Laser in situ keratomileusis: a scanning electron microscopic study

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Laser in situ keratomileusis (LASIK, or 'flap and zap') is a recently developed technique for correction of a wide spectrum of refractive errors, ranging from high myopia to high hypermetropia. This is done by resecting a thin superficial lamella of cornea with a motorised microkeratome, then ablating stromal tissue with an excimer laser, and rapidly restoring the corneal integrity by folding the lamellar flap back in place *(Figure 1)*. We carried out an electron microscopic study on donor eyes to help elucidate the underlying mechanisms of this new surgical technique.

Background

Keratomileusis was conceived by José Barraquer some 50 years ago (Barraquer 1949), in an operation which involved the microkeratome resection of a superficial corneal lamella which was reshaped on a cryolathe, and then sutured back onto the recipient. This procedure was extremely difficult to perform successfully, as the early microkeratome and cryolathe gave poorly reproducible results. Further, the excised tissue flap was devitalised by the cryolathing and had to be sutured back in place. Only spherical refractive errors could be corrected.

The technique has been gradually refined and in the last two or three years significant advances have been made. With the introduction of excimer lasers and new microkeratome systems *(Figure 2)*, (LASIK) is now a realistic procedure for surgical correction of a wide range of ametropia (Pallikaris and Siganos, 1994).

Interest in surgical correction of refractive error has developed with the introduction of radial keratotomy (RK) and excimer laser photo-refractive keratectomy (PRK). PRK is currently used to correct mild to moderate myopia (Aron-Rosa et al 1995), and the most recent generation of lasers will also effectively correct astigmatism (Gallinaro et al 1996). PRK is, however, limited in accuracy by:

- The unpredictability of the eye's healing response to the laser wound.
- The varying degrees of regression from the initially achieved refraction.
- Problems of subepithelial scarring, which can cause glare and reduce the best corrected acuity (Buratto 1994).

LASIK offers the prospect of reducing some of these problems as, instead of leaving a large open wound on the cornea at the end of surgery, the lamellar flap with its epithelium is replaced and there is extremely rapid wound healing, with minimal tissue response.

Following PRK, a patient may experience severe pain for 24-48 hours, as the cornea re-epithelialises. There then follows a protracted period of visual recovery over several months, as the epithelial and stromal reaction to the treatment gradually modifies the corneal surface. By comparison, at the completion of a LASIK proce-

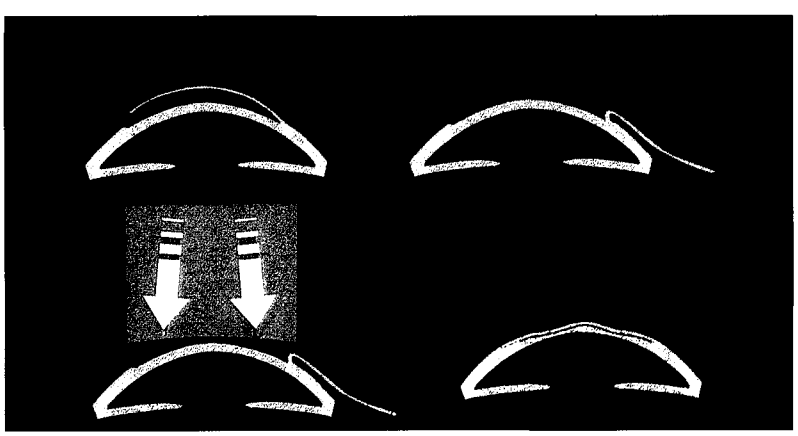


Figure 1. Diagram of LASIK for correction of hypermetropia.

a) A thin flap is resected by microkeratome.
b) The flap is folded to one side.
c) Excimer laser ablation of the superficial stroma is carried out.
d) The flap is folded back in place.

dure there is no significant epithelial defect and the patient experiences little or no post-operative pain. Because the epithelial barrier is intact over the resected lamella it is not necessary to suture the flap back, as the hydrostatic forces generated by the endothelial pump will hold it in place. By the first post-operative day the epithelium has healed across the small linear break demarcating the edge of the flap, and the reactive response of the eye to the surgical trauma is so minimal that topical steroid treatment is not generally required. Visual recovery is extremely rapid following LASIK, and by one week post-operatively a good assessment can be made of the final refractive outcome (Güell and Müller 1996). There appears to be little in the way of stromal wound healing response to the LASIK procedure, and in many patients the treated interface is hard to define, even on careful slit-lamp examination. The crescent-shaped edge of the resected flap can, however, usually be seen as there is a more obvious reactive healing process in this region.

