Quantification of Stromal Thinning, Epithelial Thickness, and Corneal Haze after Photorefractive Keratectomy Using In Vivo Confocal Microscopy

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Purpose: The authors establish, for the first time, observer-independent quantification of stromal thinning, epithelial thickness, and corneal haze after excimer laser photorefractive keratectomy (PRK) using a unique, new form of in vivo confocal microscopy.

Methods: Rapid, continuous z-scans of high-resolution confocal images, termed *confocal microscopy through focusing* (CMTF), were performed in the central corneal area of 17 patients before and 1 month after PRK for low- to moderate-grade myopia (−2.88–−9.13 diopters [D]). Corneal, epithelial, and stromal thickness measurements and an objective haze estimate were obtained from each CMTF scan by digital image analysis.

Results: Epithelial thickness averaged 51 ± 4 μm before and 45 ± 10 μm 1 month post-PRK ($P < 0.005$), whereas stromal thinning ranged from 20 to 154 μm, representing a direct estimate of the actual photoablation depth. Corneal thickness averaged 560 ± 36 μm before PRK and 462 ± 52 μm at 1 month. The change in corneal thickness correlated closely with the change in spherical equivalent refraction ($r = 0.94$, $P < 0.0001$); linear regression analysis revealed a value of 14.3 μm corneal thinning per diopter of correction. A significant correlation was found between the objective CMTF haze estimate and a clinical haze grading obtained by slit-lamp examination ($r = 0.73$, $P < 0.001$).

Conclusions: Confocal microscopy through focusing is a new, powerful in vivo tool that enables quantitative, unbiased evaluation of PRK procedures over time by providing epithelial and stromal thickness analysis, photoablation depth assessment, and unbiased haze measurement. The method is uniquely valuable in the pre- and postoperative assessment of PRK patients and for determining the optimal treatment strategy, especially in assessing refractive and visual outcomes in individual cases.

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Excimer laser photorefractive keratectomy (PRK) is a widespread surgical procedure used to correct myopia. The basic concept of PRK is to photoablate graded amounts of the anterior corneal stroma to induce a permanent and predictable change in corneal refraction. Cur-

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rently, clinical results are promising, but variable predictability of refractive and visual outcomes, regression of initially achieved refractive effect, and subepithelial haze remain major clinical concerns.[1–5] For the most part, these undesirable side effects have been attributed to variation in corneal wound healing[1,2,5,6]; however, variation in corneal photoablation rate cannot be discounted.[5,7] To determine more accurately the pathogenetic mechanisms underlying these problems, there is a basic need for an objective in vivo method to quantitatively evaluate corneal photoablation rate and sublayer wound healing after PRK.

Four-dimensional (x, y, z, time) confocal microscopy has recently proved to be a unique, noninvasive technique used to obtain high-resolution microscopic images of the human cornea in vivo.[8] Various groups previously have used confocal microscopy to evaluate histopathologic changes after PRK in humans[8] and rabbits.[9,10] Recently, we have developed a quantitative technique using digital image analysis of continuous, high-speed confocal z-scans, termed *confocal microscopy through focusing* (CMTF), to obtain precise corneal sublayer thickness measurements in vivo of the epithelium, Bowman's layer, and the stroma.[11] In the present study, we performed CMTF scans in patients undergoing PRK for myopia to measure epithelial thickness and photoablation depth (stromal thinning) 1 month after surgery and to estimate the amount and location of postoperative corneal haze.

Patients and Methods

Patients

Seventeen eyes of 17 patients (5 women, 12 men; age 42 ± 8 years; range, 23–52 years) underwent PRK for low to moderate degrees of myopia (spherical equivalent of −2.88 to −9.13 diopters [D]; Table 1). Informed consent was obtained from all patients who were recruited from a group treated under the phase II protocol approved by the Food and Drug Administration and the Institutional Review Board at the University of Texas Southwestern Medical Center at Dallas. All patients were examined before and 1 month after surgery.

Treatments and Examinations

All patients received treatment by one of two surgeons with an Aesculap-Meditec Excimer Laser (MEL 60, Model 94, Heroldsberg, Germany) according to the phase II protocol. Two patients (nos. 2 and 3) received treatment with a 5-mm diameter photoablation zone whereas a 6-mm zone was used in all other patients. Before ablation, corneal epithelium was removed manually by gentle scraping in an area just larger than the ablation zone. During surgery, ablated debris was removed by nitrogen flow. Before and at 1 month postsurgery, manifest subjective refraction was assessed and corneal clarity was graded during slit-lamp examination using standardized manufacturer-supplied control photographs: grade 0, clear; grade 1, minimal haze; grade 2, trace haze; grade 3, mild haze; grade 4, moderate haze; grade 5, marked haze.

In Vivo Confocal Microscopy

All patients underwent in vivo confocal microscopy before and 1 month after PRK using a Tandem Scanning Confocal Microscope developed by Tandem Scanning Corporation, Reston, Virginia; the preoperative examination was missed in one patient (Table 1). The system design was similar to previous reports.[8,12–14] A microscope objective (24×, 0.6 numerical aperture) with a concave applanating tip (7.5 mm radius of curvature) and movable internal lenses which provide a variable working distance (0–1.5 mm) was used. The focal plane position was controlled using an Oriel 18011 Encoder Mike Controller (Oriel Corp, Stratford, CT) interfaced to a personal computer.[13] The illumination source was a 100 W mercury arc lamp, and real-time image detection was performed using a Dage MTI SIT VE-1000 camera (Dage MTI, Michigan City, IN) and an SVHS video recorder. With the current objective and camera, the system has an approximate field-of-view of 475 × 350 μm and an optical slice thickness (z-axis resolution) of 9 μm.[12] An improved eye fixation device (Haag-Streit, Waldwick, NJ) was recently added to the system to reduce eye movements and to facilitate precise alignment of the objective to the visual axis. In postoperative corneas, this allows examination of the central and deepest part of the photoablation profile.

