Clinical Trials

Comparison of In Vivo Confocal Microscopic Findings between epi-LASIK Procedures with Different Management of the Epithelial Flaps

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Purpose. To use in vivo confocal microscopy to compare the wound-healing process in epikeratome laser-assisted in situ keratomileusis (epi-LASIK) with different management of the epithelial flaps.

Methods. This prospective study comprised 46 eyes in the on-flap group and 47 eyes in the off-flap group. Epithelial flaps were repositioned or removed in the on- and off-flap eyes, respectively. In vivo confocal microscopy was performed before surgery and at 1 and 2 weeks and 1, 3, and 6 months after surgery. Corneal epithelial thickness, basal/apical surface epithelial morphology, and stromal reactions were analyzed.

Results. Complete epithelialization by slit lamp biomicroscopy was faster for mitomycin C (MMC)-treated off-flap (4.27 ± 0.70 days) than on-flap (5.84 ± 0.08 days) eyes ($P = 0.01$). The percentage of eyes recovering to preoperative basal epithelial cell confocal morphology in the on- and off-flap groups was 87.5% and 92.3% for MMC-treated eyes and 86.3% and 90.5% in eyes without MMC treatment at 1 month after surgery. Of the studied eyes, with or without MMC, 50% and 100% returned to their preoperative apical epithelial morphology by 3 months in the on-flap and off-flap groups, respectively. Regression analysis revealed that the repositioned flap resulted in significant delay of epithelialization and apical–basal epithelial recovery ($P < 0.01$). Stromal reaction did not differ significantly at any of the time points. Corneal epithelial thickness was significantly thicker in the on-flap eyes (54.2 ± 4.5 μm) than in the off-flap eyes (26.7 ± 5.8 μm) at 1 month after surgery ($P < 0.01$).

Conclusions. On- and off-flap epi-LASIK showed comparable clinical outcomes after surgery. Off-flap epi-LASIK had more rapid re-epithelialization and normalization of epithelial morphology than did on-flap epi-LASIK, when observed by in vivo confocal microscopy. (ClinicalTrials.gov number, NCT00491439.) (*Invest Ophthalmol Vis Sci.* 2011;52:3640–3647) DOI:10.1167/iovs.10-6390

Epithelial laser in situ keratomileusis (epi-LASIK), first developed by Pallikaris et al.[1] in 2003, is a surface ablation procedure that has been reported to be safe and efficient in the treatment of myopia.[2–7] During the traditional epi-LASIK procedure, an epithelial sheet, or flap, created with an automated epikeratome is deflected and repositioned after completion of the laser ablation (on-flap).[1,2,8] The preservation of the epithelial sheet theoretically protects the underlying ablated corneal stroma and thus decreases the postoperative inflammation that results in haze formation, as occurs in conventional photorefractive keratectomy (PRK).[9–12] Although the original studies claimed the epithelial sheet to be viable for at least 24 hours after treatment,[13,14] others have reported that most basal cells in the epithelial flaps are dead cells.[15] Whether the epithelial flaps created during epi-LASIK should be repositioned is still debated. However, some prospective randomized studies have shown that epi-LASIK without retained epithelial flaps (off-flap) has comparable or even better results in regard to postoperative pain, epithelial healing, and vision recovery.[16–18] To determine the best management of epithelial flaps after epi-LASIK, more systematic, and prospective clinical studies are needed.

To our knowledge, no in vivo confocal microscopy study comparing the wound-healing process of on-flap with that of off-flap epi-LASIK has been published. We have reported in vivo confocal microscopy findings of corneal wound healing after on-flap epi-LASIK.[19] In the present study, we used in vivo confocal microscopy to investigate the clinical results and healing of corneal wounds produced by off-flap epi-LASIK and compared them with those of on-flap epi-LASIK.

Patients and Methods

Patients

From March 2005 to July 2007, 55 patients (93 eyes) with myopia or myopic astigmatism who underwent epi-LASIK surgery were enrolled in this prospective study. Informed consent for the surgery was obtained from all patients, and in vivo confocal microscopy examination was performed. The study protocol was approved by the Institutional Review Board for Human Studies of the National Taiwan University Hospital and adhered to the tenets of the Declaration of Helsinki. Inclusion criteria were age >18 years, stable refraction, no previous refractive surgery, no ocular or systemic disease that could affect

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TABLE 1. Baseline Characteristics of Eyes for Epi-LASIK

	On-flap	Off-flap	*P*
Patients, *n*	27	28	
Eyes, *n*	46	47	
Age, y (mean ± SD)	29.2 ± 5.4	28.4 ± 3.6	0.24
Range	21–47	22–40	
Sex			
Male/female	12/34	18/29	
Preoperative SE, mean ± SD (D)	−8.63 ± 2.71	−9.14 ± 2.47	0.31
Range	−4.125 to −16.5	−1.875 to −13.75	
Preoperative BSCVA mean ± SD (logMAR)	−0.05 ± 0.05	−0.05 ± 0.05	0.91
MMC-treated eyes, *n*			
Yes	24	26	0.22
No	22	21	

epithelial healing, tear break-up time >10 seconds, and Schirmer test, with anesthesia, of no less than 5 mm before surgery. Baseline characteristic of the patients in the on- and off-flap groups are shown in Table 1. Some data from the on-flap epi-LASIK patients has been reported in our previous study.[19] However, for statistical comparison between these two procedures, we again enrolled the reported on-flap epi-LASIK patients in the present study. Visual acuity, slit lamp examinations, and in vivo confocal microscopy were performed before and after the surgery.

Epi-LASIK Procedure

Epi-LASIK surgery was performed by two surgeons (WLC, FRH). From March 2005 to May 2006, all patients underwent epi-LASIK with epithelial sheets created by the Centurion Epi-Edge epikeratome (Norwood Abbey, Melbourne, VIC, Australia). Patients were enrolled in the on-flap group if the epithelial flap had been successfully created and repositioned after laser ablation. Patients with incomplete flaps, free flaps, or poor adhesion of the epithelial flap immediately after surgery had the flaps removed and were excluded from the study. From June 2006 to July 2007, all patients underwent epi-LASIK with removal of the epithelial flap created by the Amadeus II epikeratome (Advanced Medical Optics, Santa Ana, CA). Both the Centurion and Amadeus epikeratomes create epithelial sheets with a diameter of 9 mm and a hinge length from 2 to 4 mm. In off-flap epi-LASIK eyes, the epithelial sheet was separated with the use of one of the epikeratomes and was removed at the hinge with a beveled knife, except for those with free epithelial flaps, which were removed with no additional manipulation.

