Corneal Reinnervation after LASIK: Prospective 3-Year Longitudinal Study

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Purpose. To measure the return of innervation to the cornea during 3 years after LASIK.

Methods. Seventeen corneas of 11 patients who had undergone LASIK to correct myopia from −2.0 D to −11.0 D were examined by confocal microscopy before surgery, and at 1, 3, 6, 12, 24, and 36 months after surgery. In all available scans, the number of nerve fiber bundles and their density (visible length of nerve per frame area), orientation (mean angle), and depth in the cornea were measured.

Results. The number and density of subbasal nerves decreased >90% in the first month after LASIK. By 6 months these nerves began to recover, and by 2 years they reached densities not significantly different from those before LASIK. Between 2 and 3 years they decreased again, so that at 3 years the numbers remained <60% of the pre-LASIK numbers ($P < 0.001$). In the stromal flap most nerve fiber bundles were also lost after LASIK, and these began recovering by the third month, but by the third year they did not reach their original numbers ($P < 0.001$). In the stromal bed (posterior to the LASIK flap interface), there were no significant changes in nerve number or density. As the subbasal nerves returned, their mean orientation did not change from the predominantly vertical orientation before LASIK. Nerve orientation in the stromal flap and the stromal bed also did not change.

Conclusions. Both subbasal and stromal corneal nerves in LASIK flaps recover slowly and do not return to preoperative densities by 3 years after LASIK. The numbers of subbasal nerves appear to decrease between 2 and 3 years after LASIK. The orientation of the regenerated subbasal nerves remains predominantly vertical. (*Invest Ophthalmol Vis Sci.* 2004;45: 3991–3996) DOI:10.1167/iovs.04-0561

The ophthalmic division of the trigeminal nerve innervates the cornea and mediates aversion reflexes needed to avoid noxious stimuli and to preserve the normal functions and architecture of this sensitive tissue.[1] This important sensory function is disrupted when nerves are cut during photorefractive procedures such as LASIK and photorefractive keratectomy (PRK). In LASIK, the flap created with a microkeratome cuts the subbasal nerve fiber bundles and the superficial stromal nerves, although nerves that run through the hinge are spared. Stromal nerves beneath the flap in the layer that is ablated are also destroyed. This disruption is thought to contribute to dry eyes and alterations in the tear film after these procedures.[2,3]

Clinical confocal microscopy has been used to examine changes in the subbasal nerve plexus[4] and to visualize the return of nerve fiber bundles in the human cornea after LASIK in both cross-sectional studies out to 6 months postoperative,[5–7] and in qualitative[8] and quantitative[9] longitudinal studies from preoperative to 1 year postoperative. The latter study demonstrated that 12 months after LASIK, the number of subbasal nerves and stromal nerves in the flap is less than half of the preoperative number.[9] This nerve deficit is present despite the findings of normal corneal sensation by Cochet-Bonnet esthesiometry (admittedly a gross test of sensitivity compared to pneumatic esthesiometry) by 6 months after LASIK.[2–5, 10–12]

In this study the examination of reinnervation after LASIK was extended to 3 years. Clinical confocal examinations of 17 eyes that had received LASIK were assessed, and the number of subbasal and stromal nerves, their density, and their orientation from before LASIK to 3 years after treatment were determined.

Methods

Patients

Seventeen eyes of 11 patients who had bilateral LASIK were studied. Five eyes of these patients were excluded because of reoperations for undercorrections. The patients included 10 females and 1 male, aged 32 ± 9 years (mean ± SD). All patients had had a complete ophthalmologic examination before surgery to assure a normal cornea and anterior segment. Patients with previous ocular surgery, glaucoma, topical ophthalmologic treatment, or diabetes were excluded. The preoperative spheroequivalent refraction was −6.6 ± 2.4 D (range, −2.0 to −11.0 D). After LASIK none of the patients wore contact lenses. Each patient gave informed consent to participate, after the nature and possible consequences of the study had been explained. All patients had participated in an assessment of their corneal reinnervation during the first year after LASIK.[9] The study was approved by the Institutional Review Board of Mayo Clinic and followed the principles of the Declaration of Helsinki for research involving human subjects.

LASIK Procedure and Postoperative Treatment

Patients were treated according to our standard protocol for LASIK. A microkeratome (Hansatome; Chiron Vision Corp., Claremont, CA) was used to create a flap with a superior hinge and a planned thickness of 180 μm. Stroma was ablated by using an excimer laser (VISX Star 2; VISX, Santa Ana, CA) to a mean planned depth of 63 ± 26 μm (range, 18–110 μm). Postoperatively, patients administered topical corticosteroids (0.1% fluorometholone, FML; Allergan Inc., Irvine, CA) four times per day for 1 week and tapered over 2 additional weeks, and antibiotics (0.3% ofloxacin, Ocuflox; Allergan Inc.) four times per day for 5 days.

