Remodeling of the Corneal Epithelial Scaffold for Treatment of Persistent Epithelial Defects in Diabetic Keratopathy

Manami Ohta\textsuperscript{a} Yukiko Morita\textsuperscript{a} Naoyuki Yamada\textsuperscript{a} Teruo Nishida\textsuperscript{a, b} Naoyuki Morishige\textsuperscript{a, b}

\textsuperscript{a}Department of Ophthalmology, Yamaguchi University Graduate School of Medicine, Ube, Japan; \textsuperscript{b}Ohshima Eye Hospital, Fukuoka, Japan

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Corneal epithelium · Persistent epithelial defect · Diabetic keratopathy · Surgery

Abstract
\textbf{Background:} To develop a strategy based on surgical removal of a degenerated corneal epithelial scaffold for treatment of persistent epithelial defects (PEDs) in diabetic keratopathy.

\textbf{Case Presentation:} Three diabetic patients with PEDs were initially treated with eyedrops containing the fibronectin-based peptide PHSRN (Pro-His-Ser-Arg-Asn) or both the substance P-derived peptide FGLM-NH\textsubscript{2} and the insulin-like growth factor-1-derived peptide SSSR. A degenerated Bowman's layer or calcified lesion thought to be responsible for incomplete healing was surgically removed after confirmation of reactivity to the peptide eyedrops. All three patients achieved complete epithelial wound closure after surgery. Two cases treated by phototherapeutic keratectomy or lamellar keratoplasty did not show PED recurrence during 6 or 36 months of follow-up, respectively. One case treated by mechanical removal of a degenerated Bowman's layer manifested recurrence after 1 month, but resurfacing of the defect was again
achieved after repeat surgery. **Conclusion:** We propose a new strategy for treatment of diabetic PEDs based on surgical remodeling of the corneal epithelial scaffold for patients who respond to peptide eyedrops but fail to achieve wound closure.

**Background**

Diabetes mellitus can give rise to corneal epithelial disorders collectively known as diabetic keratopathy [1]. Manifestations of this condition include superficial punctate keratopathy, simple erosion, recurrent erosion, and persistent epithelial defects (PEDs). Pathophysiologic characteristics of diabetic keratopathy are associated with impairment of corneal epithelial function and include a reduced number of hemidesmosomes [2], cellular degeneration [3, 4], defective epidermal growth factor receptor signaling [5, 6], and changes to the basement membrane of the corneal epithelium such as nonenzymatic glycation [7, 8].

The goals of currently available treatments for corneal epithelial disorders include (1) protection of the corneal surface, (2) activation of corneal epithelial cells, and (3) improvement of the condition of the corneal epithelial scaffold. Conventional treatments in the first category include administration of artificial tears, hyaluronate eyedrops, or oily ointment, insertion of punctal plugs, and application of a bandage soft contact lens. More novel treatment options in the second category include topical application of autologous serum [9], nerve growth factor [10], the substance P-derived peptide FGLM-NH₂ plus insulin-like growth factor-1 (IGF-1) [11], or the fibronectin-derived peptide PHSRN [12]. Finally, treatments in the third category include topical application of fibronectin [13] and surgical removal of a degenerated Bowman’s layer.

In addition to these various treatment options, experimental treatments specific to diabetic keratopathy include topical administration of insulin [14], a galactose reductase inhibitor [15], or naltrexone [16]. Furthermore, we have previously shown that eyedrops containing the substance P-derived peptide FGLM-NH₂ plus IGF-1 [17] or PHSRN [18] facilitated corneal epithelial wound closure in diabetic rats. In addition to the effectiveness of PHSRN eyedrops for the treatment of PEDs in patients with diabetic keratopathy [12], we also found that eyedrops containing FGLM-NH₂ and either IGF-1 [19] or the IGF-1-derived peptide SSSR [20] were effective for the treatment of PEDs in patients with neurotrophic keratopathy. Despite the potential of these various peptides for the treatment of PEDs associated with diabetic keratopathy, however, we encountered some cases that responded with incomplete epithelial resurfacing.

We now present three cases of PEDs in patients with diabetic keratopathy that, despite an initial response, failed to heal during treatment with peptide eyedrops. Surgical removal of a degenerated corneal epithelial scaffold allowed complete epithelial resurfacing in all three patients.
**Case Presentation**

The application of eyedrops containing the peptides FGLM-NH₂ plus SSSR or those containing PHSRN for the treatment of PEDs was approved by the Institutional Review Board of Yamaguchi University Hospital, and adhered to the tenets of the Declaration of Helsinki. The peptides FGLM-NH₂, SSSR, and Ac-PHSRN-NH₂ were obtained from Protein Research Foundation (Osaka, Japan). The eyedrops contained either FGLM-NH₂ plus SSSR at 1 mg/mL and 0.05629 µg/mL, respectively, in phosphate-buffered saline [20] or 0.02% Ac-PHSRN-NH₂ in phosphate-buffered saline [12, 21], as previously described.

