Corneal Wound Healing after LASIK Surgery

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Introduction

As refractive surgery has grown in popularity over the last decade, the types of surgical options available to correct a patient’s refractive error have also increased in number and become more complex. A recent annual survey that evaluated the variety and volume of refractive surgery performed by American Society of Cataract and Refractive Surgery (ASCRS) practicing ophthalmologists shows this diversity of surgical options clearly.1 The ACRS survey also continues to report that LASIK is still the number one technique being performed in the United States (approximately 1.3 million LASIK procedures are performed annually in the United States). Although initial excitement about LASIK led primarily to clinical outcome studies, implementation of new cutting-edge technological instruments, and implementation of new adjunctive measures, basic science research on the anatomy, biology, and physiology of the wound healing process after LASIK obviously is still needed to fully understand what transpires postoperatively and what biomechanical alterations occur long-term. Unfortunately, basic science research has lagged behind these clinical advances. Animal–primarily rabbit–and human histopathologic studies were recently completed and appear to answer most of the questions that clinicians and patients have wanted to know about the advantages and disadvantages of LASIK surgery.2-5 Additionally, since clinical outcomes and several postoperative complications are directly related to the wound healing process, a basic description of this process is described below.

Anatomy: Histological, Ultrastructural, and Immunofluorescent Studies

Animal and human studies have shown that the cornea heals after LASIK through a series of well-defined stages and steps.2-3

1) Active wound healing.
   a) Initial epithelial and stromal injury due to LASIK results in apoptosis or necrosis of keratocytes and epithelial cells in and slightly around the wound, and extracellular tissue damage.
   b) Cellular proliferation and migration of surviving keratocytes and epithelial cells occurs, which repopulates the acellular wound regions.
   c) Cellular differentiation of keratocytes into one of three distinct morphologic cell types occurs: the migratory keratocyte, the activated keratocyte, or the myofibroblast.

2) Wound remodeling.

3) Completion of wound healing.

Although human corneal wound healing is similar to that in animal models, it also has notable differences: adult human corneas heal less aggressively, more slowly, and not as completely as animal corneas.3-4 Human studies also have shown that LASIK corneal stromal wounds remain in the active wound healing stage up to the first 6 months after surgery before entering the wound remodeling stage that occurs from 6 month to 3 to 4 years after surgery. Wound remodeling is important as it is associated with an improvement in corneal transparency and wound strength.3-5 Theoretically, the LASIK wound should be completely healed 4 years after surgery, but sub-clinical remodeling may still occur indefinitely.
Overall, the long-term result in human corneas that have had LASIK is that a hypercellular fibrotic reparative stromal scar develops and persists in wound regions where epithelial-stromal interactions occur (i.e., the LASIK flap wound margin)—presumably because chemotactic cytokines and growth factors from epithelial cells, kerocytes, and tears act locally only over short distances where the two tissues are in apposition.\(^2\)\(^3\) Whereas, a hypocellular primitive reparative stromal scar develops and persists in wound regions where kerocyte injury pathways act by themselves (i.e., the central and paracentral LASIK interface wound, Slide 1).\(^3\)\(^4\) Thus, only reparative stromal scarring develops after creation of a LASIK flap (by mechanical and femtosecond laser means) and/or excimer laser ablation, but not stromal regeneration. The two stromal wound types have functional significance in that the hypercellular fibrotic stromal scar is strong because it is composed of a dense network of collagen fibrils, but also hazy because of myofibroblastic cells populating this scar type (Slide 2).\(^4\) This latter fact was confirmed by immunofluorescent studies that identified alpha-smooth muscle actin positive kerocytes and increased collagen type III staining in the extracellular matrix of the hypercellular fibrotic scar (Slide 2, bottom-left inset).\(^3\) In comparison, the hypocellular primitive stromal scar is transparent, but weak in tensile strength; thus, explaining its potential to serve as a space for fluid, cells, and microbes to collect (Slide 3).\(^4\) A special electron microscopic staining technique supports these latter conclusions as the hypocellular primitive stromal scar was found to primarily consist of abnormally large, nonfibril-bound proteoglycans (Slide 3, bottom-right inset).\(^3\) Immunofluorescence studies further showed decreased or absent levels of all normal corneal stromal constituents other than collagen type I in the hypocellular primitive stromal scar (Slide 4).\(^3\) An additional variable to consider in this wound healing scheme is that precisely realigned wounds (i.e., sutured or unsutured LASIK flap margins with minimal gaping and no epithelial cell plugging) were found to heal more efficiently—stronger and less hazy—than poorly aligned wounds (i.e., LASIK flap margins with wide wound gaping, epithelial plugging, or incarceration of adjacent corneal tissue).

The type, location, and amount of stromal scar found in histopathologically studied post-LASIK corneas can be summarized by the following: the hypercellular fibrotic stromal scar at the LASIK flap wound margin averaged 50 to 75 microns in depth and 8 microns in thickness and the hypocellular primitive stromal scar at the central and paracentral interface wound (i.e. in the remaining deeper stromal zone away from the epithelial surface) averaged 5 microns in thickness.\(^3\) Focal or diffuse epithelial ingrowth at the flap wound margin was found in 53% of post-LASIK corneas that were evaluated by step sections through the entire cornea.\(^3\) Post-LASIK corneas with epithelial ingrowth were notably different from those without epithelial ingrowth in that they only had a 5 to 10 micron in-depth adjacent zone of hypercellular fibrotic scarring next to the epithelial ingrowth site.

Histopathologic studies performed on human post-LASIK corneas also have shown that all cases had various degrees of focal undulations of Bowman’s layer and overlying focal basal epithelial hypertrophy over the surface of the flap.\(^3\) Similarly, at the LASIK flap wound margin, all post-LASIK corneas had focal basal epithelial hypertrophy and, occasionally, epithelial hyperplasia.\(^3\) A summary diagram of the wound healing process is shown in Slide 5.

