Long-Term Corneal Morphology after PRK by In Vivo Confocal Microscopy

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PURPOSE. To examine human corneal morphology and nerve recovery 5 years after photorefractive keratotomy (PRK).

METHODS. Fourteen eyes of 14 patients (ages, 27–53 years) who underwent 6-mm diameter PRK for low to moderate myopia (spherical equivalent [SE] –2.5 to –8.0 D) were examined once 5 years after surgery. Nine healthy individuals served as control subjects. Standard biomicroscopy, manifest refraction, and visual acuity tests were performed. The morphology of the corneas was examined by in vivo confocal microscope. Thicknesses of the epithelium and stroma, as well as the density of corneal opacity (haze) were obtained from digital image analysis of the confocal microscopy through-focusing (CMTF) scans.

RESULTS. Confocal microscopy revealed increased reflectivity in the subepithelial extracellular matrix, keratocyte nuclei and processes in all patients. The mean objective haze estimate was 166.7 U (range, 50–390) in control corneas compared with a mean of 225.9 U (range, 125–430, P = 0.15) in the post-PRK corneas. The density of the subbasal nerve fiber bundles in post-PRK corneas (mean, n = 4.2; range, n = 1–7 per field of view) was not significantly lowered from that in control subjects (mean, n = 4.9; range, n = 3–6; P = 0.56). Bowman’s layer was undetectable in all post-PRK corneas. Clinically, slit-lamp–observed trace of haze in four corneas correlated positively with the ablation depth (P = 0.016) and the thickness of the haze area (P = 0.006) in the confocal microscope.

CONCLUSIONS. In vivo confocal microscopy demonstrates the presence of morphologic alterations even 5 years after PRK. However, these alterations were not associated with cellular and neural recovery and do not seem to interfere with visual performance.

Photorefractive keratotomy (PRK)1 has been a relatively safe method for correcting refractive errors.2–8 The main problems associated with PRK for myopia are the development of transient subepithelial haze, regression of myopia, and dry eyes. In cases of low to moderate myopia PRK and laser in situ keratomileusis (LASIK)9 have yielded equal clinical results.3–8 Despite the exploded popularity of laser refractive procedures, relatively few long-term (>5 years) clinical follow-up studies10–15 have been reported.

According to the present knowledge, anterior stromal fibrosis (haze) is a result of increased cellular reflectivity16 and synthesis of extracellular matrix (ECM).17–20 This new tissue appears to compensate for the photodamaged anterior stroma, thus leading to corneal resteeplening. In some cases, regression of myopia may continue even up to 5 years after PRK.16

Both PRK and LASIK severely affect the nerves in the anterior cornea, which leads to decreased sensitivity,21 impaired tear fluid secretion, and dry eye symptoms22–24 and may induce the development of relative neurotrophic epitheliopathy.25 Absence of neural control on keratocytes26 may as well interfere with wound healing. Because the degree of recovery in neural function is of decisive importance for long-term results of PRK, this topic has gained great scientific interest.

Confocal microscopy (CM) enables the evaluation of tissue and cell responses as well as epithelial and stromal thickness analysis of human corneas in vivo.27–35 In addition, the degree of regeneration of the subbasal nerve fiber bundles (SNFBs); the occurrence, density, and thickness of post-PRK haze; and the morphologic alterations in keratocytes 5 years after PRK for myopia can be assessed.

METHODS

Patients

To enroll volunteers for the study, we contacted patients who underwent surgery performed by one of the authors (TMTT) during 1993 and 1994 in a private excimer laser clinic. Twenty randomly selected patients were invited for the study; six patients were unable to attend. Each patient gave informed consent. Altogether, 14 eyes of 14 patients (5 women, 9 men; age, 40.1 ± 7.6 years; range, 27–53) were examined. Clinical outcome was followed up to 5 to 6.3 years (mean, 5.7 years) after PRK performed for low to moderate myopia (spherical equivalent [SE] –4.59 ± 1.59 D; range, –2.5 to –8.0). The initial astigmatism was less than 1.00 D in 12 eyes, and 1.25 and 1.75 D in another two eyes. Nine healthy individuals (four women, five men; age, 39.0 ± 8.5 years; range, 27–53, P = 0.741) served as control subjects for confocal microscopic evaluation. The Ethical Review Committee of Helsinki University Eye Hospital had approved the research plan, and the study design followed the tenets of the Declaration of Helsinki.
Photorefractive Keratectomy

After surgical removal of the epithelium 6-mm-wide PRKs were performed with an excimer laser equipped with software (model 20/20 B laser and version 2.7; Visx Co., Sunnyvale, CA). The mean ablation depth was 54.64 ± 14.98 μm (range, 30–80), and the purpose of each operation was to correct to attain emmetropia. The eyes were patched for 2 to 3 days after PRK. The postoperative medication included chloramphenicol ointment (Oftan Chlor; Santen, Tampere, Finland) for 1 week and, after the epithelium had healed, fluorometholone (Liquifilm-FML; Allergan, Irvine, CA), dexamethasone (Maxitrol; Alcon Laboratories, Inc., Fort Worth, TX), or prednisolone acetate (Pred Forte; Allergan) drops for 1 to 4 months.

