Corneal Stromal Remodeling After Photorefractive Keratectomy

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INTRODUCTION

The cornea is an optically clear tissue that forms the front surface of the eye.

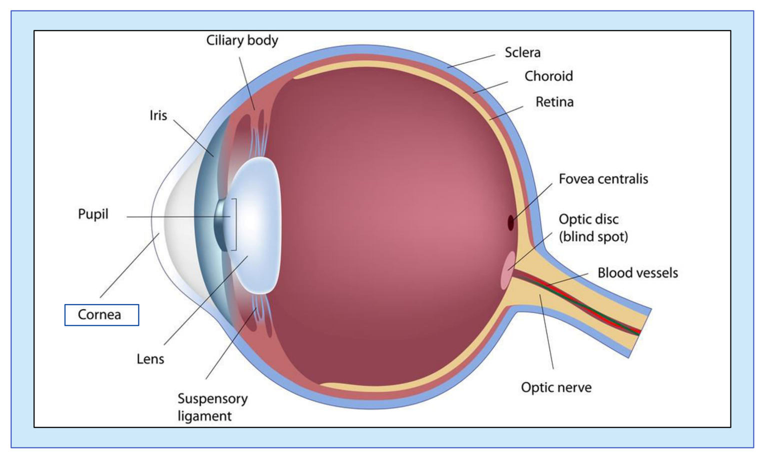

Figure 1. Cross section of the eye. (Figure adapted from http://www.airdriefamilyeyedoctors.com/images/anatomy-1280x635.jpg)

The cornea is responsible for about 2/3 of the eye's refractive power and as such, is a target for vision correction procedures. One common type of vision correction procedure is photorefractive keratectomy (PRK).

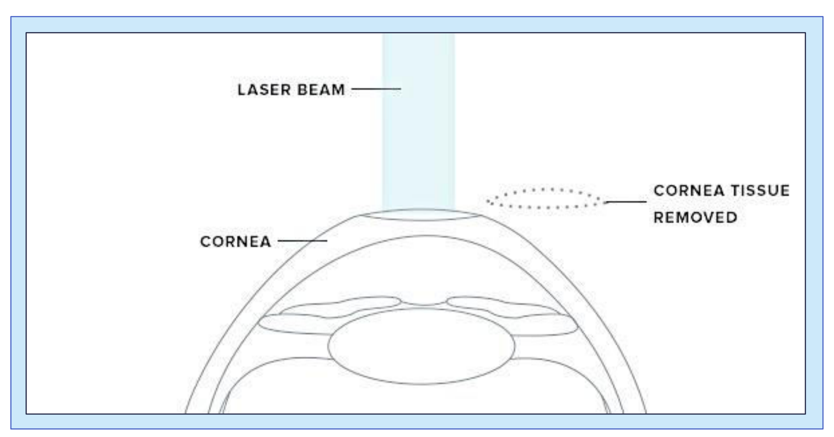

Figure 2. During PRK, an excimer laser is used to remove tissue from the anterior cornea, altering its shape and thickness. (Figure adapted from https://us.discovericl.com/why-visian-icl/prk-surgery-overview)

The stroma is a collagenous layer within the cornea that accounts for about 90% of corneal thickness. Following injury or refractive surgery (including PRK), a small percentage of patients experience a decrease in corneal transparency due to stromal fibrosis and haze.

