with prominent nuclei but no identifiable cellular borders (a high N/C ratio was found); border (+) nucleus (+): cells with cellular border and low N/C ratio.

had high N/C ratios but not the normal, large, squamous appearance. A normal, large, flat epithelium with small nuclei was observed after 1 month. Among all the eyes, only two in the group with no MMC treatment were found to have a morphologically healthy basal epithelium, as seen in case 2 throughout the observational period.

**DISCUSSION**

Normal wound healing after epithelial debridement involves three distinct but continuous phases. In the first phase, hemidesmosomes are lost and provisional attachment complexes, called focal contacts, form. The epithelial cells flatten, migrate, and slide as a single-layered intact sheet to cover the denuded surface. During the second phase, cells distal to the original wound proliferate to repopulate the wound area, and cell stratification and differentiation occur. In the third phase, hemidesmosomes reform, and extracellular matrix is synthesized and reassembled.

After epi-LASIK, healing of the corneal epithelium may differ from normal epithelial healing. The replaced epithelial flap, whether dead or alive, stays in front of the leading edge, and a
Denuded surface is lacking. In addition, preservation of the basement membrane under the epithelial flap may exert specific effects. Moreover, laser treatment of the stromal bed may induce an epithelial–stromal interaction and interfere with wound healing. Finally, pharmacologic agents used during surgery may contribute to wound healing.

We examined specific epithelial wound healing after epi-LASIK by using in vivo confocal microscopy. This method has been used to observe corneal epithelial healing in various conditions. Cho et al. found that healing superficial cells in rabbit corneas after epithelial debridement were initially elliptical and large. By the end of the second week, the cells were almost the same size and shape as normal cells. In limbal stem cell–deficient corneas, healing epithelial cells were smaller and more variable in size than were normal corneal cells. In post-PRK rabbit corneas, small superficial corneal epithelial cells with high N/C ratios were found at 1 week after surgery. The corneal epithelium recovered normal cellular morphology at 2 weeks. Although these studies provided important information about epithelial wound healing, the authors did not describe observations in different layers of corneal epithelial cells.

Discrepant results from different studies on the survival of epikeratome-created epithelial flaps have been reported. Palilikaris et al. initially concluded that the epikeratome produces epithelial flaps with a sharply separated basement membrane and intact basal cells, although occasional focal disruption of the basal lamina was also observed. Their more recent study showed that most of the epithelial cells were morphologically normal with only minor cell degeneration. Tanioka et al. demonstrated that most of the basal cells in epithelial flaps created with different epikeratome devices were PI-positive dead cells. Because of the difficulty in obtaining human corneal epithelial sheets for evaluation, these studies observed only flap survival in the first few days after epi-LASIK. Our long-term results are more similar to those of Tanioko et al. In the group without MMC treatment, 31.8% of the eyes had basal cells with normal morphology (Fig. 1A) on postoperative day 1; the incidence decreased dramatically on days 3 and 7. Those cells with morphology as shown in Figures 1B, 1C, and 1D were thought to have undergone severe pathologic changes—most likely, cell death. The healing basal epithelial cells during the first few days after surgery showed high N/C ratios without visible cellular borders (Fig. 1E). Visible

| Table 3. Stromal Reaction Represented by Intensity of Light (z-Scan Profile) |
|---|---|---|---|
| Time after Surgery | With MMC | Without MMC | P |
| Peak of stromal light intensity | | | |
| 1 mo | 65.6 ± 22.9 | 71.3 ± 27.8 | NS |
| 3 mo | 61.0 ± 24.2 | 66.8 ± 34.2 | NS |
| 6 mo | 56.25 ± 21.6 | 58.5 ± 17.1 | NS |
| Average light intensity of the anterior 50-μm depth of the stroma | | | |
| 1 mo | 59.8 ± 11.4 | 64.2 ± 20.8 | NS |
| 3 mo | 56.6 ± 14.7 | 62.7 ± 16.3 | NS |
| 6 mo | 52.3 ± 7.1 | 52.2 ± 6.7 | NS |
| Average light intensity of the whole stroma | | | |
| 1 mo | 48.3 ± 12.5 | 52.2 ± 18.4 | NS |
| 3 mo | 47.6 ± 19.2 | 49.7 ± 13.6 | NS |
| 6 mo | 41.5 ± 12.1 | 41.5 ± 19.3 | NS |

MMC, mitomycin C treatment during surgery. NS, nonsignificant by Student’s t-test.
cellular borders usually reappeared at 1 to 2 weeks after surgery (Fig. 1F). At that time, basal cellular nuclei were usually seen, but the N/C ratios markedly decreased. After 3 weeks, 72.7% of the eyes had no visible nuclei. To our knowledge, we are the first to report the appearance and subsequent disappearance of basal cellular nuclei, combined with the disappearance and subsequent reappearance of basal cellular borders during wound healing, evaluated by in vivo confocal microscopy. Although not proved, a large and visible nucleus may represent new, actively growing cells that are metabolically active and unstable. The reestablishment of cellular borders may represent reconstruction of the cellular junction, which implies a return to normal and stable conditions.

