Recurrent corneal erosion (RCE) is characterized by repeated episodes of breakdown of corneal epithelium due to a defect in adhesion to the basement membrane [1]. Episodes of RCE are heralded by the sudden onset of eye pain, usually at night or upon awakening, accompanied by redness, photophobia, and tearing. The episodes can last from hours to days and cause reduced quality of life due to eye pain or discomfort and poor or unstable vision. Slit-lamp examination reveals punctate epithelial erosions in milder cases (microform erosions) and a frank epithelial defect or even a large area of edematous nonadherent epithelium in severe cases (macroform erosion) [2].

In some patients RCE may be secondary to an epithelial basement membrane dystrophy [3,4], but the vast majority of patients develop RCE following a localized, mechanical trauma to the corneal epithelium and basement membrane. A number of treatments for RCE have been described, including use of lubricating agents, patching, wearing a bandage contact lens, anterior stroma puncture, and excimer laser phototherapeutic keratectomy (PTK). However, these treatments have reported success rates of only 50-80% [3,4]. A better understanding of the pathogenesis of RCE should prove helpful in designing more effective treatment regimens.

It has long been believed that postinjury failure of the epithelial cells to regain and maintain tight adhesive contacts with the underlying stroma plays the pivotal role in the pathogenesis of traumatic RCE. An ultrastructural study of specimens of corneal epithelium from eyes with nontraumatic RCE revealed the presence of “pale cells” in the basal layer of the corneal epithelium, an almost complete absence of hemidesmosomes, and detachment of the basement membrane from underlying corneal tissues [2]. Similarly in cases of RCE due to trauma that were studied using electron microscopy, the basement membrane over the traumatized area of cornea was found to be defective [5]. These and other ultrastructural studies have shown that there is a corneal epithelial adhesion complex that maintains attachment of the epithelium to the underlying Bowman’s layer. The complex involves four cytologic structures, namely hemidesmosomes, the lamina lucida, the lamina densa and anchoring fibrils, and several molecules, including integrins, laminin, and type VII collagen.

Each corneal epitheliopathy may involve different layers or molecules of the corneal epithelial adhesion complex [6-9]. Even though traumatic RCE is common in clinical practice, to our knowledge the exact defect or plane of cleavage that leads to separation of corneal epithelium from Bowman’s layer in cases of traumatic RCE has never been investigated.
We therefore undertook the study described here, in which we used laser scanning spectral confocal microscopy and immunofluorescence staining with antibodies to several adhesion molecules (integrin $\beta_1$, integrin $\beta_4$, laminin-5, and collagen VII) to pinpoint the defect responsible for shedding of corneal epithelium in cases of traumatic RCE.

**METHODS**

**Patients:** Between January 2003 and January 2005, eight patients with traumatic RCE scheduled for excimer laser phototherapeutic keratectomy (PTK) in the Laser Refractive Surgery Center at National Cheng Kung University Hospital were recruited for this study. All patients had a history of corneal abrasion on initial presentation and at least two subsequent episodes of recurrent corneal epithelial breakdown. A diagnosis of RCE had been made in each case based on the presence of a spontaneously occurring local epithelial defect and/or an area of loosely adherent edematous epithelium. Under slit-lamp examination for the current acute erosive episode, all eight patients had been found to have macroform erosion of the cornea that had not improved with conventional therapy, including applications of lubricating agents and a therapeutic bandage contact lens. Thus they had been scheduled for PTK.

None of the patients had pre-existing ocular or systemic diseases, such as dry-eye syndrome, anterior basement membrane dystrophy, keratoconus, diabetes, thyroid or other autoimmune disease that would affect the corneal epithelium. Informed consent for biopsy of the corneal epithelium before PTK was obtained from every patient and normal control in accordance with the tenets of the Helsinki Declaration, and the Institutional Review Board of National Cheng Kung University Hospital approved the obtaining of epithelial biopsies for this immunofluorescence study.

