Role of Keratocyte Loss on Corneal Wound Repair after LASIK

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PURPOSE. To investigate whether an initial keratocyte loss intensifies central corneal wound repair after LASIK in rabbits.

METHODS. New Zealand White rabbits received either conventional LASIK (~8 D, 6-mm diameter) or LASIK combined with a 7-mm diameter, epithelial denudation (LASIK-scrape). Animals were examined during 4 months by slit lamp and in vivo confocal microscopy to monitor changes in central corneal morphology, light backscattering (haze), and sublayer thickness. At various time points, corneas were processed for histology and stained for nuclei; F-actin; ED-A fibronectin; α-smooth muscle actin; TGF-β1, -β2, and -β receptor II; and connective tissue growth factor (CTGF).

RESULTS. In vivo confocal microscopy identified no major acellular zones or changes in cell morphology or reflectivity after conventional LASIK. By contrast, a complete loss of keratocytes was observed in the anterior 77 ± 25 μm stroma 1 week after LASIK-scrape. Highly reflective migratory fibroblasts gradually repopulated the acellular zone, and by week 8, quiescent-appearing keratocytes were observed throughout the stroma. Correspondingly, stromal light backscattering peaked at 2 weeks after LASIK-scrape (2200 ± 620 U) followed by a decline to approximately 60 U from week 8; comparable to the slightly increased reflectivity (approximately 50 U) observed after conventional LASIK (ns). Stromal thickness appeared stable 8 weeks after both LASIK and LASIK-scrape, after a re-growth of 13 ± 3 and 20 ± 11 μm, respectively (ns). In addition, both procedures induced a minor and comparable epithelial hyperplasia of 4 ± 2 and 7 ± 5 μm, respectively (ns). No myofibroblast transformation or TGF-β growth factor expression was observed below the flap after either treatment.

CONCLUSIONS. LASIK-scrape induces an anterior keratocyte loss, leading to development of temporary haze during cell repopulation. However, 8 weeks after both LASIK and LASIK-scrape, only a slightly increased reflectivity is noted at the interface. Corneal thickness is stable by week 8, and stromal regrowth and epithelial hyperplasia are comparable after both treatments. Thus, an initial loss of stromal keratocytes does not appear to intensify corneal wound repair after LASIK. (Invest Ophthalmol Vis Sci. 2004;45:3499–3506) DOI:10.1167/iovs.04-0391

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Photorefractive keratectomy (PRK) and laser in situ keratomileusis (LASIK) have become popular surgical procedures for the correction of refractive errors. However, postoperative regression of the refractive effect1–5 and development of stromal haze4,5 remain serious clinical problems related to corneal wound repair. Thus, myopic regression after PRK has been correlated to regrowth of the corneal stroma,5 and in LASIK-treated rabbits, significant stromal rethickening and epithelial hyperplasia were recently demonstrated.6 After PRK, increased light-scattering from reflective myofibroblasts and other wound-healing phenotypes is a major cause of stromal haze.5,7 By contrast, the central cornea generally remains clear after LASIK,2,4,6 although haze due to fibrosis and myofibroblast transformation develops next to the flap margin, at the basement membrane incision, in LASIK-treated rabbits.8

The differences between PRK and LASIK have led to intense research in corneal wound repair, and notably the initial loss of keratocytes has received considerable attention. Epithelial scrape injuries have been known for several years to lead to disappearance of the anterior keratocytes.9–12 Within the past decade, this cell loss has been demonstrated to occur through apoptosis,13 although the underlying mechanism is debated.14–17 Because keratocyte apoptosis appears to be the first identifiable stromal response after epithelial injury, it has been hypothesized to be the initiator of subsequent corneal wound repair,13,16 and as such is a promising target for therapeutic intervention. However, an isolated epithelial scrape injury induces only temporary stromal haze during cell repopulation without changes in corneal thickness.11 Thus, the wound repair after isolated epithelial injuries appears to be different from the changes that occur after combined epithelial-stromal injuries such as PRK or LASIK.5,6,18,19 The integrity of the epithelial basement membrane has been suggested to play a role in this difference.12,20 and development of stromal fibrosis was recently demonstrated to be restricted to the basement membrane incision at the flap margin in LASIK-treated rabbits.8 Still, a postoperative keratocyte loss may influence the magnitude of the stromal wound-healing response. Thus, in rabbits, the initial cell loss after PRK has been reported to be significantly greater than that occurring after LASIK,21 which has been hypothesized to explain the clinical differences between the two surgical procedures. Also, an epithelial scrape injury after LASIK in humans has been speculated to intensify the wound healing response with induction of corneal haze.22 Yet, it remains to be fully elucidated whether an initial keratocyte loss enhances corneal wound repair after refractive surgery.

