Epithelial Ingrowth After Laser In Situ Keratomileusis

A Histopathologic Study in Human Corneas

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Objective: To report the histopathologic findings in 4 human corneas that developed epithelial ingrowth after laser in situ keratomileusis (LASIK), at various postoperative intervals.

Methods: One specimen was obtained intraoperatively during treatment of epithelial ingrowth 2 months after LASIK (case 1). The other 3 corneal specimens were obtained after penetrating keratoplasty performed at 7 months (case 2), 20 months (case 3), and 5 years (case 4) after LASIK. The specimens were examined with both light and transmission electron microscopy.

Results: In case 1, most of the epithelial cells under the flap looked viable. However, some had begun to lose their characteristic shape and intercellular contacts. In case 2, aggregations of nonactivated fibroblasts and degrading epithelial cells could be observed. The surrounding collagen matrix differed significantly from that of the intact corneal matrix. In case 3, only completely degraded epithelial cells could be found, surrounded by collagen fibrils approximately 2 to 2.5 times larger in diameter than typical corneal collagen. In case 4, epithelial cell remnants, surrounded by a continuous layer resembling the basal membrane, were observed.

Conclusions: Corneal epithelial cells lose their characteristic morphologic features and eventually degrade in the metabolically “unusual” environment of the flap interface. Concurrently, a capsule of connective tissue similar to scar tissue forms, separating them from healthy cornea.

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Growth of epithelial cells within the flap interface is the most common flap complication of laser in situ keratomileusis (LASIK). Its incidence is reported to vary markedly from zero to 20%, depending on the surgeon. However, clinically significant epithelial ingrowth requiring removal was noted in only 0.92% to 2.2% of LASIK cases.

Currently, 2 theories exist to explain the mechanism of epithelial growth under a corneal flap. The first suggests that basal epithelial cells are implanted under the flap intraoperatively, possibly owing to the microkeratome blade, which mechanically drags cells into the interface during keratectomy. Epithelial cells from the corneal surface may also float into the interface during irrigation of the stromal bed after the ablation. It is believed that such cells have a very low potential to proliferate because they are separated from the limbal stem cells and transient amplifying cells.

The second theory states that corneal epithelium invades the interface through a defect in the flap, or in areas where the flap borders adhere poorly to the underlying stroma. Although the term “epithelial ingrowth” is falsely applied to describe every case in which epithelial cells are found under a corneal flap, it more accurately describes the growth of epithelial cells by this mechanism. This mechanism is also considered to play the main role in the pathogenesis of epithelial ingrowth after LASIK.

Although there are several case reports on epithelial ingrowth after LASIK and even more epidemiologic studies on the incidence and treatment of this complication, evidence is lacking in the literature regarding the histopathologic findings. Only 5 such reports exist, to our knowledge. This lack of histopathologic evidence, especially on the natural course of this entity, is because even severe epithelial ingrowth can be treated with excellent results, if identified early. Moreover, even though it is a common flap complication of LASIK, its overall incidence is very low. In this study, we report the histopathologic findings in 4 human corneas.
neas with epithelial ingrowth, at various postoperative intervals from 2 months to 5 years after LASIK.

METHODS

All of the eyes in this report were operated on during 1993-1995. The use of LASIK to treat myopia was still under investigation at that time. Also, prior to the introduction of alternative techniques (ie, phakic intraocular lenses) into clinical practice, LASIK was applied for the correction of a wide range of myopic errors. This explains the high degree of attempted correction in our cases. All patients were informed about the experimental nature of the LASIK procedure and the possible complications. Signed informed consent was obtained preoperatively. The Automated Corneal Shaper (Chiron Vision Inc, Claremont, Calif) and the MEL-60 Excimer Laser (Aesculap-Meditec, Jena, Germany) were used in each case.

CASE 1

A 65-year-old man underwent LASIK in the right eye (attempted correction, −19 diopters [D] in a 5-mm optical zone). The operation was uneventful except for an epithelial defect at the temporal border of the flap, which healed 3 days later, leaving a faint gray-white opacity in the interface. The patient was lost to follow-up until 2 months later, when he complained of increasing blurring of vision. The central cornea appeared opaque, and on slitlamp examination there was a thick layer of cells arising temporally and invading the interface up to the visual axis.

The flap was lifted at that time, and the layer was carefully scraped from the bed. The stromal side of the flap was sent for histopathologic study. The cornea healed uneventfully, with a grade 2 residual stromal haze, and visual acuity was restored to the preoperative level. No recurrence of the epithelial ingrowth was observed during a 2-year follow-up period.

