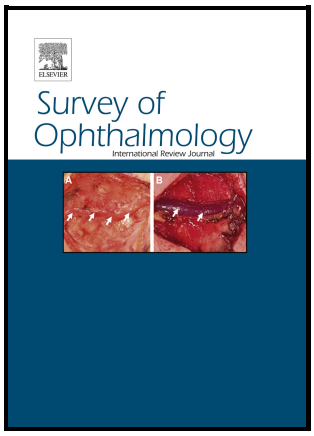
New developments in the management of persistent corneal epithelial defects

Thia Zhang Zhe, Ho Yik To, Kendrick Co Shih, Louis Tong



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Authors: Thia Zhang Zhe,[1,2,6] Ho Yik To,[3] Kendrick Co Shih,[3] Louis Tong[1,4,5,6]

[1] Singapore Eye Research Institute, Singapore

[2] National University Hospital, Singapore

[3] Hong Kong University, Hong Kong

[4] Singapore National Eye Center, Singapore

[5] Duke-NUS Medical School, Singapore

[6] Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Contact for correspondence and reprints:

Louis Tong MD PhD

Senior Consultant

Principal Clinician Scientist

Singapore National Eye Centre

The Academia, 20 College Road, Discovery Tower Level 6, Singapore 169856

Email: louis.tong.h.t@singhealth.com.sg

Tel: +65 6227 7255

Fax: +65 6225 2568

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Abstract

Persistent epithelial defect (PED) is a corneal epithelial defect that failed to heal after 2 weeks. It is a condition that carries much morbidity, and our understanding of PED remains poor, with current treatment methods often having unsatisfactory outcomes. With PED becoming more prevalent, more efforts are required to establish reliable treatment modalities.

Our reviews describe the causes of PED and the different approaches developed to manage them, as well as their associated limitations. Emphasis is placed on understanding various advances in the development of new treatment modalities. We have also described a case a woman with a background of graft-

versus-host disease (GVHD) on long-term topical corticosteroids who developed complicated PED involving both eyes.

The current approach to managing PED generally involves exclusion of an active infection, followed by treatment modalities that aim to encourage corneal epithelial healing. Success rates, however, remain far from desirable, as treatment remains challenging due to multiple underlying etiologies. In summary, advances in the development of new therapies may be able to facilitate progress in the understanding and treatment of PED.

1. Introduction

A persistent epithelial defect (PED) is defined as a corneal epithelial defect that has failed to heal after 2 weeks. This may be related to stem cell deficiency secondary to a variety of ocular diseases. Such defects should be measured precisely and their

progress monitored closely. Upon exclusion of an infective condition, healing of the epithelium should be encouraged with supplementation of biological substances such as plasma products. Neurological recovery should be promoted as well if there is an element of neurotrophic keratopathy involved. We describe the current treatment modalities and the direction of future research in the management of such patients.[99]

Here, we present a complex case of PED requiring multiple different treatment modalities in a patient with prolonged treatment for ocular graft-versus-host disease (GVHD). A Chinese-Burmese woman with a known history of GVHD following a bone marrow transplant in May, 2013, for acute myeloid leukemia who was on long-term topical corticosteroids presented with chronic eye pain and blurring of vision secondary to bilateral se-

vere dry eye disease. She had not experienced any significant injection, discharge, photophobia, diplopia, or pain on eye movement. She also denied any previous chemical injury, trauma, ocular surgery, corneal ulcers, neuropathic keratitis, eyelid procedure or intracranial surgery. Furthermore, she did not have a personal or family history of ophthalmic or systemic conditions such as diabetes mellitus.

Ocular examination revealed a best-corrected visual acuity (BCVA) of 6/24 in both eyes with normal intraocular pressure (IOP) of 17 mmHg and 15 mmHg in the left and right eye, respectively. There were no eyelid abnormalities or abnormal ocular movements in all directions of gaze. The conjunctiva was noted to be white and quiet, and the anterior chambers were assessed to be deep and quiet. Both lenses were clear bilaterally, and examination of the posterior segment – was unremarkable

bilaterally. The significant findings were that of severely reduced tear break-up time (TBUT) of roughly 3 seconds with confluent punctate epithelial erosions (PEE) and progression to corneal defect confirmed with fluorescein staining.

Subsequent development of persistent epithelial defect (PED) in the left eye was initially treated with bandage contact lenses along with levofloxacin eye drops as prophylaxis against infective keratitis. During follow-up, diffuse pigmented keratic precipitates were noted, associated with a severe anterior chamber reaction and hypopyon. Aqueous tap was thus performed and cytomegalovirus (CMV) DNA was detected by polymerase chain reaction (PCR) in the aqueous humor of both eyes, confirming the diagnosis of CMV endotheliitis. This was further complicated by corneal stromal edema and the formation of epi-

thelial bullae due to decompensation of the endothelial cells associated with a loss of pump function. She was started on anti-CMV agents topical ganciclovir 0.15% eye gel and oral valganciclovir tablets.

