Identical Excimer Laser PTK Treatments in Rabbits Result in Two Distinct Haze Responses

Russell L. McCally,[1,2] *Patrick J. Connolly,*[2,3] *Walter J. Stark,*[2] *Sandeep Jain,*[2,4] *and Dimitri T. Azar*[2,4]

Purpose. To obtain objective light-scattering measurements to test a hypothesis that identical PTK treatments cause distinct low- and high-level light-scattering responses in rabbit corneas.

Methods. An excimer laser was used to produce identical 6-mm diameter phototherapeutic keratectomy treatments (PTK) in 32 pigmented rabbits. Eyes were treated by performing a 40-μm epithelial ablation, followed by a 100-μm stromal PTK. Objective scattering measurements were made before treatment, weekly up to 5 weeks, and then biweekly to 9 weeks. Confocal microscopy was performed on several corneas at 4 and 7 weeks.

Results. Mean scattering levels split into distinct low- and high-scattering groups 2 weeks after treatment and remained distinct until week 7 ($P < 0.003$). Scattering in the low group reached a broad peak that lasted from weeks 2 to 4 at approximately 3 times the pretreatment level. Scattering in the high group peaked at 3 weeks at approximately 12 times the pretreatment level. Scattering levels diminished after reaching their peaks. Confocal images showed a band of highly reflective material in the anterior stroma that extended much deeper in corneas from the high group. The reflective band in the highly scattering corneas obscured the posterior stroma from view for up to 5 weeks.

Conclusions. Q*uantitative* scattering data obtained with the scatterometer suggest that identical PTK treatments indeed result in *distinct* low- and high-level light-scattering responses in rabbits. (*Invest Ophthalmol Vis Sci.* 2006;47:4288–4294) DOI:10.1167/iovs.05-1469

Argon fluoride excimer lasers operating at a wavelength of 193 nm are being used extensively in the United States and throughout the world for both photorefractive (PRK) and phototherapeutic (PTK) keratectomies. In contrast with laser in situ keratomileusis (LASIK), PRK does not appreciably weaken the cornea, and it is less invasive.[1,2] Nevertheless, PRK treatments frequently result in the development of increased subepithelial light scattering that gives the cornea a hazy appearance in the treated area during the first months after surgery.[3] In standard clinical practice, haze is graded subjectively via slit lamp examination by an experienced observer.[4,5] Based on this type of subjective grading, several investigators have suggested that there may be different healing responses. In a multicenter study of PRK treatments for high myopia, it was noted that mild subepithelial haze developed in all patients, but two patients experienced more significant haze.[6] Durrie et al.[7] have suggested that patients with PRK can be divided into three groups: normal responders, who have an initial hyperopic overcorrection that regresses to plano by 6 months; inadequate responders, whose initial hyperopic overcorrection does not regress adequately; and aggressive responders, whose early hyperopic overcorrection rapidly regresses to myopia.[7] The normal and inadequate responders had clear corneas or exhibited trace haze at 6 months, whereas the aggressive responders had more pronounced haze at 6 months. However, detailed studies of the possibility that different wound-healing responses are accompanied by different degrees of haze have been hampered by the use of subjective grading procedures. Comparisons of haze severity between different subjects or even between different times for the same subject are difficult with such methods. Moreover, patients in clinical studies have necessarily received different treatments, and it is known from objective haze measurements that deeper treatments result in greater haze levels.[8,9]

We have devised a standardized PTK treatment procedure in rabbits, to evaluate the development of haze in a controlled manner. This treatment model is similar to one subsequently used by Faktorovich et al.[10] We have made objective measurements of backscattered light with an instrument called a scatterometer, to determine the time course of haze development after *identical* PTK treatments.[11,12] The scatterometer is capable of making reproducible, objective measurements of corneal scattering from normal and excimer-treated eyes (McCally RL et al. *IOVS* 1993;34:ARVO Abstract 802)[13] and is different in concept from other instruments for measuring scattering that have been described.[14–18] Preliminary objective measurements of haze on a small number of rabbit eyes ($n = 8$) after identical PTK treatments have suggested the possibility that there are two distinct responses in which some eyes develop similar, relatively low levels of haze, whereas others develop much higher levels (McCally RL et al. *IOVS* 1994;35:ARVO Abstract 1299). In the present study, we used the scatterometer to investigate a larger number of eyes to test this hypothesis. We demonstrate objectively, for the first time, that there are indeed two distinct haze responses after identical PTK treatments on rabbits.

From the [1]The Johns Hopkins University Applied Physics Laboratory, Laurel, Maryland; and [2]The Wilmer Eye Institute, The Johns Hopkins Medical Institutions, Baltimore, Maryland.

Present affiliation: [3]Indianapolis Neurosurgical Group, Indianapolis, Indiana; and the [4]Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, Chicago, Illinois.

Presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Fort Lauderdale, Florida, May 1994 and 1995.

Supported in part by National Eye Institute Grants EY01019, EY12165 (RLM), and EY10101 (DTA), by a CERT grant from Chesapeake Physicians Professional Association, Baltimore, MD (DTA), and by an unrestricted grant from Research to Prevent Blindness, Inc. (DTA).

Submitted for publication November 16, 2005; revised April 17 and June 16, 2006; accepted August 18, 2006.

Disclosure: **R.L. McCally**, None; **P.J. Connolly**, None; **W.J. Stark**, None; **S. Jain**, None; **D.T. Azar**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Russell L. McCally, The Johns Hopkins University Applied Physics Laboratory, 11000 Johns Hopkins Road, Laurel, MD 20723-6099; russell.mccally@jhuapl.edu.

Materials and Methods

Animals

In conducting the experiments, we adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The pigmented rabbits used in the study were all 4 to 5 pounds and were presumed to be approximately the same age. Both eyes were treated, with a minimum interval of 1 week between treatments. Before ablation the rabbits were anesthetized with an intramuscular injection of xylazine and ketamine hydrochloride in the proportions: 60% of 20 mg/mL xylazine to 40% of 100 mg/mL ketamine by volume. In addition, a topical anesthetic (0.05% proparacaine hydrochloride) was applied to each eye approximately 10 minutes before exposure.

