Alcohol Debridement of the Corneal Epithelium in PRK and LASEK: An Electron Microscopic Study

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PURPOSE. To determine the plane of cleavage of the corneal epithelium and smoothness of underlying stroma, after alcohol debridement in photorefractive keratectomy (PRK) and laser subepithelial keratectomy (LASEK).

METHODS. The epithelial flap from six patients undergoing alcohol delamination of corneal epithelium before PRK and the epithelium and stroma from three eye bank donor eyes were fixed and processed for transmission (TEM) and scanning electron microscopy (SEM). The smoothness of the underlying stroma was studied by SEM and the plane of cleavage was determined by morphologic examination and morphometric measurements of basement membrane attached to the epithelial flap, using image-analysis software.

RESULTS. A very smooth stromal bed, ideal for PRK was seen in the stroma of all three eye bank donor eyes after alcohol delamination. The plane of cleavage was determined to be at the hemidesmosomal attachments, including the most superficial part of the lamina lucida of the basement membrane.

CONCLUSIONS. Alcohol delamination of the corneal epithelium before PRK or LASEK consistently results in a very smooth cleavage at the level of the hemidesmosomal attachments, including the superficial lamina lucida. It leaves behind a very smooth surface, which is ideal for PRK. It also allows for an intact epithelial flap to be lifted as a sheet from the corneal surface and hence is ideally suited for the LASEK technique.

(Invest Ophthalmol Vis Sci. 2003;44:510–513) DOI:10.1167/iovs.02-0488

The removal of the corneal epithelium is the first stage of excimer laser photorefractive keratectomy (PRK). A number of techniques have been described to remove the epithelium, including blunt debridement, mechanical debridement with a rotating brush, excimer laser transepithelial ablation, and alcohol delamination, followed by blunt debridement.1,2 All these techniques have the disadvantage that the epithelium is lost during the procedure, with the potential problems of delayed improvement in visual acuity caused by epithelial defects, significant postoperative pain, and formation of stromal haze. Recently, a new technique called the epiflap or laser subepithelial keratectomy (LASEK) has been described that uses 20% ethanol to delaminate the epithelial basement membrane, which is then reflected away from the central ablation zone before use of the excimer laser.3 After the procedure, the epithelial flap is replaced on the stromal bed in a fashion similar to that used in laser in situ keratomileusis (LASIK). This technique has been shown to reduce pain, significantly improve uncorrected visual acuity, and reduce stromal haze in patients with low to moderate myopia when compared with the conventional alcohol assisted epithelial debridement method.3,4 In a recent study of 222 cases, Claringbold5 showed that LASEK provides excellent refractive and visual results with no loss of best corrected visual acuity and no serious complications, over a 12-month follow-up period.

The plane of cleavage achieved with alcohol and the nature of the underlying stromal surface to which laser is applied, however, is not known. In a preliminary report,6 we demonstrated that this plane may be at the hemidesmosomal attachments and superficial part of the lamina lucida of the epithelial basement membrane. The purpose of this study was to determine by transmission electron microscopy (TEM) and morphometry the exact site of cleavage of the epithelial flap after alcohol delamination of human corneas, and to evaluate by scanning electron microscopy (SEM) the smoothness of the residual corneal surface after removal of the epithelium.

METHODS

An epithelial flap was obtained from eyes of patients undergoing PRK and three whole fresh human donor eyes unsuitable for transplantation. The technique of epithelial delamination with alcohol has been described.3 Briefly, with the patient under topical anesthesia with 1% amethocaine hydrochloride (Minims; Chauvin, Romford, UK), a 9-mm optical zone marker was applied firmly to the corneal surface, centered on the visual axis, and filled with 20% ethanol in balanced salt solution. After 30 to 40 seconds, the ethanol was absorbed with a Merocel sponge (Medtronics Merocel, Mystic, CT) with care taken to avoid spillage on the areas not being treated; the cornea washed with topical 0.5% chloramphenicol (Minims; Chauvin), four times a day for 3 weeks. With the three donor eyes, the corneas remaining after epithelial debridement were excised from the globe and also processed for electron microscopy. This study conformed to the tenets of Declaration of Helsinki for research involving human subjects, and informed consent was obtained from the participants.

