Histologic and Ultrastructural Findings in Human Corneas After Successful Laser In Situ Keratomileusis

Nicole J. Anderson, MD; Henry F. Edelhauser, PhD; Nariman Sharara, MD; Keith P. Thompson, MD; Roy S. Rubinfeld, MD; Dawn M. Devaney, OD; Nancy L'Hernault, MA; Hans E. Grossniklaus, MD

Objective: To examine the histologic and ultrastructural features of human corneas after successful laser in situ keratomileusis (LASIK) in 2 patients post mortem.

Methods: Portions of 4 corneas were processed for histology, transmission electron microscopy, and scanning electron microscopy.

Results: Case 1 had undergone LASIK 3 months prior to death and case 2 had undergone LASIK 20 months prior to death. A Hansatome (Bausch & Lomb Surgical Inc, Claremont, Calif) microkeratome with a 180-µm plate had been used for case 1 and an Automated Corneal Shaper (Chiron Corporation, Munich, Germany) with a 160-µm plate had been used for case 2. Histologically, the LASIK flap measured 160 µm and 150 µm in thickness in case 1 and case 2, respectively. Corneas from both cases exhibited minor epithelial ingrowth into the wound, reactive keratocytes at the wound margin, irregular collagen fibrils in the wound bed, and severed collagen bundles at the flap hinge. These findings were more pronounced in case 1 than in case 2, and the wound interface was virtually imperceptible in case 2. Additionally, the corneas from case 1 contained periodic acid-Schiff–positive electron dense material and wide-spaced collagen at the wound interface, and there was an absence of corneal nerves.

Conclusions: These findings show that changes caused by wound repair that are present at 3 months are minor 20 months after LASIK.

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Wound healing following laser in situ keratomileusis (LASIK) has been studied in animal models and clinically in human corneas. The changes caused by the wound healing process after LASIK have been compared with those that occur after photorefractive keratectomy (PRK). The Bowman layer is disrupted by PRK and the resulting corneal haze ensues secondary to the wound repair response. The Bowman layer is preserved, however, after LASIK in humans. The role of the Bowman layer in corneal wound healing is under investigation. Histologic and ultrastructural studies in rabbits have shown parallel collagen lamellae in the flap bed and disarranged collagen alignment with associated reactive keratocytes at the keratectomy wound margin in rabbit corneas after LASIK. The few reports of ultrastructural changes in human corneas following LASIK are in corneal button specimens after penetrating keratoplasty or in blind phthisical eyes in which LASIK was performed just prior to enucleation.

In this study, we examined the histologic and ultrastructural findings after uncomplicated, successful LASIK. Our findings show active wound healing changes 3 months after LASIK. These changes include reactive keratocytes, collagen disarray, and eosinophilic periodic acid-Schiff (PAS)–positive electron dense material at the flap interface. There are minimal changes at the flap interface 20 months after LASIK.

RESULTS

The corneas from both cases were tri-sectioned. One third was placed in 10% neutral buffered formalin and each remaining third was placed in 2.5% glutaraldehyde. The formalin-fixed portions were routinely processed through increasing concentrations of alcohol, cleared in xylene, and embedded in paraffin. Sections including the LASIK flap were stained with hematoxylin-eosin, PAS, and Bodian. One third of each cornea in the 2.5% glutaraldehyde solution was postfixed with 0.1 M cacodylate buffer and 1% osmium tetroxide, and was embedded in epoxy resin. Semithin sections were stained with uranyl acetate-
CASE REPORTS

