It has been generally recognized by clinicians for decades that persistence of a corneal epithelial defect beyond 10 days to 3 weeks after trauma, surgery, infection, or disease usually leads to scarring of the corneal stroma underlying the epithelial defect, although little has been written on the etiology of this disorder. Research over the past decade on the critical role of the epithelial basement membrane in the etiology of stromal scarring (late haze or fibrosis) after photorefractive keratectomy (PRK) or stromal scars after microbial keratitis has provided important insights into stromal opacities occurring after most trauma, surgery, infection, or toxic exposures to the cornea.1-7

The purpose of this study was to evaluate rabbit corneas with persistent epithelial defects with immunohistochemistry and transmission electron microscopy to characterize the stromal healing response that occurs with persistent epithelial defects.

**PATIENTS AND METHODS**

This study is both a case study of two laboratory cases of spontaneous persistent epithelial defects and an assessment of pathophysiology and treatment of persistent epithelial defects. Female New Zealand white rabbits (12 to 15 weeks old) weighing 2.5 to 3 kg each had -4.50 D PRK without mitomycin C using previously described methods.1 These eyes were treated with 0.5% moxifloxacin (Vigamox; Alcon Laboratories, Inc., Fort Worth, TX) four times a day following surgery and continued until the epithelium healed as detected by slit-lamp photographs, immunohistochemistry for the myofibroblast marker alpha-smooth muscle actin (α-SMA), and transmission electron microscopy.

**RESULTS:** Myofibroblasts developed at the stromal surface within the persistent epithelial defect and for a short distance peripheral to the leading edge of the epithelium. No normal epithelial basement membrane was detectable within the persistent epithelial defect or for up to 0.3 mm behind the leading edge of the epithelium, although epithelial basement membrane had normally regenerated in other areas of the zone ablated by an excimer laser where the epithelium healed promptly.

**CONCLUSIONS:** A persistent epithelial defect in the cornea results in the development of myofibroblasts and disordered extracellular matrix produced by these cells that together cause opacity within, and a short distance beyond, the persistent epithelial defect. Clinicians should treat persistent epithelial defects within 10 days of non-closure of the epithelium to facilitate epithelial healing to prevent long-term stromal scarring (fibrosis).

From Cole Eye Institute, The Cleveland Clinic, Cleveland, Ohio (SEW, CSM); the Department of Ophthalmology at University of São Paulo, São Paulo, Brazil (CSM, MRS); and the Department of Ophthalmology at Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (MRS).

Submitted: May 14, 2017; Accepted: November 20, 2017

Supported in part by U.S. Public Health Service grants RO1EY10056 (SEW) and EY015638 from the National Eye Institute, National Institutes of Health, Bethesda, Maryland, and Research to Prevent Blindness, Inc., New York, New York.

The authors have no financial or proprietary interests in the materials presented herein.

Correspondence: Steven E. Wilson, MD, Cole Eye Institute, i-32, The Cleveland Clinic, 9500 Euclid Ave., Cleveland, OH 44195. E-mail: wilsons4@ccf.org doi:10.3928/1081597X-20171128-01

**ABSTRACT**

**PURPOSE:** To analyze corneal persistent epithelial defects that occurred at 3 to 4 weeks after -4.50 diopter (D) photorefractive keratectomy (PRK) in rabbits and apply this pathophysiology to the treatment of persistent epithelial defects that occur after any corneal manipulations or diseases.

**METHODS:** Two of 168 corneas that had -4.50 D PRK to study epithelial basement membrane regeneration developed spontaneous persistent epithelial defects that did not heal at 3 weeks after PRK. These were studied with slit-lamp photographs, immunohistochemistry for the myofibroblast marker alpha-smooth muscle actin (α-SMA), and transmission electron microscopy.

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fluorescein staining with FUL-GLO Fluorescein Sodium Strips USP (Akorn, Lake Forest, IL). In one rabbit, where the epithelium developed an epithelial defect that persisted for 3 weeks after surgery, the moxifloxacin drops were continued four times a day until obvious corneal scarring was seen in the stroma beneath the epithelial defect at the slit lamp (Figure A, available in the online version of this article). At that point, the rabbit was killed with an intravenous injection of 100 mg/kg pentobarbital while the animal was under 30 mg/kg ketamine hydrochloride and 5 mg/kg xylazine intramuscular general anesthesia. The corneoscleral rim was removed and bisected through the persistent epithelial defect. Half of the corneoscleral rim was fixed in optical coherence tomography compound (Sakura FineTek, Torrance, CA) within a 24 × 24 × 5 mm mold (Fisher Scientific, Pittsburgh, PA) for immunohistochemistry for the alpha-smooth muscle actin (α-SMA) marker for myofibroblasts using previously detailed methods. The other half of the corneoscleral rim was fixed in fresh 2.5% glutaraldehyde and 4% paraformaldehyde with 0.2 M cacodylate buffer at 4°C and processed for transmission electron microscopy using previously detailed methods.