**Human epithelial biopsy in recurrent corneal erosion:** The corneal epithelial biopsy specimens from eyes with RCE were obtained immediately before PTK. In brief, after instillation of one drop of proparacaine hydrochloride 0.5% (Alcaine®; Alcon Laboratories, Inc., Fort Worth, TX) for topical anesthesia, the sheet of loose, nonadherent corneal epithelium overlying or adjacent to the acute erosive wound in each patient was excised with Vanas scissors and gently removed with jeweler’s forceps. Care was taken to avoid applying any force when mobilizing the loose epithelial sheets, and in fact the sheets were readily detached from the underlying stroma after having been cut along the border of the lesion.

Each epithelial sheet was placed on a Millipore filter membrane after removal and then embedded immediately in optimal cutting temperature (OCT) compound. It was then frozen and stored at -20°C until processing for immunofluorescence staining.

**Normal corneal epithelial sheets excised by mechanical separation as controls:** A small sheet of tightly adherent normal corneal epithelium around pterygial head during bare scleral procedure was slowly stripped off by jeweler’s forceps in a manner similar to that used in obtaining RCE samples, but slight force was found to be needed to remove an adherent corneal epithelial sheet from the normal eyes. Three epithelial

---

**Table 1. Clinical characteristics of traumatic recurrent corneal erosion**

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age/gender</th>
<th>Eye</th>
<th>Number of prior attacks</th>
<th>Duration of current episode</th>
<th>Type of trauma</th>
<th>BCVA before PTK</th>
<th>Conventional therapy before PTK</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36/F</td>
<td>OD</td>
<td>2</td>
<td>14 days</td>
<td>Fingernail</td>
<td>20/200</td>
<td>Artificial tears, ciprofloxacin, BCL</td>
</tr>
<tr>
<td>2</td>
<td>35/M</td>
<td>OD</td>
<td>3</td>
<td>6 days</td>
<td>Abrasion by paper</td>
<td>20/20</td>
<td>Artificial tears, tropicamide, patch</td>
</tr>
<tr>
<td>3</td>
<td>43/F</td>
<td>OD</td>
<td>2</td>
<td>10 days</td>
<td>Fingernail</td>
<td>20/80</td>
<td>Artificial tears, gentamycin, BCL</td>
</tr>
<tr>
<td>4</td>
<td>40/M</td>
<td>OD</td>
<td>1</td>
<td>7 days</td>
<td>Fingernail</td>
<td>20/40</td>
<td>Artificial tears, tropicamide, patch</td>
</tr>
<tr>
<td>5</td>
<td>53/M</td>
<td>OS</td>
<td>3</td>
<td>19 days</td>
<td>Abrasion by glasses frame</td>
<td>20/25</td>
<td>Artificial tears, ciprofloxacin, BCL</td>
</tr>
<tr>
<td>6</td>
<td>27/F</td>
<td>OD</td>
<td>1</td>
<td>4 days</td>
<td>Fingernail</td>
<td>20/60</td>
<td>Artificial tears, tropicamide, BCL</td>
</tr>
<tr>
<td>7</td>
<td>31/M</td>
<td>OD</td>
<td>2</td>
<td>14 days</td>
<td>Abrasion by paper</td>
<td>20/100</td>
<td>Artificial tears, norfloxicon, BCL</td>
</tr>
<tr>
<td>8</td>
<td>33/M</td>
<td>OS</td>
<td>2</td>
<td>7 days</td>
<td>Abrasion by twig</td>
<td>20/50</td>
<td>Artificial tears, patch</td>
</tr>
</tbody>
</table>

The demographic data for 8 patients in this study are listed. Causes, attack episodes, durations, best-corrected visual acuity (BCVA), and treatments of recurrent corneal erosions prior to phototherapeutic keratectomy (PTK) are documented in details. BCL, bandage contact lens.
sheets from three patients were collected and processed with the same immunostaining procedure.

