Noncontact Measurements of Central Corneal Epithelial and Flap Thickness after Laser In Situ Keratomileusis

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PURPOSE. To investigate the changes in the epithelium and flap after laser in situ keratomileusis (LASIK), when measured with optical coherence tomography (OCT).

METHODS. Twenty-eight eyes of 14 patients (age: 39.9 ± 8.6 years) underwent LASIK. The central thickness of corneal epithelium and flap were measured with a real-time 1310 nm OCT 1 day, 1 week, and 1 month after surgery. A custom software program was used to process multiple images of each eye on each visit.

RESULTS. After surgery, the corneal epithelium changed significantly (ANOVA: F(3, 81) = 12.3, P < 0.000) with not statistically significant thinning at one day (mean ± SD: 57.8 ± 5.9 μm, P = 0.26, compared with baseline: 59.9 ± 5.9 μm) and statistically significant thickening at 1 week (60.8 ± 5.8 μm, P = 0.04, compared with 1 day) and 1 month (64.6 ± 6.1 μm, P = 0.008 compared with all others). There were statistically significant changes in the corneal flap thickness (ANOVA: F(3, 81) = 4.59, P = 0.01) with thickening in the intervals between 1 day (143.3 ± 20.6 μm) and 1 week (149.7 ± 24.6 μm, P = 0.12), and between 1 week and 1 month (152.7 ± 19.3 μm, P = 0.01). There was a strong correlation (r = 0.898) between the difference of corneal thickness before and after surgery and predicted laser ablation depth.

CONCLUSIONS. OCT is a useful noncontact tool for thickness measurements of the epithelium, flap, and total cornea. After LASIK, the epithelium and flap showed thickening during the study period. (Invest Ophtalmol Vis Sci. 2004;45:1812–1816) DOI:10.1167/iovs.03-1088

Lasers in situ keratomileusis (LASIK) has been considered to be a safe, effective, and precise way to treat ametropia and resulted from advancements in technology and instrumentation, such as customized laser ablation and aberrometric techniques. In an effort to minimize the complications associated with LASIK, predict outcomes more precisely, and understand the biomechanics of the cornea after refractive surgery, the measurement of discrete corneal layers such as the epithelium and planned flap thickness has become critical. Because dimensional measurements such as confocal microscopy and high-frequency ultrasound are both contact measurements, they are not normally applicable to patients shortly after LASIK.

Using low-coherence interferometry and image-processing techniques, optical coherence tomography (OCT) has been reported to measure epithelial and total corneal thickness centrally and topographically in a repeatable and precise manner (Fonn D, et al. IOVS 2000;41:ARVO Abstract 5591). With the newly developed real-time OCT, many image frames can be obtained in a very short time, and these quick measurements offer a realistic way to image the anterior segment of the eye. The purposes of this study were to investigate the changes in epithelial and flap thickness after LASIK, when measured with real-time OCT. We determined discrete layer changes at the 1-day, 1-week, and 1-month intervals and attempted to correlate these with other factors.

METHODS

Subjects

Twenty-eight eyes of 14 myopes (12 women and 2 men, mean age 39.9 ± 8.3 years) with no history of ocular or systemic disease were recruited in the Department of Ophthalmology at the University Hospitals of Cleveland for this study. All patients were candidates for LASIK to correct myopia. Informed consent was obtained from each subject after ethics approval. All subjects were treated in accordance with the tenets of the Declaration of Helsinki. All eyes enrolled in this study were between −1.00 and −14.00 D of myopia with no more than −5.00 D of refractive astigmatism.

