

Treatment of Persistent Corneal Epithelial Defect with Autologous Serum

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Aim: To determine the efficacy and safety of topical autologous serum as an alternative treatment for persistent corneal epithelial defect and non-healing corneal ulcer.

Patients and Methods: Twenty eyes with clinical evidence of persistent corneal epithelial defect of at least 2 weeks' duration that failed to heal with conventional treatment were evaluated. Relevant patient details were recorded, including the cause and duration of persistent corneal epithelial defect, previous treatments, and past medical and ocular history. Slit-lamp examination established the size and location of persistent corneal epithelial defect and also the status of associated inflammation and neovascularisation, dry eye, and corneal sensation. One eye was excluded due to complete healing during this period. Treatment consisted of 20% autologous serum diluted in preservative-containing artificial tears applied 4 to 6 times per day for up to 4 weeks.

Results: The mean duration of persistent corneal epithelial defect prior to treatment was 52.2 days. Following the use of serum eyedrops, healing occurred in 8 eyes (40%) within ≤ 2 weeks and in 6 eyes (30%) within 2 to 4 weeks. All patients reported subjective improvement. Healing occurred in all 8 eyes with corneal anaesthesia but not in 3 eyes with underlying thermal burns. Healing was more rapid in persistent corneal epithelial defects with an initial size of ≤ 2.5 mm or eyes with postoperative persistent corneal epithelial defect. Long-term follow-up of 10 patients confirmed ocular surface stability in each patient. No major ocular or systemic side effects were observed.

Conclusion: Topical administration of preservative-containing autologous serum was found to be an effective and safe therapy for persistent corneal epithelial defect and can be used as an alternative to more aggressive surgical management.

Key words: Corneal diseases, Corneal epithelium, Eyedrops, Serum, Treatment outcome

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Introduction

The mechanism of corneal wound healing is not yet fully understood. However, it is known that the healing of corneal epithelial wounds is modulated by various growth factors, including members of the epidermal growth factor, transforming growth factor- β , hepatocyte growth factor, fibroblast growth factor, insulin-like growth factor (IGF), and platelet-derived growth factor families.¹ In addition, inflammatory cytokines such as interleukins-1 and -6 and tumour necrosis factor- α are involved in corneal wound healing and stimulate corneal epithelial migration both in vitro and in vivo.² Components of the extracellular matrix, including fibronectin, hyaluronan, laminin, and collagen type IV also facilitate epithelial

migration³⁻⁹ and substance-P and IGF-1 synergistically promote corneal epithelial migration.¹⁰ Serum contains many of these factors, which are also present in tears. The presence of lipids in serum may also act as a replacement for lipid tear components produced by the meibomian glands. In addition, serum contains high concentrations of proteins, such as prealbumin, which may contribute significantly to the stability of the tear film.¹¹

There have been reports of the topical use of autologous serum for treating diseases of the external eye, such as keratoconjunctivitis sicca, persistent epithelial defects (PED),¹² recurrent corneal erosions,¹³ and superior limbic keratoconjunctivitis,¹¹ or following complicated penetrating keratoplasty (PK)¹⁴ or laser-assisted subepithelial keratectomy.¹⁵

The present study was conducted to review and evaluate the effects of topical application of autologous serum in the management of PED.

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Patients and Methods

Patient Characteristics and Examination Procedures

In this case series, 21 eyes of 21 consecutive patients with PED, referred to the Poustchi Eye Clinic, Khalili Eye Hospital between October 2002 and August 2004, were prospectively evaluated. Inclusion criteria were clinical evidence of PED due to any cause including non-healing corneal ulcer and failure to heal with conventional treatment of at least 2 weeks' duration. Patients with progressive corneal melting, active microbial infection, acute herpes simplex or herpes zoster keratitis, drug toxicity, vitamin A deficiency, or recurrent corneal erosion were excluded from the study. Previous conventional therapy included various eyedrops such as lubricants, topical antibiotics and steroids, vitamin C, and collagenase inhibitors (doxycycline, tetracycline), eyelid surgery such as patching and tarsorrhaphy, and therapeutic contact lens use. Information about each patient is given in Table 1.