**Partially lamellar keratectomized samples as controls:** A small piece of partially keratectomized sample containing adherent corneal epithelium and minimal amount of corneal stroma adjacent to pterygial head during surgery of pterygial wide excision was taken by a surgical blade as a control for this study. The entire tissue blocks from three different patients were immediately embedded in OCT compound and frozen before processing with the same immunostaining procedure.

**Human corneoscleral rims as controls:** The control specimens were obtained from three healthy corneas that had been donated for corneal transplantation. From the block of corneal tissue that remained after corneal buttons were taken during a conventional penetrating keratoplasty procedure, a 2.0x2.0x0.7 mm block was cut from residual corneoscleral rim and the posterior portion of endothelium was excised to prevent immunostaining on endothelial adhesion complex during staining of the epithelial adhesion complex. Then, the entire tissue block was immediately embedded in OCT compound, frozen, and stored at -20 °C until processing for immunofluorescence staining.

**Immunofluorescence staining materials:** Mouse monoclonal antibodies against integrin β1 or collagen VII and fluorescein isothiocyanate conjugated (FITC) goat antimouse IgG antibody were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Mouse monoclonal antibodies against integrin β4 and laminin 5 were obtained from Accurate Chemical & Scientific Corp. (San Diego, CA). The Vectashield with 4',6-diamidino-2-phenylindole (DAPI) and propidium iodine (PI) mounting medium were purchased from Vector Laboratories (Franklin, IN).

**Immunofluorescence staining procedure:** Cryosections (5 µm) were cut and fixed in cold acetone (-20 °C) for 10 min. They were air dried and washed with phosphate-buffered saline (PBS) three times for 5 min each time. Each section was then rinsed with a blocking agent (5% bovine serum albumin in PBS) for 20 min. Every specimen was then immersed in a solution of one of the primary antibodies at a dilution of 1:100 and incubated at 4 °C overnight. After three 10 min rinses in PBS, the sections were immersed in a solution of FITC-conjugated antismouse secondary antibody at room temperature for 30 min. PI or DAPI was then used for nuclear counterstaining.

Sections from each specimen were examined by light microscopy (Olympus model CX31-P Polarizing Microscope, Olympus, Tokyo, Japan), and images were captured using an Optronics video system (Olympus DP12 Digital Camera, Tokyo, Japan) attached to the microscope. Sections from all specimens were rechecked by laser scanning spectral confocal microscopy (TCS SP2, Leica, Heidelberg GmbH, Germany) to see if there were different findings. Image management software (Photoshop version 7.0; Adobe Systems Inc., San Jose, CA) was used to create merged images of fluorescence from tissues stained with FITC-PI or FITC-DAPI.

**RESULTS**

The patient group included three women and five men between 27 and 53 years of age. Pertinent clinical findings in patients with RCE are listed in Table 1 and the results of immunofluorescence staining of epithelial biopsy specimens from patients and controls are summarized in Table 2. The results of light microscopy and laser scanning confocal microscopy were consistent for each specimen. However, with the latter technique, superimposition of immunostaining signals from epithelial cells in neighboring layers was avoided, which allowed for more clear delineation of structures in each layer.

Figure 1A shows a representative clinical picture of our RCE patients, indicating the area where we obtained loosened epithelial specimens. A phase-contrast micrograph of corneal epithelial sheet taken from the patient in Figure 1A revealed an intact epithelium comprising four-seven layers of epithelial cells but without discernable corneal stromal components (Figure 1B). In sections stained to reveal integrin β1, there was a strongly positive immunofluorescence response ob-

<p>| Table 2. Results of Immunofluorescence Staining of Corneal Epithelial Sheets |
|---------------------------------|--------|--------|--------|--------|</p>
<table>
<thead>
<tr>
<th>Patient number</th>
<th>Integrin β1</th>
<th>Integrin β4</th>
<th>Laminin 5</th>
<th>Collagen VII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial sheets from recurrent corneal erosion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mechanically separated epithelial sheet controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Partial lamellar keratectomized controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Corneoscleral rim controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

