Femtosecond Laser-Assisted Corneal Flap Cuts: Morphology, Accuracy, and Histopathology

Mike P. Holzer, Tanja M. Rabsilber, and Gerd U. Auffarth

PURPOSE. Precision in corneal flap cutting is essential in LASIK surgery. Current mechanical microkeratomes have a very good performance record; however, in a few cases, complications can occur during the microkeratome pass and flap cut. Femtosecond lasers offer an alternative to the mechanical cut and can provide additional features regarding the flap morphology. In this study, we analyzed femtosecond laser flaps regarding their morphology, cut accuracy, and histopathology.

METHODS. Forty-five fresh porcine cadaveric eyes were prepared for femtosecond laser flap cutting with the Femtec femtosecond laser system (20/10 Perfect Vision, Heidelberg, Germany). The eyes were assigned to three different thickness groups, with 120-, 140-, or 180-μm cut depth, respectively. In addition, different flap diameters ranging from 8.0 to 9.5 mm and rim edge angulations between 60° and 90° were performed. After the cut, the eyes were examined under a microscope regarding accuracy and potential defects, and flap thickness and diameter were measured. In addition, flaps were prepared for further histopathologic examination.

RESULTS. All flap cuts were easily performed without any intraoperative complications. Flap thickness measurements revealed a median (in micrometers) of 110.5 (intended thickness 120), 142.5 (intended 140), and 180.0 (intended 180), respectively. The flap diameter for an intended size between 8.0 and 9.5 mm was within a range of ±0.4 mm, the median at the maximum was 0.3 mm off. Histopathology revealed very low to almost no changes in the stromal structure of the cornea and correct hinge angulations.

CONCLUSIONS. LASIK flap cuts were easily performed without any complications. The accuracy and morphology were very precise and consistent. Histopathology revealed a smooth cut with hinge angulations, as expected. (Invest Ophthalmol Vis Sci. 2006;47:2828–2831) DOI:10.1167/iovs.05-1123

Precisely cut corneal flaps are essential for successful laser in situ keratomileusis (LASIK) treatments and calculation of ablation profiles. Even with modern mechanical microkeratomes, variations in flap thickness and morphology can be found. Differences in flap thickness can be related to several factors, including the microkeratome model used. The manufacturers have made extensive improvements in their microkeratomes over the past years, to create more precise flaps.

However, many complications of LASIK surgery are still related to the flap-cutting process. An alternative way of producing LASIK flaps involves modern lasers that are based on the femtosecond laser technology. At the moment, two femtosecond laser systems are commercially available on the market and are U.S. Food and Drug Administration (FDA) approved for the creation of LASIK flaps. One of these systems is the Femtec femtosecond laser system (20/10 Perfect Vision, Heidelberg, Germany). The wavelength of this laser is 1 μm infrared, and the spot size adjustable to several micrometers. By photodisruption of the corneal tissue—a nonthermal ablation process—the stromal layers can be divided and a LASIK flap created. The laser-induced vaporized tissue forms a cavitation gas bubble that mainly consists of H₂, N₂, and H₂O and diffuses out of the cornea through normal mechanisms. The advantage in comparison to conventional mechanical microkeratomes is that common complications of LASIK surgery like button holes, free caps, or high variations in flap thickness can be avoided, and the shape of the flap can be individualized to the needs of the patient and surgeon. The purpose of this study was to investigate the performance of the Femtec femtosecond laser system in regard to quality of flap cutting as well as accuracy, reliability, and histopathologic characteristics of corneal flaps.