As a result of the corneal epithelial defect and use of contact lens, as well as the administration of topical corticosteroids, the patient subsequently developed bacterial and fungal keratitis. The bacteria isolated were identified to be *Pseudomonas aeruginosa* and *Corynebacterium spp*, whereas the fungal culture grew yeast. Antimicrobial eye drops comprising of gentamicin, cefazolin, natamycin and vancomycin were added, but failed to achieve complete resolution of the infection. Therefore, therapeutic descemet stripping automated endothelial keratoplasty (DSAEK) and deep anterior lamellar keratoplasty (DALK) were performed. In addition to keratoplasty, amniotic membrane

transplantation (AMT) and tarsorrhaphy were performed at the same time. Unfortunately, after disintegration of the amniotic membrane, the patient's epithelial defect recurred, accompanied by neovascularization of the peripheral cornea. This required ocular surface reconstruction by a buccal mucosal graft after 3 months, with visual rehabilitation placement of a boston keratoprosthesis scheduled.

The right eye developed a PED as well, followed by a similar series of events as the left eye that unfortunately resulted in band keratopathy and scarring over of the corneal defect. The infections, however, did not require corneal grafting.

2. Causes of persistent corneal epithelial defects

Diseases that cause PED are summarized in **Table 1**. Therapy for PEDs should be designed based on the biological mechanisms that impair wound healing. Understanding the 4 major mechanisms that contribute to a PED is therefore important:

2.1. Tear disorders

The tear film is essential in maintaining a healthy ocular surface and promoting wound healing. The presence of inadequate tear film may lead to reduced lubricity and excessive friction between the eyelid and the cornea, causing mechanical and subsequently inflammatory damage. Inadequate tear film can be a result of reduced tear production, increased tear evaporation, or a mixed mechanism.

The tear film supplies oxygen and other essential nutrients while removing metabolic waste and foreign bodies from the avascular

cornea. This is necessary to support the repair and growth of a healthy epithelium. It also protects the cornea by acting as a physical barrier. In particular, the oily layer of the tear film has been shown to reflect hydrophilic invading particles.[23] The mucous layer is able to trap, absorb, and immobilize foreign particles and microbes on the ocular surface, subsequently removing them by a sweeping action of the lid towards the lower fornix.[2] Lubricating action provided by the mucous layer prevents shearing damage to the corneal epithelium during blinking.[45] Apart from physical protection, antimicrobial substances within the tear film such as lysozyme, lactoferrin, lipocalin, secretory IgA, and defensins also help defend the ocular surface from infection.[22,56,83,111,205]

The specific protein factors that play a role in preventing epithelial defects include biologically active growth factors such as epidermal growth factor (EGF), transforming growth factor-beta (TGF-β), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), nerve growth factor (NGF), fibroblast growth factor (FGF), keratinocyte growth factor (KGF), and insulin-like growth factor (IGF).[110] Apart from maintaining corneal homeostasis during normal wear and tear, these protein factors also play important roles in wound healing. Upon injury to the cornea, numerous reactions occur simultaneously that require the integration of cell proliferation, migration, differentiation, apoptosis and intercellular communication to enable rapid healing of the defect and restoration of normal vision.[110] Growth factors and cytokines present in the tear film are the key players of these processes. At the same time, a neuronal lacrimal reflex loop is

triggered immediately following corneal injury to cause the hypersecretion of tears until reepithelialization is complete, which ensures an increase in supply of growth factors for the wound healing process to take place efficiently.[110,132,221]

One particular protein factor that has been extensively studied is EGF, which upon binding to its receptor (EGFR) will result in DNA synthesis essential for corneal epithelial cell proliferation, as well as the synthesis of extracellular matrix (ECM) molecules such as fibronectin and hyaluronic acid.[26] Phosphorylation of EGFR also leads to actin cytoskeletal rearrangement, which enhances cell motility. Together with the presence of ECM molecules such as fibronectin, polarization for directional cell migration in wound healing can be achieved.[26,93,110,134] Studies have shown that topical application of stimulatory growth factors

such as EGF and KGF into tear film help speed up the epithelial wound healing process.[88,149,193]

In certain autoimmune diseases, tear dysfunction can be more severe. For example, Sjo:gren syndrome involves infiltration of inflammatory cells into the lacrimal gland causing reduced reflex tearing, as well as injuring cells responsible for producing stimulatory growth factors such as HGF and EGF.[110] These patients often have significantly decreased tear EGF concentrations.[154,165]

In addition, some tear proteins help defend the ocular surface against oxidative damage. Examples include S100A proteins, lactoferrin as well as enzymes such as superoxide dismutase (SOD), peroxidase, catalase, and mitochondrial oxidative enzymes. Reactive oxygen species (ROS) in the tear and ocular

surface become elevated when there is dry eye. With the ocular surface not being able to protect against the elevated ROS, it ultimately leads to an inflammatory reaction.[188]

2.2. Limbal stem cell deficiency

Limbal epithelial stem cells (LESC) provide a constant supply of proliferating cells necessary for corneal epithelial regeneration throughout a person's lifespan.[69,132,219] During normal corneal homeostasis, they migrate from the limbus to the central cornea, undergo rapid proliferation, and eventually differentiate into central corneal epithelial cells.[4,9,70,124,162,219]

