Early Corneal Wound Healing and Inflammatory Responses after Refractive Lenticule Extraction (ReLEx)

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PURPOSE. To compare the early corneal wound repair and inflammatory responses after refractive lenticule extraction (ReLEx) and LASIK.

METHODS. Eighteen rabbits underwent ReLEx and another 18 underwent LASIK. Each group was divided into three subgroups of six rabbits each and these were subjected to refractive corrections of −3.00 diopters (D), −6.00 D, and −9.00 D. Slit lamp photography, anterior segment optical coherence tomography (AS-OCT), corneal topography, and in vivo confocal microscopy were performed 1 day after surgery. After euthanatization, the corneas were subjected to immunofluorescent staining for fibronectin, CD11b, Ki-67, and TUNEL assay.

RESULTS. On slit lamp microscopy, all corneas appeared clear pre- and postoperatively in both ReLEx and LASIK eyes. Corneal topography showed a more significant corneal flattening after LASIK than after ReLEx as the degree of correction was increased (P = 0.916 after −3.00 D correction to P = 0.097 after −9.00 D correction). In vivo confocal microscopy showed less light-scattering particles at the flap interface after ReLEx compared with LASIK. Immunostaining of fibroconnectin showed a less abundant expression in corneas that underwent ReLEx than LASIK. The differences became more marked as the power of correction was increased. Similar trend was seen in the number of CD11b-positive cells (P = 0.476 after −3.00 D correction to P < 0.001 after −9.00 D correction). There was no marked disparity observed in cell death and proliferation between post-ReLEx and -LASIK eyes.

CONCLUSIONS. This study has shown that the ReLEx procedure may result in less topographic changes, inflammation, and early extracellular matrix deposition than LASIK, especially at high refractive correction. (Invest Ophtalmol Vis Sci. 2011;52: 6213–6221) DOI:10.1167/iovs.11-7439

Lasers in situ keratomileusis (LASIK) is the most common refractive surgical procedure performed worldwide. The surgical technique is considered to be safe and effective, induces minimal pain, and is normally followed by minimal inflammation and rapid wound healing postoperatively.1,2 The absence of myofibroblasts, which can cause corneal haze and scarring after the surgery, is also another reported advantage of LASIK.2,3

The use of femtosecond laser (FS) technology in refractive surgery, has been largely restricted to the creation of corneal flaps as an alternative to the mechanical microkeratome, while the actual refractive correction remains the remit of the excimer laser.4 A recent breakthrough of the FS technology has resulted in a novel refractive procedure called refractive lenticule extraction (ReLEx). In ReLEx, the FS is programmed to excise an intrastromal refractive lenticule (matching the patient’s refractive error) within the cornea stroma, which is then removed. Lenticule extraction may be performed in two different ways. First, femtosecond lenticule extraction (FLEX) describes a procedure very similar to LASIK, in that a superiorly hinged corneal flap is performed by the laser, the flap is opened, and the exposed lenticule is stripped away. Second, small incision lenticule extraction (SMILE) is an alternative flapless procedure, whereby a flap is not made, but the lenticule extracted through a smaller accurate vertical incision of approximately 6 mm circumferential diameter, usually placed superiorly.

In LASIK, a 193 nm UV light from the argon fluoride excimer laser produces high-energy photons that break organic molecular bonds within corneal tissue in a process called ablative photodestruction.5 The 1053 nm wavelength of light used by the FS laser, unlike argon fluoride excimer pulses, is not absorbed by corneal tissue. During the FS laser-induced optical breakdown process called photodisruption, a plasma, shockwave, and cavitation bubble are produced. An advantage offered by FS laser is that by decreasing the pulse duration, the fluence threshold for breakdown can be reduced, thus minimizing the collateral damage and bubble size.6,7 In addition, the injury resulting from the excimer laser treatment has been shown in vivo and in vitro to cause the release of various cytokines and chemokines that modulate the corneal wound healing process.8 However, the release of some cytokines and chemokines may also potentially promote the infiltration of inflammatory cells that may damage tissue and paradoxically impair the wound healing process.9,10 The sequelae of corneal wound healing can occasionally lead to undesirable complications, such as regression of the refractive outcome and haze.11,12

Clinically, Sekundo et al.13 and Blum et al.14 have reported promising outcomes from patients who underwent ReLEx for myopia correction. However, the biological changes associated with the ReLEx surgical technique are still unknown. To gain more perspective into this novel refractive procedure, we used a rabbit model to compare the early postoperative inflammatory and wound healing response, as well as the corneal topographic changes between different degrees of correction with ReLEx and LASIK.