MATERIALS AND METHODS

In an experimental laboratory setup, 45 fresh porcine eyes were used for cutting corneal flaps. Before the procedure the corneal epithelium was removed with a corneal scraper in all eyes, to have consistent smooth corneal surfaces, which is important to measure an exact thickness of the created flap. Afterward, the eyes were fixed in an interface, positioned, and attached, by applying suction energy, to the Femtec laser system (20/10 Perfect Vision). The laser spots were applied in a single-pass spiral pattern disc move from the periphery to the center of the cornea, followed by an anterior movement of the spots to cut through the anterior corneal surface. The laser settings were as follows: stromal bed energy of 3 μJ; bed spacing 8/10 (8-μm line separation and 10-μm spot separation); rim energy of 4.4 μJ; rim spacing 3/6 (3-μm line separation and 6-μm spot separation); edge angulation of 60°, 75°, or 90°; and flap diameter of 8.0, 8.5, 9.0, or 9.5 mm. The intended flap thickness was set at 120, 140, or 180 μm. After completion of the laser cut, each eye was observed under the microscope for any damage or complications due to the laser cut. A flap spanula (15470 spanula, Geuder, Heidelberg, Germany) was introduced into the interface, and, by slight movements to both sides, the flap was separated from the underlying stroma. The intent was to cut the flap completely free, to facilitate the investigation of the shape and thickness of the flap. After the flap was removed from the eye, in a subgroup of 12 eyes, the diameter was measured and recorded with a millimeter scale. Thirty eyes were used for thickness measurements, with 10 being in each thickness group. The thickness measurements were performed with a micrometer (Digimatic; Mitutoyo Inc., Kanagawa, Japan) measuring instrument (accuracy, 1 μm) by positioning the flap immediately after completion of the laser cut without any further manipulation, between two microscope coverslips. The coverslips were compressed with a locking screw with the lowest pressure possible until the first stop of the screw, and the thickness was noted.

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Table 1. Intended and Achieved Flap Diameters in Porcine Eyes

<table>
<thead>
<tr>
<th>Eyes (n)</th>
<th>Intended Flap Diameter (mm)</th>
<th>Achieved Flap Diameter (mm), median</th>
<th>Range Achieved Diameter (mm)</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>8.0</td>
<td>8.0</td>
<td>7.8-8.2</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>8.5</td>
<td>8.3</td>
<td>8.1-8.4</td>
<td>-2.35</td>
</tr>
<tr>
<td>3</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>9.5</td>
<td>9.2</td>
<td>9.2</td>
<td>-3.16</td>
</tr>
</tbody>
</table>

Statistical analysis was performed on computer (Medcalc software version 7.3.0.1; Medcalc Software, Mariakerke, Belgium) with the Wilcoxon test for unpaired nonparametric data. P < 0.05 was considered statistically significant. At the completion of surgery, three flaps were created just for histopathologic examination, without measuring them, to prevent any damage to or change in the flaps. These three and all other flaps were stored in a formalin solution container and afterward prepared for histopathology with hematoxylin and eosin (H&E) stain. Hinge angulation was measured with the histopathology slides, and stromal structure was analyzed and compared with conventional microkeratome cuts.

RESULTS

Flap cut in all eyes was uneventful, and the flaps were easy to separate from the underlying stroma. The difference between measured and attempted diameter ranged between 0 and 400 μm with a percentage difference between 0% and -3.16% (Table 1).

The flap thickness median was (in micrometers) 110.5 (intended 120), 142.5 (intended 140), and 180.0 (intended 180) (Fig. 1; Table 2). Of all the flaps, 33% were within 5 μm of the intended thickness, 53% within 10 μm, and 86% within 15 μm. The highest difference was 24 μm (120 μm intended, 96 μm achieved). Regarding the accuracy, the 180 μm thickness group showed the lowest SD with ±9.12 μm followed by the 140- and 120-μm group. However, there was no significant difference between the 120- and 140-μm groups when compared with the 180-μm group (Wilcoxon test; P > 0.05).

Histopathologic analysis of the flaps revealed very low to almost no changes in the stromal structure of the cornea. The rim edge angulations were as intended and showed a smooth, straight cut (Fig. 2). The interface (flap and surface of underlying stroma) showed a straight cut with a few remaining tissue fibers in some cases that were caused by minimal trauma when separating the flap from the underlying stroma with the flap spatula.