**Physiology and Biology: Clinical and Laboratory Studies**

**Transparency**

The normal cornea transmits 95% to 99% of incident visible light because of the stroma’s lattice-like arrangement of collagen fibrils and its similar refractive index changes, and
because the cells of the cornea are predominantly transparent. Recently, a few studies have suggested that the cells that make up part of the cornea may be the most important determinant to maintaining normal corneal transparency. For example, clinical slit-lamp examination and in vivo confocal microscopy of the normal human cornea have shown that most of the back-scattered light (ie, reflected and obliquely back-scattered light) comes from the cellular components in the cornea as opposed to the collagen fibrils or other extracellular matrix constituents (endothelial cells and epithelial cells > nerve cells > keratocytes >> extracellular matrix). Similarly, subepithelial stromal haze, which commonly develops after various types of corneal stromal injury at the epithelial-stroma junction (e.g., photorefractive keratectomy (PRK), LASIK flap margin, traumatic perforating corneal injury), also appears to result from cellular factors. Wound-response keratocytes have been shown to transform into myofibroblasts—cells that initiate wound contracture and are currently thought to be the primary source of haze after refractive surgery. A recent ex vivo confocal microscopy study tried to quantitatively measure this back-scattering of light after LASIK surgery. The ex vivo confocal study found that 30-micron optical sections of the hypocellular primitive stromal scar back-scattered similar amounts of light as the normal corneal stroma (51.9 vs. 51.8 U), whereas the hypercellular fibrotic stromal scar back-scattered 38% more light than normal (78.4 vs. 57.2 U). The cause of the latter finding was thought to be due to back-scattering actin microfilament stress bundles and/or alterations in cytoplasmic protein levels in myofibroblasts. It has been suggested, but not proven, that myofibroblast persistence and, possibly, development is caused biomechanical stress and/or epithelial basement membrane disruption.

Curvature

Postoperative refractive regression is defined as a gradual loss of initial refractive correction. It is thought to result from postoperative corneal wound healing, which causes a change in curvature of the central anterior corneal surface. Postoperative refractive regression appears to occur in human corneas predominantly over the first 6 months after surgery and, after LASIK, averages between 4% and 10% (as opposed to rabbit corneas that regress 100%) (Slide 6). Compared to LASIK, the amount of regression in human PRK corneas is higher, averaging between 10% and 17% with newer, smoother, large-ablation profiles. The cause for refractive regression after LASIK is multifactorial, but is at least partially due to all of the following: focal epithelial hypertrophy over the center of the LASIK flap, development and growth of two types of stromal scars, and, possibly, an acute anterior forward shift in the cornea (not the same as ectasia).

Based on histopathologic studies, the typical LASIK cornea regresses primarily because, on average, a 5-micron-thick hypocellular primitive stromal scar develops centrally and an 8-micron-thick hypercellular fibrotic stromal scar develops at the flap wound margin, resulting in a re-steepening of the central anterior surface of the LASIK flap after the typical myopic LASIK treatment (Slide 7). The hypercellular fibrotic stromal scar presumably has more of an impact on regression than the central hypocellular primitive stromal scar because it contains contractile myofibroblasts that cinch down on the wound margin 1 to 3 months postoperatively, which further re-steeps the central anterior surface of the LASIK flap (mechanistically similar to how conductive keratoplasty steepens the central anterior corneal surface). Occasionally, the central anterior surface of the LASIK flap may re-steepen more or less than the typical case does. The authors have found that most of these atypical regression cases were caused by poorly aligned flap margins, which sometimes result in focal epithelial hyperplasia or hypoplasia over the center of the flap.
Stability

The biomechanical breaking force of a tissue is called the cohesive tensile strength of the tissue. Normal adult human corneal stroma has been found to have a cohesive tensile strength between 22 g/mm and 36 g/mm; typically being strongest in the peripheral stroma and in the anterior third of the central stroma, which are regions of the cornea stroma that have the highest density of bridging collagen filaments and/or interweaving collagen lamellae. The weakest measurement typically was found in the central, posterior two-thirds of the stroma. The region of the corneal stroma where the LASIK flap is made (i.e. 100-180 microns in depth from the anterior corneal surface) was found to average 30 grams/mm (Slide 8A). In contrast, the cohesive tensile strength of the post-LASIK cornea was found to much weaker than normal corneas, being weakest along the lamellar interface scar—regardless of postoperative time (Slide 8B). Moreover, the mean cohesive tensile strength of the hypocellular primitive stromal LASIK scar averaged 2.4% (0.7 g/mm) of normal controls (30.1 g/mm) and showed no change in strength over time after surgery (Slide 9A); the mean peak cohesive tensile strength of the hypercellular fibrotic stromal LASIK scar gradually increased over time after surgery, reaching maximum values by 3.5 years after surgery when the average was 28.1% (8.5 g/mm) of controls (Slide 9B).

The latter wound strength study concluded that corneal stromal LASIK wounds were weaker than normal unoperated corneal stroma because normal stromal structures were not regenerated during the wound healing response. Nevertheless, the newly created collagen fibrils in the reparative stromal scars were found to intercalate between and around adjacent old cut fibrils, which is similar to what David Maurice proposed in 1987—that new collagen fibrils in the stromal scar do not reconnect end-to-end with old cut fibril ends. Histological and ultrastructural correlations performed in the wound strength study also contributed to our understanding of corneal wound healing as it showed that the amount of densely packed, intercalating collagen fibrils and, probably, the number myofibroblasts contained in the scar were most indicative of strong scars (30% to 50% of normal strength). On the other hand, stromal scars that contained epithelial cells or non-fibril-bound proteoglycans were generally the weakest (8-15% and 1-5% of normal strength, respectively).