Confocal Microscopy

Corneas were examined preoperatively and at 1, 3, 6, 12, 24, and 36 months after LASIK by using a tandem scanning confocal microscope (Tandem Scanning Corporation, Reston, VA). The specifications and operation of this instrument have been described by Patel et al.[13] and a brief description is given here. A drop of 2.5% hydroxypropyl meth-

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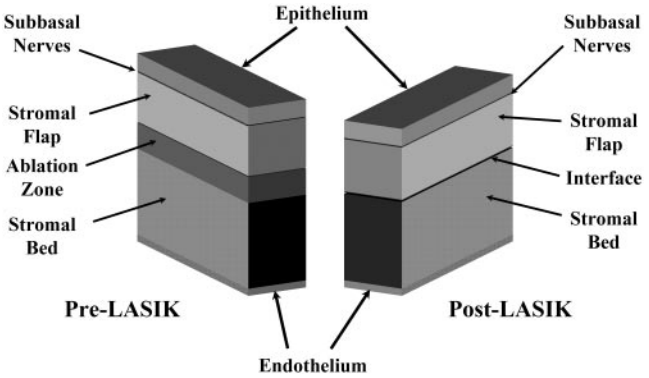

FIGURE 1. Schematic representation of the subbasal nerves, stromal flap, and stromal bed after LASIK and the corresponding layers before LASIK. The position of the interface (distance from most anterior keratocytes and from the endothelium) at 1 month was used to determine the ablation zone in the preoperative cornea.

ylcellulose (Goniosol; CIBAVision Ophthalmics, Atlanta, GA) was placed on the tip of the objective lens as an optical coupling medium, and the lens was advanced until the solution contacted the cornea. The objective lens of the confocal microscope was aligned with the center of the cornea by centering the light and dark rings of the epithelial image. After alignment, the focal plane was scanned through the cornea at approximately 72 μm/s from anterior to posterior while digital images were stored on a computer workstation (Indy; Silicon Graphics Inc., Mountain View, CA) at 30 frames/s. Each image represented a coronal section approximately 475 $\times$ 350 μm (horizontal $\times$ vertical) and was separated from adjacent images by approximately 2.4 μm. A scan through the full thickness of the cornea required 7 to 9 seconds to complete. On each visit, the cornea was scanned four to eight times. The objective lens and coupling medium were separated from the cornea between each scan. All scans were within the central 4 mm of the cornea, although not in the identical region each time. Therefore, the scans represented random, full-thickness samples of the central cornea.

Nerve Fiber Bundles

In all frames that included nerves (bright, linear objects without shadows, visible in more than one frame), the length and mean orientation of each nerve fiber bundle were measured by using a computer program developed in our laboratory. On each frame with visible nerve fibers, the operator traced the outline of each nerve by using a simple point-and-click method. Each nerve was measured only once. If its length extended across several frames, its total length was measured as if it were projected onto one frame. The program calculated the length in μm and the mean angle of orientation from the positive horizontal axis, a convention similar to that used to designate the axis of cylinder in ophthalmic lenses.[14] The depth in the cornea of stromal nerves was the distance in μm from the epithelial surface and was determined from the number of frames between the image of the epithelium and the nerve bundle. Branches longer than 50 μm were counted as separate nerves.

The number of nerves in the subbasal region (just anterior to Bowman's membrane), the stromal flap (region between the most anterior keratocyte and the flap interface), and the stromal bed (region between the flap interface and the most posterior keratocytes, excluding Descemet's membrane) were recorded from each scan. The interface was identified in all scans as a layer in the anterior stroma that contained small bright objects, presumably debris from the keratome. The distances between the corneal surface and the subbasal nerves, the interface, and the most posterior keratocytes were determined from the appearance of these structures in the video sequence, as described by McLaren et al.[15] The distances from the most anterior keratocyte to the flap interface (stromal flap thickness) and from the interface to the most posterior keratocytes, excluding Descemet's membrane (stromal bed thickness) determined from the 1-month post-LASIK scans were used to delimit the corresponding layers in the preoperative cornea (Fig. 1).

