Optical Effects of Anti-TGFB Treatment after Photorefractive Keratectomy in a Cat Model

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PURPOSE. To assess the contribution of corneal myofibroblasts to optical changes induced by photorefractive keratectomy (PRK) in a cat model.

METHODS. The transforming growth factor (TGF)-β-dependence of feline corneal keratocyte differentiation into α-smooth muscle actin (αSMA)-positive myofibroblasts was first tested in vitro. Twenty-nine eyes of 16 cats were then treated with −10 D PRK in vivo and divided into two postoperative treatment groups that received either 100 μg anti-TGFB antibody for 7 days, followed by 50 μg dexamethasone for another 7 days to inhibit myofibroblast differentiation, or vehicle solution for 14 days (control eyes). Corneal thickness and reflectivity were measured by optical coherence tomography. Wavefront sensing was performed in the awake-behaving state before surgery and 2, 4, 8, and 12 weeks after surgery. Wound healing was monitored using in vivo confocal imaging and postmortem αSMA immunohistochemistry.

RESULTS. In culture, TGFB caused cat corneal keratocytes to differentiate into αSMA-positive myofibroblasts, an effect that was blocked by coinubcation with anti-TGFB antibody. In vivo, anti-TGFB treatment after PRK resulted in less αSMA immuno-reactivity in the subablation stroma, lower corneal reflectivity, less stromal regrowth, and lower nonspherical higher order aberration induction than in control eyes. However, there were no intergroup differences in epithelial regeneration or lower order aberration changes.

CONCLUSIONS. Anti-TGFB treatment reduced feline corneal myofibroblast differentiation in vitro and after PRK. It also decreased corneal haze and fine-grained irregularities in ocular wavefront after PRK, suggesting that attenuation of the differentiation of keratocytes into myofibroblasts can significantly enhance optical quality after refractive surface ablations. (Investigative Ophthalmology & Visual Science, February 2009, Vol. 50, No. 2)

Increased awareness of the potential risk of iatrogenic keratectasia after laser in situ keratomileusis (LASIK) has led to a renewed interest in photorefractive keratectomy (PRK) and prompted the development of advanced surface ablation techniques like laser subepithelial keratomileusis (LASEK) and epi-LASIK. 1 One major disadvantage of surface ablations over LASIK is the more pronounced wound-healing response, whose functional consequences include a longer visual rehabilitation period, regression, and haze. Since these side effects can significantly limit the treatment of higher myopia (reviewed in Refs. 3–5), several attempts have been made to decrease their occurrence through preservation of the epithelial layer (e.g., LASEK and Epi-LASIK) and pharmacologic modulation of wound healing, as proposed in the early days of PRK. 6 Topical steroids have been used widely, 7 but their effects on haze and refractive regression remain controversial. 8–11 Mitomycin C, a cytostatic agent originally introduced for chemotherapy of malignant tumors, has also been shown to attenuate wound healing after PRK, particularly in cases of higher susceptibility to regression and haze (i.e., those involving the treatment of higher myopia). 12–15 However, safety concerns and side effects have been associated with the use of steroids (elevation of intraocular pressure, cataract induction, and delayed epithelial healing) and mitomycin C (cytotoxic, possibly mutagenic, and limited data on long-term keratocyte integrity), prompting the search for alternatives.

One means of modulating corneal wound healing is via the inhibition of transforming growth factor (TGFB) 16–17 multi-functional cytokine released by the lacrimal gland, the corneal epithelium, and conjunctival cells. 18 It promotes keratocyte proliferation, 19,20 migration, 21 differentiation into myofibroblasts that express α-smooth muscle actin (αSMA; reviewed in Ref. 22) and the deposition of extracellular matrix proteins. 19 TGFB has been shown to play a crucial role in the development of haze after PRK, so that application of anti-TGFB antibodies to the eye reduces both corneal reflectivity (haze) and fibrosis after PRK. 16 However, stromal regrowth still occurred, suggesting that at least in the rabbit, stromal regeneration may be controlled by TGFB-independent mechanisms. 17 Other than on haze, the optical consequences of blocking TGFB after PRK 16,17 have never yet been examined. The cat model used in the present study is unique in allowing simultaneous investigation of biological and optical aspects of corneal wound healing after PRK. 23–25 However, before testing the in situ effects of anti-TGFB treatment in the cat, we first measured the response of feline corneal keratocytes to TGFB stimulation in vitro, to verify that they behaved similarly to keratocytes from rabbits, pigs, and humans. In vivo experiments were then performed to test the hypothesis that blocking TGFB activity in the feline eye after PRK (1) decreases transformation of corneal keratocytes into contractile myofibroblasts, (2) decreases haze (corneal reflectivity) by reducing the incidence of reflective myofibroblasts in the ablation optical zone, (3) decreases refractive regression by slowing keratocyte proliferation and the
generation of new extracellular matrix in the stroma, and (4) decreases the induction of higher order aberrations (HOAs) by decreasing the fine-grained, contractile influence of myofibroblasts on the corneal surface.

