Corneal Endothelial Cell Loss 9 Years After Excimer Laser Keratorefractive Surgery

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Objective: To determine the long-term changes in the corneal endothelium after laser in situ keratomileusis (LASIK) and photorefractive keratectomy (PRK).

Methods: Twenty-nine eyes (16 patients) received myopic LASIK or PRK, with intended correction to emmetropia. Central endothelial photographs were taken before and 9 years after surgery and were analyzed by the same masked investigator after appropriate calibration for magnification. Comparisons were made by using generalized estimating equation models to account for any correlation between fellow eyes of the same patient. The annual exponential rate of cell loss was compared with cell loss during a 10-year period in 42 normal (unoperated) corneas of 42 subjects.

Results: Endothelial cell density 9 years after LASIK and PRK had decreased by 5.3% from preoperative density ($P < .001$), whereas coefficient of variation of cell area ($P = .24$) and percentage of hexagonal cells ($P = .19$) did not change. The mean annual rate of cell loss after refractive surgery (0.6% [standard deviation, 0.8%]) was not different from that in normal corneas (0.6% [0.5%], $P = .88$; minimum detectable difference $= 0.5%$; $\alpha = .05$; $\beta = .20$).

Conclusions: Laser in situ keratomileusis and PRK had no long-term effect on the corneal endothelium. Corneas that have undergone LASIK or PRK can be considered for use as donors for posterior lamellar keratoplasty procedures.


PHOTOABLATION OF THE CORNEAL STROMA BY EXCIMER LASERS IS A METHOD OF CORRECTING REFRACTIVE ERRORS. LASER IN SITU KERATOMILEUSIS (LASIK) REQUIRES THE CREATION OF AN ANTERIOR CORNEAL FLAP AND PHOTOABLATION OF THE MIDSTROMA, WHEREAS PHOTOREFRACTIVE KERATECTOMY (PRK) INVOLVES EPITHELIAL REMOVAL AND ANTERIOR STROMAL PHOTOABLATION. THE LONG-TERM EFFECTS OF PHOTOABLATION ON EPITHELIAL AND STROMAL THICKNESS,1 KERATOCYTE DENSITY,2 AND CORNEAL NERVE REGENERATION3 ARE KNOWN, BUT THE LONG-TERM EFFECT ON THE CORNEAL ENDOTHELIUM HAS NOT BEEN REPORTED. THERE HAVE BEEN CONFLICTING REPORTS OF THE EFFECT OF PHOTOABLATION ON THE CORNEAL ENDOTHELIUM IN THE SHORT-TERM, WITH MOST STUDIES FINDING NO EFFECT,4-11 THOUGH A FEW STUDIES HAVE INDICATED ENDOTHELIAL CELL LOSS HIGHER THAN AGE-RELATED PHYSIOLOGIC CELL LOSS,12-14 POSSIBLY BECAUSE OF MECHANICAL TRAUMA FROM SHOCKWAVES, LOCAL OXIDATIVE CHANGES, OR THERMAL EFFECTS.15-18

In this study, we examined changes in the corneal endothelium 9 years after excimer laser keratorefractive surgery and made comparisons with changes in the normal (unoperated) corneal endothelium during a 10-year period. In addition, we explored the relationships between endothelial cell loss and ablated and residual bed thicknesses.

METHODS

SUBJECTS

Twenty-nine eyes of 16 patients that had endothelial photographs taken before myopic keratorefractive surgery at Mayo Clinic between July 1998 and January 1999 were reexamined with endothelial photography 9 years later. Twenty eyes of 10 patients received LASIK, and 9 eyes of 6 patients received PRK. Exclusion criteria included diabetes mellitus or other significant systemic disorders, glaucoma or ocular hypertension (> 22 mm Hg), use of any ocular medications, use of any systemic medications known to have adverse effects on the cornea, and any ocular surgery (with the exception of refractive surgery enhancement procedures) in the period between examinations. A LASIK enhancement procedure was performed in 8 eyes of 5 patients for undercorrection, and a PRK enhancement procedure was performed in 1 eye.

