Correlations of Long-term Matrix Metalloproteinase Localization in Human Corneas After Successful Laser-Assisted In Situ Keratomileusis With Minor Complications at the Flap Margin

Pierre R. Fournié, MD; Gabriel M. Gordon, BS; Daniel G. Dawson, MD; Henry F. Edelhauser, PhD; M. Elizabeth Fini, PhD

Objective: To determine whether matrix metalloproteinases (MMPs) are present long-term in human corneas after successful laser-assisted in situ keratomileusis (LASIK).

Methods: Eighteen postmortem corneas from 10 patients with postoperative intervals of 2 to 8 years after LASIK surgery and 4 normal control corneas from 2 patients were collected from US eye banks and processed for histologic analysis and immunolocalization with antibodies to MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, and MMP-14.

Results: Matrix metalloproteinase 7 was present in the epithelium of all corneas. Other MMPs were localized to the wound margin in some post-LASIK corneas. Matrix metalloproteinase 9 was detected around epithelial cells trapped in the lamellar scar in 5 of 6 corneas with epithelial ingrowth. Various MMPs were detected in fibrotic tissue at the wound margin in 2 of 2 corneas with flap retraction.

Conclusions: The presence of MMPs in post-LASIK corneas correlates with an ongoing wound healing process associated with minor post-LASIK complications. Matrix metalloproteinases might contribute to instances of ongoing flap instability, and if so, judicious use of MMP inhibitors could provide benefit.

Arch Ophthalmol. 2008;126(2):162-170

Author Affiliations: Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, Florida (Drs Fournié, Dawson, and Fini and Mr Gordon); Institut national de la santé et de la recherche médicale (INSERM), U563, Université Toulouse III Paul Sabatier, and Service d’Ophthalmologie, Hôpital Purpan, CHU Toulouse, Toulouse, France (Dr Fournié); and Emory Eye Center, Emory University, Atlanta, Georgia (Drs Dawson and Edelhauser).

LASER-ASSISTED IN SITU KERATOMILEUSIS (LASIK) has certain advantages relative to other refractive surgical procedures, including little or no significant central haze, less myopic regression, and less postoperative discomfort; thus, it is currently the most frequently performed refractive surgical procedure in the world.1

In LASIK procedures, damage to the epithelial basement membrane occurs only at the flap margins, providing only minimal opportunity for direct epithelial-stromal cell interactions required to stimulate the fibrotic repair response responsible for haze.2,4 Recent studies of human postmortem corneas reveal that central and para-central LASIK wounds heal by producing a hypocellular primitive stromal scar5-7 that has a very weak cohesive tensile strength8 and displays no evidence of remodeling over time for up to 6.5 years postsurgery. It is only the region localized around the flap margin of the LASIK cornea adjacent to the surface epithelium that heals by fibrosis. This healing produces a hypercellular fibrotic stromal scar that reaches maximal tensile strength by approximately 3.5 years postsurgery, implying a much longer healing time than previously thought.5-7

Matrix metalloproteinases (MMPs) are a family of enzymes that constitute the principal mediators of tissue remodeling in physiology and pathology.8-12 As a family, they are capable of dismantling virtually any extracellular matrix structure and they also act on a large number of other substrates, including cytokines and cell adhesion molecules.13,14 Matrix metalloproteinases are typically secreted in a latent form that must undergo an internal cleavage to be activated. The MMP family currently includes more than 25 members that can be divided into 5 subfamilies based on substrate preference: collagenases (MMP-1, MMP-8, and MMP-13), stromelysins (MMP-3 and MMP-10) and matrixins (MMP-7 and MMP-26), gelatinases (MMP-2 and MMP-9), membrane-type MMPs (MMP-14 to MMP-17 and MMP-24), and others. Most MMPs occur naturally only in very low levels in normal tissue. In fact, their expression is tightly regulated, induced only when needed, so as to avoid uncontrolled and excessive tissue destruction.15 Matrix metalloprotei-
ase expression is transcriptionally up-regulated in resident tissue cells during wound healing, regeneration, and remodeling. MMPs are also brought in by invading leukocytes, which both synthesize and store MMPs. The cornea has been an important experimental model for defining mechanisms of MMP expression and role in normal and pathologic repair processes, including epithelial regeneration and failure to heal, fibrotic repair and scar remodeling, infection, and angiogenesis. Similar- ly, a number of different MMPs are produced by the fibrotic repair tissue deposited in response to keratectomies that penetrate through the epithelium and into the stroma. In this case, MMP expression continues long-term, at least 1.5 years, as documented in a rabbit penetrating keratectomy model, correlated with the lengthy process of repair tissue remodeling. Some of the reports evaluating MMP expression in more specific models of photorefractive keratectomy and LASIK have supported similar conclusions in the short-term, but the long-term studies have not been done. The purpose of the present study was to determine whether MMPs continue to be expressed long-term in human corneas after reportedly uncomplicated successful LASIK. All corneas were obtained postmortem from US corneal eye bank donors at varying intervals after LASIK surgery. We also investigated factors, including patient age, postoperative interval, and histopathologic findings, that could affect MMP expression.