**MATERIALS AND METHODS**

All animal procedures were conducted according to the guidelines of the University of Rochester Committee on Animal Research (UCAR), the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the NIH Guide for the Care and Use of Laboratory Animals.

**Cell Culture Experiments**

Corneal keratocytes were isolated from four normal, adult domestic short-haired cats, as previously described.²²⁻²⁶ Cells were plated on both 1-mm collagen IV-coated glass coverslips and six-well collagen IV-coated tissue culture plates (VWR International, West Chester, PA). Cells were seeded at a density of 10⁴ cells per well and cultured in 1× penicillin-streptomycin, gentamicin, 1× Dulbecco’s modified Eagle’s medium/nutrient mix F-12 (DMEM/F-12; 1:1) liquid, 1:1, containing t-glutamine, but no HEPES buffer or phenol red (Invitrogen, Carlsbad, CA). When the first batch of cells approached 80% confluence, they were exposed to recombinant human TGFβ (Calbiochem, San Diego, CA) ranging in concentration from 0 to 10 ng/mL to assess whether this factor caused them to differentiate into αSMA-positive myofibroblasts. The optimal dose at which TGFβ induced strong αSMA expression at 72 hours was 1 ng·mL⁻¹. New sets of primary feline corneal keratocytes were then incubated with a combination of 1 ng·mL⁻¹ TGFβ and neutralizing mouse monoclonal anti-TGFβ antibody (Clone 1D11, blocks all subtypes of TGFβ; R&D Systems, Minneapolis, MN) ranging in concentration from 0 to 2 ng·mL⁻¹. After 72 hours in culture, the experiment was stopped to assess what dose of antibody blocked TGFβ-dependent induction of myofibroblasts differentiation, as measured via αSMA expression. All cell culture experiments were repeated three times.

**Immunofluorescence.** Cultured cat corneal keratocytes plated on coverslips were rinsed once in phosphate buffered saline (PBS) and fixed in 4% paraformaldehyde in 0.1 M PBS for 6 minutes at room temperature (25°C). They were then permeabilized by incubation with 0.2% Triton X-100 in 0.1 M PBS (Sigma-Aldrich, St. Louis, MO) for 5 minutes in 0.05% trypsin with EDTA 4 Na (1×) liquid, 1:1, containing t-glutamine, but no HEPES buffer or phenol red (Invitrogen, Carlsbad, CA). After primary antibody washes, the coverslips were incubated with anti-mouse IgG tagged with AlexaFluor 488 (2 μg·mL⁻¹; Invitrogen-Molecular Probes, Eugene, OR). Finally, the cells were double-stained with propidium iodide (0.1 μg·mL⁻¹; Invitrogen, Carlsbad, CA) to identify all nuclei. Coverslips were then mounted onto slides and imaged with a 63× objective on a confocal microscope (LSM510; Carl Zeiss Meditec, Inc., Dublin, CA).

**Western Blot Analysis.** Cells were collected from cultures at various time points (0, 24, 48, 72, and 120 hours) by incubating them for 5 minutes in 0.05% Tryton X-100 in 0.1 M PBS (Sigma-Aldrich, St. Louis, MO) for 6 minutes at 25°C before incubation for 1 hour at 37°C in 2 μg·mL⁻¹ of mouse monoclonal anti-αSMA antibody (clone 1A4; Sigma-Aldrich). After primary antibody washes, the coverslips were incubated with anti-mouse IgG tagged with AlexaFluor 488 (2 μg·mL⁻¹; Invitrogen-Molecular Probes, Eugene, OR). After the blot was developed (Gel Doc system; Bio-Rad, Hercules, CA), the blot and incubated for 5 minutes at room temperature, after which the blot was developed (Gel Doc system; Bio-Rad, Hercules, CA).

**In Vivo Experiments**

A list of treatments administered and measurements collected in living cats is provided in Table 1.

