Optimal parameters to improve the interface quality of the flap bed in femtosecond laser-assisted laser in situ keratomileusis

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PURPOSE: To analyze the interface quality of the anterior stroma after femtosecond laser flap creation using atomic force microscopy.

SETTING: IRCCS Fondazione G.B. Bietti, Rome, Italy.

DESIGN: Experimental study.

METHODS: A 110 μm depth flap was created in 20 human corneal tissues using a femtosecond laser platform (Intralase iFS). Tissues were divided into 4 groups of various cutting parameters: pulse energy and spot separation of 0.75 μJ and 6 μm (Group 1), 0.65 μJ and 5 μm (Group 2), 0.55 μJ and 4 μm (Group 3), and 0.45 μJ and 4 μm (Group 4). Four additional tissue sections were cut using a motorized microkeratome (Hansatome). Atomic force microscopy (Autoprobe CP) analysis was performed on the stromal bed of each sample.

RESULTS: The corneal tissues treated with higher pulse energies and wider spot separations (Groups 1 and 2) showed a rougher stromal bed interface (root mean square [RMS] roughness Z = 0.23 ± 0.008 (SD) and 0.24 ± 0.009 μm, respectively) than tissues in Groups 3 and 4 (RMS roughness = 0.18 ± 0.006 μm and 0.18 ± 0.008 μm, respectively; P<.001, 1-way analysis of variance). The stromal surface quality of tissues treated with pulse energies of 0.55 μJ or lower and spot separations narrower than those currently used in the clinical setting. The flap interface smoothness created by the femtosecond laser was comparable to that created by the microkeratome.

CONCLUSIONS: The femtosecond stromal interface quality was improved with pulse energy lower and spot separations narrower than those currently used in the clinical setting. The flap interface smoothness created by the femtosecond laser was comparable to that created by the microkeratome.

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Laser in situ keratomileusis (LASIK) is the most popular refractive surgery technique worldwide. During the past decade, efforts to improve the safety and efficacy of the procedure have been made by clinicians and manufacturers.1-3

Femtosecond laser-assisted LASIK is gaining interest among refractive surgeons because of high repeatability in achieving the desired flap thickness and fewer intraoperative complications than reported with microkeratomes.4-6 Various clinical studies have reported a smaller variation in the targeted flap thickness with the femtosecond laser than with the microkeratome7,8 as well as a more regular flap thickness profile.9 However, irregularities of the stromal interface, even after femtosecond laser flap creation, have also been described.10 The femtosecond laser can create, with high accuracy, flaps thinner than those created by the microkeratome, allowing the surgeon to treat eyes whose corneal thickness might be a contraindication to LASIK surgery.8,9 Controversies remain concerning the advantages of the femtosecond laser over the microkeratome in improving clinical outcomes, such as postoperative visual performance and corneal higher-order aberrations (HOAs). Calvo et al.11 report that the planar configuration of the femtosecond laser flap does not offer any
advantage in corneal HOAs or visual acuity over a 3-year follow-up, whereas Lim et al.\textsuperscript{5} report a smaller degree of postoperative spherical aberration in patients who had femtosecond LASIK than in those who had LASIK with a mechanical microkeratome.

It has been shown that the more stromal surface micrometric irregularities induced by the surgery, the greater the amount of induced HOAs in the eye.\textsuperscript{12-15} A complete and correct knowledge of the ultrastructure of the corneal stroma after femtosecond flap creation could enable ophthalmic surgeons to optimize the effectiveness of femtosecond LASIK. Studies have shown images of the stromal flap bed of tissues cut using various femtosecond platforms and microkeratomes; however, they do not provide quantitative information about the surface roughness or the morphological differences between the procedures.\textsuperscript{5,8,10,11,16,17}

The aim of the present study was to analyze the interface quality of the anterior stroma after femtosecond laser flap creation. We investigated the influence of various combinations of pulse energy and spot separation on the stromal bed surface roughness using a scanning probe ultramicroscopic technique; ie, atomic force microscopy (AFM).\textsuperscript{16,18} We also developed a quantitative description of the stromal interface after mechanical flap creation to compare the morphological differences between the mechanical and femtosecond flap beds.

MATERIALS AND METHODS

Twenty fresh human donor corneas not suitable for transplantation were obtained from the Eye Bank of Rome, Italy. The mean donor age was 69.64 years ± 7.38 (SD); the mean donor cadaver time and endothelial cell density were 8.65 ± 6.39 hours and 2000 ± 319 cell/mm\textsuperscript{2}, respectively. All tissues were stored at 4°C in Eusol C solution (Alchimia Srl) and used within 48 hours. Sixteen tissues were cut by a commercial femtosecond laser (Intralase iFS 150 KHz, Abbott Medical Optics, Inc.) and 4 were cut using a motorized microkeratome (160 μm head, 9.5 mm ring Hansatome, Bausch & Lomb).

