A Microscopy Study of the Structural Features of Post-LASIK Human Corneas

Mohammad Abahussin¹,², Sally Hayes¹, Henry Edelhauser³, Daniel G. Dawson³,⁴, Keith M. Meek¹*

¹ Structural Biophysics Research Group, School of Optometry and Vision Sciences, Cardiff University, Cardiff, United Kingdom, ² Optometry Department, College of Applied Medical Science, King Saud University, Riyadh, Saudi Arabia, ³ Emory Eye Center, Emory University, Atlanta, Georgia, United States of America, ⁴ Department of Ophthalmology, University of Florida, Gainsville, Florida, United States of America

Abstract

Purpose: To study the structural features of human post-LASIK corneas.

Methods: A pair of post-mortem donor corneas, from a 55-year-old patient who underwent uncomplicated LASIK surgery five years previously, were bisected and fixed in 4% paraformaldehyde. The right cornea and one half of the left cornea were processed for light microscopy and scanning electron microscopy. One half of the right cornea was also examined by transmission electron microscopy.

Results: The flap-bed interface could be easily detected several years after LASIK and, although the flap appeared to be in close association with the stromal bed, there was a noticeable absence of reconnection between adjacent severed lamellae. Tissue gaps were evident at the flap margin, which once free of cellular components revealed the presence of a few bridging fibres.

Conclusion: Examination of corneas five years after LASIK revealed evidence of primitive reparative scar development at the wound interface, but no reconnection of severed collagen lamellae. Such findings may explain the occurrence of flap dislocation following trauma in some patients months or years after surgery.

Citation: Abahussin M, Hayes S, Edelhauser H, Dawson DG, Meek KM (2013) A Microscopy Study of the Structural Features of Post-LASIK Human Corneas. PLoS ONE 8(5): e63268. doi:10.1371/journal.pone.0063268

Editor: Rajiv R. Mohan, University of Missouri-Columbia, United States of America

Received November 13, 2012; Accepted March 30, 2013; Published May 1, 2013

Copyright: © 2013 Abahussin et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by a 5-year United Kingdom Medical Research Council Grant (G0600755 awarded to KMM). MA was the recipient of a scholarship from King Saud University, Riyadh, Saudi Arabia. KMM is a Royal Society-Wolfson Research Merit Award Holder. These funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: meekkm@cf.ac.uk

Introduction

Laser in situ keratomileusis (LASIK) has been used to correct corneal refractive error for over 20 years [1]; the surgery involves the use of a microkeratome to create a hinged corneal flap and the in situ ablation of the exposed stromal bed with an excimer laser (Figure 1A). In the literature, a large number of publications have evaluated the results of LASIK surgery in terms of its clinical outcomes. However, few have investigated the ultrastructural changes that occur within the cornea and result in the creation of a flap that can be easily dislocated many years after treatment [2,3]. Moreover, most studies that concentrate on ultrastructural changes are based on animal models [4,5,6] and one must realise that the architecture [7] and material properties [8] of animal corneas can be quite different from those of humans.

This paper examines the corneal ultrastructure of two post-mortem human corneas that underwent uncomplicated LASIK 5 years previously. The findings contribute to our understanding of the material behavior of post-LASIK corneas.

Methods

Ethics statement

The research presented in this manuscript was approved by the Human Science Ethical Committee (School of Optometry and Vision Sciences, Cardiff University, UK). The institutional review board approved the use of all corneas described in this study; a waiver of consent was given for the donor corneas as they were obtained from an eye bank (Georgia Eye Bank, Atlanta, USA). All tissue used in this study was obtained in accordance with the tenets of the Declaration of Helsinki and local ethical rules were adhered to throughout.