In order to appreciate better the nature of the microkeratome and laser wounds created in LASIK, we carried out experimental procedures in human cadaver eyes, and examined the tissue by scanning electron microscopy.

Materials and methods

A pair of human eyes that had been donated for transplantation or research, but which were not suitable for transplantation, were used in this study. Each globe was in turn held with a suction ring and a lamella flap cut with a Chiron Automatic Corneal Shaper. A Technolas Keracor 116 excimer laser was used to ablate tissue from the stromal bed and the flap was then folded back in place. The eyes were fixed in 3% glutaraldehyde solution in 0.1 M cacodylate buffer for 24 hours (pH 7.4), washed in buffer twice, then postfixed in 1% osmium tetroxide solution in the same buffer for 16 hours. The samples were subsequently washed with buffer solution and dehydrated in an ethanol series up to 100% ethanol (30, 70, 90 and 100%). The fixed cornea was taken from the ethanol and immersed in Agar 100 resin for 24 hours, then placed on a tissue holder and quickly frozen in liquid nitrogen for a few minutes, together with a razor blade. Each cornea was cracked with the razor blade and divided into two parts. These parts were given two washes of 100% ethanol to remove resin, and then critical-point dried in liquid carbon dioxide. The corneal fragments were glued onto microscope stubs and sputter-coated with gold. Examination was carried out with a scanning electron microscope (Zeiss DSM 940) operated at 30 kV.

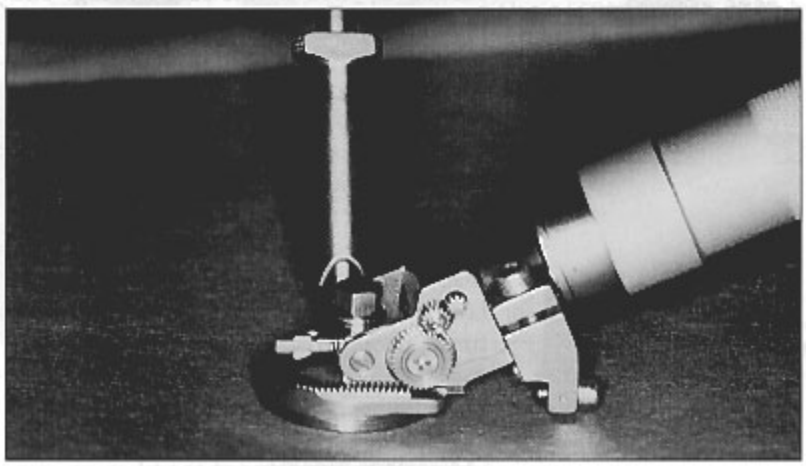
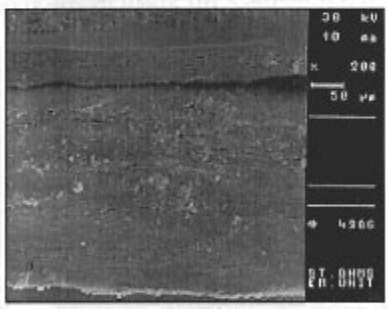


Figure 2. The assembled microkeratome, engaged in the dovetail guide of the suction ring.

Figure 3a



Results

Figure 3a (above) shows a transverse sectional view through the central area of a cornea following LASIK. The cornea has been cleaved along the mid-line by cryofracture and the plane of breakage through the superficial lamella is slightly offset from that of the underlying stroma. The lamella can be seen to be of uniform thickness, of around 50 microns. *In vivo* the thickness of the resected lamella is determined by the distance plate of the microkeratome, which was in this case 160 microns. The cornea had some post-mortem oedema at the time of resection, and will also have undergone shrinkage during the fixation and processing for electron microscopy. The underlying stroma is here 300 microns thick, this being a combination of thinning from the laser ablation and from the fixation process.

