Keratocyte Apoptosis after Corneal Surgery

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PURPOSE. Programmed cell death (apoptosis) is the controlled death of cells that occurs with minimal collateral damage to surrounding cells or tissue during development, homeostasis, and wound healing. The authors hypothesize that keratocyte apoptosis is an initiating factor in the wound-healing response after refractive surgical procedures. To evaluate the effects of different corneal manipulations, keratocyte apoptosis was examined qualitatively and quantitatively after traditional epithelial scrape–photorefractive keratectomy (PRK), transepithelial PRK, removal of a cap of superficial cornea using a microkeratome, production of a flap of superficial cornea with a microkeratome, and laser-assisted in situ keratomileusis (LASIK) compared with unwounded controls in rabbit corneas.

METHODS. Refractive surgical procedures or their components were performed in rabbit eyes. Keratocyte apoptosis was monitored using the terminal deoxyribonucleotidyl transferase-mediated dUTP-digoxigenin nick-end labeling assay to detect DNA fragmentation. Cellular morphologic changes were evaluated by electron microscope examination.

RESULTS. Keratocyte apoptosis was noted with each refractive procedure or corneal manipulation and was variable from eye to eye with each procedure. Transepithelial PRK was associated with the lowest levels of central corneal apoptosis, even if the stromal surface was scraped after the procedure. Keratocyte apoptosis is confined to the superficial stroma extending to a depth of approximately 50 µm to 75 µm after epithelial scrape–PRK and transepithelial PRK. Apoptosis was noted in the deeper central corneal keratocytes located anteriorly and posteriorly to the lamellar cut in LASIK.

CONCLUSIONS. There are qualitative and quantitative differences in keratocyte apoptosis between LASIK, epithelial scrape–PRK, and transepithelial PRK. Epithelial injury is an important factor modulating keratocyte apoptosis. The level and distribution of keratocyte apoptosis, along with subsequent repopulation by activated stromal keratocytes, are likely to be important determinants of corneal wound healing associated with variability and regression after PRK and LASIK. Transepithelial PRK induces low levels of keratocyte apoptosis, and, therefore, this approach may be useful for treating higher levels of myopia and for retreatment after regression. (Invest Ophthalmol Vis Sci. 1998;39:276-283)
evaluated differences in keratocyte apoptosis among traditional epithelial scrape-PRK; transepithelial PRK, in which the overlying epithelium is ablated with the excimer laser before PRK; LASIK; and other corneal manipulations in the rabbit model.

**Methods**

Adult New Zealand White rabbits were anesthetized with xylazine and ketamine. Each eye was included in one of the following groups: control unwounded, 10 eyes; epithelial scrape, 8 eyes; traditional PRK with epithelial scrape followed by surface ablation (epithelial scrape-PRK), 10 eyes; transepithelial PRK, 10 eyes; transepithelial PRK followed by scrape of the exposed stroma (transepithelial PRK-stromal scrape), 3 eyes; microkeratome flap repositioned without laser treatment (microkeratome flap), 6 eyes; microkeratome cap with excision of cap (microkeratome cap off), 6 eyes; LASIK, 8 eyes. This study adhered to the tenets of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

The rabbit eye was proptosed anteriorly to the eyelids before each procedure. Scraping for the mechanical epithelial scrape wound group, the epithelial scrape-PRK group, and the transepithelial PRK-stromal scrape groups was performed with a #64 blade ( Beaver; Becton-Dickinson, Franklin Lake, NJ). The diameters of the scraped epithelium or stroma were 8 mm and 6 mm, respectively, anatomically centered on the cornea. For the epithelial scrape-PRK group, a 1.5-diopter, 4-mm diameter, 20-µm deep (approximately 5% of the corneal thickness in the rabbit) ablation, anatomically centered on the cornea, was performed with the Summit Technologies (Midland, MD) Apex excimer laser after epithelial scraping. The pulse energy density was 180 mJ/cm², and the fluency was 10 Hz. In the transepithelial PRK group, the excimer laser was initially programmed with a phototherapeutic keratectomy with a 6.5-mm ablation diameter. Anatomically centered transepithelial ablation was performed on the cornea until the typical fluorescent blue of the epithelium changed to black, which was characteristic of stroma during the entire ablation using an operating microscope with low-level illumination. A 1.5-diopter, 4-mm diameter, 20-µm deep PRK ablation was then performed in the center of the epithelial ablation zone. Transepithelial PRK-stromal scraping was performed with an identical technique to transepithelial PRK alone, except the stromal surface was scraped with a #64 Beaver blade after laser ablation using the same technique as scraping in the epithelial scrape-PRK group.