Before each examination, a drop of topical anesthetic (0.5% proparacaine hydrochloride, Alcon, Inc., Humacao, Puerto Rico) was applied on the cornea and a drop of 2.5% hydroxypropyl methylcellulose (Goniosol, IOLAB Pharmaceuticals, Claremont, CA) was placed on the objective tip to serve as an immersion fluid. To obtain high-quality images, a standard confocal examination of all corneal layers was performed initially at a slow speed and with video camera gain and black levels set to automatic mode. Subsequently, video camera gain and black level were set to manual values that were kept constant at all examinations, allowing direct comparison of the scans. Then, CMTF was performed as described recently, in detail.[11] In brief, CMTF is a continuous confocal scan through the entire cornea, starting in front of the epithelium and ending below the endothelium. During CMTF scanning, the focal plane is moved at a speed of approximately 64 μm/second using the computer controlled Oriel Encoder system; thus, individual scans can be obtained within 7 to 10 seconds.[11] Because CMTF scans are video recorded at 30 frames/second, consecutive video images are separated in the z-axis by approximately 2.12 μm.[11] Three to seven complete scans were analyzed per visit. Fine saccadic eye movements, which were observed in all patients, have been shown not to affect the accuracy or reproducibility of the CMTF measurements.[11] More significant eye movements, such as loss of fixation, occurred in 15% of the CMTF scans, which were deleted from further evaluation. Total corneal examination time was approximately 4 minutes per visit.

Image Analysis and Three-dimensional Reconstruction

Confocal microscopy through focusing analysis takes advantage of the fact that different sublayers of the

Table 1. Characteristics of Patients Undergoing Photorefractive Keratectomy for Myopia

Patient No.	Pre-PRK			1-mo Post-PRK				
	Corneal Thickness (μm)	Epithelial Thickness (μm)	Spherical Equivalent (D)	Corneal Thickness (μm)	Epithelial Thickness (μm)	Spherical Equivalent (D)	CMTF–Haze Estimate (U)	Clinical Haze Grading
1	587	58	−2.88	553	44	0.50	28	0
2	618	55	−8.88	528	54	−0.38	342	0
3	501	52	−9.13	369	49	1.25	767	2
4	535	55	−3.88	499	53	0.00	211	0
5	530	53	−6.00	435	56	0.50	603	1
6	567	51	−5.63	435	45	2.25	51	1
7	518	48	−6.25	405	16	0.75	6021	4
8	590	48	−7.63	474	42	−0.38	1064	2
9	536	51	−3.88	451	45	1.25	544	0
10	593	50	−5.75	494	39	2.38	68	0
11	567	56	−6.63	468	52	1.38	147	0
12	542	51	−5.38	458	42	0.50	128	0
13	523	46	−4.50	458	48	0.13	123	0
14	619	41	−4.25	546	33	1.38	348	1
15	558	53	−6.88	466	50	0.13	562	0
16	581	50	−6.50	413	36	4.63	1548	2
17	—	—	−7.25	395	53	7.13	1042	1

PRK = photorefractive keratectomy; CMTF = confocal microscopy through focusing.

cornea reflect light at differing intensities, and that generating a depth–intensity profile allows for accurate and reproducible, quantitative determination of corneal sublayer location. To generate depth–intensity profiles, CMTF video recordings were digitized initially (approximately 210–300 sequential video frame images) and analyzed using a specially developed software program, as recently described.[11] Using this program, an average pixel intensity in the central 285 × 285 μm region was calculated for each video frame image, and the results were plotted as a function of z-axis position (each image separated by approximately 2.12 μm). Because each point along the curve corresponds to a specific video frame image, an interactive cursor can be used to mark points of interest on the curve, e.g., peak intensities, with concurrent display of the corresponding video frame image. Thus, all points on the curve can be correlated directly to high-resolution images. Specifically, the z-axis position of the epithelial surface, subepithelial nerves, anterior layer of stromal keratocytes, endothelium, and postoperative haze can be defined and marked on the curve. The focal plane position of each image also is displayed so that the thickness of structures of interest can be calculated.[11] From selected patients, z-series of CMTF images were transferred to a Silicon Graphics Indy Workstation (Mountain View, CA) for three-dimensional display. Using the ANALYZE image analysis software package (Mayo Medical Ventures, Rochester, MN), six-voxel-thick surface projections were generated in the "Volume Render" tool and the contrast was adjusted arbitrarily. It should be noted that because of the nonlinear relationship between lens position and focal plane position, the spacing between images in the CMTF z-series is not constant.[11] In addition, the voxels displayed by ANALYZE are not cubic. Thus, the simple three-dimensional reconstructions shown are somewhat distorted in the z-axis; however, it should be emphasized that correct equations were used for all quantitative calculations.

Statistics

Individual CMTF data given in Table 1 represent the average of repeated scans. Group data are given as mean ± standard deviation. In each patient, the coefficient of variation (standard deviation/mean) for repeated CMTF measurements was calculated at each visit. Statistical analyses were performed with the aid of SigmaStat for Windows Version 1.0 (Jandel Scientific, San Rafael, CA) testing normal distribution, Student's paired *t* test, Wilcoxon's signed rank test, Mann–Whitney rank sum test, Pearson product moment correlation, Spearman rank order correlation, and linear regression analysis (with y intercept set to 0).

