In Vivo Confocal Microscopy After Photorefractive Keratectomy in Humans

A Prospective, Long-term Study

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Objective: To assess corneal morphological characteristics in vivo after photorefractive keratectomy (PRK) in humans.

Methods: Eighteen eyes were examined before and after PRK by means of in vivo confocal microscopy. Epithelial, stromal, and endothelial morphological characteristics were recorded. Minimum follow-up was 12 months.

Results: Immediately after PRK, the anterior stroma showed marked intercellular edema. At 1 month, fine linear structures were noted in the anterior stroma and midstroma, and a thin hyperreflective scar was present. The linear structures and the scar tissue were more marked at 4 months but were still present up to 26 months. Anterior stromal keratocyte density increased significantly 1 and 4 months after PRK, whereas midstromal and posterior keratocytes and endothelial cell densities did not change. Basal epithelial nerves were recognizable as early as 1 month after PRK. Contact lens–related microdots in the stroma remained unaffected.

Conclusions: The stromal linear structures represent a finding that is detectable only by confocal microscopy at high magnification, is not related to previous contact lens wear, and is still visible 26 months after PRK. The extension of these structures as far as the midstroma indicates that the permanent corneal changes caused by PRK affect deeper stromal layers than the immediate subepithelial region.


Excimer laser photorefractive keratectomy (PRK) to correct myopia up to 6.0 diopters (D) has proved to be an effective refractive procedure. Although every eye initially develops a subepithelial scar (visible as haze on slitlamp examination), this scarring usually does not substantially impair visual acuity. Accordingly, the incidence of loss of best-corrected visual acuity is very low. Clinically, the haze is confined to the very anterior stroma underlying the original ablation zone. Previous studies have shown that small ablation zones and higher corrections cause a denser haze and a higher rate of visual acuity loss.

Several methods have been used to quantify the amount of scarring in the anterior stroma after PRK. Braunstein et al showed a positive correlation between a corneal light-scattering index, best-corrected visual acuity, and haze intensity after PRK. High-frequency ultrasound and Scheimpflug photography allow for epithelial and stromal thickness measurements as well as haze quantification.

Corbett et al graded haze with digitalized retroillumination images and with video slitlamp images assessed through polarizing filters.

Møller-Pedersen et al, using confocal microscopy, made a 3-dimensional reconstruction of the human cornea in vivo before and 1 month after PRK. They also quantified the anterior stromal haze.

In a previous publication, confocal microscopy was used to examine patients up to 3 years after PRK. Linear structures in the anterior two thirds of the stroma and punctate changes through the entire stromal thickness were noted. Because no preoperative confocal microscopic examinations had been performed, preexisting stromal abnormalities, possibly caused by long-term contact lens wear, could not be excluded. We therefore conducted this prospective study to examine the corneal stroma before and at regular intervals after PRK, using realtime confocal microscopy.

RESULTS

Contact lenses were worn before surgery on a regular basis in 12 eyes and occasionally...
PATIENTS AND METHODS

Twelve patients were entered into the study. Inclusion criteria were absence of corneal abnormality and willingness to participate in a follow-up of at least 12 months. Written informed consent was obtained after extensive counseling. History and clinical data were recorded in a standardized protocol. Eighteen eyes underwent PRK for myopia or myopic astigmatism. Mean preoperative spherical equivalent was -5.30 ± 2.10 D (range, -1.20 to -11.75 D). Before PRK, the epithelium was abraded manually with the use of a hockey knife in an area just larger than the ablation zone. The PRK was performed with an excimer laser (Schwind Keratom, Kleinostheim, Germany), operating at 13 Hz and a fluence of 279 mJ. Patient self-fixation and eye tracking were used. The diameters of the ablation zones ranged from 6.0 to 7.0 mm for spherical corrections, and from 3.8 to 8.1 mm for astigmatic corrections. Ablation depths ranged from 35 to 100 µm. Postoperatively, a bandage contact lens was fitted for the first days, and dexamethasone sodium (Voltaren) and tobramycin drops were administered 4 times daily. Topical corticosteroids (flurometholone) 4 times daily were begun after reepithelialization was completed (usually on the third day) and were tapered over 3 months. In cases in which regression occurred, flurometholone was resumed. In the event of a retreatment, follow-up for the purposes of this study was discontinued (2 eyes). Slitlamp examination was performed at every visit and the corneal haze was graded on a scale from 0 to 5.12

With the use of a real-time scanning flying-slit confocal microscope (Hund Microphthal, Wetzlar, Germany) in 4 eyes. Contact lenses were never used in 2 eyes. Before PRK, epithelial morphological characteristics, including the basal cell layer and the basal epithelial nerve plexus, appeared normal as viewed with confocal microscopy. In both eyes of 1 wearer of rigid gas-permeable contact lenses, an increased number of basal epithelial nerves with abnormal branching pattern were seen. Stromal nerves and endothelium were normal. In 13 eyes, very small hyperreflective dots were present throughout the entire stroma between stromal cells as well as in the cell nuclei. In 1 eye fine fibrillar structures were seen in the stroma before excimer treatment. All of these 14 eyes had worn rigid gas-permeable or soft contact lenses.