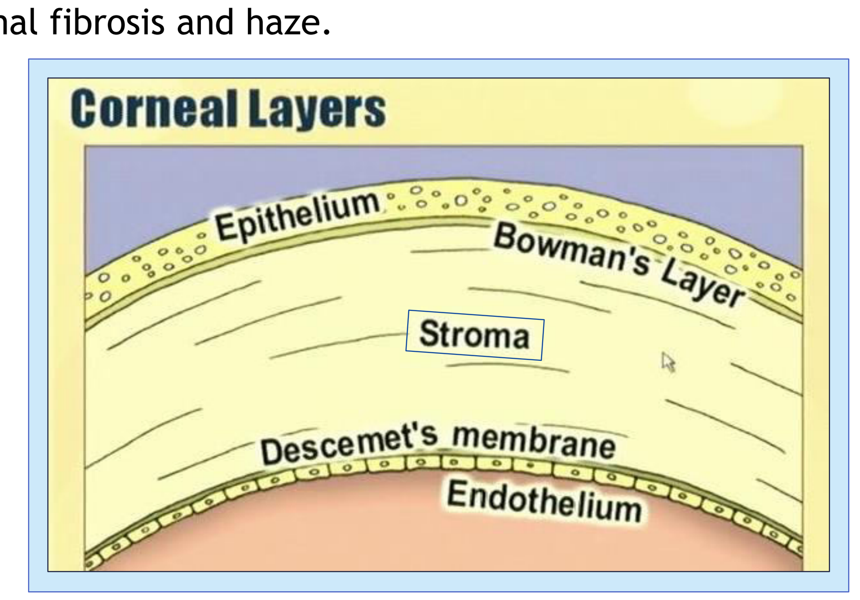

Figure 3. Cross section of the cornea. Within the stroma, sheets of collagen fibrils, called lamellae, are stacked in an orthogonal pattern. In each collagen lamellae, individual fibers are organized in a precise arrangement that is required for transparency. (Figure adapted from http://www.newvisionindia.com/wp-content/uploads/2013/01/corneal-layers-for-web.jpg)

Quiescent Keratocytes
↓
Injury or Surgery
↓
Fibroblasts
↓
Myofibroblasts

Figure 4. Haze occurs when normally quiescent keratocytes become activated and transform into fibroblasts, or further into myofibroblasts. These cells scatter more light, generate contractile forces that can disrupt the collagen architecture, and secrete abnormal extracellular matrix components.

METHODS

We performed PRK on the right eyes of 12 New Zealand white rabbits and scanned both eyes using *in vivo* confocal microscopy at various time points.

-7 days (pre-scan) → PRK → 7 days → 21 days → 90 days → 6 months

Figure 5. Time points at which rabbits were scanned using *in vivo* confocal microscopy. A subset of rabbits was also sacrificed at each post-PRK time point for *in situ* multiphoton fluorescent imaging of f-actin and second harmonic generation (SHG) imaging of collagen organization.

In house *Confo* software was used to build 3D reconstructions and measure stromal thickness and backscatter (haze).

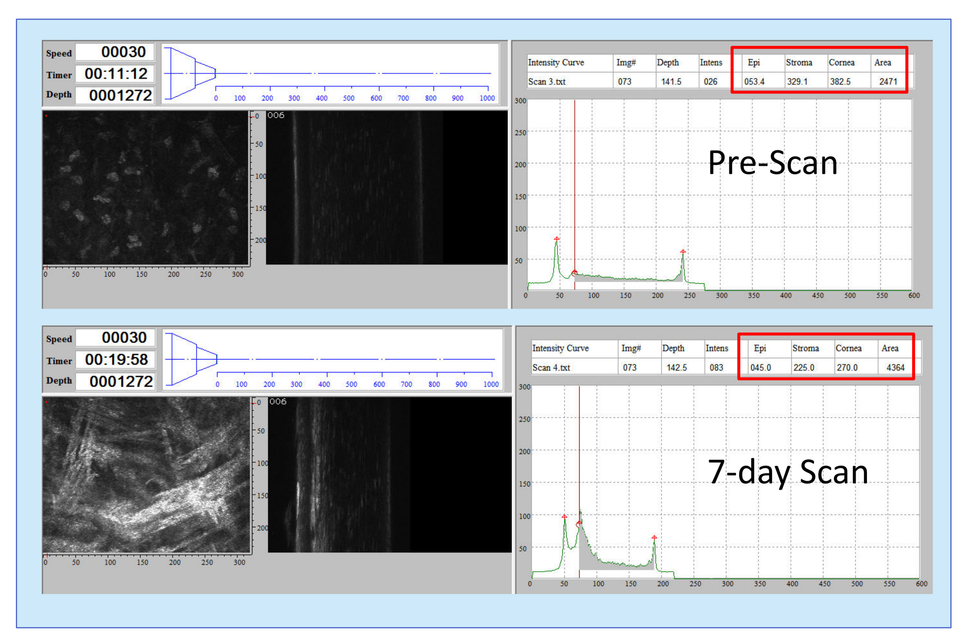

Figure 6. *Confo* software interface. By marking the locations of epithelium, stroma, and endothelium in the 3-D image stack, we can obtain the thickness of each layer. In addition, the area under the thickness v. intensity curve can be used as to assess stromal backscatter (haze).