A major problem in most experimental studies on wound healing after photorefractive surgery is that histologically detectable changes may have little relevance to the clinically important parameters (including corneal sublayer regrowth and haze development). Thus, to study corneal wound repair adequately, a combination of both ex vivo and in vivo techniques should be used. In the present study, in vivo confocal microscopy and conventional histology were combined to investigate the impact of a major keratocyte loss by epithelial removal on corneal wound repair after LASIK.
**Materials and Methods**

**Animals**

New Zealand White rabbits (weight 4.0–5.5 kg; the Danish Serum Institute, Copenhagen, Denmark) were treated in one eye with either conventional LASIK or LASIK combined with removal of the central 7-mm diameter epithelium (LASIK-scrape). Before surgery and at all examinations, animals were anesthetized with topical 0.8% oxybuprocaine and intramuscular injections of 2.0 mg/kg midazolam and 0.5 mL/kg Hypnorm (0.315 mg/mL fentanyl and 10 mg/mL fluanisone; Janssen Pharmaceuticals, Beerse, Belgium). Two drops of 0.5% chloramphenicol and 3 mg/mL ciprofloxacin were applied before surgery whereas the postoperative treatment included topical ciprofloxacin for 2 days and topical chloramphenicol and systemic buprenorphine (0.05 mg/kg) for 5 days. To avoid changes in corneal hydration, all eyes were closed immediately after the induction of anesthesia and until examination or surgery. Rabbits were killed by injection of 150 mg/kg sodium pentobarbital. All animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research, and the study was approved by the Danish Animal Experiments Inspectorate.

**Surgery**

In all animals, the nictitating membrane was removed 1 week before the refractive surgical procedure. After gentle proptosis of the eye, LASIK was performed by cutting a 9-mm diameter, hinged corneal flap with a microkeratome (Supratome; Schwind, Kleinostheim, Germany) and a new, disposable microkeratome blade (no. 19407; Schwind) for each eye. The flap was lifted, and the underlying stroma received a 6-mm diameter, −8.0 D correction by excimer laser (MEL 70 G-Scan; Asclepion, Jena, Germany). After the flap was repositioned, a bandage soft contact lens (7.4 mm radius of curvature; Igel Rx 67 Spheric UV; Ultravision International, Bedfordshire, UK) was inserted for 5 days to prevent flap dislocation.

In LASIK-scrape–treated corneas, the central, 7-mm diameter epithelium was gently removed with a hockey knife, immediately before the refractive surgical procedure. Subsequently, these animals received exactly the same treatment as rabbits treated with conventional LASIK.

**Slit Lamp Biomicroscopy and In Vivo Confocal Microscopy**

Seven LASIK- and seven LASIK-scrape–treated corneas were examined before surgery and at 1, 2, 3, 4, 6, 8, 12, and 16 weeks using slit lamp biomicroscopy and in vivo confocal microscopy. A tandem scanning confocal microscope (Tandem Scanning Corp., Reston, VA) was used to evaluate central corneal morphology and perform Confocal Microscopy Through-Focusing (CMTF) for sublayer pachymetry and assessment of light-backscattering (haze).

The contralateral, untreated eyes were examined before surgery and at 16 weeks to monitor physiological changes due to ocular growth.

To locate the photoablation center (defined as the region of minimal stromal thickness), 15 to 10, two-way CMTF-scans were performed within a 2-mm diameter zone at the corneal apex. Only CMTF-scans obtained at the photoablation center were used for further analysis of sublayer thickness and light backscattering. In brief, thickness measurements were performed by calculating the z-axis distance between in-focus images of relevant corneal structures (including the epithelium, basement membrane, interface particles, and endothelium). Increased light backscattering from structures of interest (such as the LASIK interface and reflective keratocyte phenotypes) was assessed by integrating the area below the corresponding peaks on the CMTF curve. Three-dimensional (3-D) reconstructions of selected CMTF scans were generated and representative two- (2-D) and 3-D images were contrast adjusted.

