ABSTRACT

PURPOSE: To assess the effects of LASIK with the 15-kHz IntraLase femtosecond laser on corneal endothelium.

METHODS: In a prospective, single-center clinical trial, 138 patients (mean age: 32.0±7.1 years [range: 21 to 42 years]) underwent femtosecond LASIK for the correction of myopia −0.75 to −9.00 diopters (D) and cylinder up to 3.25 D. Patients were divided into two groups: contact lens group (n=76) and non-contact lens group (n=62). Pre- and 12-month postoperative specular microscopy of the central corneal endothelium was performed. The integrity of the central endothelium was assessed in terms of endothelial cell density and percentage of hexagonality.

RESULTS: In the contact lens group, mean endothelial cell density improved significantly from 3401±292 cells/mm² to 3587±262 cells/mm² (P<.001) with a mean increase of 5.5%. The percentage of hexagonal cells was statistically significantly higher after surgery (32.5±4.0%) compared with preoperative data (31.0±5.1%) (P=.035). No statistically significant differences were noted regarding mean endothelial cell density (P=.126) or hexagonality (P=.56) before and 1 year after femtosecond LASIK in the non-contact lens group.

CONCLUSIONS: Femtosecond LASIK to correct myopia was safe for the corneal endothelium. Improvement in mean endothelial cell density and percentage of hexagonality was observed in the contact lens group. 

The ultra-short–pulse femtosecond laser is a solid-state laser used to create the corneal flap in LASIK.1,2 The femtosecond laser has been used successfully in other corneal procedures including the creation of channels for intracorneal ring segments3 and the preparation of donor and host tissues for keratoplasty.4 Numerous studies have shown that LASIK performed with a mechanical microkeratome does not have any toxic effect on healthy corneal endothelium.5-10 Surgery candidates who wear contact lenses may show post-LASIK improvement in mean central corneal endothelial cell density and percentage of hexagonal cells, explained by the discontinuation of contact lens use after surgery.5 However, LASIK performed with a mechanical microkeratome in eyes with Fuchs endothelial dystrophy or corneal guttata may cause transient corneal edema and endothelial cell loss, leading to corneal decompensation.11-14 Hence, photorefractive keratectomy or thin-flap LASIK performed with the femtosecond laser have been proposed as safer alternatives for eyes at risk for developing corneal endothelial damage.15

The present study investigated the effects of femtosecond LASIK on the corneal endothelium in myopic patients 12 months after surgery. We believe this is the first report that evaluates the implications of femtosecond LASIK in maintaining the integrity of corneal endothelium.

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The authors have no financial or proprietary interest in the materials presented herein.

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SURGICAL TECHNIQUE
before surgery; otherwise the patient was considered a lens use for at least 16 hours per week during the year (tact lens group) only if he/she had a history of contact lens wear. A patient was considered a contact lens wearer (contact lens group) only if he/she had a history of contact lens wear. All patients had a stable refractive history for 2 years. Written informed consent was obtained from all patients before surgery in accordance with the Declaration of Helsinki. Institutional review board approval was not required. Exclusion criteria were previous ocular surgery, mesopic pupil diameter >7.0 mm, central corneal endothelial cell density <2250 cells/mm², corneal dystrophy, cataract, history of uveitis or retinal detachment, and glaucoma or intraocular pressure >21 mmHg. All patients discontinued contact lens use for at least 1 week before surgery.

Preoperatively, patients had a complete ophthalmologic examination including manifest and cycloplegic refractions, uncorrected (UDVA) and corrected (CDVA) distance visual acuity measurements, videokeratography, slit-lamp microscopy, Goldmann applanation tonometry, binocular indirect ophthalmoscopy, ultrasonic pachymetry, and endothelial cell analysis. One eye of each patient (right or left) was randomly assigned to be included in the analysis. Patients were divided into two groups depending on contact lens use. A patient was considered a contact lens wearer (contact lens group) only if he/she had a history of contact lens use for at least 16 hours per week during the year before surgery; otherwise the patient was considered a non-contact lens wearer (non-contact lens group).

STUDY DESIGN
This prospective, interventional study evaluated consecutive patients who underwent bilateral simultaneous femtosecond LASIK for myopia ranging from −0.75 to −9.00 diopters (D) and cylinder up to 3.25 diopters (D). All patients had a stable refractive history for 2 years. Written informed consent was obtained from all patients before surgery in accordance with the Declaration of Helsinki. Institutional review board approval was not required. Exclusion criteria were previous ocular surgery, mesopic pupil diameter >7.0 mm, central corneal endothelial cell density <2250 cells/mm², corneal dystrophy, cataract, history of uveitis or retinal detachment, and glaucoma or intraocular pressure >21 mmHg. All patients discontinued contact lens use for at least 1 week before surgery.