**PRK Surgery.** Twenty-nine eyes from 16 normal, adult short-haired cats (fels cattus) underwent a conventional spherical PRK for −10 D with a 6-mm optical zone (OZ) and a 1.5-mm transition zone, resulting in a total treatment zone 9.1 mm in diameter and a central ablation depth of 168 μm (Planomac 4:1; Bausch & Lomb). PRK was performed by one of two surgeons (SM, JB) in cats under topical (proparacaine 0.5%; Falcon, Fort Worth, TX) and surgical (ketamine, 5 mg·kg⁻¹; medetomidine hydrochloride 0.08 mg·kg⁻¹) anesthesia, with a laser (Technolas 217; Bausch & Lomb). The ablation was centered to the pupil, which was constricted with two drops of pilocarpine 3% (Bausch & Lomb). Anti-TGFβ Treatment. After PRK, 13 eyes received a topical administration of anti-TGFβ antibody (Clone 1D11, blocks all subtypes of TGFβ; R&D Systems). Eleven of these eyes received 50 μg of anti-TGFβ antibody twice daily, a dose that was determined to be optimal based on experiments in a separate set of cats (including two from this cohort; data not shown) and on results from the literature.⁶,⁷ The contralateral eyes were treated with vehicle solution (Refresh; Allergan, Irvine, CA) to serve as the control. An additional three eyes from three cats were later added to the control group when a power analysis revealed the need for additional animals to attain statistical significance. For the first application immediately after PRK, both treatment and vehicle solutions were held in place on the cornea using a saturated, sterile, gelatin sponge (Surgipan; Ethicon, Piscataway, NJ) for 2 minutes. Each eye then received a drop of triple antibiotic solution (Neomycin, Polymyxin B Sulfate, Gramicidin; Ophthalmic Solution USP; Bausch & Lomb) for the next 7 days. Treatment eyes received 1 drop of 1 mg·mL⁻¹ anti-TGFβ antibody and 1 drop of antibiotic solution twice daily. Control eyes received one drop of vehicle solution and one drop of antibiotic solution twice daily. During the second postoperative week, treatment eyes received 1 drop of 0.1% (1 mg·mL⁻¹) dexamethasone suspension (Maxidex; Alcon, Fort Worth, TX) per day. In case of delayed epithelial closure, the first week’s treatment was continued until the epithelium closed (usually a couple of extra days). Pain in the early postoperative period was treated with IV flunixin meglumine 1.1 mg·kg⁻¹ (Banamine; Schering-Plough Animal Health, Kenilworth, NJ) three times/day for 3 days. Four eyes were excluded from this study: two received a nonoptimal dose of anti-TGFβ antibody (as part of dose-response trials), one had a severely centered optical zone, and one developed an ulcer and a sequestrum.

**Table 1. In Vivo Experiment: Eyes, Treatments, and Measures**

<table>
<thead>
<tr>
<th>PRK-Treated Eyes (n) and Test</th>
<th>Exclusion from Study</th>
<th>OCT</th>
<th>In Vivo Confocal</th>
<th>Histology</th>
<th>Wavefront Sensing</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-TGFβ treatment</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>15</td>
<td>2</td>
<td>10</td>
<td>7</td>
<td>16</td>
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Optical Coherence Tomography. A custom-built, 1310-nm anterior segment optical coherence tomograph (OCT) was used to image corneas before and 2, 4, 8, and 12 weeks after PRK. The animals were lightly anesthetized, treated with eye gel (GenTeal; Novartis, East Hanover, NJ) and placed in a head-restrained device to hold the head stable. The OCT recorded a video stream of the central 10 mm of cornea at a rate of eight frames per second. Twenty-five corneal images extracted from the video stream were analyzed and averaged per eye and time-point. Custom software was used to measure and calculate the normalized intensity profile across each corneal image, as reported previously. Briefly, a rectangular analysis area 105 μm wide and spanning the entire thickness of the cornea and gel layer was positioned 1 mm nasal to the middle of each corneal image (light gray rectangles in Fig. 4A). This nasal location was chosen to ensure that the same region of cornea was analyzed in all eyes and that it lay outside the area of saturated reflectivity associated with the specular reflection. A profile of pixel intensities was generated across the vertical extent of each analysis area, as previously described. To compensate for fluctuations in laser strength, we normalized each profile by dividing the mean pixel intensity at each vertical pixel location by the mean pixel intensity in the analyzed region of cornea. Finally, the area under the normalized intensity curve was computed for the most superficial 20% of the stroma and expressed as a cumulative intensity value in Figure 4C. The normalized backscatter intensity profiles were also used to estimate the thickness of the epithelium and stroma from each OCT image, by measuring the vertical difference between intensity peaks within each analysis area.