Clinical Examination

Preoperative and postoperative 1-month examinations included refraction, uncorrected (UCVA), and best corrected (BCVA) visual acuity, and slit lamp biomicroscopy. At 5 years after PRK, patients were examined on slit lamp to estimate corneal haze36 and UCVA, BCVA, and refraction were measured. Oral comments of the patients were also collected.

In Vivo Confocal Microscopy

After clinical examination, a tandem scanning confocal microscope (TSCM; Model 165A; Tandem Scanning Corp., Reston, VA) was used for morphologic evaluation of surface epithelial cells, basal epithelial cells, subbasal nerve plexus, most anterior keratocytes, stromal keratocytes, stromal nerves, and endothelium of the central cornea. The setup and operation of the confocal microscope has been described.30,37,38 Briefly, a 24× variable-working-distance objective lens was used. The field-of-view with this lens is 450 × 360 μm, and the z-axis resolution is 9 μm. Images were detected with a low-light level camera (VE1000; Dage, Michigan City, IN) and recorded on SVHS tape. The number of the long (defined as ≥200 μm) central SNFBs was calculated in the image with the highest density of bundles. In addition, confocal microscopy through-focusing (CMTF) scans were obtained as previously described.30,38 Video images of interest were digitized using a PC-based imaging system with custom software (University of Texas Southwestern Medical Center at Dallas), and printed (Stylus Color 800 printer; Seiko Epson Corp., Nagano, Japan). Using the custom software, the CMTF data were digitized onto the PC and intensity profile curves of the backscattering of light were calculated.38 The CMTF-curves exhibit the intensity of the backscattered light from the corneal subunits, where epithelium, subbasal nerves, most anterior keratocytes, and endothelium comprise the areas of the highest intensity (Fig. 1A).

From each scan, the epithelial, stromal, and total corneal thicknesses were measured. A quantitative estimate of the increased backscattering of light (CMTF-haze, Fig. 1B, C)30 of the operated corneas was obtained by calculating the area below the CMTF profile. This intensity peak originated from the regenenerated subbasal nerves, highly reflective ECM, and morphologically altered keratocytes. Depth and thickness of the haze area were determined as an area with bright keratocytes and increased ECM (1) and area posterior to “1” with alterations only in keratocytes (i.e., visible processes). Haze area “1” served as a subject for statistical analyses. For control corneas a corresponding area with subbasal nerves (Fig. 1A, peak c) and most anterior keratocytes (see Fig. 1A, peak c’), corresponding to the peak in the CMTF-profile, was selected. An average of seven (range, four to nine) CMTF scans was performed on each eye. The scan of one eye was excluded from the analysis because no acceptable CMTF-profile could be obtained from one patient (Table 1, patient 14).

Statistical Analyses

Statistical analyses were performed on computer (SPSS for Windows, ver. 7.0; SPSS, Chicago, IL). Continuous variables were tested by Mann-Whitney or Student’s t-test. The Pearson (r) or the Spearman ρ correlation coefficient was used to evaluate the correlations between variables. Data are expressed as the mean ± SD. Mean values of the CMTF-measurements were used for all statistical calculations.

RESULTS

Visual Outcome

Table 1 shows respectively the pre- and postoperative BCVAs, postoperative UCVAs, and the respective refractions (SEs). The postoperative UCVA was 20/20 or more in 3 (21%) of 14 eyes, and 20/40 or more in 12 (86%) of 14 eyes at 1 month; one patient missed the follow-up. At 5 years, UCVA was 20/20 or more in 8 (57%) of 14 eyes, and 20/40 or more in 10 (71%) of 14 eyes, and all but 1 eye (93%) had BCVA of 20/20 or better. Five patients gained one line of BCVA. One patient, who lost
At 5 years after PRK, two patients were unsatisfied with their regression of myopia (patients 1 and 14 in Table 1, SE −1.00 and −1.25 D, ages 39 and 27, respectively), but neither of them preferred an operation to enhance visual acuity. One patient with a pigmentary glaucoma in the operated eye had ptosis as well (Table 1, patient 3). In addition, one unsatisfied patient complained about glare and shadows in the temporal field still 5.5 years after surgery (Table 1, patient 9).

**Patients’ Comments**

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**In Vivo Confocal Microscopy**

**Corneal Morphology.** Cell morphology was evaluated from the central cornea of control subjects and patients. The surface and basal epithelium were similar in both groups.