The density of subbasal nerves, which were in a narrow layer, was the total length of nerve in this layer divided by the area of the frame (μm/mm^2). The densities of stromal nerves, which were measured throughout the depth of the stromal flap and the stromal bed, were the total length of all nerves in each region divided by the sample volume of the region, expressed as μm/mm^3. The sample volumes of the stromal flap and stromal bed were the frame area multiplied by the flap or bed thickness. We also estimated the number of visible subbasal nerves and the number of visible nerves in the stromal flap and the stromal bed. Densities and numbers of nerves in each region (subbasal, stromal flap, and stromal bed) were estimated from each scan and the average from all scans was accepted as the density or number of nerves for the visit. All scans were evaluated and measured by one observer (MPC) masked to the patient and time of the scan. Adequate scans were available (two to eight scans per eye per visit) for all patients at each examination.

Statistical Analysis

Differences in number and density of nerve fiber bundles were determined by the Friedman's test adjusted for multiple comparisons by using the Student-Newman-Keuls procedure. Data were not normally distributed, and are presented as medians and interquartile ranges (25th and 75th percentiles, Q25 and Q75, respectively). $P < 0.05$ was considered statistically significant. Mean nerve angles after treatment were compared to angles before treatment by using paired *t*-tests, or

TABLE 1. Number of Nerve Fiber Bundles per Scan per Cornea (n = 17)*

Layer	Months after LASIK							Friedman's *P* Value
	Preoperative	1	3	6	12	24	36	
Subbasal	4.0† (2.6, 5.3) [4.1 ± 2.4]	0.0‡ (0.0, 0.6)	0.0‡ (0.0, 0.8)	0.6‡ (0.0, 1.3)	1.8§ (0.4, 3.7)	2.5† (1.5, 4.8)	2.3§ (1.5, 3.5)	< 0.001
Stromal flap	0.6†§ (0.4, 1.0) [0.67 ± 0.47]	0.0‡ (0.0, 0.3)	0.3†‡ (0.0, 0.7)	0.5§ (0.0, 0.8)	0.3‡ (0.0, 0.3)	0.0‡ (0.0, 0.3)	0.3‡ (0.0, 0.5)	< 0.001
Stromal bed	0.25† (0.0, 0.5) [0.30 ± 0.35]	0.5† (0.2, 0.6)	0.3† (0.3, 0.6)	0.3† (0.3, 0.5)	0.5† (0.0, 0.8)	0.3† (0.2, 0.5)	0.5† (0.0, 0.8)	0.74

* Median (interquartile range: Q25, Q75). The means and standard deviations are listed in brackets for the preoperative corneas, which had normally distributed data.

†‡§ Medians with the same symbol in each row were not significantly different from each other (Student-Newman-Keuls procedure).

TABLE 2. Nerve Density ($n = 17$)*

Location	Months after LASIK							Friedman's *P* Value†
	Preoperative	1	3	6	12	24	36	
Subbasal ($\mu m/mm^2$)	6188† (3399, 8160) [5867 ± 3316]	503‡ (0, 1271)	0‡ (0, 1385)	1079‡ (0, 2168)	2377§ (433, 4580)	3184†‖ (2105, 6338)	3169§‖ (1636, 5266)	< 0.001
Stromal flap ($\mu m/mm^3$)	9731† (5525, 17174)	0‡ (0, 5121)	1692‡ (0, 9269)	3984†‡ (0, 12702)	3022‡ (0, 4688)	0‡ (0, 2002)	3351‡ (0, 9096)	0.002
Stromal bed ($\mu m/mm^3$)	950† (0, 2282)	1441† (592, 2847)	1596† (622, 4209)	977† (413, 2486)	2137† (0, 3662)	922† (426, 1779)	2048† (0, 3171)	0.57

* Median (interquartile range: Q25, Q75), The means and standard deviations are listed in brackets for the preoperative corneas, which had normally distributed data.

†‡§‖ Medians with the same letter in the each row were not significantly different from each other (Student-Newman-Keuls procedure).

signed-rank tests as appropriate, and adjusted for multiple comparisons by using the Bonferroni technique. Tests were checked for the effects of potential correlations between measurements from both eyes of the same subject by using generalized estimating equation (GEE) models. Given the non-normal distribution of the data, the GEE models were fitted by using the rank transformation of the data. The results of the GEE models were similar to results of the standard tests, and only the results of the standard tests are presented. All statistics were calculated by using SAS software (SAS Institute Inc., Cary, NC).