The epithelial flaps and donor corneas were fixed by immersion in 2.5% glutaraldehyde (in 0.1 M cacodylate buffer, pH 7.4) for 16 to 24 hours. Each sample was then cut into 1-mm thick slices and washed in cacodylate buffer followed by secondary fixation in 1% osmium tetroxide for 1 hour. The epithelial flaps were then processed according to the standard procedures, by dehydration in ethanol followed by infil-
tration and embedding in Epon resin before polymerization at 60°C for 16 hours. Suitable areas for TEM were selected from 0.5 μM toluidine blue-stained sections. After they were trimmed, 80-nm sections were cut and mounted on copper grids before double staining with uranyl acetate and lead citrate. A transmission electron microscope (model 1010; JEOL, Welwyn Garden City, UK) was used to observe the prepared sections. The thickness of attached basement membranes were measured at multiple points (20–50 per specimen) corresponding to the attachments of the hemidesmosomes, directly from digital images, using image analysis equipment (SIS; Soft Imaging System, GmbH, Münster, Germany). The mean ± SD of the measurements was calculated for each sample.

One half of each of the donor corneas were dehydrated through ascending concentrations of ethanol before critical-point drying (Sandri Pvt 3; Tousims Research Corp., Rockville, MD). The samples were then mounted onto aluminum stubs before coating with gold using a sputter coater (SCD 050; Bal-Tec, Balzers, Liechtenstein). A scanning electron microscope (model JSM-35; JEOL) was used to observe the prepared sections.

For control subjects, two human donor corneas were obtained from an eye bank and subjected to mechanical debridement as for PRK, using a crescent blade (Beaver 64). The epithelium in the central 7-mm zone was scraped from the periphery to the center and the surface irrigated with balanced salt solution to wash off epithelial cell debris, before fixation. The corneas were then fixed and processed for SEM, as described for TEM.

Epithelial sheets obtained by alcohol delamination from three additional patients who were not included in the morphometric analysis, were stained with trypan blue 0.4% for 1 minute and examined for cell viability by light microscopy.

RESULTS

Representative scanning electron micrographs of the donor cornea surfaces at an original magnification between ×540 to ×1860 showed the stromal bed to be extremely smooth with very little debris except at the edges (Figs. 1, 2A). In comparison, the surface of the control corneas where epithelium was mechanically scraped off showed linear splits in the basement membrane and numerous ruffled ridges, giving the surface a very roughened appearance (Fig. 2B).

A study of the epithelial flaps by TEM revealed that delamination had occurred through the superficial part of the epithelial basement membrane, so that a small amount of basement membrane was left attached to the flap and was of variable thickness and undulating in appearance. The residual base-

FIGURE 1. Scanning electron micrograph of a donor cornea from which the epithelium was removed by alcohol debridement, showing a very smooth basement membrane (BM). The edge of the remaining epithelial sheet (ES) is visible, with some debris adjacent to it. ST, stroma of the cut edge of the cornea. Bar, 20 μm.

ment membrane was thickest at sites where hemidesmosomes were present on the basal surface of the overlying epithelial cells. (Fig. 3). Occasional basal epithelial cells between hemidesmosome attachments, demonstrated vesicular projections from the basal surface after alcohol delamination (Fig. 4).

Thickness measurements were performed in six samples (Table 1). Measurements were taken at points along the basement membrane that remained attached to the epithelial sheet after alcohol delamination, and, for each sample, the maximum and minimum thickness was noted and the mean ± SD calculated. The mean thickness of the basement membrane attached to the epithelial flap in the six normal patients was 44.0 ± 8.46 nm (SD). The mean minimal thickness of the attached basement membrane was 28.7 ± 9.18 nm, and the mean maximum thickness of the attached basement membrane was 72.13 ± 26.3 (Table 1).

Thirty percent of the cells counted (1000–1500 per sample) in one sample, 50% in the second sample, and 58% in the third sample demonstrated staining with trypan blue, indicating that 42% to 70% of the cells in the epithelial sheets were viable.
DISCUSSION

A smooth surface after removal of the corneal epithelium is considered desirable before PRK. Referring to alcohol debridement, Weiss et al. state, “As application of laser ablation to an irregular surface can theoretically result in irregular astigmatism, corneal haze, a less predictable visual result or all 3, the least traumatic epithelial removal may be preferable.” They also quote McDonnell² as stating, “Although the exact cause of post operative corneal haze is unknown, the risk of haze can probably be minimized with very smooth ablation profiles, because irregular edges can stimulate epithelial and keratocyte proliferation.” A number of techniques have been described to remove the corneal epithelium, including blunt scraping with a blade, rotary brushing, alcoholic debridement, and laser. Studies using a scanning electron microscope to look at debrided corneas have shown that, apart from blunt scraping, all methods leave a relatively smooth surface for the subsequent excimer laser procedure. Unfortunately, epithelial debridement causes severe postoperative pain until reepithelialization occurs, delays visual recovery, and may play a significant role in production of stromal haze.