Table 1. Femtosecond laser parameters used to create the LASIK flap and study groups.

<table>
<thead>
<tr>
<th>Pulse Energy (μJ)</th>
<th>Spot Separation (μm)</th>
<th>Layer Separation (μm)</th>
<th>Time Duration (s)</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75</td>
<td>6</td>
<td>6</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>0.65</td>
<td>5</td>
<td>5</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>0.55</td>
<td>4</td>
<td>4</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td>0.45</td>
<td>4</td>
<td>4</td>
<td>31</td>
<td>4</td>
</tr>
<tr>
<td>Microkeratome</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5</td>
</tr>
</tbody>
</table>

Each group comprised 4 specimens.
the flap bed side facing upward and observed in balanced salt solution using V-shaped silicon nitride gold-coated cantilevers with a nominal spring constant of 0.06 N/m (Veeco Instruments, Inc.). All images were acquired with a 256 × 256 point resolution with scan rates of 2 Hz per line. A single area on the cornea was imaged twice obtaining the same results to ensure that the force exerted was not sufficient to damage the sample surface and cause artifacts. Only areas close to the center of each specimen were imaged to avoid interpreting artifacts caused by forceps manipulation at the edges of tissues as surface features. Images were processed and analyzed using the specific AFM software. Surface measurements were made on 50 reference areas of 5 μm² for each corneal sample. Considering a surface topography \( z(x_i, y_j) \) defined in a rectangular coordinating system OXYZ, with \( M \) and \( N \) the measurement points on OX and OY axes, respectively (\( i = 1, ..., M \) and \( j = 1, ..., N \)), the roughness parameters used in this study were defined as following:

1. average of the roughness within the given area (\( \text{AVE rough} \)):

\[
\text{AVE rough} = \frac{1}{MN} \sum_{j=1}^{N} \sum_{i=1}^{M} [z(x_i, y_j) - \bar{z}] \quad (1)
\]

with \( \bar{z} \) representing the mean height;

2. root-mean-square (RMS) value of the roughness within the given area (\( \text{RMS rough} \)); ie, the standard deviation of the height data:

\[
\text{RMS rough} = \sqrt{\frac{1}{MN} \sum_{j=1}^{N} \sum_{i=1}^{M} [z(x_i, y_j) - \bar{z}]^2} \quad (2)
\]

3. peak to valley (PV):

\[
\text{PV} = |S_p - S_v| \quad (3)
\]

where \( S_p \) and \( S_v \) are the highest peak and the lowest valley of the surface, respectively.

**Statistical Analysis**

The 1-way analysis of variance (ANOVA) test was used to statistically compare the differences between specimens treated with various femtosecond laser parameters. When statistical significance was found, the differences between samples were further compared using the Tukey test for pairwise comparisons. For each data comparison, the minimum difference detectable by the test was measured to understand the achieved power of the statistical analysis (\( \beta \)) into detecting the significance of the differences in the \( \text{AVE rough} \) values between samples. Differences with a \( P \) value of less than 0.05 were considered statistically significant.

**RESULTS**

The treatment time duration ranged between 15 seconds and 31 seconds (24.25 ± 7.07 seconds). The mean pressure in the artificial anterior chamber was 39.00 ± 4.36 mm Hg (range 32 to 43 mm Hg).

Complete flap creation was achieved in all specimens. Both surgeons were able to lift the flap using a LASIK spatula and a 2-pass dissection technique in all cases. The maneuver was judged by both surgeons to be easier for tissues in Groups 3 and 4.

The flap bed created using relatively higher pulse energies and 6 or 5 μm spot separations, ie, in Groups 1 and 2, showed a rougher interface than tissue treated with 0.55 μJ or lower pulse energy and 4 μm spot separation, in Groups 3 and 4 (\( P < .001, \text{ANOVA} \)). According to the surface roughness descriptors, 2 distinct groups of flap bed interface quality were delineated: The first, with higher roughness, were tissues treated using 0.75 and 0.65 μJ pulse energies (Tukey: \( P > .05 \); \( \beta = 0.88 \); Figure 1, A and B) and the second, with an even surface, were tissues cut using 0.55 and 0.45 μJ pulse energies (Tukey: \( P > .05 \); \( \beta = 0.93 \); Figure 1, C and D). Thestromal bed surface qualityof Groups 3 and 4 was comparable to that provided by the microkeratome (Tukey: \( P > .05 \); \( \beta = 0.80 \) between Groups 3 or 4 and 5; Figure 2). The mean RMS rough values of the flap bed were 0.228 ± 0.008 μm, 0.240 ± 0.009 μm, 0.187 ± 0.006 μm, 0.185 ± 0.008 μm, and 0.171 ± 0.006 μm in Groups 1, 2, 3, 4, and 5, respectively. Results of the surface roughness analysis are summarized in Table 2.