Results

In Vivo Morphology

Epithelium. The surface of all 17 preoperative corneas was covered with a normal-appearing epithelium with individual superficial cells containing highly reflective central nuclei (Fig 1A). One month postsurgery, 16 patients had regained their normal epithelial morphology (Fig 1B); however, in 1 patient (no. 7) who experienced delayed epithelial healing, the ablated stroma was covered

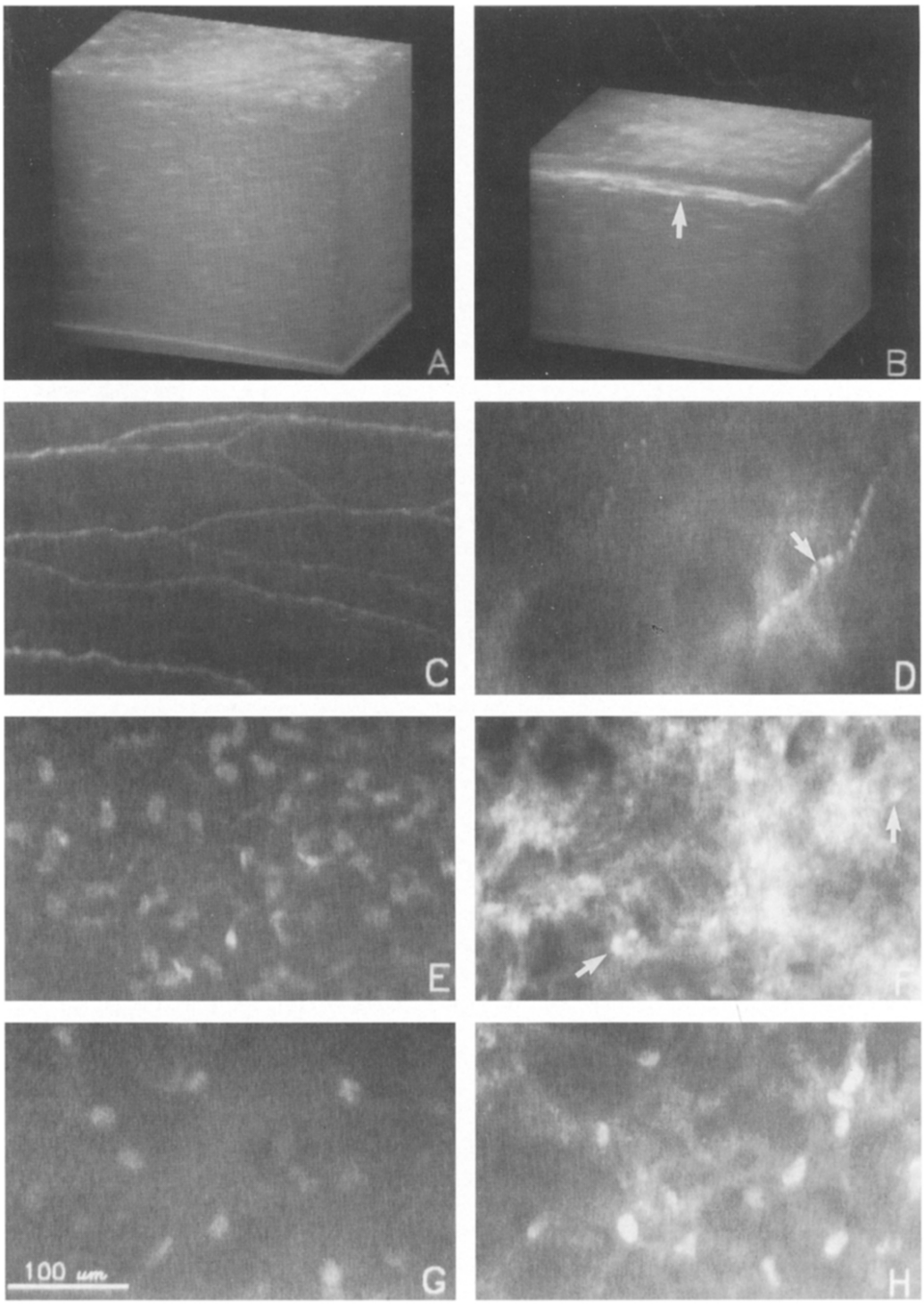

Figure 1. In vivo confocal microscopy of human corneas before and 1 month after photorefractive keratectomy (PRK). **(A)** three-dimensional reconstruction of a preoperative cornea (patient 16) with superficial epithelial cells on top and endothelium at the bottom. **(B)** three-dimensional reconstruction of the same cornea (patient 16) 1 month post-PRK with 168 μm corneal thinning and highly reflective subepithelial haze (arrow). **(C)** subepithelial nerve plexus with beaded nerve fibers in a preoperative cornea (patient 3). **(D)** subepithelial reinnervation of a beaded nerve fiber (arrow) 1 month after PRK (patient 7). **(E)** Preoperative most anterior stromal layer with a high keratocyte density and an irregular, elongated nuclear morphology (patient 16). **(F)** anterior layer of a postoperative stromal wound with highly reflective matrix material and partially visible keratocyte nuclei (arrows) surrounded by darker regions (patient 16). **(G)** preoperative corneal mid stroma with a low keratocyte density and a more rounded nuclear morphology (patient 16). **(H)** same region in the same cornea (patient 16) at the postoperative visit with highly reflective, rounded keratocyte nuclei and partially visible cell processes. Horizontal bar indicates 100 μm in images C–H.