Instruments

A real-time custom-made OCT system to measure the cornea was built at the Case Western Reserve University, as described previously.9 The system uses 1310-nm light, allowing real-time (eight frames per second) OCT imaging of the anterior segment. The image size of this OCT was set to be 960 (lateral) × 384 (longitudinal) pixels. The principles of OCT and the measurement of the cornea have been described previously (Fonn D, et al. IOVS 2000;41:ARVO Abstract 5591).10,11 The OCT probe was mounted on a slit-lamp to facilitate the measurement and a video camera was installed to monitor the eye. An internal fixation target to the imaging eye was provided to help position and stabilize the eye with respect to the imaging system. Under video guidance, the operator centered the OCT scan on the pupil, while ensuring that the OCT beam was perpendicular to the cornea apex by visualizing the specular reflex in the OCT image. The optical power incident on the cornea was 4.9 mW, which is well below the maximum exposure limit for 1310-nm light as documented by the American National Standards Institute (ANSI Z136.1-2000). The full-width half-maximum resolution of the system with the cornea was approximately 10 μm. Only the central corneal thickness was measured with the OCT in this study. Custom software was used to process multiple OCT images taken of each eye at each visit to obtain the thickness of the different corneal layers. The averaged reflectivity profile (Fig. 1B) was processed from the central 122 pixels (equal to the central 1.45 mm width as marked by the rectangle) of an entire scanned image (960 pixels/11.6-mm width and 584 pixels/3.3-mm depth) with the alignment of peak a (the air–cornea interface).
In this study, we defined total corneal thickness as the distance between the first (Fig. 1B, peak a) and last (peak d) peaks. Epithelial thickness was defined as the distance between the first (a) and second (b) peaks, as described in other studies.\textsuperscript{7,10,12} Flap thickness was defined as the distance between peaks a and c, which are located on the interface between the flap and bed, as described in Maldonado et al.\textsuperscript{10} The OCT-determined thickness is not absolute, because the exact refractive index of the examined tissues is unknown at the resolution afforded by the OCT. Hence, the exact thickness cannot be determined without precise knowledge of the refractive index.

**Procedure**

Patients scheduled to undergo bilateral treatment with LASIK for the correction of myopia and astigmatism were screened for eligibility. Eligible patients were examined before surgery to establish a baseline. A microkeratome (Hansatome; Bausch & Lomb, Rochester, NY) was used in each eye to create the corneal flap. Conventional ablation was performed with a laser (model S3, software version 4.51; Visx, Santa Clara, CA). The ablation zone was 6.5 mm with an 8.0-mm blend zone, using variable-sized moving spots. After surgery, patients returned at 1 day, 1 week, and 1 month for ophthalmic evaluations. At each visit, the central cornea was measured with the OCT. Multiple images were obtained from each eye at each visit. Custom software was used to process the raw images, and 12 sagittal scan points in the center of each image were analyzed, to yield precise measurements of each layer of the cornea.

**Data Analysis**

Data analysis was conducted on computer (Statistica; StatSoft Inc., Tulsa, OK). Analysis of variance (ANOVA) and F-tests were used to determine whether there were differences, and post hoc tests were used to determine whether there were pair-wise differences (\(P < 0.05\)). Pearson correlations were used to determine the association between the variables tested.

**Results**

After LASIK, myopia decreased significantly, and no myopic regression was found up to 1 month, as shown in Table 1. The reflectivity profile of the cornea after a LASIK procedure showed a distinct interface at a mean depth of approximately 150 \(\mu\)m, as shown in Figure 1B by the increased reflectivity (peak c) in the profile. The corneal epithelium changed significantly overall (ANOVA: \(F_{3, 81} = 12.3, P = 0.000\)). Although no statistically significant thinning of the epithelium appeared at 1 day after surgery (mean \(\pm\) SD: 57.8 \(\pm\) 5.9 \(\mu\)m, \(P = 0.26\), compared with baseline: 59.9 \(\pm\) 5.9 \(\mu\)m), there was statistically significant thickening at 1 week (60.8 \(\pm\) 5.8 \(\mu\)m, \(P = 0.04\), compared with 1 day) and 1 month (64.6 \(\pm\) 6.1 \(\mu\)m, \(P = 0.008\) compared with the others; Fig. 2).

The OCT-determined thickness is not absolute, because the exact refractive index of the examined tissues is unknown at the resolution afforded by the OCT. Hence, the exact thickness cannot be determined without precise knowledge of the refractive index.

