

# Corneal wound healing following laser in situ keratomileusis (LASIK): a histopathological study in rabbits

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## Abstract

**Aims**—To investigate the histopathological changes of rabbit corneas after laser in situ keratomileusis (LASIK) and to evaluate the corneal wound healing process.

**Methods**—A LASIK was performed on white rabbit eyes. Postoperatively, rabbits were killed on days 1 and 7, and at 1, 3, and 9 months.

**Results**—Periodic acid Schiff (PAS) positive material and disorganised collagen fibre were seen along the interface of the corneal flap even 9 months after operation.

**Conclusions**—The wound healing process still continued at 9 months after LASIK indicating that a much longer time than expected was required for corneal wound healing following LASIK.

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Laser in situ keratomileusis (LASIK) is currently gaining acceptance as an effective surgical procedure for the correction of refractive errors. Previous studies have described the satisfactory results obtained with this procedure.<sup>1-6</sup> The rapid recovery of vision, minimal risk of corneal haze, and refractive predictability are the main advantages of this surgery. However, histopathological data on the healing of the corneal wounds after LASIK are incomplete.<sup>7-9</sup> As such, some fundamental questions remain unanswered: (1) When is the corneal wound completely healed after LASIK? (2) Is the subepithelial haze seen after PRK also present after LASIK? (3) What are the biological characteristics of the cornea in the wound healing process? and (4) is the strength of the cornea or structural strength restored completely? In order to obtain answers to these questions, we analysed the cornea of rabbits histopathologically after LASIK.

## Materials and methods

The handling of animals was in accordance with the NIH guiding principles on the care and use of animals (DHEW Publication, NIH 80-23). The rabbits were anaesthetised with an intramuscular injection of xylazine (4 mg/kg) and ketamine hydrochloride (3 mg/kg). A nasally based, 160 µm thick, 7.5 mm diameter corneal flap was made using an automated microkeratome (Microlamellar Keratomileusis System, Eye Technology), and a spherical ablation of -10.0 dioptres (D) was performed on the exposed stromal bed using an EC-5000

excimer laser system (Nidek, Japan). All procedures employed a 193 nm wavelength emission, a 5 mm diameter ablation zone, 117 mJ/pulse, and a 30 Hz pulse rate.

The rabbits were killed with an overdose of sodium pentobarbital on days 1 and 7, and at 1, 3, and 9 months after the LASIK procedure. The excised cornea was divided into four pieces. A quarter was fixed in 10% formaldehyde and embedded in paraffin wax. Serial 6 µm sections were cut and stained with either haematoxylin and eosin, or periodic acid Schiff (PAS), or Masson's trichrome stain. The second quarter was frozen in OCT compound (Baxter Scientific, Columbia) for immunohistochemistry. Methods for immunohistochemistry have been described previously.<sup>10</sup> A monoclonal antibody to type IV collagen was obtained from Southern Biotechnology (Birmingham, AL, USA). The third quarter was treated for electron microscopy as described.<sup>11</sup> The remaining quarter was saved.

## Results

### 1 DAY AFTER LASIK

Tissue obtained on day 1 after LASIK and stained with haematoxylin and eosin revealed a hyperplastic epithelial plug at the edge of the incision (Fig 1A). Only a few polymorphonuclear leucocytes were observed at the wound margin. Although normal staining for type IV collagen was seen at the centre of the cornea (Fig 1B), staining for type IV collagen was absent adjacent to the wound made by the microkeratome blade passing through the epithelium into the stroma (Fig 1C). Under the electron microscopy, the corneal flap was swollen and wavy, and collagen lamellae with shrunken keratocytes were present (Fig 1D). In contrast, parallel formation of collagen lamellae with flat-shaped keratocytes were observed in the stromal bed.