For patients who were receiving frequent doses of preservative-containing eyedrops before entering the study, a 'washout period' of 1 week was scheduled, during which the dosage was reduced to the minimum. Five such eyes, none of which showed significant change in PED size during the washout period, were included (Table 1). One patient, whose large, recalcitrant PED healed completely during the washout period, was excluded from the study.

Prior to commencement of autologous serum, baseline data were obtained from all patients, including the reason and duration of PED, previous treatments, and medical and ocular history. In

addition, the patients' eyes were examined with a slit-lamp to determine the size and location of PED and status of associated inflammation and neovascularisation, dry eye, tear film break-up time (TFBUT), and corneal sensation. Twenty patients, 12 men and 8 women, with a mean age of 45.6 years (SD, 42.2 years), were included in the study (Table 1). PED was present in the right eye in 11 patients and the left eye in 9. Prior ocular treatments were bandage soft contact lenses (7 patients), unsuccessful lateral tarsorrhaphy (5 patients), and punctal occlusion for dry eye syndrome (1 patient). Toxic antiviral agents (trifluridine and acyclovir) were discontinued or changed to oral acyclovir for patients with meta-herpetic epithelial defects.

Location and size of PED and TFBUT were assessed by means of biomicroscopy after applying a fluorescein strip to the conjunctival cul-de-sac. Epithelial defects were measured in 2 dimensions using a slit-lamp micrometer — the maximum width and the width perpendicular to that dimension. The size of each PED was defined as the mean of the latter 2 measurements (Table 1). The location of PED was classified as central when mostly located in the central 4 mm of the cornea, and paracentral when located beyond this region but within the central 8 mm of the 8 mm optical zone. The site of the PED was central in 12 eyes, paracentral in 7, and involved almost the entire cornea in 1 eye. Prior to treatment, the mean duration of PED was 52.2 days (SD, 45.5 days) and the mean PED size was 2.9 mm (SD, 1.6 mm) [Table 1 and Figure 1].

Table 1. Characteristics of patients and response to treatment.

Patient number	Age (years)	Sex	Underlying condition	Duration of persistent epithelial defects* (days)	Size of persistent epithelial defects (mean) [mm]	Duration of healing [†] (days)	Rate of healing (µm/day)
1	22	Female	Post-laser-assisted subepithelial keratectomy	30	3.2 x 3.3 (3.25)	Not healed	—
2	24	Male	Thermal burn	14	4.5 x 10 (7.25)	Not healed	—
3	26	Female	Post-pars plana vitrectomy	19	4.6 x 7 (5.80)	13	446
4	63	Male	Metaherpetic ulcer	122	1.5 x 2 (1.75)	12	145
5	56	Male	Post-penetrating keratoplasty	60 [‡]	4.0 x 4.3 (4.15)	4	1037
6	75	Male	Post-penetrating keratoplasty	70 [‡]	2.5 x 4.5 (3.50)	28	125
7	27	Male	Abrading injury	15	1.0 x 1.5 (1.25)	9	138
8	71	Male	Metaherpetic ulcer	85 [‡]	1.5 x 3.5 (2.50)	25	100
9	30	Female	Shield ulcer	206	1.5 x 1.8 (1.65)	Not healed	—
10	7	Male	Thermal burn	43	2.0 x 2.7 (2.35)	Not healed	—
11	8	Male	Recurrent fungal keratitis	27	2.0 x 2.8 (2.40)	Not healed	—
12	65	Male	Metaherpetic ulcer	43	2.0 x 1.7 (1.85)	28	66
13	26	Female	Bacterial keratitis	20	3.2 x 3.2 (3.20)	20	160
14	27	Female	Acantamoeba keratitis	30 [‡]	1.0 x 2 (1.50)	16	93
15	15	Female	Glaucomatous bullous keratopathy	14	0.5 x 1.5 (1.00)	10	100
16	70	Male	Post-penetrating keratoplasty	66	2.2 x 2.5 (2.35)	23	102
17	27	Female	Thermal burn	57	3.4 x 5.1 (4.25)	Not healed	—
18	28	Male	Neurotrophic and neuroparalytic keratopathy	38	1.0 x 1.3 (1.15)	12	95
19	80	Male	Post-penetrating keratoplasty	25	3.0 x 5.5 (4.25)	10	425
20	64	Female	Post-penetrating keratoplasty	75	3.0 x 3.0 (3.00)	10	300