RESULTS

In the first month after LASIK, the median number and density of subbasal nerves and nerves in the stromal flap were reduced to zero (Tables 1 and 2, Fig. 2). Nerve fiber bundles began to return (median number began to increase) to the stromal flap by 3 months and to the subbasal region by 6 months. Subbasal nerve fiber bundles gradually returned, and by 2 years their numbers and densities were not significantly different from what they were preoperatively. However, between 2 and 3 years, nerve fiber bundles decreased to numbers and densities that were significantly less than those before treatment. In the stromal flap, the nerves increased to numbers not significantly different from those preoperative by 3 and 6 months, but then decreased at 1 year and remained significantly below pre-LASIK numbers at 2 and 3 years. In the stromal flap, nerve density was significantly less than it was preoperative at all times after LASIK except at 6 months. In the stromal bed, neither nerve number nor nerve density changed significantly after LASIK.

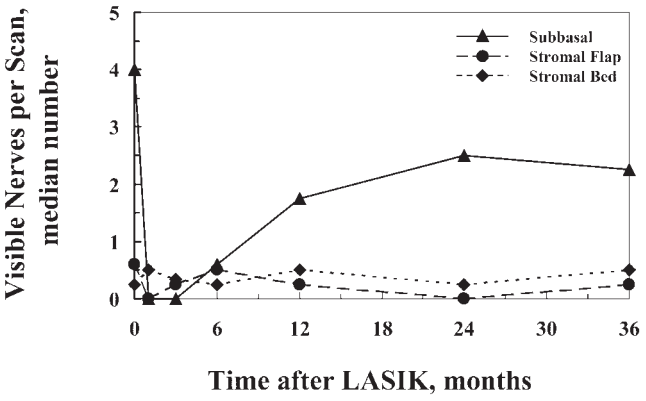

FIGURE 2. Number of nerve fiber bundles after LASIK. The subbasal nerves began to return by 6 months, but failed to reach preoperative numbers, although their numbers were not significantly different from preoperative numbers at 2 years, and decreased thereafter. Nerves in the stromal flap returned to preoperative numbers by 6 months, but then decreased again. Nerves in the stromal bed did not change.

Before LASIK, nerve fiber bundles in the subbasal region were strongly biased toward vertical orientation; their mean angle was 96 ± 20° (mean ± SD; Table 3, Fig. 3). After LASIK, the mean angles of the subbasal nerve fiber bundles did not change (Fig. 4). In the stroma, orientation was somewhat more oblique, with a mean angle of 101 ± 39° in the stromal flap and 88 ± 41° in the stromal bed. Other than a decrease in the mean angle of orientation in the stromal flap at 1 month, the mean angle was at all post-LASIK times not significantly different from the mean angle before LASIK in either the stromal flap or the stromal bed.

DISCUSSION

This investigation demonstrated that, after LASIK, nerve density in the central anterior cornea, as it appeared in confocal microscopy, recovers very slowly; the subbasal nerve layer, which we earlier noted does not recover by 1 year,[9] did not completely recover by 3 years. The slow return of innervation is consistent with histologic studies in human eyes, although the longest postoperative times for histologic examination have been 4 months,[16] and 20 months.[17] Anderson et al. [17] found a lack of corneal nerves at 3 months and a few small superficial nerves at 20 months after LASIK in two postmortem corneas. In normal corneas that have not had surgery, nerve density decreases only slightly between ages of 25 and 70,[24] and the decreased nerve density 3 years after LASIK is unlikely to represent the normal demise of nerves in our group of patients.

The partial recovery of nerves to the central cornea is not consistent with studies of corneal sensitivity, which have shown normal sensitivity to Cochet-Bonnet esthesiometry 6 months after the procedure.[2–5,10–12] Control subjects, however, responded to the lowest stimulus force provided by the Cochet-Bonnet esthesiometer, and by 6 months after LASIK, mean corneal sensitivity had returned to slightly less than this. If the smallest stimulus available from this device is well over the threshold in a normal untreated cornea, then a response to near this stimulus may not represent a complete recovery. Recovery must be assessed with an esthesiometer capable of providing a subthreshold stimulus as a control, such as the noncontact gas esthesiometers described by Murphy and coworkers[18,19] and Belmonte and coworkers.[20]

One advantage of permanent recordings of full-thickness scans by confocal microscopy is that the scans can be reevaluated as more sophisticated analyses are developed. Scans recorded preoperatively and during the first postoperative year in these 17 eyes have been evaluated for corneal nerves three