Figure 3b shows a general view of the stromal bed with the overlying lamellar flap removed. The corneal epithelium extends to the edge of the serrated cut of the microkeratome. Ridges of stromal tissue can be seen at the edge of the wound, but these taper off quite rapidly and the central area of stroma – which has been cut not only with the microkeratome but also by the laser – can be seen to be very smooth.

Figure 3c shows the central laser-ablated area under high power (500x original magnification) and there are no distinctive morphological features in this area.

Figure 3d shows a general view of the under side of the lamellar flap. The appearance is very similar to that of the bed, with ridges at the periphery, but again presents a smooth central area. This central area has not, however, been cut with the laser; only by the microkeratome blade.

Figure 3e shows a high power view of the central area on the cut surface of the flap. There was no significant difference between the findings in the two eyes examined.

Discussion

Early versions of Barraquer's microkeratome required the surgeon to advance the cutting head manually across the surface of the cornea, the movement being controlled by flanges on the microkeratome head running in dovetail guides on the suction ring. This was extremely difficult to perform evenly, as any torsional force created between the microkeratome head and suction ring could lead to jamming and severe irregularity in the cut. The Chiron automated keratome motor has an eccentric bush on the drive shaft to produce the blade oscillation, and also has a worm drive which produces translational movement for the microkeratome head, by driving a cog along a pinion track on the suction ring *(Figure 4)*.

This means that the surgeon is not required to produce the force for the translational movement of the microkeratome, and the flap resection process is entirely automated. The lamellar cut is completed in a matter of 2 or 3 seconds, and the motor is then reversed to withdraw the blade from the wound. As evident in the electron micrographs, an extremely smooth bed is produced over a large area encompassing the optical zone of the cornea. The blade does produce ridges in the most peripheral part of the bed, and a number of factors could account for this.

During the cutting movement the central part of the cornea is applanated by the thickness plate of the microkeratome, and the small offset between the thickness plate and the blade determines the thickness of the flap. The whole of the central area of cornea is forced against the thickness plate under some compression, since the intraocular pressure is typically temporarily raised to around 60mm Hg, by distortion of the globe from the action of the vacuum applied by the suction ring. Thus the central corneal tissue is held rigidly between the cutting blade and the plate, whereas the peripheral edge of the corneal flap can perhaps move slightly with the oscillations of the blade, since this area is not in contact with the thickness plate *(Figure 5)*.

Another potentially significant factor is that Bowman's zone – the most superficial layer of stroma – has an amorphous structure, in comparison to the regular arrangement of collagen fibre bundles in the underlying stroma. In addition the blade is cutting the peripheral edge of the flap obliquely to the plane of the fibres running in the stromal lamellae, whereas in the central area of the lamellar resection the cut is in the natural cleavage plane between adja-

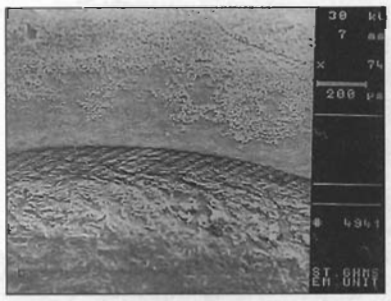


Figure 3b. Stromal bed following microkeratome resection and laser ablation.

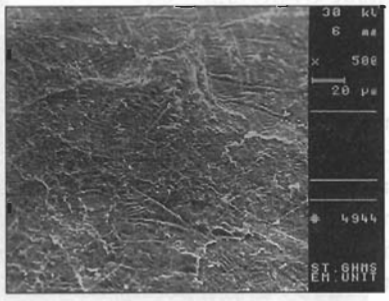


Figure 3c. Laser ablated stromal bed following LASIK.

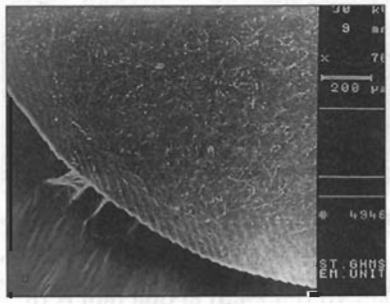


Figure 3d. View of underside of superficial lamellar flap following LASIK.