Biomechanical elastic properties (e.g. stress, strain, and Young’s modulus) of the post-LASIK cornea have also recently been evaluated in the laboratory using interferometry techniques. Early results (currently only performed in some animal models) suggest that cutting the LASIK flap alone causes reduced corneal stress and strain on the flap. This resulted in a 21% increase in the displacement of the central post-LASIK cornea after hydrostatic loading compared to normal corneas. Because the cornea is part of the eye wall and helps contain the intraocular pressure (IOP), the interferometry study provides direct evidence of why post-LASIK corneal instability and ectasia occasionally develops postoperatively and also suggests that the risk for biomechanical instability is higher after LASIK than after PRK or other surface ablative procedures. Moreover, it has also been estimated that LASIK cuts 232 million corneal fibrils as opposed to 5 million in PRK, this along with the poor wound healing of the human cornea results in an uncoupling of the LASIK flap from the residual stromal bed. Thus, the LASIK flap contributes minimally (1.5-2.0%) to the biomechanical stiffness of the corneal eyewall. Almost all of the biomechanical support of the post-LASIK corneal resides in residual stromal bed, which now has a reduced corneal stiffness, or Young’s modulus, than normal, unoperated corneas—since it is thinner. Until recently, no reliable way to determine the in vivo elastic biomechanical properties of a patient’s cornea existed. The Reichert Ocular Response Analyzer is a new promising tool that appears to determine the in vivo elastic biomechanical properties of a cornea by measuring a property called corneal
hysteresis, which represents an aggregate of corneal thickness, rigidity, hydration, and other factors.\textsuperscript{12}

The long-term biomechanical alterations found in a post-LASIK cornea help explain many clinical conundrums. First, these alterations offer an explanation why applanation tonometry IOP readings are lower after LASIK surgery than can be explained by reduced corneal thickness alone (reduced central corneal hysteresis and reduced central corneal Young’s modulus). Second, the alterations suggest a reason why corneal edema in post-LASIK corneas results in interface fluid syndrome (the hypocellular primitive stromal scar preferentially absorbs water and the weak cohesive tensile strength of this scar easily swells to form interface fluid pockets). Finally, the alterations also explain why LASIK flaps can be lifted indefinitely or why microbes and cells preferentially accumulate in the central and paracentral interface wound (the weak hypocellular primitive stromal scar heals very poorly resulting in a permanent potential space in all post-LASIK corneas).

\textit{Sensitivity}

The epithelium of the cornea is described as being the most densely innervated surface epithelium of the body with about 2,500 nerve terminals/mm\textsuperscript{2} (300 to 600 times more densely innervated than skin).\textsuperscript{7,13} As the surface area of the adult human cornea is approximately 138 mm\textsuperscript{2}, it has been estimated that each cornea contains 315,000 to 630,000 nerve endings. Most of the nerve fibers in the cornea are sensory in origin and are derived from the ophthalmic branch of the trigeminal nerve (CN\textsubscript{V})\textsubscript{1}, which supplies the eye mainly through two long ciliary nerves. Innervation of the cornea (corneal epithelium and the anterior third of the corneal stroma) arises from the long ciliary nerves that penetrate the sclera around the scleral canal and begin to branch in the outermost layers of the choroid as they pass beneath the ora serrata. The main nerve trunks are myelinated and provide branching networks that supply the sclera, episclera, and conjunctiva. The remaining branches form a circumcorneal network around the limbus called the annular plexus where 60 to 80 radial nerve trunks enter the peripheral cornea near or at the midpoint of the corneal stroma. Within 1 mm of the limbus, the stromal nerve trunks lose their myelin sheaths and perineurium, but retain their Schwann cell sheaths. The radial nerve trunks then branch extensively in the anterior third of corneal stroma interconnecting among themselves in the subepithelial plexus. Few, if any, of the nerves pass posteriorly to innervate the area of the deeper stroma and none pass through Descemet’s membrane to supply the endothelial cells.

Although only a few nerve fibers in subepithelial plexus terminate in the anterior third of the corneal stroma, parts of the stromal nerves in the subepithelial plexus have gaps in the Schwann cell sheaths that act as receptor elements. When the nerve bundles of the subepithelial plexus turn 90 degrees to penetrate the Bowman’s layer throughout the peripheral and central cornea, they lose their Schwann cell sheaths. They then again turn 90 degrees and continue parallel to the corneal surface forming the sub-basal nerve plexus, which is composed of nerve bundles that contain straight and beaded nerve fibers. Only the beaded fibers branch from the sub-basal nerve plexus to form epithelial nerves, which course between corneal epithelial cells before finally terminating into 10 to 20 unspecialized free nerve endings. The terminals typically extend up to the most superficial epithelial cell layers. Since different types of epithelial nerve fibers can be distinguished on the basis of their ultrastructure, it appears that corneal epithelial nerves are primarily A-delta and C fibers (touch and pain sensations).

In addition to their sensitivity functions, corneal nerves also appear to be important in maintaining the health of the corneal epithelium and ocular surface via trophic influences.
and/or other factors. Since the earliest experimental studies by Magendie, it has been shown that dysfunction of corneal innervation produces a degenerative condition to the corneal epithelium called neurotrophic keratitis.\textsuperscript{2,5,14} Although many disorders can cause neurotrophic keratitis, LASIK has recently gained the most attention for temporarily causing this condition.\textsuperscript{15} This usually results in short-term symptoms of dry eye after refractive surgery, which is not only due to a transient decrease in corneal sensation and epithelial trophic factors, but also from a transient decrease in tear production.\textsuperscript{2} Dry eye symptoms have been found to be more frequent, more severe, and longer in duration following LASIK than PRK, because the total surface area and depth of corneal nerve injury are greater after LASIK than PRK. Additionally, re-innervation takes more time with LASIK than PRK. In most cases, LASIK is immediately followed by loss of corneal sensation over the flap and gradual disappearance of most of the corneal nerves in the flap over the first 2 days after surgery. Significant re-growth of the cut nerves starts between 1 and 3 months after the procedure with corneal sensation typically recovering to normal levels only by 6 to 12 months postoperatively. However, the total length, morphology, and sensation of the re-grown corneal nerve fibers never completely return to normal preoperative levels and only reaches maximum levels by 1 to 2 years after LASIK. The temporary de-nervation of the post-LASIK cornea also has been associated with a transient decrease in anterior stroma cellular density, which may be due to Schwann cell degeneration and/or loss of neurotrophic influences on keratocytes.\textsuperscript{3}