CASE 2

A 32-year-old woman underwent LASIK in the left eye (attempted correction, −27 D in a 5-mm optical zone). The operation was uneventful, but there was a residual refractive error of −9 D. She was scheduled for retreatment 3 months after the initial procedure. The surgical plan was to lift the flap and complete the ablation.

The flap was manually detached from the underlying stroma up to the hinge. Attempted correction was −9 D. The procedure was complicated by eccentric ablation, accidental ablation of the nasal, stromal side of the flap in an area approximately 0.3 mm from the hinge, and a small epithelial defect at the site where detachment of the flap was initiated. After the ablation was completed, the flap was realigned and a therapeutic contact lens was fitted. The epithelial defect healed, and the contact lens was removed on the third postoperative day. However, best-corrected visual acuity was significantly decreased, from 20/60 preoperatively to 20/100. This was attributed to an eccentric pattern of ablation evident on corneal topography. Thinning of the central zone of the cornea was detected on slitlamp examination. Ultrasonic pachymetry measured 388 µm of central corneal thickness.

One month later, the vision had deteriorated further (counting fingers at 2 m), without any improvement in the topographic pattern of the cornea. In addition to this, epithelial ingrowth was evident in the interface, more prominent near the hinge. The deterioration of vision was attributed to irregular astigmatism caused by the eccentric ablation and ectasia of the thinned central cornea. For that reason, scraping of the epithelium was deferred, and penetrating keratoplasty was suggested as a means to restore visual acuity. This was eventually performed 7 months after the second LASIK procedure. The host corneal button was sent for histopathologic study.

CASE 3

A 41-year-old man underwent LASIK in the left eye (attempted correction, −12 D in a 5-mm optical zone). During keratectomy, the whole flap was completely cut off, creating a “total cap.” On the second postoperative day, the cap was dislodged and lost. Despite the use of intensive lubrication and the application of a therapeutic soft contact lens, epithelialization proceeded very slowly and a central descemetocele formed. Tectonic lamellar keratoplasty was performed on the 10th postoperative day.

The donor button was smaller in diameter compared with the original flap (7.5 mm vs 8.0 mm), leaving a 0.25-mm band of exposed stromal bed from each side of the donor cornea. Epithelialization was complete by the sixth postoperative day. Twenty months after the initial procedure, best-corrected visual acuity was counting fingers at 2 m. There was no epithelial defect, but the donor cornea was opaque, with a grade 4 haze. Also, multiple foci of epithelial ingrowth were evident in the interface but did not involve the visual axis. Penetrating keratoplasty was performed in an effort to restore vision. The host corneal button was sent for histopathologic study.

CASE 4

A 39-year-old woman underwent LASIK in the right eye (attempted correction, −15 D in a 5-mm optical zone). The operation was complicated by a thin flap (95 m). A 10-0 nylon suture was placed at the 8-o’clock position, and a therapeutic contact lens was fitted to prevent flap dislocation and to allow proper flap realignment. The suture was removed 2 days later, and contact lens wear was discontinued on the fourth postoperative day. One month after LASIK, 2 foci of epithelial accumulation were evident in the lower temporal periphery of the treatment zone. These did not enlarge on follow-up.

Seventeen months after LASIK, photorefractive keratectomy was performed to correct a residual refractive error of −7 D. Following reepithelialization, the best-corrected visual acuity had lost 3 Snellen lines (20/80 vs 20/40 preoperatively), with a refractive error of −6.5 D cylinder at 30°. A highly irregular ablation pattern was evident on corneal topography. The patient complained of severe glare and a halo effect. In an effort to restore the regularity of the central optical zone to some degree, 2 arcuate cuts were performed, 1 at 2½ years and 1 at 5 years after the initial procedure, but without any significant increase in visual acuity or improvement of subjective symptoms. Therefore, penetrating keratoplasty was performed 5 years after the LASIK procedure. The host corneal button was sent for histopathologic study.

