In Vivo Corneal Confocal Microscopy Comparison of Intralase Femtosecond Laser and Mechanical Microkeratome for Laser In Situ Keratomileusis

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PURPOSE. To assess and compare corneal modifications induced by Intralase PulsionFS femtosecond laser and mechanical microkeratome Hansatome for laser in situ keratomileusis (LASIK) using the new-generation Heidelberg Retina Tomograph II (HRT II)/Rostock Cornea Module confocal microscope.

METHODS. In this case-control study, 24 eyes of 12 patients were examined with the HRT II in the follow-up of Intralase femtosecond laser for LASIK myopic surgery. Twenty eyes of 10 patients were also examined after microkeratome Hansatome-LASIK surgery. In both groups, the patients underwent the first follow-up examination on day 7 and the last 12 months after surgery. Morphologic modifications of corneal architecture were evaluated, and comparisons were made between the two flap-formation techniques.

RESULTS. Evaluation of both groups on day 7 showed keratocyte transformation, most likely related to cellular activation beneath the interface. The flap margin after the Intralase technique appeared microscopically as a clear-cut edge that included the epithelial plug. At month 2, secondary fibrosis, adjacent to the still well-defined Intralase flap edge, was observed. This reaction diminished with time, leaving a fibrotic scar adjacent to a wound constriction originating from the surrounding stroma. The flap margin of the mechanical microkeratome had the appearance of a less clearly identified fibrotic scar with no epithelial plug.

CONCLUSIONS. This study reveals morphologic similarities between the interfaces obtained by femtosecond laser and mechanical microkeratome, probably because the same excimer laser performed the photoablation. However, the Intralase flap margin showed greater fibrotic scarring than that induced by the mechanical microkeratome. (Invest Ophthalmol Vis Sci. 2006;47:2803–2811) DOI:10.1167/ iovs.05-1207

Laser in situ keratomileusis (LASIK) is the leading technique in refractive surgical correction of ametropias. Even with the constantly evolving microkeratomes, a problem with flap creation, though rare, may have dramatic consequences. The most important intraoperative complications with corneal flap cut,2,4 are perforation of the flap (button hole), which is more frequent with steep corneas; incomplete flap formation, which results from discontinued microkeratome progression; complete cut of the flap (free cap); and irregular or very thin flap, which in most cases results from the loss of suction, insufficient aspiration, or epithelial abrasion from microkeratome blade-induced microtrauma of the flap. Fortunately, intraocular perforation during corneal flap formation no longer occurs with the latest generation microkeratomes and the systematic incorporation of plates that limit cutting depth.

The advent and recent introduction on the market of the Intralase (IntraLase Corp., Irvine, CA) femtosecond laser,3–6 which enables a lamellar corneal flap, has given us a valuable alternative for flap creation. Theoretical advantages include avoidance of the imperfections of mechanical flap formation, thinner flaps, discontinuation of procedures for preparation and maintenance of microkeratomes, decreased risk of infection, and reduced need for intraocular pressure for suction during the procedure. Moreover, this technique improves precision in determining flap diameter and thickness and may allow programming of the angulation of the flap periphery, which theoretically can provide better flap stability and thus prevent epithelial invasion. Finally, this technique offers the possibility of continuing a lamellar flap procedure in a single session after a pause in flap creation.

Many studies in the past few years involving in vivo confocal microscopy have focused on corneal modifications induced after LASIK surgery.7–18 In the present study, we used a new-generation confocal microscope, the corneal module of the Heidelberg Retina Tomograph II (HRT II; Heidelberg Engineering GmbH Heidelberg, Germany) system.19–21 This device provides high-definition histologic-like images of the ocular surface, which allowed us to observe the aspects of the corneal interface and corneal flap margin in the long-term follow-up of the femt-LASIK technique and to compare them with patterns observed after classic microkeratome-assisted LASIK treatment.

MATERIALS AND METHODS

Patients

Twenty-four eyes of 12 patients (eight men, four women) who underwent femtosecond LASIK surgery were systematically examined from July 2004 to July 2005. Mean age of the patients was 34.8 years (range, 22–53 years). The femtosecond laser used was the Intralase PulsionFS, and the excimer laser used was Technolas 217C (Bausch & Lomb Chiron Technolas GmbH, Dornach, Germany).