**TABLE 1. Manifest Refraction before and after LASIK in 28 Eyes**

<table>
<thead>
<tr>
<th></th>
<th>Preop</th>
<th>1 d Postop</th>
<th>1 wk Postop</th>
<th>1 mo Postop</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sphere</td>
<td>-3.47</td>
<td>-0.29</td>
<td>-0.31</td>
<td>-0.32</td>
</tr>
<tr>
<td>SD</td>
<td>1.66</td>
<td>0.57</td>
<td>0.53</td>
<td>0.58</td>
</tr>
<tr>
<td>Cylinder</td>
<td>-0.72</td>
<td>-0.21</td>
<td>-0.21</td>
<td>-0.32</td>
</tr>
<tr>
<td>SD</td>
<td>0.82</td>
<td>0.29</td>
<td>0.29</td>
<td>0.32</td>
</tr>
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</table>

Data are expressed as the mean in diopters.
to 489.1 ± 38.6 μm (post hoc tests: \( P < 0.05 \); Fig. 5). The bed thinned significantly (post hoc test: \( P < 0.001 \)) by 1 week after surgery (326.9 ± 30.7 μm) compared with 1 day (340.8 ± 32.9 μm) and 1 month (336.4 ± 33.1 μm) after surgery. No significant differences were found between right and left eyes in epithelial, flap, bed, and total corneal thickness at each visit (ANOVA: \( P > 0.05 \)). No change of corneal thickness was found in three eyes, and an increase was detected in another three eyes 1 day after surgery compared with the baseline, as shown in Figure 6.

**FIGURE 3.** Corneal flap thickness changed significantly after the LASIK procedure (\( P = 0.01 \)), with a significant decrease at 1 month after surgery compared with 1 day after surgery (vertical bars denote 95% confidence interval).

**FIGURE 4.** There was a poor correlation between intended flap thickness and OCT-measured flap thickness 1 day after surgery, although the flap thickness of the 180-μm subgroup was thicker overall than the 160-μm subgroup (two tailed, unpaired \( t \)-test: \( P = 0.02 \)). Note the wide range of flap thicknesses in each subgroup.

**FIGURE 5.** Total corneal thickness was significantly different before and after the LASIK procedure (\( P = 0.000 \)), with a significant decrease from 509.9 ± 31.7 to 484.2 ± 37.7 μm (\( P < 0.001 \)). Total mean corneal thickness continued to decrease by 7.6 μm at 1 week after surgery, but increased at the 1-month visit to 489.1 ± 38.6 μm (\( P < 0.05 \); vertical bars denote 95% confidence interval). The thinning of total corneal thickness was due to the thinning of the bed as measured in this study.

**FIGURE 6.** There was a strong correlation between planned laser ablation and OCT-measured thickness differences at 1 day after surgery. Note the negative change or no-change results, which indicate corneal swelling.

**DISCUSSION**

Optical coherence tomography (OCT) is a technique that applies low-coherence interferometry and image processing to show the microstructures of optical tissue with noncontact and noninvasive features based on light backscatter from the tissue.\(^5\)\(^9\)\(^{13-17}\) Previous studies have demonstrated that it is feasible to measure the thickness of different layers of the cornea, such as the epithelium, corneal flap, and total corneal thickness with a precision within 1 to 5 μm.\(^6\)\(^8\)\(^{10}\) This is similar to the precision obtained with very high-frequency ultrasound biomicroscopy\(^{16,19}\) and confocal microscopy through focusing (CMTF).\(^20\) These three instruments have been used to study the anatomic changes brought about by refractive surgery. However, the latter two methods normally are not used in the very early postoperative period, especially in patients after LASIK procedures, because they require touching the cornea. In this study, we demonstrated that real-time OCT is a useful noncontact tool to evaluate epithelial and flap thickness after LASIK surgery.