After the creation of the epithelial flaps, an excimer laser (Technolas 217z; Bausch & Lomb, Rochester, NY) was used to ablate the corneal stroma. Emmetropia was the targeted outcome in all patients. In both groups, MMC 0.02% was applied to eyes with refraction of −6.0 to −8.0 D for 20 seconds, and if refraction was more than −8.0 D, MMC was applied for 30 seconds. It was not used on eyes with myopia less than −6.0 D. The stroma was irrigated copiously with chilled physiologic saline (BSS Plus; Alcon Laboratories Inc., Fort Worth, TX) after MMC application. A bandage contact lens (Purevision; Bausch & Lomb) was immediately placed on all eyes after the operation and removed within 7 days after surgery. Gentamicin (0.3%) was applied four times daily for 1 week after surgery. Artificial tears (Refresh Plus, Allergan, Irvine, CA) and 0.1% fluorometholone were applied four times per day for 1 month and then tapered over 4 months.

Follow-up of Epithelial Healing by Slit Lamp Biomicroscopy and Visual Acuity

Slit lamp biomicroscopy was performed daily after surgery until the completion of corneal epithelialization. After removal of the bandage contact lenses, fluorescein staining was used to check for complete epithelial closure. Slit lamp examinations were also performed at 1 and 2 weeks and 1, 3, and 6 months after surgery. Spherical equivalent (SE) and uncorrected and best spectacle-corrected visual acuity (UCVA and BSCVA) were recorded during the 1-, 3-, and 6-month visits. The efficacy index (mean postoperative UCVA/mean preoperative BSCVA) and safety index (mean postoperative BSCVA/mean preoperative BSCVA) were determined using the 6-month postoperative log-MAR visual acuity data.

In Vivo Confocal Microscopy

In vivo confocal microscopy was performed by one examiner on all patients before surgery and during the first and second weeks after surgery. Examinations were also performed at 1, 3, and 6 months after surgery. The examiner was masked as to the operative procedure. One drop of 0.5% proparacaine solution and artificial tears was instilled immediately before each examination. The patient was asked to look straight ahead, and an in vivo confocal microscope (Confoscan 3.4.1; Nidek Technologies, Padova, Italy) equipped with a standard 40× water-immersion front lens captured images of the full thickness of the central cornea automatically. Each examination took approximately 1 to 3 minutes and recorded 350 images at a distance of 1.5 and 4 μm between successive images on the *z*-axis. During each visit, the measurements were repeated three times with a 4-μm *z*-interval followed by three times with a 1.5-μm *z*-interval.

Image Analysis of Corneal Basal Epithelial Cells

Images of the central cornea obtained by in vivo confocal microscopy were set up by our team and analyzed as previously described, with some modifications.[19,20] The corneal basal epithelium was classified as follows: (1) intact cellular border, without a visible nucleus (Fig. 1A), which demonstrated normal cells or cells in the late differentiation phase of wound healing; (2) poorly defined cells, including those with an elongated shape with intact cellular borders but no visible nucleus, patchy whitening of the cellular sheet, and amorphous with poorly indentified appearance, which demonstrated necrotic cells or cells undergoing severe pathologic changes (Fig. 1B); (3) basal cells with prominent nuclei and high nucleus/cytosol (N/C) ratios but no identifiable cellular borders, which demonstrated cells in an early regenerative stage during the healing process (Fig. 1C); and (4) basal cells with cellular borders and a low N/C ratio, which demonstrated cells during the healing process later than (3) but earlier than (1) (Fig. 1D). In the classification criteria established in our previous study,[19] cells with an elongated shape and intact cellular borders but no visible nucleus, patchy whitening of the cellular sheet, and amorphous with poorly identified appearance were separate categories. We combined these three categories in this study into one classification—poorly defined cells—for simplicity in data analysis.

Image Analysis of Corneal Apical Surface Cells

In the early healing phase, the epithelial thickness is thin and only surface and basal cells are present. Hence, corneal apical surface cells were defined in this study as cells above the basal cells.[19,20] Images of the apical surface cells obtained by in vivo confocal microscopy were

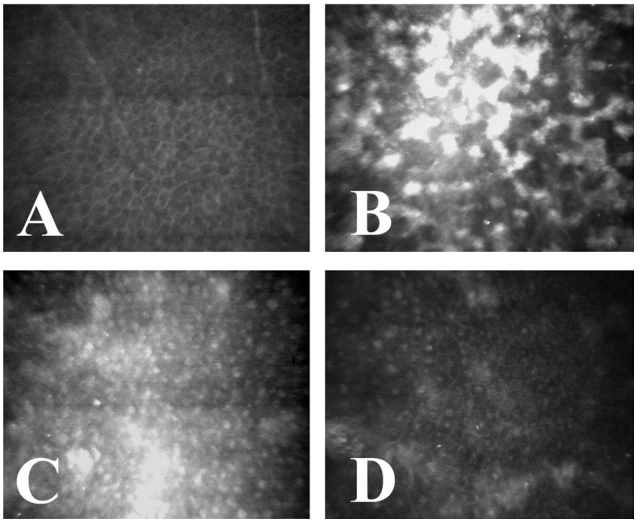

FIGURE 1. In vivo confocal photomicrographs showing the different patterns of basal epithelial cells. (**A**) Basal epithelial cells with an intact cellular border without visible nuclei. This pattern was seen in all eyes before surgery and in cells thought to be in a late differentiation phase during wound healing (image obtained before surgery). (**B**) Basal epithelial cells with amorphous and poorly identified morphology. This pattern was seen in only the on-flap group within 2 weeks after surgery. (**C**) Regenerated basal cells with prominent nuclei but no cellular borders. The N/C ratio was high. This pattern was seen in both groups during the first few weeks after surgery (image obtained 1 week after surgery). (**D**) Further regenerating cells with a decreased N/C ratio and newly formed cellular borders. This pattern was seen during the first few weeks after surgery in both groups and usually appeared later than the pattern shown in (**C**). The image was obtained 3 weeks after surgery. N/C, nucleus/cytosol ratio.