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SURGICAL TECHNIQUE
The femtosecond laser technique for flap creation has been described previously. All surgeries were performed by a single surgeon (G.M.) using a 15-kHz Intralase femtosecond laser (Abbott Medical Optics, Santa Ana, California) with a planned flap diameter of 9.0 mm and an attempted flap thickness of 100 µm. All flaps were created using a raster pattern with the hinge located in the superior position, raster energy 1.9 µJ/spot, spot separation 6 µm with interbeam separation of 11 µm, hinge angle 50°, side-cut angle 70°, and side-cut energy of 2.5 µJ. To decrease the occurrence of an opaque bubble layer in the interface, a pocket width of 2.6 mm was created. The corneal stromal ablation was performed with a Visx Star 2 excimer laser (Abbott Medical Optics), leaving a residual stromal bed of at least 250 µm in every eye.

Postoperative examinations were performed at 1, 3, and 7 days and 1, 3, 6 and 12 months, and included UDVA, CDVA, slit-lamp evaluation, and applanation tonometry. Postoperative endothelial cell analysis was performed at 12 months.

CORNEAL ENDOTHELIAL ANALYSIS
All eyes in the study underwent corneal endothelial analysis using the Topcon SP-2000P noncontact specular microscope (Topcon Corp, Tokyo, Japan) preoperatively and 12 months postoperatively. Three consecutive endothelial images of the central cornea were obtained from each eye. All images were subjected to a fully automated analysis by the IMAGEnet 2000 Endothelial Cell Analysis Module software (Topcon Corp). This method is based on a planimetric variable frame technique in which the software determines the borders of at least 100 endothelial cells per image by the detection of contrast differences between cells and intercellular borders in a black and white image. The individual endothelial cell area is measured by the software, which then calculates mean cell area and other morphometric parameters. One examiner (C.A.D.) corrected software-defined cell borders in all analyzed images in IMAGEnet. This correction entailed erasing any incorrectly drawn or incomplete cell boundaries and redrawing them as they were deemed true. The software then made the planimetric endothelial cell analysis, and the mean of 3 analyses of each eye resulted in the endothelial cell density. Only whole cells with continuous cell borders were accepted by the IMAGEnet analysis software. Edge effects (incomplete cells on edges of the images to be analyzed) did not have to be taken into account. Another important endothelial morphometric parameter provided by the IMAGEnet 2000 endothelial cell analysis module is the percentage of hexagonality, which is the ratio of cells with 6 corners to the total number of cells identified.

PRE- AND POSTOPERATIVE MAGNIFICATION RATIO OF CORNEAL ENDOTHELIAL CELLS
Noncontact specular microscopy magnifies the corneal endothelium. The amount of magnification (M) can be calculated with the formula:

\[ M = 1 + \left( \frac{e}{K/n_c} \right), \]

where the central thickness of the cornea is e, the refractive power of the anterior surface of the cornea is K, and the refractive index of the cornea is n_c (see Appendix in Nawa et al). The amount of magnification is altered after LASIK. In myopic LASIK, corneal endothelial cell density is overestimated after surgery because the image is minimized depending on
the change in corneal thickness and keratometry. The postoperative/preoperative magnification ratio of the image (R) is expressed as:

\[
R = \frac{M_2}{M_1} = \frac{[1 + (e_2 \times K_2/n_c)]}{[1 + (e_1 \times K_1/n_c)]}.
\]

This calculation assumes that the posterior radius of curvature of the cornea does not change after surgery. For example, for a \(-4.50\) D myopic eye with preoperative pachymetry of 560 \(\mu\)m and central K of 46.00 D and postoperative pachymetry of 490 \(\mu\)m and K of 42.00 D, and considering the refractive index of the cornea is \(n_c=1.3771\), the postoperative/preoperative magnification ratio of the image would be:

\[
R = \frac{M_2}{M_1} = \frac{[1 + (0.000490\times42/1.3771)]/[1 + (0.000560\times46/1.3771)]}{1.014944/1.018706} = 0.9963.
\]

Thus, the image is minimized by 0.37\% after LASIK in this specific eye. If the postoperative corneal endothelial cell density was 3600 cells/mm\(^2\), it should be corrected by \(-0.37\%\) to a real value of 3587 cells/mm\(^2\) for comparison with preoperative values.