**Histology**

Corneas were obtained for histology for up to 4 months after surgery. The tissue was processed for cryosectioning and stained as previously reported for F-actin, ED-A fibronectin, α-smooth muscle actin (α-SMA), transforming growth factor (TGF)-β1 and β2, connective tissue growth factor (CTGF), and TGF-β receptor II (TGF-βRII). In all tissue sections, colocalization of cell nuclei was performed using Hoechst 33342 (2 μg/mL; Molecular Probes, Leiden, The Netherlands). Control experiments included staining of tissue from unoperated animals, use of irrelevant isotype-matched primary antibodies, omission of primary or secondary antibodies, and preadsorption of primary antibodies with corresponding growth factors. Sections were evaluated using an inverted fluorescence microscope equipped with a zoom-adaptor (range 0.4–2.0×). Digitized images were contrast adjusted and merged.

**Statistics**

All CMTF-measurements were corrected for the change in refractive index between the immersion fluid (2.5% methylcellulose) and the cornea. Furthermore, all thickness measurements in surgically treated corneas were corrected for the growth of the nonsurgically treated fellow cornea, assuming that the physiological growth-rate was identical in both treated and untreated eyes. Statistics were performed on computer (StatsDirect, ver. 2.2.7; CamCode, Ashwell, UK), and the analyses comprised the unpaired t-test, the paired t-test, Pearson’s correlation, and linear regression analysis. Data are reported as the mean ± SD.

**Results**

**Slit-Lamp Biomicroscopy**

In the preoperative cornea (Fig. 1A), a uniform stroma with low reflectivity was observed using slit lamp biomicroscopy. However, 1 week after LASIK-scrape (Fig. 1B), a dark band was detected in the anterior stroma (star), overlying a region of slightly increased light-backscattering. Within 4 weeks, the dark band was replaced by a brightly reflecting layer (Fig. 1C, star), giving rise to corneal haze. The haze gradually dimin-
ished, and by week 16 (Fig. 1D), stromal light-scattering appeared almost similar to that of the preoperative cornea (compare Fig. 1D with 1A), apart from reflecting particles (Fig. 1D, arrow) and a slightly increased light backscattering at the LASIK interface (arrowheads).

In contrast to LASIK-scrape, few changes were observed after conventional LASIK using slit-lamp biomicroscopy. Thus, throughout the study, reflecting particles and a detectable LASIK interface were the only changes identified; similar to the findings sixteen weeks after LASIK-scrape (Fig. 1D).

**Cell Morphology**

At all time points after conventional LASIK, the central epithelial morphology was similar to that of the preoperative cornea, as previously demonstrated. By contrast, the epithelium appeared immature 1 week after LASIK-scrape with variation in cell size and reflectivity (Fig. 2A), and with prominent nuclei in the basal epithelial layers (Fig. 2B), indicating ongoing cellular proliferation. By week 2, the epithelial morphology had normalized and could no longer be distinguished from that of the preoperative cornea.

In the stroma, no major acellular zones were identified after conventional LASIK, and stromal keratocytes appeared quiescent at all time points. By contrast, three morphologically distinct regions were observed 1 week after LASIK-scrape: (1) an anterior, acellular zone (Fig. 2C) that stretched approximately 20% into the stroma and corresponded to the dark anterior region detected by slit lamp biomicroscopy (Fig. 1B, star); (2) a middle zone of highly reflecting, spindle-shaped (migratory) cells (Fig. 2D); and (3) a posterior zone (approximately 60% of the stroma) of normal-appearing, quiescent keratocytes (Fig. 2E). Over time, the acellular zone was repop-
Corneal Light-Scattering