Ablations were performed with an excimer laser (Twenty/Twenty laser system; (VisX Corp. Santa Clara, CA), using a fluence of 160 ± 10 mJ/cm^2 and a pulse repetition frequency of 5 Hz. The laser was calibrated before each treatment session by ablating a Lucite sheet according to the manufacturer's instructions. Lids were retracted with a speculum, and the epithelium was removed over a 6-mm diameter area of the central cornea by ablating to a depth of 40 μm. The exposed stroma was then irrigated with a sterile saline solution and dried with a sterile sponge. For the stromal ablations, the diameter of the treatment zone was set at 6.0 mm. PTK ablations were performed to a stromal depth of 100 μm (total depth, 140 μm). All treatments were performed without nitrogen gas blowing over the surface. After treatment, the corneas were flushed with the saline solution, and erythromycin ophthalmic ointment (U.S.P., 5 mg/g) and 1% atropine were applied.

The rabbits were treated in four groups. In the first group (six rabbits), the right eyes were exposed in a single treatment session, and the left eyes were exposed 1 week later. The second group (six rabbits) was treated similarly, except that the left eyes were treated 2 weeks after the right eyes. The rabbits in these two groups were part of a study of the effect of mitomycin C on haze development (Connolly PJ et al. *IOVS* 1994;35:ARVO Abstract 2015)[12] The eight eyes used in the present study were the control and received no drug treatment. As noted previously, it was the results from these eight eyes that suggested the possibility that there are two distinct haze responses. Two additional groups were then treated to test this hypothesis. In the third group (nine rabbits), the left eyes were treated 4 weeks after the right eyes, and in the fourth group (four rabbits), the left eyes were treated 1 week after the right eyes. One rabbit in the third group died 1 week after the first (right eye) treatment and was excluded from the study. A second rabbit in the third group developed a severe infection in its left eye in the week after its treatment and had to be killed. Thus, only the data from the right eye of this rabbit through week 4 were available for analysis. In summary, data from 31 eyes were available for the study.

Scatterometry, as described later in the text, was performed on all eyes immediately before laser treatment, at weekly intervals up to 5 weeks, and then biweekly up to 9 weeks. In addition, two rabbits were examined at various intervals up to 23 weeks. Before the scatterometer measurements, the rabbits were anesthetized as described earlier, their pupils were dilated with tropicamide, and their lids were retracted with a speculum. The eyes were irrigated with a sterile saline solution periodically during the measurement session. Some rabbits were killed at intervals throughout the study and their eyes enucleated for examination with the laser scanning confocal microscope as described later. Rabbits were killed while under anesthesia by an overdose of pentobarbital sodium administered in a marginal ear vein.

Scatterometer

The scatterometer, which consists of an appropriately modified slit-lamp microscope (Nikon, Tokyo, Japan), has been described previously (McCally RL et al. *IOVS* 1993;34:ARVO Abstract 802).[8,11–13] Figure 1 shows its salient features.

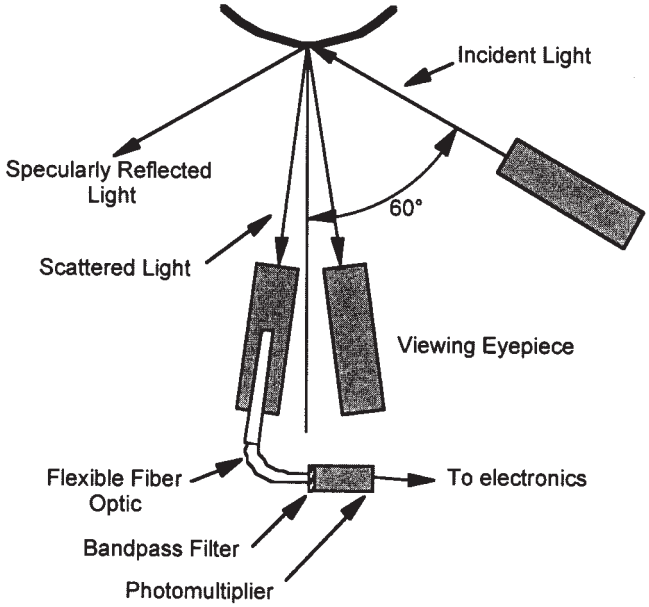

Figure 1. Schematic diagram of the scatterometer. The fiber optic is located at the image plane of the slit lamp objective and therefore acts as a field stop, defining the 1.7-mm diameter region of the cornea from which scattered light is detected. The slit is adjusted so that its width is 2.2 mm. A 550-nm, 50-nm bandwidth interference filter was used for wavelength selection. The optical arrangement assures that specularly reflected light is not detected.

All corneal scattering measurements are referenced to the scattering measured from a standard block (Spectralon; Labsphere, North Sutton, NH) with the same illumination conditions. The standard is an extremely stable, reproducible, and diffuse spectral reflectance standard. It is a nearly perfect lambertian reflector, with a reflectance of >99% over the visible spectrum. Any variations in lamp intensity, photomultiplier gain, or amplifier output are removed by taking the ratio of corneal scattering to the scattering of the standard. Herein, we report relative scattering (RS) defined as the ratio of the scattering measured from each cornea at each session (relative to the standard) to its pretreatment level (relative to the standard). Thus, the data represent the relative change in scattering caused by the laser treatment. This method of analysis has the advantage that each cornea serves as its own control.