**METHODS**

**SOURCE OF THE CORNEAS**

After approval by the Emory University institutional review board, 18 postmortem corneoscleral buttons from 10 corneal eye bank donors with a history of LASIK surgery were obtained from various eye banks in North America. A number was assigned to each cornea according to the order of inclusion. The specimens were received in Optisol-GS solution (Bausch & Lomb Surgical, Irvine, California) within 6 days of death (mean [SD] time of preservation, 3.51 [1.4] days). Review of preoperative, intraoperative, and postoperative clinical records, when available, was performed. Four postmortem normal corneas stored in Optisol-GS (mean [SD] time of preservation, 2.95 [0.35] days) from 2 patients were obtained from the Georgia Eye Bank (Atlanta) and the Lions Eye Bank (Miami) as controls.

**CORNEAL PROCESSING**

The corneoscleral buttons were oriented with the hinge superiorly and then trisected. The central portion was immediately snap-frozen in liquid nitrogen, embedded in optimal cutting temperature compound (Tissue-Tek-II; Miles Inc, Elkhart, Indiana), and stored at −70°C until sectioned. Sections (8 μm) were cut in a cryostat microtome (Leica 1850 cryostat; Leica, Deerfield, Illinois) and mounted on adhesive-coated glass slides for conventional and immunofluorescent histologic processing.

**HISTOLOGIC EXAMINATION**

The sections were fixed and stained with hematoxylin-eosin according to standard procedures. Histopathologic findings from light microscopic examination of the peripheral lamellar wound at the flap margin and central lamellar wound, flap thickness (taken from a representative area with the fewest artifacts and containing all 3 distinct types of epithelial cells: basal, wing, and superficial epithelial cells), and residual stromal bed thickness measurements using a Zeiss Axiosvert 200M inverted microscope (Carl Zeiss Meditec, Jena, Germany) were recorded.

**ANTIBODIES**

Polyclonal antibodies to MMPs were purchased from Triple Point Biologics (Forest Grove, Oregon): rabbit antibodies to interstitial collagenase (RHMMP1), gelatinase A (RHMMP2), stromelysin 1 (RP2MMP3), MMP-7 (RP2MMP7), neutrophil collagenase (RP1MMP8), gelatinase B (RP3MMP9), stromelysin 2 (RP2MMP10), and membrane-type MMP-1 (RP2MMP14). Rabbit monoclonal anti-CD11b (Mac-1) (clone M1/70.15) was obtained from Cedarlane Laboratories (Burlington, North Carolina). CD11b (Mac-1) was used as a marker for leukocytes, including macrophages and granulocytes. Rabbit polyclonal antitilamin (L9393) and mouse monoclonal α-smooth muscle actin (α-SMA) (clone 1A4) were purchased from Sigma-Aldrich (St Louis, Missouri). Rabbit IgG and mouse IgG (Chemicon, Temecula, California) were purchased as negative controls. Secondary antibodies used were Alexa Fluor 488 conjugated goat antimouse IgG (A-11001) and donkey antirabbit IgG (A-21206) from Invitrogen Molecular Probes (Carlsbad, California).

**INDIRECT IMMUNOLOCALIZATION**

Slides were to be stained were air-dried for 20 minutes at room temperature and then fixed in 100% cold acetone (−20°C) for 20 minutes. They were washed with phosphate-buffered saline (PBS) 3 times for 5 minutes each, followed by a 1-hour incubation in a humidified level chamber in 10% normal donkey (D9663, Sigma-Aldrich) or goat (G9023, Sigma-Aldrich) sera.
in PBS to block nonspecific staining. Primary antibodies were used in a dilution of 1:100 and incubated at 4°C overnight. After 3 additional washes with PBS for 5 minutes each, the Alexa Fluor 488 conjugated secondary antibody was applied for 1 hour.