RESULTS

Full thickness confocal scans indicate that cells in the wound region become activated and induce haze up to 21 days after surgery. However, backscattering gradually decreases at 90 days and 6 months after injury. Cells in the posterior stroma remain quiescent at all time points.

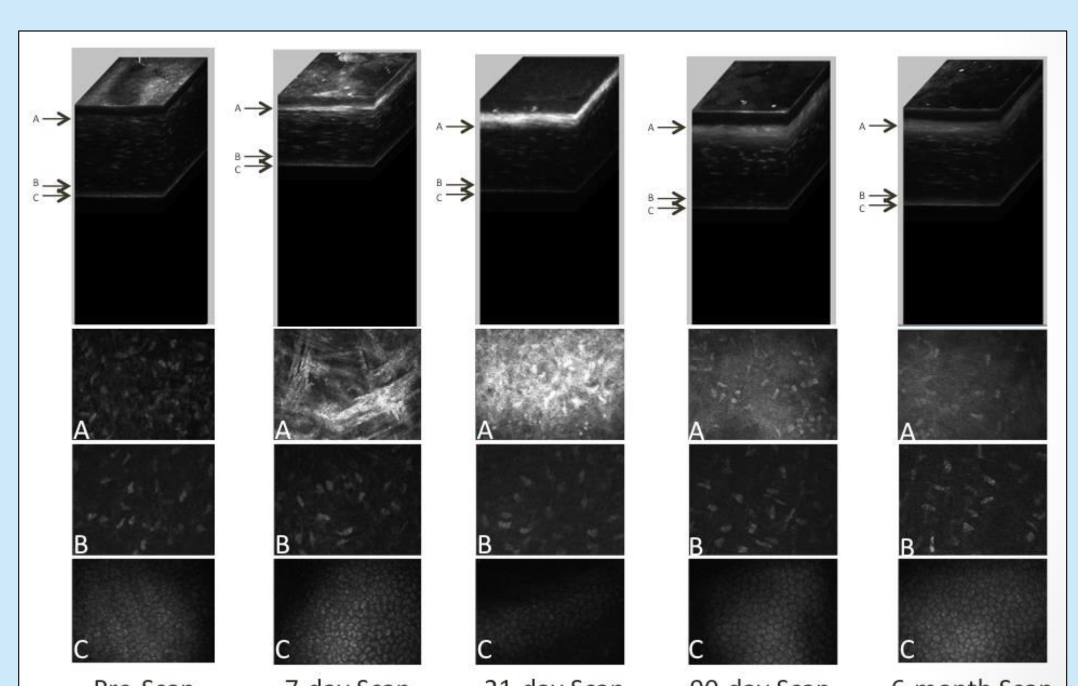

Figure 7. *In vivo* confocal images taken from the anterior stroma (A), posterior stroma (B), and endothelium (C). Top row shows full 3-D image stacks.

RESULTS

Stromal thickness first decreased, then increased. Stromal backscatter (haze) first increased, then decreased.

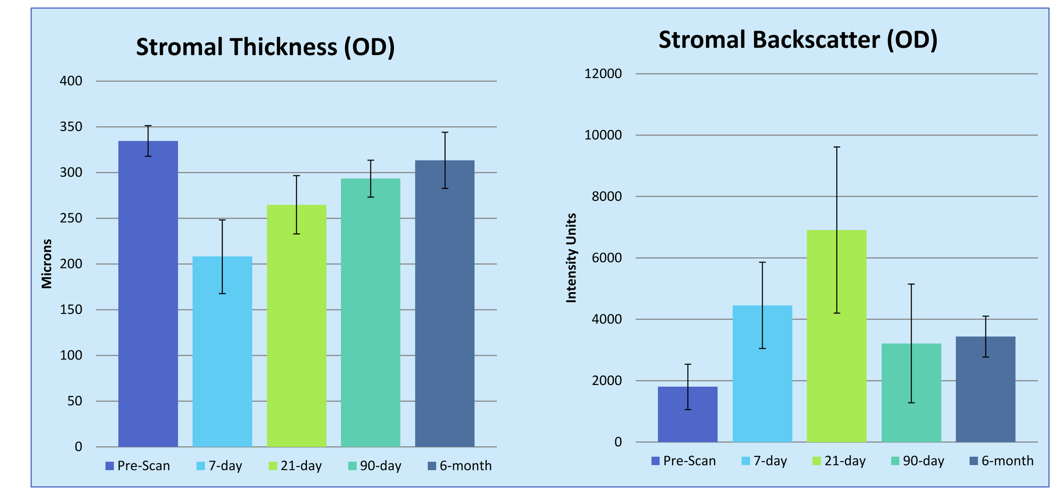

Figure 8. Thickness variation corresponds to tissue removal during surgery, followed by partial tissue regeneration. Haze increase up to 21 days is due to activation and transformation of keratocytes to myofibroblasts. Haze is significantly reduced at 90 days and 6 months after surgery.

