Comparison of Goblet Cell Density after Femtosecond Laser and Mechanical Microkeratome in LASIK

Alejandra E. Rodriguez,1 Jose L. Rodriguez-Prats,1 Islam M. Hamdi,2 Ahmed Galal,3 Mohamed Awadalla,3 and Jorge L. Alio1,4

PURPOSE. To study the effect of the LASIK procedure performed with a femtosecond laser and a manual microkeratome on the conjunctival goblet cell and epithelial cell populations.

METHODS. In this prospective, nonrandomized, masked study, 64 eyes undergoing LASIK were included: 30 with the Moria M2 (M2) microkeratome and 34 with the IntraLase femtosecond laser (IL). The preoperative spherical equivalent was $-2.0 \pm 3.8 \text{ D}$ in the M2 group and $-3.1 \pm 3.1 \text{ D}$ in the IL group. The time that the suction ring was applied on the eye was registered, and goblet cell density (GCD), epithelial cell morphology, and inflammatory cells were evaluated by conjunctival impression cytology, before and after the surgery.

RESULTS. All the patients in both groups showed a decrease in GCD after LASIK ($P < 0.001$) that recovered after 6 months. At 1 week, 1 month, and 3 months, GCD was lower with IL than with M2 ($P < 0.019$, $P < 0.001$, and $P < 0.024$, respectively). The mean period that the suction ring was applied was longer in the IL than in the M2 group ($P < 0.001$). There was a high correlation between the decrease in GCD and the suction time ($R = 0.8$), and the preoperative spherical equivalent ($R \equiv 0.4$).

CONCLUSIONS. Impression cytology showed a greater reduction in goblet cell populations after IL than after M2, probably because of the length of time that the suction ring exerted pressure on the conjunctiva. These changes in the goblet cells may contribute to the development of the ocular surface syndrome after LASIK procedures. (Invest Ophthalmol Vis Sci. 2007;48:2570–2575) DOI:10.1167/iovs.06-1259

LASER IN SITU KERATOMELEISIS (LASIK) is the most common refractive procedure for the correction of mild to moderate myopia1–4 and different devices are used to create the corneal flap before the LASIK ablation. Mechanical microkeratomes are more commonly used and a rapid visual recovery is observed with minimum discomfort for the patients and reproducibility of the refractive effect.5 The femtosecond laser is an automated flap creation device for LASIK and is a safe and effective alternative to traditional mechanical microkeratomes.6 It may provide greater safety, reproducibility, predictability, and flexibility of the flap-making procedure and minimize interface deposits, epithelial defects, and striae.7 To create the corneal flap, either with manual microkeratomes or the femtosecond laser, it is necessary to apply a suction ring to the perilimbal conjunctiva. The ring which provides a vacuum on the anterior part of the eye that is needed to maintain a tight grip on the globe, so as to facilitate the smooth and perfect passage of the blade and thus create a flap without adverse effects.8–10 In preliminary research, we studied the relation of goblet cell density (GCD) with the application of the suction ring when a mechanical microkeratome was used.11

In the past few years, many studies have reported dry eye as the most frequent complication after LASIK12–15 and many theories of pathogenesis have been proposed for this phenomenon. Among these are the damage of the corneal nerve plexus,16–17 toxicity of topical medication,14 tear film instability,18,19 and the reduction of conjunctival GCD.11,19–21

The study of ocular surface cell populations has been greatly facilitated by impression cytology.12–25 This technique has enabled the study of many diseases, including dry eye syndrome,20–23 ocular surface neoplasia,27 and ocular surface infections.20,21 Dry eye classifications have been made to evaluate the degree of harm to the ocular surface, by using specific parameters—among them, GCD per square millimeter (GCD/mm²), description of epithelial cells, degree of keratinization, and power of cohesion. This technique has been used to study the modifications to the ocular surface after LASIK with different microkeratomes; however, this technique has not yet been applied when a femtosecond laser is used.