Quantitative changes in corneal light-scattering were examined using CMTF light-intensity profiles. In preoperative corneas, distinct peaks were generated by the superficial epithelium, the epithelial basement membrane, and the endothelium (Figs. 4A, H), whereas no specific peaks were identified in the stroma. After conventional LASIK (Figs. 4B–G), the only major change in corneal light-scattering was an additional small peak generated by the interface (arrowheads). Over time, the reflectivity of this peak diminished from 142 ± 97 U by week 1 (Fig. 5) to a near-constant level of approximately 50 U from week 8. In contrast to conventional LASIK, distinct changes in the CMTF light-intensity profiles were observed after LASIK-scrape (Figs. 4I–N). Thus, by week 1, migratory cells gave rise to a prominent peak (x’) located below the acellular, low-reflecting region in the anterior stroma, corresponding to the 3-D observations (compare Fig. 4I with 3F). Over time, this peak (x’) shifted anteriorly, and by week 3 (Fig. 4K), the migratory cells had reached the basement membrane, indicating that repopulation of the acellular zone was complete. The migratory cells caused an increase in stromal light-scattering from 1 400 ± 940 U by week 1 to 2 200 ± 620 U by week 2 (Fig. 5). Subsequently, reflectivity declined, suggesting transformation from a reflective to a more transparent keratocyte phenotype. By week 8, stromal reflectivity in LASIK-scrape-treated corneas reached a near-constant level of approximately 60 U (Fig. 5), comparable to the reflectivity (approximately 50 U) observed 8 weeks after conventional LASIK (ns). In both groups, this slightly elevated reflectivity was caused by particles persisting at the interface (Figs. 2H, 4, arrowheads).

Keratocyte Loss

No major keratocyte loss was identified by in vivo confocal microscopy at any time point after conventional LASIK. By contrast, an acellular region of 77 ± 25 μm was detected in the anterior stroma 1 week after LASIK-scrape (Fig. 1B, 2C, 3F, and 4I). By week 2, the thickness of this zone had decreased significantly to 37 ± 15 μm (P < 0.05), and after 3 weeks it could no longer be detected (Figs. 1C, 3G, 4K–L); demonstrating complete repopulation of the anterior stroma.

Changes in Stromal Thickness

In preoperative corneas, stromal thickness averaged 339 ± 23 μm with no significant difference between LASIK- (335 ± 26 μm) and LASIK-scrape- (344 ± 19 μm) treated eyes. After both treatments, minimal stromal thickness was not observed until week 2, due to edema during the first week (Fig. 6). The photoablation depth (defined as the difference in stromal thickness before and 2 weeks after surgery) averaged 89 ± 8 μm after conventional LASIK. By contrast, the photoablation depth measured 70 ± 11 μm after LASIK-scrape (P < 0.01), a difference that may be related to changes in corneal hydration due...
changes in epithelial thickness after LASIK and LASIK-scrape, measuring 139 ± 38 and 147 ± 46 μm, respectively (ns). Stromal thickness appeared stable by 8 weeks after a stromal regrowth of 13 ± 3 μm after LASIK, comparable to the 20 ± 11 μm regrowth observed after LASIK-scrape (ns). After either treatment, no significant correlation was identified between the amount of stromal regrowth and the preoperative corneal or epithelial thickness, the photoablation depth, or the cumulative haze development. All CMTF measurements were corrected for the preoperative corneas (A, H), the superficial epithelium (a), epithelial basement membrane (b), and endothelium (d) gave rise to prominent peaks in the light-intensity profiles, whereas no specific peaks could be identified in the corneal stroma (c). At all time points after conventional LASIK (B-G), the interface gave rise to a small peak (arrowheads). One week after LASIK-scrape (I), a low reflective region (y') corresponding to the acellular zone was identified between the basement membrane (b) and a prominent peak (x') caused by highly reflecting, migratory cells. By week three (K), the acellular zone could no longer be detected, indicating complete cellular repopulation. Stromal reflectivity gradually decreased, and by week 16 (N) the light-intensity profile appeared almost similar to that of the preoperative cornea. At all time points, a small peak (arrowheads) was generated by the interface. All scans are aligned relative to the position of the endothelium.

Changes in Epithelial Thickness

The preoperative epithelial thickness measured 45 ± 3 μm, with no significant difference between LASIK (44 ± 3 μm) and LASIK-scrape–treated (47 ± 3 μm) animals. After conventional LASIK, an epithelial hyperplasia of 4 ± 2 μm (P < 0.01) was detected from week 1 (Fig. 6). After LASIK-scrape, the preoperative epithelial thickness was restored by week 2, followed by development of a 7 ± 5 μm hyperplasia (P < 0.01) between weeks 2 and 6, similar to the changes observed after conventional LASIK (ns). The epithelial hyperplasia after either treatment did not correlate significantly with the preoperative corneal or epithelial thickness, the photoablation depth, or the cumulative haze development. In nonsurgically treated, fellow corneas, no changes in epithelial thickness were detected.