**Case 1**

A 63-year-old man who had had type 2 diabetes for 8 years was referred to Yamaguchi University Hospital for treatment of a PED in his left eye (Table 1; Fig. 1). He had been treated with artificial tear eyedrops, oily ointment, and a bandage soft contact lens for 4 months. He had also been treated for severe proliferative diabetic retinopathy, with the result that his visual acuity at his first visit to our hospital was non-light perception in the left eye. He also manifested an intraocular pressure (IOP) of 21 mm Hg, corneal sensation as measured with a Cochet-Bonnet esthesiometer of <5 mm, and tear secretion as determined by Schirmer's test of only 4 mm in this eye. Slit-lamp biomicroscopy revealed an epithelial defect on a degenerated Bowman's layer at the lower central cornea as well as hematocornea due to prolonged anterior chamber hemorrhage. We initiated treatment of the PED with eyedrops containing FGLM-NH₂ + SSSR and those containing levofloxacin four times a day. We also inserted a punctal plug into the lower punctum and applied a bandage soft contact lens to improve the condition of the ocular surface in the left eye. The epithelial defect had shrunk to 40% of its original size after 28 days, but no further improvement was evident. We changed the eyedrops containing FGLM-NH₂ + SSSR to those containing PHSRN and continued treatment for another 20 days, but no further epithelial migration was achieved. We considered the degenerated Bowman's layer to be responsible for prevention of complete healing, and so we removed it surgically. Postsurgical resumption of treatment with PHSRN eyedrops resulted in complete closure of the epithelial defect after 8 days. Pathological analysis revealed calcification of the excised Bowman's layer. The patient experienced PED recurrence in his left eye 2 months after complete healing of the initial defect. We again performed surgical removal of the degenerated Bowman's layer and applied PHSRN eyedrops, resulting in complete healing of the PED. Additional recurrence was not evident during follow-up for 3 months after the second surgery.

**Case 2**

A 28-year-old man who had had type 1 diabetes for 13 years was referred to Yamaguchi University Hospital for treatment of a PED in his right eye that had not responded to conventional treatments including artificial tear eyedrops, rebamipide eyedrops, oily ointment, and a bandage soft contact lens over a period of 5 months after pars plana vitrectomy for proliferative diabetic retinopathy (Table 1; Fig. 2). His visual acuity at the first visit was 20/280 OD, and he manifested an IOP of 19 mm Hg, corneal sensation of 10 mm, and tear secretion of 34 mm in his right eye. Slit-lamp biomicroscopy detected an epithelial defect on a degenerated Bowman's layer at the lower central cornea of the right eye. We initiated treatment with FGLM-NH₂ + SSSR eyedrops four times a day in the right eye. The epithelial defect shrunk to
30% of its original size but did not heal completely. We therefore performed phototherapeutic keratectomy to remove the degenerated Bowman’s layer and continued application of FGLM-NH₂ + SSSR eyedrops. Complete wound closure was achieved 7 days after surgery. Recurrence of an epithelial defect was not observed during follow-up for 6 months after surgery.

**Case 3**

A 46-year-old man who had type 2 diabetes for 15 years was referred to Yamaguchi University Hospital because of a PED due to diabetic keratopathy in his right eye (Table 1; Fig. 3). The PED had been treated for 1 month with conventional therapies including artificial tear eyedrops, hyaluronate eyedrops, rebamipide eyedrops, oily ointment, and a bandage soft contact lens. Visual acuity was 20/600, IOP was 11 mm Hg, corneal sensation was 40 mm, and Schirmer test value was 5 mm in the right eye. Slit-lamp biomicroscopy showed an epithelial defect in the right eye. Topical application of FGLM-NH₂ + SSSR eyedrops resulted in an initial slight improvement in the epithelial defect in the right eye, but the defect subsequently increased in size. Diabetic anterior uveitis was then detected in the right eye, and so betamethasone eyedrops were administered four times a day. The FGLM-NH₂ + SSSR eyedrops were also replaced with PHSRN eyedrops, but no improvement in the PED was apparent. Given that a white deposit thought to reflect calcification was detected in the area of the epithelial defect, we performed lamellar keratoplasty on the right eye. No recurrence was detected and corneal transparency was maintained in the right eye during follow-up for 3 years.