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METHODS

Animals

Thirty-six 12- to 15-week-old New Zealand White rabbits (3–4 kg body weight) were obtained from National University of Singapore and housed under standard laboratory conditions. Eighteen rabbits in each group were subjected to ReLEx and LASIK procedure. Each group (ReLEx and LASIK) was divided into three subgroups consisting of six rabbits that underwent either −3.00 D, −6.00 D or −9.00 D refractive corrections. One eye of each rabbit, selected at random, was subjected to surgery. In each group, three contralateral eyes were used as unoperated controls and the other three contralateral eyes underwent ReLEx with the corneal flap left intact (not lifted). Animals were anesthetized with xylazine hydrochloride (5 mg/kg intramuscularly; Troy Laboratories, Smithfield, Australia) and ketamine hydrochloride (50 mg/kg intramuscularly; Parnell Laboratories, Alexandria, Australia). The rabbits were euthanized under anesthesia 1 day after the surgery by overdose intracardiac injection of sodium pentobarbital. All animals were treated according to the guidelines of the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. The protocol was approved by the Institutional Animal Care and Use Committee of SingHealth.

Femtosecond LASIK Procedure

Our rabbit experimental model for LASIK was used as previously described and of which procedures were performed by J.S.M. and R.I.A.13 LASIK flaps were created by using a 500 kHz femtosecond laser (VisuMax, Carl Zeiss Meditec, Jena, Germany). The laser parameters were as follows: 110 μm flap thickness; 7.9 mm flap diameter; 170 nJ power; and spot distance and tracking spacing of 4.8 μm/4.8 μm for lamellar and 2 μm/2 μm for flap side cuts, respectively. After the flap was lifted, the underlying stroma received a 6.5-mm optical zone ablation using an excimer laser (Technolas; Bausch & Lomb, Rochester, NY); spot size 2.0 mm diameter, fluence 120 mJ/cm2, and repetition rate 50 Hz. When the flap was repositioned, a bandage contact lens (Bausch & Lomb) was applied and the eyelid was closed with a temporary tarsorraphy using a 6-0 silk suture.

Refractive Lenticule Extraction (ReLEx) Procedure

ReLEx was performed using a femtosecond laser (Visumax; Carl Zeiss Meditec) as described by Sekundo et al.13 The laser was virtually centered on the pupil. A small (S) curved interface cone was used in all cases. In order, the main refractive and nonrefractive femtosecond incisions were performed in the following automated sequence: the posterior surface of the lenticule (spiral pattern), the anterior surface of the lenticule (spiral out pattern), followed by a side cut of flap. The femtosecond laser parameters were: 120 μm flap thickness, 7.5 mm flap diameter, 175 nJ power for lenticule and 7.5 mm flap diameter, 175 nJ power for flap side cut. After completion of the laser sequence, a Siebel spatula was inserted under the flap near the hinge and the flap was lifted, the refractive lenticule was then grasped with forceps and extracted. The flap was then repositioned. A bandage contact lens (Bausch & Lomb) was placed over the flap and the eyelid was closed with a temporary tarsorraphy. Three contralateral eyes in each group underwent the same ReLEx procedure using the settings as described, however the flaps were not lifted and hence, no tarsorraphy was done.

Slit Lamp Photography, Optical Coherence Tomography, and Corneal Topography

Slit lamp photographs and anterior segment optical coherence tomography scans (AS-OCT) were captured before surgery and on Day 1 after surgery. Slit lamp photographs were taken with a zoom photo slit lamp (Nikon FS-3V; Nikon, Tokyo, Japan). Corneal cross-sectional visualization was performed using an AS-OCT (Visante; Carl Zeiss Meditec) and corneal topography was captured by using a hand-held videokeratograph (Oculus, Lynnwood, WA).