In eyes with recurrent corneal erosion (RCE), the manifestation of key adhesion molecules (integrin β1, integrin β4, laminin 5, and collagen VII) in epithelial sheets from the recurrent corneal erosion or the control eyes is summarized. Compared to control eyes, the most striking difference in affected eyes is the paucity of collagen VII, showing either patchy (discontinuous) staining or negative staining in immunofluorescence. Staining was characterized as positive (+) with a continuously linear pattern for antibody, patchy (+/-) with a discontinuous pattern for antibody, or negative (-) for antibody.
served intercellularly in the basal and suprabasal layers of epithelial sheets from eyes with RCE (Figure 2A). In controls of normal epithelial sheets of mechanical separation, partially keratectomized corneal samples, and corneal tissue from corneoscleral rims, immunofluorescence to integrin β1 showed a positive response from corresponding areas (Figure 2B-D, respectively). Staining for integrin β4 yielded a linear pattern and localized only at the basal layer of RCE epithelial sheet (Figure 3A). The pattern and location of positive response were compatible with hemidesmosome distribution. The same linear pattern of integrin β4 was observed in the basal layer of all controls as well (Figure 3B-D). In confocal microscopic images, a punctate pattern, which is typical of hemidesmosome distribution, can be discerned continuously throughout the whole basal side of epithelial sheets (Figure 3A). Similarly, staining to reveal laminin 5 showed a positive response along a line corresponding to the basement membrane in epithelial sheets of RCE (Figure 4A) and in control epithelia of mechanical separation (Figure 4B). Staining of normal cornea tissue from partial lamellar keratectomy (Figure 4C), and of corneal tissue from corneoscleral rims (Figure 4D).

In contrast, staining to reveal collagen VII showed quite different patterns of distribution in epithelial sheets of RCE and epithelia from controls. Short lines or patches of positive response to staining for collagen VII were present in the basal area of epithelial sheets from three of the eight patients with

Figure 1. Clinical picture and micrograph of recurrent corneal erosion. A: A flank epithelial defect was stained green with sodium fluorescein. An area of loosened epithelium surrounding the flank epithelial defect was outlined with arrow heads. Biopsy of recurrent corneal erosion (RCE) was performed in this area. B: Light micrograph of RCE sample. An epithelial sheet comprising 4-7 layers of cells without apparent stromal components was noted. The scale bar represents 20 μm.

Figure 2. Integrin β1 in epithelial sheets from eyes with recurrent corneal erosion. A: Integrin β1 immunostaining of the nonadherent corneal epithelium in recurrent corneal erosion (RCE) showed strongly positive results (green) at the basal and suprabasal layers of detached epithelial sheet (arrows; nuclei counterstained red with propidium iodine). B: A similar pattern of integrin β1 was observed in mechanically separated normal epithelial sheets, showing positive pericellular staining at suprabasal and basal layers (arrows). C: Integrin β1 immunofluorescence showed positive pericellular staining mainly at basal layer in partially lamellar keratectomized corneal tissue (arrows). The dotted line represents the inferior contour of lamellar keratectomy sample. D: Integrin β1 immunofluorescence staining is observed at suprabasal and basal cell layers of adhesion complex in corneoscleral rim (arrows). The scale bar represents 20 μm. A,D: Light micrographs. B,C: Confocal scanning micrographs.
Figure 3. Integrin $\beta_4$ in the epithelial sheets from the eyes with recurrent corneal erosion. 

A: Staining for integrin $\beta_4$ was positive with a linear manifestation (arrows) and localized only at basal epithelial cells of recurrent corneal erosion epithelial sheet (nuclei counterstained red with propidium iodine). 

B: Staining for integrin $\beta_4$ demonstrated a typical punctate pattern in a linear fashion (arrows), consistent with hemidesmosome arrangement in mechanically separated normal epithelial sheets. 

C: Integrin $\beta_4$ was deposited in a punctate pattern in basal epithelial cells (arrows) throughout the whole length of sample in partially lamellar keratectomized corneal tissue. The dotted line represents the inferior contour of lamellar keratectomy sample. 