For patients undergoing LASIK, mean age at surgery was 34 years (standard deviation [SD], 8 years; range, 23-47 years) and mean pre-
operative spheroequivalent refractive error was −6.2 diopters (D) (SD, 1.4 D; range, −4.0 to −9.25 D). Eight of the 10 patients were current contact lens wearers, whereas the other 2 patients had worn contact lenses in the past. For patients undergoing PRK, mean age at surgery was 39 years (SD, 6 years; range, 31–44 years) and mean preoperative spheroequivalent refractive error was −3.5 D (SD, 1.7 D; range, −1.25 to −5.75 D). Five of the 6 patients were current contact lens wearers and the other patient had worn contact lenses in the past.

This study adhered to the tenets of the Declaration of Helsinki and was approved by the Mayo Clinic institutional review board. Informed consent was obtained from all subjects after explanation of the nature and possible consequences of the study.

REFRACTIVE SURGERY PROCEDURES

For LASIK, the flap was created by using a mechanical microkeratome (Hansatome; Bausch & Lomb, Rochester, New York), with a flap diameter of 8.5 mm or 9.5 mm and an intended thickness of 180 µm. For PRK, the epithelium was removed by using the laser-scratch technique. For both LASIK and PRK, the stroma was ablated with a VISX Star excimer laser (VISX, Santa Ana, California). Emmetropia was attempted in all cases by using an ablation zone that ranged from 6.0 × 6.0 mm for spherical corrections to 4.5 × 6.0 mm for some astigmatic corrections.

ENDOTHELIAL CELL ANALYSIS

Before surgery, the central corneal endothelium was photographed with a noncontact specular microscope (Konan Non-Robo SP8000; Konan Medical Inc, Hyogo, Japan). With the patient’s head stabilized by using the chin and forehead rests, the observer aligned the microscope with the center of the cornea and used the automatic function to capture a focused image of the central endothelium. Images were stored to digital media. Images consisted of 640 × 480 pixels (horizontal × vertical), which corresponded to a field size of 284 × 364 µm (0.103 mm²) based on calibration measurements of a micrometer slide.

Nine years after surgery, the endothelium of the same cornea was photographed by using a contact in vivo confocal microscope (ConfoScan 4; Nidek Technologies, Greensboro, North Carolina). With the patient’s head stabilized, the observer aligned the objective lens, which was coated with optical coupling medium (GenTeal Gel; Novartis Pharmaceuticals Corp, East Hanover, New Jersey), with the center of the cornea. Digital images of the central endothelium were recorded. Images consisted of 768 × 576 pixels and were rescaled to 640 × 480 pixels for analysis; image field size was 434 × 320 µm (0.139 mm²) based on calibration measurements of the same micrometer slide used to calibrate the specular microscope.

Digital images were transferred to an image analysis system (KSS-400; Konan Medical USA, Torrance, California), and the center of each endothelial cell was digitized by 1 observer who was masked to the identity of the images. Fifty to 100 cells were counted in each endothelial photograph depending on image quality. The analysis program calculated the mean cell area and its reciprocal, endothelial cell density (ECD), the coefficient of variation (standard deviation ÷ mean) of cell area, and the percentage of hexagonal cells.

MEASUREMENT OF ABLATED AND RESIDUAL BED THICKNESSES

A Tandem Scanning Confocal Microscope (Tandem Scanning Corporation, Reston, Virginia) was used to examine corneas in vivo before and at 1 month after surgery, as described in detail previously.19 All confocal scans were manually reviewed, and scans with the least lateral ocular movement and with no anteroposterior movement of the cornea relative to the objective were selected for analysis. An intensity profile of backscattered light was generated from the confocal images of the selected scan.30,31 Peaks in the light intensity profiles of preoperative and postoperative corneas corresponded to the superficial epithelium, the endothelium, the subbasal nerve plexus, and the most anterior keratocytes.31,22,23 The video image corresponding to each intensity peak was displayed. Profiles generated from corneas after LASIK also showed a peak corresponding to the lamellar interface, and this was typically confirmed by the presence of interface debris in the corresponding video image.32

The thickness of the ablated tissue was calculated as the difference between preoperative and postoperative stromal thickness. Before PRK, stromal thickness was defined as the distance from the subepithelial plexus to the endothelium (and therefore included the Bowman layer and Descemet membrane); after PRK, and before and after LASIK, stromal thickness was defined as the distance from the most anterior keratocyte to the endothelium (after PRK, the Bowman layer was not present; and after LASIK, the Bowman layer remained unchanged). Residual bed thickness was defined as the distance between the most anterior keratocyte and the endothelium after PRK and as the distance between the lamellar interface and the endothelium after LASIK.