In Vivo Confocal Microscopy

In vivo confocal microscopy was performed before surgery and on postoperative Day 1, using retina tomography (HRT3; Heidelberg Engineering GmbH, Heidelberg, Germany). A carborum gel (Vidisc; Mann Pharma, Berlin, Germany) was used as immersion fluid. All corneas were examined centrally with at least 3 z-axis scans through from epithelium to endothelium. In vivo confocal micrographs were analyzed (Heidelberg Eye Explorer version 1.5.1 software; Heidelberg Engineering GmbH). Semi-quantitative analysis of the reflectivity level of the flap interface was performed by measuring the mean gray value of the reflective particles using Image software (developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij/index.html).

Tissue Fixation and Sectioning

For immunofluorescent staining, the corneas were excised from the globe and embedded in OCT cryo-compound (Leica Microsystems, Nussloch, Germany). Frozen tissue blocks were stored at −80°C until sectioning. Serial sagittal corneal 10-μm sections were cut using a cryostat (Microm HM550; Microm, Walldorf, Germany). Sections were placed on polylysine-coated glass slides and air dried for 15 minutes.

Immunofluorescent Staining

Sections were fixed with 4% paraformaldehyde (Sigma, St. Louis, MO) for 15 minutes, washed with 1X PBS, blocked with 4% bovine serum albumin (Sigma) in 1X PBS, 0.15% Triton X-100 (Sigma) for 1 hour, and incubated with either mouse monoclonal antibody against cellular fibronectin (Millipore, Billerica, MA) diluted 1:400, mouse monoclonal antibody against CD11b (BD Pharmingen, Franklin Lakes, NJ) diluted 1:100 in the blocking solution, or with prediluted mouse monoclonal antibody against Ki-67 (Invitrogen, Carlsbad, CA) at 4°C overnight. After washing with 1X PBS, the sections were incubated with goat anti-mouse Alexa Fluor 488-conjugated secondary antibody (Invitrogen) at room temperature for 1 hour. Slides were then mounted with medium containing DAPI (UltraCruz Mounting Medium; Santa Cruz Biotechnology, Santa Cruz, CA). For negative controls, nonimmune serum was used in place of the specific primary antibody. Sections were observed and imaged with a fluorescence microscope (Zeiss Axiosplan 2; Zeiss, Oberkochen, Germany).

TUNEL Assay

To detect apoptotic cells, a fluorescence-based TUNEL assay (In Situ Cell Death Detection Kit; Roche Applied Science, Indianapolis, IN) was used according to the manufacturer’s instructions.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) where appropriate. The P value was determined using the two-tailed Student’s t-test (Excel 2007; Microsoft). Data were considered to be statistically significant when P < 0.05.

RESULTS

Slit Lamp Photography and AS-OCT

One day after the surgery, the central cornea of all rabbits in either ReLEx or LASIK groups remained clear on slit lamp examination (Fig. 1A). The flap side cut outline could be seen on Day 1 after both surgical techniques. In both groups, increasing the degree of refractive correction did not seem to
visualize. In the post-ReLEx corneas, the keratocytes only showed moderate light-scattering particles (Fig. 2A, control). The flap interface itself was acellular and characterized by light-scattering particles. More intense and abundant reflective particles were observed in the corneas that underwent LASIK compared with those that underwent ReLEx, particularly in the corneas that had -6.00 D and -9.00 D correction (Fig. 2B). Semiquantitative analysis of the intensity of the reflectivity at the flap interface is depicted in a bar graph (Fig. 2C).

In corneas that underwent ReLEx with the flap left intact, the reflective particles could be seen at the posterior and anterior incisions of the lenticule (Fig. 2D). The density and intensity were comparable to those in the corneas where the lenticule was removed. Keratocytes with moderate light-scattering nuclei were present in the lenticule’s lamellae of the undisturbed ReLEx procedure (Fig. 2D).

**Corneal Topography**

As expected with myopic correction, flattening of the central corneas could be seen in the both post-LASIK and ReLEx corneas (Table 1). The average $K$ of the nonoperated corneas was $44.7 \pm 1.2$ D. Keratometric changes were similar in the corneas that underwent -3.00 D LASIK and ReLEx; $K_{\text{min}}$ and $K_{\text{max}}$ after LASIK were $39.2 \pm 3.3$ D and $39.6 \pm 1.9$ D, respectively compared with $38.6 \pm 1.1$ D and $40.0 \pm 1.2$ D, respectively after ReLEx. The average $K$ was $39.4 \pm 2.3$ D after LASIK and $39.3 \pm 0.5$ D after ReLEx ($P = 0.916$).

