The Effect of Chronic Corneal Epithelial Debridement on Epithelial and Stromal Morphology in Dogs

Ellison Bentley, Sean Campbell, Heung M. Woo, and Christopher J. Murphy

PURPOSE: To determine the effect of chronic corneal epithelial debridement on epithelial and stromal morphology and extra-cellular matrix components, and to compare those changes to those in spontaneous chronic corneal epithelial defects (SCCED) in dogs.

METHODS: Axial corneal epithelial wounds, 10 mm in diameter, were created weekly for 8 weeks in five normal adult laboratory beagles. Slit lamp biomicroscopy and corneal pachymetry were performed weekly before wounding. Three days after the last debridement the dogs were killed humanely, and corneas were processed for light and electron microscopy and immunohistochemistry for collagen IV, collagen VII, fibronectin, and laminin.

RESULTS: No significant changes in corneal thickness were found. All samples demonstrated epithelial dysmaturaration adjacent to the wound edge, and, in four of five, a narrow zone of nonadherent epithelium formed adjacent to the exposed stroma. All samples had a stromal acellular zone in the area of the defect and continuing for a short distance under the adjacent attached epithelium. Experimentally wounded dogs did not form the superficial hyaline acellular lamina found in 92% of dogs with SCCED. Laminin, collagen IV, and fibronectin were present on the stromal surface in all samples, and collagen VII was present in four of five samples. Transmission electron microscopy (TEM) demonstrated the presence of basement membrane on the surface of the exposed stroma.

CONCLUSIONS: Epithelial changes are similar between experimentally wounded dogs and dogs with SCCED. The stromal acellular zone that forms in experimentally wounded dogs is distinct from the hyaline lamina observed in dogs with SCCED. The difference in the acellular stromal layers between chronically wounded dogs and dogs with SCCED may be of relevance to our understanding of the pathophysiology of persistent epithelial defects. (Invest Ophthalmol Vis Sci. 2002;43: 2136–2142)

Spontaneous chronic corneal epithelial defects (SCCED) in dogs are commonly observed in companion animal veterinary practices and have similarities to recurrent erosions and neurotrophic epithelial defects in humans. Clinically, affected dogs are middle aged and have varying degrees of blepharo-spasm and loosely adherent epithelium. The clinical course of wound healing is prolonged, with some defects persisting beyond 180 days.

In dogs, superficial keratectomy is often used as a primary or secondary treatment for SCCED, because of its high success rate. The use of this technique has allowed the morphol-ogy of these chronic erosions to be studied in detail. Reported light microscopic findings include a dysplastic epithelium in a loosely adherent or nonadherent sheet adjacent to the ero-sion.5,10 Basement membrane components are not present or are discontinuous on the stromal surface of the defect, as demonstrated by transmission and scanning electron micros-copy and immunohistochemistry. Most samples have a thin, superficial, abnormally smooth, hyalinized-appearing acellular zone on the surface of the stroma in the area of the erosion.5,10 Variable amounts of fibroplasia and leukocytic infiltrate have been noted.10 Previous work in rabbits to evaluate the effect of chronic epithelial injury found that, after several weeks, a superficial stromal acellular zone also formed, and the epithelium became hyperplastic.11 The purpose of the present study was to examine the effect of chronic corneal epithelial debride-ment on epithelial and stromal morphology in normal dogs and to compare those changes with those previously found in dogs with spontaneous chronic corneal epithelial defects (SCCED).

METHODS

Experimentally Wounded Dogs

Five normal adult (1–2 years old) female laboratory beagles were used in this study. Ophthalmic examinations, including slit lamp biomicroscopy, indirect ophthalmoscopy, and applanation tonometry, were nor-mal for all dogs. Schirmer tear test results were normal (21.25 ± 5.0 mm/min) in all dogs.

Dogs were heavily sedated with intramuscular morphine (1 mg/kg) and acepromazine (0.1 mg/kg). Slit lamp biomicroscopy and corneal pachymetry (Pach-Pen; Mentor, Norwell, MA) were performed weekly before wounding. Several drops of 0.5% proparacaine were applied to each eye before measurement of corneal thickness and wounding. Three pachymetry measurements were obtained in the central cornea, and the average was used for calculations. A 10-mm-diameter area of the central cornea of the right eye of each dog was outlined with a dull trephine, and the epithelium was removed with an excimer laser spatula (Visitec, Sarasota, FL). Wounding was performed once weekly for 8 weeks. After wounding, both eyes of all dogs were treated with a neomycin-polymyxin-gramicidin (Bausch & Lomb, Tampa, FL) solu-tion twice daily. All dogs were treated in accordance with the tenets of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the research was approved by the Animal Care and Use Committee of the University of Wisconsin-Madison.