Mean preoperative refraction in spherical equivalence was –4.65 D (range, –2.25 to –8.25 D) measured with an autorefractometer (Topcon RM-8800; Topcon America Corp., Paramus, NJ). The mean preoperative pachymetry was 538 μm (range, 500–580 μm) measured by Orbscan II (Bausch & Lomb). The programmed corneal flap thickness was 100 μm for four eyes, 110 μm for 16 eyes, and 120 μm for four eyes, and the mean programmed diameter of the optical zone of all 24 eyes was 5.6 mm (range, 5.5–6 mm).

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All patients undergoing the femto-LASIK procedure were examined by the same operator (BS) with the in vivo HRT II/Rostock Cornea Module (HRT II/RCM; Heidelberg Engineering GmbH) confocal microscopy, as follows: 22 eyes on day 7, 14 eyes at month 2, 10 eyes at month 6, and 8 eyes 12 months after surgery.

Twenty eyes of 10 patients (six men, four women) were included in the second group after treatment with a classic mechanical microkeratome-assisted LASIK operation. Mean age was 31.3 years (range, 20–49 years). The microkeratome used to create the corneal flap was the Hansatome (Bausch & Lomb), and the laser used for the photoblation was the same excimer laser (Technolas 217C; Bausch & Lomb). Mean preoperative refraction in spherical equivalence was −4.75 D (range, −3.25 to −6.25 D). Mean preoperative pachymetry was 540 μm (range, 520–575 μm). The programmed thickness of the corneal flap was 160 μm for 16 eyes and 180 μm for four eyes, and the mean programmed diameter of the optical zone was 5.7 mm (range, 5.5–6 mm). The 8.5-mm ring was used in all patients. All 20 eyes were examined by the same operator (BS) using the same technique: 18 eyes on day 7, 14 eyes at month 2, 12 eyes at month 6, and 10 eyes 12 months after surgery.

In this case-control study, all patients in the femto-LASIK and the classic microkeratome-LASIK technique groups underwent single refractive surgery with no intraoperative or postoperative complications, as assessed by repeated slit lamp examinations and refraction measurements. With the exception of the difference in average attempted corneal flap thickness, both groups were fully comparable; the criterion for inclusion in either of the two groups was consultation with the surgeon with access to IntraLase technology.

All HRT II images were analyzed in a masked manner by two additional examiners (CB, VI) to compare corneal patterns in each of the two studied groups, with particular attention paid to the flap and stromal aspects, including keratocyte activation and keratocyte-apparent density.

Because the HRT II confocal microscope is a minimally invasive technique and the choice of treatment technology was not determined for the purpose of our study, our institutional review board (Quinze-Vingts National Ophthalmology Hospital, Paris, France) approved this investigation. Systematic consent was obtained from all participants after explanation of the nature of the study. All participants were treated in accordance with the Declaration of Helsinki.

Flap Creation Technique with the Femtosecond Laser

The femtosecond laser is a solid state laser. The laser creates plasma inside the corneal stroma without interference with the epithelium. Plasma expands into small cavitation bubbles (microvacitation). This photodisruption phenomenon vaporizes small volumes of corneal stroma. Each impact of 1 to 3 μm produces a cavitation bubble of a maximum 10 μm in diameter. Flap periphery can be programmed to have an angulation with respect to the flap plane (30°–90°). In theory, this angulation provides better overlapping of the flap after replacement. Our patients benefited from this technique, performed according to a standard procedure. After laser application, the resected corneal flap was elevated with a smooth spatula. Then refractive ablation with the excimer laser was performed. The stromal bed was irrigated with balanced salt solution (BSS; Alcon Laboratories, Fort Worth, TX) before flap replacement to eliminate possible debris. After surgery, combination tobramycin 0.3%/dexamethasone 0.1% suspension (Tobradex; Alcon Laboratories) was prescribed to all patients 3 times a day for 8 days. In addition, carboxymethylcellulose sodium 1% eye drops (Celuvisc; Allergan Laboratories, Irvine, CA) were prescribed 3 times a day for 15 days.

