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Assessment of Keratocyte Activation Following LASIK With Flap Creation Using the IntraLase FS60 Laser

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Abstract

PURPOSE—To assess the response of corneal keratocytes to the IntraLase FS60 femtosecond laser using attenuated steroids.

METHODS—Thirty patients (30 eyes) who underwent LASIK with the IntraLase FS60 were assessed by clinical examination and confocal microscopy 3 months postoperatively. Postoperative steroid regimen was Econopred Plus (Alcon Laboratories Inc) every hour for 1 day and four times daily for 7 days.

RESULTS—No cornea had clinically significant flap interface haze. Two corneas had trace haze at the interface detected by slit-lamp examination; both showed significant keratocyte activation by confocal microscopy. Overall, some degree of keratocyte activation was detected at the flap interface in 10 of 30 eyes. The measured interface reflectivity was 328.8 ± 85.0 confocal backscatter units (CBU) in eyes with activated keratocytes and 88.9 ± 74.5 CBU for the remaining 19 eyes ($P < .001$).

CONCLUSIONS—With attenuated steroids, keratocyte activation was found in a significant number of eyes, although interface haze was subclinical. A higher steroid dosage might therefore be indicated.

Femtosecond laser ablation for LASIK flap creation (IntraLase; IntraLase Corp, Irvine, Calif) was introduced in late 2001 and is growing rapidly in use. Previous studies have shown that IntraLase provides more consistent flap thickness with fewer complications than mechanical microkeratomes, and results in better visual outcome in most patients.^{1–4} IntraLase entered its fourth generation with the introduction of the IntraLase FS60. The FS60 laser generates 60,000 pulses per second, allowing for faster procedure time as compared to the third-generation FS30 laser. Because the Intra-Lase FS60 laser is relatively new, its impact on corneal wound healing has not been well documented. In this study, we use quantitative three-dimensional confocal microscopy to assess the keratocyte response to LASIK with flap creation using the IntraLase FS60 laser.

PATIENTS AND METHODS

Clinical and confocal microscopy assessments were conducted on 30 eyes of 30 patients who underwent LASIK with flaps created by femtosecond laser ablation using the IntraLase FS60 laser between September 12, 2006 and March 16, 2007, at the University of Texas Southwestern Medical Center at Dallas, Texas. Five experienced surgeons (R.W.B., H.D.C.,

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S.M.V., V.V.M., J.P.M.) performed the surgical procedures. Corneas were examined clinically and by confocal microscopy through focusing (CMTF) 3 months postoperatively to assess interface haze and keratocyte activation.⁵ This study was approved by the Institutional Review Board at University of Texas Southwestern Medical Center, and informed consent was obtained.

LASIK WITH INTRALASE FS60

Twenty-nine myopic eyes (mean attempted correction: -4.35 ± 3.31 diopters [D]) and 1 hyperopic eye (attempted correction: $+1.87$ D) underwent LASIK with flap creation using the IntraLase FS60. The planned flap diameter ranged from 8.5 to 9.4 mm, planned flap thickness was 110 to 120 μm , hinge angle was 50° , raster energy was 1.3 μJ , raster line separation was 8 μm , raster spot separation was 8 μm , and side cut energy was 2.1 μJ . Hinges were placed superiorly for all flaps, and a standard pocket was created off the resected plane.

After flap creation, all eyes were allowed to rest for 10 minutes to allow absorption of the gases from the microcavitation bubbles. The flap was then separated and reflected superiorly. For photoablation, patients were treated with either the Star S4 Custom View excimer laser (VISX, Santa Ana, Calif), the LADARVision4000 Custom Excimer Laser System (Alcon Laboratories Inc, Ft Worth, Tex), or the ALLEGRETTO WAVE Excimer Laser (WaveLight Laser Technologie AG, Erlangen, Germany).

POSTOPERATIVE TREATMENT

The protocol for steroid treatment was as follows: one drop of Econopred Plus (Alcon Laboratories Inc) every hour while awake for the first 24 hours, then four times daily for 1 week. Patients also used 1 drop of Vigamox (Alcon Laboratories Inc) 4 times a day for the first week.

POSTOPERATIVE EXAMINATION

Three months postoperatively, slit-lamp examination was used to assess interface haze. In vivo CMTF was performed using a constant camera gain and offset value as previously described.⁶ The thickness of the corneal epithelium and the LASIK flap were calculated from the CMTF intensity curve, and individual images from the CMTF scans were evaluated for qualitative assessment of changes in keratocyte morphology and/or reflectivity.⁵ The area under the CMTF peak corresponding to the flap interface on the intensity curve was also calculated as previously described⁷; this measurement incorporates all factors that might contribute to interface backscatter such as keratocyte activation, edema, interface particles, and debris. The area is expressed in arbitrary confocal backscatter units (CBU), defined as $\mu\text{m} \times \text{pixel intensity}$.