FIGURE 1. Flap thickness deviation from intended flap thickness in the three thickness groups investigated.

A different approach to cutting corneal flaps is the use of laser energy. Ultrashort laser pulses such as those emitted by pico- and femtosecond lasers have been tested, and the femtosecond laser technology has shown the most promising results in flap separation. At the moment, two different companies distribute such femtosecond lasers for LASIK flap cuts: IntraLase (Irvine, CA) and 20/10 Perfect Vision. Both are based on the same technology and are mainly used to cut LASIK flaps. Different investigators report good experiences and results for the IntraLase system.8–11 A difference between the two laser systems is the interface that is used to dock the patient’s eye to the laser. The Femtec laser needs less suction energy because the interface has a curvature similar to the cornea.

To the best of our knowledge, this is the first report on the morphology and accuracy of LASIK flaps created with the Femtec laser system that includes histopathology. The flap diameter measured in all eyes in our study had a median difference between 0% and 3.16% or ±0.3 mm from the intended diameter. Because the main outcome measurement of the study was flap thickness, the flap diameter was measured in only a small subgroup. All other flaps were measured for thickness immediately after surgery without further manipulation. Further studies with a higher number of flaps would therefore be necessary to confirm these results. However, similar findings for the accuracy of the IntraLase femtosecond laser are reported by Binder,12 who found a range differing up to 0.6 mm from the intended flap diameter and a standard deviation between 0.12 and 0.26 mm. In general, femtosecond lasers are much more precise regarding flap diameter when compared with mechanical microkeratomes. Binder reported a standard deviation of 300 μm of the attempted diameter using mechanical microkeratomes.15

DISCUSSION

The invention of the mechanical microkeratome was an important step in enhancing the popularity of LASIK as a safe and painless corneal refractive surgery procedure. Since the first introduction of a microkeratome to cut corneal flaps for LASIK surgery by Pallikaris et al.,5 many changes have been made by the different manufacturers to produce more reliable and consistent flaps. The current microkeratomes have a very precise and reliable performance and show standard deviations in flap thickness between 15 and 35 μm.1 However, the accuracy of mechanical microkeratomes is due to their specifications and their method of cutting limited; thus, they still can cause some complications during the cutting process. Among these are incomplete cuts, free caps, decentered flaps, button holes, inconsistent flap thickness, epithelial defects, induction of higher-order corneal aberrations, and others.2,6
Even more important is the thickness of LASIK flaps in precisely predicting the residual stromal bed after excimer laser ablation and in preventing complications like corneal ectasia. In a large prospective, multicenter study, Solomon et al. found that the thickness SD of LASIK flaps created with modern microkeratomes from the current manufacturers ranges between 15 and 35 μm. These are good and precise results for mechanical microkeratomes, and improvements to these systems for even more accurate cuts seem to be limited due to the nature of the systems. To cut more precisely, computer-assisted lasers such as femtosecond lasers are an alternative. The IntraLase femtosecond laser system, which is in use by several surgeons, shows promising results for precision cutting. Kezirian and Stonecipher compared the IntraLase femtosecond laser with the Hansatome (Bausch & Lomb, Tampa, FL) and Carriazo-Barraquer (CB; Moria SA, Antony, France) microkeratomes regarding flap thickness, epithelial integrity, and visual outcome. They found a standard deviation of 14 μm for the IntraLase and 26 and 29 μm, respectively, for the CB and Hansatome microkeratomes. Epithelial damage was found with the use of both mechanical microkeratomes, but not with the use of the of the femtosecond laser. Visual outcome, however, was not different between the groups that they studied. Binder found, in his analysis of femtosecond laser flaps, a thickness standard deviation between 12 and 18.5 μm. In our study we found standard deviations between 9.1 and 11.1 μm, depending on whether the intended flap thickness was 120, 140, or 180 μm. These results are comparable to those published in the peer-reviewed literature for the IntraLase femtosecond laser. It is important to note that our study was totally experimental and standardized to achieve accurate and reliable data and to detect the limitations of this laser system. In contrast to clinical studies, no ultrasound pachymetry was used, since it is difficult to measure exactly the same spot on the cornea before and after surgery. An alternative to ultrasound might be optical low-coherence reflectometry, which is implemented in some excimer laser systems as online pachymetry and more accurate than ultrasound. However, it is also necessary to measure the same spot before and after surgery. In our study, we chose to separate the flap from the cornea and measure the thickness over the whole surface by putting it between two coverslips immediately after the laser cut was finished. These coverslips were then compressed with the lowest pressure possible to avoid dehydrating the flaps, and the pachymetry measured was noted. The thickness measurements may have been influenced by this method, and the flaps may have been measured thinner than they originally were. However, this method still gives the best thickness accuracy over the total flap. The other possible methods mentioned would measure only the thickness at one specific spot, and the accuracy would be lower.