TABLE 3. Mean Angle of Visible Nerve Fiber Bundles

	Months after LASIK						
Layer	**Preoperative**	**1**	**3**	**6**	**12**	**24**	**36**
Subbasal	96 ± 20	99 ± 23	78 ± 20	84 ± 38	104 ± 25	94 ± 25	96 ± 30
n	17	7	5	12	14	17	16
Stromal flap	101 ± 39	66 ± 18*	81 ± 54	81 ± 40	92 ± 49	93 ± 47	68 ± 33
n	15	7	10	11	11	6	10
Stromal bed	88 ± 41	78 ± 49	81 ± 55	82 ± 61	89 ± 42	101 ± 44	89 ± 44
n	10	13	13	13	11	12	12

Nerve angle in degrees ± SD (ophthalmic lens convention). *n,* Number of corneas with visible nerves.
* Significantly different from pre-LASIK, $P = 0.04$.

times. First, a qualitative analysis devised by Linna et al.[5] was used to measure the number of corneas in which a particular type of subbasal nerve was seen at each postoperative examination.[21] Second, the numbers of subbasal and stromal nerves per scan were measured.[9] Third (the present study), the density and orientation as well as the numbers of subbasal and stromal nerves were measured, and additional examinations at 2 and 3 years were performed. One masked examiner (different for each report) evaluated all scans. The number of nerves estimated in the present study was similar to those reported previously,[9] and small differences could be attributed to the subjective nature of identifying nerves and differences between observers.

Preoperative estimates of nerve densities in this study were similar to densities expressed as length of nerve per area of view and reported by Jacot et al.[22] in rat corneas, and Oliveira-Soto and Efron[14] in human corneas (Table 4). Numbers of nerves per frame were similar to those found by Rosenberg et al.[23] in their normal control corneas. Small differences between Oliveira-Soto's results in humans and ours may in part be related to the difference between the sizes and shapes of the fields of view of the different confocal microscopes. However, densities reported by Grupcheva et al.,[24] expressed in the same way, were approximately one tenth of those reported by us and by Jacot et al.[22] and Oliveira-Soto and Efron.[14] Grupcheva et al.[24] assessed nerve length in each image by using a method that reduced the effective depth of the focal plane and included only 10% to 20% of the length of visible nerve fibers. In contrast, we and Oliveira-Soto and Efron[14] included the entire visible length of nerve fibers in the image. Differences between these methods have been discussed.[25] Differences in nerve density that arise from the variations in shape, magnification of images, and selection criteria from different confocal microscopes are important when comparing densities across studies, but they should not affect conclusions in longitudinal studies, such as this one, that record images by using the same microscope and method throughout the study.

The median subbasal nerve density increased from very few within 1 month after surgery to approximately half of the normal densities at 2 years, and although this appears well below normal, it was not significantly different from normal. The median density then decreased slightly in the third year, to a density that was again significantly less than it was before LASIK. It is not clear if this decrease in the third year means that the recovery reached an upper limit and receded, that it was part of the normal variation of corneal innervation, or that our ability to assess nerve density by using this technique is limited. Additional studies are needed to determine the true nature of these changes and to determine whether recovery will extend beyond 3 years and nerve density will eventually reach the pre-LASIK density.

The reason for a decrease in subbasal nerve density after a partial recovery is not clear. Erie et al.[26] also noted a decrease in keratocyte density during the third year in the same 17 corneas. Direct innervation of a keratocyte by a stromal nerve has been demonstrated by Müller.[27] If stromal nerves and keratocytes interact, then the populations of nerves and keratocytes may be dependent on each other. For example, if keratocytes are required to maintain the maximal density of nerve fibers, or if keratocytes are maintained by neurotrophic factors, then the population of one would decrease when the population of the other decreases. Both populations could also

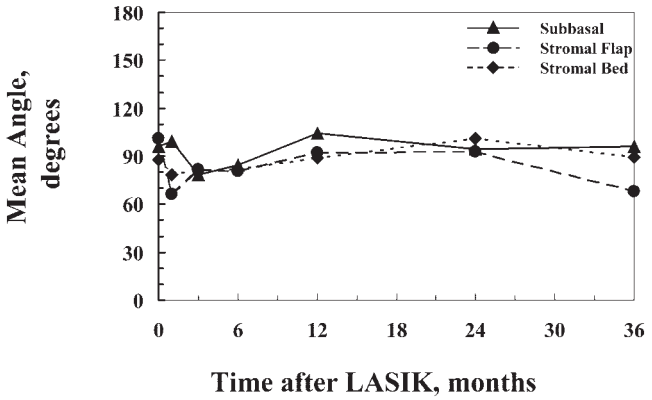

FIGURE 3. Mean orientation of the nerve fiber bundles. The mean orientation of the nerves did not change, except at 1 month the mean angle in the stromal flap was less than it was before LASIK ($P = 0.04$). See Table 3 for number of eyes with visible nerves at each time.