set up by our team and analyzed as previously described, with some modifications.[19,20] The corneal apical epithelium was classified as follows: (1) large, flat, superficial epithelia with small nuclei (Fig. 2A) that demonstrated normal cells or cells in the late differentiation phase of wound healing; (2) poorly defined cells, including elongated superficial epithelial cells, large, flat epithelial cells with multidirectional elongation, exfoliating superficial epithelial cells, and amorphous, poorly identified cells, which demonstrated necrotic cells or cells undergoing severe pathologic changes (Fig. 2B); (3) cells with high N/C ratios, but without the normal large, squamous appearance, which demonstrated cells in the early regenerative phase of the healing process (Fig. 2C). In our previous study,[19] we had classified superficial cells with elongated shape, large flat shape with multidirectional elongation, exfoliating morphology, and amorphous poorly identified shapes in separate categories. As for the basal cells, these categories are combined in this study into the classification, poorly defined cells, for ease of data analysis.

Measurement of Corneal Epithelial Thickness

A *z*-scan curve was recorded for all areas where the basal epithelium and the apical surface epithelial cells could be clearly visualized. The corneal epithelial thickness measured was indicated (by the software) as the depth value on the *z*-axis and was the distance between the innermost basal epithelium and the most superficial apical surface epithelial cells. Three repeated measurements were obtained at 1.5-μm *z*-intervals for analysis.

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, as previously described.[19] Briefly, the *z*-scan system using the profile for scattered light assessment was used for analysis. Three measurements obtained at 4-μm *z*-intervals were obtained and used for analysis. Three parameters proposed by our previous study[19,20] were used to represent the stromal reaction: (1) the peak of the light intensity in the whole stroma, (2) the average light intensity of the anterior stroma within the anterior 50 μm of depth, and (3) the average light intensity of the whole stromal layer. A representative image of a strong postoperative stromal reaction is presented in Figure 2D.

Statistical Analysis

Results of corneal epithelial thickness and stromal reaction measurement are expressed as the mean ± SD. The Mann-Whitney U or χ^2 test was used to compare baseline characteristics between groups when appropriate. Three different wound-healing parameters analyzed were (1) the complete epithelial healing time (CEHT) observed by slit lamp, (2) the status of basal epithelial cell morphology (BECM), and (3) the status of apical epithelial cell morphology (AECM), observed by in vivo confocal microscopy. Linear mixed models were used to analyze the associating factors for CEHT, and generalized mixed models with logistic links were used to analyze the associating factors for BECM and AECM. Explanatory covariates include flap management and MMC application. Linear mixed models were used to determine the associations between the postoperative 6-month SE of on- and off-flap groups with each of the three aforementioned wound-healing parameters. Age, sex, surgeon, and preoperative SE were adjusted in all models. Because of the collinearity between flap management and epikeratome type, the latter was excluded from regression analysis. A random variable was used in each model to catch the correlations of both eyes from the same individuals. Student's *t*-tests were used to compare the epithelial thickness and stromal reactions between the groups. $P < 0.05$ was considered statistically significant (SAS version 9.1; SAS Institute Inc, Cary, NC).

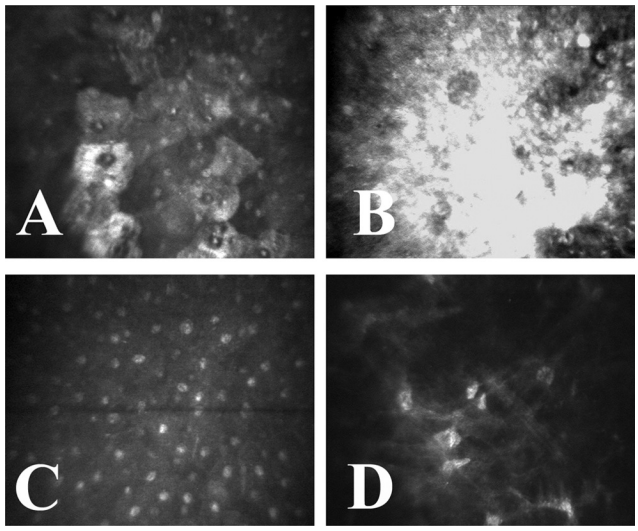

FIGURE 2. In vivo confocal photomicrographs showing the different patterns of corneal apical surface epithelial cells (**A**–**C**) and stromal reaction (**D**). (**A**) Pattern of normal squamous superficial cells found in preoperative eyes and in the late healing stages after both on- and off-flap epi-LASIK (image obtained before surgery). (**B**) Pattern of amorphous cells seen only during the first 2 weeks after flap-on EpiLASIK. Difficulty in identifying these cells was thought to be caused by the severe pathologic change in the epithelial flap (image obtained 1 week after surgery). (**C**) Pattern of nonsquamous epithelial cells with a high N/C ratio that were thought to be regenerating cells without full differentiation found after 1 week after surgery in both the on- and off-flap groups. This pattern was seen in most eyes between weeks 2 and 4 after surgery (image obtained 3 weeks after surgery). (**D**) The presence of strong stromal reaction due to the presence of active stromal fibroblasts.

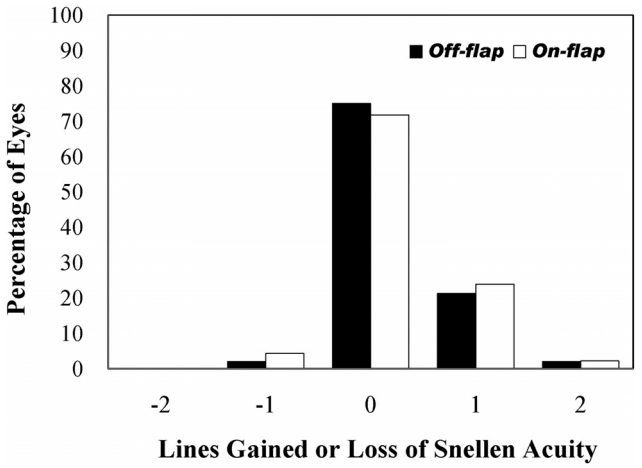

FIGURE 3. Changes in number of lines of BSCVA at 6 months.