For the microkeratome flap group, an automated corneal shaper (System ALK; Chiron Ophthalmics, Claremont, CA) was used with a LASIK ring to produce a flap 130-µm thick that was anatomically centered on the cornea. Suction is frequently difficult to maintain in rabbit eyes with the corneal shaper. Therefore, we used the largest rabbits available, and, with the eye proposed, the surgeon maintained posterior pressure on the LASIK ring while the pass was made with the microkeratome head. The flap was retracted with a spatula, and the surface was irrigated with balanced salt solution before returning the flap to its original position. For the LASIK group, the flap was created in an identical manner, and a 1.5-diopter, 4-mm diameter, 2-µm deep PRK ablation was performed on the exposed stromal bed anatomically centered on the cornea. The bed and flap were irrigated with balanced salt solution, and the flap was returned to its original position on the cornea. For the microkeratome cap-off group, the cap was created with the microkeratome, reflected, and excised at the hinge with scissors.

A temporary tarsorrhaphy was performed using a 5-0 nylon suture (Alcon, Fort Worth, TX) after the procedure in each group. Anesthesia was maintained with supplemental injections. At 4 hours after the procedure, the tarsorrhaphy was removed and the corneoscleral rim excised with surgical scissors and forceps. The animal was euthanized. Four hours was selected as the end point for terminal deoxynucleotidyl transferase-mediated dUTP-digoxigenin nick-end labeling (TUNEL) analysis, because 2 to 4 hours after wounding is the time at which apoptosis of keratocytes detected by TUNEL assay is maximal after mechanical scrape injury, PRK, or LASIK in the rabbit (Wilson SE, unpublished data) or mechanical scrape injury in the mouse.4 By electron microscopy, cellular morphologic changes consistent with apoptosis are detectable immediately after epithelial injury (Wilson SE, unpublished data).*

Two corneas from each group were also evaluated by transmission electron microscopy. Corneas were fixed overnight in 3% gluteraldehyde, 1% paraformaldehyde. Electron microscopy (JEM 1200 EX; JEOL, Peabody, MA) was performed as previously described.12

Corneas for histologic analyses were embedded in OCT compound (Miles Laboratories, Elkhart, IN) in the center of a 10-mm diameter mold so that the center of the cornea could be located for sectioning and frozen in liquid nitrogen for TUNEL assay staining. Sections 7-µm thick that extended transversely across the central cornea were stained according to the manufacturer's instructions (ApopTag assay; Oncor, Gaithersburg, MD) with the peroxidase-based TUNEL assay.4 The TUNEL assay detects fragmentation of the DNA associated with apoptosis.13-15 Our previous study demonstrated that keratocyte cells, which became stained in the TUNEL assay, undergo cellular morphologic changes, detected by electron microscope, that are consistent with apoptosis. Hematoxylin and eosin staining was also performed on sections from each cornea. Photographs were obtained with a fluorescent-light microscope (Nikon, Melville, NY).

The total number of cells in seven nonoverlapping, full-thickness columns extending from the anterior stromal surface to the posterior stromal surface were manually counted for each specimen. The diameter of each column was the 400 X Optiphoto-2 (Nikon) microscopic field. The seven columns in which counts were performed were selected at random from the central cornea for each specimen. Depths were approximated with a micrometer. Variations were expressed as standard errors of the mean (SEM), and statistical comparisons between the groups were performed with the analysis of variance test. P values less than 0.05 were considered statistically significant.

**Results**

Quantitative comparisons between the different procedures, of the number of keratocyte cells that became stained in the TUNEL assay for seven columns of 400X fields extending transversely across the central cornea, are provided in Figure 1. Because the diameter of the laser ablation was 4 mm, the scrape and/or ablation was anatomically centered on each cornea in each group, and the cornea was centered in the mold
at the time of embedding, the central treatment zone of each TUNEL assay (Fig. 2B), although there was notable variability in FIGURE 1. Terminal deoxyribonucleotidyl transferase-mediated dUTP-digoxigenin nick-end labeling (TUNEL)-positive procedures and are provided for the difference between the (for example, comparing PRK with LASIK).