Confocal Microscopy

Freshly enucleated eyes were mounted and immersed in saline solution (BSS; Alcon Surgical) as described by Masters[19] for examination with a laser scanning confocal microscope (LSM-10; Carl Zeiss Meditec, Inc., Dublin, CA). The microscope was fitted with a 40×, 0.75 numerical aperture, water-immersion objective (Carl Zeiss Meditec, Inc.). The internal argon ion laser (488 nm) was used to illuminate the cornea. The microscope was operated in the *z*-scanning mode in which single 512-pixel line scans were made at 1-μm intervals throughout the depth of the cornea, thus yielding a cross-sectional view of the cornea. Dimensions of corneal features were obtained with software utilities provided with the microscope's computer operating system.

Results

All eyes developed haze and, by slit lamp observation, all eyes had re-epithelialized at the time of the first scatterometer measurement 1 week after treatment. The rate of re-epithelialization was not measured, as it would have required staining that could interfere with the scattering measurements. The RS from the eight eyes in first two treatment groups that suggested that

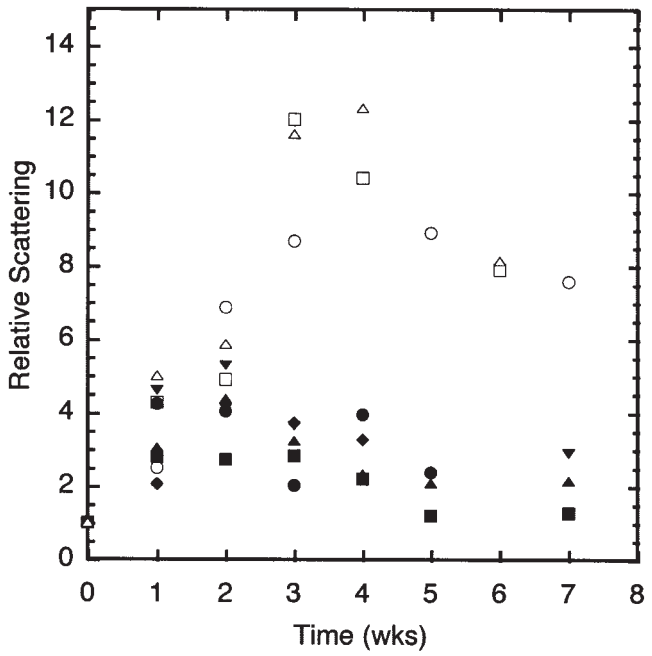

FIGURE 2. Relative scattering measured from the center of the treated area at various times after identical PTK treatments. The points are the scattering measurements from individual eyes of the eight rabbits in the first two treatment groups, normalized by the baseline measurements made before treatment in the same eyes. In this method of plotting, each rabbit eye serves as its own control. It was these data that suggested the hypothesis that identical treatments cause distinct low- and high-level light-scattering responses in rabbit corneas.

there were possibly two *distinct* haze responses are plotted in Figure 2. In these corneas, there was some overlap in RS up to 2 weeks after treatment. At later times, RS appeared to separate into two distinct groups. Five corneas (Fig. 2, solid symbols) all had similar development of haze and achieved peak scattering of ~2.5 to ~5 times greater than the scattering before treatment. The remaining three corneas (Fig. 2, open symbols) also developed haze over a similar time course, except that they achieved higher RS, ranging from ~9 to ~12 times greater than their pretreatment levels.

To test the hypothesis that there were two distinct haze responses, we treated the right and left eyes of two additional groups of rabbits, as described in the Methods section. These additional data were combined with the data from the eight corneas shown in Figure 2. Based on the data in Figure 2, which show a clear separation into high and low groups at 3 weeks, the combined 3-week data were plotted as a histogram to examine if a distinct separation occurs in the larger data set. The histogram in Figure 3 shows a clear separation, with 20 eyes having RS < 6 (mean, 3.39) and 9 eyes having RS > 8 (mean, 12.0). The eyes with RS < 6 at 3 weeks were then assigned to the low-scattering group at all time points and those with RS > 8 were assigned to the high-scattering group. The means, standard deviations, number of corneas, and probabilities for all the time points are listed in Table 1, and the mean RS values are plotted in Figure 4. Corneas in the high-scattering group reached their peak scattering intensity 3 weeks after surgery. Corneas in the low-scattering group reached the peak scattering 2 to 3 weeks after surgery. The peak in their scattering levels was much broader and flatter than that in the high-scattering group. At 9 weeks, there were too few corneas remaining to obtain meaningful statistics; however, the trend of splitting into a low and high-scattering group was still evident. The slight apparent increase in mean scattering in the low group at 9 weeks probably is a result of the very small remaining sample size ($n = 2$). Because there was no certainty that the data were distributed normally, the probabilities in Table 1 were calculated with the Wilcoxon-Mann-Whitney test (KaleidaGraph software; Synergy Software, Reading, PA). At 1 week, the mean responses in the two groups were statistically indistinguishable; however, from weeks 2 through 7, the difference in scattering between the two groups was significant ($P \leq 0.005$; cf., Table 1).

Confocal microscope images were obtained from some of the corneas immediately after they were killed. The confocal microscope is particularly valuable for viewing the cellular response in the treated corneas and for determining the locations in the cornea that show increases in reflectivity.[18,20–23] However, it is important to note that confocal microscopes detect and record specularly reflected light, not scattered light, which is measured by the scatterometer. Thus, although quantitative measures are possible with the confocal microscope,[21] the measured quantity differs fundamentally from that measured by the scatterometer. No attempt was made to quantify reflectivity in the confocal images used in this study. Figure 5a is a *z*-scan cross-sectional image of a cornea from the low-scattering group 4 weeks after treatment. It shows a band of reflective material beneath the epithelium as well as some reflective material within the epithelium. The stroma beneath the reflective band is similar in appearance to untreated corneas (not shown). Figure 5b is a cross-sectional image from a cornea in the high-scattering group 4 weeks after treatment that shows a broader band of very highly reflective material beneath the epithelium (the gain was lower for this image than for the image in Fig. 5a). There also was some reflective material within the epithelium. The basement membrane was uneven, and the thickness of the epithelium was variable. The band of highly reflective material obscured the posterior stroma. Obscuration of the posterior stroma by the subepithelial reflective band was typical for the corneas in the high-scattering group 4 and 5 weeks after treatment and was a limitation of confocal microscopy in relatively opaque corneas. Figure 5c is from a cornea in the low-scattering group 7 weeks after treatment. The band of reflective material had largely resolved in this particular cornea and normal-appearing keratocytes populated the entire depth of the stroma. Other corneas in the low-scattering group had reflective bands at 7 weeks and even at later times; indeed, one rabbit from the low-scattering