D: A punctate pattern of integrin $\beta_4$ was observed in a linear expression at the basal epithelial layer as well in adhesion complex of the corneoscleral rim (arrows). The scale bar represents 20 $\mu$m. 


Figure 4. Laminin 5 in the epithelial sheets from the eyes with recurrent corneal erosion. 

A: Staining for laminin 5 showed a line of positive response along the base of recurrent corneal erosion epithelial sheet (nuclei counterstained red with propidium iodine). 

B: Linear expression of laminin 5 was along the basal side of mechanically separated normal epithelium (arrows). 

C: Staining for laminin 5 also had a positive response and manifested as a linear expression along the basal aspect of epithelium in partially lamellar keratectomized corneal tissue (arrows). The dotted line represents the inferior contour of lamellar keratectomy sample. 

D: Immunofluorescence staining for laminin 5 exhibited a linear pattern (arrows) at the interface of epithelium and stroma in the corneoscleral rim. The scale bar represents 20 $\mu$m. 

Figure 5. Collagen VII in the epithelial sheets from eyes with recurrent corneal erosion. Immunostaining for collagen VII resulted in two reference patterns of response in epithelial sheets from the eyes with recurrent corneal erosion. These responses were either a discontinuous line of positive staining along the basal aspect (A, arrows) or no staining at all (B). Nuclei were counterstained blue with 4',6'-diamidino-2-phenylindole. C: A continuous linear pattern for the staining of collagen VII (arrows) at the basement membrane complex was noted in mechanically separated normal epithelial sheet, indicating that the plane of cleavage is deeper than the collagen VII layer in the traumatized corneal epithelium of normal eye. D: A uniform staining of the basement membrane complex for collagen VII was observed as a continuous line (arrows) in the partially lamellar keratectomized corneal tissue, with the above epithelium and underlying stroma counterstained blue. The dotted line represents the inferior contour of lamellar keratectomy sample. E: A continuous linear pattern for the staining of collagen VII (arrows) in the basement membrane complex was discernible in the normal corneoscleral rim. F: A picture taken at a higher magnification and limited to a single plane by confocal microscopy in the normal corneoscleral rim also revealed a continuous linear pattern for the collagen VII staining (arrows) in the basement membrane complex. The green-stained anchoring fibril layer seemed to be slightly undulating under such a high magnification. The scale bar represents 20 μm. A,C,D,F: Confocal scanning micrographs. B,E: Light micrographs.
RCE viewed both by light microscopy and confocal scanning microscopy. In pictures limited to show a monolayer epithelium by confocal scanning microscopy, the incomplete pattern of collagen VII expression was even more clearly evident (Figure 5A). No areas of positive staining for collagen VII were seen in epithelial sheets from the other five patients with RCE (Figure 5B). However, in sections of epithelial sheets derived from mechanical separation, epithelium in partially lamellar keratectomized corneal tissue and epithelium from corneoscleral rim of normal eyes, an intense and uniform expression was observed in collagen VII staining in a continuous linear pattern along the basement membrane when examined by confocal scanning microscopy (Figure 5C,D,F) and light microscopy (Figure 5E). Collectively, all these data indicate that the cleavage plane in RCE is exclusively located at collagen VII anchoring fibrils.

DISCUSSION

Corneal epithelium consists of five to seven layers of epithelial cells and rests on the basement membrane just anterior to Bowman’s layer. Cell adhesion molecules of the integrin family are largely responsible for the adhesion of the corneal epithelium to the stroma. The adhesion complex of corneal epithelium is composed of, from the outermost to the innermost, keratin 3/12, Integrin α3β1, plectin, BP230, BP180, integrin α6β4, laminin 5, and type VII collagen anchoring fibrils (Figure 5) [10]. Of them, integrin α3β1 exists intercellularly and at cell-matrix junction, forming focal adhesion [11]. BP180 and integrin α6β4 are both integral membrane components of hemidesmosome [12]; laminin 5, a ligand of integrins α3β1 and α6β4, is a major constituent for lamina lucida [13]; and type VII collagen fibrils, an essential component of lamina densa, penetrate deeply into Bowman’s layer to ensure a firm adhesion of the epithelium to the underlying connective tissue [14].