RESULTS

Patients

In the on-flap epi-LASIK group, a total of 46 eyes completed examinations at 1 week, 2 weeks, and 1 month after surgery, and 42 and 40 eyes completed the 3- and 6-month follow-up visits, respectively. All patients (47 eyes) in the off-flap epi-LASIK group completed the 1- and 2-week and 1-, 3-, and 6-month follow-up examinations. The mean postoperative 6-month spherical equivalent (SE) of the on- and off-flap groups were -0.28 ± 0.96 D (range, -1.25 to 1.75) and -0.19 ± 0.39 D (range, -1.0 to 0. 5), respectively ($P > 0.05$). After adjustment for age, sex, and preoperative SE, the SE at 6 months after surgery did not correlate significantly with flap management, MMC use, or CEHT, observed by slit lamp biomicroscopy, nor did it correlate with BECM recovery time or AECM recovery time, according in vivo confocal microscopy data ($P > 0.05$).

Visual Acuity

Figure 3 shows the change in lines of BSCVA for each group at 6 months after surgery. No eye had greater than one line loss of visual acuity. BSCVA was unchanged or increased in 98% and 95% of the eyes in the on- and off-flap epi-LASIK groups, respectively. The efficacy index was 0.99 for both the on- and off-flap groups. The safety index for the on- and off- flap groups was 1.04 and 1.03, respectively.

Complete Epithelial Healing Time

Slit lamp examinations and fluorescein staining of the cornea for on-flap eyes revealed that an average of 5.84 ± 0.08 and 4.41 ± 0.07 days were necessary for complete epithelial healing for on-flap eyes, with and without MMC application, respectively. In the off-flap group, the CEHTs were 4.27 ± 0.70 and 3.32 ± 0.85 days for MMC-treated and untreated eyes, respectively. Regression analysis revealed that preservation of the epithelial flap ($P = 0.01$) was significantly associated with delayed epithelial healing.

BECM by In Vivo Confocal Microscopy

Table 2 summarizes the percentage of different patterns of BECM after epi-LASIK, as determined by in vivo confocal microscopy. Before surgery, intact cellular borders without visible nuclei were observed in all eyes and were recognized as normal BECM. After surgery, in the MMC-treated eyes, normal BECM was seen in 7.7% and none of the eyes at 1 week, 73.1% and 37.5% at 2 weeks, 92.3% and 87.5% at 1 month, and in all eyes at 3 and 6 months of the off- and on-flap epi-LASIK groups, respectively. In eyes that were not treated with MMC, the percentage reaching normal BECM was 19.0% and 13.6% at 1 week after surgery, 76.2% and 36.4% at 2 weeks, 90.5% and 86.3% at 1 month, and 100% at both 3 and 6 months, in the off- and on-flap epi-LASIK groups, respectively. A mixed-models regression analysis indicated that eyes in the off-flap group healed significantly faster (1 month) than those in the on-flap group, when judged by in vivo confocal microscopy of BECM ($P < 0.01$; Table 3).

Poorly defined cells with different morphologies and no discernible nuclei were found in 25% and 23% of the eyes in the on-flap epi-LASIK group with MMC and without MMC treatment at 1 week, respectively. This pattern was not found in both the MMC-treated and untreated off-flap epi-LASIK group, since the epithelial flap was removed immediately after surgery. In the MMC-treated eyes, regenerating basal cells with prominent nuclei, but no cellular borders, were seen in 41.6% and 23.1% of the eyes at 1 week after surgery in the on- and

TABLE 2. Percentage of Different Patterns of Basal Epithelial Cells after Epi-LASIK Classified by In Vivo Confocal Microscopy

Time after Surgery	Border (+) Nucleus (−)	Poorly Defined Cells	Border (−) Nucleus (+)	Border (+) Nucleus (+)	Total Eyes (*n*)	Border (+) Nucleus (−)	Poorly Defined Cells	Border (−) Nucleus (+)	Border (+) Nucleus (+)	Total Eyes (*n*)
On-flap*[19] *with MMC Treatment						***Off-flap with MMC Treatment***				
1 Week	0 (0)	25 (6)	41.6 (10)	33.3 (8)	24	7.7 (2)	0 (0)	23.1 (6)	69.2 (18)	26
2 Weeks	37.5 (9)	8.3 (2)	0 (0)	54.2 (13)	24	73.1 (19)	0 (0)	3.8 (1)	23.1 (6)	26
1 Month	87.5 (21)	0 (0)	0 (0)	12.5 (3)	24	92.3 (24)	0 (0)	0 (0)	7.7 (2)	26
3 Months	100 (22)	0 (0)	0 (0)	0 (0)	22	100 (26)	0 (0)	0 (0)	0 (0)	26
6 Months	100 (21)	0 (0)	0 (0)	0 (0)	21	100 (26)	0 (0)	0 (0)	0 (0)	26
On-flap*[19] *without MMC Treatment						***Off-flap without MMC Treatment***				
1 Week	13.6 (3)	22.7 (5)	31.8 (7)	31.8 (7)	22	19.0 (4)	0 (0)	14.3 (3)	66.7 (14)	21
2 Weeks	36.4 (8)	4.5 (1)	9.1 (2)	50.0 (11)	22	76.2 (16)	0 (0)	4.8 (1)	19.0 (4)	21
1 Month	86.3 (19)	0 (0)	0 (0)	13.7 (3)	22	90.5 (19)	0 (0)	0 (0)	9.5 (2)	21
3 Months	100 (20)	0 (0)	0 (0)	0 (0)	20	100 (21)	0 (0)	0 (0)	0 (0)	21
6 Months	100 (19)	0 (0)	0 (0)	0 (0)	19	100 (21)	0 (0)	0 (0)	0 (0)	21

Data are the percentage (number) of eyes. Column heads from left to right: (1) border (+) nucleus (−): intact cellular borders without visible nuclei; (2) poorly defined cells: elongated cells with intact cellular borders, but no visible nuclei; amorphous cells; and patchy whitening of the cell sheet; (3) border (−) nucleus (+): basal cells with prominent nuclei, but no identifiable cellular borders (a high N/C ratio was observed); (4) border (+) nucleus (+): cells with cellular border and low N/C ratio.