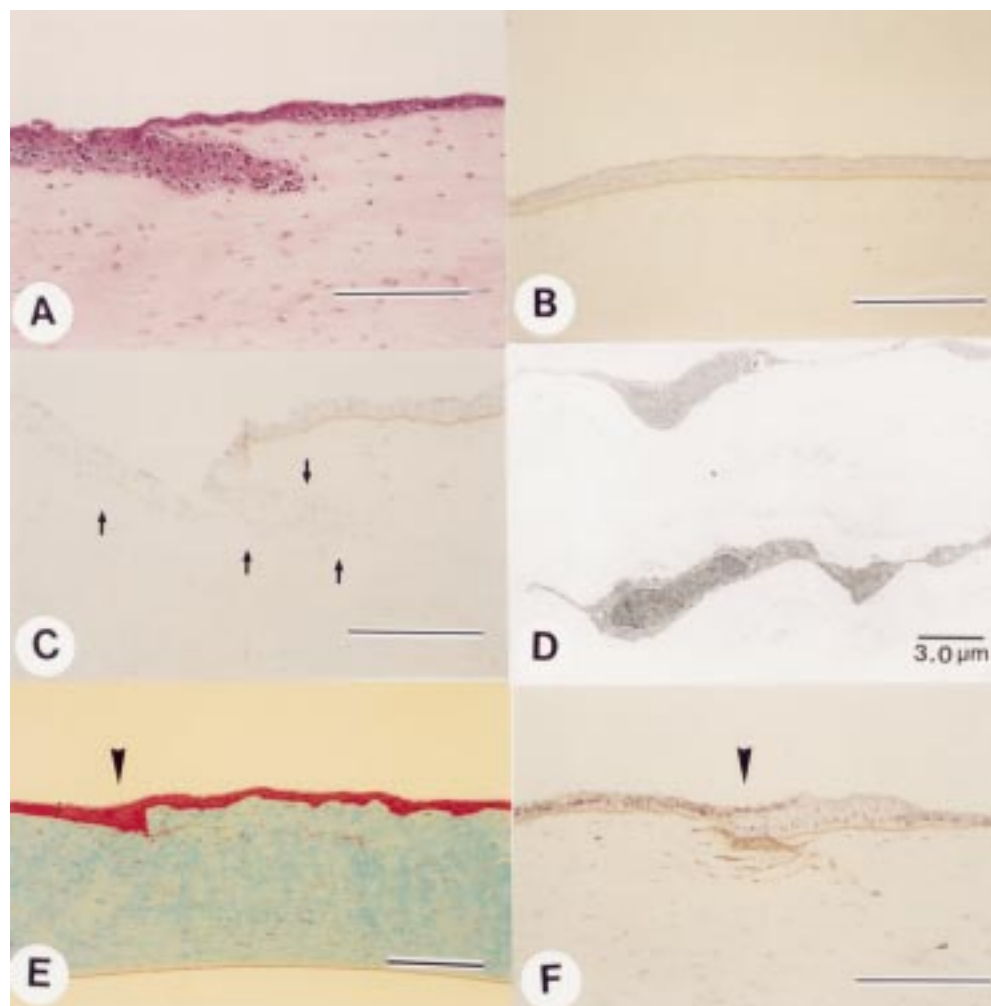
### 7 DAYS AFTER LASIK

Seven days after the LASIK procedure, the epithelial plug was smaller (Fig 1E). At the wound margin, diffuse staining for type IV collagen was seen directly under the epithelial layer indicating that the wound healing of the epithelial basement membrane was not yet complete (Fig 1F). Activated keratocytes were present beneath the cut edge. Although no staining for type IV collagen was observed along the lamellar incision, positive staining for type IV collagen was seen around the portion of epithelial ingrowth (Fig 2A).

