The Healing Process at the Flap Edge

The healing process may influence the development of corneal ectasia after LASIK.

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Post-LASIK keratectasia, a progressive deformation of the cornea occurring after surgery, is associated with severe visual disturbance. In most reports, eyes develop a progressive central or inferior corneal steepening associated with a significant increase in myopia and a progressive thinning of the cornea. This typically occurs months to several years after the primary procedure; however, it may occur sooner if the patient undergoes a secondary enhancement. Generally, corneal ectasia follows higher attempted corrections, but it may also transpire following correction of low myopia.1,2

Post-LASIK ectasia seems to result from three potential causes: (1) underlying defective corneal disease or biomechanics, such as keratoconus or its forme fruste, (2) too much tissue removal, which is probably rare except in cases of high myopia, or (3) the lack of biomechanical support from the LASIK flap, generally because the flap is deep and contributes little or no strength to the cornea.

We posed the following question: Why don’t lamellar keratoplasty patients become ectatic, even when the lamellar bed is dissected to Descemet’s membrane? We postulated that the difference in outcomes between LASIK and lamellar keratoplasty is due to the strength contributed by the fibrotic scarring of sutures. To test our theory, we dissected flaps in rabbit corneas similar to LASIK flaps.4 We then sutured half of the flaps—removing the sutures before increasing the intraocular pressure (IOP)—and compared the bulging of the corneas with the other half of the eyes, which had not been sutured.

SURGICAL PROCEDURE

A total of 20 New Zealand white rabbits received intramuscular xylazine 10 mg/kg, ketamine HCl 50 mg/kg, and topical anesthesia (proparacaine HCl). A lid speculum was placed into the rabbits’ left eye, and a corneal flap (160 µm) with a nasal hinge position was made in one of the eyes with a Chiron Automatic Corneal Shaper (Bausch & Lomb, Rochester, New York). A new blade was used for each eye. The flap was lifted with a Lindstrom spatula (ASICO LLC, Westmont, Illinois) and immediately replaced into its original bed. The rabbits were then divided in two groups. In group A, the flap was allowed to dry in position for 3 minutes until it adhered to the stromal bed. The flap was left without sutures. In group B, the flap was sutured with 12 interrupted 10-0 nylon sutures (Alcon Laboratories, Inc., Forth Worth, Texas) placed around 300º. In all eyes, a tarsorrhaphy using a single 5-0 nylon suture (Alcon Laboratories, Inc.) was left in place for the first 24 hours. All eyes were treated with prednisolone acetate 1% and moxifloxacin drops four times daily for 1 week and buprenorphine 0.05 mg/kg intramuscularly for 2 days. In group B, the stitches were removed under general anesthesia after 3 weeks.

IMMUNOHISTOLOGY

Six weeks after surgery, all rabbit eyes were enucleated and the entire corneas were excised, bisected, and embedded in an optimal cutting temperature compound (Miles Inc., Elkhart, Indiana). Cryostat sections (8 µm) were prepared from each cornea, air dried, and then stored at -80°C. Alpha-SMA, a marker of stromal myofibroblasts, is an important factor in wound healing. We stained for rabbit corneal myofibroblasts (RCM), and the corneas were exami...
Furthermore, the biomechanical strength of the posterior stroma is less than that of the anterior stroma. Because the load-bearing function of the anterior stroma is disabled after keratotomy, only the weaker, deep stroma is left to maintain corneal integrity.9

Recently, we have noticed a trend toward less invasive corneal graft surgery. Newer techniques, including deep anterior lamellar keratoplasty (DALK), remove approximately 80 to 90% of the corneal tissue and spare Descemet’s membrane and the endothelium.10-13 No corneal ectasia following DALK was reported in our studies, and few reports of corneal ectasia following this procedure have been reported in the literature.14 In fact, deep lamellar surgery has been postulated as a means to treat patients with keratoconus.15

In another study,16 we used in vivo confocal microscopy to follow the healing process after deep lamellar keratoplasty. Not only did hyperplastic epithelial cells plug the edge of the incision, but many activated keratocytes were found beneath the edge of the cut, aggregating into a dense cluster mainly around the sutures. Activated keratocytes, or repair-fibrocytes, are involved in the production of repair extracellular matrix.17,18

We postulate that the strength of the lamellar keratoplasty wound is derived from the sutures, which enhance the healing response at the wound’s edge. Our study clearly showed more corneal steepening after increasing the IOP from baseline to 25 mm Hg when no sutures were used compared with when corneal sutures were used. Therefore, we hypothesize that sutures induce a stronger healing at the edge of the corneal flap, increasing its mechanical strength and perhaps preventing the late ectasia seen in some LASIK eyes.