In Vivo Corneal Confocal Microscopy

All patients were examined with the newly developed in vivo HRT II/RCM confocal microscope. The HRT II is a confocal scanning laser ophthalmoscope primarily used for analysis of the posterior segment of the eye (optic nerve head and macula). With the addition of the corneal module developed in collaboration with scientists at the University of Rostock (Germany), the HRT II is converted to an in vivo confocal microscope of the eye surface. It allows the in vivo study of all corneal layers, both in the center, as did first-generation confocal microscopes, and at the periphery, giving high definition images. The laser source used in the HRT II/RCM is a diode laser with a wavelength of 670 nm. The acquired two-dimensional image is defined by 384 × 384 pixels covering an area of 400 μm × 400 μm with lateral digital resolution of 1 μm/pixel and depth digital resolution of 2 μm/pixel.

Before in vivo confocal microscopy examination, one drop of a topical anesthetic, oxybuprocaine 0.4% (Novesine 0.4%; MSD-Chibret, Paris, France) and one drop of a gel tear substitute, carbomer 0.2% (Lacrigel, carborurer 9800NF, Europhia, Monaco), with a refraction index similar to that of the cornea, were instilled in the lower conjunctival fornix. The patient was then seated at an examination table with the head in the headrest. The patient fixated on a small, bright, red light with the contralateral eye. The eye was adjusted by means of the live image and was controlled by a charge-coupled device (CCD) color camera (640 × 480 pixels, RGB, 15 frames/s). All acquired images were visualized directly on a computer screen (PC Pentium IV, Windows XP) and were stored on a hard disc. The x-y position of the image and the section depth were controlled manually. The objective of the microscope was an immersion lens (Olympus, Hamburg, Germany), magnification ×60, covered by a polymethylmethacrylate cap. The focal plane of analysis could be displaced manually over the entire cornea. In contrast to the classic confocal microscope, the corneal HRT II allows lateral displacements, thus permitting examination of the flap periphery.

The examination was performed in the sagittal axis (anteroposterior axis) so that, as the operator proceeded, corneal epithelium, subepithelial neural plexus, anterior stroma, posterior stroma, and endothelium were successively examined. The flap periphery was examined in the same manner when the patient gazed laterally. For all eyes studied, several confocal microscopic images were taken of all corneal layers. Each eye was examined for less than 15 minutes, and no complications related to in vivo confocal microscopy evaluation were noted. The results of corneal morphology were compared after femto-LASIK and classic microkeratome-LASIK surgery in all central and peripheral corneal layers, with particular attention paid to interface and flap margin areas.

RESULTS

Corneal Epithelium

After femto-LASIK and microkeratome-assisted LASIK, corneal epithelium morphology showed no particular change up to 12 months. In most of the eyes examined by femto-LASIK (22 of 24) and microkeratome-LASIK (18 of 20) procedures, some images exhibited a discontinuous Bowman layer—precisely in the center—in alternation with anterior stromal keratocytes (Fig. 1). This aspect could thus correspond to microfolds in the Bowman layer. These presumed microfolds were not clearly identified at the biomicroscopic examination. Quantification of the presumed microfolds was difficult for two main reasons. First, given the high microscopic magnification of the cornea, the entire Bowman layer could not be observed for a precise count of the microfolds. Second, during examination, it was difficult to stabilize the image at the Bowman layer because of ocular movements (even if they were minor, they were amplified by the high magnification) on the part of the patient. These presumed microfolds were more easily observed at the center of the cornea than at the periphery because centrally the Bowman layer is perpendicular to the optical axis. However, for the examiner and for the two other examiners who analyzed all images in a masked manner, no major differences were observed when comparing the aspect and the density of the
the microfolds after the femto-LASIK or the microkeratome-assisted LASIK technique.

Nerve Fibers in the Subbasal Region

The subbasal nerve fiber bundles were not observed in any patient on either day 7 or 2 months later with either the femto-LASIK or the microkeratome-assisted LASIK technique. At the 6-month follow-up, subbasal individual nerve fiber regeneration was visible in all patients in both groups (Fig. 2).

Nerve Fiber Bundles in the Corneal Stroma

Nerve regeneration was visible in the corneal stroma from the 2-month follow-up and beyond in both groups (Fig. 3).

Corneal Stroma Changes

In the anterior stroma, for the femto-LASIK and the microkeratome-assisted LASIK groups, the most anterior stromal layer (including the flap) showed no modification of keratocyte morphology during the entire 12-month examination period.