In Vivo Confocal Imaging. Confocal imaging of the central cornea was performed in two animals before and 2, 4, 8, and 12 weeks after PRK. After OCT imaging, the anesthetized cats were imaged with the Heidelberg Retina Tomograph with the Rostock Cornea Module (Heidelberg Engineering, Dossenheim, Germany). A drop of eye gel (GenTeal; Novartis) was placed on each cornea and on the contact cap. Correct alignment was attained, and after the focus was set on the epithelium, several automated scans, each 58 m in depth, were performed until the endothelium became visible. Scans were recorded as digital video sequences and stored on a PC for analysis.

Wavefront Analysis in Awake-Fixating Cats. Cats were trained to fixate on single spots of light presented on a computer monitor as previously described. Wavefront measurements were performed in each eye before surgery and 2, 4, 8, and 12 weeks after PRK with a custom-built Hartmann-Shack wavefront sensor. The wavefront sensor was aligned to the visual axis of one eye while the other eye fixated a spot on the computer monitor. At least 10 spot array patterns were collected per imaging session per eye. From each spot array pattern, wavefront errors (WFEs) were calculated using a 2nd- to 10th-order Zernike polynomial expansion according to the published standards for reporting aberration data of the eye. The measurements were centered on the ablation OZ by shifting a 6-mm centroiding area equivalent; spherical aberration RMS (the RMS of all coma terms C
\[n\]) was expressed as a cumulative intensity value in Figure 4C. The normalized backscatter intensity profiles were also used to estimate the thickness of the epithelium and stroma from each OCT image, by measuring the vertical difference between intensity peaks within each analysis area.

RESULTS

Cell Culture Experiments

Primary cultures of cat corneal keratocytes failed to express αSMA and exhibited a dendritic morphology typical of quiescent corneal keratocytes. When TGFβ was added to the serum-free medium, feline keratocytes altered their morphology, retracting some of their dendritic processes, multiplying, and acquiring a distinct myofibroblastic phenotype, which was accompanied by intracellular expression of αSMA after 72 hours of culture (Fig. 1A). The minimum concentration of TGFβ required for this differentiation and for strong αSMA expression at 72 hours was 1 ng · mL⁻¹, as confirmed by immunofluorescence and Western blot analysis (Figs. 1A, 1C). Addition of anti-TGFβ antibodies to the incubation medium blocked expression of αSMA in a dose-dependent manner, as shown by immunofluorescence (Fig. 1B) and Western blot (Fig. 1D).

In Vivo Experiments

Clinical Course and Slit Lamp Findings after PRK. Of the 10 eyes treated with 1 mg · mL⁻¹ anti-TGFβ twice daily, only one exhibited slightly delayed epithelial healing, which required us to start dexamethasone treatment 2 days late. All other eyes showed uneventful follow-up, with full epithelial closure by day 7.

Immunohistochemistry. Two cats were killed at 2 weeks after PRK, five at 4 weeks after PRK and four at 12 weeks after PRK for the purpose of performing corneal histology. Two animals were kept for long-term optical follow-up and no ex vivo histology was performed on their corneas. Two separate, normal, adult cats were also killed to serve as nonsurgical control subjects. After the cats were euthanized, the corneas were excised and drop fixed in 1% paraformaldehyde in 0.1 M PBS (pH 7.4) for 10 minutes. They were then transferred to 30% sucrose in 0.1 M PBS and stored at 4°C for 2 days. After they were embedded in OCT Compound (Tissue Tek; Sakura Finetek, Zoeterwoude, The Netherlands), serial, 20-μm-thick cross-sections were cut on a cryostat (2800 Frigocut E; Leica, Nussloch, Germany), mounted on microscope slides and stored in a −20°C freezer until ready to stain.

Slides containing three corneal sections each were air dried and rinsed in 0.1 M PBS. Two of the sections on each slide were incubated overnight at 4°C with 2 μg · mL⁻¹ mouse monoclonal anti-αSMA antibody (clone 1A4; Sigma-Aldrich). The third section was incubated with 0.1 M PBS as a negative control. After washing off the primary antibody with 0.1 M PBS, the sections were then reacted with antirabbit IgG tagged with AlexaFluar 488 (2 μg · mL⁻¹; Invitrogen-Molecular Probes), followed by propidium iodide (0.1 μg · mL⁻¹; Invitrogen), to label the cell nuclei. The double-labeled sections were imaged with a fluorescence microscope (AX70; Olympus, Lake Success, NY), and photomicrographs were collected via a high-resolution video camera interfaced with a computer (ImagePro software; Media Cybernetics, Bethesda, MD).