The difference in keratometric measurements became more evident as we increased the power of refractive correction. When the degree of correction was increased to -6.00 D, the $K_{\text{min}}$ and $K_{\text{max}}$ after LASIK were $37.6 \pm 3.1$ D and $38.0 \pm 2.0$ D, respectively, compared with $37.9 \pm 2.1$ D and $41.0 \pm 2.9$ D, respectively, after ReLEx. The average $K$ was $37.8 \pm 2.0$ D after LASIK and $39.5 \pm 2.2$ D after ReLEx ($P = 0.196$). After -9.00 D correction, the $K_{\text{min}}$ and $K_{\text{max}}$ post-LASIK were $34.3 \pm 4.0$ D and $35.5 \pm 2.1$ D, respectively, compared with $35.6 \pm 0.4$ D and $38.9 \pm 2.2$ D, respectively, after ReLEx. The average $K$ was $34.9 \pm 2.9$ D after LASIK and $37.3 \pm 1.2$ D after ReLEx ($P = 0.097$).

The keratometric changes were depicted by the corneal topography. As the degree of refractive correction was increased, we could clearly see a more flattened zone (blue zone on topography) on corneas after LASIK compared with those after ReLEx (Fig. 3).

**Immunohistochemistry**

Expression of fibronectin appeared in the central cornea on day 1 after ReLEx and LASIK surgery. The expression was distinct and consistent along the laser injury site. We observed a marginal difference in the staining intensity between the corneas that underwent -3.00 D ReLEx and -3.00 D LASIK. However, the difference became more marked when the degree of correction was increased to -6.00 D and -9.00 D. Fibronectin expression was relatively stronger at the site of laser injury and more apparent at the stromal bed after LASIK, in comparison with that after ReLEx (Fig. 4A). Similar features were also seen in the peripheral flap area (Fig. 4B).
In the corneas that did not undergo flap elevation, weak expression of fibronectin was observed along the posterior and anterior dissection plane of the lenticule after the ReLEx procedure (Fig. 4C). Similar staining intensity could be found at the peripheral flap (Fig. 4D).

Immunostaining of CD11b, an early inflammatory marker, showed a distinct difference between post-ReLEx and post-LASIK eyes, primarily in the central cornea. There was little to no CD11b-positive cells detected in the central cornea after −3.00 D ReLEx and only a little was seen after −3.00 D LASIK. However, there was a significant elevation of the number of cells expressing CD11b in the post-LASIK corneas when the power of correction was increased to −6.00 D and −9.00 D. Whereas in the post-ReLEx corneas, the CD11b-positive cells remained low even when higher power of correction was performed (Fig. 5A). There was an elevated number of cells expressing CD11b in the peripheral area of the flap compared with that in the central, probably representing peripheral recruitment and migration of inflammatory cells. This observation applied to both post-ReLEx and post-LASIK eyes. Lowering or increasing the power of correction did not seem to affect the number of the inflammatory cells after ReLEx (Fig. 5B). Quantification of CD11b-positive cells is depicted in a bar graph (Fig. 5C).

When the corneal flap was not lifted after ReLEx, there were no inflammatory cells seen in either the cornea center (Fig. 5D) or the flap margin (Fig. 5E).