In contrast, more posteriorly, the interface was identified by the absence of keratocytes and the presence of small particles of variable brightness. In both groups, a few very bright particles were visible in all patients and at all examinations. The interface morphology was similar with both techniques (Fig. 4A–C) during the entire 12-month examination period.

At the day 7 examination in the femto-LASIK group, hyper-reflective zones appeared in an equidistant arrangement, measuring only a few microns. The dimensions of these zones and the distance between them perfectly matched the theoretical dimensions of the femtosecond laser spot impacts (the spot measures 3–5 μm) and the distance between spots programmed by the surgeon (the distance separating spots varies between 8 and 16 μm). Thus, the aspect found in our study could be the tissular response to the femtosecond laser impacts (Fig. 5). It was not possible to use confocal microscopy to identify whether these presumed impacts were located at the posterior side of the flap or at the stromal bed. These hyper-reflective spots were also seen outside the laser ablation zone, which is a logical occurrence because the IntraLase laser zone is much larger than the excimer laser zone, but once again it was not possible to determine the exact location of these presumed spots. These images were not seen during the follow-up visits, and they spontaneously disappeared between day 7 and month 2. Moreover, these images were not observed in any cornea of the microkeratome-LASIK group. Finally, epithelial invasion at the interface was not observed in either group during the entire 12-month examination period.

In the posterior stroma, the most important findings were seen below the interface. Keratocytes showed high reflectivity. This aspect could be the expression of keratocyte activation or migration. These keratocytes were more easily identified with visible processes and bright nuclei than were quiescent keratocytes. Activation seemed to be the most important at the day 7 examination (Fig. 6A–D). No cellular-free zone with complete loss of keratocytes, suggesting apoptosis, was found below the flap. At the 2-month examination, keratocytic activation diminished, and at the 6- and 12-month examinations, inactive, normal keratocytes were observed. Moreover, at the same time intervals, fibrillary processes were observed. These processes could be considered nerve regeneration, though they seemed to be in contact with keratocyte cell bodies (Fig. 7A–D). Thus, it was difficult to define the nature of these processes more accurately.

For the examiner, no difference in keratocyte activation intensity was found between the two techniques. Keratocytes were not quantified because they could not be accurately counted at the same depth and in same areas in all eyes. The cap covering the microscope lens measures 12 mm of diameter, has a surface area of 75 mm², and covers practically the whole corneal surface. Because of that and because of the high resolution of the microscope, it was difficult for the examiner
to know where the images were located on the microscopic level; thus, it was impossible to analyze the number and the topography of the activated keratocytes and to reexamine a precise area.

Both masked examiners agreed that it was impossible for them to determine any difference between the two techniques based on the HRT II images. Therefore, it can be said that morphologic transformation of keratocytes occurred and seemed similar according to both techniques. However as previously described, precise quantitative comparison of the activated keratocytes could not be achieved. More posterior stromal layers underneath the zone of modification, corresponding to a thickness of approximately 100 μm behind the interface, showed no significant change.

**Endothelium**

The endothelium showed no particular change in the femto-LASIK group or the microkeratome-assisted LASIK group during the entire 12-month examination period.

**Periphery of the Flap**

Flap edge creation is theoretically precise with the IntraLase. This precision was confirmed on the images provided by the corneal HRT II, which showed, at the day 7 examination, a very well-defined periphery within the corneal stroma (Figs. 8A, 8B). Regular borders defined the flap edge, with epithelial cells filling the empty spaces at the flap margin.

In contrast, flap periphery created by the Hansatome on day 7 showed a less clearly defined periphery with epithelial cell invasion (Fig. 9). In most patients, these epithelial cells disappeared at the 2-month examination, leaving an irregular zone of fibrotic wound healing (Fig. 10). Nevertheless, in 4 of 14 eyes examined at month 2, some epithelial cells were observed in the periphery.