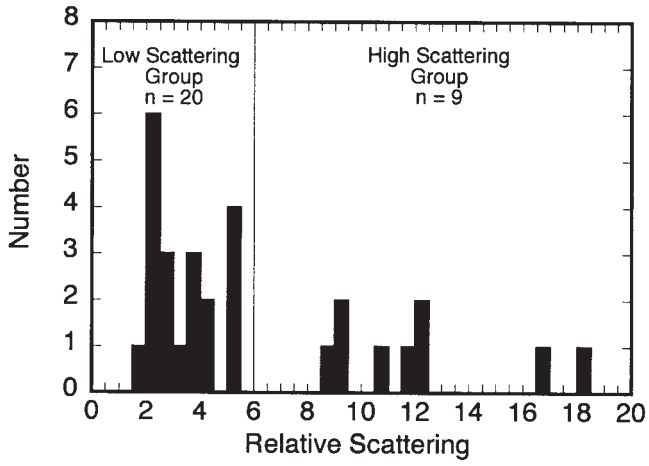

FIGURE 3. A histogram of the numbers of corneas as a function of relative scattering at 3 weeks after the identical PTK treatments. The histogram clearly shows that the data separate into distinct low- and high-scattering groups, with the high-scattering group showing greater variability.

TABLE 1. Mean Relative Light-Scattering Values, Standard Deviations, Number of Corneas, and Probabilities for the Low- and High-Scattering Groups

Time (wk)	Low Group			High Group			
	Mean	SD	*n*	Mean	SD	*n*	*P*
1	3.00	1.27	21	3.46	0.86	10	0.17
2	3.32	1.04	21	6.93	3.38	10	0.0004
3	3.39	1.19	20	12.03	3.29	9	<0.0001
4	3.08	1.30	17	11.04	3.70	8	<0.0001
5	2.76	1.12	13	9.93	6.02	6	<0.0001
7	2.43	1.00	8	8.42	5.19	5	0.0031
9	3.85	2.17	3	6.70	3.09	2	0.40

group was followed up for 23 weeks, and the subepithelial reflective band still had not resolved (not shown). Figure 5d is from a cornea in the high-scattering group 7 weeks after treatment. The bright-scattering band persisted, but it did not obscure the posterior stroma from view as it did in corneas in the high-scattering group 4 and 5 weeks after treatment.

DISCUSSION

We made objective measurements of haze as it developed after identical PTK treatments in rabbits. We found that 2 weeks after surgery the relative scattering intensities split into two distinct groups. These two groups, a low-scattering group consisting of about two thirds of the treated corneas, and a high-scattering group, remained distinct from each another up to at least 7 weeks after treatment. Haze in the high-scattering group peaked 3 weeks after treatment and then diminished, whereas haze in the low-scattering group had a blunted peak that lasted 2 to 4 weeks after treatment before diminishing. All the corneas within the low-scattering group responded in a similar manner, both in the time course of their haze development and in the levels of scattering attained relative to their pretreatment levels. All corneas in the high-scattering group also had a similar time course of haze development; however, they exhibited more variability in their relative scattering intensities.

Scattering induced by PTK or PRK reaches its maximum level much sooner after treatment in rabbits than it does in humans, where maximum haze levels occur 2 to 6 months after treatment.[3,15,16,22] Other potentially important differences between rabbit and human corneas are: rabbit cornea lacks a Bowman's layer[24,25]; the lamellae in the anterior human cornea interweave,[26–28] whereas those in rabbit do not[29,30]; the den-

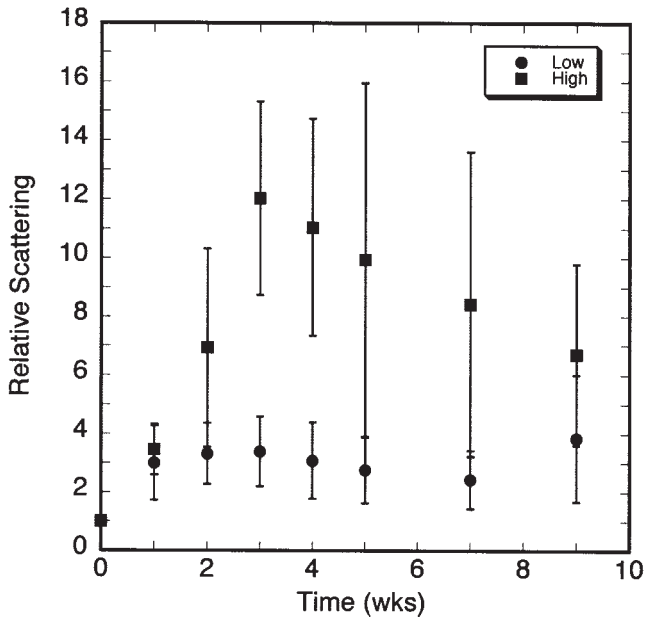

FIGURE 4. Mean relative scattering levels and standard deviations at various times after identical PTK treatments. After 2 weeks, the mean scattering levels split into distinct low and high-scattering groups. The groups remain statistically distinct up to 7 weeks ($P < 0.005$; cf., Table 1).