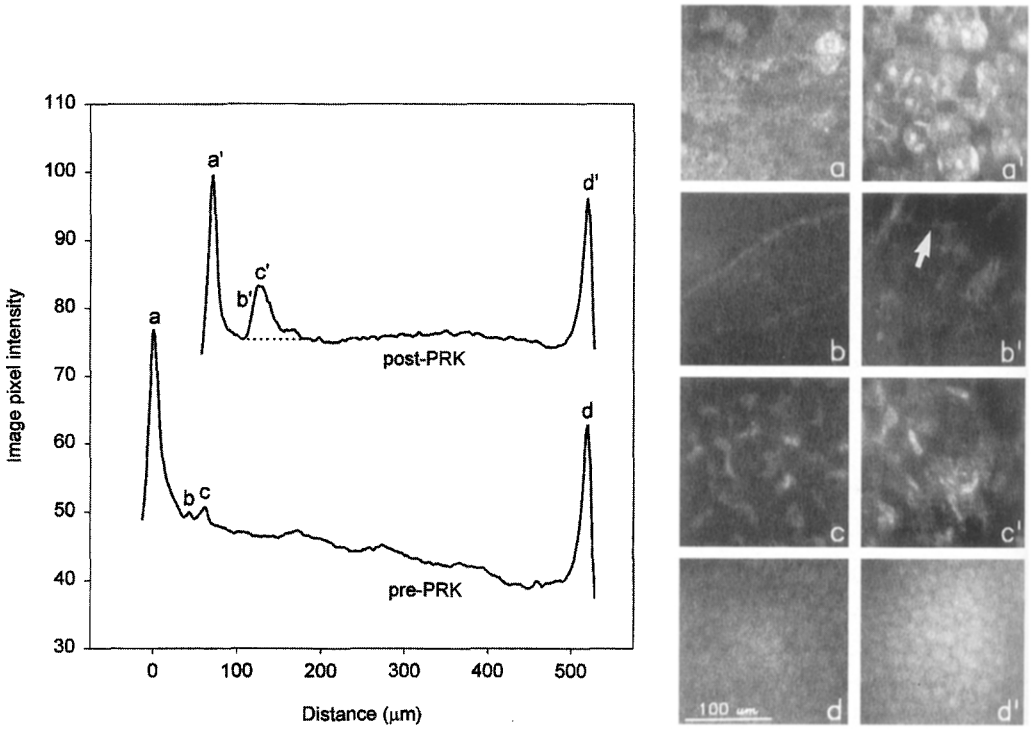

Figure 2. Corneal confocal microscopy through focusing (CMTF) scans of the same patient (no. 13) before and 1 month after PRK. At the postoperative visit, the cornea was graded clinically clear (grade 0 haze). Bottom: preoperative scan with four main peaks and corresponding images obtained by high-speed CMTF. Peaks and images correspond to superficial epithelium **(a)**, subepithelial nerve plexus **(b)**, anterior layer of keratocytes **(c)**, and endothelium **(d)**. Top: postoperative scan with three main peaks and corresponding images obtained by high-speed CMTF. Peaks and images correspond to superficial epithelium **(a′)**, subepithelial haze **(c′)**, and endothelium **(d′)**. The first in-focus image in the subepithelial haze region with a thin regenerating nerve fibre (arrow) represents the epithelial-stromal interface **(b′)**. The dotted line on *peak* **(c′)** indicates the haze peak baseline. For graphical purposes, the postoperative CMTF scan is offset arbitrarily by 35 units in the y-axis (image pixel intensity). The endothelial peaks are further aligned at the same depth in the x-axis to illustrate the postoperative decrease in corneal and stromal thickness. Horizontal bar indicates 100 μm.

by elongated surface epithelial cells, suggestive of migrating epithelium. No quantitative analysis of epithelial cell size or morphology was performed.

Subepithelial Nerves. Below the epithelium, a highly reflective nerve plexus was observed in all preoperative corneas, with the majority of the nerve fibers appearing beaded (Fig 1C). At the 1-month visit, 9 of 17 patients (53%) had detectable, delicate nerve fibers at the epithelial–stromal interface, indicating that reinnervation had started (Fig 1D).

Stroma. In preoperative corneas, the most anterior layer of stromal keratocytes had a high density with an elongated, irregular nuclear morphology (Fig 1E). In contrast, the anterior stroma in postoperative corneas showed the appearance of highly reflective matrix material overlying partially visible keratocyte nuclei (Fig 1F). Interspersed between reflective regions were darker areas, suggestive of normal, optically transparent matrix (Fig 1F). Keratocytes in the preoperative mid stroma had a low density and a rounded nuclear morphology (Fig 1G), whereas the same region at the postoperative visit showed highly reflective, condensed keratocyte nuclei with partially visible cell processes (Fig 1H).

Endothelium. No apparent changes in the normal endothelial cell density and morphology were observed beneath the ablated area at the 1-month visit; however, no quantitative evaluation was performed.

Confocal Microscopy Through Focusing.