In the first days after PRK, before the epithelium healed, decreased cellularity and enlarged spaces between nuclei, especially in the anterior stroma, were detected by confocal microscopy (Figure 1, A and B). From the fourth day, broader linear structures with an apparent relationship to the keratocytes were seen in the anterior stroma, and some edema was still present (Figure 1, C, D, E, and F).

At 1 month postoperatively, the morphological characteristics of the epithelium were restored, and the basal cell layer did not appear to be more polymorphic than preoperatively (Figure 2, A and B). The basal epithelial nerve plexus was visible only in 1 eye (Figure 3, A) and a thin subepithelial scar, recognizable as a highly reflective layer, was seen in every eye. In the anterior stroma, immediately under the subepithelial scar and reaching into the midstroma, fine linear structures were seen between the keratocytes. These structures, which were not seen before PRK, were oriented in every direction in the same plane, sometimes displayed hyperreflective bumps, and were clearly visible in 5 eyes. Some keratocytes were spindle shaped, and their nuclei appeared more reflective. The endothelium did not show any changes when compared with preoperative images.

The most marked changes on confocal microscopy were seen 4 months postoperatively. A subepithelial hyperreflective scar was present in every eye. The reflectivity of the scar, although not quantified, was variable and correlated with the subjective grade of haze observed at the slitlamp. The basal epithelial nerve plexus was either absent or obscured by the subepithelial scar in 11 eyes and could be seen only in 7 eyes (Figure 3, B). The 2 eyes with the preoperative abnormal branches of the basal epithelial nerves showed regenerated nerves with morphological characteristics similar to the pre-PRK pictures. The fine hyperreflective linear structures seen in the stroma at 1 month had become more pronounced and were clearly visible in 9 eyes. These structures were more frequent immediately under the subepithelial scar, but also reached into the midstroma (Figure 3, C, D, and F). Some highly reflective cells with elongated nuclei were seen in the anterior stroma. The fine punctate changes observed preoperatively in the 13 eyes with a history of contact lens wear did not change substantially. The endothelium appeared normal, and the polymegathism noted before surgery in some eyes of contact lens wearers remained unaltered.

The cornea was examined before PRK and at 1, 4, 12, and, in 5 cases, 24 months postoperatively. Four corneas were also examined during the first week after surgery. The clinical use of this confocal microscope has been described previously.13-15 Briefly, images are taken through a gel-immersed objective (×23, ×40, and ×50 magnification) and are recorded on an S-VHS videotape at a speed of 25 frames per second. Optical section thickness varies between 16 µm (×25 objective) and 10 µm (×50 objective).16 Single images can be retrieved from the tape and pictures can be taken directly from the screen or with a digital image processor.

One of us (B.E.F.) reviewed the taped images in a non-masked fashion on 3 occasions. Counts were performed manually, directly from the monitor on epithelial basal cells, on anterior, midstroma, and posterior keratocytes, and on endothelial cells. Preoperative anterior keratocyte densities were calculated by counting the cells in a layer about 40 µm below the Bowman layer, ie, 4 frames below the basal cell layer. Postoperative counts were made approximately in the same layer for comparative purposes, counting the cells immediately below the subepithelial scar. Midstroma keratocytes were defined as keratocytes in sections adjacent to stromal nerves, several frames away from the epithelium or endothelium. Posterior keratocytes were defined as those found in the last keratocyte layer, just anterior to the Descemet membrane. Postoperative data were compared with preoperative values by the paired t test. P<.05 was considered statistically significant. Mean ± SD follow-up was 17.4 ± 7.5 months, and mean age at surgery was 34.7 ± 12 years.

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Confocal microscopy at 12 months showed a subepithelial scar in 9 eyes (Figure 3, E). The basal epithelial nerve plexus was detectable in 13 eyes. The linear structures in the stroma were still present up to 26 months postoperatively (Figure 3, F), as were the microdots. In 4 eyes, the linear structures were absent or not detectable during the entire follow-up.