In the second rabbit, the persistent epithelial defect had not healed by 3 weeks after -4.50 D PRK and the eye was being treated with 0.5% moxifloxacin four times a day following surgery. The area of the persistent epithelial defect developed a dense scar, despite the epithelium then closing by 4 weeks after surgery. At 4 weeks after surgery, the rabbit was killed and the cornea was removed and processed for transmission electron microscopy.

RESULTS

In the first rabbit cornea with persistent epithelial defect removed at 3 weeks after surgery, a rolled leading epithelial edge characteristic of persistent epithelial defects was noted (Figure 1A) in immunohistochemistry for the α-SMA marker for myofibroblasts and 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI) that stains the nuclei of all cells. It was not possible to cut sections including the leading edge of the epithelium without this portion of the epithelium artifically separating from the underlying stroma due to poor adhesion. There were α-SMA+ myofibroblasts present near the stromal surface, even beyond the rolled leading edge (Figure A). Greater numbers of α-SMA+ myofibroblasts were noted in the anterior stroma of the cornea (Figure 1B) within the stroma underlying the prior epithelial defect.

Transmission electron microscopy confirmed myofibroblasts in the anterior stroma underlying the epithelial defects (Figures 2A-2B) and a distance of approximately 0.3 mm beyond the leading edge of the epithelial defect but not in the adjacent cornea where the epithelium had closed normally within the PRK-ablated zone. Within the epithelial defects and approximately 0.3 mm peripheral, there was no epithelial
basement membrane lamina lucida and lamina densa detectable by transmission electron microscopy with magnifications up to 43,000× (lamina lucida and lamina densa, when present, are clearly seen at 20,000× magnification). Normal lamina lucida and lamina densa were noted in the areas within the PRK-ablated zone where the epithelium healed normally within 5 to 10 days after surgery (Figure 2C).
In the second rabbit with a persistent epithelial defect at 3 weeks after -4.50 D PRK that subsequently closed by 4 weeks after surgery, transmission electron microscopy at 4 weeks after PRK (Figure 2D) showed layers of myofibroblasts with large amounts of rough endoplasmic reticulum within the area of the cornea where the persistent epithelial defect had been located. No epithelial basement membrane lamina lucida and lamina densa were detected within this area, even with magnifications up to 55,000×.

**DISCUSSION**

Persistent corneal epithelial defects that do not close within 10 days to 2 weeks after trauma, surgery, or infection and many other etiologies commonly develop scarring of the anterior corneal stroma underlying the epithelial defect, even if the epithelium subsequently heals. Two rabbit corneas were evaluated in this study after a spontaneous persistent epithelial defect did not heal for at least 3 weeks after -4.50 D PRK, where late haze does not occur when the epithelium closes in the typical 5 to 8 days after surgery.1,4 These corneas demonstrated that the underlying mechanism of scarring is myofibroblast-mediated fibrosis, which is similar to that noted after -9.00 D PRK1 or *Pseudomonas* keratitis7 in rabbits. In those studies, the epithelium healed within 10 days after surgery or sterilization of the ulcer with antibiotics, respectively, but normal mature epithelial basement membrane did not regenerate for 2 months—likely due to deficient keratocyte contributions of components such as laminins and nidogens to the nascent epithelial basement membrane.4,5,8 In the case of a persistent epithelial defect that never heals, no epithelial basement membrane could be present within the area of the epithelial defect because even nascent epithelial basement membrane is not produced without overlying epithelium. Absence of epithelial basement membrane on the stromal surface within the persistent epithelial defect of cornea 1 in this study was confirmed by transmission electron microscopy (Figure 2). In rabbit 2 of this study, even when the epithelium closed between 3 and 4 weeks after PRK, no epithelial basement membrane could be detected with transmission electron microscopy within the area of the prior persistent epithelial defect (Figure 2D).