Statistical Analysis

Intergroup differences in reflectivity, corneal thickness, and wavefront aberrations were compared with a paired (or two-sample), two-tailed Student's t test. If data were not normally distributed according to a Kolmogorov-Smirnov-Lilliefors test, a Wilcoxon-Mann-Whitney U test was used instead. Finally, where appropriate, a 2 (condition) × 4 (postoperative time point) mixed factorial ANOVA was also performed. A probability of error of P < 0.05 was considered statistically significant. All statistical tests were performed with commercial software (SPSS 11.0; SPSS Inc., Chicago, IL).

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Two weeks after PRK, there was increased reflectivity at the epithelial–stromal interface, which was more prominent in control than in treated eyes (Figs. 2B, 2F). The number of migratory fibroblasts increased, with some sprouting processes in both groups. Below the hypocellular zone (10 to 20 μm below the epithelial–stromal interface), activated, reflective keratocytes with distinct cell processes were clustered (Fig. 3B). In control eyes, the epithelial–stromal interface appeared to contain more pronounced cellular elements (Fig. 2F)—likely to be myofibroblasts—and the layer below the epithelial–stromal interface was populated by activated keratocytes, which appeared more densely packed than in treated eyes (Fig. 3F). The entire layer of activated cells and increased reflectivity was ~90 μm thick in both control and anti-TGFβ-treated eyes.

Three and 4 weeks after PRK, both treatment and control eyes showed further increases in the reflectivity of the epithelial–stromal interface relative to their preoperative state. Once again, this reflectivity appeared greater in control eyes (Figs. 2C, 2G). Eyes treated with anti-TGFβ contained some bright stromal cells below the epithelium, but they were less dense than in control eyes and appeared separated by large, optically clear spaces (Figs. 3C, 3G). The subepithelial layer of activated...
especially at 2 and 4 weeks after PRK. There were spindle-shaped migratory fibroblasts (arrow) and the cluster of reflective activated keratocytes in the right eye at 2 weeks after PRK. Clustering was still present in anti-TGFβ-treated eyes 4 weeks after PRK, but was not seen in control eyes. As is evident in this set of images, control eyes exhibited greater cell density and greater reflectivity at this corneal depth than did eyes treated with anti-TGFβ/dexamethasone, at all postoperative time points.

Corneal Backscatter Reflectivity. OCT-derived preoperative reflectivity of the anterior 20% of the cornea was relatively low and did not differ significantly between cat eyes destined for anti-TGFβ treatment or control groups (Figs. 4A–4C). PRK increased reflectivity in the anterior 20% of the stroma of both groups (P < 0.05; Figs. 4A–C). However, this increase was significantly lower in treated than in control eyes, with a peak difference at 4 weeks after PRK (P < 0.05; Figs. 4A–C).

Corneal Thickness Changes. Before surgery, the mean central corneal thickness of treatment eyes was 587 ± 55 μm (control eyes: 582 ± 40 μm), the mean stromal thickness was 537 ± 67 μm (control eyes: 538 ± 52 μm), and the mean epithelial thickness was 67 ± 15 μm (control eyes: 59 ± 8 μm). There were no significant intergroup differences in any of these measures before surgery (P > 0.05; Figs. 4D, 4E). The epithelial layer was scraped off during PRK, but 2 weeks later, central epithelial thickness had not only regenerated, but in the control group, it was 36 ± 37 μm thicker than it had been before surgery (P < 0.005). This effect was not observed in eyes treated with anti-TGFβ until 4 weeks after PRK, after which, epithelial thickness returned to preoperative values in both groups (Fig. 4D). An ANOVA showed no significant effect especially at 2 and 4 weeks after PRK. There were spindle-shaped migratory fibroblasts (arrow) and the cluster of reflective activated keratocytes in the right eye at 2 weeks after PRK. Clustering was still present in anti-TGFβ-treated eyes 4 weeks after PRK, but was not seen in control eyes. As is evident in this set of images, control eyes exhibited greater cell density and greater reflectivity at this corneal depth than did eyes treated with anti-TGFβ/dexamethasone, at all postoperative time points.
of treatment or postoperative time on epithelial thickness. PRK removed approximately 168 μm of central stroma. Two weeks later, the central stroma had regenerated half that loss in control eyes, but was still 141 μm thinner than before surgery in treated eyes ($P < 0.01$; Fig. 4E). An ANOVA revealed a main effect of treatment ($F_{1,11} = 4.96, P = 0.048$) across the entire postoperative period with control eyes maintaining a significantly thicker stroma after surgery than eyes treated with anti-TGFβ (Fig. 4E).