**Conclusion**

Although the enthusiasm associated with LASIK surgery is high due to its good clinical success in permanently correcting refractive errors, basic science research has now more clearly shown that LASIK, like most surgical procedures, has both short-term and long-term advantages and disadvantages. After considering all these factors, a prospective LASIK patient, with the help of the ophthalmologist, can decide whether LASIK surgery is in his or her best interest.
Slide 1. Photomicrograph of the histology and clinical slit lamp photos (insets) of a 5-year post-LASIK cornea. Four long-term histological findings were present in this representative post-LASIK cornea: epithelial modifications (i.e. basal epithelial hypertrophy and/or hyperplasia); Bowman’s layer undulations; hypercellular fibrotic stromal scarring; and hypocellular primitive stromal scarring. Note that the only histological finding that could consistently be seen on slit-lamp examination was the area of the hypercellular fibrotic stromal scar (black outlines and arrowheads). Toluidine blue x25.
Slide 2. Transmission electron micrographs of a 4-month post-LASIK cornea showing a representative hypercellular fibrotic stromal wound margin scar (arrow). The initial 42-micron length of the scar was 5.1 micron thick (between arrowheads). The extracellular matrix of this scar is consistent with fibrosis because it predominantly was composed of a dense network of disorganized collagen fibrils (arrows in main and top-right inset). Arrowhead in the top-right inset shows focal electron dense material that progressively disappears from this scar over 3 to 4 years after LASIK (ie, remodeling). The bottom-left inset is an immunofluorescent photomicrograph using anti-alpha-smooth muscle actin antibody (light green) and propidium iodide as a nuclear counterstain (red). Bar indicates 1 micron. Main transmission electron microscopy photo x4,750.
**Slide 3.** Transmission electron micrographs of a 6-month post-LASIK cornea showing a representative hypocellular fibrotic stromal scar that resides in the central and paracentral interface wound. The thickness of the central scar varied from 5.8 micron thick (between arrowheads on the left) and 1.1 micron thick (between arrowheads on the right). High magnification views of this scar (top-right inset) showed that it is primarily composed of electron dense granular material (arrows on top-right inset) with sparsely interspersed collagen fibrils (arrowhead on top-right inset). A tangential cupromeronic blue-stained TEM (bottom-right inset) showed that most of the electron-granular material was an abnormally-large (360 nm x 20 nm), non-fibril-bound type of proteoglycan. Bar indicates 1 µm. Main transmission electron microscopy photo x4,750.
Slide 4. Summary diagram of the immunofluorescent studies performed on human post-LASIK corneas.

Slide 5. Diagram of normal corneal healing pathways (black) and pathologic pathways (blue). Pharmacologic adjunctive medications (red) are included.
Slide 6. Summary diagram demonstrating the biologic reason why regression occurs after myopic LASIK surgery. The post-LASIK cornea immediately after surgery is depicted in black solid lines. The light blue solid lines represents the typical stromal scar deposition, which occurs over the first 6 months after surgery; the light blue dashed lines represents how this scarring affects the central anterior surface of the LASIK flap (i.e. partial myopic regression). Similarly, the red solid and dashed lines represent the occasional case that regresses in the hyperopic direction (usually, in these cases, the flap wound margin is poor aligned).
Slide 7. Chronology of the deposition and persistence of the hypocellular primitive stromal scar (between arrowheads) are depicted in transmission electron micrographs from 1-month post-LASIK to 3-years post-LASIK. (A) A 1-month post-LASIK cornea showed early deposition of the stromal scar, a few migratory keratocytes, and numerous highly activated keratocytes (not shown in main picture). Inset is from a 2-month post-LASIK cornea, which showed highly activated keratocytes more clearly. (B) A 6-month post-LASIK cornea showed even more deposition of the stromal scar than at 1 to 2 months after surgery and less activation of the keratocytes. (C) A 1-year post-LASIK cornea showed no more deposition of the stromal scar than at 6 months after surgery. Notice that the keratocytes are now quiescent with more than normal amount of cytoplasmic vacuoles (inset). (D) A 3-year post-LASIK cornea showed again no more deposition of the stromal scar than at 6-month after surgery; more importantly, it persisted and did not appear to remodel or regenerate into normal corneal stroma. The inset in (D) is from a normal cornea to show what a normal, quiescent keratocyte looks like. Bar indicates 1 micron. Main transmission electron microscopy photos x4,750.
Slide 8. Representative cohesive tensile strength line graphs of a normal cornea (A) and a post-LASIK cornea (B) are shown. Both tracings are plotted from left to right. (A) The tracing showed the interlamellar cohesive tensile strength of a normal, control cornea mechanically separated in a lamellar fashion. All control corneas were similar to the graph shown here. The cornea from limbus to limbus was measured between the arrows and the tracing outside the arrows represents the sclera. (B) Cohesive tensile wound strength tracing of a 5-year post-LASIK cornea showed that the weakest point of separation of post-LASIK cornea was at the lamellar interface scar. All LASIK corneas had tracing similar to this one. The location of separation in the LASIK wound is labeled in the graph with accompanying arrows and the quantitative results are labelled in italic print for this case.
A scatter graph of all the cohesive tensile strength measurements plotted versus the time after LASIK in the region of the hypocellular primitive stromal scar (open circles) is shown in (A) and the hypercellular fibrotic stromal scar (black solid circles) is shown in (B). Best curve fit lines (dashed lines) were made for each of the two data sets. No evidence of a gain in tensile wound strength over time was measured in the hypocellular primitive scar best fit curve (A), whereas a gradual increase was measured up to 3.5 years after LASIK in the hypercellular fibrotic scar (B) before plateauing. Square represents mean value of 5 normal controls.
References


Refractive Surgery (LASIK and others): Early and Late Histopathologic Findings and Complications

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2005 Market Scope Data

• U.S. Market
  – 40 million myopes
  – 22 million hyperopes
  – 27 million presbyopes
  – 36 million cataracts

• Refractive Surgery
  - 1.3 million LASIK procedures performed in U.S. in 2005 (3.2 million in the world)
  – 97% of the refractive surgery market is excimer laser based
    70-90% LASIK vs. 10-30% Surface Ablation
Purpose:

• To examine the histologic, ultrastructural, and immunohistologic finding of human post-LASIK corneas.
• To examine the physiology of these corneas compared to normals.
• To compare and correlate these findings to clinical studies.

Methods:

• Corneas from 223 eyes from 117 post-mortem patients who had LASIK surgery between 1 week and 10 years prior to death were evaluated using LM, TEM, SEM, or IHC techniques.