Three days after the last epithelial debridement (week 8), dogs were humanely killed, and corneas were divided for fixation in forma-lin, for light microscopy and immunohistochemistry, and in 2% glutar-aldehyde, for transmission electron microscopy (TEM). Samples were taken from the cut edge of the corneal sections. TEM was performed as previously described.

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sions, identified by complete ophthalmic examination, had been present for a minimum of 3 weeks with no known underlying cause. The average duration of defects was 6.35 ± 4.6 weeks (SD). Samples were fixed in either formalin (n = 23) or 2% glutaraldehyde in phosphate buffer (n = 25) and were processed and analyzed as described earlier.

**RESULTS**

**Experimentally Wounded Dogs**

All abrasions healed within 5 days of wounding. By the fourth week after initial wounding, all dogs showed development of a subepithelial corneal haze axially that was opaque enough to obscure posterior corneal and iridal detail (Fig. 1). This haze persisted until the end of the experiment. Anterior chamber cell or flare was not noted at any time point. Corneal thickness was not significantly different between chronically wounded eyes and control eyes at any time point, nor was any significant difference noted between time points. At the time of death, 3 days after the last wounding, the defects were approximately 3 mm in diameter.

**Light Microscopy**

Four of the five samples had a narrow zone of nonadherent epithelium immediately adjacent to the exposed stroma and a mild epithelial neutrophilic infiltrate. All samples exhibited epithelial dysmaturation adjacent to the exposed stroma (Fig. 2).

![Figure 2](image-url)

**Comparison of Experimentally Wounded Dogs with Dogs with SCCED**

In previous work in our laboratory, we examined keratectomy samples from dogs with spontaneous chronic corneal epithelial defects. Superficial lamellar keratectomy was performed by board-certified veterinary ophthalmologists in 48 eyes of 46 dogs as a therapeutic procedure over a 6-year period (1993–1999). All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All superficial, nonseptic corneal erosions, identified by complete ophthalmic examination, had been present for a minimum of 3 weeks with no known underlying cause. The average duration of defects was 6.35 ± 4.6 weeks (SD). Samples were fixed in either formalin (n = 23) or 2% glutaraldehyde in phosphate buffer (n = 25) and were processed and analyzed as described earlier.

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A mild suppurative stromal infiltrate (8.33 cells/high-power field) and generalized moderate stromal fibroplasia were present in all samples. All samples had a stromal acellular zone that was present in the area of the exposed stroma, as well as under adjacent attached epithelium and was 79 ± 23 μm (SD) thick (Fig. 3).

**Transmission Electron Microscopy**

Basement membrane was present on the surface of the exposed stroma of all images when imaged with TEM. TEM also revealed the presence of normal-appearing stromal fibers in the area of the stromal acellular zone (Fig. 4).

**Immunohistochemistry**

Laminin, collagen IV, and fibronectin were present on the surface of the exposed stroma in all five samples. Collagen VII was present on the surface of the exposed stroma in four of five samples (Fig. 5). The appearance of collagen IV, laminin, and collagen VII was virtually identical. Fibronectin, however, was present in a slightly thicker band with a slight degree of extension into the anterior stroma (Figs. 5C, 5D).

**Comparison of Experimentally Wounded Dogs with Patients with SCCED**

In 81% (39/48) of samples from patients with SCCED, a zone of nonadherent epithelium was demonstrated, and in 94%, epithelial dysmaturation was observed, both of which were more extensive than that found in experimentally wounded dogs (Fig. 6; Table 1). Immunolocalization of laminin, collagen IV, and collagen VII revealed that most samples had no evidence of these components on the surface of the exposed stroma (22/41, 25/37, 22/37, respectively). When these components were present, it was usually as discontinuous segments. In contrast, fibronectin was present on most samples in the area of the erosion (33/37). In 31/48 (65%) samples, a leukocytic infiltrate, with both neutrophils and lymphocytes was present. Neutrophils were the most common cell type identified, with 17 samples with a mild neutrophilic infiltrate, 6 with a moderate neutrophilic infiltrate, and 3 with a severe neutrophilic infiltrate. Two samples had a mild lymphocytic-plasmacytic infiltrate, 1 sample had a moderate lymphocytic-plasmacytic infiltrate, and 2 had a severe lymphocytic-plasmacytic infiltrate. Varying degrees of stromal fibroplasia were noted in 57/48 samples, ranging from superficial mild fibroplasia (n = 5), to superficial moderate fibroplasia (n = 3), to superficial severe fibroplasia (n = 18). A distinct superficial stromal hyaline acellular lamina was present in the area of the erosion in 44 of 48 samples. The average thickness was 4.4 ± 1.56 μm (SD; Fig. 6). TEM revealed 15 of 15 samples examined to have either no basement membrane or only patchy, discontinuous segments of basement membrane on the surface of the erosion, with an amorphous substance admixed with the stromal fibrils anteriorly (Fig. 7).