Preoperative. In preoperative corneas, four main peaks were identified in the CMTF scans, corresponding to major reflections originating from, respectively, the superficial epithelial cells (*peak a*), subepithelial nerve plexus (*peak b*), anterior layer of keratocytes (*peak c*), and endothelium (*peak d*) (Fig 2). As previously described,[11] these four reflections can be used as valid structural landmarks for defining, respectively, the anterior corneal surface (*peak a*), the epithelial–stromal interface (*peak b*), the posterior surface of Bowman's layer (*peak c*), and the posterior corneal surface (*peak d*) (Fig 2). Thus, preoperative corneal thickness (C_{pre}), epithelial thickness (E_{pre}), and stromal thickness (S_{pre}) were calculated by the following equations:

$$C_{pre} = z_d - z_a$$
$$E_{pre} = z_b - z_a$$
$$S_{pre} = z_d - z_b$$

where z_a, z_b, and z_d were the peak focal plane positions in the z-axis of, respectively, the surface epithelium, subepithelial nerves, and endothelium.

Postoperative. One month after excimer laser photoablation, three main peaks were generated in the CMTF scans, corresponding to the superficial epithelial cells (*peak a′*), subepithelial haze (*peak c′*), and endothelium (*peak d′*) (Fig 2). In 9 of the 17 patients, the epithelial–stromal interface (*b′*) was defined by the regenerating subepithelial nerves detected at the 1 month visit (Figs 1D and 2). Although these regenerating nerves were too delicate to produce a distinct CMTF peak, their exact z-axis position could be defined on the CMTF curve using the previously described interactive computer program.[11] In the remaining eight patients without detectable subepithelial nerves, the epithelial–

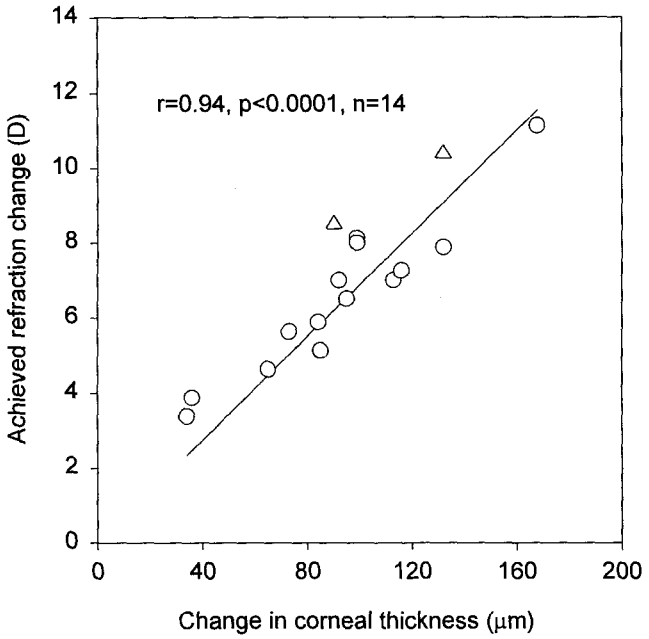

Figure 3. In photorefractive keratectomy (PRK) patients operated with a 6-mm diameter photoablation (○), the change in corneal thickness correlated significantly with the achieved change in spherical equivalent refraction (r = 0.94, $P < 0.0001$, n = 14); Linear regression analysis revealed a value of 14.3 μm corneal thinning per diopter (with y intercept set to 0). Two patients were operated with a 5-mm diameter zone (△).

stromal interface (b') was defined by the first in-focus CMTF image in the subepithelial haze region (Fig 2). Thus, postoperative corneal thickness (C_{post}), epithelial thickness (E_{post}), and stromal thickness (S_{post}) were calculated by the following equations:

$$C_{post} = z_{d'} - z_{a'}$$
$$E_{post} = z_{b'} - z_{a'}$$
$$S_{post} = z_{d'} - z_{b'}$$

where $z_{a'}$, $z_{b'}$, and $z_{d'}$ were the focal plane positions in the z-axis of, respectively, the superficial epithelial cells, regenerating subepithelial nerves (or first in-focus haze image), and corneal endothelium. Stromal thinning (S_{thin}) was calculated as the difference between stromal thickness at the pre- and postoperative visit:

$$S_{thin} = S_{pre} - S_{post}.$$

Corneal haze was quantified by calculating the area of the postoperative CMTF peak generated by the subepithelial haze (*peak c'* in Figure 2). The haze area estimate combines information on both haze thickness (peak width) and haze intensity (peak height). Initially, the haze peak baseline was defined by manually marking the start and end points (Fig 2). Then the area under the curve was integrated using a newly developed extension of the previously reported software program.[11] In preoperative corneas, the area of the anterior keratocyte peak (*peak c* in Fig 2) was recorded as a background value representing the normal, anterior stromal reflection. The amount of haze was expressed in CMTF haze units, which were arbitrarily defined as μm × pixel intensity. It is recognized that the peak intensity measured using CMTF can vary with changes in detector sensitivity, illumination intensity, microscope alignment, etc. Care was taken before each examination to ensure that all lens surfaces were clean and that the microscope was perfectly aligned. The light source also remained unchanged during this study. Furthermore, to maintain the detector at the same level of sensitivity, the video camera was checked to ensure that manual settings for black level, kilovolts, and gain remained constant for all scans. However, detector sensitivity and the illumination intensity still may degrade slightly over time because of use. Because patients were evaluated randomly during this study, degradation in either detector sensitivity or illumination intensity also would have a random effect. Therefore, we concluded that the variation in intensity between scans would be related primarily to the reflectivity of the object scanned.

