Corneal Healing after Uncomplicated LASIK and Its Relationship to Refractive Changes: A Six-Month Prospective Confocal Study

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Purpose. To investigate corneal healing and the factor(s) possibly responsible for refractive changes after laser in situ keratomileusis (LASIK).

Methods. Twenty eyes of 10 patients who underwent LASIK for myopia were examined clinically and by real-time confocal microscopy for 6 months. Epithelial and posterior stromal thicknesses and the thickness of the keratocyte activation zone were measured, and refractive changes were compared with these values. Keratocyte morphology, flap thickness, and subbasal nerve fiber bundle morphology after LASIK were also investigated.

Results. No significant change was detected over time in epithelial thickness after LASIK treatment; however, the posterior stromal thickness was found to be significantly higher 1 month after surgery. A slight but statistically significant negative correlation was detected between the thickness of the keratocyte activation zone and the spherocylindrical refraction after LASIK. The subbasal nerve fiber bundle’s morphology returned to its preoperative appearance 6 months after LASIK, but in the flap stroma the nerve fiber bundle morphology remained abnormal at 6 months after LASIK surgery.

Conclusions. A weak but significant negative correlation between the thickness of the keratocyte activation zone and spherocylindrical refraction was found after LASIK. The different refractive properties of activated keratocytes may be responsible for the myopic shift after LASIK. Further studies are needed to clarify this hypothesis. (Invest Ophthalmol Vis Sci. 2004;45:1334–1339) DOI:10.1167/iovs.03-1025

Laser in situ keratomileusis (LASIK) is a relatively new technique for correction of myopia. A hinged flap (consisting of epithelium, Bowman’s layer, and anterior stroma) is created first, and the exposed stroma is photoablated after the flap is folded back. Although many studies have been published on the clinical outcome after LASIK,1–4 relatively few reports address the biological changes associated with the procedure.5–9 The advent of in vivo confocal microscopy has furnished us with the means of improving imaging of wound healing in the living cornea.8,10 The most dramatic morphologic difference between the pre- and postoperative confocal microscopy examination has been reported to occur in kerocytes that settle immediately behind the flap interface. The oval and brightly reflecting kerocyte nuclei appear larger than preoperative nuclei, and processes can easily be visualized, suggesting that the cells are activated.10,11 Keratocyte activation was strongest at 1 to 2 weeks and persisted until 3 months after LASIK surgery.11,12 Similarly, activated kerocytes have been reported after photorefractive keratotomy (PRK).10,13 Neither LASIK nor PRK has been shown superior in efficacy outcomes14,15; however, LASIK has some advantages, such as minimal postoperative pain, faster clinical and functional recovery, less regression of refractive status, and less haze formation.14–16 A recent confocal microscopic study revealed that keratocyte-mediated regrowth of the photoablated stroma was a key biological factor responsible for post-PRK refractive instability in humans treated with PRK.17 It is logical to think that keratocyte activation can be a determining factor for the refractive changes after LASIK treatment.

The purpose of this study was to investigate the factor(s) responsible for the refractive changes after LASIK. For this purpose, epithelial thickness, posterior stromal thickness, and the thickness of the keratocyte activation zone were measured by confocal microscopy, and we sought to establish a correlation between refractive changes and these measurements. We also investigated keratocyte morphology, flap thickness, and subbasal nerve fiber bundle morphology after LASIK.

Methods

Design

This prospective, interventional cohort study was begun after approval was obtained from the LSU Health Sciences Center institutional review board. Each patient gave written informed consent, and the research followed the tenets of the Declaration of Helsinki.

Patients

Twenty eyes of 10 patients who underwent LASIK for myopia were included in the study. All eyes had normal anterior ocular segments, intraocular pressure (<20 mm Hg), and fundi. Contact lens wear was discontinued 2 weeks (soft lenses) or 3 weeks (hard lenses) before the LASIK operation. There were six women and four men (mean age, 35.4 ± 8.7 years). All patients were 21years of age or older and had stable refractive errors at least 1 year before the laser procedure. Patients who had undergone reoperation, those with diabetes mellitus or glaucoma, or those using any topical ophthalmic medication were excluded. Patients with corneas thinner than 500 μm centrally and/or with a severe systemic disorder that could cause them to miss examinations were also excluded.