Samples were mounted with the Vectashield mounting medium with 4',6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, California) for nuclear counterstaining. Negative control sections were processed identically but incubated

---

**Table 2. Histologic Findings**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Margin of the Flap</th>
<th>Flap Thickness, µm</th>
<th>Residual Stromal Bed Thickness, µm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1: “Normal Healing”</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2Ra</td>
<td>No specificity, good alignment of BL ends</td>
<td>110</td>
<td>345</td>
</tr>
<tr>
<td>4R</td>
<td>No specificity, good alignment of BL ends</td>
<td>115</td>
<td>385</td>
</tr>
<tr>
<td><strong>Group 2: Epithelial Ingrowth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Major epithelial ingrowth, good alignment of BL ends with a small gap at the break</td>
<td>145</td>
<td>335</td>
</tr>
<tr>
<td>4Lb</td>
<td>Minor epithelial ingrowth with islands of epithelial cells in the scar, good alignment of BL ends</td>
<td>155</td>
<td>345</td>
</tr>
<tr>
<td>7L</td>
<td>Major epithelial ingrowth, depressed BL end with a small gap at the break</td>
<td>154</td>
<td>315</td>
</tr>
<tr>
<td>9R</td>
<td>Major epithelial ingrowth, good alignment of BL ends with a small gap at the break</td>
<td>170</td>
<td>330</td>
</tr>
<tr>
<td>9L</td>
<td>Minor epithelial ingrowth with microscopic foci of epithelial cells in the scar, good alignment of BL ends</td>
<td>140</td>
<td>295</td>
</tr>
<tr>
<td>10</td>
<td>First flap: epithelial ingrowth, good alignment of BL ends; second flap: no specificity, good alignment of BL ends</td>
<td>First flap: 135; second flap: 40</td>
<td>330</td>
</tr>
<tr>
<td><strong>Group 3: Epithelial Hyperplasia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3R</td>
<td>Epithelial hyperplasia, depressed BL end</td>
<td>140</td>
<td>350</td>
</tr>
<tr>
<td>3L</td>
<td>Epithelial hyperplasia, depressed BL end</td>
<td>125</td>
<td>318</td>
</tr>
<tr>
<td>5L</td>
<td>Epithelial hyperplasia, good alignment of BL ends with a small gap at the break</td>
<td>120</td>
<td>460</td>
</tr>
<tr>
<td>6R</td>
<td>Epithelial hyperplasia, elevated BL end</td>
<td>110</td>
<td>335</td>
</tr>
<tr>
<td>6L</td>
<td>Epithelial hyperplasia, depressed BL end</td>
<td>100</td>
<td>350</td>
</tr>
<tr>
<td>7R</td>
<td>Minor epithelial hyperplasia, good alignment of BL ends</td>
<td>110</td>
<td>405</td>
</tr>
<tr>
<td>8R</td>
<td>Minor epithelial hyperplasia, good alignment of BL ends</td>
<td>125</td>
<td>340</td>
</tr>
<tr>
<td>8L</td>
<td>Minor epithelial hyperplasia, depressed BL end</td>
<td>120</td>
<td>390</td>
</tr>
<tr>
<td><strong>Group 4: Flap Retraction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2L</td>
<td>BL end curled inward with mild flap retraction</td>
<td>120</td>
<td>335</td>
</tr>
<tr>
<td>5R</td>
<td>BL end curled inward with significant flap retraction</td>
<td>115</td>
<td>390</td>
</tr>
</tbody>
</table>
with strain-specific IgG as primary antibodies. Samples were
examined using a Zeiss Axiovert 200M inverted fluorescence
microscope and images were captured using a Zeiss AxioCam
MRc5 camera attached to the microscope. Camera and micro-
scope settings were controlled by Axiovision software version
4.1 (Carl Zeiss Meditec). To evaluate the effect of LASIK, 4 re-
gions were evaluated: (1) the LASIK flap wound margin, (2)
the corneal stroma in the LASIK flap, (3) the paracentral and
central lamellar wound regions, and (4) the residual stromal
bed. Normal control corneas were evaluated in the central, para-
central, and peripheral regions. The LASIK flap wound mar-
gin was also examined using a Leica TCS SP2 confocal micro-
scope (Leica Microsystems, Bannockburn, Illinois) with a total
magnification of \( \times 400 \).