STATISTICAL ANALYSIS

Corneal ECD, coefficient of variation of cell area, and the percentage of hexagonal cells were compared before and 9 years after surgery. Comparisons were made by using generalized estimating equation models to adjust for any correlation between fellow eyes of the same patient.21 P < .05 was considered statistically significant.

We calculated the annual rate of corneal endothelial cell loss by assuming cell loss occurred as a first-order exponential process according to the following relationship: \( ECD_t = ECD_0 e^{-kt} \), in which \( ECD_t \) is ECD before surgery, \( ECD_0 \) is ECD at 9 years after surgery, \( k \) is an exponential rate constant, and \( t \) is the time between examinations in years. We compared the annual exponential rate of cell loss after keratorefractive surgery with cell loss in 42 normal corneas that had not been operated on from 42 subjects who were examined twice during a 10-year period, as previously reported.21 At the first examination, the normal subjects’ mean age was 50 years (SD, 17 years; range, 20–74 years); during the 10 years between repeat examinations, none of the normal subjects wore contact lenses or developed diabetes, and none had any ocular surgery.21 In the previous article,21 because the exponential rate constants (\( k \)) were small, they approximated and were used as the annual exponential rates of cell loss (this approximation gives an error of 0.001% per year when the rate of decline is 0.5% per year).

Correlations between the percentage of endothelial cell loss at 9 years after refractive surgery and the preoperative spheroequivalent refractive error, measured ablation, and residual bed thicknesses at 1 month after surgery were assessed by using the Pearson correlation coefficient if the data were distributed normally and the Spearman test if they were not; significances were completed by using generalized estimating equation models. Data are presented as mean (SD) unless otherwise indicated.

RESULTS

For all 29 eyes, ECD was 5.3% lower at 9 years after refractive surgery than preoperative ECD (\( P < .001 \)),

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### Table 1. Endothelial Cell Density and Morphology Before and After Keratorefractive Surgery

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (SD), Cells/mm²</th>
<th>MDD</th>
<th>P Value</th>
<th>Coefficient of Variation of Cell Area</th>
<th>Mean (SD)</th>
<th>MDD</th>
<th>P Value</th>
<th>% of Hexagonal Cells</th>
<th>Mean (SD)</th>
<th>MDD</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Eyes (n=29)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Preoperative</td>
<td>2837 (336)</td>
<td>&lt;.001</td>
<td></td>
<td>0.34 (0.05)</td>
<td>0.03</td>
<td>.24</td>
<td></td>
<td>54 (8)</td>
<td>5.7</td>
<td>.19</td>
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<tr>
<td>At 9 years</td>
<td>2685 (351)</td>
<td></td>
<td></td>
<td>0.33 (0.03)</td>
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<td></td>
<td></td>
<td>56 (5)</td>
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<tr>
<td>LASIK Eyes (n=20)</td>
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</tr>
<tr>
<td>Preoperative</td>
<td>2925 (303)</td>
<td>&lt;.001</td>
<td></td>
<td>0.33 (0.05)</td>
<td>0.04</td>
<td>.98</td>
<td></td>
<td>55 (9)</td>
<td>7.4</td>
<td>.77</td>
<td></td>
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<tr>
<td>At 9 years</td>
<td>2741 (308)</td>
<td></td>
<td></td>
<td>0.33 (0.03)</td>
<td></td>
<td></td>
<td></td>
<td>56 (5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRK Eyes (n=9)</td>
<td></td>
<td></td>
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<tr>
<td>Preoperative</td>
<td>2641 (340)</td>
<td>.45</td>
<td>.98</td>
<td>0.36 (0.03)</td>
<td>0.009</td>
<td></td>
<td></td>
<td>51 (5)</td>
<td>.01</td>
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<td></td>
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<tr>
<td>At 9 years</td>
<td>2559 (423)</td>
<td></td>
<td></td>
<td>0.32 (0.04)</td>
<td></td>
<td></td>
<td></td>
<td>58 (5)</td>
<td></td>
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</tr>
</tbody>
</table>

Abbreviations: LASIK, laser in situ keratomileusis; MDD, minimum detectable difference for nonsignificant comparisons (α=.05, β=.20); PRK, photorefractive keratectomy.

a Comparisons between preoperative and 9-year measurements were made by using generalized estimating equation models to account for any correlation between fellow eyes of the same patient.