The average preoperative spherocylindrical refraction was −5.87 ± 3.45 D (range, −1.75–11.00 D) and the planned ablation depth was 59.8 ± 27.1 μm (range, 16–110 μm). Each patient was examined in the pre- and postoperative period. Preoperative examina-
tions were performed 1 to 3 days before surgery. Postoperative examinations were performed 1 day, 3 days, 1 week, 1 month, 3 months, and 6 months after surgery. Each examination included latent and manifest refraction measurement, uncorrected and corrected near and distance visual acuity measurement, slit lamp microscopy, and videokeratography. Confocal microscopic examinations were performed at the preoperative period and 1 week, 1 month, 3 months, and 6 months after LASIK.

PRK and LASIK Procedures
All LASIK procedures were performed in eyes under topical anesthesia, using an excimer laser (20/20; VISX, Santa Clara, CA). A corneal flap was produced with an automated corneal shaper (ACS) microkeratome (ALK-E; Chiron Vision, Irvine, CA). The flap diameter was 8.5 mm and the intended thickness was 160 μm. Suction was monitored during the procedure with a Barraquer tonometer. Patients fixated on a target during the ablation. The stromal bed was irrigated with room temperature balanced salt solution before and after flap replacement to eliminate residual debris. The flap was allowed to dry in place for at least 3 minutes to facilitate adhesion at the end of the operation. After the LASIK procedure, the eyes were not occluded. Antibiotic (tobramycin 0.3%; Tobrex; Alcon, Fort Worth, TX) and corticosteroid (fluorometholone 0.1%; FML; Allergan Inc., Irvine, CA) were prescribed to all patients, four times a day for the first 5 days.

Confocal Microscopy
The eyes were examined with a tandem scanning confocal microscope (Advanced Scanning, New Orleans, LA) with a 20× water-immersion objective. Methylcellulose (Gonisol; CIBA Vision Ophthalmics, Atlanta, GA) was used as an optical coupler between the cornea and the tip of the water-immersion objective. The microscope objective lens was disinfected with 70% isopropyl alcohol wipes before and after the examination. Images were displayed in real time on a monitor (Sony Medical Monitor; Sony, San Diego, CA) and recorded through a CCD camera (Kappa Optoelectronics, Gleichen, Germany) onto digital videotape for later playback and analysis. The video images of interest were printed in color (Epson Stylus Color 800; Seiko Epson, Nagano, Japan) without any image enhancement. Video sequences were reviewed at least twice and evaluated in a masked fashion.

From each scan, the flap thickness, defined as the distance between the surface epithelium, and the flap interface, characterized by accumulation of interface particles (Fig. 1), were measured. Epithelial thickness, defined as the distance between superficial epithelium and basal epithelial nerve plexus, was also measured, as were posterior stromal thickness, defined as the distance between endothelium and flap interface, and thickness of the keratocyte activation zone, defined as the stromal thickness that contained keratocytes with bright nuclei and visible processes (Fig. 2).

Statistical Analysis
Statistical analyses were performed on computer (SPSS for Windows, ver. 10; SPSS Sciences, Chicago, IL). Normality was tested by the Shapiro Wilk test. Flap thickness, epithelial thickness, posterior stromal thickness, and the thickness of the keratocyte activation zone, and the change in spherocylindrical refraction at different examination points were compared with each other by using a one-way ANOVA test. Tukey’s post hoc test was used to detect statistically significant differences between values. The correlation between postoperative spherocylindrical refraction and the epithelial thickness, posterior stromal thickness, and the thickness of the keratocyte activation zone was analyzed by partial correlation. Data are expressed as the mean ± SD, and the differences are considered statistically significant when P < 0.05.