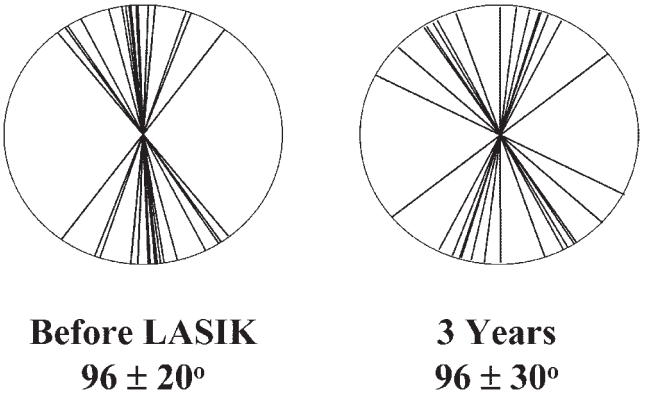

FIGURE 4. Mean orientation of the central subbasal nerve fiber bundles before LASIK and at 3 years. Each *line* represents the mean angle of subbasal nerves in the central 3 to 4 mm of the cornea of one subject. At 3 years the overall mean angle was not different from pre-LASIK, although angles were distributed over a slightly greater range.

TABLE 4. Comparison of Nerve Fiber Bundle Density in Normal Corneas by Different Investigators (Mean ± SD)

Author	Jacot[15]	Grupcheva[17]	Oliveira-Soto[11]	Calvillo et al. (This Study)
Number of corneas		25	14	17
Subjects	Rats	Humans	Humans	Humans
Type of study	Histology	Confocal	Confocal	Confocal
Type of confocal microscope	None	Slit scanning	Slit scanning	Tandem scanning
Subbasal nerve density	7,950 ± 860 $\mu m/mm^2$	632 ± 288 $\mu m/mm^2$	11,110 ± 4290 $\mu m/mm^2$	5,867 ± 3,316 $\mu m/mm^2$
Subbasal nerve orientation			109 ± 43°	96 ± 20°
Stromal nerve density	3,900 ± 360 $\mu m/mm^2$		3,691 ± 1,036 $\mu m/mm^2$	12,866 ± 11,203 $\mu m/mm^3$, stromal flap 1,594 ± 2,212 $\mu m/mm^3$, stromal bed
Stromal nerve orientation			96 ± 54°	101 ± 39° stromal flap 88 ± 41° stromal bed

be responding to other unknown factors that, when diminished, reduce populations of both nerves and keratocytes. These changes do not seem to reflect a universal response to injury; after PRK, nerves recover to near preoperative densities by 2 years and density does not decrease in the third year.[28]

The long-term consequence of the nerve deficit after LASIK is unknown. Normal innervation is not necessary for corneal clarity; transplanted corneas can have severe nerve deficits[29] and hypoesthesia[30] for many years but remain clear. Whether the decreased keratocyte density[31] and the eventual loss of stromal clarity in some patients with corneal transplants is related to this loss of nerve fibers is unknown.

The loss and recovery of innervation after LASIK likely depends on the location of the flap relative to the path of most nerves into the central cornea. In an earlier study Müller et al.[32] suggested that the corneal nerve bundles enter the cornea at the nasal and temporal limbus and extend primarily in a horizontal orientation. If this were true, cutting LASIK flaps with a superior hinge would sever more nerves than those with a nasal hinge. Two studies have compared corneal sensitivity after LASIK in patients with flaps cut on the nasal versus superior sides. Donnenfeld et al. [2] found greater loss of sensitivity in corneas with superior hinges, confirming the original suggestion of Müller et al.,[32] while Kumano et al.[11] found the opposite result. In a more recent investigation, Müller et al.[33] observed that the corneal nerves, in agreement with previous histologic studies,[29,34] enter the cornea radially from all directions rather than primarily nasally or temporally, and extend across the central cornea primarily in a vertical direction. Thus, superior and nasal hinges should sever similar numbers of nerves, and alternative explanations must be sought for the opposing findings in the two previous clinical studies.[2,11]

The orientation of central subbasal nerves in the normal preoperative corneas in this study was primarily vertical as noted by Oliveira-Soto and Efron,[14] and this is consistent with the recent model suggested by Müller et al.[33] based on histologic analysis. After LASIK, as nerve fibers returned to this region, they still had a vertical component, but many nerves were oblique (Fig. 4). This perhaps reflects the direction of new growth across the interface into this region horizontally from the sides.

