Slitlamp, Specular, and Light Microscopic Findings of Human Donor Corneas After Laser-assisted In Situ Keratomileusis

V. Vinod Mootha, MD; Dan Dawson, MD; Amit Kumar, MD; Joel Gleiser, MD; Clifford Qualls, PhD; Daniel M. Albert, MD

Objective: To examine slitlamp, specular, and light microscopic features of human donor corneas known to have undergone laser-assisted in situ keratomileusis (LASIK).

Methods: Twenty-six donor corneas known to have undergone LASIK prospectively underwent slitlamp examination with particular attention to the presence of a flap edge, as well as specular microscopy with particular attention to the presence of highly reflective particles in the stroma corresponding to the LASIK interface. Central endothelial cell density and pachymetry were obtained. They were compared with 26 control donor corneas without LASIK. Eleven LASIK donor corneas were processed for histology. Twenty-six donor corneas with no known prior keratorefractive surgery also underwent similar slitlamp examination and specular microscopy to serve as controls.

Results: Twelve (46%) of 26 LASIK donor corneas had an obvious flap edge, and 10 (39%) had a subtle flap edge by slitlamp examination. Four (15%) had infiltrates by slitlamp examination, of which 3 were confirmed by histopathologic examination. Highly reflective particles were seen by specular microscopy in the stroma of 23 (88%) of 26 LASIK donor corneas, but only 1 (4%) of 26 control donor corneas had a single highly reflective particle in the stroma (P<.001). The mean central endothelial cell counts were similar: 2138 cells/mm² in the LASIK group compared with 2250 cells/mm² in the controls (P=.39). Vacuolization and pyknosis of keratocytes was a consistent histopathologic finding after LASIK. Metallic particles at the interface were not detected by histology.

Conclusions: Detection of a flap edge by slitlamp examination may detect at least half of the donor corneas that may have undergone LASIK. The detection of highly reflective stromal particles may form an effective basis for screening for LASIK donor corneas using specular microscopy and requires further study.


IN 2001, THE 80 EYE BANKS OF THE Eye Bank Association of America provided tissue for 46532 corneal transplantation procedures. With millions of procedures already performed in the United States, the increasing popularity of laser-assisted in situ keratomileusis (LASIK) threatens the donor cornea pool. Corneas that have had any previous refractive surgery are not suitable for transplantation because of structural and optical compromise to the tissue. Michaeli-Cohen et al described 2 patients who underwent penetrating keratoplasty by surgeons who were unaware that the donor eyes had previous LASIK. In one of these cases, separation of the corneal lamellae was noted at the time of surgery.

Eye banks rely primarily on the medical and social history interview to detect donors who have had previous refractive surgery. New screening techniques are required to protect the donor cornea pool.

The in vivo confocal microscope consistently demonstrates the presence of variable reflectivity particles in the corneal interfaces of all patients after LASIK. Pisella et al speculate that the highly reflective interface particles consist of metallic particles originating from the microkeratome blade and the less reflective particles may represent cellular debris or plastic particles. Gokmen et al used the presence of the highly reflective particles found in all examined patients with the in vivo confocal microscope as a basis to measure the thickness of the LASIK flap. In an-
other recent confocal microscope study,12 highly reflective particles were found in the anterior stroma in 78 of 85 confocal scans performed after LASIK. Like the confocal microscope, the specular microscope is a reflected light microscope. The specular microscope is readily available at eye banks and is used to study the endothelial cell count density and morphologic structure in donor corneas. This study attempted to determine the usefulness of the specular microscope to detect the presence of the highly reflective particles in the region of the central stroma corresponding to an interface in donor corneas known to have had LASIK. To our knowledge, this study is the first to examine the endothelium of donor corneas after LASIK surgery. The specular microscope was also used to assess central endothelial cell density and central corneal pachymetry measurements. This study also attempted to determine the usefulness of the slitlamp to identify the LASIK flap edge in postmortem eyes.