F-actin and ED-A Fibronectin Expression

During the first 3 weeks after LASIK and LASIK-scrape, expression of ED-A fibronectin was observed as a thin layer at the interface (Fig. 7A, arrowheads) compared with the lack of fibronectin in normal, untreated corneas. At all time points after conventional LASIK, no changes in the preoperative distribution of F-actin was detected. In contrast, elongated cells with distinct F-actin expression (Fig. 7B, arrows) were identified below the acellular zone (star) from 1 to 3 weeks after LASIK-scrape, indicating transformation into migratory fibroblasts.

Myofibroblast Transformation and TGF-β Pathway Signaling

After both LASIK and LASIK-scrape, α-SMA expression was identified in the central cornea at any time point, indicating that myofibroblast transformation did not occur. Correspondingly, no expression of TGF-β1 or β2, CTGF, or TGF-βRII was detected below the flap after either treatment, suggesting that
neither treatment induced activation of TGF-β signaling pathways in the central corneal stroma.

It is important to note that after both LASIK and LASIK-scrape, a much more intense wound-healing response was observed at the flap margin compared with the corneal center. Thus, in a narrow region immediately peripheral to the flap edge, TGF-β expression, myofibroblast transformation, extracellular matrix deposition, and wound contraction were observed. This fibrotic wound repair at the flap margin appeared similar in both LASIK and LASIK-scrape–treated animals and was recently characterized in a separate paper.8

**DISCUSSION**

In the present study, characteristic changes in central corneal morphology were observed during the first 8 weeks after LASIK-scrape. The changes included an initial, massive loss of keratocytes in the anterior stroma followed by gradual repopulation of the acellular region by highly reflecting, migratory fibroblasts. By week 3, repopulation was complete and corneal morphology gradually normalized. These morphologic events appeared similar to the changes previously reported after simple epithelial debridement.9–12 However, in addition to these putative cellular events in the anterior stroma, minor changes were observed at the interface after LASIK-scrape. Thus, a thin layer of extracellular material (including ED-A fibronectin) was deposited at the interface during the first few weeks, and reflecting particles (recently demonstrated to consist of plastic fragments from the microkeratome blade)26 could be detected at all time points. These minor morphologic changes at the interface appeared similar after conventional LASIK versus LASIK-scrape.

The morphologic changes after LASIK and LASIK-scrape were accompanied by corresponding changes in corneal reflectivity. Thus, after conventional LASIK, corneas appeared clear, apart from slightly increased light-scattering (up to approximately 140 U) at the interface. In contrast, the highly reflecting migratory fibroblasts after LASIK-scrape gave rise to a temporary haze development of up to 2200 ± 620 U during repopulation of the acellular region, comparable to previous reports on corneal light-scattering after simple epithelial debridement.31 Yet, by week 8, corneal transparency was restored, and only a slight increase in interface reflectivity was noted, which was due to persisting particles. Thus, the changes caused by cell loss and repopulation were fully resolved 8 weeks after surgery, at which time point LASIK-

![Figure 5](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932925/)  
**Figure 5.** Quantitative changes in stromal light-scattering after LASIK (n = 7, ○) and LASIK-scrape (n = 7, □) as measured using CMTF. Two weeks after LASIK-scrape, stromal reflectivity peaked at approximately 2200 U and declined to a near-constant level of approximately 60 U from week 8. After conventional LASIK, the maximum increase in stromal reflectivity (approximately 140 U) was observed by week 1, followed by a decline to a constantly elevated level of approximately 50 U at week 8.

![Figure 6](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932925/)  
**Figure 6.** Stromal and epithelial thickness measurements before and after LASIK (n = 7, closed symbols) and LASIK-scrape (n = 7, open symbols). After both treatments, minimal stromal thickness was observed until week 2 due to initial edema. From week 2, stromal thickness gradually increased and appeared stable 8 weeks after surgery. Stromal regrowth was not significantly different between the two treatments. Conventional LASIK induced a slight epithelial hyperplasia from week 1. After LASIK-scrape, preoperative epithelial thickness was restored by week 2, followed by a minor hyperplasia between weeks 2 and 6. All thicknesses are given relative to their preoperative value (Index).
scrape-treated corneas were indistinguishable from corneas treated with conventional LASIK. Overall, the initial changes in morphology and reflectivity after LASIK-scrape appeared very similar to those after an isolated epithelial injury, whereas the minimal but permanent changes at the interface were similar to those induced by conventional LASIK. The wound repair after LASIK-scrape thus seemed to consist of two separate parts: temporary changes in the anterior stroma caused by removal of the epithelium and changes at the interface in the corneal midstroma due to LASIK-surgery.