Cause of traumatic and atraumatic recurrent corneal erosion: The presumptive cause of RCE following traumatic injury is failure of the wounded epithelial cells to regain tight adhesion to the underlying stroma, whereas in eyes with epithelial basement membrane dystrophy, RCE results from loss of adhesion due to a defect in the basement membrane [1]. Tripathi and Bron [2] reported that when specimens from eyes with nontraumatic RCE were examined by electron microscopy, there was an absence of hemidesmosomes. Thus, it is reasonable to surmise that nontraumatic RCE is due to an alteration or a defect of integrin α6β4, a major component of hemidesmosomes. In contrast, in all of the cases we report of RCE caused by traumatic insult and without apparent corneal dystrophy, neither integrin α6β4 (shown as β4 subunit) nor integrin α3β1 expression (shown as β1 subunit) was affected. Because integrin α6β4 plays a critical role in hemidesmosomal adhesion between basal epithelial cells and lamina lucida, the fact that in our study there was no break in the line of immunofluorescence representing expression of integrin α6β4 in any of the sections suggests that adhesion of hemidesmosomes was unaffected in these cases of traumatic RCE. We also found no defect in the layer that stained positive for laminin 5. Nevertheless, we did find that epithelial sheets from patients with traumatic RCE had either discontinuous segmental staining or no staining of type VII collagen fibrils, suggesting that separation or cleavage between corneal epithelium and Bowman’s layer in cases of traumatic RCE is due to disruption of collagen fibrils that anchor the basement membrane to Bowman’s layer.

Treatments for recurrent corneal erosion: Conventional medical therapies for traumatic RCE include patching or application of a bandage contact lens and use of a lubricating agent or hypertonic saline. More recently, topical application of an inhibitor of matrix metalloproteinase (MMP) activity has been advocated. Use of this therapy is based on the results
of studies in which MMP-2 expression and MMP-9 expression were found to be upregulated in human corneal basal epithelial cells in cases of posttraumatic RCE [14,15]. Because collagen VII is known to be a substrate for MMPs [14], MMPs could play a major role in degradation of the epithelial anchoring system and could explain our finding that the cleavage plane in cases of traumatic RCE involved collagen fibrils anchoring basement membrane to Bowman’s layer. This might also explain why inhibitors of MMP-9 and doxycycline may produce rapid resolution of RCE and prevent further recurrence in some cases of traumatic RCE that have been unresponsive to other therapies [16].

Various surgical interventions have been reported to promote corneal wound healing and augment collagen VII fibrils anchoring to the underlying Bowman’s layer. Anterior stromal micropuncture with a needle [17], superficial keratectomy with diamond bur [3], Nd:YAG [18], and PTK [3], have been used for years to treat RCE that has not responded to medical treatments. Anterior stromal micropuncture can promote new basement membrane formation, without excessive overproduction, within 28 days [19]; the plugs of epithelium within the punctures could function as “spot welds” to bind the loosened sheet of epithelium to the underlying stroma [17]. When superficial keratectomy is performed with an excimer laser, the irregular basement membrane is removed and fibroblasts in the anterior stroma are stimulated to form new anchoring fibrils for firmer epithelial adhesion [20]. All those surgical interventions reported are aimed to promote corneal wound healing and augment collagen VII fibril anchoring to the underlying Bowman’s layer.

**Factors predisposing to corneal erosion along various cleavage planes:** There are various corneal epitheliopathies that predispose to corneal erosions along various cleavage planes, as illustrated in Figure 6. For example, long-term contact lens wearers may have a lower density of corneal epithelial cells and thus of hemidesmosomes and so may suffer loss of epithelial cells due to cleavage superficial to the basement membrane [6]. Recently, Espana et al. [9] showed that corneal epithelium exposed to 20% ethanol during laser-assisted subepithelial keratectomy (LASEK) cleaves between the lamina lucida and lamina densa within the basement membrane.