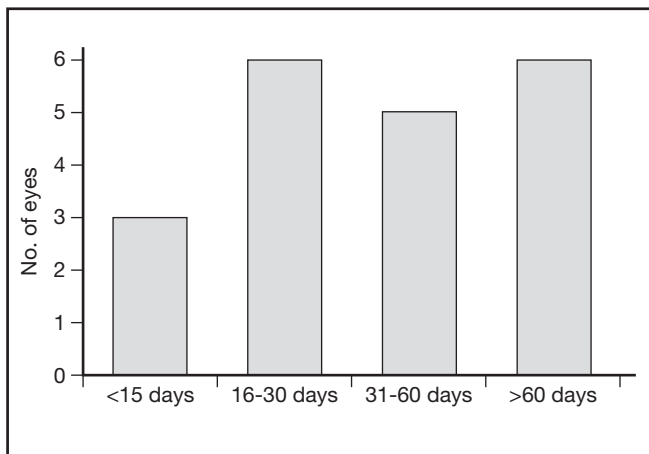
* Prior to treatment.

[†] In response to treatment.

[‡] Including 1-week washout period.

Autologous Serum for Persistent Corneal Epithelial Defect

Figure 1. Pretreatment duration of persistent epithelial defects.



Thirteen eyes (65%) were classified as 'dry eye' on the basis of an abnormal TFBUT of ≤ 10 seconds. The corneal sensitivity test was performed by touching the patient's central corneal zone with the tip of a cotton swab and a normal response was defined as a blink reflex.

Prior to treatment, 8 eyes (40%) had corneal anaesthesia (no response), 9 eyes (45%) had corneal hypoaesthesia (ability to feel contact but blink reflex absent), and 3 eyes (15%) had normal corneal sensation. Five eyes (25%) showed no corneal stromal inflammation, 8 eyes (40%) had mild inflammation (superficial infiltration to a depth $< 50\%$ with density comparable to nebular opacity), 7 eyes (35%) had moderate inflammation (superficial infiltration with density comparable to macular opacity), and no eye had more severe inflammation. Classification of neovascularisation as mild, moderate, or severe was based on the presence of superficial vessels running < 1 mm, 1 to 3 mm, and > 3 mm onto the cornea, respectively. Neovascularisation was absent from 9 eyes (45%), mild in 6 eyes (30%), moderate in 4 eyes (20%), and severe in 1 eye (5%).

This detailed evaluation, including both subjective and clinical assessments, was repeated twice weekly during treatment with autologous serum at the outset, then more frequently when closure of the PED appeared imminent, until healing was complete. Six to twelve months after discontinuation of autologous serum therapy, all patients were called to attend for re-examination.

Preparation of Autologous Serum Eyedrops

Venesection was performed at the cubital fossa under aseptic conditions and 10 mL of blood was collected in a sterile container. After 2 hours at room temperature to allow clotting, blood was centrifuged at 4000 rpm for 10 minutes.