At the 2-month examination, biomicroscopic evaluation of the femto-LASIK group showed a visible flap periphery and a hyperreflective adjacent stroma with irregularly arranged fibrils, suggesting a wound healing process. The periphery was still clearly delimited by epithelial cells. No activated or abnormally large keratocytes were visible. Paradoxically, although at the biomicroscopic level the periphery was more clearly delimited with IntraLase, we observed a more substantial wound healing process than was observed with the mechanical microkeratome, as assessed by the presence of white fibrotic strands in the stroma adjacent to the flap margin. This result observed with the confocal microscope matched biomicroscopic patterns (Figs. 11A, 11B).

At the 6- and 12-month examinations, the cicatricial reaction of the femto-LASIK group seemed more organized, leaving, in certain areas, fibrotic tissue adjacent to the epithelial plug. Conversely, in other areas, the cicatricial reaction seemed to decrease, leaving only thin stromal fibrosis. These patterns suggested the occurrence of stromal contraction at the incision level with a variable disappearance of the epithelial plug. At the incision bed, complete fibrosis was seen without epithelial cells (Figs. 12A–E). The cicatricial stromal fibrosis was clearly seen even at the 12-month postoperative follow-up.

At the 6- and the 12-month follow-up, examination of the microkeratome-assisted LASIK group showed minimal cicatricial fibrosis in the periphery, without epithelial cells. Epithelial cells were observed in some areas of the periphery in only 3 of 10 eyes examined 12 months after surgery.

**DISCUSSION**

Laser creation of a corneal flap during refractive surgery is an alternative to mechanical microkeratomes. To date, many studies investigating in vivo corneal confocal microscopy after LASIK have been published. These studies have
mainly demonstrated the presence of microfolds of the Bowman layer, particles of variable dimensions and reflectivity at the interface, and morphologic modifications of the keratocytes.

Recent studies using electron microscopy or histopathologic or immunohistochemical investigations have made it possible to compare results with those obtained by in vivo confocal microscopy. They have proven that after LASIK, corneas show basal epithelial cell hypertrophic changes over valleys produced by underlying Bowman layer undulations. The in vivo confocal microscopy performed in the present study, despite the higher resolution than obtained with first-generation confocal microscopes, did not show such histologic modifications in the epithelial cells. However, aspects of Bow-

![Figure 6](imageurl)  
**FIGURE 6.** Demonstration on day 7 examination of the likely tissular response of the laser impacts of IntraLase laser (arrowhead) and the underlying presence of keratocytes with high reflectivity and visible cytoplasmic processes (A, arrow). Activated keratocytes at the stromal bed under the interface (B). Similar keratocyte modifications after classic microkeratome-assisted LASIK surgery (C). Quiescent keratocytes of a normal control cornea (D). HRT II images; magnification, 400 × 400 μm.

![Figure 7](imageurl)  
**FIGURE 7.** Fibrillary processes (arrow) observed 6 months after IntraLase-LASIK surgery (A) and mechanical microkeratome-LASIK surgery (B), which could be considered nerve regeneration, though they seem to be in contact with keratocyte nuclei (arrowhead). Fibrillary processes (arrow) observed 12 months after IntraLase-LASIK surgery (C). Quiescent keratocytes are seen under the interface 6 months after IntraLase-LASIK surgery (D). HRT II images; magnification, 400 × 400 μm.
man layer microfolds were consistently observed, in accordance with histopathologic studies.28–30 Interestingly, no clinical implication was found in relation to the Bowman membrane microfolds.

Studies of in vivo confocal microscopy of corneal reinnervation after microkeratome-assisted LASIK9,13,16,17,27 have shown that in the subbasal and stromal flap regions, the number of nerve fiber bundles decreased by more than 90% 1 week after LASIK. Subbasal and stromal flap nerve fiber bundles gradually increased from 3 months to 1 year after surgery, but they remained less abundant than the preoperative value. In our study, flap creation by IntraLase did not seem to cause any delay or enhancement in regeneration of subepithelial nerve fibers because all 10 eyes examined at month 6 showed at least some nerve regeneration.

It has been found7,13,18,25,26 that keratocytes tend to disappear around the wound interface, presumably because of keratocyte apoptosis. At the same time, other keratocytes show activation, namely reflective nuclei and visible cytoplasmic processes, compared with normal, weakly reflective nuclei.