An important function of intact mature epithelial basement membrane is to modulate the passage of activated epithelium-derived transforming growth factor beta (TGFβ) and platelet-derived growth factor into the stroma.1,2,4,9 If the regenerated epithelial basement membrane is defective, these growth factors penetrate into the stroma from the overlying epithelium and drive the development of fibrosis-producing myofibroblasts from both keratocyte-derived and bone marrow cell–derived precursor cells.4,9 In corneas with a persistent epithelial defect, where the epithelium never closes, these profibrotic growth factors likely pass into the exposed stroma from the tears10,11 after release from corneal epithelial cells peripheral to the epithelial defect,12,13 and perhaps from conjunctival epithelium14 and lacrimal gland.15 Thus, these growth factors bind the corresponding receptors on the stromal myofibroblast precursor cells and trigger the development of vimentin + α-smooth muscle actin + desmin + (V+A+D+) mature myofibroblasts.16

Once the myofibroblasts develop in the anterior stroma, opacity is caused by the opaque myofibroblasts themselves17,18 and the disordered extracellular matrix these myofibroblasts produce.4 Note that for the persistent epithelial defect shown in Figure A, transmission electron microscopy (Figure 2) showed myofibroblasts and fibrosis development at only 3 weeks after surgery. Thus, some α-SMA+ myofibroblasts developed but the surrounding stroma was just beginning to show a disorder of the normal arrangement of the collagen fibrils if one compares the sharpness of the collagen fibrils in the area of the cornea outside the persistent epithelial defect (Figure 2C), where no myofibroblasts have developed, to that within the persistent epithelial defect (Figures 2A-2B), where myofibroblasts are present and excreting excessive disorganized collagen type1,19 and collagen type37 and other extracellular matrix materials, not normally present in the corneal stroma.20 These newly deposited collagens disrupt the precise organization of the fibrils responsible for stromal transparency.20

The longer the epithelial defect persists, the more myofibroblasts will be generated, the greater the amounts of disordered extracellular matrix secreted by these cells, and the greater the disruption of the normal corneal stromal structure within the stroma underlying the persistent epithelial defect. In rabbit 2, although the epithelium eventually closed at 4 weeks after PRK, many subepithelial myofibroblasts persisted in the area of the former persistent epithelial defect because normal overlying epithelial basement membrane did not regenerate. This points to the importance of facilitating timely persistent epithelial defect closure to promote regeneration of normal epithelial basement membrane and resulting apoptosis of myofibroblasts to allow keratocytes to repopulate the anterior stroma and reabsorb extrinsic extracellular matrix material. This process can ultimately lead to the restoration of corneal transparency.

A detailed discussion of the different etiologies and treatments for persistent epithelial defect is beyond...
the scope of this article. However, regardless of the underlying etiology, most persistent epithelial defects should initially be approached with standard treatment modalities such as ocular surface lubrication, bandage contact lenses, tarsorrhaphy, autologous serum drops, and/or amniotic membranes, with increasing aggressiveness beyond 8 to 10 days after surgery, trauma, or infection. Table A (available in the online version of this article) lists some of the most common conditions associated with persistent epithelial defect and references articles with specific treatment strategies. However, general comments regarding measures to reduce scarring (fibrosis) in the stroma underlying the persistent epithelial defect are of interest.

The leading edge of the epithelium at the persistent epithelial defect is commonly “rolled” and “stalled,” impeding subsequent growth of epithelium across the defect (Figure 1A). “Freshening the edges” with a scalpel blade can facilitate epithelial healing. In addition, the stromal surface within the persistent epithelial defect is typically irregular and may bind molecules from the tear film that further impede epithelial healing (Figure 2B). This surface irregularity may also retard epithelial closure and, even if the epithelial defect subsequently closes, may interfere with regeneration of normal epithelial basement membrane and increase haze (fibrosis) (Figure 2D). In our experience, to address this problem, it has been useful to freshen the edges of the persistent epithelial defect for approximately 1 mm peripheral with a scalpel blade and possibly perform limited phototherapeutic keratectomy and masking-smoothing with the excimer laser using a beam diameter that is the size of the augmented epithelial defect.