In the present study, postoperative/preoperative magnification ratio of every measurement was considered and postoperative endothelial cell density was calculated accordingly.

**STATISTICAL ANALYSIS**

Statistical analysis was performed with MINITAB Release 14 (Minitab Inc, State College, Pennsylvania) for Windows. The Student \(t\) test for two samples and the Student \(t\) test for paired samples were used. A \(P\) value <.05 was considered statistically significant.

**RESULTS**

This study included 138 eyes of 138 patients (65 men and 73 women), aged 21 to 42 years (mean: 32.0±7.1 years). Seventy-six eyes were in the contact lens group and 62 eyes were in the non-contact lens group. Contact lens wearers and non-contact lens wearers were not homogeneous populations regarding central corneal endothelial cell density or morphology. Preoperatively, the non-contact lens group showed statistically significantly higher mean endothelial cell density (\(P<.0001\)) and mean percentage of hexagonal cells (\(P=.012\)) compared with the contact lens group.

Refractive status and visual acuity before and 12 months after femtosecond LASIK are shown in Tables 1 and 2. No differences were noted between groups in terms of refractive error, UDVA, or CDVA before or after surgery. Average ablation depth was 48.2±23.1 µm (range: 19.0 to 113.2 µm) in the contact lens group and 45.2±29.8 µm (range: 12.0 to 123.2 µm) in the non-contact lens group (\(t\) test, \(P=.517\)).

**CONTACT LENS GROUP**

A summary of corneal noncontact specular microscopy data obtained before and 12 months after femtosecond LASIK, including central corneal endothelial cell density and percentage of hexagonality, is shown in Table 3. Twelve months after femtosecond LASIK, mean endothelial cell density improved significantly (3587±262 cells/mm\(^2\)) compared with preoperative data (3401±292 cells/mm\(^2\)) (\(P<.001\)). Mean endothelial cell density increased by 5.5\% on average. The percentage of hexagonal cells was statistically significantly higher after surgery (32.5±4.0\%) compared with preoperative data (31.0±5.1\%) (\(P=.035\)).

**NON-CONTACT LENS GROUP**

One year after femtosecond LASIK, mean endothelial cell density was slightly lower (3564±232 cells/mm\(^2\)) than preoperatively (3605±219 cells/mm\(^2\)), with a mean loss of 1.1\%. This difference was not statistically significant (\(P=.126\)). The percentage of hexagonal cells was slightly lower (34.4±11.2\%) compared to preop-
eratively (35.1±11.71%) (P=.56). No statistically significant differences regarding mean endothelial cell density or hexagonality before and 1 year after femtosecond LASIK in the non-contact lens group were noted (Table 3).

**DISCUSSION**

In the present study, correction of up to 9.00 D of myopia using femtosecond LASIK did not cause any deleterious effects on corneal endothelial cell density or endothelial morphology. Previous in vivo specular microscopic studies in patients who underwent LASIK using a mechanical microkeratome reported insignificant changes in central endothelial cell density.5-10

The main finding of our study is that femtosecond LASIK was safe for the corneal endothelium 12 months after surgery. Response of the corneal endothelium to femtosecond LASIK was different according to whether the patient was a contact lens wearer. In the contact lens group, mean endothelial cell density increased significantly by 5.5%, together with a higher percentage of hexagonality, whereas in the non-contact lens group, mean endothelial cell density and hexagonality were nearly unchanged. In the only scientific report addressing endothelial toxicity of the femtosecond laser in vivo on human eyes, Smith et al15 found no statistically significant change in central corneal endothelial cell density following thin-flap (100 µm) femtosecond LASIK for myopia, concluding that the technique was safe with regards to endothelial integrity. However, the authors acknowledged that their study was limited by the relatively short follow-up period (3 months) and the lack of endothelial cell morphology analysis. Choi et al18 studied the effects of femtosecond laser in enucleated porcine eyes and showed that the femtosecond laser stromal lamellar dissection and side cutting was safe for the central endothelium and caused only minimal damage to the peripheral endothelium, regardless of the laser emission pattern. Cheng et al19 evaluated the endothelial cell viability after femtosecond laser preparation of posterior lamellar disks for Descemet-stripping endothelial keratoplasty and concluded that the femtosecond laser did not cause damage to the corneal endothelium. Our results are in accordance with these studies and show femtosecond LASIK to be a safe technique for the corneal endothelium.