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**Figure 1** (A) Haematoxylin and eosin staining section on day 1 after LASIK. (bar 200  $\mu$ m). (B) Normal epithelial basement membrane structure is observed at the centre of the cornea. (Anti-type IV collagen, postoperative day 1, bar 200  $\mu$ m.) (C) At the wound margin, epithelial basement membrane structure is absent (arrows). (Anti-type IV collagen, postoperative day 1, bar 200  $\mu$ m). (D) Electron microscopy showing wavy keratocyte with swelling the stroma in the corneal flap (postoperative day 1). (E) One week after LASIK. Masson trichrome staining shows the epithelial plug is smaller than on day 1 after the surgery. Arrowhead indicates the wound edge (bar 200  $\mu$ m). (F) Diffuse staining for type IV collagen is noted beneath the epithelium at the wound margin. Arrowhead indicates the wound edge (postoperative day 7, bar 200  $\mu$ m).

#### 1 MONTH AFTER LASIK

Haematoxylin and eosin stained sections demonstrated that the wound margin recovered to the level of a normal cornea. The section stained with PAS, however, showed positively stained material deposited along the lamellar interface (Fig 2B).

#### 3 MONTHS AFTER LASIK

Haematoxylin and eosin stained sections appeared almost normal at 3 months after surgery (Fig 2C). Surprisingly, although staining for type IV collagen at the wound margin became more linear, diffuse staining for type IV collagen was seen even at this post LASIK time (Fig 2D).

#### 9 MONTHS AFTER LASIK

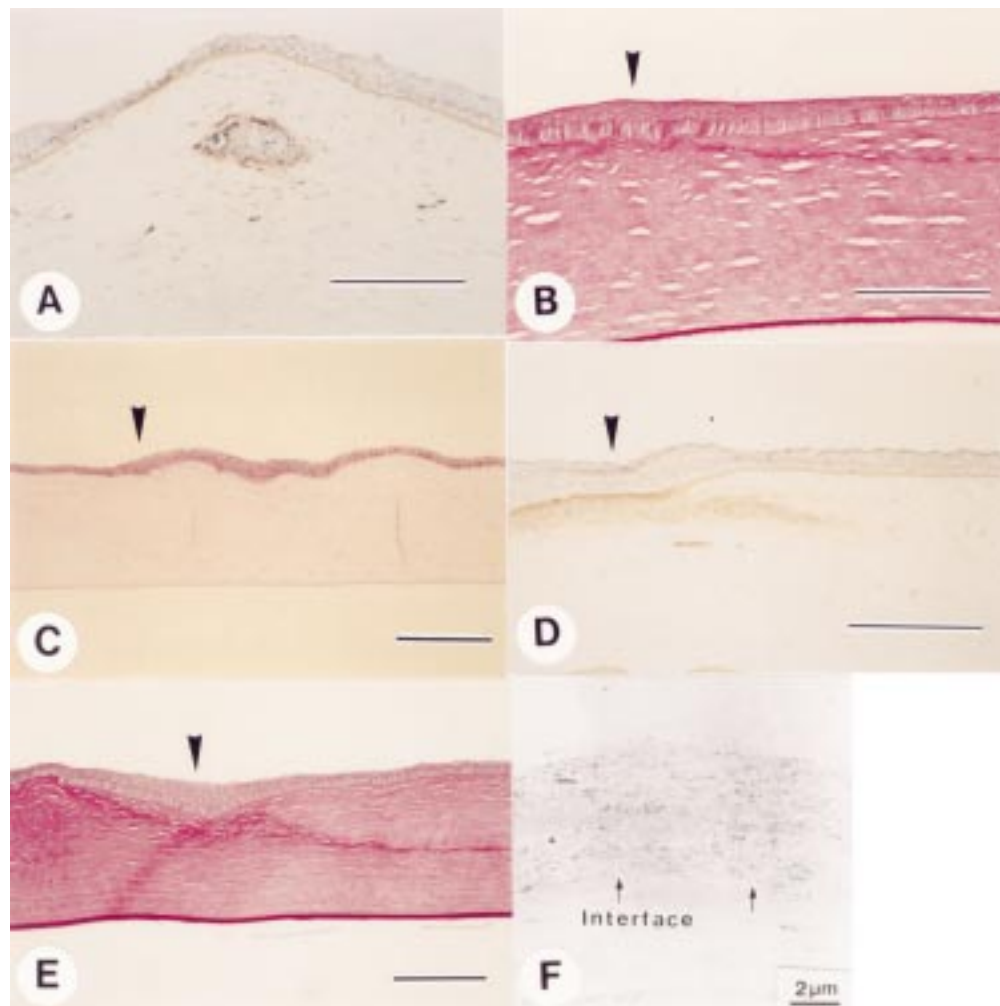
PAS staining demonstrated that PAS positive material was still deposited along the lamellar incision (Fig 2E). Electron microscopy revealed that the intact collagen bundles had disappeared at the interface and an approximately 5  $\mu$ m thick disorganised collagen fibre layer was seen along the interface (Fig 2F).