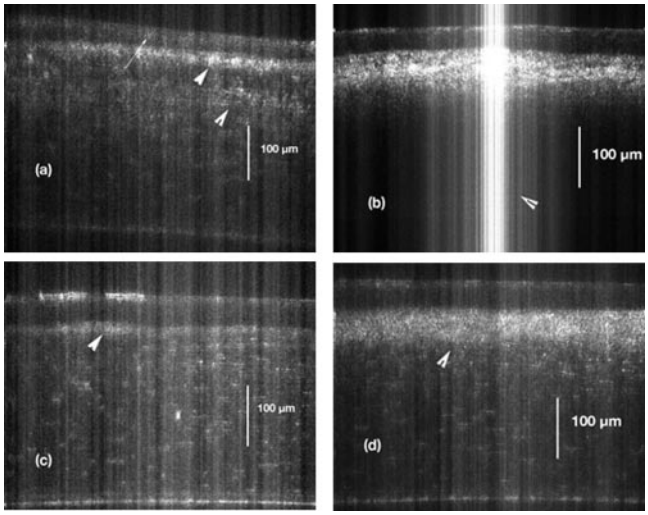

FIGURE 5. Laser scanning confocal microscope *z*-scan images of corneas at different times after identical PTK treatments. The vertical lines in the images, which are very bright in (**b**), were caused by a reflection artifact in the microscope optics. The extremely high reflectivity of this particular cornea exacerbated the artifact. Relative intensities should not be compared between the different images. (**a**) Cornea from the low-scattering group 4 weeks after treatment. Relative scattering for this cornea was 2.6. *Small arrow*: scattering centers inside the epithelium. The epithelial thickness was 40 μm. There was a narrow reflective band beneath the epithelium and a more diffuse band that extended nearly halfway through the cornea (*large arrows*). (**b**) Cornea from the high-scattering group 4 weeks after treatment. Relative scattering for this cornea was 13.8. The basement membrane was irregular, and the epithelial thickness varied between 34 and 43 μm. The wide reflective band beneath the epithelium obscured the underlying stroma and endothelium from view. (**c**) Cornea from the low-scattering group 7 weeks after treatment. Relative scattering for this cornea was 1.4. The epithelial thickness averaged ~46 μm. The very narrow subepithelial reflective band (*large arrow*) has nearly resolved in this particular cornea. (**d**) Cornea from the high-scattering group 7 weeks after treatment. Relative scattering for this cornea was 4.2. The epithelial thickness averaged ~46 μm. A broad band of reflectivity persisted in the anterior stroma, but it did not obscure the underlying stroma and endothelium.

sity of the stromal neural plexus is greater in rabbits[31]; and, rabbit and human keratocytes have some differences in the levels of expression of certain water-soluble proteins.[32] Because of these differences one should exercise caution in extrapolating the results of this study on rabbits to humans.

Based on subjective evaluations of haze intensity, several groups have noted different haze levels after PRK.[6,7] Part of this variability may be due to the patients' having received different treatments. Indeed, it has been established that deeper treatments result in higher objective haze levels.[8,22] Therefore, because the patients in these studies had received various treatments, it would not have been possible to determine whether identically treated corneas would exhibit the type of distinct wound-healing responses or haze levels observed in this study.

The underlying mechanism for two distinct responses to identical PTK treatments is not yet understood. However several factors, including rate of re- epithelialization,[33–35] behavior of the plasminogen-activator–plasmin system (O'Brart DPS et al. *IOVS* 1994;35:ARVO Abstract 1723),[36–39] variable levels of collagen IV after surgery,[40] keratocyte apoptosis,[41–44] and the relationship between transforming growth factor (TGF)-β and myofibroblast transformation[10,42,45–47] may play roles, either individually or collectively. Individual variations in healing may also lead to a degree of unpredictability in the outcomes.[21]

The low or high haze response did not appear to be specific to a particular rabbit. Of the 12 rabbits in groups 3 and 4 whose right and left eyes were treated in separate sessions, 6 had opposite responses in the pair eye. This observation also tends to exclude age as a factor and, as noted previously, all the rabbits in the study were assumed to be close in age. We also investigated whether the high or low response was associated with the pretreatment scattering level, but no significant correlation was found.

In this study, left eyes were treated in intervals of 1 to 4 weeks after the right eyes It has been reported that in eyes that are subjected to either scrape injuries[48] or PRK[49] 1 week apart, the epithelium of the second eye heals faster than that in the first eye. However we found that the low or high haze response did not depend on any particular treatment session. Each treatment session except one yielded eyes with both high- and low-scattering responses. All the eyes treated in that particular session (which were the left eyes in group 2) developed low scattering levels; however, these eyes were treated 2 weeks after the right eyes. Rask et al.[49] found that the epithelial healing rate was not significantly different in eyes with a two or more week interval between treatments.