### Table 1. Keratometric (K) Changes 1 Day after ReLEx and LASIK

<table>
<thead>
<tr>
<th>Procedure</th>
<th>K&lt;sub&gt;min&lt;/sub&gt;</th>
<th>K&lt;sub&gt;max&lt;/sub&gt;</th>
<th>Average K</th>
<th>P</th>
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<td>Control</td>
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<td>44.7 ± 1.2</td>
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<td>−3.00 D LASIK</td>
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<td>0.916</td>
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<td>40.0 ± 1.2</td>
<td>39.3 ± 0.5</td>
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<tr>
<td>−6.00 D LASIK</td>
<td>37.6 ± 3.1</td>
<td>38.0 ± 2.0</td>
<td>37.8 ± 2.0</td>
<td>0.196</td>
</tr>
<tr>
<td>−6.00 D ReLEx</td>
<td>37.9 ± 2.1</td>
<td>41.0 ± 2.9</td>
<td>39.5 ± 2.2</td>
<td></td>
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<tr>
<td>−9.00 D LASIK</td>
<td>34.3 ± 4.0</td>
<td>35.5 ± 2.1</td>
<td>34.9 ± 2.9</td>
<td>0.097</td>
</tr>
<tr>
<td>−9.00 D ReLEx</td>
<td>35.6 ± 0.4</td>
<td>38.9 ± 2.2</td>
<td>37.3 ± 1.2</td>
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**Cell Proliferation and Cell Death**

Ki-67 staining was observed in some cells on day 1 after both LASIK and ReLEx. We did not observe much difference in the...
number of cells expressing this proliferation marker after both surgeries and after increasing the degree of correction. There were very few cells expressing Ki-67 in the central flap (Fig. 6A). The expression was mainly restricted to the epithelial cells at the peripheral site of the corneal flap (Fig. 6B). Similar observation could be made on the corneas with intact flaps (Figs. 6C and 6D). Ki-67 was also primarily present in the epithelial cells of the flap margin.

TUNEL assay detected DNA fragmentation adjacent to the keratotomy site in the central flap (Fig. 7A), as well as peripheral flap (Fig. 7B). An increased number of apoptotic cells in the flap periphery in comparison with the flap center could be observed after ReLEx and LASIK. However, there was no significant disparity in the number of apoptotic cells after both procedures, even after elevating the power of refractive correction. Quantification of TUNEL-positive cells is depicted in a graph (Fig. 7C).

Cell death was also detected in the corneas with intact flaps, adjacent to the laser incision in the cornea center (Fig. 7D) and the flap margin (Fig. 7E). DNA fragmentation was distinct along the posterior and anterior aspects of the lenticule incisions after the ReLEx procedure.

**DISCUSSION**

LASIK is the most popular refractive procedure worldwide based on good clinical results in myopia treatment up to −12.00 D correction, with minimal pain and relatively short visual recovery time. Despite these advantages, there...
are some complications associated with the technique and subsequent wound healing process and inflammation; diffuse lamellar keratitis, epithelial ingrowth, stromal rethickening causing regression, haze, keratoectasia, flap dislocation, and dry eye symptoms.\textsuperscript{17–20} The refractive lenticule extraction (ReLEx) technique and its variant small incision

\textbf{FIGURE 4.} Expression of cellular fibronectin in the corneas on Day 1 after ReLEx and LASIK. Each group was subjected to a different degree of correction: $-3.00$ D, $-6.00$ D, and $-9.00$ D. Fibronectin was detected along the incision line in (A) the cornea center and (B) the periphery of flap. In the post-ReLEx corneas with flap not elevated, fibronectin was also expressed along the incision line at (C) the flap center and (D) the flap margin. Arrowheads show the lenticule’s posterior incision line and arrows show the lenticule’s anterior/ flap interface incision line. Sections were counterstained with DAPI, which stained the nuclei (blue). Scale bars, 50 $\mu$m.

\textbf{FIGURE 5.} Expression of CD11b in the corneas on Day 1 after ReLEx and LASIK. Each group was subjected to a different degree of correction: $-3.00$ D, $-6.00$ D, and $-9.00$ D. CD11b-positive cells was observed along the incision line in (A) the cornea center and (B) the periphery of the flap. (C) Mean number of CD11b-positive cells in the flap center and periphery depicted in the bar graph. Error bars represent SD and asterisks (*) indicate that $P < 0.05$. In the post-ReLEx corneas with intact flap, there were no CD11b-positive cells seen along the incision line at either (D) the flap center or (E) the flap margin. Sections were counterstained with DAPI, which stained the nuclei (blue). Scale bars, 50 $\mu$m.
lenticule extraction (SMILE) have recently been proposed as alternatives to conventional LASIK and may offer benefits in terms of reduced tissue removal, better biomechanical stability, better flap strength, reduced risk of flap dislocation, and less dry eye symptoms. However, until now little has been known about the immediate inflammatory and wound healing responses after ReLex.