A trend toward more horizontal fibers may have been masked by the ophthalmic lens convention (0° to 180°) used to specify angle. For example, if nerve fibers were distributed about the horizontal axis, the mean angle would be close to 90°, although the SD would be greater than 43°. Measurement of angle from the horizontal (regardless of slope) would better indicate if nerves were vertical or horizontal. The mean angle from horizontal would range from 0° to 90°; a mean near 0° would represent a predominantly horizontal distribution, whereas a mean near 90° would represent a predominantly vertical distribution. Expressed in this way, our mean nerve angle from the horizontal before LASIK was 73 ± 12°, and at 1, 2, and 3 years after LASIK was 60 ± 17°, 55 ± 20°, and 58 ± 15° ($P = 0.003$, $P = 0.007$, and $P = 0.02$ vs. preoperative, paired *t*-test, adjusted for six comparisons by the method of Bonferroni). At the other times this variable was not significantly different from what it was before LASIK. Although the nerve angle was significantly less by this convention at 1 to 3 years after LASIK, nerve orientation remained predominantly vertical, as illustrated in Figure 4.

Our study was limited to the central 3 to 4 mm of the cornea, and outside this area, but still under the flap, nerve growth may be in directions other than what we measured and at other orientations. Observations across the entire LASIK flap are required to describe the complete path of reinnervation.

In summary, the corneal nerves that are lost during LASIK slowly regenerate, but do not return to preoperative densities by 3 years. The return is characterized by variations in the regeneration rate, with a decrease in number during the third year. As nerve fibers return to this tissue, they maintain their preoperative orientation, which is predominantly vertical for subbasal nerves and more random for stromal nerves. The long-term consequences of these changes on corneal clarity and vision are unknown.

References

1. Mensher JH. Corneal nerves. *Surv Ophthalmol*. 1974;19:1–18.
2. Donnenfeld ED, Solomon K, Perry HD, et al. The effect of hinge position on corneal sensation and dry eye after LASIK. *Ophthalmology*. 2003;110:1023–1029.
3. Benitez-del-Castillo JM, del-Rio T, Iradier T, Hernandez JL, Castillo A, Garcia-Sanchez J. Decrease in tear secretion and corneal sensitivity after laser in situ keratomileusis. *Cornea*. 2001;20:30–32.
4. Auran JD, Koester CJ, Kleiman NJ, et al. Scanning slit confocal microscopic observation of cell morphology and movement within the normal human anterior cornea. *Ophthalmology*. 1995; 102:33–41.
5. Linna TU, Vesaluoma MH, Perez-Santonja JJ, Petroll WM, Alio JL, Tervo TM. Effect of myopic LASIK on corneal sensitivity and morphology of subbasal nerves. *Invest Ophthalmol Vis Sci*. 2000; 41:393–397.
6. Perez-Gomez I, Efron N. Change to corneal morphology after refractive surgery (myopic laser in situ keratomileusis) as viewed with a confocal microscope. *Optom Vis Sci*. 2003;80:690–697.
7. Slowik C, Somodi S, Richter A, Guthoff R. Assessment of corneal alterations following laser in situ keratomileusis by confocal slit scanning microscopy. *Ger J Ophthalmol*. 1997;5:526–531.
8. Kauffmann T, Bodanowitz S, Hesse L, Kroll P. Corneal reinnervation after photorefractive keratectomy and laser in situ keratomileusis: an in vivo study with a confocal videomicroscope. *Ger J Ophthalmol*. 1997;5:508–512.
9. Lee BH, McLaren JW, Erie JC, Hodge DO, Bourne WM. Reinnervation in the cornea after LASIK. *Invest Ophthalmol Vis Sci*. 2002; 43:3660–3664.