2000 = 4 2001 = 7 2002 = 13 2003 = 51
2004 = 57 2005 = 56 2006 = 35
Gross examination

- 89% had a visibly detectable hazy semi-circular wound margin
- 100% had visibly detectable incision lines
Light microscopy (LM): Epithelium

• All corneas had focal areas of basal epithelial cell hypertrophy on the flap surface—over low points caused by random Bowman’s layer undulations.
• All flap margins had basal epithelial cell hypertrophy and/or epithelial hyperplasia
• All corneas had a 2 to 8 µm thick lamellar interface scars
  - Most easily seen on PAS stained sections
  - Most difficult to see after 3 years postop or longer, especially on toluidine blue-stained sections
LM: Stroma cont’d

- More hypercellular and thicker scar found at the wound margin

- Hypocellular, thinner scar found in the paracentral and central regions of the scar
Other notable LM findings

- Variable epithelial ingrowth found in ~50% of cases
- Variability in the alignment of Bowman’s Layer
## Quantitative LM

<table>
<thead>
<tr>
<th>Pathologic finding</th>
<th>Mean thickness +/- SD</th>
<th>Range of thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flap Thickness</td>
<td>160 +/- 23 µm</td>
<td>110 to 216 µm</td>
</tr>
<tr>
<td>Residual stromal bed thickness</td>
<td>399 +/- 54 µm</td>
<td>290 to 460 µm</td>
</tr>
<tr>
<td>Endothelial cell density</td>
<td>2,307 +/- 974 µm</td>
<td>1,828 to 2,843 cell/mm²</td>
</tr>
<tr>
<td>Epithelial ingrowth</td>
<td>291 +/- 209 µm</td>
<td>30 to 900 µm</td>
</tr>
</tbody>
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# Quantitative LM: Epithelium

<table>
<thead>
<tr>
<th>Pathologic finding</th>
<th>Mean thickness +/- SD</th>
<th>Range of thickness</th>
<th>Mean cell layer +/- SD</th>
<th>Range of cell layers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral epithelial thickness</td>
<td>33 +/- 6 µm</td>
<td>24 to 44 µm</td>
<td>5.0 +/- 0.9</td>
<td>4 to 7</td>
</tr>
<tr>
<td>Marginal epithelial thickness</td>
<td>43 +/- 9 µm</td>
<td>26 to 64 µm</td>
<td>5.8 +/- 1.4</td>
<td>4 to 9</td>
</tr>
<tr>
<td>Central epithelial thickness</td>
<td>29 +/- 7 µm</td>
<td>20 to 44 µm</td>
<td>4.9 +/- 0.9</td>
<td>4 to 6</td>
</tr>
</tbody>
</table>
Keratocyte density


Reduced keratocyte density
- 32%* (4.3%/yr)
- 42%* (7.2%/yr)
- 42%* (8.4%/yr)

Full-thickness 26% reduction

*13 to 19% of the cell loss occurred over the first 6 months postop.
Keratocyte density cont’d
Summary of TEM findings

Two stromal scar types

(1) Hypercellular fibrotic scar†
   *mean thickness: 7.8 +/- 4.1 µm (range 1.1 - 16.4)
   *ultrastructure = mildly disorganized dense network of normal
   diameter+ (26.6 +/- 3.0 nm) collagen fibrils

(2) Hypocellular primitive scar†
   *mean thickness: 4.5 +/- 3.1 µm (range 0.4 – 11.4)
   *ultrastructure = mainly composed of electron dense
   material with scattered smaller diameter (21.1 +/- 0.8 nm) collagen
   fibrils

†activated keratocytes only present ≤ 6 months after surgery
+Normal collagen fibril diameter 25.9 +/- 1.4 nm
4 Months Postop:
TEM of wound margin

- Initial 42 μm length of wound heals with a hypercellular fibrotic stromal scar (thickness 2 μm)
- Bars = 0.5 μm
3 Years Postop: TEM of wound margin

- Initial 70 µm length of wound heals with a hypercellular fibrotic stromal scar (thickness 5 µm)
- Bars = 1 µm
6 Months Postop:
TEM of paracentral wound

- Hypocellular primitive stromal scar (thickness 5.5-7.0 µm)
- Bar = 1 µm
6 Months Postop:
TEM of central wound

- Hypocellular primitive stromal scar (thickness 2.2-5.6 µm)
- Bar = 1 µm
5 Years Postop:
TEM of central wound

- Hypocellular primitive stromal scar (thickness 5.0 µm)
- Bars = 1 µm
Chronology of cell types in the scar

3 months

6 months

1 year

Normal
Chronology of extracellular matrix deposition

- 1 month
- 6 months
- 1 year
- 3 years
Electron dense granular material = large proteoglycans (360 nm x 20 nm) and collagen molecules/microfibrils
Laser Capture Microscopy

- Mildly lower concentrations of monosulfated and disulfated keratan sulfate
- Three-fold higher concentration of Δdi-6S dermatan sulfate
Keratoepithelin (βig-h3 gene product)
Other notable TEM findings

Other notable findings
- Empty spaces filled with fibrillar material
- Histocytes filled with fibrillar material
- Foci of microscopic epithelial cell implantation
- Wide-spaced banded collagen
- Plastic particles
Summary of Immunofluorescence Studies

↑ collagen III & α-SMA (myofibroblasts)
± collagen I, V, VI, DS, and KS
↑ MMP 9 (epithelial ingrowth cases only)

± collagen I
↓ collagen VI & KS
↓↓ collagen V
Absent collagen type III and DS
5 Years Postop:
IF for collagen type 1 and type 3

Green (left)       Collagen type 1
Green (right) Collagen type 3
4 Months Postop:
IF for alpha-smooth muscle actin and corresponding region on PAS

Green (left)  Alpha-SMA positive staining
PAS(right)  Keratocytes around marginal interface
5 Years Postop:

IF for alpha-smooth muscle actin with propidium iodide counterstaining

Red (left)  Propidium iodide nuclear counterstain
Combined (center)
Green(right)  Alpha-SMA positive staining
Transparency

- Light scattering animal studies

Circle of haze after PRK

Semi-circle ring of haze after LASIK

Transparency: Confocal microscopy

• Our study

Hypocellular primitive scar:
Back-scattered light (51.9 U) was similar to normal corneal stroma (51.8 U)

Hypercellular fibrotic scar:
Unable to measure except one case (79.1 U). Normal wound margin 60.3 U. 31% more back-scattered light than normal per 30 um section (100-300% more light scattering than normal).
Curvature: Regression