The Table summarizes the data on the mean densities of the anterior, midstromal, and posterior keratocytes as well as endothelial cells. Anterior keratocytes increased in number after PRK, reaching statistical significance at 1 (P = .02) and 4 (P = .04) months. The densities of the posterior and midstromal keratocytes and of the endothelial cells did not change significantly. Basal cell densities could not be calculated in the majority of the eyes because the quality of the pictures of this layer was not sufficient. (Preliminary results on basal cell densities have been reported previously by our group.17)
This prospective study using in vivo confocal microscopy demonstrated that, in addition to the previously described post-PRK subepithelial scar that forms in the ablated area, another type of abnormality is present, consisting of fine linear stromal structures that extend from the immediate subepithelial area to the midstroma. This finding is not visible with the slitlamp.

Few confocal microscopic studies have been published on corneal appearance after PRK, and they have not reported these linear stromal structures in humans. This discrepancy may result from the different imaging capabilities of the confocal microscopes used.

It is interesting that the stromal linear structures have not been seen in previous histological studies. The linear structures are oriented in every direction in the plane parallel to the corneal curvature, and are therefore difficult to detect with standard histological cutting techniques. In a recent article, similar linear stromal structures were reported in rabbits that had undergone PRK but did not receive postoperative corticosteroids. The spindle-shaped structures disappeared after 3 weeks and were believed to be migratory fibroblasts or keratocytes. This interpretation was supported by a gradually decreasing reflectivity of the anterior stroma 7 weeks after PRK, suggesting that the fibroblasts had reached a stationary state. Because of the different response to corneal injury in rabbits, comparison with human data must be made cautiously. In the above recent study, for example, there was a progressive increase in stromal thickness starting 2 weeks after PRK, and an almost total restitution of thickness by 6 months, illustrating that the rabbit cornea does not heal like the human one. An additional difference was the lack of postoperative treatment with topical corticosteroids, which could in part explain the rapid disappearance of the linear structures. Previous studies with rabbits did not report any stromal linear structures.

The nature of these stromal structures in humans remains unknown. Activated keratocytes are probably not involved, because the linear changes were seen up to 26 months after PRK, by which time keratocyte nuclei appear normal again. Lymphocytes would appear more round and nonlinear. A possible explanation for the linear structures is that pathological collagen or lipofuscin is deposited in the stroma during the healing process. We have observed similar stromal structures in cornea transplants and after microbial keratitis, so this finding may not be specific to PRK and could reflect a nonspecific response to long-term low-grade inflammation. If this hypothesis is valid, it could explain the fact that the linear structures were more pronounced 4 months after PRK (ie, after discontinuation of topical corticosteroids) than at 1 month. In a previous retrospective study, 2 types of linear structures were detected with confocal microscopy after myopic PRK: very fine needle-like structures and somewhat thicker rods. In this study, we observed only 1 type of linear stromal structure. The laser used in the retrospective study was an older scanning excimer laser, and the postoperative corticosteroid regimen was different, which may explain the discrepancy in the findings. The small punctate changes seen throughout the entire stroma were probably the result of long-term contact lens wear; they were preexistent in eyes that had worn contact lenses and did not alter after PRK. We speculate that the dots may represent deposition of lipofuscin. The presence of these changes even after discontinuation of contact lens wear has been reported.

It was possible to visualize the basal epithelial nerve plexus in only 7 eyes at 4 months and in 13 eyes at 12 months postoperatively. This was either because the plexus was still absent or because the hyperreflectivity of the subepithelial scar masked the fine nerves. The presence of the epithelial nerves at 4 months is in agreement with previous studies and with clinical observations, showing variable recovery of corneal sensitivity 3 months after PRK. Auran et al measured the velocity of nerve growth in the basal epithelial plexus in humans by means of a confocal microscope and found it to vary between 7 and 17 µm daily. Linna and Tervo reported on somewhat abnormal basal epithelial nerves years after PRK. The interpretation of their results is difficult, because no confocal microscopy had been performed before PRK.

In a rabbit model, Ishikawa et al showed a transient increase in density of intraepithelial nerves, which correlated with hypersensitivity. At 210 days, the nerve pattern had returned to normal. Trabucchi et al found a morphologically disorganized subepithelial plexus at 1 month. Pallikaris et al made similar observa-

**Figure 2.** A, Basal epithelial cell layer before −3.0 diopter/−1.0 diopter × 180° photorefractive keratectomy. B, Same eye, 1 month after photorefractive keratectomy. Bars indicate 50 µm.
tions. Those results are in disagreement with a report by Tervo et al., describing reinnervation in rabbits 3 to 12 months after PRK and the presence of abnormal branching of stromal nerves as late as 1 year postoperatively. In a study on monkey corneas treated with PRK with −1.50-D and −3.00-D ablations, the number of activated keratocytes under the treated zone increased up to 4 months and decreased thereafter. Similar results had been previously reported by Fantes et al. In rabbits, an acellular zone in the anterior stroma under the ablated area has been observed for 3 weeks after PRK. Later, an increased number of fibrocytes populated the same area.