TABLE 3. Analysis Results of the Associating Factors with Generalized Mixed Models for Recovery to Normal BECM and AECM by Postoperative 1 Month as Observed by In Vivo Confocal Microscopy

	Normal BECM at 1 Month		Normal AECM at 1 Month	
	OR (95% CI)	*P*	OR (95% CI)	*P*
On-flap vs. off-flap	0.13 (0.05–0.34)	<0.01	0.16 (0.05–0.50)	<0.01
MMC+ vs. MMC−	0.62 (0.25–1.55)	0.30	0.48 (0.18–1.29)	0.15

off-flap groups, respectively. In both the MMC-treated and untreated eyes at the first postoperative week, there were more eyes showing cellular borders and low N/C ratios in the off-flap epi-LASIK group than in the on-flap group.

AECM by In Vivo Confocal Microscopy

Results classified according to different AECM seen by in vivo confocal microscopy are shown in Table 4. Normal AECM with large, flat apical surface epithelia was seen in 7.7% and 14.3% of the eyes in the off-flap epi-LASIK group, with and without MMC treatment, respectively, as early as the first postoperative week. This pattern of normal squamous epithelium was observed in all the eyes by the third postoperative month in the off-flap group, whereas only 50% of the eyes in the on-flap group showed this pattern, with or without MMC treatment. Poorly defined cells were seen only in the on-flap group. Cells with a high N/C ratio, but without a normal, large, squamous appearance were still seen in 50% of the on-flap MMC-treated and untreated eyes at 3 months after surgery, whereas no eyes exhibited this pattern for the MMC-treated and untreated off-flap groups. Recovery to normal AECM by 1 month was significantly delayed in the on-flap eyes ($P < 0.01$) as shown in Table 3.

Corneal Epithelial Thickness

In the on-flap group, the epithelial thickness before surgery and at 1, 3, and 6 months after surgery was 54.2 ± 7.3, 54.2 ± 4.5, 56.1 ± 6.7, and 58.1 ± 5.1 μm, respectively. In the off-flap group, the corneal epithelial thickness before surgery and at 1, 3, and 6 months after surgery was 56.5 ± 5.5, 26.7 ± 45.8, 53.8 ± 7.2, and 56.9 ± 5.4 μm, respectively. A significant difference in corneal epithelial thickness between the groups was found only at 1 month after surgery ($P < 0.01$).

Stromal Reaction Observed by In Vivo Confocal Microscopy

The stromal reactions of the two groups based on three parameters are shown in Table 5. No significant difference was found between the groups for all parameters at 1, 3, and 6 months after surgery.

DISCUSSION

Normal corneal epithelial wound repair after epithelial debridement involves three distinct phases.[21] In the first phase, hemidesmosomal attachments disappear in preparation for epithelial cell migration.[22,23] Lamellipodia and filopodia are formed later, and epithelial cells begin to migrate covering the denuded wound surface.[21,24,25] In the second phase, proliferation of cells distal to the original wound occurs along with centripetal migration and differentiation.[26,27] In the last phase, hemidesmosomes re-form, and the extracellular matrix reassembles.[21,26] Theoretically, this wound repair process occurs after off-flap epi-LASIK. A different mode of healing occurs after on-flap epi-LASIK, because a denuded surface is lacking, and the replaced epithelial flap, whether dead or alive, stays in front of the leading edge.[13–15] In off-flap epi-LASIK, the epithelial flap is removed, leaving a denuded corneal surface similar to that after PRK, except with a supposedly smoother surface and a more regular wound border created by the epikeratome.[1,6,14,17,28–30] Slit lamp observation of wound healing in our study revealed that off-flap epi-LASIK eyes healed approximately 1.3 days faster than on-flap eyes. Similarly, others have reported faster re-epithelialization after off-flap epi-LASIK than after on-flap.[17,18] Torres et al.[31] reported that eyes treated with epi-LASIK require more time for epithelial healing (4.75 ± 1.44 days) than do PRK-treated eyes (3.95 ± 1.39 days). Together, these studies indicate that replacement of the epithelial flap during epi-LASIK may adversely affect the epithelial wound-healing process.

To our knowledge, we are the first to describe in vivo confocal microscopy findings of epithelial wound healing in off-flap epi-LASIK eyes. Analysis of basal epithelial cell morphology showed a slower recovery to normal morphology in the on-flap epi-LASIK eyes than in the off-flap eyes. Initially, the

TABLE 4. Percentage of Different Patterns of Apical Surface Cells Classified by In Vivo Confocal Microscopy

Time after Surgery	Normal Squamous	Poorly Defined Cells	Nonsquamous with Large N/C	Total Eyes (*n*)	Normal Squamous	Poorly Defined Cells	Nonsquamous with Large N/C	Total Eyes (*n*)
On-flap*[19] *with MMC Treatment					***Off-flap with MMC Treatment***			
1 Week	0 (0)	79.2 (19)	20.8 (5)	24	7.7 (2)	0 (0)	92.3 (24)	26
2 Weeks	12.5 (3)	33.3 (8)	54.2 (13)	24	34.6 (9)	0 (0)	65.4 (17)	26
1 Month	37.5 (9)	0 (0)	62.5 (15)	24	84.6 (22)	0 (0)	15.4 (4)	26
3 Months	50.0 (11)	0 (0)	50.0 (11)	22	100 (26)	0 (0)	0 (0)	26
6 Months	52.4 (11)	0 (0)	47.6 (10)	21	100 (26)	0 (0)	0 (0)	26
On-flap*[19] *without MMC Treatment					***Off-flap without MMC Treatment***			
1 Week	0 (0)	81.8 (18)	18.2 (4)	22	14.3 (3)	0 (0)	85.7 (18)	21
2 Weeks	13.6 (3)	50.0 (11)	36.4 (8)	22	52.4 (11)	0 (0)	47.6 (10)	21
1 Month	54.5 (12)	0 (0)	45.5 (10)	22	90.5 (19)	0 (0)	9.5 (2)	21
3 Months	50.0 (10)	0 (0)	50 (10)	20	100 (21)	0 (0)	0 (0)	21
6 Months	57.9 (11)	0 (0)	42.1 (8)	19	100 (21)	0 (0)	0 (0)	21

Data are percentage (number) of eyes. Columns heads from left to right: (1) normal squamous: large, flat epithelium with small nucleus/cytosol (N/C) ratio; (2) poorly defined cells: elongated apical surface cells, exfoliating or necrotic cells, and amorphous cells; (3) nonsquamous with large N/C: cells with large N/C ratio but without normal large, squamous appearance.