Conjunctival GCD and epithelial cell morphology constitute an objective way to evaluate the status of the ocular surface, in that conjunctival GC are responsible for the production of gel forming mucin, MUC5AC,30 and corneal and conjunctival epithelial cells are responsible for the production of transmembane mucins such as MUC1, 2, and 4.31–33 The most important role of these mucins is to stabilize the tear film, given that the gel forming mucin constitutes the main component of the mucous layer, which is the innermost layer of the film.34 Alterations in the population of goblet cells and modifications of epithelial cells after refractive surgery contribute to the development of the ocular surface syndrome (OSS). This term, OSS, has recently been introduced to describe dry eye and covers a variety of conditions leading to a physical and functional breakdown of the tear film.35

The purpose of the present study was to evaluate (1) the modifications in GCD, nucleus-cytoplasm (N/C) ratio, and inflammatory cells after surgery when the flap is created with a femtosecond laser or with a mechanical microkeratome; (2) the comparison of GCD, N/C ratio, and inflammatory cells between both techniques before and after surgery; (3) the relationship between GCD before and after the time that the suction ring exerts pressure on the conjunctiva, and (4) the relationship between the GCD and the spherical equivalent (SE).

METHODS

This prospective masked study included 64 eyes undergoing myopic or hyperopic LASIK. The study was performed in the Department of Investigative Ophthalmology & Visual Science, June 2007, Vol. 48, No. 6

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Research and Development, Vissum, Instituto Oftalmológico de Alicante, Spain, in 2005. All procedures conformed to the tenets of the Declaration of Helsinki, and written informed consent was obtained from each patient after institutional review board approval.

Eyes were divided into two groups, depending on the method of flap creation: 30 eyes scheduled for LASIK with a mechanical microkeratome (M2 group; M2 microkeratome; Moria, Antony, France) and 34 eyes scheduled for LASIK with a femtosecond laser (IL group; IntraLase Corp., Irvine, CA).

The main outcome measures included the GCD, N/C ratio, and inflammatory cells before and after the surgery when the flap was created with a femtosecond laser or with the mechanical microkeratome, and comparison of all these measures between both techniques. SE and suction time were also recorded.

All patients fulfilled the following inclusion criteria: mean refractive error between −2 and −9 D (SE) for myopia, between 2 and 4 D (SE) for hyperopia, and mean keratometry between 39 and 47 D.

Before surgery, all patients underwent full examinations, including recording of subjective symptoms and systemic and topical medica-
tions and a slit lamp examination. Patients were asked to remove soft contact lenses (CLs) for at least 1 week before surgery and to remove rigid lenses at least 1 month before. Cleaning of their eyelids twice daily for 4 days before surgery with treated towels (Lephanet towels; polysorbate 20, poloxamer 184, hyaluronate; Thea Laboratories, Clermont-Ferrand, France) was also required. Participants were excluded from the study for the following reasons: previous ocular surgery, active ocular infections, pregnancy, cataracts, neurologic damage, complications after LASIK such as diffuse lamellar keratitis, conjunctivitis, infectious keratitis, ocular allergic reactions, or LASIK enhancement.

Postoperative ocular surface evaluation was performed on all patients including subjective symptoms and biomicroscopic examination under the slit lamp. In the postoperative period, patients received a topical antibiotic and steroid, tobramycin 0.3%- dexamethasone 0.1% (Tobradex; Alcon Cusi, SA, Barcelona, Spain) for 1 week, four times daily for treatment after M2 LASIK and six times daily for IL LASIK. Also, patients applied unpreserved carboxymethyl cellulose sodium 0.5% artificial tears (Viscosfresh 0.5; Allergan, Dublin, Ireland) or hyaluronic acid 0.15% (Hyabak; Thea Laboratories) three times daily, for at least the first 3 months after surgery.

Data from the patients’ clinical history, such as age, sex, type of ablation, use of CLs, period of CL use, topical or systemic treatments, and SE, were recorded.

Impression Cytology

Samples were taken before surgery as control samples. Four more examinations were performed to detect the degree of damage and rate of recovery of the epithelium. Impression cytology was performed with polyethersulfone membrane filters, 0.20 μm (Supor-200; Sigma-Aldrich, Madrid, Spain) cut into 5 × 4-mm strips. Two samples were taken from each eye at each visit from the peribulbar conjunctiva: the first from the superior bulbar (SB) conjunctiva at 12 o’clock and the other from the inferior temporal bulbar (ITB) conjunctiva. Each filter was a different shape, to enable its identification in the sample vial. We used one vial per eye. After instillation of one drop of topical anes-
thesia of oxybuprocaine and tetracaine (Colicuris Anestésico Doble; Al-
con-Cusi) in each eye, filter papers were placed on the conjunctiva, pressed uniformly for 5 to 10 seconds, removed, placed in the tube containing the fixative solution (alcohol 96%), and stored at 4°C. Finally, the samples were stained with periodic acid-Schiff (PAS) according to Tseng’s method.24

Assessments of GCD by impression cytology were performed in the IL and the M2 groups immediately before surgery and at 1 week (± 2 days), 1 month (± 1 week), 3 months (± 2 weeks), and 6 months (± 3 weeks) after surgery.