**Ex Vivo Histology.** Normal, unoperated cat corneas exhibited a classic histologic structure and a complete absence of αSMA staining within the stroma (Fig. 5). Two and 4 weeks after PRK, the subablation zone was identifiable by the absence of a clear Bowman’s layer, and the expression of αSMA below the epithelium. In control eyes, the band of αSMA immunoreactivity was thick and continuous, whereas in eyes treated with anti-TGFβ, it formed a much thinner layer, interrupted by αSMA-negative zones (Fig. 5). Qualitative inspection of PI-staining revealed an apparent decrease in keratocyte density after PRK relative to surgical corneas. However, among PRK-treated corneas, the density of subepithelial stromal cells always appeared higher in control corneas than in corneas treated with anti-TGFβ. Twelve weeks after PRK, stromal αSMA immunoreactivity decreased relative to its levels 4 weeks after PRK in both treatment groups. However, PI staining continued to show apparently higher cell density in the subablation zone of control eyes (Fig. 5).

**Wavefront Analysis.** Before surgery, there were no statistically significant differences in lower or higher order wavefront aberrations between the two experimental groups ($P > 0.05$; Figs. 6, 7). Two weeks after PRK, the mean change in spherical equivalent was 4.79 ± 0.86 D in the anti-TGFβ group and 5.04 ± 2.11 D in the control group (Table 2; Fig. 6A). Similar amounts of astigmatism ($j_{13}$ and $j_{21}$) were induced in the two groups after PRK ($P > 0.05$; Table 2, Figs. 6B, 6C).

With respect to HOA, control eyes exhibited a dramatic and significant increase in total higher order RMS (HOA RMS) 2 weeks after PRK relative to preoperative levels ($P < 0.05$, Fig. 7A). This increase was statistically greater in controls than in eyes treated with anti-TGFβ ($P < 0.05$, Table 2, Fig. 7A). An ANOVA revealed a main effect of post-PRK time ($F_{3,21} = 3.38, P = 0.037$), as well as a significant interaction of time with treatment group for HOA RMS ($F_{3,21} = 4.71, P = 0.011$). Controls also exhibited marked, significant increases in coma, spherical and residual HOA RMS at 2 weeks after PRK, ($P < 0.05$, Fig. 7B–D). These aberrations then decreased, with the exception of spherical aberration, which remained high (Fig. 7C). Eyes that received anti-TGFβ treatments did not exhibit the peak increase in HOA observed in control eyes at 2 weeks after PRK (Table 2, Fig. 7). Coma RMS, residual HOA RMS, and total HOA RMS remained close to preoperative levels throughout the post-PRK period examined. Only spherical aberration approximated levels attained in controls, particularly at later time points. A consequence of the greater magnitude of HOA in control eyes was a greater irregularity of the wavefront relative to anti-TGFβ treated eyes. This peaked 2 weeks after surgery, but was still clearly visible at later time points (Fig. 8).

**DISCUSSION**

Several major new findings emerged from the present study. First, cat corneal keratocytes reacted similarly to keratocytes from other species when exposed to TGFβ and its blocking antibodies. Second, after PRK, myofibroblast differentiation in the feline eye could be decreased by topical application of antibodies against TGFβ. Third, decreasing myofibroblast differentiation after PRK significantly decreased nonspherical, higher-order aberrations without affecting refractive outcome. In short, the present experiments identify, for the first time, a...
specific contribution of myofibroblast activity to changes in ocular optics after laser refractive surgery.

Comparative Behavior of Feline Keratocytes in Culture

Given that earlier studies had suggested some important interspecies differences in corneal wound healing, a fundamental finding in the present study was that primary feline corneal keratocytes exhibited a dendritic morphology, lacked αSMA immunoreactivity, and reacted to the administration of TGFβ in a manner that was consistent with that of rabbit, human, and bovine keratocytes. Furthermore, our cell culture experiments did not support the concept that differences in corneal wound healing that might be observed in our cat model of PRK would be related to differential TGFβ regulation of feline keratocyte differentiation into αSMA-positive myofibroblasts. Having established this behavioral baseline in vitro, we were able to proceed with in vivo experiments in which the TGFβ regulatory pathway was manipulated after PRK in cats. Specifically, we tested the hypothesis that myofibroblasts, through their contractile ability, are significant contributors to the induction of higher (rather than lower) order ocular aberrations after PRK.