Clinical data
–Regression data from large studies using large, smooth ablation profiles
  -Biologic regression of 2-10% of initial refractive effect

–Longitudinal studies

<table>
<thead>
<tr>
<th>Post-op time period</th>
<th>Change in Refraction</th>
<th>Change in Keratometry</th>
<th>Change in Pachymetry</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 wk – 1 mo</td>
<td>-0.36 D</td>
<td>+0.39 D</td>
<td>4 µm</td>
</tr>
<tr>
<td>1 mo – 3 mo</td>
<td>-0.28 D</td>
<td>+0.28 D</td>
<td>5 µm</td>
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<tr>
<td>3 mo – 6 mo</td>
<td>-0.38 D</td>
<td>+0.17 D</td>
<td>6 µm</td>
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<tr>
<td>6 mo – 1 yr</td>
<td>+0.05 D</td>
<td>+0.19 D</td>
<td>0 µm</td>
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<tr>
<td>1 yr – 2 yr</td>
<td>-0.07 D</td>
<td>+0.05 D</td>
<td>2 µm</td>
</tr>
<tr>
<td>Total</td>
<td>-1.04 D</td>
<td>+1.08 D</td>
<td>17 µm</td>
</tr>
</tbody>
</table>
Curvature: Regression and Stability

Long-term studies

- Alio JL, et al. Tens years after LASIK and PRK for the correction of Myopia: Is the cornea stable. AAO poster #255
  - Corneal keratometry was stable after 1 year postop for both PRK and LASIK groups.
  - Myopic regression averaged -1.05 D for PRK and -1.65 D for LASIK at 10 years after surgery.
  - Both procedures showed good stability and durable results 10 years after surgery, with a trend toward higher regression for LASIK.

  - Early (1 to 6 months after surgery) hyperopic shift regression was reduced with the larger 6 mm optical zone treatment.
  - Refractive stability was maintained from 1 year to 12 years after PRK surgery.
• Our study (up to 10 years postop).
  1) No epithelial hyperplasia over central flap, only focal basal epithelial cell hypertrophy in valleys of the microstriae = 4 +/- 2 µm.
  2) Keratocyte-mediated stromal scar = 5 +/- 3 µm
  3) Cannot evaluate possible forward shift in cornea
Stability: Wound strength

• Qualitative tensile strength—formalin or glutaraldehyde fixed
  - Artifactual flap detachments (partial or total) occurred in at least one slide in 34% of cases examined
  - Artifactual flap margin detachments only occurred in the hyp cellular scar in corneas ≥ 3 years old

<table>
<thead>
<tr>
<th>Postop Time</th>
<th>2 mon</th>
<th>4 mon</th>
<th>6 mon</th>
<th>19 mon</th>
<th>24 mon</th>
<th>27 mon</th>
<th>30 mon</th>
<th>3 yrs</th>
<th>3 yrs</th>
<th>3.5 yrs</th>
<th>5 yrs</th>
<th>5 yrs</th>
<th>5 yrs</th>
<th>5 yrs</th>
<th>6 yrs</th>
<th>6.5 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen</td>
<td>++/++</td>
<td>-/+</td>
<td>+/-</td>
<td>-</td>
<td>-/+</td>
<td>++/++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>OF</td>
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<td>++/++</td>
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<td>-/+</td>
<td>OF</td>
</tr>
<tr>
<td>Paraffin</td>
<td>+/-</td>
<td>-/</td>
<td>+/-</td>
<td>-/</td>
<td>-/</td>
<td>-</td>
<td>-/</td>
<td>-/+</td>
<td>-/+</td>
<td>-/</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>Epoxy Resin</td>
<td>++/-</td>
<td>-/+</td>
<td>-/+</td>
<td>-/</td>
<td>-/-</td>
<td>-</td>
<td>-/-</td>
<td>+/-</td>
<td>+/+</td>
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<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
</tbody>
</table>

Artifactual partial flap separation from sectioning
Hyp cellular scar

Hyper cellular scar
Artifactual partial flap separation from sectioning
Hyp cellular scar
Stability: Wound strength cont’d

• Quantitative tensile wound strength study (QTWSS)—Optisol fixed
  — Identification of the LASIK-flap and the LASIK-hinge by a dissection microscope (Olympus SZ-40, Tokyo, Japan) using Optisol-GS stored specimens
  — Placement of the corneascleral button anterior side up on a convex polyethylene surface
  — Cutting of a 4-mm limbus-to-limbus corneoscleral strip centered on the hinge (double-bladed knife)
Stability: Wound strength cont’d

• QTWSS
  — Fixation of the anterior and posterior lamellar of the corneoscleral strip to two 5-mm-long hooks (base plate, strip extensiometer)
Stability: Wound strength cont’d

• QTWSS

Normal control cornea

Representative tensile strength tracing of controls

LASIK cornea (3.0 yrs. post-op)

Representative tensile strength tracing of LASIK wounds

Mean strength of hypocellular primitive stromal scar = 0.5 grams/mm (1.7%)

Peak strength of hypercellular fibrotic stromal scar = 8.0 grams/mm (26.5%)
QTWSS: Results

LASIK cohesive tensile wound strength measurements (i.e. breaking force)

Hypercellular scar avg. 28.1%

Hypocellular scar avg. 2.4%
Table 3. Summary of wound strength data in human LASIK eyebank corneas