Tissue

Two healthy corneo-scleral buttons from a 55 year old male donor who underwent uncomplicated bilateral LASIK surgery five years prior to death were provided by the Georgia Eye Bank for the purposes of this study. No other ocular history details, such as refractive errors and corneal topography maps, were available...
from the eye bank. The corneo-scleral buttons were stored for 7 days in Optisol-GS solution (Bausch and Lomb Rochester, NY) before being fixed in 4% paraformaldehyde for one week. The corneo-sclera buttons were then bisected and half of the left cornea and both halves of the right cornea sent to the UK. On arrival, the samples were stored for a further two weeks in 4% paraformaldehyde before being processed for light microscopy and scanning electron microscopy. The right cornea was also processed for transmission electron microscopy.

**Light Microscopy**

A 2 mm limbus to limbus section was cut from each cornea (Figure 1) and embedded in wax. Thin corneal cross sections (7 µm thick) were cut from the wax blocks and mounted on slides. The corneal sections were wax cleared and dehydrated before being stained with Haematoxylin and Eosin and examined under a light microscope (DMRAZ, Leica Milton, UK).

**Scanning electron microscopy**

One half of each corneo-scleral button (Figure 1) was immersed in 2.5% glutaraldehyde for 2 hours. The tissues were then placed into a solution of 5% NaOH for 2 days at room temperature (20–25 °C) in order to remove most of the cellular elements (including epithelial cells) whilst keeping the collagen lamellae intact [9]. The samples were postfixed in 1% osmium tetroxide (O₃O₄) for 3 hours and stained by immersion in 0.5% aqueous solution of uranyl acetate for 60 minutes to enhance visualization. The tissue samples were subsequently dehydrated in graded ethanol (ranging from 50% to 100%) and immersed in hexamethyl disilazane (HMDS). Finally, the tissues were coated with a layer of gold in a sputter coater (Polaron system, Quorum Technologies, UK) to enhance structure visualisation. A high vacuum Phillips XL 20 scanning electron microscope (FEI Company, Eindhoven, Netherlands) was used to study the corneal tissue structure at different magnifications.

**Transmission Electron Microscopy and Light Microscopy**

Tissue samples taken from the middle of the flap and the residual stromal bed (Figure 1) were immersed in 2.5% glutaraldehyde; after 2 hours, the corneal tissue was removed from the glutaraldehyde and postfixed in 1% osmium tetroxide (O₃O₄) followed by 0.5% uranyl acetate. The tissue was then dehydrated in graded ethanol (ranging from 50% to 100%) and embedded in pure Epon resin. Ultrathin sections were cut and stained with uranyl acetate and lead citrate prior to examination in a Phillips EM 208 transmission electron microscope (FEI Company, Eindhoven, Netherlands).

**Results**

Light microscopy images showed that the flap-bed interface was clearly visible five years after LASIK surgery (Figure 2) and, although some epithelial cells were missing (due to tissue storage and processing), Bowman’s layer and Descemet’s membrane appeared intact.

When viewed by scanning electron microscopy, the flap was seen to be in close association with the stromal bed, except at the flap margin (Figures 3 and 4), where a tissue gap measuring 10–20 µm in width was observed (Figure 3C–D). Closer examination of the flap and stromal bed interface revealed collagen lamellae to be in disarray with no reconnections formed between adjacent...
severed lamellae (Figure 3C and 4C). The wound gap at the flap margin showed evidence of a few bridging fibres, but appeared empty of cellular components (Figure 3D). At the primary incision site, chatter lines caused by the action of the mechanical microkeratome were clearly seen in the residual stromal bed (Figure 4D).

Transmission electron microscopy images showed stromal collagen lamellae lying essentially parallel to the corneal surface on either side of the wound interface (Figure 5). The interface wound, which measured 1–2 μm thick, appeared free from collagen fibrils or bridging components, but contained primitive scar tissue similar to that reported previously [10] (Figure 5).