Methodological Issues

Unlike prior experiments with neutralizing antibodies against TGFβ in rabbits, we used a prolonged, sequential treatment approach in our cat experiments, with neutralizing antibodies for the first postoperative week and dexamethasone for a second week (after epithelial closure). Although dexamethasone reduces TGFβ1 and -β2 mRNA levels in healing tissues, it was not administered during the first week after PRK for two reasons (1) because steroids can impair epithelial closure and (2) because epithelial disruption is a major source of signals for myofibroblast differentiation. Once the epithelium closed, dexamethasone was administered for 1 week to provide a more upstream, prolonged anti-TGFβ effect than antibody application alone. However, differentiation between antibody and steroid action was not the purpose of our study. Thus, control eyes received only vehicle solution, and we did not include a third, steroid-only treatment group.
Spherical aberration (SA) RMS (RMS of all C_n2) previously demonstrated in rabbit models of lamellar keratectomy.16,17 myofibroblasts in the subablation zone. A similar effect was recognized only the cellular component of this reaction, and whether used alone, is likely to underestimate the extent of “abnormal” or “reactive” stroma in a given piece of tissue. OCT imaging showed higher reflectivity in the anterior 20% of the stroma in control corneas relative to the treated group throughout the follow-up period, confirming previous results with anti-TGFβ treatment in rabbits, which was also shown to decrease reflectivity and haze in the cornea.16,17,42 Whereas the epithelial layer regenerated to approximately the same extent in both treatment groups, anti-TGFβ treatment decreased stromal regrowth throughout the postoperative period in the cat. This contrasts with previous results in rabbits treated with the 1D11 anti-TGFβ antibody, albeit only three times a day for 3 days after PRK.17 In rabbits, this treatment decreased haze and αSMA expression in the subablation zone, but did not impede stromal regrowth.17 It is conceivable that

**TABLE 2. Treatment Effects at 2 Weeks after PRK**

<table>
<thead>
<tr>
<th></th>
<th>Anti-TGFβ (n = 4)</th>
<th>Control (n = 7)</th>
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<tbody>
<tr>
<td><strong>M (D)</strong></td>
<td>4.79 ± 0.86</td>
<td>5.04 ± 2.11</td>
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<tr>
<td></td>
<td>(4.04 to 5.68)</td>
<td>(2.56 to 7.65)</td>
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<tr>
<td><strong>J_0 (D)</strong></td>
<td>0.09 ± 0.09</td>
<td>0.27 ± 0.48</td>
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<tr>
<td></td>
<td>(&lt;0.03 to 0.20)</td>
<td>(&lt;0.38 to 0.84)</td>
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<tr>
<td><strong>J_4s (D)</strong></td>
<td>0.20 ± 0.13</td>
<td>0.30 ± 0.52</td>
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<tr>
<td></td>
<td>(0.05 to 0.37)</td>
<td>(&lt;0.20 to 1.14)</td>
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<tr>
<td><strong>Total HOA RMS (μm)</strong></td>
<td>0.20 ± 0.18*</td>
<td>0.82 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>(0.036 to 0.562)</td>
<td>(0.477 to 1.232)</td>
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<tr>
<td><strong>Coma RMS (μm)</strong></td>
<td>0.08 ± 0.28†</td>
<td>0.48 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.13 to 0.46)</td>
<td>(&lt;0.17 to 0.71)</td>
</tr>
<tr>
<td><strong>Spherical aberration RMS (μm)</strong></td>
<td>0.21 ± 0.07</td>
<td>0.41 ± 0.40</td>
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<tr>
<td></td>
<td>(0.139 to 0.279)</td>
<td>(&lt;0.156 to 1.001)</td>
</tr>
<tr>
<td><strong>Residual HOA RMS (μm)</strong></td>
<td>0.06 ± 0.1*</td>
<td>0.49 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.025 to 0.201)</td>
<td>(0.196 to 0.874)</td>
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<tr>
<td><strong>6th- to 10th-order RMS (μm)</strong></td>
<td>0.01 ± 0.01</td>
<td>0.10 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.022 to 0.297)</td>
<td>(0.014 to 0.255)</td>
</tr>
<tr>
<td><strong>Log BCVSOTF</strong></td>
<td>-0.27 ± 0.34</td>
<td>-0.67 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.65 to 0.18)</td>
<td>(&lt;1.15 to -0.46)</td>
</tr>
</tbody>
</table>

Results are shown as change of refraction. VSOTF, HOAs for a 6-mm pupil diameter relative to baseline. All data are expressed as the mean ± SD (minima and maxima in parentheses). Data in bold indicate statistically significant differences between control eyes and those that received anti-TGFβ treatment. BCVSOTF, visual Strehl ratio, based on the optical transfer function (simulation for best spectacle correction); total RMS, RMS of 2nd- to 5th-order aberrations; HOA RMS, RMS of 3rd- to 5th-order aberrations; coma RMS, RMS of 3rd- and 5th-order coma terms; residual HOA RMS, RMS of all noncoma, nonspherical HOA.