<table>
<thead>
<tr>
<th>Donor #</th>
<th>Eye</th>
<th>Time after LASIK (Years)</th>
<th>Cohesive tensile wound strength</th>
<th>Histopathology at the flap margin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Central flap thickness (µm)</td>
<td>Central and paracentral (grams/mm)</td>
<td>Flap margin (grams/mm)</td>
</tr>
<tr>
<td>1</td>
<td>OD</td>
<td>0.4</td>
<td>85</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>OS</td>
<td></td>
<td>83</td>
<td>0.44</td>
</tr>
<tr>
<td>2</td>
<td>OD</td>
<td>0.5</td>
<td>106</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>OS</td>
<td></td>
<td>106</td>
<td>0.33</td>
</tr>
<tr>
<td>3</td>
<td>OD</td>
<td>1.5</td>
<td>160</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>OS</td>
<td></td>
<td>145</td>
<td>0.75</td>
</tr>
<tr>
<td>4</td>
<td>OD</td>
<td>2.0</td>
<td>244</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>OS</td>
<td></td>
<td>198</td>
<td>1.25</td>
</tr>
<tr>
<td>5</td>
<td>OD</td>
<td>2.0</td>
<td>137</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>OS†</td>
<td></td>
<td>123</td>
<td>1.0</td>
</tr>
<tr>
<td>6</td>
<td>OD</td>
<td>2.5</td>
<td>127</td>
<td>0.75</td>
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<tr>
<td></td>
<td>OS</td>
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<tr>
<td>7</td>
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<td></td>
<td>OS</td>
<td></td>
<td>242</td>
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</tr>
<tr>
<td>8</td>
<td>OD</td>
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<td>0.33</td>
</tr>
<tr>
<td></td>
<td>OS</td>
<td></td>
<td>178</td>
<td>0.5</td>
</tr>
<tr>
<td>9</td>
<td>OD</td>
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<td>187</td>
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<tr>
<td></td>
<td>OS</td>
<td></td>
<td>149</td>
<td>1.0</td>
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<tr>
<td>10</td>
<td>OD</td>
<td>4.5</td>
<td>187</td>
<td>0.75</td>
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<tr>
<td></td>
<td>OS</td>
<td></td>
<td>153</td>
<td>0.75</td>
</tr>
<tr>
<td>11</td>
<td>OD</td>
<td>5.0</td>
<td>205</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>OS</td>
<td></td>
<td>180</td>
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</tr>
<tr>
<td>12</td>
<td>OD</td>
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<td>145</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>OS</td>
<td></td>
<td>6.5</td>
<td>1.75</td>
</tr>
<tr>
<td>13</td>
<td>OD</td>
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<td>167</td>
<td>0.75</td>
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<tr>
<td></td>
<td>OS</td>
<td></td>
<td>187</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Mean±SD: 3.1±1.6 168±51 0.7 ±0.33 7.18±4.15 165±116 46±35

SD, standard deviation; †, laser microkeratome LASIK corneas; *, hinge/partial hinge; P, peripheral cornea; L, limbus; OD, right eye; OS, left eye.
QTWSS: Histopathology and ultrastructure

- Normal cornea
- LASIK wound - central
- LASIK wound - paracentral
- LASIK wound – flap margin
- LASIK cornea
- Mechanial microkeratome LASIK cornea
- Laser microkeratome LASIK cornea
QTWSS: Ultrastructure
QTWSS: Ultrastructure
QTWSS: Ultrastructure
Stability: Wound strength cont’d

• This chronically weak interface scar explains why you can always re-lift these LASIK flap presumably indefinitely (12 yrs postop per GOW).

• This also explains why traumatic cases of LASIK flap dehiscence have been reported out to 4 years postop.
Stability: Biomechanics

• 2005 AAO symposia: Best of Anterior Segment
  “Stabilizing Refractive Outcome and Understanding
  Biomechanics” by John Marshall, Ph.D.

  - PRK/LASEK/Epi-LASIK cuts on average 5 million collagen fibrils.
    Refractive outcome is typically stable over 12 years of follow-up.

  - LASIK cuts on average 232 million collagen fibrils; when replaced or, even
    when, completely healed, the LASIK flap doesn’t appear to contribute
    significantly to biomechanical integrity of the cornea.

  - Therefore, LASIK uncouples normal corneal biomechanics 45-fold more
    than surface ablative procedures.

  - LASIK is the refractive surgical procedure at the highest risk for causing
    long-term corneal biomechanical instability and, at worst, iatrogenic
    keratectasia.
Stability: Biomechanics cont’d

- Elasticity
  — Change in electronic speckle pattern interferometry (ESPI) in sheep corneas to an increase in IOP of 0.15 mm Hg.
    - Normal control
      - Mean maximum displacement of 1.50 µm
    - LASIK flap replaced
      - Mean maximum displacement of 1.78 µm (18.7% > normal)
    - LASIK flap not replaced
      - Mean maximum displacement of 1.81 µm (20.7% > normal; 1.7% > flap replaced)

Stability: Biomechanics cont’d

- Elastic system definitions
  - **Stress** = force per cross-sectional area (i.e. IOP).
  - **Strain** = change in length per unit original length (i.e. innate properties of the corneal eyewall).
  - **Young’s modulus** = ratio of stress to strain (i.e. a measure of a material’s rigidity or resistance to a change in length).


    Normal corneas = 57 N/mm²
    Keratoconus corneas = 28 N/mm² (range 20-37 N/mm²) or 49.1% of normal (range 35-65% of normal)

  --Estimated Young’s modulus from Marshall’s and our work
    LASIK (74% of pre-op)
    PRK (92% of pre-op)
    PRK with MMC (93% of pre-op)
**Stability: Biomechanics cont’d**

### Summary Table of post-LASIK and post-PRK ectasia cases

<table>
<thead>
<tr>
<th>Case #</th>
<th>Specimen #</th>
<th>Thickness (µm)</th>
<th>BL Breaks</th>
<th>Other notable findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Thinnest TCT</td>
<td>LASIK Flap</td>
<td>RS B</td>
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<tr>
<td>1</td>
<td>OP03-294</td>
<td>200</td>
<td>104</td>
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<td>96</td>
<td>144</td>
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<td>3</td>
<td>OP03-1629</td>
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Stability: Biomechanics cont’d
Stability: Biomechanics cont’d

LASIK cohesive tensile wound strength measurements (i.e. breaking force)

Hypercellular scar avg. 28.1%

Hypocellular scar avg. 2.4%
Stability: Biomechanics cont’d

Summary Diagrams of Normal Corneal Tensile Strengths

- Bowman’s layer supports 4% of eye wall stress
- Anterior third of corneal stroma (excludes BL) supports 44% of eye wall stress
- Posterior two-thirds of corneal stroma (excludes DM) supports 50% of eye wall stress
- Descemet’s membrane supports 2% of eye wall stress
Stability: Edema

Published Clinical Reports of interface fluid syndrome (IFS)
- High intraocular pressure
  21 reports
  67 eyes of 41 patients
- Endothelial cell dysfunction
  10 reports
  13 eyes of 12 patients