Serum (2 mL) was removed with a syringe and deposited into a sterile dropper bottle preloaded with 8 mL preservative-containing

artificial tears (hydroxypropyl methylcellulose, dextran 70, and benzalkonium), which served as diluent. At this dilution (20%), serum maintains adequate viscosity and provides a relatively high concentration of growth factors.¹⁶ Because fibronectin has limited biological stability,¹⁷ each patient was provided with 1 bottle of autologous serum, to be used for 1 week only, in a container with a coolant bag. As vitamin A is easily degraded by light,¹² patients were instructed to store the bottle in a refrigerator at approximately 4°C.

Autologous Serum Application

The patients were instructed to instill 1 drop of the autologous serum preparation in the affected eye 6 times per day during waking hours. In addition, all less toxic pre-existing medications such as chloramphenicol and cycloplegic agents, with or without systemic and/or topical steroid, and systemic antiherpetic agents were continued at the lowest possible dosages. Systemic vitamin C and collagenase inhibitors were discontinued and autologous serum was used instead of lubricants. When healing of the PED was almost complete, the serum eyedrops were administered 4 times daily until healing was complete. Most topical medications were discontinued after resolution of the PED but instillation of the serum drops 3 to 4 times daily continued for an additional week.

Patients were asked to report any new ocular symptoms that developed in the intervals between visits, to contact the investigators immediately if their symptoms worsened or an adverse reaction developed, and to compare their symptoms before and after autologous serum application.

Statistical Analysis

To allow assessment of the effect of each variable that may have influenced the rate of healing and effectiveness of treatment, patients were divided into 2 subgroups based on the extent to which they exhibited each of these variables.

The rate of healing was calculated by dividing the pretreatment size of the PED (in μm) by the duration of healing, that is, the number of days taken to achieve complete healing. For each variable, the rates of healing for the 2 subgroups of patients were compared using the Mann-Whitney *U* test (Table 2). The relative effectiveness of treatment in each subgroup pair was compared using the Fisher exact test. Statistical analyses were conducted using the Statistical Package for the Social Sciences. A *p* value < 0.05 was considered statistically significant.

Results

The PEDs in 14 of the 20 eyes evaluated (70%) healed completely within 4 to 28 days (mean, 15.8 days; SD, 7.7 days) [Table 1].

Table 2. Comparison of mean rate of healing in various paired subgroupings of patients whose persistent epithelial defects healed in response to treatment.

Patient subgroupings compared	Mean rate of healing (µm/day)	p Value
Age (years)		
≤40	179.5	
>40	287.3	
Location		
Central	264.4	
Paracentral	217.7	
Size of persistent epithelial defects (mm)		
≤2.5	110.3	
>2.5	415.5	
Prior duration of persistent epithelial defects (days)		
≤30	235.7	
>30	245.1	
Dry eye		
Absent	197.8	0.80
Present	265.1	
Corneal sensation		
Absent	285.8	1.00
Present*	181.5	
Stromal infiltration		
Absent	290.4	0.23
Present	117.8	
Neovascularisation		
Absent	300.2	0.54
Present	182.0	

*Including reduced corneal sensation.

Healing occurred in 8 eyes (40%) within ≤2 weeks and in 6 eyes (30%) within 2 to 4 weeks. All patients, even those with failed PED healing, reported subjective improvement. In all eyes in which healing was complete, the healing process progressed at a mean rate of 240 µm/day (SD, 260 µm/day) [Table 1].

No major ocular or systemic side effects of treatment were observed during this trial. The only adverse effect associated with autologous serum use was punctate epithelial erosion caused by the preservative in 11 of 14 healed eyes, but this disappeared when serum drops were discontinued at the end of the treatment period.

Statistical analysis indicated that the duration, size, and location of PED, associated dry eye, stromal infiltration, and neovascularisation did not significantly affect the outcome of the

treatment (healed versus not healed). All 8 eyes with corneal anaesthesia healed successfully (p = 0.04). Among healed eyes, the mean rate of healing in eyes with postoperative PED was 405.5 µm/day compared with 117.8 µm/day for the remaining eyes (p = 0.03). Postoperative PED began immediately after PK or pars plana vitrectomy. Healing was also more rapid when the size of the PED was ≤2.5 mm (Table 2).