The keratocytes with DNA fragmentation were confined to the proximity of the anterior 50 μm of the central corneal stroma after epithelial scrape-PRK (Fig. 2C).

There was a reduction in the number of cells undergoing apoptosis in the central cornea with transepithelial PRK (Figs. 1, 2D) compared with those undergoing scraping of the epithelium (P = 0.0004) or epithelial scrape-PRK (P = 0.04). The number of keratocytes undergoing apoptosis in the transepithelial PRK group was not different from the unwounded control group (P = 0.99). The few cells that became stained during the TUNEL assay after transepithelial PRK were in the proximity of the anterior 50 μm of the central corneal stroma. Scraping the stromal bed using a #64 Beaver blade after transepithelial PRK did not increase keratocyte apoptosis (9, 9, and 11 TUNEL assay-positive cells in the three specimens, respectively). In a preliminary experiment, we used transmission electron microscopy to demonstrate that the transepithelial method we used removed all the epithelium overlying the phototherapeutic keratectomy ablation before the refractive PRK ablation (not shown).

The difference in the number of keratocytes undergoing apoptosis after removal of a cap of central cornea with the microkeratome and epithelial scrape injury to the corneal surface was statistically significant (P = 0.03). There were fewer than one fourth the number of TUNEL assay-positive cells per cap-off specimen than in the epithelial scrape group (Figs. 2G, 2H). The stromal surface was relatively smooth after the microkeratome cut, and epithelial injury was noted only near the point at which the blade passed through the epithelium (Fig. 2E). TUNEL assay-positive keratocyte cells were present in high numbers in the anterior stroma at the point in the peripheral cornea at which the microkeratome blade passed through the epithelium (Fig. 2F).

Apoptotic cells were also detected in the central cornea after production of a flap with the microkeratome without subsequent laser application. The cells that stained positively during the TUNEL assay in the microkeratome flap group (not shown) were distributed anteriorly and posteriorly to the lamellar incision in a manner identical to that of the LASIK group.

More keratocyte cells underwent apoptosis in the central cornea after LASIK than in the unwounded control, but the difference did not reach statistical significance (P = 0.18; Fig. 1). There was, however, marked variability in the total number of keratocyte cells that became stained during the TUNEL assay in the LASIK group. Two specimens had no cells undergoing apoptosis detected in the central cornea, and two had 84 and 85 cells, respectively. Keratocyte cells that had DNA fragmentation with LASIK were deeper in the cornea and were distributed within a zone approximately 50 μm anteriorly and posteriorly to the lamellar cut (Fig. 2L). Similar numbers of keratocyte cells in the central cornea stained positively during the TUNEL after LASIK and epithelial scrape-PRK, but the distribution of the cells was different (Figs. 2I, 2J, 2K, 2L). The most superficial cells in the central cornea that became stained during the TUNEL assay after LASIK were greater than approximately 50 μm from the corneal surface—in the epithelial scrape-PRK group, most cells were less than 50 μm from the surface. Epithelial injury was noted at the edge of the flap (Fig. 2I) and keratocyte cells undergoing apoptosis were more superficial (Fig. 2J) and increased in number in the peripheral...
cornea than in the central cornea. This increase in apoptosis near the point at which the microkeratome passed through the epithelium was similar in the microkeratome cap-off, microkeratome flap, and LASEK groups.

Transmission electron microscope analysis of corneas from each group, except the three corneas that had scrape after transepithelial PRK, confirmed that keratocyte cells in locations in which staining occurred during the TUNEL assay had cellular morphologic changes consistent with apoptosis (not shown). Keratocyte cellular changes that were noted at 4 hours after each procedure included cell shrinkage, chromatin condensation and fragmentation, and cellular blebbing with the formation of membrane-bound structures that appeared to be apoptotic bodies, as found in our previous study. Keratocyte cells with these characteristics were found in each group, except the unwounded control group, and this finding confirms our TUNEL results. The number and distribution of keratocyte cells undergoing apoptosis were, however, different among the groups and consistent with the results obtained with the TUNEL assay. In the epithelial scrape, epithelial scrape–PRK, and transepithelial PRK groups, keratocyte cells that had morphologic changes consistent with apoptosis were confined to the proximity of the anterior 50 μm to 75 μm of the corneal stroma. Few cells were detected within this zone that did not have morphologic changes of cell death at 4 hours in the group with epithelial scrape injury. In the transepithelial PRK group, however, there were many normal-appearing keratocyte cells in the superficial corneal stroma.