Refractive Changes

Before surgery, all 17 patients had low to moderate degrees of myopia, with an average spherical equivalent of −5.96 ± 1.74 D (range, −2.88−−9.13 D; Table 1). One month after PRK, the average spherical equivalent was 1.38 ± 1.93 D (range, −0.38−7.13 D), with an initial overcorrection seen in 15 of the 17 patients (Table 1). The overcorrection was profound in two patients, 7.13 D (no. 17) and 4.63 D (no. 16), indicating poor predictability in these two patients at the 1 month visit.

Corneal Thickness

Total corneal thickness was determined with an average coefficient of variation of 1.2% for individual patient measure-

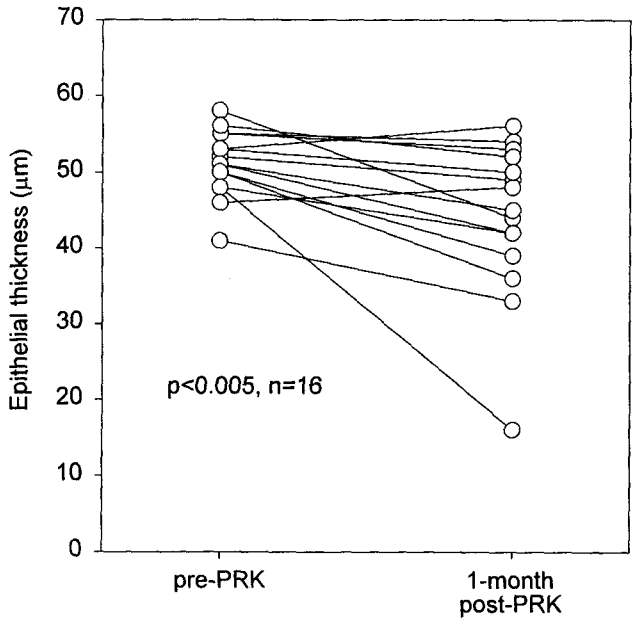

Figure 4. Epithelial thickness was estimated from confocal microscopy through focusing (CMTF) scans before and 1 month after photorefractive keratectomy and was significantly thinner at the postoperative visit ($P < 0.005$, n = 16).

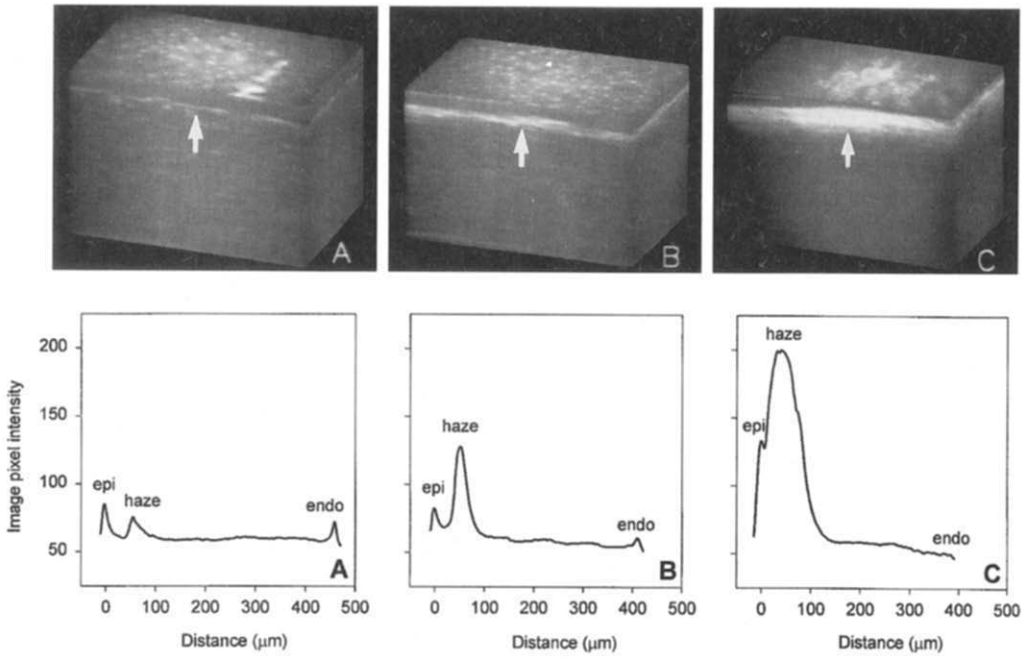

Figure 5. Three-dimensional reconstructions and corresponding confocal microscopy through focusing (CMTF) scans of three human corneas 1 month after photorefractive keratectomy. **(A)** cornea no. 9 was clinically clear (grade 0 haze). **(B)** cornea no. 16 had clinical grade 2 haze. **(C)** cornea no. 7 had clinical grade 4 haze. Note the increased subepithelial reflectivity in all three corneas (arrows). In the corresponding CMTF scans, a profound increase in both haze thickness (haze peak width) and haze intensity (haze peak height) is seen with increasing clinical grades (average CMTF haze estimates: **(A)** 544 units, **(B)** 1548 units, **(C)** 6021 units).

ments, indicating a high degree of repeatability. Preoperative corneal thickness had a mean of 560 ± 36 μm (range, 501–619 μm) whereas corneal thickness 1 month after surgery averaged 462 ± 52 μm (range, 369–553 μm; Table 1). In patients operated with a 6-mm diameter zone, a close correlation was found between the change in corneal thickness and the change in spherical equivalent refraction (Pearson product moment correlation, $r = 0.94$, $P < 0.0001$, n = 14) (Fig 3); linear regression analysis revealed an average decrease in corneal thickness of 14.3 μm per diopter of correction.

