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 keratectomy (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 –8.00 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, the changes appear to be resolved by cellular and neural recovery and do not seem to interfere with visual performance.

Photorefractive keratectomy (PRK) has been a relatively safe method for correcting refractive errors. 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) have yielded equal clinical results.

Despite the exploded popularity of laser refractive procedures, relatively few long-term (>3 years) clinical follow-up studies have been reported.

According to the present knowledge, anterior stromal fibrosis (haze) is a result of increased cellular reflectivity and synthesis of extracellular matrix (ECM) by (activated) keratoctyes. Corrections of high myopia (i.e., deeper ablations) enhance formation of haze. Haze reaches its maximum between the second and third months, but then resolves spontaneously by 6 months to 1 year. The PRK-induced haze may continue to disappear, even after the first postoperative year. Although, cases of late-onset haze have also been described.

Regression of myopia after PRK is thought to result from restenesis of ECM by activated fibroblasts and altered keratocytes. This new tissue appears to compensate for the photoablated anterior stroma, thus leading to corneal resteecheling. In some cases, regression of myopia may continue even up to 5 years after PRK.

Both PRK and LASIK severely affect the nerves in the anterior cornea, which leads to decreased sensitivity, impaired tear fluid secretion, and dry eye symptoms and may induce the development of relative neurotrophic epitheliopathy. Absence of neural control on keratocytes 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. 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 (TMT) 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 –8.00 D). 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, 59.0 ± 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 a correction 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; 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 haze 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. Briefly, a 2× 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 (VE 1000; 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. 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. 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) of the operated corneas was obtained by calculating the area below the CMTF profile. This intensity peak originated from the regenerated 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
In Vivo Confocal Microscopy

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, pigmentary glaucoma and 0.5 haze (Table 1, patient 9), with a regression of myopia (patients 1 and 14 in Table 1, SE –1.00 and –1.25 D). One of these three eyes presented with the same –1.0 D refraction already at 1 month (Table 1, patient 1). Two eyes developed a regression during the 5 months after surgery (Table 1, patients 4 and 14).

Slit Lamp Examination

At 1 month, haze scores from 1 to 2 were observed in six corneas, and the remaining corneas appeared to be clear or with a trace of haze (Fantes haze scale grade 0.5). At 5 years, a haze of 0.5 was noted in four (28.6%) corneas and the other 10 with a trace of haze (Fantes haze scale grade 0.5). At 5 years, a wider range of haze scores from 1 to 2 was observed in six corneas.

Objective Haze Estimate

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), showed BCVA of 20/25 at 1 month (refraction, +0.13) and only 20 of 32 at 6 years (+0.5) after surgery. He also presented with ptosis, pigmentary glaucoma and 0.5 haze (Table 1, patient 3). Six (43%) of 14 eyes were within ±0.25 D and 11 (79%) of 14 within ±0.75 D of the intended emmetropia (SE), and 3 eyes (21%) had a residual refractive error from –1.0 to –1.25 D. One of these three eyes presented with the same –1.0 D refraction already at 1 month (Table 1, patient 1). Two eyes developed a regression during the 5 months after surgery (Table 1, patients 4 and 14).

Corneal Morphology

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 10 (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 anterior layer 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).

In Vivo Confocal Microscopy Through-Focusing

Thickenss 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 (47 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 1067

DISCUSSION

In vivo confocal microscopy is a relatively new tool for analyzing corneal morphology and innervation, in both diseased and postsurgical corneas.\(^{29,30,32-34,59-41}\) The present confocal microscopic study clearly reveals that even the biomicromicroscopically clear corneas show morphologic changes in the subbasal nerve plexus, subepithelial keratocytes, and ECM 5 years after PRK.

Regeneration of Subbasal Nerves

In previous CM studies faint subbasal nerves were observed in the central cornea already at 1 to 4 months after PRK.\(^{30,33}\) Linna et al.\(^{34}\) showed that, after LASIK, corneal sensitivity improves as the subbasal nerves regenerate so that both functional and nerve morphology seem to be normalized in 6 months. PRK, in contrast, has been shown to induce morphologic alterations in nerves up to 3 years after surgery.\(^{29}\) After 5 years, 71% of post-PRK corneas showed a branching pattern of regenerated subbasal nerves closely resembling that observed in normal control corneas. This indicates that after such a long period many patients have regained centrally the original pattern of innervation. As some surgical corneas still showed a reduced nerve fiber bundle density, it is likely that in those cases complete neural recovery will not be reached. Whether the number of recovered SNFBs correlates with complete functional recovery is under investigation. Frequent reports of dry eye during the first months after PRK seem to be related to neural damage.\(^{22}\) 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).

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 \(\mu\)m (range, 27.1–44.4). 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 \(\mu\)m (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 \(\mu\)m 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 (\(r = -0.165\), \(P = 0.595\)), or the thickness of the haze area (\(r = 0.156\), \(P = 0.610\)).

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.

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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 pseudomembranous Bowman-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 myoblasts 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 assumed 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; Visx) 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, Pettroll WM, Cavanagh HD, Jester JV. Confocal microscopic characterization of wound healing after

Confocal Microscopy 5 Years after PRK for Myopia 1069