In this study, we looked at the histologic findings of 11 of these LASIK donor corneas. One other histologic study13 of human corneas after successful LASIK included only 4 corneas. Other reports of histologic changes in human corneas involved corneal button specimens after penetrating keratoplasty for LASIK-related complications14−16 or phthisical blind eyes in which the procedure was performed just before enucleation.17

**METHODS**

This project was approved by the Human Research Review Committee of The University of New Mexico Health Sciences Center. With use of the medical and social history interview, participating eye banks of Tissue Banks International (Baltimore, Md) identified potential donor corneas that had undergone LASIK. After consent for research was obtained, the corneas were removed by in situ excision and were placed in vials with Optisol-GS corneal storage medium (Chiron Ophthalmics, Irvine, Calif) by eye bank technicians using established protocols of the Eye Bank Association of America.18 Central endothelial cell counts were obtained by the participating eye bank responsible for harvesting the cornea. The corneas were sent to the New Mexico Lions Eye Bank, Albuquerque, using protocols established by the Eye Bank Association of America.19 The donor corneas were stored at 4°C until further examination.

Twenty-six donor corneas known to have undergone LASIK prospectively underwent slitlamp examination with particular attention to the presence of a flap edge. A cornea fellowship-trained investigator (V.V.M.) used the slitlamp examination to rate the LASIK flap edge as “obvious,” “subtle,” or “absent.” The LASIK donor corneas with an absent flap edge were confirmed to have had the refractive surgery by histopathologic examination. These corneas then underwent thorough specular microscopy with the Konan Eyebank Keratoanalyzer (Konan Medical, Tokyo, Japan) with attention to the presence of highly reflective particles in the region of the stroma that would correspond to an interface. The field of view of the specular microscope used in this study was 0.2 × 0.4 mm. The vial with the cornea positioned with its endothelial surface down was placed in the vial adapter and chamber holder assembly. The z-axis knob adjustment was used to focus on the central endothelium. Endothelial cell count measurements (using the center method technique with the built-in software of the Keratoanalyzer) were obtained in each donor cornea if not obtained at the harvesting eye bank. The coefficient of variation (standard deviation of the endothelial cell area divided by the mean cell area ×100) and percentage of hexagonal cells were calculated by the software of the Keratoanalyzer specular microscope. Central pachymetry measurements were obtained on each cornea optically by focusing from the endothelium to the epithelium using the z-axis knob. The z-axis knob was used to search for highly reflective central stromal particles starting from a depth of 150 µm from the endothelium. The paracentral stroma was examined by use of the x-axis and y-axis knobs on the microscope platform. The central stroma was examined at 25-µm increments toward the epithelium. The specular microscope was sharply focused on any highly reflective particle in the stroma, and its depth from the endothelium was noted. The maximum number of particles in any field was counted and compared with the controls, an absent flap edge was observed in 26 donor corneas. Twenty-six donor corneas already present at the New Mexico Lions Eye Bank with no known history of prior keratorefractive surgery underwent similar slitlamp examination and specular microscopy to serve as controls.

Eleven LASIK donor corneas were placed in 10% neutral buffered formalin and processed for permanent sections. Five-micron serial sections from the center of the donor corneas were obtained. They were examined under light microscopy (original magnification, ×20 to ×400) after being stained with hematoxylin-eosin and periodic acid–Schiff.

The refractive data of the LASIK donors, including the preoperative manifest refraction, preoperative pachymetry, date of LASIK, microkeratome used, loose flap, and amount of ablation, were obtained if possible. The collected data were entered into an Excel spreadsheet (Microsoft Corp, Redmond, Wash), and analyses were performed with SAS statistical software, version 8.2 (SAS Institute, Cary, NC).

**RESULTS**

Demographic information on the LASIK donor corneas and controls is given in **Table 1**. The mean donor age among the LASIK corneas (50 years) was younger than that among the controls (66 years) (P < .001). On average, specular microscopic examination of the endothelium occurred 4.8 days after death in the LASIK group compared with 2.5 days in the controls (P = .01). The available preoperative refractive and operative data on the LASIK donor corneas are given in **Table 2**.

Slitlamp examination of the LASIK donor corneas revealed that 12 (46%) of 26 had an obvious flap edge (**Figure 1A**), 10 (39%) had a subtle LASIK flap edge (**Figure 1B**), and 4 (15%) had an absent flap edge. Among the controls, an absent flap edge was observed in 26 (100%) (P < .001 compared with controls, Fisher exact test). Four donor corneas (15%) had evidence of infiltrates by slitlamp examination.