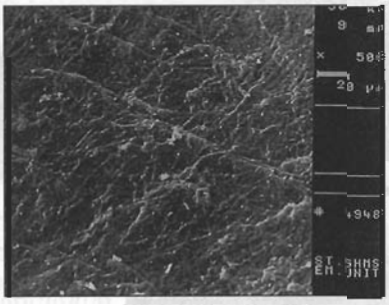


Figure 3e. Central undersurface of flap following LASIK.

cent lamellae.

In clinical practice, the consequence of these findings may be that the irregular ridges around the edge of the flap stimulate reactive fibrosis and visible scarring at the edge of the wound. The irregularity of the wound edge may also lead to poor apposition of the tissue, with the potential for epithelial cells to invade the tissue plane beneath the lamella, a known occasional complication of LASIK. We have demonstrated the smoothness of the central cut area, which ensures that when the flap is replaced the surfaces lie in close apposition, and thus probably incite little in the way of keratocyte response to the tissue disruption. This lack of tissue response is of particular relevance in the correction of hypermetropia, where large areas of mid-peripheral ablation are required in order to produce central steepening. Most workers have found that results of PRK for hypermetropia are disappointing, because the large area of cornea treated with the unusual undulating profile produced seem to provoke excessive tissue reaction, leading to regression, scarring, and irregular astigmatism. For this indication, and also for the higher degrees of myopia, LASIK seems to offer distinct advantages over PRK. The benefits for the treatment of lower degrees of myopia are less clear-cut, as for this indication both PRK and RK can give good results.

Figure 4. Disassembled microkeratome.
The motor drive unit (top left) screws into the headpiece (top right). At the end of the motor drive shaft is a worm drive to actuate the gear train for the translational movement, and an eccentric bush which engages with the drive plate to oscillate the blade (bottom right). The end-stop adjustment ring (bottom left) is used to arrest the microkeratome before the flap is fully resected, leaving a small 'hinge' attachment to the underlying stromal bed.

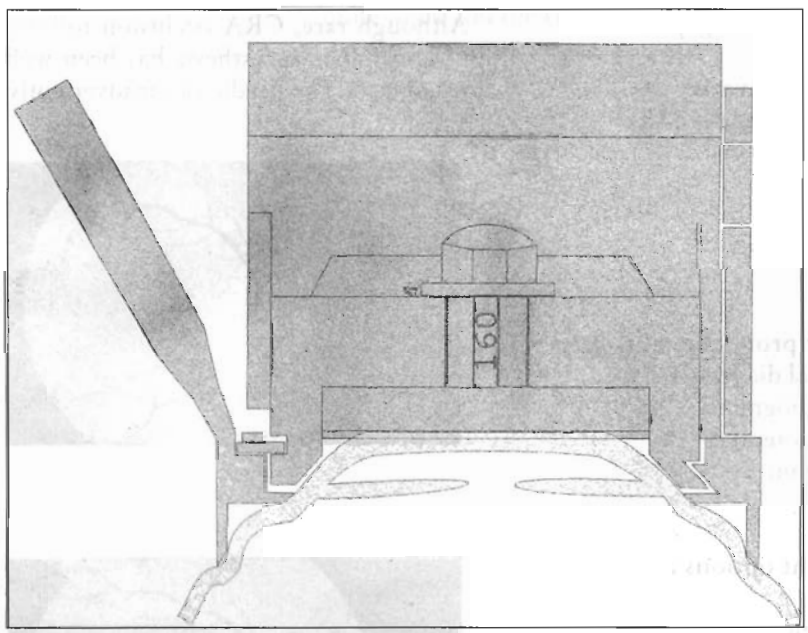


Figure 5. Schematic cross-section of the microkeratome cutting the corneal flap.
The central area of the cornea is applanated by the microkeratome thickness plate. This holds the blade at a depth of 160 microns away from the corneal surface. Only the edges of the cut tissue are not held in compression against the thickness plate.

Conclusion

Keratomileusis using the Chiron microkeratome and excimer laser in situ ablation (LASIK) produces corneal wounds, the cut surfaces of which are very smooth. This no doubt contributes to the rapid visual recovery and early refractive stabilisation of LASIK surgery.

Donor eyes were provided by the Keratec Eye Bank (Registered Charity No: 803386). The Eye Laser Academy provided their facilities for the LASIK surgery, and the scanning electron microscopy was carried out at The Electron Microscopy Unit of St George's Hospital Medical School.

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