Compared with a conventional OCT with its long acquisition time, the real-time OCT used in this study quickly acquired multiple images for data analysis. Briefly, the benefits using this system include real-time imaging and the reduction of misalignment and patient motion artifacts while imaging dynamic ocular events.\(^7\) For this study, custom software was developed to process the raw images and generate mean backscatter profiles (Fig. 1), from which dimensional results were directly ob-
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thickness decreased 1 day after surgery, but this was not statistically significant. After that, epithelial thickness was found to increase up to 1 month (Fig. 2). Epithelial hyperplasia has been reported to occur after LASIK\textsuperscript{59,60} and PRK.\textsuperscript{41,42} The mechanism of epithelial hyperplasia remains unknown. It may be a part of the wound-healing process or a response to the biomechanical changes of the cornea, such as tissue loss, redistribution of corneal tension,\textsuperscript{43} and innervation.\textsuperscript{44,45} Linna et al.\textsuperscript{44} found a major loss of innervation in the epithelial and superficial stromal layers, except for the area at the flap hinge, in rabbit corneas 3 days after LASIK. These findings were confirmed in human eyes and associated by Linna et al. with a loss of corneal sensitivity.\textsuperscript{45}

Spadea et al.\textsuperscript{7} reported that increased epithelial thickness played a role after LASIK in regression of myopia in patients with high myopia. However, we could not demonstrate a relationship between epithelial hyperplasia and regression of myopia, because there was not a statistically significant regression in our sample (Table 1). The reasons may be different study groups (low myopia in this study versus high myopia in Spadea et al.) and follow-up period (1 month in the present study vs. more than 12 months in Spadea et al.). It is also likely that epithelial hyperplasia has little impact in patients with low myopia after LASIK. The factor of bed thinning (Fig. 5) combined with epithelial hyperplasia should be considered among the range of biomechanical responses of the cornea and the measurement of total cornea thickness.

In summary, real-time OCT was demonstrated to be a valuable tool for noncontact measurements of epithelial and flap thicknesses after LASIK, although flap thickness could not be correlated with other ocular parameters investigated in our study. This method can be used to monitor the changes in specific corneal layers during wound healing after refractive surgery, aiding efforts to understand the biomechanics of LASIK and PRK.

Acknowledgments

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References

8. Muscat S, McKay N, Parks S, Kemp E, Keating D. Repeatability and reproducibility of corneal thickness measurements by optical co-

Corneal flap thickness was first measured with OCT by Maldonado et al.,\textsuperscript{10} who found it a useful tool to perform this task in a noncontact and noninvasive manner, with high precision. However, in Maldonado’s report, the authors did not mention how many longitudinal scans were analyzed by placing the cursors on the peaks and the epithelial layer was not differentiated after surgery. In the present study, multiple longitudinal scans (122 scans) were processed to yield the reflectivity profile and the epithelial thickness was obtained at all visits. Ultrasound subtraction pachymetry (subtracting the intraoperative corneal bed pachymetry measurement from the intraoperative total corneal pachymetry) was often used in previous studies investigating the corneal flap and its impacting factors.\textsuperscript{22–26} The major problem with this method is its indirect measurement, which provides an estimate of the bed thickness. The swelling of the corneal bed may cause inaccurate measurements of the corneal flap, which may account for the varying reports of factors that influence flap thickness.\textsuperscript{22–26} We found no strong relation between the flap thickness and other measured parameters, using the direct measurement provided by our OCT. Other aspects of the microkeratome and the eye may affect the flap thickness, however, and further studies may be needed.

Epithelial thickening was evident in this study when measured with OCT by Vivo to study myopes who undergo LASIK, Erie et al.\textsuperscript{2} found that epithelial thickness was increased by 22% 1 month after LASIK compared with its preoperative baseline and maintained the increased thickness up to 1 year after LASIK. Spadea et al.\textsuperscript{7} demonstrated that epithelial thickness increased within the first week after LASIK, with a maximum increase of approximately 6.5 μm by the third month. In this study, epithelial thickness was found to increase up to 1 month (Fig. 2). Epithelial hyperplasia has been reported to occur after LASIK\textsuperscript{59,60} and PRK.\textsuperscript{41,42} The mechanism of epithelial hyperplasia remains unknown. It may be a part of the wound-healing process or a response to the biomechanical changes of the cornea, such as tissue loss, redistribution of corneal tension,\textsuperscript{43} and innervation.\textsuperscript{44,45} Linna et al.\textsuperscript{44} found a major loss of innervation in the epithelial and superficial stromal layers, except for the area at the flap hinge, in rabbit corneas 3 days after LASIK. These findings were confirmed in human eyes and associated by Linna et al. with a loss of corneal sensitivity.\textsuperscript{45}

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