Crater-like features or linear cracks of variable depth and length were observed in all femtosecond specimens treated with the femtosecond laser (Figure 1). The width of the craters was between 1.5 μm and 4.3 μm diameter, and the depth ranged between 0.3 μm and 1.5 μm. Deeper craters (\( P < .05 \)) were measured in tissues in Groups 1 and 2 (0.90 ± 0.35 μm) than in tissues in Groups 3 and 4 (0.59 ± 0.20 μm). No discernible differences in the crater’s width were found between samples treated with various laser parameters. Undulations and granules of variable dimensions spread on the stromal bed interface were more frequently observed in samples in Groups 1 and 2. Pools of collagen fibers were observed in specimens in Groups 3 and 4 only. The stromal flap bed interface created with the microkeratome was relatively even, with elongated features resembling collagen fiber bundles (Figure 2).

**DISCUSSION**

Although femtosecond laser-assisted LASIK is an established refractive procedure worldwide, experimental work is needed to improve the flap bed surface smoothness.

Corneal photodisruption can be considered a multi-cause mechanical effect starting with laser-induced
optical breakdown—the tissue is ultimately split by mechanical forces.\textsuperscript{19-24} By tightly focusing the near infrared laser radiation, intensities greater than 1 TW/cm\textsuperscript{2} arise inside the laser focus. When the fluence at the laser focus reaches a threshold, it transforms matter of normal state to plasma, which is similar to gas (mainly water vapor and carbon oxides) in which a certain portion of the particles are ionized. Two other physical effects are associated with plasma formation—shock wave generation and cavitation. Since the diameter of cavitation bubbles may reach up to a few millimeters, macroscopic photodisruptive effects inside the tissue are believed to primarily originate from the action of cavitation. Whereas plasma-induced ablation is spatially confined to the breakdown region (ie, the focal volume of the laser beam), bubble-induced damage (ie, the linear extent of the damage zone) has been determined to scale with the cube root of the contained energy.\textsuperscript{21,24} Complete intrastromal lamellar dissection with smooth stromal interface requires contiguous femtosecond photodisruption in turn necessitating a match between the plasma volume and cavitation bubble size or, equivalently, a match between the pulse energy and the spot separation. The pulses must impact the tissue outside the bubble. In the optimum case, the ratio of

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Group & PV (\textmu m) & RMS Rough (\textmu m) & AVE Rough (\textmu m) \\
\hline
1 & 1.333 \pm 0.04 & 0.228 \pm 0.008 & 0.180 \pm 0.007 \\
2 & 1.380 \pm 0.05 & 0.240 \pm 0.009 & 0.197 \pm 0.008 \\
3 & 1.068 \pm 0.03 & 0.187 \pm 0.006 & 0.152 \pm 0.005 \\
4 & 1.078 \pm 0.04 & 0.185 \pm 0.008 & 0.150 \pm 0.006 \\
5 & 1.065 \pm 0.04 & 0.171 \pm 0.006 & 0.141 \pm 0.006 \\
\hline
\end{tabular}
\caption{Atomic force microscopy analysis of the stromal bed interface quality.}
\end{table}
the cavitation diameter at the moment of arrival of the next pulse and the plasma diameter should approach unity. If the spot separation is too large for the chosen pulse energy, tissue islands are left behind and must be broken mechanically, increasing surface irregularity. If spot separation is too narrow for the chosen pulse energy, the cavitation bubbles can merge together and affect the subsequent laser beam. As a result, the cut becomes irregular, as reported in previous studies in which relatively high pulse energies were combined with narrow spot separations between subsequent laser pulses.19,25–30

In this study, we investigated the optimal combination of pulse energy and spot separation to improve the stromal bed interface quality after LASIK flap creation using a commercial femtosecond platform. Starting from the parameters proposed by the manufacturer to cut the flap (i.e., 0.75 μm with 6 μm separation), we selected 3 additional groups of lower energy settings and narrower spot separations, taking into consideration the above theoretical and experimental studies19–25 other than the total time duration of the cut.

The human corneal samples were imaged using a nondestructive scanning microscopy technique, namely AFM. Atomic force microscopy allows investigation of the corneal specimens in a liquid medium closer to their native state, with minimal preparation of the tissues permitting the preservation of their hydration without altering the surface architecture.14,15,18,19,25 Furthermore, AFM allows the acquisition of 3-dimensional images of the corneal stroma and a direct quantitative surface analysis.