DATA ANALYSIS

The slides were evaluated for staining intensity, cellular local-
ization of staining, and the presence of any distinctive staining
patterns. Immunoreactivity intensity was evaluated using a semi-
quantitative scale with the negative control as baseline (− = none
or same as background, + = weak [trace or slightly perceptible
above background], ++ = moderate, and +++ = strong). Re-
liability was assessed by having 2 evaluators independently rate
the staining intensities at different times. The average of the 2
observers’ scores was used. Concordance among the observers
was high.

RESULTS

CLINICAL FINDINGS

A complete history of the LASIK surgeries could not be
obtained for the majority of the 18 specimens. Clinical
data that were available for every specimen included pa-
tient age, cause of death, a history of uncomplicated
LASIK, and the postoperative interval after LASIK
(Table 1). Patient 10 underwent LASIK with 2 enhance-
ments by flap relifting 6 and 12 months after initial sur-
gery and a third enhancement by recutting a flap (Figure 1E). The 10 patients
ranged in age from 34 to 62 years (mean [SD], 50.30 [7.15]
years). The postoperative interval after LASIK ranged from 2 to 8 years.

HISTOLOGIC FINDINGS

Hematoxylin-eosin–stained light microscopy evalua-
tions revealed a lamellar interface scar that was rela-
tively easy to find (Figure 1) in all the specimens. The mean (SD) central thickness of the LASIK flap was 130 (21) µm. The mean (SD) residual stromal bed thickness

### Table 3. Immunostaining Findings in the LASIK Wound Margin (Groups 1-4) and in the Control Group

<table>
<thead>
<tr>
<th>Patient</th>
<th>MMP-1</th>
<th>MMP-2</th>
<th>MMP-3</th>
<th>MMP-7</th>
<th>MMP-8</th>
<th>MMP-9</th>
<th>MMP-10</th>
<th>MMP-14</th>
<th>Laminin</th>
<th>α-SMA</th>
<th>Mac-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>2R&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Normal</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>4R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Normal</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>3R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Normal</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>4L&lt;sup&gt;c&lt;/sup&gt;</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Normal</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>7R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>9R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>9L&lt;sup&gt;d&lt;/sup&gt;</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>1R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>1L</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2R&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>3L</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>4R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>7R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>8R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>8L</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>5R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Control 1R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Control 1L</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Control 2R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Control 2L</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Abbreviations: LASIK, laser-assisted in situ keratomileusis; MMP, matrix metalloproteinase; SMA, smooth muscle actin; −, no staining intensity or same as
background; +, weak staining intensity (trace of slightly perceptible above background); ++, moderate staining intensity; ++++, strong staining intensity.
<sup>a</sup>CDC11b.
<sup>b</sup>Right cornea.
<sup>c</sup>Left cornea.
<sup>d</sup>First flap/second flap.
was 353 (39) µm (Table 2). At the LASIK flap wound margin, some corneas had variable amounts of epithelial ingrowth into the lamellar wound (Figure 1C), microscopic foci or islands of epithelial cells actually in the scar, and variability in the alignment of the cut end of Bowman’s layer at the wound margin (eg, good alignment with a small gap at the break [Figure 1C], depressed or elevated ends [Figure 1B], and ends curled inward with various flap retraction and stromal defects [Figure 1D]). Epithelial ingrowth into the flap margin was observed in 6 of the 18 corneas (33.3%) examined using serial sections. Variable amounts of epithelial hyperplasia (Figure 1B) were commonly present at the wound margin, filling the gaps in Bowman’s layer. The histologic findings in the wound margin are summarized in Table 2. Corneas were grouped according to their main histopathologic finding at the wound margin.

**IMMUNOSTAINING FINDINGS**

Staining of normal control corneas was the same in the central, paracentral, and peripheral regions: only MMP-7 was detectable in 2 of the 4 normal control corneas with at least ++ staining intensity, localized in the epithelium (Table 3). A number of different MMP proteins were immunodetected in the wound margin of LASIK corneas. Semiquantitative staining intensity results in the wound margin are summarized in Table 3. No staining...
was observed at the paracentral or central scar regions. All other regions in the corneal stroma were unstained. The negative control serving as reference for immuno-fluorescence intensity evaluation is shown for each Figure. Matrix metalloproteinase 9 immunostaining with a level of at least $\times H11001/H11001$ was observed in 5 (patients 1, 7L, 9R, 9L, and 10) of the 6 corneas with epithelial in-growth. Immunoreactive MMP-9 protein was detected around epithelial cells trapped in the lamellar scar (Figure 2C and Figure 3B). The most MMPs within the defect area at the wound margin were detected in corneas from the 2 patients (patients 2L and 5R) (Figure 4) with the ends of Bowman’s layer curled inward with flap retraction. This area was also positive for CD11b (Mac-1), a marker for granulocytes or macrophages, in the corneas of these 2 patients (Figures 4E). In 3 corneas, $\alpha$-SMA, a marker for myofibroblast differentiation, was detected within the margin defect of the right cornea of patient 5 (Figure 4I) and of the left cornea of patient 2 and in keratocytes between both flap cuts of patient 10 (Figure 3E-F).