Normal  LASIK  PISK/PIIK  Interface Pocket of Fluid
Stability: Edema cont’d
Laboratory Model of IFS

Donor Post-LASIK Corneas, n=12

Corneal Perfusion Chamber

Three Hours of Perfusion
BSS (Control)
0.9% NaCl (Endothelial Cell Damage)
55 mm Hg IOP (High IOP)

Histopathology

Pre-test Confocal Microscopy

Post-test Confocal Microscopy
Laboratory Model of IFS: Histology

- Normal LASIK Scar
- Thickened LASIK Scar (Early Edema)
- Focal Fluid Pockets (Moderate Edema)
- Diffuse, Confluent Pocket (Severe Edema)
Previous Supportive Work on IFS

PREFERENTIALLY ABSORBS WATER:
Abnormally large, non-fibril-bound proteoglycans in interface scar

SWELLS EASILY:
Weak cohesive tensile strength

Cupromeric-blue Stained TEM (Frontal View)
Stability: Edema cont’d

- **Stage 0**: Normal LASIK Cornea
- **Stage 1**: Mild Interface Fluid Syndrome (thicker scar without inflammation)
- **Stage 2**: Moderate Interface Fluid Syndrome (focal fluid pockets with hydropic degeneration of surrounding keratocytes +/- focal minimal inflammation)
- **Stage 3**: Severe Interface Fluid Syndrome (diffuse, confluent pocket with hydropic degeneration of surrounding keratocytes +/- minimal inflammation)

High IOP

Endothelial Cell Damage
Unable to evaluate corneal nerves because they degenerate completely by 24 to 48 hours after being cut.

Our study did find pathologic evidence that was associated with transient de-innervation.
Sensitivity cont’d

- Basal tear secretion is maintained by low frequency impulse activity in corneal and conjunctival nerves, which does not elicit conscious sensations.

- When ocular surface dries or is irritated, sensory afferent impulse activity increases and evokes reflexly augmented tear secretion. Tearing can also be evoked by emotional response centers in cerebral area (not shown).

Belmont C. Eye dryness sensations after refractive surgery: impaired tear secretion or “phantom” cornea. JCRS (in press).
40% of patients complain of ocular dryness after LASIK

- LASIK causes a decrease in basal tear secretion because corneal sensory nerves never re-constitute completely back to normal. The dry ocular surface stimulates intact afferent nerves, evoking a dry sensation.

- LASIK injures corneal nerves, which regenerates producing microneuromas and hyperalgesic nerve terminals. Despite adequate basal tear secretion or artificial tear agents, abberant impulses result in a dry sensation.

Belmont C. Eye dryness sensations after refractive surgery: impaired tear secretion or “phantom” cornea. JCRS (in press).
LASIK
(n = 223)
-Most popular refractive surgical technique since 1999

Avg. wound healing: 9 µm centrally (stroma 5 µm; epithelium 4 µm)
23 µm at the wound margin (str 12 µm; epi 11 µm)
# LASIK pathologic-clinical correlation

<table>
<thead>
<tr>
<th>Pathologic finding</th>
<th>Clinical correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compensatory outward epithelial hyperplasia at the wound margin</td>
<td>Smooths contour of anterior corneal surface</td>
</tr>
<tr>
<td>Focal inward basal epithelial cell hypertrophy over flap surface</td>
<td>Fills in valleys produced by Bowman's layer undulations and Bowman's layer breaks</td>
</tr>
<tr>
<td></td>
<td>Minor or secondary role in regression of refractive effect (early component 1 wk to 1 month)</td>
</tr>
<tr>
<td></td>
<td>Post-operatively may increase flap thickness measurements</td>
</tr>
<tr>
<td>Focal undulations of Bowman's layer in the flap</td>
<td>Represents how the flap corneal stroma adjusts to fill in the gap produced by the missing ablated tissue</td>
</tr>
<tr>
<td>Hypercellular fibrotic wound margin scar</td>
<td>Hazy gray semi-circular ring may be clinically apparent (primarily due to myofibroblasts)</td>
</tr>
<tr>
<td></td>
<td>Strongest portion of scar (holds flap in place)</td>
</tr>
<tr>
<td></td>
<td>Major role in regression of refractive effect</td>
</tr>
<tr>
<td>Hypocellular primitive central scar</td>
<td>Clinically invisible scar</td>
</tr>
<tr>
<td></td>
<td>Weakest portion of scar (allows easy lifting of flap for an indefinite time period postoperatively)</td>
</tr>
<tr>
<td></td>
<td>Moderate role in regression of refractive effect</td>
</tr>
<tr>
<td></td>
<td>Increased capacity to absorb water and swell</td>
</tr>
<tr>
<td></td>
<td>Potential space for cells or microbes to collect</td>
</tr>
<tr>
<td>Transient de-nervation</td>
<td>Transient decrease in corneal sensation/decrease in tear production/neurotrophic epitheliopathy</td>
</tr>
<tr>
<td></td>
<td>Transient decrease in anterior keratocyte density</td>
</tr>
</tbody>
</table>
PRK ($n = 2 + 4$)
-2nd most popular refractive technique

Avg. wound healing: 32 µm centrally (stroma 20 µm and epithelium 12 µm)
Astigmatic keratotomy/LRI (n = 6)
Refractive lens surgery (n = 2)
Conductive keratoplasty (n = 2)

- Basal epithelial cell hypertrophy
- Hypercellular fibrotic stromal scar
- Hypocellular/primitive stromal scar

Stromal edema from corneal perforation
Electron dense granular material with lipids and persistently activated keratocytes

Intrastromal corneal ring segments (n = 2)
Newer or future options

- **Phakic IOLs**
- **Custom excimer laser ablation algorithms**
  - Wavefront-guided, wavefront-optimized, topography-guided, Q-factor-guided.
- **Advanced surface ablation**
  - LASEK
  - Epi-LASIK
  - Pharmacologic adjuncts
    - Topical steroids
    - Prophylactic MMC use (0.02-0.002% soaked sponge applied to ablated surface for 15 seconds to 2 minutes).
    - Anti-TGF-beta drops
    - 5-FU?