Recent studies14,31,32 evaluating rabbit corneas after LASIK with an in vivo tandem scanning confocal microscope showed that all keratocytes above and below the interface remained quiescent without activation or transformation. Histologic analysis of the same corneas detected no expression of α-smooth muscle actin, suggesting that myofibroblasts were not involved in the central wound healing response. The quiescent aspect of keratocytes found below the interface in rabbit eyes was not observed in our study. In our analyses, regardless of the method of flap formation (IntraLase or Hansatome), all corneas showed transformed morphology of the keratocytes located below the flap and the presence of highly reflective cytoplasmic processes. In no examination during our study was keratocytic transformation in the corneal flap observed. There did not seem to be any difference in the aspect of keratocyte transformation of the posterior stroma regarding the method of flap creation.

In histologic studies of postmortem corneas after uncomplicated LASIK surgery,9,13,27,33 keratocytes in or next to the entire length of all stromal wounds had the morphologic appearance of activated keratocytes. The proportion of activated keratocytes diminished over time from 1 month to 6 months after surgery. Keratocyte cell count showed a significant reduction in the anterior portion of the stroma and increased up to 12 months after surgery, whereas the posterior stroma showed no change in the number of keratocytes up to 12 months after surgery. In our study, keratocyte density was not evaluated because of the difficulty of accurately distinguishing and counting cells in a three-dimensional network within the stroma.

Globally, in our study, the interfaces were similar with either flap formation technique (IntraLase or Hansatome), most likely because photoablation was performed with the same excimer laser in both groups. With the in vivo confocal microscope, the interface was identified by the presence of reflective particles in all patients regardless of flap dissection technology used. Histologically,28,29 the interface is described as a variably thick, 1- to 9-μm hypocellular stromal scar containing extracel-

**Figure 8.** Examination on day 7 shows a clearly limited and pronounced periphery within the corneal stroma with epithelial cells (arrow) filling the empty spaces at the flap margin (A). Day 7 examination of the flap margin at the Bowman layer (B). HRT II images; magnification, 400 × 400 μm.

**Figure 9.** Flap periphery created by the Hansatome shows a less clearly identified periphery with epithelial cell invasion on day 7 (arrow). HRT II image; magnification, 400 × 400 μm.

**Figure 10.** Disappearance of epithelial cells at 2-month examination after Hansatome-LASIK treatment, leaving an irregular fibrotic wound. HRT II image; magnification, 400 × 400 μm.
the microkeratome. Other authors suggest that these particles (polyether imide), also originating from the interface during the stage of active wound healing (particles coming from the microkeratome blade). This observation contradicts the hypothesis of metal or plastic served at the interface when the flap was made with IntraLase. A study, the brightest hyperreflective particles were also observed in keratocytes and viable or necrotic epithelial cells. In our study, particles after LASIK seemed to be intracytoplasmic vacuoles (lacunar matrix material and variably reflective cellular structures. Studies have shown, with the use of confocal microscopy, interface particles of variable dimensions and reflectivity.

Various hypotheses have been proposed to explain the origin of the most reflective particles found at the interface. Metal particles from the microkeratome blade have been suggested, probably because of their highly brilliant aspect at confocal microscope imaging. A more recent hypothesis posits that plastic particles (polyether imide), also originating from the microkeratome. Other authors suggest that these particles could be ocular surface debris such as lipid products, implanted corneal epithelial cells, synthetic material such as sponge particles, powder from surgical gloves, or inflammatory cells. Dawson et al. suggest that transient particles found in the interface during the stage of active wound healing (≥6 months after surgery) may be stress fiber bundles in migratory keratocytes, extracellular empty spaces filled with fibrillar material, histiocytes filled with vacuoles full of fibrillar material, or clear vacuoles in activated keratocytes. The permanent interface particles after LASIK seemed to be intracytoplasmic vacuoles in keratocytes and viable or necrotic epithelial cells. In our study, the brightest hyperreflective particles were also observed at the interface when the flap was made with IntraLase. This observation contradicts the hypothesis of metal or plastic particles coming from the microkeratome blade.

Examination of the LASIK flap margin with confocal microscopy has not been widely undertaken, probably because of technical issues raised by first-generation confocal microscopes. Vesalouma et al. showed the presence of basal epithelial cells forming a plug inside the cut in the wound gap. Wound constriction had been completed in most cases by 3 to 6 months. The presence or absence of an epithelial plug in the wound gap could depend on good flap alignment of anterior keratocyte layers over the flap margin.