Table 1. Clinical Data of the Two Groups

<table>
<thead>
<tr>
<th>Data</th>
<th>M2</th>
<th>IL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>33 ± 8</td>
<td>38 ± 10</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>22–48</td>
<td>23–59</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>12/18</td>
<td>20/14</td>
</tr>
<tr>
<td>Type of ablation</td>
<td>Myopic</td>
<td>Hyperopic</td>
</tr>
<tr>
<td></td>
<td>22 (73%)</td>
<td>28 (82%)</td>
</tr>
<tr>
<td>Hyperopic</td>
<td>8 (27%)</td>
<td>4 (12%)</td>
</tr>
<tr>
<td>Mixed astigmatism</td>
<td>0</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>Contact lenses</td>
<td>Users</td>
<td>14 (46%)</td>
</tr>
<tr>
<td></td>
<td>Mean period used ± SD (y)</td>
<td>12 ± 9</td>
</tr>
<tr>
<td>Mean spherical equivalent ± SD</td>
<td>−2.0 ± 3.8</td>
<td>−3.1 ± 3.1</td>
</tr>
<tr>
<td>Total</td>
<td>−5.9 ± 2.6</td>
<td>−4.1 ± 2.4</td>
</tr>
<tr>
<td>Myopic</td>
<td>3.2 ± 0.7</td>
<td>2.4 ± 0.9</td>
</tr>
<tr>
<td>Hyperopic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Conjunctival Goblet Cell Density in the Two Groups

<table>
<thead>
<tr>
<th>Group/Bulbar Site</th>
<th>Pre-surgery</th>
<th>1 Week</th>
<th>1 Month</th>
<th>3 Months</th>
<th>6 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>M2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior</td>
<td>542 ± 105</td>
<td>212 ± 82</td>
<td>250 ± 103</td>
<td>315 ± 117</td>
<td>359 ± 113</td>
</tr>
<tr>
<td>Inferior</td>
<td>481 ± 136</td>
<td>259 ± 97</td>
<td>257 ± 78</td>
<td>388 ± 107</td>
<td>459 ± 199</td>
</tr>
<tr>
<td>Average</td>
<td>418 ± 103</td>
<td>232 ± 85</td>
<td>338 ± 104</td>
<td>353 ± 99</td>
<td>391 ± 136</td>
</tr>
<tr>
<td>IL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior</td>
<td>351 ± 154</td>
<td>215 ± 152</td>
<td>170 ± 106</td>
<td>253 ± 160</td>
<td>332 ± 171</td>
</tr>
<tr>
<td>Inferior</td>
<td>449 ± 133</td>
<td>198 ± 71</td>
<td>223 ± 170</td>
<td>306 ± 114</td>
<td>420 ± 105</td>
</tr>
<tr>
<td>Average</td>
<td>401 ± 117</td>
<td>173 ± 48</td>
<td>144 ± 102</td>
<td>274 ± 94</td>
<td>366 ± 138</td>
</tr>
</tbody>
</table>

Data are expressed as the mean number of cells per square millimeter ± SD.
Microscopic Examination

All samples were examined in the laboratory by optic microscopy (Optiphot HFX-IIA; Nikon, Tokyo, Japan) at 100× and 400×, observing the GCD per square millimeter, N/C ratio, and inflammatory cells. GCD was calculated in separate samples (superior and inferior), and count was averaged between both. Inflammatory cells were scored on the following scale: 0, none; 1, few; 2, some; 3, many; and 4, abundant. These parameters represent the degree of damage inflicted on the ocular surface.

Surgery

All procedures were performed by the same experienced LASIK surgeon (JLRP), who used an excimer laser (Esiris; Schwind, Frankfurt, Germany) in all cases. The same flap diameter (9.5 mm), hinge size (4 mm), and flap location (superior) were applied in both groups.

The two groups were the M2 group, with 30 eyes undergoing LASIK with the M2 microkeratome (Moria) targeting a flap thickness of 130 μm according to a previously described method,9,30 and the IL group, with 34 eyes undergoing LASIK with the IntraLase FS Laser (IntraLase Corp.) targeting a flap thickness of 120 μm. The cut was made with the femtosecond laser at 15 KHz.