Although investigating the ultrastructural basis for haze was not a direct part of this study, several comments are warranted. Several investigators have reviewed potential contributing factors to haze.[22,50–52] Among the potential histopathological features are: increased numbers of keratocytes,[53–56] vacuoles within and around keratocytes,[55,56] myofibroblast generation,[42,45] discontinuous and convoluted basement membrane structures,[56–58] and disorganized fibrillar and lamellar structures.[55,56,58–60] Understanding the cause(s) of increased scattering requires an understanding of the structural factors that underlie the transparency of normal cornea. In the normal rabbit cornea, the stroma accounts for approximately 80% of the small amount of green light scattered at 120°. The other 20% is from the surface of the epithelium and from the endothelium.[61] In the normal cornea, under *nonspecular* illumination conditions, the matrix of collagen fibrils is the primary source of the small amount of stromal scattering that is observed.[50,62,63] Under specular scattering conditions the keratocytes, which normally lie rather flat between the lamellae, act like small mirrors and become the predominate source of scattering.[50,62–64] Transparency of the normal cornea results from three factors: the cornea is thin; the individual collagen fibrils are weak scatterers; and interference among the waves scattered from different parallel fibrils reduces the scattering by about one order of magnitude from that which would occur if the fibrils scattered independently of one another.[50,65–68] Inspection of micrographs[55,56,58] and confocal images[23,45] of excimer-treated corneas indicates that activated keratocytes (or myofibroblasts) have a different morphology than those in normal cornea and they are frequently tilted at a variety of angles, possibly as a result of the disrupted lamellar structure. This would have the effect of creating a variety of specular angles, one for each different tilt angle, thus possibly increasing the cellular contribution to scattering. In addition, there is evidence that the refractive index of activated keratocytes (or myofibroblasts) is different from that of normal keratocytes, which could alter their contribution to scattering.[69] Vacuoles within and around keratocytes may act like the voids or "lakes" that are observed in the fibril distribution of highly scattering, edematous corneas. Such voids introduce spatial fluctuations in the index of refraction and therefore could lead to increased light scattering.[66,67,70] Indeed, it has been demonstrated that similar voids in the fibril distribution are responsible for the increased scattering in cold-swollen rabbit corneas.[66,67,70] Light scattered from different fibrils in the disorganized lamellae that lack the orderly parallel arrangement of fibrils characteristic of normal cornea cannot interfere. Thus, the approximately 10-fold reduction in scattering that results from the destructive interference that occurs in normal cornea would be lost and the fibrils would act as independent scatterers. Finally, proteoglycans in the ground substance may be altered during the healing process.[55] Such alterations might change the refractive index difference between the fibrils and ground substance, which would either increase or decrease the fibrillar scattering contribution, depending on whether the difference increased or decreased.

It is important to recognize that the scatterometer measures back-scattered light, whereas it is light scattered in near-forward directions that influences visual performance via its effects on contrast visual acuity and glare sensitivity.[71] The relationship between back- and forward-scattered light is complex even in normal cornea[62,72,73] and would not be known a priori in scarred cornea. Similarly, the relationship between specularly reflected light and forward-scattered light is unknown. Therefore, care must be exercised in interpreting scatterometer or confocal measurements, especially in drawing any conclusions as to how they may relate to visual performance. For example, Lohmann et al.[71] observed a direct correlation between forward- and back-scattered light in excimer laser–treated patients when both scatter components were at their maximum levels. In the same study, however, no correlation was found between forward- and back-scattered light in contact lens or spectacle wearers. Indeed, the study found that several soft contact lens wearers had very low levels of back-scattered light but had considerable problems with forward-scattering. It is therefore important to continue developing relationships between the scattering determined with the scatterometer and other tests such as measurements of visual acuity and of contrast and glare sensitivity.

We have used the scatterometer in the clinic on both in patients undergoing PTK and those having PRK and have found a positive correlation between higher haze levels and decreased best corrected visual acuity.[8] Another study found a correlation between measured haze and visual acuity using 5% contrast visual acuity charts.[15] The development of relationships between other tests of visual function would offer the potential for evaluating what constitutes clinically significant haze and would be of practical importance, because scatterometer measurements are less time consuming than are tests of

visual performance. Moreover, scatterometer measurements may offer the possibility of objectively determining the effectiveness of pharmacological treatment regimens to reduce haze.[11,12]

Acknowledgments

The authors thank Wallace Chamon for help and advice during the early stages of the research. The late Bernard F. Hochheimer provided expert advice during development of the scatterometer.