### Table 2. Endothelial Parameters at 9 Years After LASIK in 20 Eyes With or Without an Enhancement Procedure

<table>
<thead>
<tr>
<th>Enhancement</th>
<th>No. of Eyes</th>
<th>Endothelial Cell Density</th>
<th>Coefficient of Variation of Cell Area</th>
<th>% of Hexagonal Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (SD), Cells/mm²</td>
<td>MDD</td>
<td>P Value</td>
</tr>
<tr>
<td>No enhancement</td>
<td>12</td>
<td>2670 (340)</td>
<td>410</td>
<td>.89</td>
</tr>
<tr>
<td>Enhancement</td>
<td>8</td>
<td>2847 (234)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: LASIK, laser in situ keratomileusis; MDD, minimum detectable difference for nonsignificant comparisons (α=.05, β=.20).

a Comparisons were made by using generalized estimating equation models to account for any correlation between fellow eyes of the same patient.

whereas no differences were found for coefficient of variation of cell area or percentage of hexagonal cells (Table 1). In eyes that underwent LASIK, ECD decreased by 6.3% (P<.001), and in eyes that underwent PRK, coefficient of variation of cell area (P=.009) and percentage of hexagonal cells (P=.01) improved during the 9-year period. At 9 years after LASIK, ECD in eyes that underwent an enhancement procedure did not differ from that in eyes that did not (Table 2).

For all 29 eyes after refractive surgery, the mean annual rate of endothelial cell loss was 0.6% (0.8%; range, –1.1% to 2.2%). This did not differ significantly from the mean annual rate of cell loss in 42 normal (unoperated) corneas of 42 adults (0.6% [0.5%]; P=.88; minimum detectable difference = 0.5%; α=.05; β=.20). Mean coefficient of variation of cell area at the first examination of the normal corneas (0.26 [0.05]) was lower than the corneas at 9 years after refractive surgery (0.33 [0.05]; P<.001), and percentage of hexagonal cells at the first examination of the normal corneas (67% [8%]) was higher than that in the corneas at 9 years after refractive surgery (56% [5%]; P<.001).

Mean measured ablation depth in eyes that underwent surgery was 55 µm (35 µm) and residual bed thickness was 329 µm (55 µm) (Table 3). There was no correlation between the percentage of endothelial cell loss from preoperative and preoperative spherov equivalent refractive error (r=0.01, P=.96), measured ablation (r=0.17, P=.39), or residual bed thickness (r=−0.25, P=.20).

### Table 3. Measured Ablation and Residual Bed Thicknesses After LASIK and PRK Measured by Confocal Microscopy In Vivo

<table>
<thead>
<tr>
<th>Measurement</th>
<th>All Eyes (n=29)</th>
<th>LASIK (n=20)</th>
<th>PRK (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual bed</td>
<td>329 (55)</td>
<td>302 (31)</td>
<td>389 (49)</td>
</tr>
<tr>
<td>Measured ablation</td>
<td>55 (35)</td>
<td>66 (34)</td>
<td>31 (19)</td>
</tr>
</tbody>
</table>

Abbreviations: LASIK, laser in situ keratomileusis; PRK, photorefractive keratectomy.

The annual rate of endothelial cell loss after LASIK and PRK did not differ from the annual rate of age-related endothelial cell loss in normal, unoperated corneas. Although our study was small, we had sufficient statistical power to detect a 0.5% difference in the rate of cell loss between these 2 groups of eyes. Our results support the findings of numerous short-term studies that found no significant endothelial cell loss after LASIK and PRK. There have been few studies that have examined endothelial cell loss at 5 or more years after excimer photoablative surgery. Kato et al noted a 1.2% cell loss at 5 years after LASIK and indicated this was
within physiologic age-related cell loss. We did not find a relationship between endothelial cell loss and either the thickness of ablated tissue or the residual bed thickness, indicating that the deeper stromal ablations with LASIK than with PRK did not affect the endothelium. A safe residual bed thickness was respected in all cases, which probably protects the endothelium, as demonstrated by animal studies in which endothelial cell loss only occurred when the corneal stroma was photoablated within 40 µm of the Descemet membrane.