When the metallic suction ring of the microkeratome is applied to the ocular globe, it creates a vacuum that holds the eye in place during the rotation of the head of the microkeratome. In our study, the duration of application was recorded in seconds. In the case of the FS Laser, the time was registered from the moment the suction ring was applied to the globe until it was removed from the eye after the FS laser flap was created.

Statistical Analysis

Commercial software was used for data analysis (SPSS ver. 10.0; SPSS, Chicago, IL). Descriptive statistics were calculated as the mean ± SD and range. Student’s t-test was used to compare two independent means, the Pearson correlation coefficient (R) to correlate two variables in the same group, and the χ² test to compare discrete variables. P indicates the level of significance where P<0.05 = NS (not significant), <0.05 = S (significant).

RESULTS

Clinical data of patients in the M2 and IL groups, including age, sex, type of ablation, use of CLs, period of CL use, systemic treatments and SE, are shown in Table 1. Only two patients in the M2 group were receiving systemic medication in the form of anxiolytic drug treatment, and two of the IL group were receiving menopausal hormone therapy. None of the patients included in either group received any topical treatment before surgery.

 Conjunctival GCDs of both groups are shown in Table 2. All patients, in both groups, showed a decrease in GCD until the third month after LASIK. In the M2 group, we found an S decrease at 1 week (P<0.001) and at 1 month (P<0.001); at 3 months there was an S difference compared with preoperative (PO) GCD (P=0.037); and at 6 months we found an increase in the PO GCD, but it was NS (P=0.82). In the IL group there was an S decrease at 1 week (P<0.001), 1 month (P<0.001), and 3 months (P<0.001) after LASIK, and at 6 months we found an NS difference (P=0.78) compared with PO values.

![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933239/) &middot; Variation in GCD after LASIK on the ITB conjunctiva. At 1 week and 3 months after LASIK, the IL group showed a greater decrease in the GCD than did the M2 group: *P=0.039 and **P=0.027, respectively.

![Figure 2](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933239/) &middot; Sample of SB conjunctiva at 1 month after LASIK, showing a decrease in GCs and GCs of irregular sizes. The GCD in this sample was 180/mm². White arrowheads: normal GCs; black arrowheads: very small GCs. PAS stain (Optiphot HFX-IIA; Nikon, Tokyo, Japan). Original magnification, ×400.
When we compared GCDs of both groups before surgery, we found that they were similar, with NS differences in either the SB conjunctiva (P = 0.82) or in the ITB area (P = 0.94). In the SB conjunctiva, we found a greater decrease in the IL than in the M2 group at 1 month after LASIK (P = 0.041; Fig. 1). At 1 week and 3 and 6 months, there were NS differences (P = 0.93, P = 0.15, and P = 0.50), respectively. In the ITB conjunctiva, there was a greater decrease in the IL group at 1 week (P = 0.039) and 3 months (P = 0.027) after LASIK (Fig. 2). At 1 and 6 months, there were NS differences (P = 0.40 and P = 0.50, respectively).

A common observation after LASIK in 25% of the M2 and 23% of the IL samples was that the GCs both decreased in number and were of irregular size, either smaller or larger than commonly observed goblet cells, and frequently had no mucus inside (less intensely PAS-positive, or PAS-negative stain; Fig. 3). Chronic dry eye developed in all these patients, according to the criteria of Murube and Rivas,20 (data not shown) after surgery, without symptoms of ocular dryness before the operation. However, GCD before the surgery was slightly lower than average (293 ± 76/mm²), and 6 months after the surgery, it remained lower than average (207 ± 71/mm²).

The N/C ratio of conjunctival epithelial cells is shown in Table 3. After LASIK the N/C ratio of these cells decreased with both flap creation techniques (Fig. 4). With M2, this variation in the N/C ratio after surgery was S (P < 0.001 to P = 0.012), and with IL these variations were S at 1 month and 6 months (P = 0.022 and P = 0.008) and NS at 1 week and 3 months (P = 0.37 and P = 0.29). When we compared the N/C ratio between both methods after LASIK, there were no significant differences (P = 0.11 to P = 0.49) at any time.

Snake-like chromatin was observed in many samples, but the level was NS. No other alterations in the nucleus or cytoplasm of conjunctival epithelial cells were observed.