**Subbasal Nerves.** Subbasal nerves were observed in every control and post-PRK cornea. In control corneas the number of the long SNFBs in the field of view ranged from 3 to 6 (mean, 4.9 ± 1.1), being slightly higher than in the surgically treated corneas (range, 1–7; mean, 4.2 ± 2.1, P = 0.562, Mann-Whitney). There was also a wider range of SNFBs in the post-PRK corneas, of which five (36%) had only one or two long nerve fiber bundles. Normal corneas showed with a typical pattern of subbasal nerve branching (Fig. 1A), whereas in the post-PRK corneas (71%) of 14 showed similar branching. In control corneas, the nerve plexus was detected anterior to the nonreflecting, 14-μm-thick (range, 10–21) Bowman’s layer, which separated the nerve plexus from the anterior keratocytes. After PRK, however, the SNFBs appeared in the same optical section with the most anterior keratocytes (Fig. 1B, 1C, 2B) resulting in a high reflective zone. In all patients, Bowman’s layer was absent.

**Stromal Cells.** Control corneas showed a typical clustering of anterior keratocytes. Their evenly reflected nuclei were oval-shaped and were well separated from the surrounding cells, because cell processes were not visualized and the extracellular space reflected only minimally.

In the surgical corneas, the most anterior keratocytes appeared to be unevenly distributed, and even clinically clear corneas still showed signs of opacity: increased fibrosis-like ECM reflectivity, bright keratocyte nuclei (Fig. 2B), and visible keratocyte processes. Subtle morphologic alterations (i.e., thin, faint keratocyte processes) in the post-PRK keratocytes were also visualized posterior to the area with enhanced ECM reflectivity (Fig. 2F). Compared with control corneas, however, these changes were so subtle that they could not be considered to differ significantly from the control (Fig. 2E).

The posterior stromal keratocytes, ECM, and endothelium appeared morphologically identical with the control corneas, except one post-PRK cornea that showed microdots in both the anterior and posterior stroma (results not shown) and three postoperative corneas with posterior stromal microdots (Fig. 2H, arrows). Another two post-PRK corneas showed anterior microdots (depth 80 and 110 μm, respectively). At this stage the significance of these particles remains unclear.

**Confocal Microscopy Through-Focusing**

**Thickness Measurements.** The mean epithelial, stromal, and total corneal thicknesses are shown in Table 2. As expected, the stroma was significantly thinner in the corneas that had undergone PRK. Although the epithelial thickness did not differ from that in control corneas, the total corneal thickness was lower in the surgical corneas as well.

Three patients exhibited regression during the follow-up. Two showed normal epithelial thicknesses (>7 and 53 μm), but no CMTF-reading could be obtained in the third (Table 1, patient 14), with a regression of −1.25 D.  

**Objective Haze Estimate.** In control corneas, the CMTF peaks corresponding to the post-PRK haze area originated from
Confocal Microscopy 5 Years after PRk for Myopia

**Differences were noted in Bowman's layer and the anterior keratocyte area.** (A) Normal subbasal nerve plexus with the nonreflecting Bowman's layer. In post-PRK corneas, the most anterior keratocytes (arrowhead) and some hyperreflective ECM were imaged together with the regenerated subbasal nerves (arrow). Evenly distributed anterior keratocyte nuclei formed clusters (C, arrow) in a control cornea, whereas after PRK (D) they still showed hyperreflectivity and uneven distribution (arrow). (E, F) Midstromal keratocytes. Only minor changes were observed in the post-PRK corneas (F, arrow), but they could not be distinguished significantly from an untreated cornea (E). The posterior cornea appeared unaffected in both groups (G, H). Posterior microdots (arrow, diameter, -5 μm), however, were present in two control patients and three surgical patients.

**Subbasal nerves (Fig. 1A’, peak c) and the most anterior keratocytes (Fig. 1A’, peak c’), including the Bowman’s layer in between.** The peaks in the CMTF profiles of post-PRK corneas were related to the subepithelial haze area (Figs. 1B, 1C, 2B, 2D). They comprised mainly the regenerated subbasal nerves, increased cellular reflectivity, and ECM changes.

The difference in the mean CMTF intensity curves of the backscattering of light between control subjects (166.7 ± 95.0 U; range, 50–390) and patients with PRK (225.9 ± 83.9 U; range, 125–430) was insignificant (P = 0.15, t-test). In control patients, the thickness of the increased intensity area (subbasal nerves, Bowman’s layer, and the most anterior keratocytes) giving the peak was 34.8 ± 5.3 μm (range, 27.1–44.45). The mean thickness of the area producing subepithelial haze in patients who underwent PRK (subbasal nerves, haze, and the most anterior keratocytes) was 33.6 ± 6.2 μm (range, 20.4–46.4) Furthermore, in 11 surgically treated corneas a further zone of altered keratocytes, which appeared as visible, abnormal keratocyte processes, was observed posterior to the true haze peak. Thus, the total thickness of the area with morphologic alterations varied from 35.7 to 136.6 μm after PRK.