**DISCUSSION**

Normal dogs undergoing recurrent debridement of the epithelium show some epithelial changes that are similar to those in dogs with spontaneous chronic corneal epithelial defects (SCCED). In both spontaneous and experimentally created chronic epithelial defects, the epithelium becomes dysplastic.
adjacent to the defect and forms a nonadherent sheet adjacent to the defect. In SCCED, however, the zone of epithelial dysmaturation and the extent of nonadherence are greater than in normal dogs undergoing recurrent debridement. This finding suggests that a degree of epithelial dysmaturation and the presence of a limited zone of epithelial nonattachment at the leading edge of the migrating epithelial sheet is part of the normal wound-healing process. The more extensive zone of nonadherent epithelium surrounding the bared stroma in dogs with SCCED probably indicates an exaggerated attempt of the epithelium to migrate over the wound, combined with an inability to reform functional adhesion complexes. Most samples from dogs with SCCED showed no intraepithelial infiltrate, whereas most samples from experimentally wounded dogs had a mild intraepithelial suppurative infiltrate. These findings are somewhat different from those in a previous study of human epithelium, in which intraepithelial polymorphonuclear leukocytes were observed with recurrent erosions that were theorized to be a source of excess matrix metalloproteinases.

In this study, normal dogs with experimentally created chronic epithelial defects retained the basement membrane on the surface of the exposed stroma. In dogs with SCCED, the basement membrane is not retained on the stromal surface. In the normal cornea, the basement membrane remains attached to the underlying stroma in superficial trauma or scrape injuries. Debridement with an excimer laser spatula does not remove the basement membrane from the underlying stroma in normal dogs (Bentley E, Woo HM, unpublished data, 2000). This implies that basement membrane dynamics in dogs with SCCED are disrupted because normal adhesion complexes do not form. It is likely that an increase in degradative processes occurs in the basement membrane in dogs with SCCED. Studies in humans demonstrate an increase in matrix metalloproteinase (MMP)-2 in the debrided epithelium of human patients with recurrent erosions.

The stromal changes in experimentally wounded dogs are distinct from those in dogs with SCCED. All experimentally wounded dogs had a suppurrative stromal infiltrate and generalized fibroplasia. Only 65% of dogs with SCCED had a stromal infiltrate, which was predominantly suppurrative, but also included lymphocytes and plasma cells. This implies that the SCCED-affected dogs have a more chronic inflammatory response than the experimentally wounded dogs, which is probably due to the nonhealing nature of their defects, compared with the recurrent trauma of epithelial debridement in experimental dogs. The stromal fibrosis in experimentally wounded dogs is most likely responsible for the stromal haze observed. Dogs with SCCED had varying degrees of stromal fibroplasia, ranging from no fibroplasia, through superficial fibroplasia, to generalized fibroplasia. This variability in fibrosis correlates with the variable degree of stromal haze observed in dogs with SCCED. Experimentally wounded dogs showed development of a thick superficial stromal acellular zone composed of normal-appearing stromal fibers. Dogs with SCCED had a thin superficial stromal acellular lamina that appeared hyalinized on light microscopy and had an amorphous substance between the stromal fibers demonstrated by TEM (Figs. 6 and 7). This