TABLE 5. Stromal Reaction Represented by Intensity of Light (*z*-scan profile)

Time after Surgery	On-flap[19]	Off-flap	*P* (Student's *t*-test)
Peak of stromal light intensity			
1 Month	65.6 ± 22.9	68.4 ± 24.3	NS
3 Month	61.0 ± 24.2	62.1 ± 14.6	NS
6 Month	56.25 ± 21.6	58.7 ± 16.1	NS
Average light intensity of the anterior 50-μm depth of the stroma			
1 Month	59.8 ± 11.4	61.0 ± 18.2	NS
3 Month	56.6 ± 14.7	59.4 ± 15.1	NS
6 Month	52.3 ± 7.1	54.7 ± 5.6	NS
Average light intensity of the whole stroma			
1 Month	48.3 ± 12.5	49.4 ± 17.3	NS
3 Month	47.6 ± 19.2	47.5 ± 14.1	NS
6 Month	41.5 ± 12.1	41.7 ± 16.9	NS

regenerated basal epithelial cells exhibited prominent large nuclei without cellular borders. As the cells recovered, the cellular borders appeared and the N/C ratio decreased. These cells with nuclei and cellular borders may represent reconstruction of the cellular junction, which implies a return to a more normal stable condition. At 1 week after surgery, the percentage of cells with cellular borders and nuclei was lower in the on-flap group than in the off-flap group, indicating a slower return to relatively stable morphology. By the second postoperative week, in the MMC-treated eyes, approximately 73% of the off-flap epi-LASIK eyes exhibited normal basal cell morphology, whereas only 38% of the on-flap eyes showed this well-differentiated pattern. A higher percentage of eyes showing normal basal cell morphology was also seen in the off-flap, MMC-untreated eyes compared with the on-flap, MMC-untreated eyes. As for apical surface cells, no eye in the on-flap group had achieved normal squamous morphology at 1 week after surgery, with or without MMC treatment, even though an intact epithelial flap was present. Comparatively, approximately 10% of the eyes in the off-flap group (MMC-treated and untreated combined) exhibited this normal apical cell pattern. More eyes with normal apical surface cell morphology were also seen in the off-flap group than in the on-flap group from 2 weeks to 6 months after surgery. Regression analysis also revealed a faster recovery to normal preoperative basal and apical cell morphology at 1 month after surgery in the off-flap eyes. From our results, we may conclude that even though the on-flap cases had an intact, repositioned epithelial flap after surgery, the cells did not have a normal morphology, and the repositioned flap may have retarded the recovery to normal basal and apical surface epithelial morphology.

In the in vivo confocal microscopic examinations, the on-flap group had normal epithelial thickness at 1 month after surgery, whereas the off-flap group had a significantly thinner epithelium. Eyes that underwent off-flap epi-LASIK regained normal epithelial thickness by approximately 3 months after surgery. Because our previous study demonstrated that most epithelial cells of the flap are dead in on-flap LASIK,[19] it was interesting to explore the reasons why these dead cells in on-flap LASIK contribute to the epithelial thickness during the early operative period. Wang et al.[17] reported a more regular and smooth corneal surface in an off-flap group during wound healing, whereas a central epithelial raphe was observed in eyes that underwent on-flap epi-LASIK. They attributed the finding in on-flap epi-LASIK eyes to epithelial remodeling, rather than epithelium regrowth as occurs in off-flap cases.[17,32,33] This hypothesis may partially explain our findings. In off-flap epi-LASIK, where a denuded surface is created after the flap is removed, certain aspects of the wound-healing process may be comparable to those of PRK.[32,33] In vivo confocal microscopy studies of epithelial morphology during corneal wound healing have not been reported for PRK. However, Erie[34] recorded the changes in epithelial thickness by in vivo confocal microscopy after PRK. He found that after PRK, the central epithelial thickness returned to preoperative thickness by 1 month after surgery, continued to thicken by 21% during the first year, and then remained stable for the next 3 years. In comparison, we found a thinner epithelial flap in the off-flap cases at 1 month after surgery. Epithelial thickness in our study returned to the preoperative condition and stabilized at 3 and 6 months after surgery instead of continuing to thicken, as described by Erie. Since significant and prolonged keratocyte activation after PRK has been shown to influence corneal epithelial thickness,[34–37] the rapid stabilization of epithelial thickness in off-flap epi-LASIK in our study may be due to the relatively smoother corneal surface that results from this procedure compared with PRK and the resultant lower level of keratocyte activation.