**Haze and Ablation Depth.** A positive correlation was observed between the clinical haze score and depth of the laser ablation (r = 0.629, P = 0.016). The CMTF haze estimate (r = 0.084, P = 0.785) or the thickness of the haze area (r = 0.434, P = 0.139) showed no correlation with the ablation depth. Neither did the clinical haze score reveal correlation with the CMTF haze estimate (r = 0.312, P = 0.300). However, the clinical haze score correlated positively with the thickness of the haze area (r = 0.713, P = 0.006). The number of the SNFBs showed no correlation with the ablation depth (r = -0.128, P = 0.662), CMTF haze estimate (r = 0.153, P = 0.618), or the thickness of the haze area (r = 0.084, P = 0.785). The amount of regression of myopia, evaluated as the refractive change from the intended emmetropia, revealed no correlation with the ablation depth (r = 0.100, P = 0.734), epithelial thickness (r = 0.175, P = 0.567), CMTF haze estimate (r = -0.163, P = 0.595), or the thickness of the haze area (r = 0.156, P = 0.610).

**Discussion**

In vivo confocal microscopy is a relatively new tool for analyzing corneal morphology and innervation, in both diseased and postsurgical corneas. In previous CM studies faint subbasal nerves were observed in the central cornea already at 1 to 4 months after PRK. Linna et al. showed that, after LASIK, corneal sensitivity improves as the subbasal nerves regenerate so that both functional recovery is under investigation. Frequent reports of dry eye during the first months after PRK seem to be related to neural damage. However, after 5 years, one patient with a very well-regenerated subbasal nerve plexus in the central cornea still showed subjective dry eye (Table 1, patient 13).
Bowman’s Layer

PRK performed on a cornea with previous neural damage, such as after penetrating keratoplasty or after LASIK, appears to lead to excessive formation of haze. The absence of Bowman’s layer in every cornea after PRK may impair neural orientation and regrowth. This and the closer topographical relationship between stromal and epithelial cells in post-PRK corneas may also alter epithelial-stromal cell interaction. In the present study, the absence of the nonreflecting Bowman’s layer was judged by the occurrence of the subbasal nerve bundles in the same optical section as the one with the most anterior keratocytes.

The mechanical stabilizing properties of Bowman’s layer have appeared to be insignificant. Muller et al. suggested, however, that ablation of the most anterior interwoven collagen lamellae may reduce corneal stability. In the corneal incision wound after radial keratotomy, a pseudolamellar Bowman’s-layer-like formation appeared underneath an epithelial plug. After PRK, such zones have not been identified, and in our study the subbasal nerves were observed at the same level of the most anterior keratocytes. Most probably, Bowman’s layer is not essential, and only a newly formed basal membrane is sufficient to serve as a substrate for migration of regenerating nerves. Unfortunately, a confocal microscope is unable to visualize a nonreflecting basal membrane.

Keratocytes, Haze, and Regression

Transformation of keratocytes into highly reflective myofibroblasts as well as newly formed and irregularly deposited ECM, cause more backscattering of light, which contributes to the formation of subepithelial haze. The question remains why the epithelium regains its original morphology and the anterior stroma becomes so hazy. Imbalance in the interaction between epithelial and stromal cells and neural dysfunction are presumed to be involved in these changes.

With biomicroscopy, only 4 of 14 patients showed a faint haze, but with confocal microscopy, areas of hyperreflective keratocytes and ECM were identified in all of them. Unexpectedly, these changes were so subtle that no significant increase in the CMTF intensity scan were produced (Figs. 1A–C). Because CMTF reflectivity in control subjects was comparable with that of PRK-treated corneas, it can be concluded that regeneration of corneal tissue must have been relatively complete. However, CMTF is performed in the central cornea, and it therefore may not correlate well with the clinically observed haze when the latter is unevenly distributed over the whole cornea. Furthermore, the present study was performed with an excimer laser (model 20/20B; Viss) and there may be differences in the haze formation induced by newer lasers.

The present study indicates that corneal morphology is not reconstructed completely after PRK. However, the history of the PRK-treated patients clearly shows that optical performance is hardly impaired, even after 5 years.

Absence of Bowman’s layer and the irregular interface between the epithelium and stroma may interfere with the reflectivity and thus with the visualization of the nerves in the subbasal plexus. Despite their altered topography subbasal nerves continue to regenerate during the years. The aforementioned changes in the anterior cornea may have clinical consequences in terms of mechanical stability and wound healing, especially when patients are challenged by, for example, UV-light, infections, or inflammations.

References

17. Moller-Pedersen T, Li HF, Pettro WM, Cavanagh HD, Jester JV. Confocal microscopic characterization of wound healing after