Nerve Fiber Bundles
Nerves appeared as long, narrow structures and those longer than 50 μm were counted. The nerve fiber bundles located in the subbasal region (Fig. 3), in the stromal flap (distance from the most anterior keratocyte to the flap interface), and in the posterior stroma (Fig. 4) were evaluated. A cornea was considered positive when at least one
nerve fiber bundle was noted within any of the areas under study. The difference between the preoperative and the postoperative periods was analyzed with a $\chi^2$ test. The differences were considered statistically significant when $P < 0.05$.

RESULTS

One patient did not return for the 3- or 6-month post-LASIK examinations, and two patients did not return for the 6-month examination. Thus, for the preoperative period, 1 week and 1 month after LASIK, we analyzed data from 20 corneas of 10 patients, but for the 3-month examination we analyzed data from 18 corneas of 9 patients, and for the 6-month examination we analyzed 16 corneas of 8 patients. All data were distributed normally.

The morphology of the first keratocytes observed behind the flap interface was different from the morphology before surgery. The oval and brightly reflecting keratocyte nuclei and the cell processes could be visualized easily, suggesting that the cells were activated. We did not detect any activated keratocytes anterior to the keratome cut (Fig. 5). Nineteen (95%) of the 20 corneas showed activated keratocytes at 1 week as did 10 (50%) of 20 corneas at 1 month. We were able to detect activated keratocytes 3 months after LASIK surgery in 2 (10%) of 20 corneas. We found that the thickness of the keratocyte activation zone at 1 week was $21.54 \pm 3.00$ μm; at 1 month, $8.75 \pm 1.61$ μm; and at 3 months, $0.65 \pm 1.38$ μm. We did not detect any activated keratocytes at 6 months (Fig. 6).

The mean epithelial thickness was found to be $44.77 \pm 6.13$ μm during the preoperative period. At 1 week after LASIK treatment, the mean epithelial thickness was $45.81 \pm 7.01$ μm; at 1 month, $43.06 \pm 6.37$ μm; at 3 months, $46.25 \pm 7.26$ μm; and at 6 months, $44.00 \pm 3.85$ μm. We did not detect any significant change in epithelial thickness any time point after LASIK treatment (Fig. 7).

The posterior stromal thickness measured $337.75 \pm 18.57$ μm 1 week after LASIK treatment. One month after surgery, it...
Similarly, no significance was observed. At 3 months after LASIK, the posterior stromal thickness and the spheroequivalent refraction after LASIK (*Statistically significant difference from the 1-week postoperative thickness (P = 0.01). Error bars indicate SD. was significantly higher (370.42 ± 29.22 μm, P = 0.01). At the subsequent examinations, no further significant increase was observed. At 3 months after LASIK, the posterior stromal thickness was 365.93 ± 29.69 μm and at 6 months, 381.11 ± 30.28 μm. Both the 3- and 6-month measurements were significantly higher than the 1-week postoperative value (P = 0.026 and P = 0.01, respectively, Fig. 8).

The mean spheroequivalent refraction was found to be −0.34 ± 0.18 D one week after treatment. One month after LASIK treatment, the mean spheroequivalent refraction changed to the myopic side considerably (−0.57 ± 0.22 D), but the difference did not reach statistical significance (P = 0.06). Three months after treatment, the mean spheroequivalent refraction was −0.56 ± 0.18 D, and at 6 months, −0.56 ± 0.24 D (Fig. 9).

The mean flap thickness was 145.40 ± 9.55 μm 1 week after LASIK. The flap thickness did not change significantly between examination points.

We did not detect any significant correlation between the spheroequivalent refraction and epithelial thickness, when measured at different examination points (r = 0.068, P = 0.650). Similarly, no significant correlation was found between the posterior stromal thickness and the spheroequivalent refraction (r = −0.099, P = 0.54); however, a slight but statistically significant negative correlation was detected between the thickness of the keratocyte activation zone and spheroequivalent refraction after LASIK (r = −0.278, P = 0.049).