CASE 1

A 49-year-old man had uncomplicated LASIK performed in July 2000. A preoperative consensus refraction (2 manifest refractions and 1 cycloplegic refraction) was $-3.00 + 3.00 \times 178$ OD and $-3.25 + 3.24 \times 172$ OS. His best-corrected visual acuity was 20/20 OU. Corneal thickness measurements were obtained using ultrasonic pachymetry and measured 533 µm OD and 530 µm OS. Keratometry values determined by computed corneal topography were $43.75 \times 173/41.75 \times 83$ OD and $43.75 \times 174/42.75 \times 84$ OS. The patient was corrected for full distance acuity in both eyes. A 9.5-mm Hansatome (Bausch & Lomb Surgical Inc, Claremont, Calif) microkeratome with a plate thickness of 180 µm was used to construct the flap in both eyes. Laser ablation was performed with the Summit Autonomous (Summit, Waltham, Mass), achieving an ablation depth of 30.0 µm OD and 25.0 µm OS. On postoperative day 1, the patient’s visual acuity was 20/20 OU without correction and he had an uncomplicated course. At his last postoperative visit (2 weeks after LASIK), his manifest refraction was $-0.25 + 0.75 \times 001$ OD and $-0.25 + 0.50 \times 038$ OS. The patient died from an assault just prior to his 3-month postoperative visit and the corneas were obtained post mortem. The death-to-preservation time in Optisol-GS (Chiron Ophthalmics, Irvine, Calif) was 4 hours 35 minutes.

CASE 2

A 55-year-old man had uncomplicated LASIK performed in March 1999. A manifest refraction to determine the laser inputs was $-2.75$ OD and $-3.00 + 0.75 \times 76$ OS. His best-corrected visual acuity was 20/20 OU. Corneal thickness measurements using ultrasonic pachymetry measured 542 µm OD and 534 µm OS. Keratometry values were determined by computed corneal topography and measured 44.34 OD and $44.64 \times 83/43.78 \times 173$ OS. The patient was corrected for full distance in both eyes. An Automated Corneal Shaper (Chiron Corporation, Munich, Germany) with a plate thickness of 160 µm was used to make a flap in both eyes. Laser ablation was performed with the VISX Star Excimer Laser (VISX Inc, Santa Clara, Calif), achieving an ablation depth of 30.0 µm OD and 25.0 µm OS. The patient’s visual acuity was 20/20 uncorrected on the first postoperative day and he had an uncomplicated course. At the time of his last examination (1 year postoperatively), his manifest refraction was plano OD and plano $+ 0.50 \times 30$ OS. The patient died from congestive heart failure 20 months after LASIK and the corneas were obtained post mortem. The death-to-preservation time in Optisol-GS medium was 4 hours 10 minutes.

Figure 1. Case 1, left eye. A, There is a break in the Bowman layer (between arrowheads) and minor epithelial ingrowth present (arrow) (hematoxylin-eosin, original magnification $\times 100$). B, The ultrastructure of the area shown in A shows epithelium (epi) extending into the wound around the edge of the disrupted Bowman layer (arrowhead) (hematoxylin-eosin, original magnification $\times 4750$).
endoplasmic reticulum. The collagen lamellae in the corneal bed and undersurface of the flap exhibited a whorled pattern in both corneas from case 1, which was not present in both corneas from case 2 (Figure 6). A Bodian stain showed no corneal nerves in case 1 and rare, scattered, short corneal nerves in case 2. The deep stroma, the Descemet membrane, and the endothelium were normal in both cases. The endothelial cell counts performed on the hematoxylin-eosin–stained slides averaged 10 and 12 for cases 1 and 2, respectively.