Previous studies have shown improvement in endothelial cell density after mechanical LASIK.5,20 The main factor explaining the improvement is likely to be the discontinuation of contact lens wear, although other corneal metabolic modifications may have also contributed. In a report assessing whether discontinuation of contact lens wear allowed recovery of endothelial integrity, Odenthal et al21 found significant recovery in terms of the coefficient of variation of cell area and hexagonality, concluding that endothelial integrity changes caused by rigid or soft contact lens wear were partly reversible. Collins et al7 demonstrated no statistically significant change in mean endothelial cell density or percentage of hexagonal cells 3 years after LASIK with a mechanical microkeratome. However, the mean coefficient of variation was significantly lower after surgery. A repeated measures analysis showed that this significant improvement could not be explained by cessation of contact lens wear after LASIK and multivariate analysis did not identify any factors that were predictive of change in cell density, coefficient of variation, and percentage of hexagonal cells.

According to our results, mean endothelial cell density in the non-contact lens group did not change significantly 12 months after femtosecond LASIK, with a mean reduction of 1.1%, which is a value next to the normal physiological loss. Human central endothelial cell density decreases at an average rate of approxi-

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**TABLE 2**

**Corrected Distance Visual Acuity Before and 12 Months After Femtosecond LASIK in the Non-contact Lens and Contact Lens Groups**

<table>
<thead>
<tr>
<th></th>
<th>Preoperative</th>
<th>Postoperative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LogMAR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decimal</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>.999</td>
<td>.169</td>
</tr>
</tbody>
</table>

**TABLE 3**

**Central Endothelial Noncontact Specular Microscopy Before and 12 Months After Femtosecond LASIK in the Non-contact Lens and Contact Lens Groups**

<table>
<thead>
<tr>
<th></th>
<th>Non-contact Lens Group</th>
<th>Contact Lens Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>.126</td>
<td>.035</td>
</tr>
<tr>
<td>Preop</td>
<td>3605±219</td>
<td>3401±292</td>
</tr>
<tr>
<td>Postop</td>
<td>3564±232</td>
<td>3587±262</td>
</tr>
</tbody>
</table>

**ECD = endothelial cell count (cells/mm²), H = hexagonality**
approximately 0.6% per year in normal corneas throughout adult life.22

Pérez-Santonja et al5 showed significant postoperative improvement of endothelial cell density after mechanical microkeratome LASIK in patients with myopia up to 18.50 D, with a mean increase of 2.3%. This effect was especially important in patients who were contact lens wearers before surgery, showing a mean increase of 2.36% and 3.74% in endothelial cell density, 3 and 6 months after LASIK, respectively.5 In our study, a higher mean increase of 5.5% in endothelial cell density was obtained in contact lens wearers 12 months after femtosecond LASIK for myopia up to 9.00 D. This difference may be attributed to the differing follow-up period of both studies or to the differing amount of myopia treated. However, both systems, mechanical microkeratome and femtosecond laser, have demonstrated no significant effect in endothelial cell density of myopic patients who were non-contact lens wearers before surgery. Therefore, it seems that the beneficial effects of LASIK in endothelial cell density are related to the discontinuation of contact lens use.

One limitation of the present study is that specular microscopy was not carried out in the immediate postoperative period, whereas previous studies have reported acute reversible endothelial damage after mechanical microkeratome LASIK,13 which could also be the case for femtosecond LASIK in specific eyes. Factors other than laser ablation (femtosecond or excimer laser), such as elevated intraocular pressure, could also play a role in producing endothelial toxicity in eyes at risk. In any case, even if there had been acute changes in the corneal endothelium after femtosecond LASIK, our patients did not have consequences over 12-month follow-up.

In the present study, the SP-2000P was always managed by the same examiner using the preset factory values of magnification. Van Schaick et al22 have shown that these parameters may not be exact, resulting in errors in endothelial cell density values of up to 9%, but the percentage of hexagonal cells is not affected by calibration defects because it does not depend on cell size.9

The method that we chose to calculate endothelial cell density was the automated IMAGEnet analysis with manual correction of incorrectly drawn cell borders. Doughty et al24 showed that for accurate estimates of endothelial cell density, samples of at least 75 cells were needed. This parameter was followed in the present study as all images processed had more than 75 cells (at least 100 cells). Van Schaick et al22 also investigated the validity of several semiautomated methods compared with the gold standard manual endothelial cell density (manual counting of cells on a video print).