The data of the inflammatory infiltrates before and after surgery are shown in Table 4. Many eyes had high levels of inflammatory cells (scores >3) PO: 67% of the eyes in the M2 group and 25% in the IL group. When we compared both groups, we found NS differences during the follow-up period (P > 0.08, χ² test). In 99.8% of the eyes, the type of inflammatory cells found were lymphocytes, both before and after LASIK, except for one sample in which we observed neutrophils.

Suction time was 22.4 ± 7.9 (10–50) seconds in the M2 group and 108.5 ± 34.8 (68 to 225) seconds in the IL group, a significant difference (P < 0.001). There was a correlation between the time that the suction ring exerted pressure on the conjunctiva and the difference in GCD after surgery: at 1 week (R = 0.3) and at 1 month (R = 0.32) in the M2 group and at 1 month in the IL group (R = 0.5). If we compare both techniques, we observe a high correlation between the time necessary to fix the globe to create the flap and the decrease in the GCD at 1 month (R = 0.8; Fig. 5).

In myopic eyes, we found a good correlation between the decrease in GCD and the PO SE in the M2 group at 1 week (R = 0.61) and at 1 month (R = 0.43; Fig. 6). In the IL group, there was no correlation at 1 week (R = 0.12). However, we observed a good correlation at 1 month (R = 0.48; Fig. 7). The data of the hyperopic patients could not be statistically analyzed because there was an insufficient number of patients.

Regarding CL use, we found an S difference in the GCD before the surgery between users and nonusers of CLs (M2 group: P = 0.06 and IL group: P = 0.037). At 1 month after LASIK, there were no significant differences in any of the groups (P = 0.357).

**DISCUSSION**

LASIK induced changes in the GCD and N/C ratio of epithelial cells. In this study, the results showed the decrease in GCD to be related to suction time and the SE before surgery. We observed in myopic eyes that the decrease in GCD with both flap creation techniques for the LASIK procedures was related to the degree of preoperative myopia and the depth of the laser treatment. This effect has been observed in other studies20,37 and suggests that the destruction of the corneal tissue due to LASIK directly or indirectly affects the population of conjunctival GCs. Conjunctival GCD and N/C ratio of epithelial cells

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**Table 3. N/C Ratio of Conjunctival Epithelial Cells in the Two Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Before Surgery</th>
<th>1 Wk</th>
<th>1 Mo</th>
<th>3 Mo</th>
<th>6 Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>M2</td>
<td>0.50 ± 0.19</td>
<td>0.33 ± 0.08</td>
<td>0.31 ± 0.07</td>
<td>0.41 ± 0.09</td>
<td>0.33 ± 0.10</td>
</tr>
<tr>
<td>IL</td>
<td>0.37 ± 0.12</td>
<td>0.35 ± 0.11</td>
<td>0.29 ± 0.10</td>
<td>0.35 ± 0.13</td>
<td>0.30 ± 0.09</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ratio ± SD.

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**Table 4. Inflammatory Cells after LASIK**

<table>
<thead>
<tr>
<th>Score</th>
<th>Before Surgery</th>
<th>1 Week</th>
<th>1 Month</th>
<th>3 Months</th>
<th>6 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>M2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8.3</td>
<td>44.0</td>
<td>50.0</td>
<td>40.0</td>
<td>44.8</td>
</tr>
<tr>
<td>1</td>
<td>8.3</td>
<td>0.0</td>
<td>0.0</td>
<td>6.7</td>
<td>10.4</td>
</tr>
<tr>
<td>2</td>
<td>16.7</td>
<td>8.0</td>
<td>7.1</td>
<td>33.3</td>
<td>20.7</td>
</tr>
<tr>
<td>3</td>
<td>45.8</td>
<td>44.0</td>
<td>35.7</td>
<td>13.3</td>
<td>24.1</td>
</tr>
<tr>
<td>4</td>
<td>20.8</td>
<td>4.0</td>
<td>7.1</td>
<td>6.7</td>
<td>0.0</td>
</tr>
<tr>
<td>IL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>43.8</td>
<td>54.4</td>
<td>37.4</td>
<td>53.3</td>
<td>19.4</td>
</tr>
<tr>
<td>1</td>
<td>0.0</td>
<td>15.6</td>
<td>12.5</td>
<td>16.7</td>
<td>32.2</td>
</tr>
<tr>
<td>2</td>
<td>31.2</td>
<td>18.8</td>
<td>18.8</td>
<td>13.3</td>
<td>35.5</td>
</tr>
<tr>
<td>3</td>
<td>15.6</td>
<td>21.9</td>
<td>18.8</td>
<td>16.7</td>
<td>12.9</td>
</tr>
<tr>
<td>4</td>
<td>9.4</td>
<td>9.4</td>
<td>12.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Data are the percentage of patients with each score before surgery and at the postsurgical time points shown. Score: 0, none; 1, few; 2, some; 3, many; 4, abundant.
were significantly reduced after procedures with both the M2 microkeratome and the IL. These findings confirm previous reports on other microkeratomes and refractive surgery procedures.\(^{11,19-21}\) The IL produced a greater decrease in GCD than did the M2 manual microkeratome; however, the N/C ratio decreased with both techniques but without significant differences between them.