**COMMENT**

Studies in rabbits have shown a minimal corneal wound healing response following LASIK. In rabbits, well-arranged regular collagen lamellae are present at the interface, with collagen lamellar irregularities and reactive keratocytes appearing only at the edge of the flap. In one of these studies, prominent epithelial plugs and keratocytes appeared early in the postoperative period and were no longer present at the wound margin at 2½ and 5 months after surgery. The activated keratocytes were in close proximity with the epithelium and were no longer observed at 5 months when the epithelial plugs were much smaller. Fibronectin and tenascin were shown only at the incision site of the microkeratome in rabbit corneas, suggesting an active wound healing process at the flap edge. Periodic acid-Schiff–positive material has been identified at the wound interface in rabbits as late as 9 months after LASIK.
In vivo confocal microscopy has been used to study stromal changes in human corneas after LASIK.\textsuperscript{18,19} Keratocyte activity at the interface seems to peak in the early postoperative course (1-2 weeks), and by 6 months there appears to be a loss of keratocytes in the anterior portion of the flap.\textsuperscript{19} Up until now, histologic confirmation of these findings in humans has been limited to diseased corneas requiring penetrating keratoplasty\textsuperscript{14-16} and blind eyes that underwent LASIK just prior to enucleation (Table).\textsuperscript{17} Wright and coworkers\textsuperscript{14} described one patient with epithelial ingrowth leading to penetrating keratoplasty 6 weeks after undergoing LASIK. In the areas free of epithelial ingrowth, the interface was nearly invisible, with only slight irregularities in stromal lamellar thickness at the base of the LASIK treatment area and a few apoptotic keratocytes seen by transmission electron microscopy. Jabbur and coworkers\textsuperscript{15} recently described a cornea removed after penetrating keratoplasty for ectasia, which occurred following LASIK. The cornea showed interruption of the Bowman layer and mild anterior cellularity.\textsuperscript{15} Geggel and Talley\textsuperscript{16} described a patient with iatrogenic keratoectasia examined 22 months after undergoing LASIK. That case exhibited central stromal thinning and the flap interface exhibited a thin line of PAS-positive material 22 months postoperatively.\textsuperscript{16} Latvala and coworkers\textsuperscript{17} studied the effects of LASIK on blind, phthisical eyes at 8 days, 54 days, and 4 months prior to enucleation. All eyes contained epithelial plugs at the interface and exhibited fibronectin and tenascin in the wound. The fibronectin and tenascin were identified only at the flap margin and were associated with the epithelial plugs at 4 months after LASIK.\textsuperscript{17}

Our findings describe the histologic and ultrastructural findings 3 and 20 months after successful LASIK.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure4.png}
\caption{Case 2, left eye. There is no perceptible interface between the flap and bed in this specimen. There is focal thickening of the basement membrane of the epithelium (arrowheads) (hematoxylin-eosin, original magnification $\times 100$).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure5.png}
\caption{A, Case 1, right eye. B, Case 2, right eye. There are reactive keratocytes (arrows) surrounded by wavy collagen lamellae in the right cornea from case 1 (A) and the right cornea from case 2 (B) in the area of the flap hinge (original magnification $\times 4750$).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure6.png}
\caption{Scanning electron micrographs. A, Case 1, left eye. The stromal bed exhibits irregular whorls of collagen lamellae (original magnification $\times 100$). B, Case 2, right eye. The stromal bed is smooth, without a whorling pattern of collagen lamellae (original magnification $\times 100$).}
\end{figure}
Clinicopathologic Studies of Human Corneas After LASIK*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Wright et al14</th>
<th>Jabbur et al15</th>
<th>Geggel and Talley16</th>
<th>Latvala et al17</th>
<th>Current Case 1</th>
<th>Current Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
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<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Time after LASIK</td>
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<td>5 mo</td>
<td>22 mo</td>
<td>8 d, 54 d, 4 mo</td>
<td>3 mo</td>
<td>22 mo</td>
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<td>Tissue</td>
<td>Cornal button</td>
<td>Corneal button</td>
<td>Corneal button</td>
<td>Enucleation</td>
<td>Postmortem cornea</td>
<td>Postmortem cornea</td>
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<tr>
<td>Epithelial plugs</td>
<td>Present</td>
<td>Not mentioned</td>
<td>Faint delination</td>
<td>Reactive keratocytes</td>
<td>PAS-positive electron</td>
<td>PAS-positive electron</td>
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<tr>
<td>Interface</td>
<td>Irregular collagen and widely spaced collagen</td>
<td>FPAS-positive electron</td>
<td>PAS-positive electron dense material</td>
<td>Many reactive keratocytes</td>
<td>Normal collagen structure</td>
<td>Few reactive keratocytes</td>
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<tr>
<td>Nerves</td>
<td>Degenerated superficial nerves at 8 d; nerves present at 54 d and 4 mo</td>
<td>Nerves not identified</td>
<td>Rare superficial nerves present</td>
<td>PAS-positive electron dense material</td>
<td>Normal collagen structure</td>
<td>Normal collagen structure</td>
</tr>
</tbody>
</table>

*LASIK indicates laser in situ keratomileusis; PAS, periodic acid–Schiff; and ellipses, not applicable.