The keratocyte loss observed after LASIK-scrape was comparable to the cell loss previously reported after epithelial debridement. Apoptosis has been reported to be the main mechanism leading to this loss of keratocytes. However, the factors inducing the apoptotic response remain to be elucidated and are subject to intense debate. Thus, in two recent studies, tear fluid was elegantly demonstrated to induce a major keratocyte loss in mouse corneas, whereas epithelial factors appeared to be of minor importance. Yet, substances from the wounded epithelium have also been suggested to be main contributors to apoptosis. The loss of keratocytes has been reported to be the first observable change after epithelial or epithelial-stromal injuries. Apoptosis has therefore been suggested to be the initiator of the subsequent corneal wound repair. The magnitude of the initial cell loss (through apoptosis or necrosis) after PRK or LASIK has further more been hypothesized to explain differences in wound repair and clinical outcome, with PRK inducing a more intense wound repair as well as more haze and stromal regrowth than LASIK. Although a few studies have reported a minor loss of keratocytes after conventional LASIK in rabbits, no acellular zones were detected in the present study using in vivo confocal microscopy. Yet, by 8 weeks, a 13 ± 4 μm stromal regrowth and a 4 ± 2 μm epithelial hyperplasia were detected. These changes were comparable to the 20 ± 11 μm regrowth and 7 ± 5 μm hyperplasia observed after LASIK-scrape. Thus, the morphologic changes and the major keratocyte loss induced by epithelial debridement in LASIK-scrape-treated rabbits did not per se appear to intensify the corneal wound repair in terms of stromal or epithelial regrowth.

In this study, neither LASIK nor LASIK-scrape induced TGF-β signaling or myofibroblast transformation in the corneal center. Thus, neither treatment seemed to provoke fibrotic stromal wound repair. Yet, myofibroblast transformation and development of central corneal fibrosis have been demonstrated after combined epithelial-stromal injuries such as PRK. The postoperative loss of keratocytes has been hypothesized to initiate this fibrotic response. However, in the present study of LASIK-scrape-treated rabbit eyes, the combined epithelial-stromal injury caused a massive keratocyte loss, but no fibrosis developed. This observation clearly demonstrates that factors other than loss of keratocytes control the development of stromal fibrosis. In previous studies, the basement membrane has been demonstrated to bind certain growth factors, suggesting that it acts as a barrier to profibrotic substances from the epithelium or tear fluid. Furthermore, TGF-β signaling, myofibroblast transformation, and fibrotic wound repair were recently demonstrated at the LASIK flap margin, strictly localized to the epithelial basement membrane incision. In LASIK-scrape-treated rabbits, the lamina densa of the epithelial basement membrane remained intact over the central cornea, contrary to most other epithelial-stromal injuries. Thus, the present findings support the hypothesis that the integrity of the epithelial basement membrane may be important for the development of stromal fibrosis.

All results taken together, the major keratocyte loss in LASIK-scrape-treated rabbits induced only transient corneal wound repair during cellular repopulation, similar to the response observed after simple epithelial debridement. Thus, in contrast to other epithelial-stromal injuries, LASIK-scrape was not associated with enhanced regrowth, stromal fibrosis, or development of prolonged corneal haze. This firmly demonstrates that the intensity of the wound repair after stromal injury is not determined by the magnitude of the initial keratocyte loss, as hypothesized by some authors. By contrast, the epithelial basement membrane may be hypothesized to regulate the stromal wound repair intensity, with fibrosis being localized to regions with overlying basement membrane defects. Transplantation of amniotic membrane (that contains a thick basement membrane) has been suggested as a treatment for various pathologic conditions at the corneal surface and has been reported to promote epithelial healing and reduce stromal scarring. Moreover, the amniotic membrane has been found to reduce inflammation and haze development after PRK. Although, the mechanisms underlying the potential beneficial effects of the amniotic membrane are unclear, it may be speculated that it mimics the function of the epithelial basement membrane.

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References


15. Zhao J, Nagasaki T. Lacrimal gland as the major source of mouse tear factors that are cytotoxic to corneal keratocytes. Exp Eye Res. 2003;77:297–304.