Increased reflectivity can be easily detected along the laser-ablated site using in vivo confocal microscopy. The localization of the lenticule’s anterior cut and lenticule’s posterior cut was noticeable in each of the post-ReLex eyes in which the flap was left undisturbed, where reflective particles were evident. The intensity of the reflectivity remained similar after the removal of the lenticule. This may imply that there was relatively similar level of apoptotic cells or denatured collagen before and after the lenticule removal, and that the lenticule manipulation and removal action did not induce more wounding to the underlying tissue.

**FIGURE 6.** Expression of Ki-67 in the corneas on Day 1 after ReLex and LASIK. Each group was subjected to a different degree of correction: −3.00 D, −6.00 D, and −9.00 D. (A) Only a few Ki-67-positive cells were detected in the epithelium of the cornea center. (B) In contrast, Ki-67-positive cells were relatively abundant in the epithelium of the flap edge. Similar Ki-67 staining pattern could be observed in the post-ReLex corneas with intact flap at (C) the flap center or (D) the flap margin. Sections were counterstained with DAPI, which stained the nuclei (blue). Scale bars, 50 μm.

**FIGURE 7.** DNA fragmentation detection by TUNEL assay in the corneas 1 day after ReLex and LASIK. Each group was subjected to a different degree of correction: −3.00 D, −6.00 D, and −9.00 D. Cell death was detected along the incision line in (A) the cornea center and (B) the periphery of flap. (C) Mean number of TUNEL-positive cells in the flap center and periphery depicted in the bar graph. Error bars represent SD. In the post-ReLex corneas with flap not elevated, cell death was also observed along the incision line at (D) the flap center and (E) the flap margin. Arrowheads show the lenticule’s posterior incision line and arrows show the lenticule’s anterior/flap interface incision line after ReLex. Sections were counterstained with DAPI, which stained the nuclei (blue). Scale bars, 50 μm.
stroma. Interestingly, a different phenomenon was observed in the post-LASIK eyes where there was an increase in light reflecting particles as we increased the degree of refractive correction. Because the reflectivity was not that different between the post-ReLEx corneas with intact flaps and those with lenticules removed, we can speculate that the excimer laser may have caused more damage to the stroma with greater attempted corrections (compare -3.00 D and -9.00 D correction). Greater levels of correction require more tissue to be ablated and longer exposure to the excimer laser, hence delivering more energy to the cornea. For -3.00 D correction, a total energy of approximately 4.10 J is delivered to the cornea, and it increases to approximately 8.07 J and approximately 11.94 J for -6.00 D and -9.00 D correction, respectively (data were obtained from Bausch & Lomb). In contrast, with the ReLEx procedure, the laser simply cuts a different shaped lenticule, requiring an energy of only about 0.58 J (data were obtained from Carl Zeiss Meditec) and the energy levels do not differ significantly between attempted corrections.

Through in vivo confocal microscopy, the deposited acellular and amorphous stromal layer at the keratotomy level showed an elevated light-scattering. Fibronectin was consistently expressed along the incision line on immunohistochemistry, including weak expression along the lenticule’s posterior and anterior cut in post-ReLEx eyes with intact flap. The removal of the lenticule and increment of the refractive correction did not seem to alter the fibronectin expression. However, treatment with the excimer laser in LASIK caused an elevated intensity of fibronectin staining when compared with the eye in the same group where the corneal flap remained intact. The little accumulation of fibronectin in the central area of the flap interface suggests a reduced wound healing reaction after ReLEx if compared with that after LASIK, especially at higher refractive correction. We also found that fibronectin was expressed at a higher level at the flap margin of LASIK compared with ReLEx, which could be ascribed to the wider treatment zone in LASIK, which normally includes a 1.0-mm blend zone in addition to the optical zone. A conventional blend zone is not necessary in ReLEx as the lenticule is shaped accordingly to allow a smooth transition between the laser-treated zone and the untreated area of the cornea.