Numerous cells with morphologic changes consistent with apoptosis were detected with transmission electron microscopy in the periphery near the point at which the blade passed through the epithelium in each of the groups in which a cut was made with a microkeratome (not shown). Few polymorphonuclear cells were noted at 4 hours after any of the surgical procedures. After a microkeratome flap was produced, occasional cells with signs of apoptosis anterior and posterior to the lamellar interface in the center of the cornea were noted, although the laser was not applied. These cells were typically within approximately 25 μm to 50 μm of the cut. There were, however, numerous normal keratocytes in the stroma anterior and posterior to the lamellar interface (not shown). In the microkeratome cap-off group, there were few keratocytes noted in the center of the cornea that had morphologic changes consistent with apoptosis. The majority of the keratocyte cells in the central cornea at 4 hours after cap removal were normal, even in the most anterior stroma that was the bed of lamellar cut before cap removal. These cells can be detected by hematoxylin and eosin staining (Fig. 2G). In the LASEK group, more keratocytes appeared to be undergoing apoptosis in the central cornea along the lamellar cut than in the microkeratome flap group, which is consistent with the TUNEL assay findings. There were, however, also numerous normal keratocytes along the lamellar cut in the LASEK group.

**DISCUSSION**

The rapid disappearance of keratocytes in the superficial cornea after epithelial scrape injury is mediated by programmed cell death (apoptosis). Many of the keratocytes that undergo apoptosis do so within 4 hours of epithelial scrape, although changes can be detected by electron microscopy immediately after the injury and for periods of time extending to more than 10 days (Wilson SE, unpublished data). Gao and coworkers have also demonstrated that keratocyte apoptosis can occur for several days after PRK. New keratocytes replenish the anterior stroma for a period of several days after epithelial injury but these keratocytes tend to be present at increased density and to have morphologic changes, such as increased endoplasmic reticulum, suggesting they are activated. Activated keratocytes have been associated with increased collagen deposition and collagen disorganization that correlates with haze and regression after refractive surgical procedures such as PRK. These keratocytes also produce increased levels of growth factors such as hepatocyte growth factor, which may promote epithelial hyperplasia and, therefore, regression of the effect of surgery by stimulating proliferation and inhibiting differentia tion of the corneal epithelial cells. The source of the activated keratocytes that replenish the anterior stroma has not been reported in the literature, but preliminary results from James Zieske of the Schepen’s Eye Research Institute, Boston, MA, (personal communication) and our laboratory (unpublished data) indicate that they are derived from mitosis of remaining keratocyte in the posterior and peripheral corneal stroma.

A recent study has suggested that the epithelial–stromal apoptosis system may function as an antiviral defense mechanism to limit the extension of pathogens such as herpes simplex, varicella zoster, and the smallpox virus from the corneal epithelium to the underlying stroma. We hypothesized that activation of this system by refractive surgical procedures, such as PRK, may initiate the subsequent wound-healing response. Furthermore, we speculate that clinical differences in the wound-healing response between PRK and LASIK, a procedure in which a flap of corneal epithelium and stroma is raised before laser ablation, may be attributable to differential activation of this epithelial–stroma communication system. The present study demonstrates that there are qualitative and quantitative variations in keratocyte apoptosis after alternative refractive surgical procedures or their component manipulations.