The femtosecond laser–treated tissues revealed similar morphological features but with significant differences in surface roughness in relation to the combination of laser parameters used for flap creation (Table 1). The stromal characteristics of femtosecond specimens were related to the spot energy and spot separation used to create the flap. The stromal bed obtained using 0.55 and 0.45 μJ pulse energies (Groups 3 and 4) exhibited the most regular stromal surface (P < .001). In addition, the femtosecond parameters used in Groups 3 and 4 offered the same flap bed smoothness as the microkeratome.

Granules of various dimensions and divots or linear cracks of various size and depth were observed in all specimens. A more bumpy surface and more marked variations were found in specimens in Groups 1 and 2, as shown by the higher roughness values. Irregularities related to higher pulse energies and wider spot separations may suggest an increased contribution of secondary, thermal or mechanical, effects to photodisruption. We were able to visualize collagen fibers only in specimens treated with pulse energy of 0.55 μJ or lower. The possibility of imaging preserved collagen fiber structure is an indirect sign of less thermal and mechanical damage to the corneal stroma.19,20,24 Low pulse energy and narrower spot separation may have decreased the occurrence and the strength of the bridges at the bed interface.30 Two experienced surgeons subjectively evaluated that the flaps in specimens in Groups 3 and 4 were lifted more easily than those in Groups 1 or 2. The corneal tissues cut with the microkeratome demonstrated a relatively even surface, but no significant differences in the tissue cut were found with 0.55 μJ or lower pulse energy.

The repeatability of AFM measurements was very high, considering that the standard deviation was lower than 4% of the mean in all samples. Obtaining reproducible data is fundamental in any surface roughness analysis to verify the reliability of results. Atomic force microscopy is a technique that can achieve a subnanometer lateral resolution. We were able to detect differences in the average surface roughness (0.04 μm) between samples treated with different laser parameters. The slight average surface roughness difference of 0.01 μm between the tissues cut with 0.55 μJ or lower pulse energy and those cut by the microkeratome were not statistically significant. However, it is not clear whether the experimental results may reflect an improvement of the surgical outcome in terms of flap interface quality and, accordingly, visual performance in patients.

Possible limitations of the present study are that the tissues were obtained from elderly donors (eye bank) and that postmortem tissue edema may have interfered with the overall quality of the femtosecond cut. The different cut depth between the femtosecond and the microkeratome groups (targeted at 110 μm and 160 μm, respectively) should also be taken into consideration when comparing surface roughness between groups. Since only small areas (≤20 μm² in this study) of the corneal surface were resolved by AFM to attain stable tip–tissue interactions (and therefore high-resolution images), morphologic features larger than the single area scanned could not be properly detected. A larger field of view of a single acquisition could be achieved by scanning electron microscopy (SEM); nevertheless, SEM requires gross preparation of tissues (often masking the surface morphology of the photodisrupted stroma by metal coating), has lower lateral resolution than AFM, and does not provide direct quantitative measurements of the sample’s surface submicron-sized features. The SEM could, however, provide information about the LASIK flap margins.24 Sonigo et al.,7 for example, point out the differences in fibrotic scarring at the margin of the flap between the Intralase and a mechanical microkeratome, showing a higher fibroblast reaction in the femtosecond group.

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In this study in an experimental setting, we demonstrated a procedure to optimize the stromal bed quality with a commercial femtosecond platform. We found the smoothest stromal bed quality in femtosecond corneal tissues treated with pulse energies of 0.55 μJ or lower associated with 4 μm spot separations. Setting these combinations of laser parameters, the stromal bed quality was comparable to that obtained by the microkeratome. The results from our study cannot be automatically generalized to other commercial femtosecond laser platforms or even the same platform with different scanning frequencies. Hu et al.31 provided confocal microscopy evidence of fewer particles in the interface and a reduction in overall interface reflectivity in flaps created with higher frequency femtosecond laser platforms (ie, femtosecond 30 KHz compared with femtosecond 15 KHz). The potential advantage of a smooth flap bed consists is obtaining a homogeneous stromal surface on which the excimer laser can work to optimize the final surgical outcome.

WHAT WAS KNOWN
- Femtosecond LASIK can produce corneal flaps thinner and with more reproducible thickness measurement than mechanical microkeratomes; however, it creates a stromal bed surface rougher than mechanical LASIK.

WHAT THIS PAPER ADDS
- Modifying energy level and spot separation in the femtosecond laser machine setting can provide stromal bed smoothness similar to the one obtained with the mechanical microkeratome.

REFERENCES


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