**Table 1. Demographics of Laser-assisted In Situ Keratorefractive Surgery (LASIK) Donor Corneas and Controls**

<table>
<thead>
<tr>
<th>Demographic</th>
<th>LASIK (n = 26)</th>
<th>Controls (n = 26)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female ratio</td>
<td>16:10</td>
<td>12:14</td>
<td>.40</td>
</tr>
<tr>
<td>Age, mean, y</td>
<td>50</td>
<td>66</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Death to preservation time, h</td>
<td>11.7</td>
<td>8.8</td>
<td>.10</td>
</tr>
<tr>
<td>Days after death of specular microscopy</td>
<td>5.8</td>
<td>6.0</td>
<td>.90</td>
</tr>
<tr>
<td>of stroma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days after death of specular microscopy of endothelium</td>
<td>4.8</td>
<td>2.5</td>
<td>.01</td>
</tr>
</tbody>
</table>

*All t tests except for Fisher exact test of sex.
Specular microscopy findings of the LASIK donor corneas and controls are presented in Table 3 and Table 4. The maximum number of highly reflective particles observed in any observed field in 26 LASIK donor corneas was as follows: none (0 particles), 3 (12%); mild (1-2 particles), 10 (39%); moderate (3-6 particles), 12 (46%); and severe (≥7 particles), 1 (4%). Twenty-three (88%) of 26 LASIK donor corneas revealed highly reflective stromal particles in the region of the interface (Figure 2). Only 1 control cornea (4%) had an isolated highly reflective intrastromal particle. The particles were found at a mean±SD minimal depth of 30.3±95 µm to a maximum depth of 362±101 µm from the endothelium. The endothelial cell density was 2138 cells/mm² in the LASIK corneas compared with 2250 cells/mm² in the control group (P=.39 [P=.41 adjusted for age]) (Figure 3). The coefficient of variation was 42 in the LASIK corneas and 33 in the controls (P<.001). The percentage of hexagonal cells was 45% in the LASIK corneas compared with 55% in the controls (P=.001) (Table 3). The mean central pachymetry measurement was 506 µm in the LASIK group compared with 528 µm in the control group (P=.06).

Histopathologic results are summarized in Table 5. Pathological findings confirmed the presence of a lamellar flap in all examined LASIK donor corneas. All interface scars showed vacuolization and pyknosis of keratocytes along the lamellar cut (Figure 4). Metallic particles were not found on the serial sections taken from the center of the donor corneas in any of the examined interfaces. Epithelial facets (minor epithelial down-growth) were occasionally seen at the entrance to the lamellar cut. Three of the 4 donor corneas that had peripheral infiltrates had evidence of nongranulomatous inflammatory anterior stromal infiltrates. One cornea stained for gram-positive cocci on the surface epithelium by Brown-Brenn stain and another cornea with Gomori methenamine silver stained positive for a small number of yeast organisms on the surface epithelium. The endothelium was unremarkable in all examined cases except for 1 pair of donor corneas with decreased numbers of endothelial cells.

**COMMENT**

Until better screening technology is developed and implemented by eye banks, the slitlamp and specular microscopy findings of the LASIK donor corneas and controls are presented in Table 3 and Table 4. The maximum number of highly reflective particles observed in any observed field in 26 LASIK donor corneas was as follows: none (0 particles), 3 (12%); mild (1-2 particles), 10 (39%); moderate (3-6 particles), 12 (46%); and severe (≥7 particles), 1 (4%). Twenty-three (88%) of 26 LASIK donor corneas revealed highly reflective stromal particles in the region of the interface (Figure 2). Only 1 control cornea (4%) had an isolated highly reflective intrastromal particle. The particles were found at a mean±SD minimal depth of 30.3±95 µm to a maximum depth of 362±101 µm from the endothelium. The endothelial cell density was 2138 cells/mm² in the LASIK corneas compared with 2250 cells/mm² in the control group (P=.39 [P=.41 adjusted for age]) (Figure 3). The coefficient of variation was 42 in the LASIK corneas and 33 in the controls (P<.001). The percentage of hexagonal cells was 45% in the LASIK corneas compared with 55% in the controls (P=.001) (Table 3). The mean central pachymetry measurement was 506 µm in the LASIK group compared with 528 µm in the control group (P=.06).