In 6 eyes, the PED failed to heal despite 2 to 4 weeks of autologous serum therapy (Table 3). All 3 eyes with underlying thermal burns did not respond to autologous serum. These underwent surgical management and, in 2 eyes, near-total conjunctivalisation occurred during the follow-up period. All 6 patients in whom treatment failed were older than 40 years (p = 0.04).

During follow-up of PEDs that healed, no obvious change was observed in the status of any neovascularisation present. Among eyes in which stromal infiltration was present initially, 8 showed no significant change and 3 showed total resolution with residual scarring.

Six to 12 months after discontinuation of autologous serum treatment, only 10 of the 14 patients for whom therapy was successful attended for re-examination. All these patients had a stable ocular surface without any sign of recurrence of epithelial defect.

Discussion

There is some evidence that growth factors and proteins found in serum may help the healing of epithelial defects. However, the concentration of biologically active molecules differs in serum and tears. Recent experimental and clinical research into the use of neuropeptides and growth factors has opened up new perspectives on the treatment of PED.^{1-10,12-14,16-22}

When the disease is first diagnosed, treatment should be focused on the specific problem causing PED. While most PEDs heal with conventional management such as eyelid taping, lubricants, discontinuation of toxic antibiotics and antiviral medications, soft bandage contact lenses, or tarsorrhaphy, some remain refractory to standard therapy.^{23,24} Sometimes surgical procedures such as creating a conjunctival flap and lamellar or

Table 3. Probable cause of failure when persistent epithelial defects did not heal in response to treatment.

Patient number	Underlying condition	Probable cause of failure
1	Post-laser-assisted subepithelial keratectomy	Subepithelial fibrosis*
2	Thermal burn	Advanced limbal stem cell deficiency†
9	Advanced vernal keratoconjunctivitis	Some degree of limbal stem cell deficiency and subepithelial fibrosis
10	Thermal burn	Advanced limbal stem cell deficiency
11	Recurrent fungal keratitis on graft	Severe inflammation following recurrence of keratitis
17	Thermal burn	Advanced limbal stem cell deficiency

*Whitish tapered epithelium at the leading edge of the epithelial defect.

† Diagnosis was clinical and based on pallor of limbal palisades.

penetrating keratoplasty become necessary to preserve the anatomic integrity of the globe. More recently, the use of amniotic membrane transplantation and a scleral lens have been reported. When there is corneal stem cell deficiency, limbal stem cell transplantation is the recommended primary treatment.¹⁶

The concentration of the autologous serum in drops used for treating PED has varied among studies. Fox et al used 30% serum diluted with normal saline and all patients improved subjectively and objectively.²⁵ Satisfactory outcomes were also achieved by Tsubota et al,¹⁶ who used 20% serum diluted with saline, and Poon et al,¹² who used 50% serum and included chloramphenicol in the diluent because of their concern about microbial contamination of their serum bottles. No significant complication has been reported as a result of using serum drops, except for 1 case report of immunoglobulin deposition in the cornea as an annular infiltrate (immune ring).²⁶ The autologous serum diluted to 20% with preservative-containing artificial tears used in the present study did not result in any adverse symptoms or signs, except for punctate epithelial keratopathy secondary to preservative toxicity in some patients.

Antibiotics, steroids, and antiherpetic drugs have been reported to suppress epithelial resurfacing.²³ Therefore, discontinuation of these drugs with initiation of autologous serum eyedrops might be expected to affect the results.¹⁷ In the present study, in an attempt to promote epithelial healing, lubricants were discontinued, topical antibiotics were changed to the less toxic chloramphenicol, systemic treatment with steroid (in some cases) and antiherpetic agents (in all cases) were substituted for topical treatment, and the dosage of any remaining topical drops was reduced to a minimum.