No structures, which would induce subepithelial opacity, were observed throughout this wound healing period.

#### Discussion

Several experimental studies have evaluated portions of the corneal wound healing after LASIK. Amm *et al*<sup>7</sup> examined the clinical and histological differences between photorefractive keratectomy (PRK) and LASIK in rabbits. They reported that LASIK ensured quick wound healing with minimal tissue proliferation which is in contrast with the anterior stromal disorganisation after PRK. Perez-Santoja *et al*<sup>8</sup> examined immunoreactivity for the extracellular matrix proteins fibronectin and tenascin. Their results showed activated keratocytes were no longer identified at the wound margin 2.5 and 5 months after LASIK. However, fibronectin and tenascin immunoreactivities could still be observed.

Based on the present data combined with these previous reports on the biological response of the cornea after LASIK, the inflam-



**Figure 2** (A) Positive staining for type IV collagen is seen around the epithelial ingrowth portion on day 7 after LASIK (bar 200 µm). (B) PAS positive material is noted along the lamellar interface. Arrowhead indicates the wound edge (postoperative month 1, bar 200 µm). (C) Haematoxylin and eosin staining section 3 months after LASIK showing almost normal appearing cornea. Arrowhead indicates the wound edge (bar 200 µm). (D) Diffuse staining for type IV collagen is seen beneath the epithelium at the wound margin. Arrowhead indicates the wound edge (postoperative 3 months, bar 200 µm). (E) PAS stained section 9 months after LASIK. Note that the PAS positive material still present along the interface. Arrowhead indicates the wound edge (bar 200 µm). (F) Electron microscopy at 9 months after LASIK. Disorganised extracellular matrix is deposited along the interface (arrows).

matory reaction is slight and the healing reaction is weak. Such characteristics of the biological response of the cornea after LASIK are considered to have merits and demerits—that is, the cornea after LASIK is stable in terms of the refractive changes because the inflammatory and wound healing reaction are weak, and there is no subepithelial haze because epithelial cells and keratocytes on the optical axis are not activated. On the other hand, as observed in this study, the wound healing was delayed with LASIK, particularly in the interface area. A disorganised extracellular matrix was deposited along the lamellar incision even 9 months after LASIK suggesting that wound healing process might be sustained for longer periods than expected. It should be emphasised that the integrity or structural strength of the wound may not be completely restored at that time. Such drawbacks of LASIK have not been pointed out in previous reports. Thus, additional questions on the drawbacks of LASIK must be answered.

With regard to epithelial ingrowth, Helena *et al*<sup>12</sup> presented four eyes (three cases) of epithelial ingrowth within the lamellar interface after LASIK. All of them developed an interface opacity at the epithelial ingrowth portion. Unfortunately, the authors did not perform histological studies so the components of the scar tissue were not determined. In our present study, staining of type IV collagen was not seen in the ordinary interface area, but type IV collagen was positive around the epithelium in the area where the epithelial ingrowth occurred. This is an interesting phenomenon and has not been described before. This suggests that the deposition of the basement membrane components is accountable for the haze produced on the interface by the epithelial ingrowth.

In summary, these results indicated that the wound healing process after LASIK is not associated with inflammation and is slow and not completed even 9 months after the surgery. Interspecies differences may exist in the wound

healing response so we must use caution in extrapolating these data to human cases.

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