The limits of agreement with manual endothelial cell density indicated that the IMAGEnet-corrected endothelial cell density was the most accurate of all assessment methods, with the least individual measurement errors.23 These authors found that corrected IMAGEnet endothelial cell density had a high validity when very accurate endothelial cell density measurements were needed, such as in the present study. It is remarkable that corrected IMAGEnet endothelial cell density values were systematically higher than manual endothelial cell density values.23

We found that hexagonality improved significantly in the contact lens group whereas in the non-contact lens group hexagonality was virtually unaffected with a trend toward lower values. Cheung and Cho25 showed that for the same set of images captured by one examiner, the Topcon IMAGEnet system was reliable in determining endothelial cell density and was fairly reliable in determining morphometric parameters such as percentage of hexagonality of coefficient of variation (standard deviation of cell size divided by mean cell size). They also demonstrated that when images were captured on different days or by different examiners, values for endothelial cell density and average cell size were repeatable and reproducible, but not for coefficient of variation and percentage of hexagonality. Doughty and Aakre26 also used the Topcon SP-2000P specular microscope to generate coefficient of variation data for the corneal endothelium similar to those of a manual method. The limits of agreement between the two methods ranged from −4.9% to +7.9%, reflecting the extreme sensitivity of the coefficient of variation calculation to even a few different cell area values. This poor agreement needs to be considered and caution should be exercised when using coefficient of variation to monitor endothelial changes in comparative studies,25,26 which is why we did not include the coefficient of variation parameter in the present study.

Femtosecond LASIK had no toxic effects on the central corneal endothelium in myopic eyes 12 months after surgery. This finding may not be true for corneas with dystrophy or low endothelial cell density readings, as it happens with LASIK using a mechanical microkeratome.

**AUTHOR CONTRIBUTIONS**

Study concept and design (G.M.); data collection (C.A.D., T.F.B.); analysis and interpretation of data (G.M., C.A.D., H.F.S., J.J.); drafting of the manuscript (G.M., C.A.D.); critical revision of the manuscript (H.F.S., T.F.B., J.J.); statistical expertise (C.A.D.); supervision (G.M., C.A.D., H.F.S.)
REFERENCES


Please see author queries on page 7
AUTHOR QUERIES

Reference 17 cannot be verified. Please confirm accuracy.

The P value (P<.001) in Abstract results differs from Results and Table 3 (P<.0001). Please advise.

Second paragraph of Patients and Methods, please provide method of randomization. “One eye of each patient (right or left) was randomly assigned to be included in the analysis.”

Second paragraph of Discussion, please clarify meaning of together in “In the contact lens group, mean endothelial cell density increased significantly by 5.5%, together with a higher percentage of hexagonality, whereas in the non-contact lens group, mean endothelial cell density and hexagonality were nearly unchanged.”

Page 4, right column, please clarify next to the normal in sentence, “According to our results, mean endothelial cell density in the non-contact lens group did not change significantly 12 months after femtosecond LASIK, with a mean reduction of 1.1%, which is a value next to the normal physiological loss.” Do you mean a value consistent with normal physiological loss?

Page 5, left column, please clarify This effect was especially important in sentence, “This effect was especially important in patients who were contact lens wearers before surgery, showing a mean increase of 2.36% and 3.74% in endothelial cell density, 3 and 6 months after LASIK, respectively.” Why was it especially important?

Page 5, left column, please clarify “Factors other than laser ablation (femtosecond or excimer), such as elevated intraocular pressure, could also play a role in producing endothelial toxicity in eyes at risk.” At risk for what? Decreased endothelial cell density?

Page 5, left column, please provide the initials of the examiner. Are they C.A.D.? “In the present study, the SP-2000P was always managed by the same examiner using the preset factory values of magnification.”

Page 5, right column, please clarify similar to those of a manual method “Doughty and Aakre26 also used the Topcon SP-2000P specular microscope to generate coefficient of variation data for the corneal endothelium similar to those of a manual method.”

Page 5, last paragraph, please clarify sentence “This finding may not be true for corneas with dystrophy or low endothelial cell density readings, as it happens with LASIK using a mechanical microkeratome.”

Original Table 1 repeated data shown in original Table 4. Therefore, Tables 1 and 4 were combined. The tables were renumbered and reordered. Okay as edited?