Amniotic membranes have a special role in potentially reducing or preventing fibrosis in persistent epithelial defect. Amniotic membranes can downregulate TGFβ signaling. Thus, in addition to promoting epithelial closure in eyes with persistent epithelial defect, amniotic membranes may reduce fibrosis by interfering with TGFβ modulation of myofibroblast development from precursor cells. Recently, morselized formulations of amniotic membranes have been described that can be applied topically to eyes with persistent epithelial defect and these formulations could also potentially downregulate TGFβ-driven myofibroblast development in the stroma underlying a persistent epithelial defect.

Finally, the clinician should always consider the possibility that herpes simplex virus infection underlies the persistent epithelial defect. Therefore, when persistent epithelial defects, therapeutic debridement, ultraviolet-riboflavin cross-linking, traumatic or spontaneous abrasions, or any other intervention or disease remain at 10 days after PRK, we typically add empirical oral anti-herpes simplex virus therapy to other modalities to promote timely epithelial healing.

More research should be focused on other pharmacologic agents to modulate TGFβ-mediated scarring in persistent epithelial defect. Whatever therapeutic modalities are used for treatment of persistent epithelial defect, if the epithelium can be triggered to close, then the epithelial basement membrane can eventually regenerate. This, in turn, can lead to myofibroblasts apoptosis and disordered extracellular matrix reabsorption by repopulating keratocytes so that stromal transparency is restored over a period of months to years, as it can be after PRK and microbial corneal ulcers.

**AUTHOR CONTRIBUTIONS**

Study concept and design (SEW, CRM, MRS); data collection (SEW, CRM, MRS); analysis and interpretation of data (SEW, CRM, MRS); writing the manuscript (SEW, CRM, MRS); critical revision of the manuscript (SEW, CRM, MRS); supervision (SEW)

**REFERENCES**


Figure A. Slit-lamp photographs of a persistent epithelial defect (PED) in the cornea of rabbit 1 at 3 weeks after -4.50 diopter photorefractive keratectomy. (A) The epithelial defect is delineated by arrows and can be noted to have a thickened epithelial leading edge around the perimeter of the PED. The bare stroma within the PED is opaque and the opacity in the stroma extends a small distance peripheral to the leading edge of the PED. (B) Fluorescein staining of the same cornea shows the PED (original magnification ×10).
<table>
<thead>
<tr>
<th>Disorder Leading to PED</th>
<th>Treatment*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurotropic cornea(^{25})</td>
<td>Topical nerve growth factor</td>
</tr>
<tr>
<td>Diabetes mellitus(^ {26})</td>
<td>Optimization diabetic control</td>
</tr>
<tr>
<td>Herpetic keratitis(^ {27,28})</td>
<td>Antiviral treatment, judicious corticosteroids</td>
</tr>
<tr>
<td>Corneal dystrophies(^ {29,30})</td>
<td>PTK+MS, DALK, PKP</td>
</tr>
<tr>
<td>Salzmann’s nodular degeneration(^ {31})</td>
<td>Mechanical stripping of fibrous tissue, PTK+MS</td>
</tr>
<tr>
<td>Limbal stem cell deficiency(^ {32,33})</td>
<td>Limbal stem cell transplantation, scleral contact lenses</td>
</tr>
<tr>
<td>Chemical injuries(^ {34})</td>
<td>Limbal stem cell transplantation, DALK</td>
</tr>
<tr>
<td>Dry eye and Sjögren syndrome(^ {35})</td>
<td>Topical cyclosporine A, systemic treatment for Sjögren syndrome</td>
</tr>
<tr>
<td>Anesthetic abuse(^ {36})</td>
<td>Discontinue anesthetic</td>
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<tr>
<td>Medicamentosa</td>
<td>Discontinue offending medication</td>
</tr>
<tr>
<td>Graft vs host disease(^ {37})</td>
<td>Systemic immunosuppression, topical cyclosporine A</td>
</tr>
<tr>
<td>Stevens–Johnson syndrome(^ {38})</td>
<td>Corticosteroids, other immunosuppressives</td>
</tr>
<tr>
<td>Atopic keratoconjunctivitis(^ {39})</td>
<td>Topical corticosteroids, antihistamines</td>
</tr>
</tbody>
</table>

PED = persistent epithelial defects; PTK+MS = phototherapeutic keratectomy with masking smoothing; DALK = deep anterior lamellar keratoplasty; PKP = penetrating keratoplasty

*Other than lubricants, serum drops, tarsorrhaphy, bandage contact lenses, and amniotic membrane.