In situ confocal imaging post-injury shows that cells (F-actin) and collagen often co-align within the stroma.

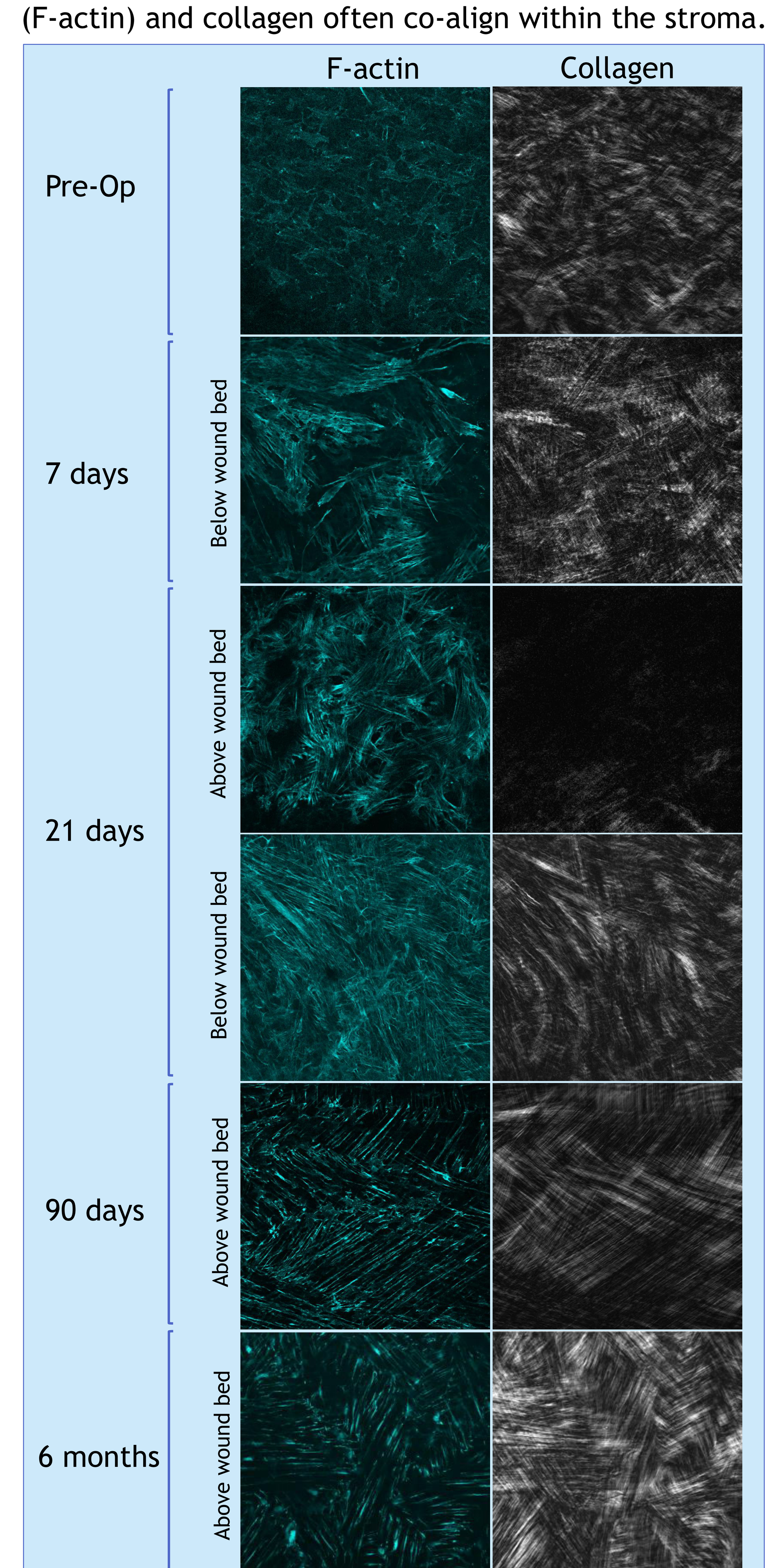

Figure 9. These *in situ* confocal images show differences in F-actin and collagen organization at different locations within the stroma. "Above wound bed" refers to the area on top of the ablated stromal tissue. "Below wound bed" refers to the area located directly beneath the ablated tissue. Note that the disorganized cells and fibrotic tissue present at 21 days become reorganized into a lamellar pattern at 90 days and 6 months after surgery.

DISCUSSION & CONCLUSIONS

Images acquired using both *in vivo* and *in situ* confocal imaging suggest that stromal wound healing occurs in two phases. During the initial phase (7 to 21 days), keratocytes located beneath the area of injury, where native collagen is intact, do not transform into myofibroblasts or form fibrotic tissue.

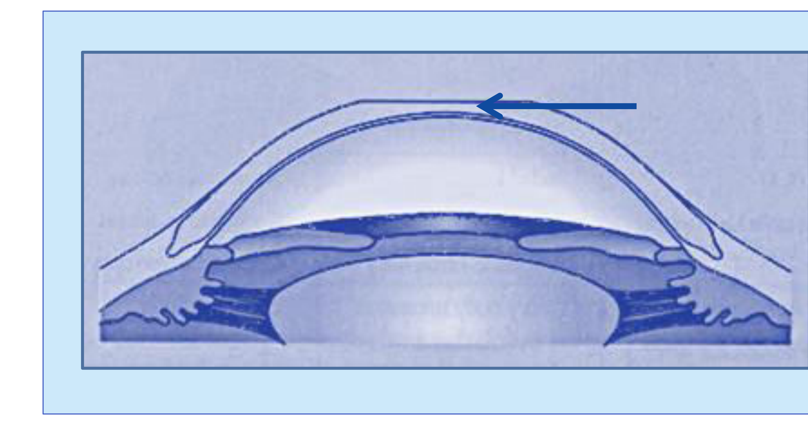

Figure 10. "Beneath the area of injury." In this region, native collagen is intact. Cells migrate into this region but fibrotic tissue does not form.