**COMMENT**

Matrix metalloproteinases are expressed by resident tissue cells in response to injury and remodeling stimuli and are also produced by inflammatory cell types that invade the tissue during remodeling events. Collagenase is the prototype member of the MMP family, its activity first demonstrated in the involuting tadpole tail.41,42 When applied shortly thereafter to the cornea, this discovery gave the first insight into the enzymatic basis of corneal ulceration.43-45 Work in the 1970s characterized corneal collagenase in normal and pathologic repair.36,46 With the molecular cloning of collagenase, and expansion of the MMP family, and development of molecular and antibody probes,10 investigation in the cornea was renewed and expanded into new territory.37-47 Much subsequent work has defined complex roles for MMPs in normal and pathologic corneal repair and remodeling processes.2-16 The results of studies using transgenic mice (knockouts or overexpression) emphasize the high functional redundancy of MMPs.48 The ablation of a particular MMP can lead to higher expression levels of other MMPs, presumably to compensate for the loss. In view of this fact, it is thought that the multiplicity of MMP forms is an indicator of the extreme importance of these enzymes for the maintenance and repair of tissues.8

This study of adult human corneas that underwent uneventful LASIK 2 to 8 years before evaluation revealed that MMPs, when present, are localized exclusively to the flap margin. Matrix metalloproteinase presence was correlated with specific histopathologic findings identified at the wound margin. There was no observable correlation between patient age or postoperative interval with the severity or type of MMP activity in corneas that underwent LASIK.

Of the 33% ($n = 6$) of corneas with epithelial ingrowth observed in the current series, 83% ($n = 5$) had at least $++$ immunostaining for MMP-9, which was positively correlated with basement membrane interruption or irregularities visualized via laminin staining. This is consistent with the capacity for MMP-9 to cleave epithelial basement membrane components, such as collagen types IV and VII and laminin.8,11 New basement membrane components such as laminin and type IV collagen are deposited underneath migrating corneal epithelial cells19 and around corneal epithelial cells implanted into
A causal relationship between overexpression of MMP-9 in the corneal epithelium and basement membrane dissolution/failure to heal has been demonstrated in experimental models. In these studies, the corneal epithelium gave the appearance of an invading front, dissolving the basement membrane and subsequently penetrating the underlying stroma in its path. Matrix metalloproteinase 9 associated with a disrupted basement membrane around ingrowing epithelium suggests a similar chronic, ongoing remodeling and invasion process and might help explain the previous findings identifying the weakest wound margin scars as those with epithelial ingrowth.7

Matrix metalloproteinase 7 immunostaining is observed around trapped epithelial cells with the same intensity as in control corneas. Matrix metalloproteinase 7 was immunolocalized in previous studies to the epithelial layers of unwounded and wounded corneas in rat.26 In this study, MMP-7 was also found in the epithelium of corneas from groups 1 and 3 but not specifically at the stromal wound margin. This is in comparison with corneas of groups 2 and 4 where MMP-7 was localized under the epithelial surface: around trapped epithelial cells in epithelial ingrowth (group 2) and in the overlying wound stroma in group 4. We thus did not mention MMP-7 in the wound margin of groups 1 and 3 in Table 3.

Figure 4. Immunofluorescence of the laser-assisted in situ keratomileusis flap margin of patient 5R with flap retraction. A-E, Positive staining for matrix metalloproteinase (MMP) 3 (B), MMP-7 (C), MMP-8 (D), and Mac-1 (CD11b) (arrows) (E) was observed within the margin defect compared with the negative control (rabbit IgG) (A). F and G, No MMP-9 staining (F) or continuous epithelial basement membrane laminin (G) were observed. I and H, Note the presence of α-smooth muscle actin (I) within the defect compared with the negative control (mouse IgG) (H) (A-I, original magnification ×400).
Unlike most MMPs, MMP-7 (matrilysin) is constitutively present in uninjured epithelia, including in the intestine, airways, and cornea. In the small intestine, MMP-7 has been shown to function in host defense by activating the latent form of defensins, a family of antimicrobial peptides that are also found in the cornea. In models of airway and corneal injury, MMP-7 expression is up-regulated in migrating epithelial cells, and the MMP-7 activity is required for repair of airway wounds. These observations indicate that matrilysin serves key functions in both epithelial defense and repair.