The recovery of normal morphology took longer in apical surface cells than in basal cells. In both the MMC and non-MMC treatment groups, most eyes had abnormal apical surface epithelial cells during the first few weeks. Abnormalities included elongated, amorphous, multidirectional, or unidentifiable morphology. Abnormal cells were thought to have undergone severe pathologic changes—most likely, cell death. In the intermediate stage of healing between 3 weeks and 3 months, most eyes had small and compact apical surface cells, with high N/C ratios but no squamous appearance (Fig. 2C). These cells were thought to be in higher metabolic, earlier differentiation stages than normal squamous cells.

The exact process of epithelial healing after epi-LASIK is still not clearly understood despite the information provided by in vivo confocal microscopy. Gradual sloughing of the damaged epithelial sheets may have occurred, followed by replacement with multilayered, highly active, new and growing cells. Because no denuded surface area was seen ahead of the leading edge (as noted in corneal debridement or PRK), we can easily explain why we saw no single layer of migrated cells, as is observed in phase I of normal epithelial healing. Features indicating the highly metabolic and undifferentiated status of the new growing cells were the increased N/C ratios of the basal and apical surface cells, the disappearance of cellular borders in basal cells, and the disappearance of the large squamous cells on the apical surface. However, we cannot rule out the possibility that new growing cells from the periphery of the cornea may have mixed with surviving cells on the flap and that together they composed the final cellular sheet.

Several of our findings need further clarification. First, our observational period and methods differed from those of previous studies. In published reports about epi-LASIK, healthy corneal epithelial sheets were observed only in the first 24 hours, and immunohistochemistry and electron microscopy were used for the examination. Our cross-sectional views of the epithelial sheets, long observation times, and in vivo observations may add important information. Second, use of...
different epikeratomes could lead to different cutting levels and affect the survival of the cellular sheet, although Tanioka et al.\textsuperscript{16} reported dead cells in epithelial flaps prepared with different epikeratomes. Third, because no similar study has been published, we have justified the grading scheme of corneal epithelial wound healing on the basis of the presumed correlation with severity just by morphologic interpretation. Furthermore, histologic studies are needed to confirm our hypothesis. Fourth, during postoperative week 1, most examinations were performed with therapeutic bandage contact lenses in place. To rule out the possibility that contact lens fitting might change the in vivo microscopic appearance of corneal epithelial cells, we did a preliminary test in rabbit eyes and found that placement of a contact lens did not change the appearance of the healing basal and apical surface epithelial cells (data not shown). We also compared the appearance of the basal and apical cells in the patients before and after removal of the contact lenses, and found no difference in apical cellular appearance (data not shown). Fifth, the stromal reaction based on the reflectivity tied to the light intensity in this study should be interpreted carefully. Potentially strong confounding factors may exist and make the results difficult to analyze. Sixth, to inhibit potential haze, we used MMC and a chilled physiologic saline solution in patients with high degrees of myopia. MMC has been widely accepted as an adjunctive therapy for corneal haze after higher myopic corrections by PRK and LASEK.\textsuperscript{30–34} It has been hypothesized that MMC inhibits fibroblast proliferation and differentiation, consequently blocking myofibroblast formation through its various potent effects.\textsuperscript{35,36} Although epi-LASIK is intended to create a cellular sheet with an intact basement membrane, the morphologically unhealthy epithelial cells we found may still be able to induce an epithelial–stromal reaction as in PRK or LASEK. There are only limited studies reported on the use of MMC in epi-LASIK. In this study, we found that higher myopic groups (>-6.0 D) treated with MMC may have similar stromal reactions compared with the low myopic group (-6.0 D) without MMC treatment. Our results support the widely accepted view that MMC can prevent corneal haze formation in surface ablation for higher myopic corrections. We also found that the group with MMC treatment had a higher incidence of early corneal basal epithelial damage compared with the group without MMC treatment. In addition, the only two eyes that had morphologically intact basal epithelial morphology during the entire observational period were without MMC treatment. Although we tried to avoid direct contact between the MMC solution and the epithelial sheets and we protected the epithelial sheet during vigorous irrigation of the stromal bed with the chilled saline solution, mechanical or pharmacologic cellular damage caused by MMC or irrigation itself may still have occurred during surgery. Because almost all eyes in the groups with and without MMC treatment have severe cellular damage of the apical surface epithelial cells within 2 weeks after surgery, it is difficult to evaluate the effect of MMC on these cells. We found no differences in corneal epithelial thickness between the two groups at 1, 3, and 6 months after surgery. The healing patterns of basal and apical cells were also similar at 3 and 6 months after surgery. The effect of MMC on corneal epithelial wound healing was thus thought to occur only in the early postoperative period.

In conclusion, our study demonstrated several important new findings. First, we believe this is the first human study in which in vivo confocal microscopy was used to study early and late wound healing after surface ablation, specifically epi-LASIK. Second, the corneal epithelial flaps after epi-LASIK were not as healthy as previously thought, as cells underwent severe pathologic changes within or after 24 hours. Third, at least several weeks were required for the epithelial cells to return to their normal morphology, and apical surface cells needed more time than basal cells. Finally, the stromal reaction was the same in the eyes with higher myopia treated with MMC as in the eyes with lesser myopia without MMC treatment. Because cellular conditions cannot be properly assessed by morphologic analysis alone, sequential evaluations using apoptosis assays, immunohistochemical staining, and/or electron microscopy are needed to provide further information about the corneal wound-healing process after epi-LASIK.

References