We did not find changes in endothelial cell morphology when we combined data from eyes that received LASIK or PRK, but we did notice an improvement in the coefficient of variation of cell area and percentage of hexagonal cells in the small number of eyes that underwent PRK. While the improvement in morphology after PRK could be attributed to cessation of contact lens wear, we would have expected a similar improvement in the eyes that underwent LASIK, because most of these patients had also worn contact lenses prior to surgery. Contact lens wear is known to induce morphologic changes in the corneal endothelium without affecting cell density, but it is not known how quickly, if at all, the morphologic changes reverse after cessation of lens wear. Improvement in endothelial cell morphology has been described after LASIK, though Collins et al were unable to associate this with the cessation of contact lens wear in a multivariate analysis.

The importance of the findings in our study relates to using corneas that have undergone LASIK or PRK as donor tissue. When keratorefractive surgery started to increase in popularity, the Cornea Donor Study was initiated in the United States to address the concern that the supply of donor tissue would diminish because donor corneas would be unusable for penetrating keratoplasty after refractive surgery. While the latter was clearly a concern for penetrating keratoplasty, in recent years, posterior lamellar keratoplasty techniques (such as Descemet-stripping with endothelial keratoplasty) have become the preferred method for treating corneal endothelial dysfunction. Because posterior lamellar keratoplasty does not involve transplantation of the anterior cornea, eye banks in the United States do accept donor corneas that have had excimer laser keratorefractive surgery for posterior lamellar keratoplasty. Our finding of no difference in endothelial cell loss after keratorefractive surgery compared with normal eyes suggests that corneas after keratorefractive surgery should be suitable for posterior lamellar keratoplasty. The increased polymegathism and pleomorphism that we found in corneas after refractive surgery compared with normal corneas that have not been operated on could result from a small sample size or might represent increased stress to the endothelium related to prior contact lens wear. Nevertheless, eye banks do not exclude potential corneal donors because of increased polymegathism or pleomorphism or a history of contact lens wear. Although none of the eyes that underwent LASIK in our study had flaps created with a femtosecond laser, femtosecond laser preparation of donor tissue for posterior lamellar keratoplasty has not been associated with endothelial cell loss; nevertheless, long-term data of endothelial cell loss after femtosecond laser-assisted LASIK are warranted to ensure that the combination of femtosecond and excimer laser energy are not detrimental to the endothelium. Whether eye banks should accept corneas that have had previous PRK with adjuvant mitomycin C is unknown; some studies have indicated no effect of PRK with mitomycin C on the endothelium, whereas other studies have found detrimental effects, including increased endothelial cell loss. However, determining which potential donor corneas have had PRK is a challenge for eye banks. The major limitation of our study was the small number of eyes available for analysis. Nevertheless, we had adequate statistical power to be confident that cell loss after refractive surgery is no greater than physiologic age-related cell loss. Our enhancement data were limited by sample size and statistical power, and no conclusions can be drawn from our data. Although we used specular microscopy to photograph the corneal endothelium before surgery and confocal microscopy after surgery, we previously reported that careful calibration of both instruments for image magnification resulted in interchangeable data. With both specular and confocal microscopy, only a very small proportion of the corneal endothelium can be visualized, introducing variation of repeated cell density measurements and limiting the analysis to 1 region of the cornea (central cornea in this study). Isager et al found that magnification of specular microscopes decreased less than 1% (for most cases of excimer refractive surgery) with decreased corneal thickness, which could result in an overestimation of postoperative ECD. We did not adjust our data for corneal thickness, but if we had, the cell loss at 9 years would have been approximately 0.5% more than indicated, which would not have altered any of our conclusions.

In summary, corneal endothelial cell loss at 9 years after LASIK and PRK (without mitomycin C) did not differ from age-related cell loss found in normal corneas. Eye banks and surgeons can consider donor corneas that have had LASIK or PRK for posterior lamellar keratoplasty.

Submitted for Publication: January 6, 2009; final revision received February 16, 2009; accepted February 20, 2009.
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Financial Disclosure: None reported.
Funding/Support: This study was supported by grant EY 02037 from the National Institutes of Health, by Research to Prevent Blindness Inc (Dr Patel as an Olga Keith Wiess Special Scholar, and an unrestricted grant to the Department of Ophthalmology, Mayo Clinic, Rochester), and by the Mayo Foundation.
Previous Presentation: This paper was presented in part at the European Association for Vision and Eye Research Annual Meeting, Portorož, Slovenia, October 4, 2008; and at the XXVI Congress of the European Society of Cataract and Refractive Surgeons, Berlin, Germany, September 13-17, 2008.
REFERENCES