The main disadvantage of treatment with serum eyedrops is the inconvenience of preparing and storing autologous serum. Recently, however, Tsubota et al demonstrated that the concentrations of vitamin A, epidermal growth factor, and transforming growth factor- β were maintained for up to 1 month at 5°C and 3 months at -20°C.²⁷

Tsubota et al reported the success of corneal transplantation following autologous serum therapy in patients with no tears, who had end-stage ocular cicatricial pemphigoid or Stevens-Johnson syndrome.²⁷ This supports the suggestion that biologically active tear components can be replaced by using autologous serum. Their study demonstrated that treatment with autologous serum improved symptoms and signs in patients with dry eye. In addition, some patients were freed from the need to use other lubricants.

Tsubota et al have reported the efficacy of autologous serum application for 16 eyes with PED due to various underlying diseases,

which had persisted for at least 2 weeks before treatment (mean, 6.8 months).¹⁶ Healing occurred within 28 days of the initiation of treatment in 62.5% of these patients.

Poon et al used autologous serum to treat 15 eyes with PED that had been present for 48.2 days on average.¹² Nine of these eyes (60%) healed within a mean of 29 days.

Similarly, in the present study, complete healing was observed within 28 days in 70% of the eyes, while subjective improvement was reported by all patients. Healing appeared to be facilitated in eyes with an adequate limbal stem cell population and no subepithelial fibrosis.

Since PED unresponsive to conventional therapy is relatively rare in clinical practice, all related clinical studies, including the present study, have been uncontrolled. The present study revealed that failure of autologous serum therapy was not associated with prior defect of longer than 30 days, PED size larger than 2.5 mm, central location, or the presence of dry eye, inflammation, or neovascularisation. Unexpectedly, older patients (older than 40 years) had a more stable and intact epithelium following treatment, which may be related to the presence of more severe underlying disease in younger patients in this series. All PEDs associated with corneal anaesthesia healed following autologous serum therapy, suggesting that autologous serum can restore neurally secreted growth factors needed for healing.

As was expected, healing occurred more rapidly in smaller PEDs than large PEDs. In addition, eyes with postoperative PED were found to heal more rapidly than other eyes. This suggests that autologous serum therapy should be the method of choice for treating long-standing epithelial defects that arise after PK, pars plana vitrectomy (particularly in patients with diabetes), or photorefractive keratectomy.

All PEDs secondary to thermal burns failed to heal. Apparently, autologous serum could not counteract the advanced stem cell deficiency in these patients. It seems that subepithelial fibrosis at the leading edge of the epithelial defect, which was present in 2 eyes with failure to heal, can form a barrier to epithelial migration. Severe stromal inflammation can also block epithelial healing. Hence, patients with PEDs associated with severe subepithelial infiltration are not good candidates for autologous serum therapy.

Serum itself has bacteriostatic properties because it contains antimicrobial agents such as immunoglobulin and lysozyme, which act in a complementary manner.²⁸ However, the inclusion of a preservative offers additional protection against bacterial contamination. In the present study, topical administration of preservative-containing autologous serum was found to be an effective therapy for PED despite toxic effects of the preservative. It appears that the trophic effects of autologous serum could

overcome this toxicity. Based on long-term follow-up of patients, autologous serum may have a long-lasting effect on ocular surface stability that persists despite discontinuation of treatment.

Corneal PEDs are variable and unpredictable in their response to different therapeutic modalities; some resolve spontaneously, some heal slowly, and some never resurface. Application of autologous serum eyedrops, which contain effective concentrations of many essential components of tears, appears to facilitate the restoration of ocular surface integrity in patients with PED and may be considered as a valuable adjunct in the management of these recalcitrant cases.

The results of the present study, which appears to represent the first study to evaluate the effect of preservative-containing autologous serum on the healing of PED, correlate well with previous reported studies.^{12,16} As autologous serum appears to be effective and safe, this novel therapy may be a preferable alternative to more aggressive treatments generally administered to patients with PED.

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