10. Kanellopoulos A, Pallikaris I, Donnefeld E, Detorakis S, Koufala K, Perry HD. Comparison of corneal sensation following photorefractive keratectomy and laser in situ keratomileusis. *J Cataract Refract Surg*. 1997;23:34–38.
11. Kumano Y, Matsui H, Zushi I, et al. Recovery of corneal sensation after myopic correction by laser in situ keratomileusis with a nasal or superior hinge. *J Cataract Refract Surg*. 2003;29:757–761.
12. Pérez-Santonja JJ, Sakla JF, Cardona C, Chipont E, Alió J. Corneal sensitivity after photorefractive keratectomy and laser in situ keratomileusis for low myopia. *Am J Ophthalmol*. 1999;127:497–504.
13. Patel SV, McLaren JW, Hodge DO, Bourne WM. Normal human keratocyte density and corneal thickness measurement by using confocal microscopy in vivo. *Invest Ophthalmol Vis Sci*. 2001;42:333–339.
14. Oliveira-Soto L, Efron N. Morphology of corneal nerves using confocal microscopy. *Cornea*. 2001;20:374–384.
15. McLaren JW, Nau CB, Erie JC, Bourne WM. Corneal thickness measurement by confocal microscopy, ultrasound, and scanning slit methods. *Am J Ophthalmol*. 2004;137:1011–1020.
16. Latvala T, Barraquer-Coll C, Tervo K, Tervo T. Corneal wound healing and nerve morphology after excimer laser in situ keratomileusis in human eyes. *J Refract Surg*. 1996;12:677–683.
17. Anderson NJ, Edelhauser HF, Sharara N, et al. Histologic and ultrastructural findings in human corneas after successful laser in situ keratomileusis. *Arch Ophthalmol*. 2002;120:288–293.
18. Murphy PJ, Patel S, Marshall J. A new noncontact corneal aesthesiometer (NCCA). *Ophthalmic Physiol Opt*. 1996;16:101–107.
19. Murphy PJ, Lawrenson JG, Patel S, Marshall J. Reliability of the noncontact corneal aesthesiometer and its comparison with the Cochet-Bonnet aesthesiometer. *Ophthalmic Physiol Opt*. 1998;18:532–539.
20. Belmonte C, Acosta MC, Schmelz M, Gallar J. Measurement of corneal sensitivity to mechanical and chemical stimulation with a CO_2 esthesiometer. *Invest Ophthalmol Vis Sci*. 1999;40:513–519.
21. Mitooka K, Ramirez M, Maguire LJ, et al. Keratocyte density of central human cornea after laser in situ keratomileusis. *Am J Ophthalmol*. 2002;133:307–314.
22. Jacot JL, Glover JP, Robison WG Jr. Computer analysis of corneal innervation density using a novel double stain in rat corneal whole mounts. *J Anat*. 1997;191:191–199.
23. Rosenberg ME, Tervo TM, Immonen IJ, Müller LJ, Gronhagen-Riska C, Vesaluoma MH. Corneal structure and sensitivity in type 1 diabetes mellitus. *Invest Ophthalmol Vis Sci*. 2000;41:2915–2921.
24. Grupcheva C, Wong T, Riley A, McGhee C. Assessing the subbasal nerve plexus of the living healthy human cornea by in vivo confocal microscopy. *Clin Exp Ophthalmol*. 2002;30:187–190.
25. Oliveira-Soto L, Efron N. Assessing the cornea by in vivo confocal microscopy. *Clin Exp Ophthalmol*. 2003;32:83–86.
26. Erie JC, Nau CB, McLaren JM, Hodge DO, Bourne WM. Long-term keratocyte deficits in the corneal stroma after LASIK. *Ophthalmology*. 2004;111:1356–1361.
27. Müller L, Pels L, Vrensen GFJM. Ultrastructural organization of human corneal nerves. *Invest Ophthalmol Vis Sci*. 1996;37:476–488.
28. Erie JC. Corneal wound healing after photorefractive keratectomy: a 3-year confocal microscopic study. *Trans Am Ophthalmol Soc*. 2003;101:287–328.
29. Tervo T, Vannas A, Tervo K, Holden BA. Histochemical evidence of limited reinnervation of human corneal grafts. *Acta Ophthalmol*. 1985;63:207–214.
30. Rao GN, John T, Ishida N, Aquarella JV. Recovery of corneal sensitivity in grafts following penetrating keratoplasty. *Ophthalmology*. 1985;92:1408–1411.
31. Bourne WM. Cellular changes in transplanted human corneas. *Cornea*. 2001;20:560–569.
32. Müller L, Vrensen GFJM, Pels L, Nunes-Cardozo B, Willekens B. Architecture of human corneal nerves. *Invest Ophthalmol Vis Sci*. 1997;38:985–994.
33. Müller LJ, Marfurt CF, Kruse F, Tervo TMT. Corneal nerves: structure, contents and function. *Exp Eye Res*. 2003;76:521–542.
34. Maurice DM. The cornea and sclera. In: Davson H, ed. *The Eye*, Vol. 1B. 3rd ed. Orlando: Academic Press; 1984:1–158.