Various MMPs were detected at flap margins with flap retraction (patients 2L and 5R). In both cases, Mac-1 (CD-11b), used as a marker for macrophages and granulocytes, was colocalized, suggesting a chronic inflammatory process. This can explain the expression of various MMPs, particularly the presence of the neutrophil collagenase (MMP-8), which is synthesized specifically by polymorphonuclear neutrophils. Also, α-SMA-positive cells, a marker of the "myofibroblast," were observed in the stroma layer near the edge of the flap in these 2 corneas. A similar α-SMA expression pattern was reported in human corneas up to 6 years after LASIK surgery. Myofibroblasts expressing α-SMA are associated with a highly fibrotic wound phenotype characterized by a significant deposition of repair-type extracellular matrix, significant hypercellularity, and extensive repair tissue contraction. In the cornea, myofibroblasts are also associated with repair tissue opacity. When repair tissue deposition reaches its peak, the fibrotic phenotype and myofibroblast markers first begin to resolve. In a fully healed wound, there are few if any myofibroblasts. Long-term expression in some post-LASIK corneas emphasizes the chronic, ongoing nature of the fibrotic wound healing process.

Our study represents the first investigation, to our knowledge, of MMP expression in a series of clinically uneventful human LASIK refractive procedures. Reports of MMP expression following complicated LASIK procedures are also rare and consist mainly of case reports. Maguen et al studied 2 corneas that underwent a complicated LASIK procedure. They reported anterior stromal expression of MMP-1, MMP-2, and MMP-7 in the epithelium in a torn flap and an ectatic cornea. They did not report presence of MMPs following a single uneventful LASIK procedure performed several years earlier. This is consistent with our study as we found that MMPs are present only in corneas with specific histopathologic findings.

In the absence of keratoconus or forme fruste keratoconus, progressive post-LASIK keratectasia might be the result of biomechanical instability or a chronic disease process with progressive enzymatic degradation. An imbalance in proteolytic breakdown and repair could lead to a progressive stromal melting. The spatial localization of MMPs at the wound margin seen in our study does not support a role in ectasia, a process that primarily involves central changes in the post-LASIK cornea. On the other hand, epithelial ingrowth and cicatrization changes at the flap edges could relate to reduced resistance of some LASIK corneas to shearing trauma, resulting in late flap displacements.

In conclusion, to our knowledge, this is the first study to report that MMP proteins can be detected in some post-LASIK corneas even after many years. The presence of MMPs correlates with an ongoing, highly localized wound healing process at the flap margin, associated with minor post-LASIK complications. We cannot say with certainty whether epithelial ingrowth, flap retraction, or the ongoing repair process this signifies is the cause or the consequence of MMP presence. However, we propose that minor defects in the surgically created flap might interfere with perfect alignment with the underlying cornea following surgery. This would predispose to slippage, creating space for epithelial ingrowth and a chronic requirement for fibrotic repair. Like other chronic repair processes, MMP expression might become excessive because of ever-amplifying feedback loops, and this could gradually evolve into a contributing factor in failure to heal. In such situations, judicious and timely use of appropriate MMP inhibitors might provide some benefit, inhibiting epithelial ingrowth and enabling fibrotic repair tissue to accumulate sufficiently to "tack" the flap in place.

Submitted for Publication: February 25, 2007; final revision received June 3, 2007; accepted June 10, 2007.

Correspondence: M. Elizabeth Fini, PhD, McKnight Vision Research Center, Bascom Palmer Eye Institute, Miller School of Medicine, University of Miami, 1638 NW 10th Ave, Miami, FL 33136 (efini@med.miami.edu).

Author Contributions: Dr Fournie and Mr Gordon are co–first authors and contributed equally.

Financial Disclosure: None reported.

Funding/Support: This work was supported by National Eye Institute grants R01-EY012651 (Dr Fini), P30-EY014801 (Dr Fini), and R01-EY00933 (Dr Edelhauser), unrestricted grants from Research to Prevent Blindness (to University of Miami and Emory University), a Senior Scientific Investigator Award (Dr Fini), and the Walter G. Ross Foundation (Dr Fini).

References