**Conclusion**

We here describe three cases of corneal PEDs in patients with diabetes that were treated by surgical removal of a degenerated epithelial scaffold. This treatment strategy was based on the assumption that such degeneration was responsible for prevention of epithelial wound closure during treatment with FGLM-NH₂ + SSSR or PHSRN eyedrops. Our results thus suggest that an improvement in the condition of the epithelial scaffold in addition to corneal epithelial stimulation may be required for complete epithelial wound closure in some patients with diabetic keratopathy.

All three patients had previously received conventional therapies such as hyaluronate eyedrops, artificial tears, oily ointment, and a bandage soft contact lens. Given the lack of response to such treatments, we initially treated the patients with FGLM-NH₂ + SSSR or PHSRN eyedrops. The efficacy of eyedrops containing those peptides have previously been reported in our reports [22–27]. Although all three patients showed corneal epithelial migration in response to the peptide eyedrops, complete healing was not achieved. We speculated that failure to attain wound closure was attributable to the presence of white-gray lesions suggestive of calcification within the area of the epithelial defect. We therefore excised the degenerated epithelial scaffold in each case by different procedures including mechanical removal of Bowman’s layer, phototherapeutic keratectomy, and lamellar keratoplasty. Each of the surgical procedures was followed by rapid wound closure.

In each of the three cases, we confirmed the reactivity of corneal epithelial cells to peptide drugs that directly stimulate corneal epithelial migration. The pathogenesis of PEDs due to diabetic keratopathy may include both loss of epithelial migratory activity and an abnormal
epithelial scaffold. We previously showed that FGLM-NH$_2$ + SSSR eyedrops [17] and PHSRN eyedrops [18] facilitated corneal epithelial migration in a rat model of diabetes. Furthermore, both types of eyedrops promoted corneal epithelial wound closure in patients with PEDs [12, 19, 20]. However, treatment of PEDs in diabetic patients with such peptide eyedrops is likely to be insufficient in the presence of other abnormalities of the cornea that impede epithelial migration in response to such drugs. Confirmation of the reactivity of the corneal epithelium to peptide eyedrops allowed us to continue this treatment after surgical removal of the degenerated epithelial scaffold and thereby to achieve complete resurfacing of PEDs.

**Statement of Ethics**

The Internal Review Board of Yamaguchi University Hospital approved the application of FGLM-NH$_2$ + SSSR eyedrops (Ref. # H-18-77-6) and PHSRN eyedrops (Ref. # H20-120). Written informed consent was obtained from patients before peptide administration. Written informed consent was obtained from the patients for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal. The clinical data of this paper is accessible at Yamaguchi University Hospital. However, the data will not be shared because they are clinical information of the individuals; thus, it is protected by the Law of Act on the Protection of Personal Information.

**Disclosure Statement**

Co-author Teruo Nishida has a patent of PHSRN eyedrops. The other authors declare that they have no competing interests.

**Author Contributions**

M.O. wrote the manuscript and mainly cared for the patients. Y.M. performed surgeries and cared for the patients. N.Y. performed surgery. T.N. suggested the treatment strategies for corneal epithelial defect. N.M. determined the treatment strategies, wrote the manuscript, and organized this report. All authors read and approved the final manuscript.

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Fig. 1. Slit-lamp photographs for case 1. The corneal epithelial defect was completely closed 8 days after removal of the degenerated Bowman’s layer. “Pretreatment” corresponds to the time before initial application of peptide eyedrops.

Fig. 2. Slit-lamp photographs for case 2. The corneal epithelial defect was completely closed 7 days after surgery.
Fig. 3. Slit-lamp photographs for case 3. The corneal epithelial defect on the graft was rapidly resurfaced after lamellar keratoplasty.

Table 1. Characteristics of the study patients

| Characteristic                  | Case 1    | Case 2    | Case 3    |
|--------------------------------|-----------|-----------|-----------|
| Age, years/sex                 | 63/male   | 28/male   | 46/male   |
| Duration of diabetes, years    | 8         | 13        | 15        |
| HbA1c at first visit, %        | 6.3       | 8.5       | 10.0      |
| Initial reactivity to FGLM-NH₂⁺ | ++        | ++        | +         |
| SSSR or PHSRN                  |           |           |           |
| Recurrence                     | yes       | no        | no        |
| Follow-up, months              | 4         | 6         | 36        |