*P < 0.01 (intergroup differences Student’s t-test).
†P < 0.05 (intergroup differences Student’s t-test).
the difference in treatment regimen, and to a lesser extent, in method of measuring stromal thickness (OCT in the present study versus confocal microscopy through focusing in Møller-Pedersen et al.\(^\text{17}\)), can explain our different results. However, the possibility remains that cats and rabbits differ in the TGFβ-dependence of cellular mechanisms controlling their stromal regrowth.

**Effects of Anti-TGFβ Treatment on Ocular Wavefront Aberrations after PRK**

The present study showed for the first time, that cat eyes treated with anti-TGFβ after PRK exhibit less nonspherical wavefront aberrations than control eyes. While cats exhibit a general undercorrection relative to humans when PRK is performed over a 6-mm OZ,\(^\text{24}\) PRK-induced HOAs in the feline model are comparable in magnitude and type to those induced by this procedure in humans.\(^\text{24,25,43}\) While HOA RMS, coma RMS, and residual HOA RMS peaked in the early post-PRK period, levels of these HOA remained close to preoperative levels in eyes treated with anti-TGFβ. Only the amounts of spherical aberration and lower order aberrations induced by PRK were similar in control and anti-TGFβ-treated eyes after surgery. Thus, myofibroblast contractility appears to alter corneal shape in an irregular, non–radially symmetric manner. A possible explanation for this is the development of local irregularities in corneal curvature due to small-scale, myofibroblastic contraction of the stroma\(^\text{44}\) that remains uncompensated by epithelial filling-in. However, anti-TGFβ treatment did not mitigate the induction of spherical aberration, suggesting this to be a phenomenon dominated by factors that do not significantly implicate myofibroblast differentiation. Previous work suggested that loss of laser ablation efficiency in the corneal periphery and changes in corneal biomechanics may play a more important role in the induction of spherical aberration after laser refractive surgery.\(^\text{43}\) The present data show that myofibroblast differentiation and contractility are unlikely to contribute significantly to this phenomenon.

**Implications for Clinical Practice**

The present experiments demonstrated that immediate pharmacologic modulation of corneal wound healing after PRK with agents whose main effect is to block TGFβ activity decreases keratocyte differentiation into contractile myofibroblasts. Of novel interest is that the decreased incidence of myofibroblasts in the post-PRK cornea decreased HOA induction and caused faster optical rehabilitation after PRK. In contrast to treatment with mitomycin C, which decreases repopulation of the acellular zone after PRK via nonspecific, cytotoxic effects,\(^\text{15}\) the treatment used in our study was aimed directly at the transformation of keratocytes into myofibroblasts. Although steroids indirectly block myofibroblast differentiation by downregulating TGFβ mRNA in the eye, they bear the risk of nonspecific side effects, such as delayed re-epithelialization\(^\text{72}\) and increased intraocular pressure (reviewed in Ref. 46). Thus, treatment with anti-TGFβ antibodies in the early postoperative phase after PRK has the advantage of an immediate myofibroblast blocking action without the side effects of steroids or mitomycin C. Clinically, a pharmacologic treatment that shortens recovery time after PRK is highly desirable because it potentially mitigates the main problems associated with this procedure: wound-healing–associated delay of visual recovery and optical instability. On the other hand, such treatment is expensive, requires special handling to avoid antibody degradation, and is only partially effective. Ongoing research in our laboratory is focusing on alternative, nonimmunogenic substances to specifically block other aspects of myofibroblast function via both TGFβ- and non–TGFβ-regulated pathways.

In conclusion, reduction of myofibroblast differentiation after PRK decreases HOA induction and increases retinal image quality. By improving visual outcome, pharmacologic modulation of corneal wound healing with myofibroblast-blocking agents could strengthen the value and feasibility of surface ablation procedures as an alternative to lamellar procedures like LASIK.

**References**