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The results of this study suggest that 46% (12/26) of LASIK donor corneas have an obvious flap edge. In this unmasked study, 39% (10/26) of the LASIK donor corneas had a subtle flap edge and 15% (4/26) had no detectable flap edge; these would probably be missed by routine slitlamp examination. Eye bank technicians, however, must be trained to recognize a LASIK flap. Retroillumination slitlamp techniques are useful in the detection of a LASIK flap. In this study, clear cornea cataract wounds, astigmatic keratotomy scars, arcus senilis changes, and posterior embryotoxon changes were seen that could mimic a LASIK flap to an inexperienced slitlamp observer. Careful slitlamp examination of the endothelial surface can help distinguish a clear corneal wound from a LASIK flap. Table 3. Specular Microscopy Results

<table>
<thead>
<tr>
<th>Finding</th>
<th>LASIK (n = 26)</th>
<th>Controls (n = 26)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of highly reflective stromal particles</td>
<td>23 (88)</td>
<td>1 (4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Presence of less reflective stromal particles</td>
<td>2 (8)</td>
<td>2 (9)</td>
<td>.67</td>
</tr>
<tr>
<td>Presence of clear stroma</td>
<td>1 (4)</td>
<td>21 (81)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Endothelial cell density, cell/mm²</td>
<td>2138</td>
<td>2250</td>
<td>.39</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>42</td>
<td>33</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Percentage of hexagonal cells, %</td>
<td>45</td>
<td>55</td>
<td>.001</td>
</tr>
<tr>
<td>Pachymetry, mean ± SD, µm</td>
<td>506 ± 80</td>
<td>528 ± 51</td>
<td>.06</td>
</tr>
</tbody>
</table>

*Data are number (percentage) unless otherwise indicated.
†Comparison of laser-assisted in situ keratomileusis (LASIK) donor cornea with controls, Fisher exact test for binary data and t-tests for continuous data.

Figure 2. A, Specular microscopy image focused 255 µm from the endothelium reveals a severe amount of highly reflective stromal particles. This 47-year-old donor cornea underwent laser-assisted in situ keratomileusis (LASIK) 9 months before death. The field of view is 0.2 × 0.4 mm. B, A more representative specular micrograph of highly reflective stromal particles. The maximum number of highly reflective particles observed was 3 particles, found a distance of 489 µm from the endothelium. This 47-year-old donor had LASIK 2 years before death. The particles range in size from approximately 5 to 20 µm.

Figure 3. A, Specular micrograph of endothelium from a 46-year-old laser-assisted in situ keratomileusis (LASIK) donor cornea 1 day after death. Cell density and morphologic structure appear normal. B, Specular micrograph of a 45-year-old LASIK donor cornea endothelium 5 days after death. Moderate and diffuse changes are found in cell appearance and morphologic structure, with notable distortion of hexagonal shape and scattered areas of blackening consistent with endothelial cell edema.

Table 4. Highly Reflective Intrastromal Particles in 26 Laser-assisted In Situ Keratomileusis Donor Corneas

<table>
<thead>
<tr>
<th>Finding</th>
<th>Mean ± SD (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum particles in any observed field</td>
<td>3.00 ± 2.56 (0-13)</td>
</tr>
<tr>
<td>Depth from endothelium, µm</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>303 ± 95 (145-539)</td>
</tr>
<tr>
<td>Maximum</td>
<td>362 ± 101 (167-558)</td>
</tr>
</tbody>
</table>


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The mean ± SD density of variable reflectivity particles (all particles, including highly reflective and less reflective) at the interface by in vivo confocal microscopy has been reported to be 504 ± 101 particles/mm² at 8 days after the LASIK procedure and decreases during the first 3 months after surgery to stabilize at 332 ± 100 particles/mm² at 1 week after the LASIK procedure and did not change significantly with time. In our investigations with the specular microscope, the density measurements of the highly reflective particles were not performed. However, the maximum number of highly reflective particles in any observed field (0.08 mm²) averaged only 3 particles. In comparison to the confocal microscope, the resolution of standard eye bank specular microscopes is far less. In our study, we were probably detecting only a small number of the most highly reflective of the variable reflectivity particles seen in vivo by the confocal microscope. The poorer resolution of the specular microscope may also account for why 12% (3/26) of the LASIK donor corneas had no detectable highly reflective stromal particles, despite a thorough search. Also, the eye bank specular microscope used in this study was designed for examination of a small area of the central corneal endothelium (0.2 × 0.4 mm) and may miss peripheral interface particles in a typical 8- or 9-mm LASIK flap.