Recently, a new technique has been described that allows the removal of the corneal epithelium by way of a hinged epithelial flap after alcohol delamination. This has been shown to be a safe procedure that does not require the use of a microkeratome, and patients have a faster visual rehabilitation and reduced postoperative corneal haze, compared with conventional alcohol debridement.³,⁴

Representative scanning electron micrographs presented in this study show the exposed corneal surface to be extremely smooth at a magnification of between ×540 and ×1860, with little residual debris. This compares favorably with the ×100 images of corneal surfaces obtained after conventional debridement techniques.⁵

The plane of separation within the cornea after alcohol delamination of the epithelial flap was previously unknown. SEM studies by Griffith et al.² and Weiss et al.³ indicate that alcohol debridement of human corneas exposes Bowman’s membrane, whereas, because rabbit corneas have no Bowman’s membrane, the anterior stroma is exposed. However, the present study has shown that alcohol delamination in fact occurs within the epithelial basement membrane. Transmission electron micrographs clearly show residual basement membrane, with a mean thickness of 44.0 nm, attached to the epithelial flap. The residual basement membrane also had an undulating appearance, coinciding with the position of hemidesmosomes on the intracellular basal surface of the underlying basal epithelial cell.

The normal corneal epithelial basement membrane has a mean thickness of 350 nm (range, 110–550 nm).¹¹ When examined by electron microscopy, it consists of two layers: the lamina lucida immediately underlying the basal epithelial cell layer and the deeper lamina densa. Immunohistochemical analysis has shown it to contain the structural elements collagen VII and heparan sulfate, as well as components involved in attachment of the overlying cell to the underlying stroma by hemidesmosomes.¹²,¹³ These include: laminin 5, fibronectin, and entactin-nidogen.¹⁴

Hemidesmosomes are specialized transmembrane cell–matrix junctions between the cytoskeleton of epithelial cells and the extracellular matrix of basement membranes. The principal component of the hemidesmosome involved in cell–matrix adhesion is the integrin heterodimer α6β4,¹⁴ a transmembrane protein that can attach to laminin in the basement membrane. It is known that the binding of integrins is a calcium-dependent process.¹⁵ For many years, workers have prepared isolates of

Table 1. Thickness Measurements of Basement Membrane that Remained Attached to the Epithelial Sheet after Alcohol Delamination

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<th>Sample number</th>
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Figure 3. (A) Transmission electron micrograph of a sheet of corneal epithelial cells removed by application of alcohol before PRK. The stratified structure is well preserved, and the cell morphology is conserved producing a smooth plane of cleavage along the base of the basal epithelial cells. (B) Transmission electron micrograph of the base of the basal cells showing the hemidesmosome attachments that have been cleaved together with the superficial part of the lamina lucida of the basement membrane. Bar: (A) 10 µm; (B) 0.5 µm.

Figure 4. Transmission electron micrograph of the base of the basal epithelial cells cleaved by alcohol application, between hemidesmosome attachments, demonstrating membrane-bound vesicles projecting outward. Bar, 5 µm.
corneal epithelium by using EDTA to disrupt hemidesmosome binding to basement membrane.16 Other workers have used 1 M sodium chloride for the same purpose.17 In both instances, electron microscope studies of the appearance of the basal aspects of the epithelial cells were similar to those presented in this study. It therefore appears that dilute alcohol affects the binding of hemidesmosomes to the underlying basement membrane in a reproducible fashion and allows the production of an extremely smooth surface for subsequent PRK. By returning the delaminated epithelial flap to the stromal surface, this newly characterized technique has been shown to allow faster visual rehabilitation and to reduce the production of corneal haze.3,4 The relatively high viability of epithelial cells after treatment with alcohol for 30 to 40 seconds, as demonstrated by trypan blue staining, is also clinically borne out by the observation that the epithelial flaps readhered to the stromal bed without sloughing off in the postoperative period.5

We therefore suggest that alcohol debridement is superior to mechanical debridement and is the preferred technique for removing epithelium before PRK or for lifting an epithelial flap before LASEK.

References