Limited information is found in the literature regarding the histopathology of femtosecond laser flaps. In the selected cases where we performed histologic analyses, we found a regular stroma with no to very mild changes in the structure of the stroma. When compared to LASIK cuts with mechanical microkeratomes, femtosecond laser cuts showed slightly more

### Table 2. Detailed Results of Thickness Measurements for the Three Investigated Flap Thickness Groups

<table>
<thead>
<tr>
<th>Intended Flap Thickness</th>
<th>Eyes (n)</th>
<th>Median</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Difference Intended − Achieved</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>180 μm</td>
<td>10</td>
<td>180.0</td>
<td>179.60</td>
<td>9.12</td>
<td>−0.40</td>
<td>167</td>
<td>194</td>
</tr>
<tr>
<td>140 μm</td>
<td>10</td>
<td>142.5</td>
<td>143.70</td>
<td>10.69</td>
<td>3.70</td>
<td>131</td>
<td>159</td>
</tr>
<tr>
<td>120 μm</td>
<td>10</td>
<td>110.5</td>
<td>112.10</td>
<td>11.06</td>
<td>−7.90</td>
<td>96</td>
<td>135</td>
</tr>
</tbody>
</table>

Data are in micrometers, unless marked otherwise.

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**Figure 2.** Corneal flaps created with the Femtec femtosecond laser system. (A) A 140-μm flap showing a hinge angulation of 90°. (B) A 180-μm flap with regular shaped stroma and normal keratocytes. H&E staining; original magnification, ×20.

**Figure 3.** Corneal flap cut with a mechanical microkeratome (Ama- deus; AMO Inc., Santa Ana, CA). (A) Arrow: incision site. (B) Hinge area (H). H&E staining; original magnification, ×10.
remaining collagen fibrils along the cut. This result seems to be related to the way the laser separation of the corneal stroma is performed. However, the clinical outcomes of LASIK surgeries after femtosecond laser flap creation show comparable to even better results in comparison to mechanical microkeratomes.9,11,16,17 More important for the postoperative outcome and fast visual recovery seems to be the low to minimal changes in the stroma and the low inflammatory response of the cornea.18 However, LASIK complications like diffuse lamellar keratitis (DLK) or epithelial ingrowth can also occur with femtosecond lasers and even new syndromes like transient light sensitivity have been reported.19

In summary, the results regarding morphology, accuracy, and histopathology of the corneal flaps created with the Femtenc femtosecond laser system presented in this study are very promising and support the clinical use of this laser system for refractive surgery. Additional applications of femtosecond laser technology in the field of corneal surgery will arise. Among these are corneal lamellar and penetrating keratoplasty, which has been described in some reports already.20,21 Another surgical procedure that will profit from femtosecond laser technology is the creation of corneal tunnel incisions for intracorneal ring segment surgery.10 Further potential applications could be intrastromal refractive surgery using femtosecond laser technology.22–24 Certainly, more studies are necessary to demonstrate that such a refractive correction can be performed with similar or better precision than excimer laser ablation of the cornea for refractive surgery.

References

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