In contrast, keratocytes that migrate on top of the area of injury, where native collagen has been removed, initially align randomly and produce fibrotic tissue, causing haze. Over time, cells appear to remodel this fibrotic tissue, producing a structure similar to the native stroma in both structure and transparency.

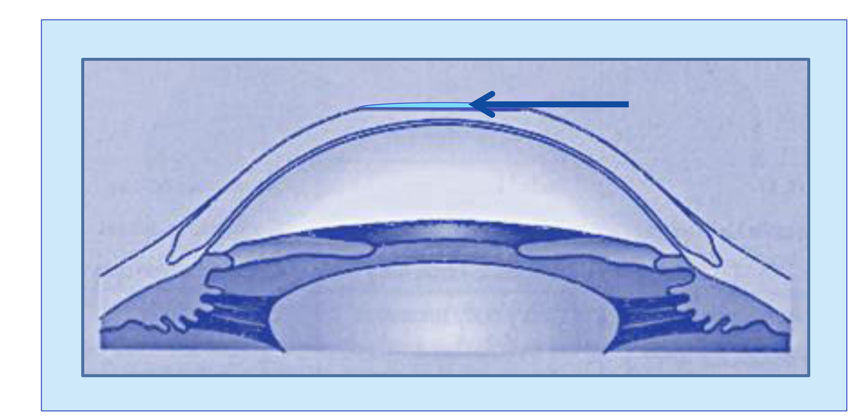

Figure 11. "Above the area of injury." In this region, native collagen has been disrupted and healing occurs in two phases. Initially, keratocytes migrate onto this surface and align randomly, producing fibrotic tissue. Over time, keratocytes appear to remodel the fibrotic tissue into a lamellar structure that resembles the native stromal collagen.

Understanding the underlying mechanism of this process could lead to treatments for reversing corneal scarring, which is a leading cause of blindness worldwide.

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ACKNOWLEDGEMENTS

We are grateful for the support of the UT Southwestern Summer Medical Student Research Program.