References

1. Jacobs JM, Taravella MJ. Incidence of intraoperative flap complications in laser In situ keratomileusis. *J Cataract Refract Surg*. 2002;28:23–28.
2. Melki SA, Azar DT. LASIK complications: etiology, management, and prevention. *Surv Ophthalmol.* 2001;46:95–116.
3. Lee YG, Chen WYW, Petroll WM, Cavanagh HD, Jester JV. Corneal haze after photorefractive keratectomy using different epithelial removal techniques. *Ophthalmology*. 2001;108:112–120.
4. Fantes EF, Hanna UD, Waring GO, Pouliquen Y, Thompson K, Salvodelli M. Wound healing after excimer laser keratomileusis (photorefractive keratectomy) in monkeys. *Arch Ophthalmol*. 1990;108:665–675.
5. McDonald MB, Frank JM, Klyce SD, et al. Central photorefractive keratectomy for myopia: the blind eye study. *Arch Ophthalmol*. 1990;108:799–808.
6. Sher NA, Barak M, Dana S, et al. Excimer laser photorefractive keratectomy in high myopia: a multicenter study. *Arch Ophthalmol.* 1992;110:935–941.
7. Durrie DS, Lesher MP, Cavanaugh TB. Classification of variable clinical response after photorefractive keratectomy for myopia. *J Refract Surg*. 1995;11:341–347.
8. Braunstein RE, Jain S, McCally RL, Stark WJ, Connolly PJ, Azar DT. Objective measurements of corneal light scattering after excimer laser keratectomy. *Ophthalmology*. 1996;103:439–443.
9. Møller-Pedersen T, Cavanagh HD, Pertoll WM, Jester JV. Corneal haze development after PRK is regulated by volume of stromal tissue removal. *Cornea*. 1998;17:627–639.
10. Faktorovich EG, Badawi DY, Maloney RK, Ariyasu RG. Growth factor expression in corneal wound healing after excimer laser keratectomy. *Cornea*. 1999;18:580–588.
11. Jain S, Hahn T, McCally RL, Azar DT. Antioxidants reduce corneal light scattering after excimer keratectomy in rabbits. *Lasers Surg Med*. 1995;17:160–165.
12. Jain S, McCally RL, Connolly PJ, Azar DT. Mitomycin-C reduces corneal light scattering after excimer keratectomy. *Cornea*. 2001; 20:45–49.
13. McCally RL, Hochheimer BF, Chamon W, Azar DT. A simple device for measurements of haze following excimer laser ablation of cornea. SPIE (International Society for Optical Engineering) Bellingham, WA: Ophthalmic Technologies III; 1993:20–25.
14. Chang S, Maurice DM, Ramirez-Florez S. Quantitative measurement of corneal haze after myopic PRK. *J Refract Surg*. 1996;12:412–416.
15. Lohmann CP, Garrtry DS, Muir MK, Timberlake GT, Fitzke FW, Marshall J. Corneal haze after laser refractive surgery: objective measurements and functional implications. *Eur J Ophthalmol*. 1991;1:173–180.
16. Lohmann CP, Timberlake GT, Fitzke FW, Garrtry DS, Kerr-Muir M, Marshall J. Corneal light scattering after excimer laser photorefractive keratectomy: the objective measurements of haze. *Refract Corneal Surg*. 1992;8:114–121.
17. Maldonado MJ, Arnau V, Navea A, et al. Direct objective quantification of corneal haze after excimer laser photorefractive keratectomy for high myopia. *Ophthalmology*. 1996;103:1970–1978.
18. Møller-Pedersen T, Vogel M, Li HF, Petroll WM, Cavanagh HD, Jester JV. Quantification of stromal thinning, epithelial thickness, and corneal haze after photorefractive keratectomy using in vivo confocal microscopy. *Ophthalmology*. 1997;104:360–368.
19. Masters BR. Specimen preparation and chamber for confocal microscopy of the *ex vivo* eye. *Scan Microsc*. 1993;7:645–651.
20. Li HF, Petroll WM, Møller-Pederson T, Maurer JK, Cavanagh HD, Jester JV. Epithelial and corneal thickness measurements by in vivo confocal microscopy through focusing (CMTF). *Curr Eye Res*. 1997;16:214–221.
21. Møller-Pedersen T, Li HF, Petroll WM, Cavanagh HD, Jester JV. Confocal microscopic characterization of wound repair after photorefractive keratectomy. *Invest Ophthalmol Vis Sci*. 1998;39: 487–501.
22. Møller-Pedersen T. On the structural origin of refractive instability and corneal haze after excimer laser keratectomy for myopia. *Acta Ophthalmol Scand Suppl*. 2003;81:6–20.
23. Møller-Pedersen T. Keratocyte reflectivity and corneal haze. *Exp Eye Res*. 2004;78:553–560.
24. Maurice DM. The cornea and sclera. In: Davson H, ed. *The Eye*. Orlando, FL: Academic Press; 1984:1–158.
25. Wilson SE, Hong J-W. Bowman's layer structure and function. *Cornea.* 2000;19:417–420.
26. Müller LJ, Pels E, Vrensen GFJM. The specific architecture of the anterior stroma accounts for maintenance of corneal curvature. *Br J Ophthalmol*. 2001;85:437–443.
27. Radner W, Mallinger R. Interlacing of corneal lamellae in the midstroma of the human cornea. *Cornea*. 2002;21:598–601.
28. Radner W, Zehetmayer M, Aufreiter R, Mallinger R. Interlacing and cross-angle distribution of collagen lamellae in the human cornea. *Cornea*. 1998;17:537–543.
29. Maurice DM. Mechanics of the Cornea. In: Cavanagh HD, ed. *The Cornea: Transactions of the World Congress on the Cornea III.* New York: Raven Press; 1988:187–193.
30. Maurice DM, Monroe F. Cohesive strength of corneal lamellae. *Exp Eye Res*. 1990;50:59–63.
31. Ojeda J, Ventosa JA, Piedra S. The three-dimensional microanatomy of the rabbit and human cornea: a chemical and microdissection-SEM approach. *J Anat*. 2001;199:567–576.
32. Jester JV, Budge A, Fisher S, Huang J. Corneal keratocytes: phenotypic and species differences in abundant protein expression and in vitro light scattering. *Invest Ophthalmol Vis Sci*. 2005;46:2369–2378.
33. Crosson CE, Klyce SD, Beuerman RW. Epithelial wound closure in the cornea: a biphasic process. *Invest Ophthalmol Vis Sci*. 1986; 27:464–473.
34. Lu L, Reinach PS, Kao WW-Y. Corneal epithelial wound healing. *Exp Biol Med.* 2001;226:653–664.
35. Serrao S, Lombardo M, Mondini M. Photorefractive keratectomy with and without smoothing: a bilateral study. *J Refract Surg*. 2003;19:58–64.
36. Csutak A, Silver DM, Tözsér J, Facskó A, Berta A. Plasminogen activator activity and inhibition in rabbit tears after photorefractive keratectomy. *Exp Eye Res*. 2003;77:675–680.
37. Csutak A, Tözsér J, Békési L, Hassan Z, Berta A, Silver DM. Plasminogen activator activity in tears after excimer laser photorefractive keratectomy. *Invest Ophthalmol Vis Sci*. 2000;41:3743–3747.
38. Drew AF, Schiman HL, Kombrinck KW, Bugge TH, Degen JL, Kaufman AH. Persistent corneal haze after excimer laser photokeratectomy in plasminogen-deficient mice. *Invest Ophthalmol Vis Sci*. 2000;41:67–72.
39. Kao WW-Y, Kao CW-C, Kaufman AH, et al. Healing of corneal epithelial defects in plasminogen- and fibrinogen-deficient mice. *Invest Ophthalmol Vis Sci*. 1998;39:502–508.
40. van Mohrenfels CW, Reischl U, Lohmann CP. Corneal haze after photorefractive keratectomy for myopia; role of collagen IV mRNA typing as a predictor of haze. *J Cataract Refract Surg*. 2002;28: 1446–1451.
41. Helana MC, Baerrveldt F, Kim W-J, Wilson SE. Keratocyte apoptosis after corneal surgery. *Invest Ophthalmol Vis Sci*. 1998;39:276–283.
42. Mohan RR, Hutcheon AEK, R. Choi, et al. Apoptosis, necrosis, proliferation, and myofibroblast generation in the stroma following LASIK and PRK. *Exp Eye Res*. 2003;76:71–87.
43. Wilson SE, Kim W-J. Keratocyte apoptosis: implications on corneal wound healing, tissue organization, and disease. *Invest Ophthalmol Vis Sci*. 1998;39:220–226.