The effect of MMC on modifying wound-healing processes after refractive surgery has been widely reported.[38–40] The application of MMC may help to decrease the keratocyte activation related to haze formation after corneal surface laser procedures such as PRK and epi-LASIK.[7,9,11,12,40,41] Our previously reported study on in vivo confocal microscopic evaluation of corneal wound healing for on-flap epi-LASIK eyes showed that MMC usage may cause more damage to the epithelial flaps.[19] This possibility was also noted in the present study, as our regression analysis of the in vivo confocal microscopy data indicated a delayed recovery to preoperative basal and apical epithelial cell morphology in MMC-treated eyes, although the difference did not reach statistical significance. Although epithelial morphologic recovery may be affected by MMC application, haze formation as analyzed by the stromal reaction after surgery was not significantly different between the off- and on-flap epi-LASIK groups at any of the time points analyzed. In both groups, the stromal reaction was highest during the first postoperative month and gradually decreased over the next 6 months. To date, only one other study has compared the clinical outcomes of on- and off-flap epi-LASIK eyes, with and without MMC application.[42] Interestingly, Kim et al.[42] found that both postoperative spherical equivalent and corneal haze at 1 year after surgery were not affected by how the flap was managed or whether MMC was applied. For low to moderate myopia, Kalyvianaki et al.[16] and Sharma et al.[18] both reported that on- and off-flap epi-LASIK had comparable postoperative haze scores and visual acuity.[16,18] However, for moderate to high myopia, Wang et al.[17] reported a lower level of haze, as determined by slit lamp biomicroscopy, at 3 months after surgery for off-flap epi-LASIK eyes. The off-flap group was also found to have a faster visual recovery and better visual quality, as determined by lower wavefront aberrations, compared with those in the on-flap group. In our study, we did not

find a lower level of stromal reaction in the off-flap group, as in the report by Wang et al.[17]

There are few points of our study that should be further clarified. First, we attempted to elucidate the possible role of MMC in wound healing by stratifying the on- and off-flap groups into those with and without MMC treatment. We also adjusted our statistical analysis to better compare the two groups with the same background. However, observer-masked, randomized studies with one eye undergoing the on-flap procedure and the contralateral eye undergoing the off-flap procedure with the same type of MMC application may provide more comprehensive results. Second, two kinds of epikeratomes were used in this study. The Centurion Epi-Edge epikeratome was used in all eyes in the on-flap group, whereas some eyes in the off-flap group had flaps made with the Amadeus epikeratome. Both epikeratomes successfully created epithelial flaps on more than 80% of the eyes in this study. However, Choi et al.[43] reported that different cleavage planes of corneal epithelium may be created by different epikeratomes. It is still possible that the results reported here were partially influenced by the use of different epikeratomes. Further studies using the same epikeratome to compare the wound-healing process of on- and off-flap epi-LASIK may give more definite results.

In conclusion, on- and off-flap epi-LASIK in this study demonstrated comparable postoperative clinical outcomes, as shown by the BSCVA, the refractive outcome, and the postoperative stromal reactions. The off-flap epi-LASIK–treated eyes had a faster re-epithelialization time than did the on-flap eyes. Both apical surface cells and basal epithelial cells returned to normal preoperative morphology, as determined by in vivo confocal microscopy, faster in the off-flap epi-LASIK eyes. MMC usage generally retarded epithelial cell recovery to normal morphology in both the on- and off-flap eyes.

Off-flap epi-LASIK offers safety comparable to that of the on-flap technique, specifically in regard to corneal surface wound healing. Although further clarification of our conclusions using immunohistochemical staining, apoptosis assays, or electron microscopy are necessary to understand the fundamental differences in corneal wound healing between on- and off-flap epi-LASIK eyes, this article offers preliminary insight, through in vivo confocal microscopy imaging, into the differences in wound-healing processes involved.