Epithelial Thickness

The average coefficient of variation for epithelial thickness measurements in individual patients was 6.6%, indicating high repeatability. In preoperative corneas, epithelial thickness varied from 41 to 58 μm, with an average of 51 ± 4 μm (Fig 4, Table 1). For the whole group, the epithelium was significantly thinner at 1 month, with a mean of 45 ± 10 μm (range, 16–56 μm; Student's paired *t* test, $P < 0.005$, n = 16; Fig 4, Table 1). Two of the 16 patients had a slightly thicker epithelium at 1 month, whereas 14 patients had a thinner epithelium, indicating that full recovery had not yet occurred. An extremely thin epithelium (16 μm) was observed in one patient (no. 7) who experienced delayed epithelial healing after PRK (Fig 4 and Table 1). Exclusion of this patient (no. 7) from the analysis did not reduce the statistical significance.

Corneal Haze

Clinical Grading. Before surgery, all 17 patients had normal and clear corneas. One month after surgery, eight patients (47%) had clinically significant, diffuse subepithelial haze, as detected by slit-lamp examination; the remaining nine patients had clinically clear corneas (Table 1). Seven of the eight patients with haze (88%) were graded 1 to 2, whereas one patient (no. 7) developed grade 4 haze, possibly because of noncompliance with the postoperative steroid medication regimen and delayed epithelial healing resulting from prolonged contact lens wear. Three-dimensional reconstructions of CMTF scans from corneas with grade 0, grade 2, and grade 4 haze revealed a profound increase in subepithelial haze thickness and intensity with increasing grades (Figs 5A–C).

Confocal Microscopy Through Focusing Haze Estimate. Using CMTF scans, postoperative corneal haze was quantified in individual patients with an average coefficient of variation of 35%, which is significantly higher than that for corneal and epithelial thickness measurements, suggesting actual, discrete regional variation in corneal haze thickness and intensity. One month after surgery, all 17 corneas had a significantly increased subepithelial reflectivity (mean 800 ± 1412 units, range, 28–6021 units) when compared with their preoperative, anterior stromal reflection (normal background value; mean, 24 ± 3 units, range, 20–30 units; Wilcoxon signed rank test, $P < 0.0001$; Fig 6). In the eight patients with clinical, slit-lamp detectable haze, the CMTF haze estimate averaged 1431 ± 1911 units (range, 51–6021 units), whereas in the nine patients with clinically clear corneas, subclinical haze was detected by CMTF (239 ± 199 units; range, 28–562 units; Fig 6, Table 1). Clinically clear and hazy corneas had significantly different CMTF haze estimates (all 17 patients: Mann–Whitney rank sum test, $P < 0.02$; without the patient with grade 4 haze: Mann–Whitney rank sum test, $P < 0.03$, n = 16; Fig 6). Moreover, a significant correlation was found between the postoperative CMTF haze estimate and the clinical haze grading (all 17 patients: Spearman rank order correlation, $r = 0.73$, $P < 0.001$; without the patient with grade 4 haze: Spearman rank order correlation, $r = 0.67$, $P < 0.005$, n = 16; Fig 6).

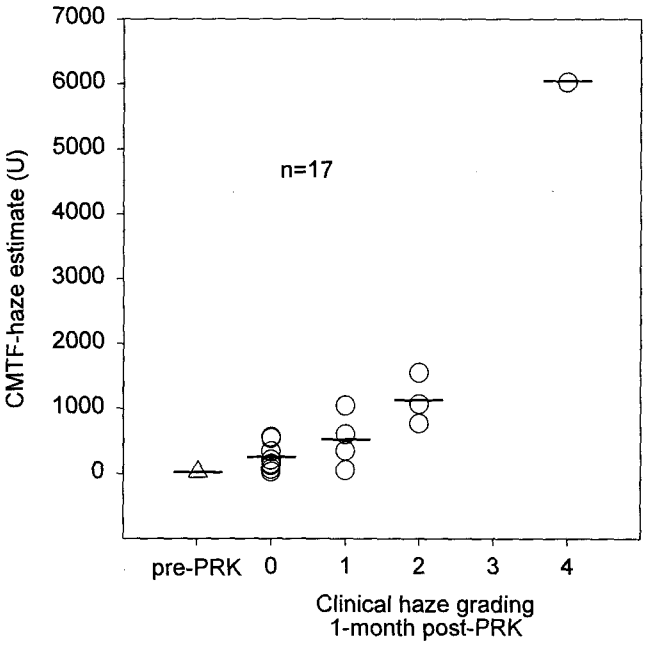

Figure 6. Corneal clarity assessment in 17 patients before and 1 month after photorefractive keratectomy. Using postoperative confocal microscopy through focusing (CMTF) scanning, all 17 corneas had a significantly increased CMTF haze estimate (○) when compared with their preoperative, anterior stromal reflection (normal background value) (△) ($P < 0.0001$). Moreover, a significant correlation was found between the postoperative CMTF haze estimate and the clinical haze grading (all 17 patients: $r = 0.73$, $P < 0.001$; without the patient with grade 4 haze: $r = 0.67$, $P < 0.005$, n = 16). Horizontal bars indicate group means.

Stromal Thinning

The achieved stromal thinning at the 1-month visit averaged 87 ± 34 μm for all patients, with a range of 20 to 154 μm. These data represent a direct measure of the actual stromal photoablation depth at the time of surgery, modified by any increase in postoperative stromal thickness caused by wound healing and tissue edema. No apparent association was found between stromal thinning at 1 month and either the CMTF haze estimate or the clinical haze grading, indicating that deeper photoablations in the present study did not produce greater corneal opacification 1 month postsurgery.