FIGURE 5. (A) Micrograph of normal canine cornea shows immunolocalization of collagen IV in the basement membrane (arrow). (B) Immunolocalization of collagen IV on surface of exposed stroma in canine cornea wounded weekly for 8 weeks (arrow). The appearance was similar for collagen VII and laminin. (C) Micrograph of normal canine cornea shows immunolocalization of fibronectin in the basement membrane (arrow). (D) Immunolocalization of fibronectin on the surface of the exposed stroma and extending slightly into the superficial stroma in a canine cornea wounded weekly for 8 weeks (arrow). Bar, 20 μm.
Previous work with chronic epithelial debridement in rabbits demonstrated thinning of the wounded cornea and thickening of the contralateral control corneas. The investigators speculated that chronic epithelial injury was involved in the pathogenesis of keratoconus. In this study in adult dogs, however, no change in corneal thickness was noted after 8 weeks of wounding. The rabbits used in the prior study were immature at 8 weeks of age, and immaturity may have played a role not seen in the adult dogs used in the current study. It seems likely that the thickening of the contralateral cornea in the immature rabbits was a normal process that repeated wounding prevented in the surgically altered eye. It is also possible that there are species differences in the reaction to chronic corneal epithelial debridement not previously realized.

Kim et al., in a previous study of chronic epithelial wounding, also noted keratocyte apoptosis underlying the corneal epithelium, and others have documented apoptosis associated with corneal injury. Preliminary studies confirm that keratocyte apoptosis occurs under the wounded epithelium in this canine model. Apoptosis occurs at significantly lower frequency in dogs with SCCED than in the experimentally chronically wounded dogs. Further studies are needed to determine whether alterations in apoptosis play a role in the differences in stromal alterations between experimentally wounded dogs and dogs with SCCED. Other studies have shown that the specific architecture of the anterior corneal stroma maintains its rigidity, even under conditions of extreme swelling. Others have postulated that this rigidity leads to an increase in the anterior stromal pressure after stromal swelling due to removal of epithelium. It may be that this increase in anterior stromal pressure leads to keratocyte death, rather than apoptosis secondary to removal of the epithelium. Other work has also shown that the tears play a role in keratocyte apoptosis after wounding.

In summary, the changes associated with SCCED cannot be attributed solely to the presence of a chronic corneal epithelial defect. The extent of epithelial dysmaturation and epithelial

**Table 1. Comparison of Morphologic Characteristics of Canine Spontaneous Chronic Corneal Epithelial Defects and Experimentally Created Chronic Corneal Epithelial Defects**

<table>
<thead>
<tr>
<th>Morphologic Characteristic</th>
<th>Experimentally Wounded Dogs (%)</th>
<th>SCCED Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial lip</td>
<td>80 (4/5)</td>
<td>81 (39/48)</td>
</tr>
<tr>
<td>Epithelial dysmaturation</td>
<td>100 (5/5)</td>
<td>94 (45/48)</td>
</tr>
<tr>
<td>Epithelial leukocytic infiltrate</td>
<td>80 (4/5)</td>
<td>38 (18/48)</td>
</tr>
<tr>
<td>Continuous basement membrane confirmed by TEM</td>
<td>100 (5/5)</td>
<td>0 (0/15)</td>
</tr>
<tr>
<td>Stromal fibroplasia</td>
<td>100 (5/5)</td>
<td>77 (37/48)</td>
</tr>
<tr>
<td>Stromal leukocytic infiltrate</td>
<td>100 (5/5)</td>
<td>65 (31/48)</td>
</tr>
<tr>
<td>Superficial hyaline stromal acellular lamina</td>
<td>0 (0/5)</td>
<td>92 (44/48)</td>
</tr>
<tr>
<td>Thick stromal acellular zone†</td>
<td>100 (5/5)</td>
<td>0 (0/48)</td>
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† Characterized by normal-appearing corneal stroma without keratocytes, as compared with the thin superficial hyalinized-appearing zone in patients with SCCED.

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**Figure 6.** (A) Light micrograph of canine cornea after 8 weeks of weekly epithelial debridement shows a small epithelial lip with dysmaturation (arrow), a large superficial stromal acellular zone (arrowheads), and generalized stromal fibroplasia. (B) Light micrograph of a dog with SCCED showing a large epithelial lip with extensive epithelial dysmaturation (black arrow), a thin superficial hyaline stromal acellular lamina (arrowheads), and a thin band of superficial stromal fibroplasia (white arrow). Hematoxylin and eosin. Bar, 20 μm.
nonadherence is much greater in SCCED than in chronically wounded dogs. The degree of fibroplasia and neutrophilic infiltrate is more consistent in chronically wounded dogs. Findings in SCCED not noted in chronically wounded dogs are the presence of an anterior hyaline stromal lamina, the absence of normal basement membrane constituents, and the previously reported changes in peptidergic innervation.

References