References

1. Pallikaris IG, Katsanevaki VJ, Kalyvianaki MI, Naoumidi II. Advances in subepithelial excimer refractive surgery techniques: Epi-LASIK. *Curr Opin Ophthalmol.* 2003;14:207–212.
2. Pallikaris IG, Kalyvianaki MI, Katsanevaki VJ, Ginis HS. Epi-LASIK: preliminary clinical results of an alternative surface ablation procedure. *J Cataract Refract Surg.* 2005;31:879–885.
3. Dai J, Chu R, Zhou X, Chen C, Qu X, Wang X. One-year outcomes of epi-LASIK for myopia. *J Refract Surg.* 2006;22:589–595.
4. Gan D, Zhou X, Dai J, et al. Outcomes of epi-LASIK for the correction of high myopia and myopic astigmatism after more than 1 year. *Ophthalmologica.* 2009;223:102–110.
5. Hondur A, Bilgihan K, Hasanreisoglu B. A prospective bilateral comparison of epi-LASIK and LASEK for myopia. *J Refract Surg.* 2008;24:928–934.
6. Katsanevaki VJ, Kalyvianaki MI, Kavroulaki DS, Pallikaris IG. One-year clinical results after epi-LASIK for myopia. *Ophthalmology.* 2007;114:1111–1117.
7. Gamaly TO, El Danasoury A, El Maghraby A. A prospective, randomized, contralateral eye comparison of epithelial laser in situ keratomileusis and photorefractive keratectomy in eyes prone to haze. *J Refract Surg.* 2007;23:S1015–1020.
8. Matsumoto JC, Chu YS. Epi-LASIK update: overview of techniques and patient management. *Int Ophthalmol Clin.* 2006;46:105–115.
9. Caubet E. Course of subepithelial corneal haze over 18 months after photorefractive keratectomy for myopia [corrected]. *Refract Corneal Surg.* 1993;9:S65–70.
10. Carr JD, Patel R, Hersh PS. Management of late corneal haze following photorefractive keratectomy. *J Refract Surg.* 1995;11:S309–313.
11. Corbett MC, Prydal JI, Verma S, Oliver KM, Pande M, Marshall J. An in vivo investigation of the structures responsible for corneal haze after photorefractive keratectomy and their effect on visual function. *Ophthalmology.* 1996;103:1366–1380.
12. Ramirez-Florez S, Maurice DM. Inflammatory cells, refractive regression, and haze after excimer laser PRK. *J Refract Surg.* 1996;12:370–381.
13. Katsanevaki VJ, Naoumidi II, Kalyvianaki MI, Pallikaris G. Epi-LASIK: histological findings of separated epithelial sheets 24 hours after treatment. *J Refract Surg.* 2006;22:151–154.
14. Pallikaris IG, Naoumidi II, Kalyvianaki MI, Katsanevaki VJ. Epi-LASIK: comparative histological evaluation of mechanical and alcohol-assisted epithelial separation. *J Cataract Refract Surg.* 2003;29:1496–1501.
15. Tanioka H, Hieda O, Kawasaki S, Nakai Y, Kinoshita S. Assessment of epithelial integrity and cell viability in epithelial flaps prepared with the epi-LASIK procedure. *J Cataract Refract Surg.* 2007;33:1195–1200.
16. Kalyvianaki MI, Kymionis GD, Kounis GA, et al. Comparison of Epi-LASIK and off-flap Epi-LASIK for the treatment of low and moderate myopia. *Ophthalmology.* 2008;115:2174–2180.
17. Wang QM, Fu AC, Yu Y, et al. Clinical investigation of off-flap epi-LASIK for moderate to high myopia. *Invest Ophthalmol Vis Sci.* 2008;49:2390–2394.
18. Sharma N, Kaushal S, Jhanji V, Titiyal JS, Vajpayee RB. Comparative evaluation of 'flap on' and 'flap off' techniques of Epi-LASIK in low-to-moderate myopia. *Eye (Lond).* 2009;23:1786–1789.
19. Chen WL, Chang HW, Hu FR. In vivo confocal microscopic evaluation of corneal wound healing after epi-LASIK. *Invest Ophthalmol Vis Sci.* 2008;49:2416–2423.
20. Chen WL, Lin CT, Ko PS, et al. In vivo confocal microscopic findings of corneal wound healing after corneal epithelial debridement in diabetic vitrectomy. *Ophthalmology.* 2009;116:1038–1047.
21. Zieske JD, Gipson IK. Agents that affect corneal wound healing: modulation of structure and function. In: Albert DM, Jakobiec FA, eds. *Principles and Practice of Ophthalmology.* Philadelphia: WB Saunders; 2000;364–372.
22. Crosson CE, Klyce SD, Beuerman RW. Epithelial wound closure in the rabbit cornea: a biphasic process. *Invest Ophthalmol Vis Sci.* 1986;27:464–473.
23. Okada Y, Saika S, Shirai K, et al. Disappearance of desmosomal components in rat corneal epithelium during wound healing. *Ophthalmologica.* 2001;215:61–65.
24. Gipson IK, Kiorpes TC. Epithelial sheet movement: protein and glycoprotein synthesis. *Dev Biol.* 1982;92:259–262.
25. Wilson SE, Liu JJ, Mohan RR. Stromal-epithelial interactions in the cornea. *Prog Retin Eye Res.* 1999;18:293–309.
26. Gipson IK, Spurr-Michaud SJ, Tisdale AS. Anchoring fibrils form a complex network in human and rabbit cornea. *Invest Ophthalmol Vis Sci.* 1987;28:212–220.
27. Cotsarelis G, Cheng SZ, Dong G, Sun TT, Lavker RM. Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. *Cell.* 1989;57:201–209.
28. Youm DJ, Tchah H, Choi CY. Comparison of early postoperative clinical outcomes of photorefractive keratectomy and lamellar epithelial debridement. *J Cataract Refract Surg.* 2009;35:703–709.
29. Kollias A, Ulbig MW, Spitzlberger GM, et al. Epi-LASIK using the Amadeus II microkeratome: evaluation of cut quality using light and electron microscopy. *J Cataract Refract Surg.* 2007;33:2118–2121.
30. Soma T, Nishida K, Yamato M, et al. Histological evaluation of mechanical epithelial separation in epithelial laser in situ keratomileusis. *J Cataract Refract Surg.* 2009;35:1251–1259.
31. Torres LF, Sancho C, Tan B, Padilla K, Schanzlin DJ, Chayet AS. Early postoperative pain following Epi-LASIK and photorefractive

keratectomy: a prospective, comparative, bilateral study. *J Refract Surg.* 2007;23:126–132.

32. Fantes FE, Hanna KD, Waring GO 3rd, Pouliquen Y, Thompson KP, Savoldelli M. Wound healing after excimer laser keratomileusis (photorefractive keratectomy) in monkeys. *Arch Ophthalmol.* 1990;108:665–675.
33. Hanna KD, Pouliquen YM, Savoldelli M, et al. Corneal wound healing in monkeys 18 months after excimer laser photorefractive keratectomy. *Refract Corneal Surg.* 1990;6:340–345.
34. Erie JC. Corneal wound healing after photorefractive keratectomy: a 3-year confocal microscopy study. *Trans Am Ophthalmol Soc.* 2003;101:293–333.
35. Wilson SE, Mohan RR, Ambrosio R Jr, Hong J, Lee J. The corneal wound healing response: cytokine-mediated interaction of the epithelium, stroma, and inflammatory cells. *Prog Retin Eye Res.* 2001;20:625–637.
36. Liu JC, McDonald MB, Varnell R, Andrade HA. Myopic excimer laser photorefractive keratectomy: an analysis of clinical correlations. *Refract Corneal Surg.* 1990;6:321–328.
37. Gauthier CA, Holden BA, Epstein D, Tengroth B, Fagerholm P, Hamberg-Nystrom H. Factors affecting epithelial hyperplasia after photorefractive keratectomy. *J Cataract Refract Surg.* 1997;23:1042–1050.
38. 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;112:208–218;discussion 219.
39. Talamo JH, Gollamudi S, Green WR, De La Cruz Z, Filatov V, Stark WJ. Modulation of corneal wound healing after excimer laser keratomileusis using topical mitomycin C and steroids. *Arch Ophthalmol.* 1991;109:1141–1146.
40. Kim TI, Pak JH, Lee SY, Tchah H. Mitomycin C-induced reduction of keratocytes and fibroblasts after photorefractive keratectomy. *Invest Ophthalmol Vis Sci.* 2004;45:2978–2984.
41. O'Doherty M, Kirwan C, O'Keeffe M, O'Doherty J. Postoperative pain following epi-LASIK, LASEK, and PRK for myopia. *J Refract Surg.* 2007;23:133–138.
42. Kim ST, Koh JW, Yoon GJ, Yang SW. Clinical outcomes of epi-LASIK: 1-year results of on- and off-flap procedures with and without mitomycin-C. *Br J Ophthalmol*;94:592–596.
43. Choi SK, Kim JH, Lee D, et al. Different epithelial cleavage planes produced by various epikeratomes in epithelial laser in situ keratomileusis. *J Cataract Refract Surg.* 2008;34:2079–2084.