There seems to be active wound healing at 3 months that virtually disappears at 20 months after uncomplicated LASIK. The wound repair response includes altered collagen alignment, reactive keratocytes, and PAS-positive electron dense material in the wound interface. Although it is possible that the pattern of collagen lamellae at the interface in case 1 is an artifact, we feel that is unlikely since both corneas looked the same and the same technique was used to prepare all 4 corneas. At 20 months postoperatively, there were occasional areas of separation between the flap and interface, and only a few reactive keratocytes at the interface, most notably at the flap hinge. Microscopic epithelial plugs were present at 3 and 20 months following LASIK. A Bodian stain was negative for corneal nerves at 3 months although it was positive for rare, short superficial corneal nerves at the edge of the flap at 20 months. These findings are consistent with the previous reports that transection of the anterior stromal epithelial nerve plexus after LASIK results in reduced corneal sensation 3 to 6 months after LASIK. Studies in rabbits19 and humans20 have shown an absence of superficial stromal epithelial nerves shortly after flap creation and laser ablation. The nerves appear to sprout and regenerate to almost normal density as early as 2 months postoperatively. In this study, we found a lack of corneal nerves at 3 months and a few small superficial nerves at 20 months after LASIK. These findings may account for clinical variability in the return of corneal sensation after LASIK.21-22

Previous studies have also shown variability in flap thickness after LASIK. Microkeratomes have plates to cut flaps of various thickness. This is important when considering the amount of tissue to be ablated; at least 250 µm of corneal thickness should be retained to minimize the possibility of keratodystasia.23-26 Our study is consistent with a previous study that showed overestimation of flap thickness.21 In case 1, a 180-µm plate resulted in a 160-µm-thick flap, and in case 2, a 160-µm plate resulted in a 150-µm-thick flap. Latvala and coworkers16 showed a flap thickness of 60 to 100 µm for an intended 140-µm-thick flap. It is unknown if this is due to undercutting by the microkeratome or due to tissue processing. The endothelial cell counts in cases 1 and 2 were 10 and 12, respectively, which are comparable to age-matched controls.27 Histologic and ultrastructural changes following LASIK show a wound healing response occurring at the flap-bed interface. This wound healing response decreases with time, resulting in minimal changes at 20 months after LASIK in our cases. Additional pathologic studies of corneas after successful LASIK are needed since variabilities in surgical technique, different surgeons, different lasers, and different patients cannot be discounted in our limited study.

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Corresponding author: Hans E. Grossniklaus, MD, L. F. Montgomery Ophthalmic Pathology Laboratory, BT428 Emory Eye Center, 1365 Clifton Rd, Atlanta, GA 30322 (e-mail: opth heg@emory.edu).

REFERENCES


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**ARCHIVES Web Quiz Winner**

Congratulations to the winner of our November quiz, Michael Sullivan Mee, OD, FAAO, Northeastern State University Oklahoma College of Optometry, Tahlequah. The correct answer to our November challenge was tick bite of the conjunctiva. For a complete discussion of this case, see the Clinopathologic Reports, Case Reports, and Small Case Series section in the December ARCHIVES (Love MC, Platt L, Westfall CT. Lone-star tick bite of the conjunctiva. Arch Ophthalmol. 2001;119:1854-1855).

Be sure to visit the Archives of Ophthalmology World Wide Website (http://www.archophthalmol.com) and try your hand at our Clinical Challenge Interactive Quiz. We invite visitors to make a diagnosis based on selected information from a case report or other feature scheduled to be published in the following month’s print edition of the ARCHIVES. The first visitor to e-mail our Web editors with the correct answer will be recognized in the print journal and on our Web site and will also receive a free copy of the Clinical Eye Atlas, published by AMA Press.