An important observation of this study is the effect of a microkeratome cut followed by the removal of a cap of epithelium and anterior stroma on keratocyte apoptosis. Within 4 hours of epithelial scrape in the rabbit, nearly all the keratocytes within 50 μm to 75 μm of the anterior stromal surface had undergone apoptosis. Thus, there was a high density of TUNEL assay-positive cells in the anterior stroma after epithelial scraping. Few, if any, normal cells could be detected in this zone by electron microscopy or by staining with hematoxylin and eosin. The effect of epithelial scrape wound injury is similar in mice, rabbits, and primates. If a cap of epithelium and anterior stroma was removed with a microkeratome, however, there were fewer than 25% the level of TUNEL assay-positive cells in the central cornea compared with the epithelial scrape injury. The difference was statistically significant (P = 0.03). This was also noted by electron microscopy and hematoxylin and eosin staining (Fig. 2G). This finding supports the hypothesis that apoptosis of the keratocytes is triggered by the release of factors from the injured epithelium. Recent studies have suggested interleukin-1 and Fas ligand as potential modulators of this response. The level of apoptosis in the central cornea would have been similar after epithelial scrape and removal of the corneal cap with a microkeratome if keratocyte cell death was caused by exposure of the cells to the atmosphere or tears, osmotic changes in the stroma, or loss of...
FIGURE 2. Terminal deoxynucleotidyl transferase-mediated dUTP-digoxigenin nick-end labeling (TUNEL) assay to detect DNA fragmentation consistent with apoptosis. (A) A normal unwounded rabbit cornea showing stain from the TUNEL assay. The horizontal bars denote the junction between the epithelium (e) and the stroma (s). No keratocyte cells became stained during the TUNEL assay. Magnification, 400X. (B) At 4 hours after a mechanical scrape injury of the corneal epithelium, superficial keratocytes in the central cornea became stained during the TUNEL assay (arrows). Magnification, 1000X.
(C) In the epithelial scrape-PRK group, in which the epithelium was removed by scraping before surface ablation, TUNEL keratocytes that became stained were not in the central anterior stroma. Magnification, 1000×. (D) In transepithelial PRK, fewer keratocyte cells in the anterior central cornea were stained during the TUNEL assay. In this lower power view, only a single keratocyte cell (arrow) stained positively for DNA fragmentation. Magnification, 200×. (E) Hematoxylin and cosin staining of the periphery of a cornea in which the corneal cap was removed after the microkeratome cut. Intact epithelium is noted to the left, peripheral to the point (arrow) at which the blade passed through the epithelium into the stroma. To the right, keratocytes can be noted immediately beneath the stromal surface. Magnification, 400×. (F) TUNEL assay in the periphery of a cornea in which the corneal cap was removed after the microkeratome cut. Intact epithelium is to the right, and the stromal bed after cap removal is to the left. The long arrow indicates the point at which the microkeratome blade passed through the epithelium into the stroma. Note the damage evident in the epithelium at this point and several keratocyte cells that stained positively for DNA fragmentation beneath the point of epithelial injury (small arrows). Similar patterns of apoptosis were noted in each of the groups in which the microkeratome was used to make a lamellar cut. Magnification, 400×. (G) Hematoxylin and cosin staining of the surface of the lamellar bed (large arrows) in the central cornea 4 hours after removal of the cap by cutting with a microkeratome. Note that numerous superficial keratocytes can be noted (small arrows). Magnification, 400×. (H) TUNEL assay of the central cornea, 4 hours after removal of the cap by cutting with a microkeratome, detected few cells with DNA fragmentation. Only one keratocyte (small arrow) was noted in this section from the central cornea along the lamellar surface (large arrows). Magnification, 400×. (I) Hematoxylin and cosin staining of the periphery of a cornea that had LASIK. Note the intact epithelium on the flap. The lamellar cut passed from right to left into the stroma, but the potential space cannot be visualized beyond the arrow. Magnification, 400×. (J) TUNEL section showing stain that was adjacent to that shown in I. The diagonal arrow indicates the same point as the arrow in I. Several keratocytes stained positively during the TUNEL assay (vertical arrows) along the lamellar cut. Note that these staining cells are more prominent during direct microscopic observation, because the color of the stained cells was brown and the corneal stroma was clear. Magnification, 1000×. (K) Hematoxylin and cosin staining of the center of a cornea 4 hours after LASIK. The junction of the epithelium with the underlying stroma is indicated by the horizontal bars. The large arrows indicate the point at which the lamellar cut passed through the stroma. It may not be visible in this section but could be clearly noted at higher magnification. Magnification, 400×. (L) TUNEL assay of the center of a cornea 4 hours after LASIK. The horizontal bars indicate the junction of the epithelium and the underlying stroma. Note that the keratocyte cells that stained positively during the TUNEL assay (small arrows) are distributed on both sides of the lamellar cut (indicated by the large arrows). Note there are no keratocyte cells adjacent to the epithelium staining with the TUNEL assay. Magnification, 400×.
Another possibility is that the laser itself somehow triggers difficulty in ascertaining an endpoint for the epithelial ablation at wound-healing response in the treatment of high myopia are PRK may attenuate the subsequent wound-healing response. Thus, apoptosis was not related to mechanical injury caused by the scraping. We speculate that the photoablative effect of the excimer laser on the corneal epithelium may limit the signaling of cytokines from the epithelium to the keratocytes through photodisruption of the signaling molecules themselves. An important property of the excimer laser is its capacity to disrupt molecules at the level of the carbon-carbon bond by photoablation.20,21 Apoptotic anterior keratocytes were also likely excised by the subsequent refractive ablation of the anterior stroma. The latter does not itself account for the difference, however, because there was more keratocyte apoptosis after epithelial scrape–PRK with an identical excimer laser ablation. This observation made with the TUNEL assay corresponded with what was seen by electron microscopy in comparing the epithelial scrape–PRK and transepithelial PRK groups. There were some keratocyte cells in the central superficial stroma that underwent apoptosis after transepithelial PRK. One explanation for this observation is that there might have been incomplete photodisruption of apoptosis-signaling molecules. Alternatively, limited quantities of signaling cytokines could have been released into the tear film from injured epithelial cells at the margin of the transepithelial ablation. Another possibility is that the laser itself somehow triggers apoptosis in keratocytes in the remaining anterior stroma after either method of PRK.