Discussion

The present study demonstrates for the first time that in vivo CMTF is capable of objectively and noninvasively measuring epithelial and stromal thickness, photoablation depth (stromal thinning), and postoperative corneal haze in patients undergoing excimer laser PRK for myopia. We previously evaluated the CTMF methodology and specifically showed that confocal corneal thickness measurements correlate closely with ultrasound pachymetry ($r = 0.96–0.995$), indicating high accuracy.[11,13] In the present study of PRK patients, the microscope objective was aligned carefully to the visual axis by a special fixation target to facilitate measurements in the deepest part of the photoablation profile. The fixation target also helped patients keep their eyes steady during examination, although 15% of the scans had to be deleted because of significant eye movements. By averaging the quantitative data obtained from three to seven complete CMTF scans per visit, total corneal and sublayer thickness measurements were obtained with high precision. To demonstrate corneal thinning and subepithelial haze after PRK, three-dimensional reconstructions of pre- and postoperative CMTF scans were performed. To the best of our knowledge, this is the first presentation of three-dimensional reconstructions of normal and photoablated human corneas in vivo.

During PRK surgery, a volume of the corneal stroma is removed analogous to a biological contact lens with a central thickness corresponding to the maximum ablation depth. The amount of central corneal thinning determined in this study was found to be directly proportional with the achieved refractive change with a relationship of 14.3 μm corneal thinning per diopter (for a 6-mm diameter photoablation). This finding is in reasonable agreement with previous theoretical calculations of 13 μm/D.[15,16] The present experimental in vivo data thereby validates the fundamental concept of PRK that graded amounts of anterior stromal thinning can induce predictable changes in refractive power. Although the achieved corneal thinning is correlated highly with the change in corneal refraction, considerable variation has been shown for predicted versus achieved change in refraction. As suggested in our study, there is a substantial range of residual refractive error (−0.38–7.13 D) at 1 month after PRK. Other more extensive studies also have shown considerable variation in achieved results over a range of attempted corrections.[1–5] The underlying cause of this variation is unclear but may be related to individual differences in corneal photoablation rate (tissue removed per laser pulse) and epithelial–stromal wound healing response (addition of new tissue).

In the present study, the epithelium was significantly thinner at 1 month post-PRK, indicating that full recovery had not yet occurred at this time point. Further temporal CMTF examinations of these patients may allow us to monitor whether epithelial thickening after PRK continues beyond the normal, preoperative thickness, leading to epithelial hyperplasia, as previously indicated.[17,18] The stromal wound healing response after PRK involves keratocyte proliferation and activation leading to degradation, removal, and repair of extracellular matrix components.[6] Overall, stromal wound healing and remodelling gradually lead to stromal thickening (addition of new tissue)[19] which, together with epithelial hyperplasia, currently is believed to be the main factor responsible for the refractive regression (loss of initially achieved refractive effect) after PRK.[5,6,16,18–20] This hypothesis has not been established definitively in man, although our data indicate that a 14.3-μm tissue addition (epithelial or stromal thickening) may lead to 1 D of regression. Direct CMTF measurements of epithelial and stromal thickness in individual PRK patients over time may allow us to differentiate between epithelial hyperplasia and stromal thickening in vivo and thereby evaluate the importance of each factor in the loss of refractive effect.

In the present study, 8 of 17 corneas had clinically significant, slit-lamp detectable haze (grades 1–4), whereas the

remaining nine corneas were graded clear (grade 0 haze) at the 1-month visit. A significant correlation was found between the clinical haze grading and the objective CMTF haze estimate using the calculated area under the haze peak intensity curve. In contrast to the slit-lamp examination, however, CMTF haze assessment detected an abnormal subepithelial haze peak in all 17 patients. Furthermore, the detection of abnormal CMTF haze values in patients assessed clinically as grade 0, albeit lower than observed for clinically significant cases, suggests that CMTF haze measurements may be more sensitive in detecting haze than slit-lamp examination. Although the haze peak intensity profile may be influenced by many factors, e.g., illumination, detector, alignment, the reproducible and consistent intensities measured in normal individuals, averaging 24 ± 3 units over a 2-month period, suggest that when extraneous factors are controlled, CMTF haze estimates provide an objective, quantitative measurement of postoperative PRK haze. Only a few objective clinical haze assessment techniques have so far been reported—high-frequency ultrasound,[21] Scheimpflug photography,[22] and light scattering measurements.[23–25] However, none of these methods are widely available in the clinic, and corneal haze assessment post-PRK still is dependent primarily on subjective examination.

In summary, CMTF is a new, powerful tool for noninvasive, unbiased evaluation of the effects of excimer laser PRK on individual human corneas over time. Confocal microscopy through focusing offers multiple in vivo features, such as (1) epithelial and stromal thickness analysis, (2) assessment of photoablation depth (stromal thinning), (3) haze thickness and intensity analysis, (4) location and depth of intrastromal opacities, and (5) visualization of cellular morphology and documentation of histopathologic changes within the corneal sublayers. By these features, CMTF offers valuable information that enables a careful preoperative assessment and a differentiated evaluation of the postoperative course of individual PRK patients. Thus, CMTF may form a unique basis for careful titration of steroids and other drugs to modulate subepithelial haze, refractive regression, and final refractive outcome after PRK procedures.

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