We found a significant increase in the number of anterior keratocytes, with a peak at 1 and 4 months and a subsequent return to a normal count. Increases in the number of anterior keratocytes after PRK have been observed in both human and animal studies. The peak in anterior keratocyte density at 1 and 4 months correlates well with the higher reflectiv...
ality of the subepithelial scar and the higher clinical haze score at that stage. This suggests that keratocytes or other cells are involved in the scar formation. However, because of the high reflectivity of the scar, it was impossible to determine whether keratocytes were present within the scar. Some authors have detected a transient decrease in anterior keratocytes in the first weeks after PRK or after epithelial abrasion. We also observed such transient decreases, but because this study examined few eyes with confocal microscopy in the first postoperative days, it was not possible to perform a statistical analysis for this early post-PRK phase. The marked intracellular and intercellular edema that is present in the anterior stroma in the first days after PRK may simulate a decrease in keratocyte density, i.e., the observed decrease may not be a reflection of keratocyte necrosis or migration. This interpretation is supported by the absence of necrotic stromal cells (pyknotic nuclei) in the first days after PRK.

No significant changes in the densities of the midstroma and posterior keratocytes or of the endothelial cells were noted after PRK. The unchanged central endothelial cell counts are in agreement with other human studies.

In conclusion, this prospective in vivo confocal microscopic study has documented linear stromal structures in the post-PRK cornea. These findings, located in the anterior two thirds of the stroma, were seen in 14 of the 18 eyes studied and remained visible up to 26 months. The clinical significance of the newly observed stromal structures is not known, but their presence indicates that the permanent corneal changes caused by PRK in fact affect deeper stromal layers than the immediate subepithelial region. This study also confirms previous publications showing a restored epithelial structure at 1 month after PRK, a basal epithelial nerve plexus visible at 4 months, and a hyperreflective subepithelial scar correlating with the clinical evolution of haze. A significant increase in the density of anterior stromal keratocytes was also substantiated.

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| Mean Cell Densities | 
|---------------------|------------------|
|                     | Preoperative     | 1 mo | 4 mo | 12 mo |
| Anterior keratocytes| 408 ± 71         | 479 ± 67* | 477 ± 99* | 434 ± 85 |
| Midstromal keratocytes| 324 ± 151       | 334 ± 68  | 333 ± 66  | 304 ± 55  |
| Posterior keratocytes| 423 ± 99        | 410 ± 101 | 439 ± 114 | 403 ± 49  |
| Endothelial cells   | 2678 ± 311       | 2511 ± 346 | 2704 ± 243 | 2658 ± 256 |

*P < .05, paired t test.

REFERENCES

Anticardiolipin Antibodies Are Frequently Present in Patients With Idiopathic Intracranial Hypertension

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Background: Anticardiolipin antibodies (ACL-Ab) are associated with various neurologic syndromes, but idiopathic intracranial hypertension (IIH) has only rarely been reported in this context.

Objectives: To delineate the frequency and clinical and radiological features of, as well as the cause-and-effect relationship between, ACL-Ab and IIH.

Methods: We analyzed the medical records of patients with IIH hospitalized between January 1989 and September 1995. All patients underwent magnetic resonance imaging or magnetic resonance venography or angiography. Excluded were patients with intracranial hypertension due to dural sinus thrombosis or traumatic, structural, neoplastic, or infectious disorders. Patients who were found on at least 2 separate occasions to have increased IgG titers of ACL-Ab were identified and compared with patients without ACL-Ab.

Results: Six (43%) of 14 patients with IIH had ACL-Ab. No differences in clinical, laboratory, or radiological variables could be found between patients with and without ACL-Ab. Only 3 of the 11 ACL-Ab–positive patients had previous systemic or neurologic abnormalities associated with ACL-Ab.

Conclusions: Anticardiolipin antibodies may cause IIH through mechanisms unrelated to major venous thrombosis. Idiopathic intracranial hypertension is frequently associated with ACL-Ab and can be the presenting symptom of the antiphospholipid syndrome. There are no major clinical, laboratory, or radiological features that distinguish between patients with IIH with and without ACL-Ab. (1998;55:817-820)

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