During the preoperative examination, all corneas had a good subbasal nerve plexus. However, 1 week after LASIK, we detected 1 (5%) of 20 corneas with subbasal nerve fiber bundles longer than 50 μm. One month after LASIK, one cornea had subbasal nerve fiber bundles longer than 50 μm. Three months after, 9 (50%) of 18 corneas showed subbasal nerve fiber bundles. Six months after the surgery, all corneas had subbasal nerve fiber bundles. The χ² test revealed significant differences between the percentage of preoperative corneas with subbasal nerve fiber bundles, and the same percentages after LASIK treatment at all examination times except 6 months (all P < 0.01).

Before surgery, we detected that 16 (80%) of 20 corneas contained nerve fiber bundles in the anterior stroma, which would correspond with the flap stroma in the post-LASIK period. One week after treatment, only 5 (25%) corneas had nerve fiber bundles in the flap stroma. The difference was statistically significant (P = 0.0005, χ² test). When confocal microscopic examination was performed 1 month after LASIK, no corneas had nerve fiber bundles in the flap stroma. The difference between the preoperative percentage and percentage at 1 month was statistically significant (P = 0.00000002, χ² test). Three months after LASIK, only 1 cornea showed nerve fiber bundles in the flap stroma (0.4%), which is significantly different from the preoperative finding (P = 0.003, χ² test). We detected nerve fiber bundles in the posterior stroma in 8 (50%) of 16 corneas 6 months after LASIK surgery. The percentage of corneas with nerve fiber bundles in the posterior stroma did not change significantly at any examination point.

**DISCUSSION**

The keratocyte morphology behind the flap interface at 1 week after LASIK operation was different from the preoperative morphology. We saw many cells with thick and visible processes, and oval, brightly reflective keratocyte nuclei. These cells were assumed to be activated keratocytes. Such activated keratocytes have been associated with the healing process in primates after PRK. The incidence of activated keratocytes diminished after 1 week, but we still detected activated keratocytes in 10 of 22 corneas 1 month after LASIK surgery. This finding is consistent with the findings of Vesaluoma et al.

Moller-Pedersen et al. demonstrated that activated keratocyte-mediated rethickening of the photoablated stroma is a key biological factor responsible for post-PRK regression of myopia. They demonstrated that the corneal rethickening causes myopic regression mediated almost solely by stromal rethickening; only a minor contribution appeared to originate from restoration of the postoperative epithelial thickness. In the present study, we found a significant thickening in the posterior stroma between 1 week and 1 month after surgery. Meanwhile, the spheroequivalent refraction changed considerably to the myopic side between these time points (−0.34 vs. −0.56 D).
It is logical to think that the posterior stromal rethickening seen 1 month after LASIK was related to the activated keratocytes, since the highest value for the thickness of the activated keratocyte zone was found at the 1-week postoperative examination point, and it is well known that activated keratocytes are associated with the healing process after excimer laser treatment.\(^\text{2,21}\) Normally it would be expected that a 10- to 15-\(\mu\)m rethickening of the posterior stroma produced a 1-D myopic shift, but the much greater rethickening observed in the present study created only a small amount of refractive change. This finding may suggest that the cornea simply swells after LASIK treatment, and anterior curvature does not change despite a high degree of thickening. However, the proposed mechanism is just a speculation at this time, because we did not have any pachymetry data or corneal curvature measurement to support the hypothesis.

Mitooka et al.\(^\text{2,2}\) reported that, although keratocyte density decreases in the anterior half of the retina, keratocyte density in the posterior stroma was not complete up to 6 months after LASIK and decreased abruptly thereafter. Although we did not detect a significant correlation between the thickness of the photoablated posterior stroma and the anterior corneal thickness, we found a weak but significant negative correlation between the thickness of the keratocyte activation zone and the refractive index.\(^\text{20}\) This finding may suggest that the cornea simply swells after LASIK treatment, and anterior curvature does not change after LASIK, as reported previously.\(^\text{21,22}\) We did not find any effect of LASIK on anterior stromal nerve fiber bundles, as expected.

In conclusion, we found a weak but still significant negative correlation with the thickness of the keratocyte activation zone and the refractive index. The different refractive properties of activated keratocytes may be responsible for the myopic shift after LASIK. Further studies with more subjects and concomitant corneal curvature analysis will help to explain the underlying mechanism of refractive error shifts that occur after LASIK surgery.

References