44. Wilson SE. Analysis of keratocyte apoptosis, keratocyte proliferation, and myofibroblast transformation responses after photorefractive keratectomy and laser in situ keratomileusis. *Trans Am Ophthalmol Soc*. 2002;100:411–433.
45. Jester JV, Petroll WM, Cavanagh HD. Corneal stromal wound healing in refractive surgery: the role of myofibroblasts. *Prog Retin Eye Res*. 1999;18:311–356.
46. Nakamura K. Interaction between injured corneal epithelial cells and stromal cells. *Cornea* 2003;22:S35–S47.
47. Chen C, Michelini-Norris B, Stevens S, et al. Measurement of mRNAs for TGFb and extracellular matrix proteins in corneas of rats after PRK. *Invest Ophthalmol Vis Sci*. 2000;41:4108–4116.
48. Estil S, Haaskjold E, Bjerknes R, Refsum SB. A previous abrasion in the contralateral eye influences the cell kinetics during healing of a central corneal abrasion. *Acta Ophthalmol Scand*. 2001;79:389–393.
49. Rask R, Jensen PK, Ehlers N. Epithelial healing in the second eye after corneal abrasion. *Acta Ophthalmol Scand*. 1996;74:232–234.
50. Farrell RA, McCally RL. Corneal transparency. In: Albert DM, Jakobiec FA, eds. *Principles and Practice of Ophthalmology.* 2nd ed. Philadelphia: WB Saunders; 2000:629–644.
51. Erie JC. Corneal wound healing after photorefractive keratectomy: a 3-year confocal microscopy study. *Trans Am Ophthalmol Soc* 2003;101:293–333.
52. Netto MV, Mohan RR, Hutcheon RAEK, Ziesky JD, Wilson SE. Wound healing in the cornea. *Cornea*. 2005;24:509–522.
53. Erie JC, Patel SV, McLaren JW, Maguire LJ, Ramirez M, Bourne WM. Keratocyte density in vivo after photorefractive keratectomy in humans. *Trans Am Ophthalmol Soc*. 1999;97:221–236.
54. Ramirez-Florez S, Maurice DM. Inflammatory cells, refractive regression, and haze after excimer laser PRK. *J Refract Surg*. 1996; 12:370–381.
55. Rawe IM, Zabel RW, Tuft SJ, Chen V, Meek KM. A morphological study of rabbit corneas after laser keratectomy. *Eye*. 1992;6:637–642.
56. Wu WCS, Stark WJ, Green WR. Corneal wound healing after 193-nm excimer laser keratectomy. *Arch Ophthalmol*. 1991;109: 1426–1432.
57. Fountain TR, Cruz Zdl, Green WR, Stark WJ, Azar DT. Reassembly of corneal epithelial adhesion structures after excimer laser keratectomy in humans. *Arch Ophthalmol*. 1994;112:967–972.
58. Goodman GL, Trokel SL, Stark WJ, Munnerlyn CR, Green WR. Corneal healing following laser refractive keratectomy. *Arch Ophthalmol*. 1989;107:1799–1803.
59. Connon C, Marshall J, Patmore AL, Brahama A, Meek KM. Persistent haze and disorganization of anterior stromal collagen appear unrelated following phototherapeutic keratectomy. *J Refract Surg*. 2003;19:323–332.
60. Tuft S, Marshall J, Rothery S. Stromal remodeling following photorefractive keratectomy. *Lasers Ophthalmol*. 1987;1:177–183.
61. McCally RL, Farrell RA. The depth dependence of light scattering from the normal rabbit cornea. *Exp Eye Res*. 1976;23:69–81.
62. Freund DE, McCally RL, Farrell RA. Effects of fibril orientations on Light scattering in the cornea. *J Opt Soc Am A*. 1986;3:1970–1982.
63. McCally RL, Farrell RA. Light scattering from cornea and corneal transparency. In: Masters BR, ed. *Noninvasive Diagnostic Techniques in Ophthalmology.* New York: Springer-Verlag; 1990:189–210.
64. Maurice DM. A scanning slit specular microscope. *Invest Ophthalmol*. 1974;13:1033–1037.
65. Cox JL, Farrell RA, Hart RW, Langham ME. The transparency of the mammalian cornea. *J Physiol* (*Lond*). 1970;210:601–616.
66. Farrell RA, McCally RL. On corneal transparency and its loss with swelling. *J Opt Soc Am*. 1976;66:342–345.
67. Farrell RA, McCally RL, Tatham PER. Wavelength dependencies of light scattering in normal and cold swollen rabbit corneas and their structural implications. *J Physiol* (*Lond*). 1973;233:589–612.
68. Hart RW, Farrell RA. Light scattering in the cornea. *J Opt Soc Am*. 1969;59:766–774.
69. Jester JV, Møller-Pederson T, Huang J, et al. The cellular basis of corneal transparency: evidence for corneal crystallins. *J Cell Sci*. 1999;112:612–622.
70. Benedek GB. The theory of transparency of the eye. *Appl Opt*. 1971;10:459–473.
71. Lohmann CP, Fitzke F, O'Brart D, Muir MK, Timberlake G, Marshall J. Corneal light scattering and visual performance in myopic individuals with spectacles, contact lenses, or excimer laser photorefractive keratectomy. *Am J Ophthalmol*. 1993;115:444–453.
72. Feuk T, McQueen D. The angular dependence of light scattered from rabbit corneas. *Invest Ophthalmol*. 1971;10:294–299.
73. Freund DE, McCally RL, Farrell RA. Direct summation of fields for light scattering by fibrils with applications to normal corneas. *Appl Opt*. 1986;25:2739–2746.