PREPARATION OF HISTOLOGIC SPECIMENS

The explants were prefixed in cold 2.5% glutaraldehyde in 0.1 M of cacodylate buffer (pH 7.4). After short prefixation, they were placed in the same fresh fixative overnight. Postfixation was performed in 2% osmium tetroxide in a 0.1M cacodylate buffer (pH 7.4) for 1 hour at 4°C. The specimens were then dehydrated in a series of alcohol solutions of gradually increasing concentration and in propylene oxide, and embedded in epoxy resin. Sections (1-µm) were prepared and stained with 1% toluidine blue and a modified trichrome stain for light microscopic examination. Selected areas were thin-sectioned and stained with uranyl acetate and lead citrate for electron microscopic examination. Electron photomicrographs were pre-
RESULTS

CASE 1

Two months after LASIK, most cells that were found under the flap were cells of the epithelial type, with their inherent morphologic characteristics (Figure 1). Some of these cells had desmosomal contacts, a morphologic feature that is characteristic of epithelial cells. Cells that lacked the characteristic shape of the epithelial cells, as well as most of their intercellular contacts, were also observed (Figure 2). Nevertheless, all of the ingrown cells looked viable; no pyknotic nuclear alterations, vacuolization of the cytoplasm, or alterations in the cell membrane were observed.

CASE 2

Seven months after LASIK, multicellular aggregations were detected under the flap, consisting partially of nonactivated fibroblasts with poor rough endoplasmic reticulum. Other cells, most probably of epithelial origin but with substantially different morphologic characteristics, were also observed. The structure of the latter evidenced various degrees of degradation, from pyknotic nuclear changes and cytoplasm vacuolization to complete absence of distinguishable cellular organoids (Figure 3). The matrix surrounding the cellular island differed from that of the intact stroma primarily in the chaotic arrangement and the variable diameter of the collagen fibrils as well as the presence of amorphous electron-dense inclusions (Figure 3).

CASE 3

Twenty months after LASIK, multiple cellular groups, usually small in size, were found under the flap (Figure 4). These cellular islands consisted mostly of epithelial cells.
that demonstrated complete degradation. As a result, almost every cellular island contained numerous “cavities.” Most probably, these cavities were formed at the places of degraded epithelial cells. The surrounding extracellular matrix demonstrated even more obvious structural differences from normal. The diameter of the collagen fibers was 2 to 2.5 times larger compared with the intact stromal collagen (Figure 5).

**CASE 4**

Five years after the LASIK procedure, the cellular islands under the flap did not contain any viable epithelial cells. Isolated homogeneous masses without any evidence of cellular organoids or contacts were observed in the interface at the site of epithelial accumulation. The shape of these fragments suggested their epithelial origin. Furthermore, a layer resembling a basal membrane was observed surrounding the area of “mummified” epithelial cells (Figure 6).

**COMMENT**

We described the histopathologic findings in 4 cases with epithelial accumulation under the corneal flap after LASIK. All cases had some common characteristics, which may have played a role in the occurrence of epithelial ingrowth. First, the attempted correction was high, resulting in very deep and irregular ablations. All 4 operations were done during a time when the efficacy limits of the LASIK procedure were not set. Currently, LASIK is deferred for myopia higher than 15 D, for it has been shown in such cases that the benefit to risk ratio is very low.³ The same surgeon, at the initial stages of his learning curve, performed all 4 LASIK procedures. Well-known risk factors for epithelial ingrowth after LASIK are limited surgical experience and poor surgical technique.¹ ⁶ ⁸ The incidence of epithelial ingrowth decreases dramatically with increased surgical experience. In some series, it has been shown to fall to even 0%.² A final common characteristic of our cases was the occurrence of flap complications intraoperatively. Flap complications have been reported to be a significant risk factor for epithelial ingrowth. These complications include early epithelial defects, flap edema, and inflammation.⁴

Clinical trials on the management of epithelial ingrowth as well as experience gained from following up LASIK patients for almost a decade now have shown that the decision to treat epithelial ingrowth should be made as soon as it is diagnosed, to avoid progression and stromal melting.² ⁴ ⁶ Specific indications exist for treatment. Any ingrowth that is either progressive, involves the visual axis, or is complicated by stromal haze and/or melting is termed “clinically significant” and as such should be treated.³ ⁷ Ingrowth was treated in only 1 of the 4 cases (case 1). In the rest, treatment was not undertaken because this was deemed unnecessary, owing to the coexistence of other, more significant complications that deteriorated vision.

From 2 months to 5 years after the LASIK procedure, the epithelial cells that had grown into the interface between the flap and the residual stromal bed in each case underwent gradual degradation and finally died. We believe that the gradual degradation of the ingrown epithelial cells is a result of the “hostile” environment they encounter within the corneal stroma. In their normal ana-
tomic position, on the surface of the avascular cornea, the epithelial cells obtain oxygen and various nutrients necessary for maintaining cellular metabolism from the tear film. Within the hypoxic environment of the corneal stroma, and without basic nutrients, epithelial cells lose their ability to proliferate, and gradually degrade. The normal, avascular cornea does not contain any cells capable of phagocytosis. This may explain the “conservation” of epithelial cell remnants for up to 5 years.