Monocytes that expressed CD11b were used as a marker for inflammatory infiltration in this study. There was a significant increase of the number of CD11b-positive cells along the center of the laser-ablated site as the power of refractive correction was increased after LASIK compared with after ReLEx: 24.07 ± 3.39 vs. 2.96 ± 2.57 J (P = 0.001) after -6.00 D correction and 31.48 ± 3.39 vs. 3.33 ± 3.35 J (P < 0.001) after −9.00 D correction. Inflammatory infiltration was almost negligible even after −9.00 D ReLEx correction. In the corneas with non-lifted flap, there were no inflammatory cells seen after ReLEx, which suggests that the eximer laser treatment in LASIK stimulates a higher degree of inflammation, by releasing more cytokines and chemokines that recruit the inflammatory cells to the injury site.

Inflammatory cells were abundant in the periphery of the flap in post-ReLEx eyes. Similar findings in post-LASIK eyes have been reported in the literature.1,2 This is understandable as both techniques create an incision through the epithelium and basement membrane at the flap edge. In the healthy and intact cornea, the basement membrane can function to bind cytokines,23 suggesting that it may act as a physical barrier for signaling molecules that are produced by the epithelial cells or tear fluid.24 Thus, when the barrier is compromised, the underlying stroma is exposed to the signaling molecules and the inflammatory cell infiltration is augmented. In more advanced ReLEx techniques such as SMILE, with a much reduced incision size, these may be reduced further due to less epithelial disruption.

Cell proliferation was only detected at the flap margin and occurred primarily in the epithelial cells at and surrounding the epithelial plug. This staining pattern was seen both after ReLEx and LASIK procedure, as well as before and after the flap was lifted. Our observation in post-LASIK eyes is similar to those previously reported by Meltendorf et al.3 and Netto et al.21

Cellular apoptosis observed after ReLEx in this study was comparable to the cell death previously reported after LASIK.3,21,22 Apoptosis has been suggested to be the initiator of the subsequent corneal wound healing cascade.25 Apoptosis keratocytes underlying the ablation zone has been proposed to be caused by the release of IL-1, TNF-α, and Fas ligand from the injured corneal epithelium.25–27 However, in the absence of epithelial damage or displacement, femtosecond laser-assisted LASIK and ReLEx still induce keratocyte cell death. Femtosecond laser-induced reactive oxygen radicals has been proposed as a contributor to the keratocyte apoptosis.28 Therefore, it is not surprising that DNA fragmentation was detected along the posterior and anterior cut of the lenticule in the cornea with intact flap after ReLEx. These TUNEL-positive cells were possibly located at both the direct anterior and posterior of lenticule anterior cut, as well as the lenticule posterior incision line. The cell death seen after lenticule removal might be the residual apoptotic keratocytes, which were not removed along with the lenticule or might be from the combination of apoptotic cells at the direct front of the lenticules anterior incision and directly at the back of the lenticules posterior incision.

In the present study, we also investigated the keratometric changes after ReLEx. The difference after −3.00 D LASIK and ReLEx treatments was minimal. However, the differences became more significant as we increased the degree of correction. The differences in topographic changes observed in this study may be due to less tissue being ablated or perhaps an undercorrection after ReLEx surgery compared with LASIK. We attempted to measure the residual corneal thickness, but our results appeared inconsistent or inaccurate, with some corneas measuring thicker postoperatively in comparison with preoperatively. A similar observation was made by Wang et al.,29 who found the thickening of corneas 1 day and 1 week after LASIK. Similarly, retinoscopic values on the first few days postoperatively are also normally not accurate.30 A follow-up study is now under way in our laboratory to investigate the long-term effects of ReLEx procedure, and the corneal thickness and retinoscopic measurements will then be addressed.

In summary, we have shown that there is a less reactive wound healing response and inflammatory infiltration, early on after ReLEx in comparison with LASIK, suggesting that ReLEx using just the femtosecond laser may result in less corneal inflammation, which may impact favorably on visual results and outcomes. These disparities were only significant at higher refractive corrections, and may be related to marked differences in energy delivery to the cornea with each treatment. This may also explain the higher variability in efficacy and predictability with LASIK for high myopia. In addition, eyes having ReLEx at higher corrections showed less corneal flattening compared with similar levels of correction with LASIK. This may represent less tissue loss to achieve the same refractive correction with ReLEx. We are currently undertaking further long-term animal studies to further elucidate these findings.
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