A study conducted in rabbits has shown the presence of myofibroblasts at the LASIK flap edge, suggesting an active wound contraction. Other studies have shown the lack of inflammatory induction after manual epithelial debridement compared with an intense cellular reaction after basement membrane disruption. In the intact cornea, the epithelial basement membrane has been reported to bind cytokines, suggesting that it may act as a barrier. Thus, when basement membrane integrity is compromised at the LASIK flap edge, molecules with mitogenic and chemotactic effects may enter the stroma and induce the activation of keratocytes, thus leading to fibrotic wound repair. Peripheral to the LASIK flap edge, strict localization of fibrosis occurs, contrary to the minimal fibrosis below the central flap. In a human corneal histopathologic study after LASIK, focal basal epithelial cell hypertrophy was observed, as were keratocytes differentiated in myofibroblasts adjacent to the wound margin. In the present study, fibrotic tissue at the corneal periphery was more abundant after femto-LASIK than after microkeratome-LASIK surgery. Flap formation with microkeratome produces an incision in the corneal tissue; in this way, after flap repositioning, the edges are in tight contact. On the contrary, it can be hypothesized that flap edge formation by IntraLase produces tissue ablation, leaving an empty space that fills with epithelial plug. Interaction between epithelium and stroma—i.e., signaling molecules produced from the epithelium or tear fluid that have a mitogenic and chemotactic effect on keratocytes—is therefore probably greater. As a result, greater scarring reaction is produced. This much greater scarring reaction after femto-LASIK procedure is visible biomicroscopically and with confocal microscopy.

Wound constriction of the flap periphery that seemed similar to the wound healing described after radial keratotomy was observed in the present study. Findings were stromal fibrosis that started at the bottom of the incision and persistence of an epithelial plug. In fact, beginning with the 6-month examination, we found a wound constriction originating at the bottom of the incision. This wound constriction was less markedly visible, and it was even absent in areas presenting a large separation of the flap margins. In no patient who had undergone femto-LASIK surgery, however, was epithelial invasion at the interface level observed. An epithelial plug in a fully healed radial keratotomy incision presumably creates a stress concentration at the incision site that may predispose the cornea to breakage after trauma. Similarly, one could imagine a more fragile flap adhesion at the periphery, though the angulation of the flap periphery theoretically provides very good overlapping and, therefore, better adhesion. Further insight with larger series will be required to determine whether the presence or the absence of epithelial cells at the flap margin is associated with more or fewer
complications, such as whether an increased incidence of epithelial cell invasion is associated with weaker flap adhesion. It would be interesting to correlate wound healing findings with biomechanical properties of the treated corneas and to compare microkeratome and Intralase techniques. In a histologic study evaluating the cohesive tensile strength of human LASIK corneal wounds, Schmack et al. found that the central and paracentral stromal scars on average are 2.4% as strong as normal corneal stroma. Conversely, the LASIK flap wound margin healed by producing a 10-fold stronger peripheral hyperreflective fibrotic stromal scar that on average was 28.1% as strong as normal corneal stromal, but there was marked variability.

In conclusion, in this in vivo study, we analyzed at an almost histologic level the results of LASIK surgery performed after femtosecond laser Intralase flap dissection over a 12-month follow-up period and compared interfaces resulting from this new, very promising technology and a classic, widely used microkeratome, the Hansatome. Based on interface examination shortly after surgery, both methods produced similar results, except for a visible aspect of presumed femtosecond laser spots in the first few days. This similarity between the two techniques seems logical because photoablation was performed by the same excimer laser. Moreover, flap creation by Intralase did not seem to show a difference in the stromal bed morphology when compared with the microkeratome. The brightly reflecting particles at the interface called into question their presumed origin because they were observed by both techniques of flap formation. Flap periphery performed by Intralase appeared microscopically more clearly defined. However, after month 2, it seemed to produce a more visible cicatricial reaction than the microkeratome. At the 6- and 12-month examinations of the femto-LASIK group, wound constriction with expulsion of the epithelial plug, more or less large depending on the initial separation of the margins, seemed to be produced. Further studies will be necessary to confirm the theoretical superiority of Intralase technology compared with standard mechanical microkeratomes.

References