Currently, 2 theoretical mechanisms exist for epithelial growth under a corneal flap. The first suggests that the cells are implanted under the flap intraoperatively. The second theory states that the corneal epithelium invades the interface through a defect in the flap or in areas where the flap borders adhere poorly to the stroma.

Our observations did not provide sufficient data to allow us to support one mechanism over the other. However, based on the finding that epithelial cells lose their ability to proliferate within the flap interface, we can hypothesize that if the first mechanism is correct, an extremely large number of implanted cells would be required to produce significant corneal opacification. Also, accumulations of this origin are not expected to increase, but rather, to remain stable or even decrease in size. Therefore, we agree that the epithelial cells in the interface communicate with the limbal stem cells, especially in cases where the area of epithelial ingrowth is enlarging over time. This communication can only be established through a connection with the surface epithelium in a continuous sheet, as suggested by the second mechanism (Figure 7).

Some authorities have hypothesized that epithelium growing in the lamellar bed after LASIK may initiate a stromal wound healing response, analogous to that stimulated by the epithelium after photorefractive keratectomy. There is histologic evidence that cytokines released by the corneal epithelium activate keratocytes, which in turn synthesize a new layer of extracellular matrix beneath the corneal epithelium after photorefractive keratectomy. In cases of epithelial ingrowth after LASIK, new extracellular matrix may be formed by an analogous mechanism, and may even grow to form a fibrous cocoon around the epithelium itself.

Our findings support that hypothesis. The reaction of the cornea to the presence of a group of degrading cells in its stroma seems to be similar to its reaction to a foreign body: the cellular islands are encapsulated into a structure that resembles a fibrous capsule. The cells that form this capsule belong to the fibroblast family and probably originate from keratocytes. The capsule may be easily distinguished from normal corneal collagen by the chaotic orientation and the large diameter of the collagen fibrils.

Chaotic orientation and different diameters of collagen fibrils may produce increased light scattering and corneal haze. Corneal haze is often detected surrounding foci of epithelial accumulation and can cause visual symptoms, such as glare or halos, common in patients with significant epithelial ingrowth even outside the visual axis.

In our cases, encapsulation of the epithelial ingrowth was evident at 7 months postoperatively. However, we believe that this capsule was formed much earlier. In the first case, as early as 2 months after LASIK, residual stromal haze was evident postoperatively even though the epithelial layer was easily separated from the surrounding stroma. This haze in the area of the previous ingrowth may represent the remaining capsule.

Our findings support the clinical recommendation that significant ingrowth is most successfully treated within 1 month from its diagnosis or, if very severe, as soon as it is diagnosed, before scar tissue develops or stromal melting occurs.

Several methods have been proposed in the literature for the treatment of epithelial ingrowth. Most authors agree that vigorous scraping of the epithelial accumulation and the surrounding fibrous tissue from both the bed and the stromal side of the flap is sufficient to remove epithelial ingrowth. This should be done as early as possible and should be combined with apposition of the LASIK flap to the stromal bed as tightly as possible (even with the use of sutures). The recurrence rate, however, is reported to be as high as 44%. This has led to the proposal of various alternative treatments. Phototherapeutic keratectomy, mitomycin C, and ethanol all have been proposed as adjunctive therapy to the area of epithelial ingrowth as a means to destroy proliferating epithelial cells. Since our results show that epithelial cells within the lamellar interface lose their ability to proliferate, we believe that such therapies have no effect other than to cause additional tissue damage.

Epithelial ingrowth is a rare but potentially serious complication of LASIK. It can be treated with excellent results if identified early. Our study provides histologic evidence on the natural course of epithelial accumulation under a corneal flap. In the process of epithelial ingrowth, the corneal epithelial cells in the interface lose their characteristic morphologic features and finally degrade. The cornea perceives the ingrown epithelial cells as foreign bodies; thus, a capsule of connective tissue, similar to scar tissue, forms, separating the cells from healthy stroma. Our findings agree with the clinical observation that epithelial accumulation is most successfully treated if removed before the formation of scar tissue.

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REFERENCES


Correction

Error in Acknowledgments. In the Epidemiology feature titled “The Risk and Natural Course of Age-Related Maculopathy,” published in the April issue of the ARCHIVES (2003;121:519-526), there were omissions from the acknowledgments.

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