The reduction in keratocyte apoptosis after transepithelial PRK may attenuate the subsequent wound-healing response. Clinical studies have suggested there is a more diminished wound-healing response and regression after transepithelial PRK than in epithelial scrape–PRK (Johnson D, New Westminster, British Columbia, personal communication).22 Our clinical observations have been similar (Wilson S, unpublished data), but conclusive studies demonstrating the clinical association between transepithelial PRK ablation and diminished wound-healing response in the treatment of high myopia are unavailable. Ablation algorithms used for transepithelial PRK should be derived independently from those used for epithelial scrape–PRK with initial mechanical scraping. Anecdotal reports of overcorrection with transepithelial PRK could be related to a diminished wound-healing response rather than difficulty in ascertaining an endpoint for the epithelial ablation at the epithelial–stromal interface. It should be noted that less than complete ablation of the epithelium overlying the intended refractive ablation (for example, by scraping the final island of epithelium after epithelial ablation) could spare apoptotic cytokines from photoablation and increase the level of apoptosis and, therefore, the wound-healing response with transepithelial PRK (Kim WJ, Shah S, Wilson, SE, unpublished data, 1997). Thus, the method of transepithelial PRK must be consistent when deriving an algorithm for treatment. This study provides a fundamental rationale for considering transepithelial PRK for high myopic corrections or retreatment after regression or scarring after epithelial scrape–PRK, in which it is desirable to limit the subsequent wound-healing response.