We also hypothesize that corneas with post-LASIK ectasia may be sutured just as lamellar grafts are, thereby promoting wound healing in an attempt to increase the mechanical stability of corneas that are compromised by flap creation. Several mechanisms for this probably exist, of which the most likely combination of events is a rearrangement of
CORNEAL TOPOGRAPHY
At 6 weeks, corneal topography was performed at the rabbits' baseline IOP (14 mm Hg) and two artificially increased IOPs (25 and 45 mm Hg). Before the topography was taken, an anterior chamber maintainer (Eagle Labs, Rancho Cucamonga, California), implanted through a paracentesis performed at the 6-o'clock position, was connected to an infusion bottle that was raised to achieve the desired IOP. The average of three readings within 5% confidence was used to achieve the correct IOP measurement (Tono-Pen applanation tonometry; Medtronic Ophthalmic, Minneapolis, Minnesota).

The headrest of the corneal topography unit (Keratron portable topographer; Opulon EyeQuip, Ponte Vedra, Florida) was applied gently to the rabbits' heads, ensuring steady positioning for reproducible maps. Moving the unit toward the eye, the corneal vertex automatically triggered the image capture as the system detected the vertex in the correct position. Six maps of the same eye were captured and automatically transferred to a computer for processing. The system then performed a repeatability check and any difference between the maps was plotted in diopeters. If the difference between corresponding points was more than 1.00 D, it was automatically deleted. The average of the simulated keratometry at a 3-mm optical zone from the six maps was recorded as the eye's keratometric (K) value.

CELL NUMBER ANALYSIS
Photographs of the tissue sections were acquired. The percentage of alpha-SMA positive cells was calculated with respect to a fixed area (10 μm²) for each sample, and a statistical analysis was performed.

In both groups, three sets of six topographies of each eye were performed. The first set was at the baseline IOP and the second and third sets were at the artificially increased IOPs. A delta K1 value, indicating the difference in the simulated K value at baseline and at 25 mm Hg was calculated for all eyes. The mean steepening effect was 2.74 ± 0.38 D in the unsutured group and 1.08 ± 0.27 D in the sutured group (P < 0.05; Figure 1). Similarly, a delta K2 value, indicating the difference in the simulated K value at baseline and at 45 mm Hg, was registered. It showed a mean steepening effect of 3.02 ± 0.87 D in the unsutured group and 0.75 ± 0.44 D in the sutured group (P < 0.05). An additional increase in IOP (25–45 mm Hg) did not produce any further steepening effect in either group (delta K2; Figure 2).

EXPRESSION OF ALPHA-SMA
Increased expression of alpha-SMA cells was observed along the peripheral flap interface in all eyes. Interestingly, higher expression of alpha-SMA cells was observed in the sutured flap group, especially in areas where the previous nylon sutures were placed (Figure 3).

An association between total stromal cells and the number of positive alpha-SMA positive cells in the peripheral interface flap area was determined. The percentage of alpha-SMA positive cells was then compared with the total diamino-2-phénylindole (DAPI) positive cells in both groups. Six weeks after surgery, the peripheral flap interface in group B (sutured group) consisted of 14.3 ± 14.15% of positive alpha-SMA compared with 4.18 ± 3.76% in group A (unsutured group).

Increased severing of corneal lamellae is the likely cause of biomechanical instability following LASIK. Collagen lamellae are more densely interfused in the superficial third of the stroma compared with the deeper two thirds of the stroma.
lamellae—possibly due to altered adhesion—associated with increased activity of degradative enzymes and ground substance. The use of sutures may induce a foreign body reaction at the flap edge, simulating an infiltr of inflammatory cells, a transformation of myofibroblasts, and a synthesis of new ground stromal substance. Our study clearly shows a significant increase in the amount of positive alpha-SMA cells in the peripheral stromal flap interface in previously sutured flaps. An increase in myofibroblasts at the flap’s edge may induce a stronger wound healing reaction that may act as a belt, preventing the bulging effect of the central cornea when the IOP is increased.

The use of sutures to increase the wound-healing response may, however, induce astigmatism and unpredictable refractive results. Growth factors, such as transforming growth factor-beta (TGF-beta); the use of tissue adhesive agents; or even temporary sutures, such as tacs, that take place of sutures at the wound periphery may provide adequate strength to the peripheral wounds and reduce the risk of ectasia. As expressed in the cornea, TGF-beta regulates extracellular matrix deposition, cell proliferation, and migration.

The cornel flap created with a mechanical microkeratome is a meniscus-shaped flap—meaning it is thinner in the center and thicker at the edge—that produces almost no healing response. Alternatively, the femtosecond laser produces a flap with uniform thickness from edge to edge. Femtosecond LASIK flaps are created in a two-part resection, first by a horizontal resection plane and second by a partial cylindrical arc cut. The surgeon can adjust the angle of these two intersecting resections, cutting more collagen fibers when using steep angles and possibly inducing a stromal healing response similar to that of deep lamellar keratoplasty. If, in fact, the strength of the central peripheral wound is essential (in addition to the residual stromal bed thickness and absence of preoperative corneal pathology) in preventing ectasia after a lamellar cut, this research will have important implications for both refractive and lamellar surgery.

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