Specular microscope reflectivity is a function of a change in index of refraction at a particular interface in the tissue examined. Air bubbles on the corneal surface, the interface between the epithelium and corneal storage medium, and epithelial irregularities or debris may induce specular reflectivity. Care must be taken not to mistake these artifacts for highly reflective particles within the substance of the stroma seen at the LASIK interface. Less reflective stromal debris is not a reliable indicator of a microkeratome cut, as this was occasionally seen in the control corneas. Interestingly, we cannot account for the presence of a single, highly reflective particle in the stroma of a control cornea from a 70-year-old female donor who died of cancer. The fellow cornea from this patient had a few highly reflective particles at the level of the epithelium.

In addition to evaluating the donor cornea endothelium, the specular microscope may be a useful instrument to screen for previous LASIK surgery by the detection of the highly reflective stromal particles corresponding to the interface. However, the manual search for highly reflective stromal particles can be tedious, as 50% (13/26) of the LASIK donor corneas had a mild
amount of particles or none. Masked studies are required to determine the true sensitivity and specificity of existing specular microscopes to screen for donor corneas that have had LASIK. The results of this study suggest that the detection of highly reflective interface debris may form an effective basis for screening using existing specular microscopes until better screening technologies are developed.

In vivo specular microscopic investigations in patients who have had LASIK have demonstrated no significant change in central endothelial cell counts. However, a study reported a transient endothelial dysfunction marked by increased pleomorphism with definite loss of hexagonality immediately after LASIK surgery. In our study, the mean central endothelial cell count density was slightly lower in the LASIK group (2138 cells/mm²) compared with the control donor corneas (2250 cells/mm²). This small difference was, however, not statistically significant even when the age discrepancy in the 2 groups was considered (P = .39 for age). However, the coefficient of variation (indicator of polymegathism) was slightly higher in the LASIK group at 42 compared with 33 in the controls (P < .001). The percentage of hexagonal cells (indicator of pleomorphism) was slightly lower in the LASIK group (45%) compared with the controls (55%) (P = .001). On average, the endothelium was analyzed with the specular microscope 4.8 days after death in the LASIK donor corneas compared with 2.5 days in the controls (P = .01). This 2-day delay was attributable to the processing and shipping time required to send the tissue from participating eye banks to the New Mexico Lions Eye Bank. This project was designed primarily for the specular microscopic examination of the stroma in donor corneas. Further studies with proper matching of age, death to preservation time, and delay before specular microscopy of the endothelium are required to assess the effects of LASIK surgery on central endothelial cell count density, morphologic structure, and viability.

Although the LASIK donor corneas were slightly thinner (506 µm) compared with the control corneas (528 µm), this difference was not statistically significant (P = .06). Pachymetry measurements with use of specular microscopy may not be as reliable as ultrasound technology. However, the pachymetry results in this study and the potential artifacts induced by epithelial defects and variable stromal edema of corneas in the storage media suggest that pachymetry using specular microscopy may not be an effective basis for screening for previous LASIK surgery.

The histologic findings on the LASIK donor corneas failed to identify metallic particles in any of the interfaces examined to be the source of the highly reflective particles seen by specular and confocal microscopy. However, the consistent histopathologic finding of vacuolization and pyknosis of keratocytes along the lamellar cut may account for some of the variable reflectivity particles seen by confocal and specular microscopy. The pyknotic nuclei may be related to the previous LASIK surgery, but postmortem changes cannot be ruled out. Vacuolization and pyknosis of keratocytes have been confirmed by other histologic studies and may account for the decreased keratocyte density in the stroma adjacent to the LASIK lamellar cut demonstrated by the in vivo confocal microscope. Further research is required to elucidate the nature and clinical significance of the keratocyte changes.

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Figure 4. Example of pyknosis and vacuolization of keratocytes in corneal interface (hematoxylin-eosin, original magnification ×400).
REFERENCES