The reduction in GCD after IL was probably due to several factors such as toxicity of topical medication, damage of the cornal and conjunctival nerves, inflammation, or mechanical trauma produced by the suction ring. To find differences between the two groups, we compared both techniques, procedures, management of patients, and time of suction. Both groups were homogeneous regarding age, type of ablation, CL users, and preoperative SE. The only difference was gender-related; in the IL group there were more women, although before surgery the GCD in both groups were very similar (\(P = 0.82\), and thus this finding seems to be irrelevant. All procedures in each group were performed by the same experienced LASIK surgeon, to avoid possible differences in the procedure for making the flap. Topical medication was very similar in both groups. Iodine solution was used only on the periocular skin, never on the conjunctiva, and the management of the ocular surface after LASIK was very similar. All patients used unpreserved artificial tears after surgery and received the same treatment after surgery with tobramycin 0.3%-dexamethasone 0.1% for 1 week—the only difference being that the application after M2 surgery was four times daily and after IL surgery was six times daily. There was a more frequent application of anti-inflammatory medication in the IL group because in the first patients, in whom we had used similar doses, we had observed a postoperative inflammation that was more severe with the IL than with the M2 (Alió JL, unpublished data, 2005). Because inflammation on the ocular surface can result in decreased conjunctival GCD, the greater decrease in the IL group could be related to this effect, in that a longer suction time may also affect the ocular surface and induce inflammation changes. Such changes are frequently found in the form of traumatic conjunctivitis during the early follow-up of these patients. Chronic inflammatory infiltrates of lymphocytes were observed on the conjunctiva of both groups of patients (scores >3) before surgery, possibly because most of the patients were long-term CL users and had chronic inflammation. There were no significant differences between both groups after surgery. Regarding the trauma caused by the suction ring on the conjunctiva, we observed that the time required to create the flap by the IL was much longer than was required with the M2, and we found a high correlation between this time and the decrease in GCs after surgery. This damage may be related to the more fragile nature of the GC, which, being a secretory cell, has a less stable cell membrane that degranulates with vacuum pressure. In contrast, corneal nerve density decreased after LASIK, whether microkeratomes or bladeless flap creation techniques are used (Éric JC et al. IOVS 2006;47:EAbstract 516). However, in this study, there were no differences between treatments, which supports the idea that the corneal nerves are damaged by the LASIK effect, regardless of the technique used to create the flap. The decrease in the GCD may have occurred due to the effect of the suction ring on the conjunctival nerves that regulate GC secretion.\(^{38,39}\)

In conclusion, analyses of the ocular surface by impression cytology before and after LASIK showed a correlation between the degree of correction performed with LASIK and decreased GCD. These changes in GCD may contribute to the development of the ocular surface syndrome that occurs after LASIK. The greater reduction in the GC population with IL than with

![Figure 5](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933239/ on 09/15/2018)

**Figure 5.** Correlation between the decrease in the GCs and the suction time at 1 month after LASIK with the M2 microkeratome and the IL femtosecond laser.

![Figure 6](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933239/ on 09/15/2018)

**Figure 6.** Correlation between the decrease in the GCs and the preoperative SE at 1 week and 1 month after LASIK with the M2 microkeratome.

![Figure 7](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933239/ on 09/15/2018)

**Figure 7.** Correlation between the decrease in the GCs and the preoperative SE at 1 week and 1 month after LASIK with the IL femtosecond laser.
the M2 microkeratome, however, is more likely to be related to the suction time than to the technology.

References