In LASIK a flap of stroma and overlying epithelium is raised with a microkeratome before laser ablation of the exposed stromal bed. In PRK the stromal surface is ablated without raising a flap. An observation of this study was the similar mean levels of keratocyte apoptosis in LASIK and epithelial scrape–PRK. There was also notable variation in the level of apoptosis with both procedures. A major difference occurred, however, between the two procedures in the location of the keratocyte apoptotic response. Keratocyte apoptosis after PRK involves only the anterior corneal stroma, typically to approximately 50 μm to 75 μm of stromal depth in the rabbit model, with the cells uniformly affected near the anterior stromal surface. Keratocyte apoptosis in the central rabbit cornea after LASIK has a different distribution. It is noted anterior and posterior to the lamellar cut. Keratocyte apoptosis is undetected in the subepithelial stroma after LASIK, as long as the overlying epithelium is not injured by the microkeratome. The central corneal epithelium overlying the pupil should be carefully preserved in LASIK. Apoptosis is noted throughout 50% or more of the anterior stroma if the central epithelium is intentionally damaged after LASIK (Wilson SE, unpublished data). A pattern of keratocyte apoptosis similar to that in LASIK is noted with the microkeratome cut alone. However, there was a trend toward more keratocyte apoptosis in LASIK (Fig. 1) in which an ablation is performed within the lamellar interface. We speculate that cytokines released from the peripheral epithelium injured by the microkeratome cut can diffuse into the lamellar interface and trigger apoptosis of the bordering keratocytes. If this is the mechanism, then the increase with LASIK compared with the microkeratome flap alone could be a result of the presence of an augmented potential space within the lamellar interface attributable to differences in curvature between the posterior surface of the flap and the lamellar bed after laser ablation. This could allow more tear fluid, along with released cytokines, to pass into the lamellar interface. Variability in this diffusion pathway could theoretically account for a complete absence of apoptosis in some LASIK specimens and significant apoptosis in others. Another potential mechanism is that corneal epithelium or epithelial debris could be deposited in the lamellar interface by the microkeratome blade or fluid backwash from the fornix during the LASIK procedure. Although we did not detect any intact epithelial cells within the interface in the few corneas examined by electron microscopy after LASIK or flap production, only a relatively small total area of cornea could be examined by electron microscopy, and epithelial growth within the interface is a fairly common complication of LASIK.23 Clinically observable epithelial growth within the interface may only occur in a small proportion of cases that have transfer of epithelial debris in the interface. Thus, our data suggest there is a significant wound-healing response within the stroma in some eyes after LASIK. This has been observed clinically, with the level of regression after LASIK varying from essentially zero to as much as 50% in individual cases.24,25 It seems clear that LASIK is associated with less regression than epithelial scrape–PRK in cases with attempted corrections above 8 to 10 D.24,25 Although stromal remodeling is a factor, a recent study (Marshall J, St. Thomas’ Hospital, London, unpublished data) suggested that regression, occurring after PRK, is also attributable to epithelial hypertrophy. Because the central corneal wound-healing response in...
LASIK is deeper within the stroma than in PRK, we hypothesize that there is a diminished effect of activated keratocytes on the overlying epithelium and, therefore, less tendency toward epithelial hypertrophy leading to regression with LASIK.17 Although the present study was performed in rabbits, superficial keratocytes have also been shown to disappear after corneal epithelial scrape wounding in mice,4 rats,1 and primates.3 We demonstrated that the disappearance of keratocytes in mice was mediated by apoptosis.3 In the present study, we confirmed that this process is mediated by programmed epithelial hypertrophy leading to regression with LASIK.17 LASIK is deeper within the stroma than in PRK, we hypothesize overlying epithelium, and, therefore, less tendency toward size that there is a diminished effect of activated keratocytes on cell death in rabbits. It is likely that the response is similar in human corneas. Thus, the variations in keratocyte apoptosis noted among different refractive surgical procedures performed in rabbits are likely to be relevant to human surgery.

There is no direct evidence that controlling keratocyte apoptosis will influence the wound-healing response and, therefore, the outcome of refractive surgical procedures. However, it seems reasonable to suggest this will be the case. Keratocyte apoptosis is detectable immediately after corneal epithelial wounding.4 Thus, in the absence of blood vessels and other tissue components that contribute to wound healing in skin and other tissues, keratocyte apoptosis and subsequent repopulation of the anterior stroma by activated keratocytes could be major determinants of the exuberance of the wound-healing process. However, there are clinical situations, such as inadvertent loss of a cap during a LASIK procedure, in which pronounced wound healing and scarring may occur despite a tendency toward reduced keratocyte apoptosis in the corneal bed noted in a similar situation in this study. There are other components of the wound-healing response that may, under certain circumstances, not be controlled by a reduction in early keratocyte apoptosis. This indicates that there may be additional activators of wound healing that remain unknown.

Future studies should be directed toward assessing the correlation between keratocyte apoptosis and optical or corneal topographic changes occurring after refractive surgery and controlling the wound-healing response after refractive surgical procedures. The results of this study suggest that transepithelial ablation is a promising technique for limiting keratocyte apoptosis and, therefore, the subsequent wound-healing response after PRK. In addition, pharmacologic methods for preventing the initial keratocyte apoptotic response associated with PRK or LASIK could improve the predictability of both procedures. Promising targets are the cytokines and growth factors that mediate interactions between epithelial cells and keratocytes.

References