With normal aging the basement membrane undergoes progressive thickening that will eventually lead to insufficient length of collagen fibrils to anchor the basement membrane to Bowman’s layer [7]. The result is that older patients are more susceptible to epithelial defects involving cleavage below the basement membrane, such as could occur during laser in situ keratomileusis (LASIK) [8]. The study we report here showed that for patients with traumatic RCE, the cleavage plane is located at the deepest level of the epithelial adhesion complex (below the basement membrane) and also involves collagen fibrils that anchor the basement membrane to Bowman’s layer. However, the underlying mechanism for recurrent RCE is impairment in collagen fibrils rather than thickening of the basement membrane as with aging.

**Cleavage plane of normal epithelium with mechanical separation:** The reason that we employed various types of controls in the current study is to avoid the confounding factor that the cleavage plane of RCE might arise from artefacts of mechanical separation during the surgery. Intriguingly, we found that the splitting plane in mechanically separated epithelial sheets from normal individuals was actually located below anchoring fibrils and above Bowman’s layer, as the immunostaining of collagen VII deposited in a continuous and linear fashion along the basal side of epithelial sheets (Figure 5C). This finding excludes the possibility that the cleavage plane of collagen VII in RCE results from surgical intervention. Interestingly, this observation is compatible with the cleavage plane resulting from mechanical separation with a blunt blade (epithelial separator) in epi-LASIK procedure [21]. Using electron microscopy, Pallikaris et al. [21] showed well preserved hemidesmosomes, lamina densa, lamina lucida, and lamina reticularis (anchoring fibrils) in the epithelial disks mechanically excised during epi-LASIK surgery. The fact that normal corneal epithelium tends to cleave at the level below anchoring fibers upon mechanical separation might imply that the whole adhesion complex, from hemidesmosomes through basement membrane to anchoring fibers, may work as a compact unit. Accordingly, adherent strength between the epithelial adhesion complex and Bowman’s layer, a distinct acellular matrix composed of interwoven type I and type V collagen fibrils of a small diameter [22], may be relatively weaker. Further investigation is needed to verify this postulation.

Aside from the aforementioned types I, V, and VII collagen fibrils, type VI collagen has also been reported to form a similar anchoring function in the ocular tissue, such as iris and ciliary body [23]. Although collagen VI has a wider distribution in the interfibrillar matrix of the corneal stroma and Bowman’s layer [24], type VII and type VI collagen fibrils are similar in the followings: (1) both of them have similar distribution at anchoring plaques of the subbasement membrane layer of the cornea and interact with collagen type IV of basement membrane [24-26]; and (2) both are susceptible to MMP-2 degradation [14,27]. To date, collagen type VI has not yet been reported to be associated with traumatic RCE; however, it has been found to be altered in a variety of diseases, such as hereditary Bethlem myopathy and cutis laxa in skin [26,28]. Based on the analogy between type VI and VII collagens, it is reasonable to surmise that that alteration in collagen VI might influence the tensile strength of corneal epithelial adhesion, and thus be associated with RCE. Our future study focusing on the role of collagen VI expression in traumatic RCE will further clarify pathogenesis of such corneal epitheliopathy.

In conclusion, epithelium of posttraumatic RCE splits between basement membrane and Bowman’s layer. The poor epithelial-to-stroma adhesion results from poor anchoring of collagen VII fibrils to Bowman’s layer, while hemidesmosomes indicated by immunolocalization of integrin β4 remained intact. To better understand RCE and develop more effective treatments of this disorder, further elucidation of its underlying pathophysiology is merited.
ACKNOWLEDGEMENTS

This study was supported by a grant from National Cheng Kung University Hospital Research Grant Foundation (NCKUH-55-2005). Part of the study was presented at the 20th congress of the Asia-Pacific Academy of Ophthalmology, Kuala Lumpur, Malaysia, in April 2005.

REFERENCES