RESULTS

In most of the eyes (19 of 30), the corneal stroma appeared quiet by confocal microscopy, with little or no keratocyte activation, and only a subtle increase in reflectivity at the interface (Figs A and B). In the remaining 10 eyes, keratocyte activation was detected by confocal microscopy, as indicated by highly reflective cell nuclei. The response ranged from a few activated cells limited to the level of the flap interface (Figs C and D), to more significant activation that extended up to 20 μm beyond the interface plane (Figs E and F). The measured interface reflectivity was 328.8 ± 85.0 CBU in the eyes with activated keratocytes and 88.9 ± 74.5 CBU for eyes with quiescent keratocytes ($P < .001$). There was no correlation between interface reflectivity and attempted correction ($R=0.15$, $P=.44$). Furthermore, although there were only a small number of eyes, no obvious association was noted between keratocyte activation and the laser used for photoablation, or the surgeon performing the procedure.

Two eyes had trace haze at the interface detected by slit-lamp examination, and both of these corneas had significant keratocyte activation (see Figs E and F). The measured interface reflectivities for these eyes were 415.3 CBU and 388.2 CBU, respectively. Thus in general, the amount of interface reflectivity and haze correlated with the degree of keratocyte activation. However, there were no visual complaints or changes in best spectacle-corrected visual acuity associated with keratocyte activation in this study.

For all eyes combined, the interface reflectivity 3 months postoperatively was 171.6 ± 139.1 CBU. Epithelial thickness was $53.3 \pm 7.2 \mu\text{m}$, and the difference between actual and intended flap thickness was $+8.8 \pm 8.1 \mu\text{m}$, indicating that the flaps were slightly thicker than intended.

DISCUSSION

Confocal microscopy allows assessment of the cellular response of the cornea to infection, injury, and surgical procedures. Confocal microscopy following photorefractive keratectomy (PRK) has demonstrated that the development of clinical corneal haze is correlated with the activation of corneal keratocytes and transformation to a fibroblast or myofibroblast phenotype.⁷ These activated cells are more reflective than quiescent corneal keratocytes, and synthesize extra-cellular matrix components that also reduce corneal transparency. In general, the degree of keratocyte activation and clinical haze following LASIK is markedly less than that induced by PRK and has been linked with higher raster energies.⁸

In the current study, we found keratocyte activation in 10 of 30 eyes by confocal microscopy 3 months after LASIK with flap creation using the IntraLase FS60 laser. This is a higher proportion than previously observed in a recent study using the FS30 laser, in which only 1 of 15 patients had keratocyte activation 3 months after surgery.⁵ The average interface reflectivity for FS60 eyes (171.6 ± 139.1 CBU) was also higher than that of FS30 eyes (104.8 ± 91.2 CBU). These measurements were made using identical camera gain settings. Because similar raster energies were used in these two studies ($1.2 \mu\text{J}$ for the FS30 vs $1.3 \mu\text{J}$ for the FS60), this difference is likely due to the fact that an extended steroid dosing regimen was used in the FS30 study (every hour for 24 hours and four times daily for 4 weeks, in contrast to every hour for 24 hours and four times daily for 1 week with the FS60). In both studies, the measured backscatter was significantly less than that previously measured following PRK using the same confocal system,⁷ and no patient was found to have clinically significant haze or transient light sensitivity syndrome. Increased steroid use is likely to further prevent keratocyte activation with the FS60 laser.

Confocal microscopy can also be used to distinguish the epithelial and stromal components of the flap, and to measure flap thickness. Epithelial thickness was $53.3 \pm 7.2 \mu\text{m}$ 3 months postoperatively, which is close to the established "normal" epithelial thickness ($\sim 51 \mu\text{m}$) in humans,⁷ and suggests that no significant hyperplasia took place. The difference from intended flap thickness for the FS60 was $+8.8 \pm 8.1 \mu\text{m}$, which is consistent with previous confocal measurements demonstrating improved accuracy and reproducibility of IntraLase as compared to mechanical microkeratomes.

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Figure 1.

Confocal images obtained 3 months after LASIK with IntraLase FS60. **A)** Single image taken from a confocal microscopy through focusing (CMTF) stack in patient 8 at the flap interface. Quiescent keratocytes are observed (arrows), as well as isolated interface particles (circles). **B)** Single image taken 20 μm below the flap interface in patient 8. Quiescent keratocytes are observed. **C)** Single image taken from a CMTF stack in patient 3 at the flap interface. Note highly reflective nuclei (arrowheads), indicating keratocyte activation. **D)** Single image taken 20 μm below the flap interface in patient 3. Quiescent keratocytes are observed (arrows). **E)** Single image taken from a CMTF stack in patient 27 at the flap interface. Note highly reflective nuclei and diffuse areas of increased interface reflectivity. **F)** Single image taken 20 μm below the flap interface in patient 27. Both activated (arrowheads) and quiescent (arrows) keratocytes are observed. Horizontal field width = 400 μm