**Discussion**

Typically, the postoperative results of LASIK surgery are excellent and pain-free with most patients seeing well enough to work or drive without correction the very next day. However, the images presented here show that the LASIK flap can be readily detected 5 years after surgery since only a primitive reparative stromal scar is formed. Unfortunately, due to the limited information supplied to eye banks, most of the clinical details of the patient were unknown, but the results clearly demonstrate the lack of long-term wound healing at the flap margins and across the flap bed. It should however be noted the patient was 50 years old at the time of surgery and there is a significant trend for increased refractive failure with increasing patient age suggesting that stromal changes with age may have influenced post-operative healing [11,12].

Scanning electron microscopy revealed that the superficial flap edges and the interface wound are visually distinct up to five years after the LASIK procedure and that the flap edges appear empty and separated from the adjacent corneal tissue by a gap of 10–20 μm. This gap was almost certainly formed during scanning electron microscopy processing, as immersion of the tissue in NaOH causes epithelial ingrowth to be removed whilst keeping the collagen lamellae intact. Moreover, it is possible that the tissue fixation process and the subsequent tissue shrinkage may have increased the gap width. It is interesting to note that even after NaOH treatment some cellular material remained at the limbus. The clusters of cells appeared to be basal epithelial cells which are clearly more adherent to the corneal surface than other cells.
Remarkably, even the chatter marks caused by the mechanical microkeratome blade as the flap was created were still visible in the stromal bed (at the primary incision site) once the covering cell layers were removed by NaOH. Previous studies identifying the presence of chatter marks caused by mechanical microkeratomes have shown that the marks are most prominent at the flap margin [13] and become more pronounced after blade reuse [14]. It has been suggested that the tissue irregularities may be related to the development of pressure ridges ahead of the blade [13]. Regardless of the cause, the observed chatter marks provide a remarkable visual demonstration of the lack of connective tissue wound healing at the flap edges following LASIK surgery. In a detailed ultrastructural study of post-mortem LASIK donor corneas, Dawson et al. [10] found two types of reparative stromal wound healing responses associated with the LASIK surgery: a hypercellular fibrotic scar at the flap wound margin (which serves as an adhesive to hold the corneal flap in place) and a weaker transparent, hypocellular primitive scar in the remaining para-central and central areas of the lamellar wound [10]. Our study has shown that collagen lamellae around the wound interface run essentially parallel to the corneal surface. However, the fact that collagen lamellae do not bond with each other at the location of the primitive scar confirms that corneal wound healing after LASIK happens only superficially (in the epithelial cell layers). This helps to explain reports that the biomechanical properties of the post-LASIK cornea are primarily determined by the residual stromal bed and that the LASIK flap contributes only minimally to the overall strength and stability of the cornea [15,16]. In fact, Schmack et al. [17] showed that on average the central and paracentral hypocellular primitive stromal scar regains only 2.4% of normal stromal strength and displays no evidence of remodelling even 6.5 years after LASIK surgery. The lack of reconnection between severed collagen lamellae at the flap/stromal bed interface also provides an explanation for reported instances of flap dislocations following trauma, which may occur many years after the LASIK surgery [2,3].

In summary, this study used a technique that removes cellular constituents to highlight the collagenous architecture of the

Figure 4. Scanning electron microscopy images showing the primary incision site in a post-mortem LASIK cornea. The flap edge can be easily seen (yellow arrows in A). Examination of the primary incision point (white arrows in B) reveals chatter marks caused by the action of the mechanical microkeratome (white arrows in C and D). Collagen lamellae appear disorganised at the flap/stromal bed interface (blue arrows in C). Original magnifications for A, B, and C are \( \times90 \), \( \times600 \), \( \times1050 \) and \( \times500 \) respectively.

doi:10.1371/journal.pone.0063268.g004
corneal stroma and in doing so identified the ultrastructural features present in human corneas after LASIK surgery. The images presented here clearly show that the LASIK flap does not integrate with the residual stromal bed, even 5 years after surgery.

Acknowledgments

The authors thank Dr Anthony Han and Dr Rob Young for their assistance with microscopy and Dr. Andrew Quantock for useful discussions and advice.

Author Contributions

Revised the paper for intellectual content: HE DGD. Final approval of the version to be published: SH MA HE DGD KMM. Conceived and designed the experiments: MA KMM HE DGD. Performed the experiments: MA. Analyzed the data: MA KMM SH. Wrote the paper: SH MA KMM.

References

1. Reinstein D, Archer T, Gobbe M (2012) The history of LASIK. Journal of Refractive Surgery 28: 291–298.
2. Holt D, Süder S, Mifflin M (2012) Surgical management of traumatic LASIK flap dislocation with macrostriae and epithelial ingrowth 14 years postoperatively. Journal of Cataract and Refractive Surgery 38: 357–361.
3. Kim H, Silverman C (2010) Traumatic dislocation of LASIK flaps 4 and 9 years after surgery. Journal of Refractive Surgery 26: 447–452.
4. Anm M, Wetzl W, Winter M, Uschoff D, Duncker G (1996) Histopathological comparison of photorefractive keratectomy and laser in situ keratomileusis in rabbits. Journal of Refractive Surgery 12: 758–766.
5. Park CK, Kim JH (1999) Comparison of wound healing after photorefractive keratectomy and laser in situ keratomileusis in rabbits. Journal of Refractive Surgery 25: 842–850.
6. Kamma-Lorger C, Boote C, Hayes S, Alten J, Boyton M, et al. (2008) Collagen ultrastructural changes during stromal wound healing in organ cultured bovine corneas. Experimental Eye Research 88: 953–959.
7. Hayes S, Boote C, Lewis J, Sheppard J, Ahalusin M, et al. (2007) Comparative study of fibrillar collagen arrangement in the corneas of primates and other mammals. Anatomical Record-Advances in Integrative Anatomy and Evolutionary Biology 290: 1542–1550.
8. Hoetzell D, Altmann P, BuzaK K, Choe K (1992) Strain extensometry for comparison of the mechanical response of bovine, rabbit, and human corneas. Journal of Biomechanical Engineering 114: 202–215.
9. Komai Y, Ushiki T (1991) The three-dimensional organisation of collagen fibrils in the human cornea and sclera. Investigative Ophthalmology and Visual Science 32: 2244–2250.
10. Dawson DG, Kramer TR, Grossniklaus HE, Waring GO, Edelhauser HF (2005) Histologic, Ultrastructural, and Immunofluorescent Evaluation of Human Laser Assisted In Situ Keratomileusis corneal wounds. Arch Ophthalmol 123: 741–756.
11. Feltham M, Wong R, Wolle R, Stapleton F (2008) Variables affecting refractive outcome following LASIK for myopia. Eye 22: 1117–1123.
12. Huang D, Stuhi R, Carr J, Thompson K, Waring G (1999) Multiple regression and vector analysis of laser in situ keratomileusis for myopia and astigmatism. Journal of Refractive Surgery 15: 538–549.
13. Hamill M, Kohnen T (2002) Scanning electron microscopic evaluation of the surface characteristics of 4 microkeratome systems in human corneas. Journal of Cataract and Refractive Surgery 28: 328–336.
14. Behrens A, Langenbucher A, Kus M, Rummelt C, Seitz B (2000) Experimental evaluation of two current-generation automated microkeratomes: the Hansatome and the Superatome. American Journal of Ophthalmology 129: 59–67.
15. Roberts C (2000) The cornea is not a piece of plastic. Journal of Refractive Surgery 16: 407–413.
16. Chan C, Boxer Wachler B (2006) Corneal ectasia and refractive surgery. International Ophthalmology Clinics 46: 13–25.
17. Schmack I, Dawson D, McCarey B, Waring G, Grossniklaus H, et al. (2005) Cohesive Tensile Strength of Human LASIK Wounds With Histologic, Ultrastructural, and Clinical Correlations. Journal of Refractive Surgery 21: 433–445.