The stem cell niche of the corneal epithelium is found in the corneoscleral junction, the limbus. As such, any disease of the limbal area has the potential to destroy the regenerative ability of

epithelial cells. Recent studies using tagging of molecules expressed predominantly in the limbus demonstrated the migration of individual limbal cells, presumably LESC, to the center of the cornea.[9,69,70] In the event of corneal injury, LESC increases its proliferative rate by about 8- to 9-fold in the limbus and 2-fold in the peripheral and central regions, giving rise to numerous transient amplifying cells with high proliferative and migratory capacities.[41,119,124,132] The indispensable role played by LESC in corneal wound healing is supported by a number of studies.[9,27,30,48,208]

Limbal stem cell deficiency (LSCD), partial or total, may result from genetic or acquired conditions. Examples include autoimmune diseases such as Stevens-Johnson syndrome, ocular cicatricial pemphigoid, and external trauma to the ocular surface by thermal or chemical insults.[195] LSCD has detrimental effects on

the ocular surface [30] such as stromal neovascularization, in-growth of conjunctival cells, eventual corneal opacity and significant visual loss.[16,107]

Limbal transplantation has been proposed as a potential solution to treat PED caused by LSCD, with studies having shown that transplantation of LESC is able to restore wound healing and achieve re-coverage of the entire corneal epithelium.[208] Transplanted limbal cells, especially if the limbal tissue is included, may be able to completely repopulate an area of defect after the central epithelium is removed.[30,36,101] Limbal transplantation will be discussed in greater detail in the later sections.

2.3. Inflammation

In severely dry eyes, exposure of the ocular surface to hyperosmolar tears results in oxidative stress, disruption of DNA, and

cell cycle arrest.[13,226] This activates a signaling cascade that ultimately leads to the increased expression of various pro-inflammatory mediators, such as IL-1β, TNF-α, IL-6, IL-8, adhesion molecules, and matrix metalloproteinases (MMP). The increased level of MMPs not only contribute to extracellular matrix degradation and epithelial loss, but also cleaves the protective anti-oxidative proteins normally present in the tear film.[12,38,47,156,200] Moreover, inflammatory cytokines recruit and activate immune cells to the ocular surface epithelium, including antigen presenting cells and T cells, which further augments the inflammatory response causing more damage. T-helper-1 derived cytokine IFN-γ in particular has been shown to facilitate pathogenic immune cells in promoting the apoptosis and squamous metaplasia of the ocular surface epithelia.[226] Epithelial cell loss resulting from inflammatory activities may compromise the barrier function of the epithelium, leading to corneal oedema and making it

even more difficult for new epithelial cells to grow over the raised, thickened corneal surface.

Inflammation destroys corneal nerves directly via inflammatory mediators and indirectly via mechanical insult due to epitheliopathy.[42,200] Corneal sensation and its neural pathways are important in promoting corneal wound healing and stimulating tear secretion (to be explained in detail in the following section), as such there is a reduced supply of trophic factors and reduced tears, inducing further damage as described above.

2.4. Neurotrophic keratopathy

The corneal epithelium is one of the most densely innervated tissues in the body. Innervation is mainly provided by the nasociliary nerve of the ophthalmic branch (V1) of the trigeminal nerve, with the inferior cornea occasionally innervated by sensory

nerves of the maxillary branch (V2). In addition, there is autonomic innervation from the superior cervical ganglion (sympathetic) and ciliary ganglion (parasympathetic) as well.[145] Corneal nerves are thermal, mechanical, and chemical sensors, important in maintaining the integrity of the epithelial cell layer by modulating the blink response, stimulating the production of tears and releasing neurotrophins for homeostasis, as well as in response to injury.[50,71,132,234]

Neurotrophic keratopathy is a rare degenerative disorder with significant morbidity affecting less than 1.6 per 10,000 people that remains notoriously difficult to manage.[150] A recent definition for neurotrophic keratopathy is “a disease related to alterations in corneal nerves leading to impairment in sensory and trophic function with consequent breakdown of corneal epithe-

lium, affecting the health and integrity of the tear film, epithelium and stroma".[50] Neurotrophic keratopathy can be classified into 3 stages using the Mackie classification scheme, where Stage 1 is characterized by epithelial alterations, Stage 2 involving PED frequently found in the superior half of the cornea, and Stage 3 determined by the presence of corneal ulcers that can potentially progress to perforations or stromal melt.[20]

Studies using animal models have demonstrated abnormal and delayed epithelial wound healing as a consequence of corneal denervation.[8,15,20,220] On the other hand, stimulation of corneal nerve regeneration after nerve damage led to enhanced epithelial wound healing, which further highlights the importance of corneal innervation in the wound healing response.[39] As such, while current treatment options range from lubrication alone to various

medical and surgical modalities, recent advances in therapy are aimed at re-innervating the cornea.[150]

Several theories have been put forward to explain the role of corneal nerves in proper epithelial healing that include the loss of neuronal lacrimal reflex loop resulting in corneal surface dryness,[14] reduced blinking response with impaired corneal sensitivity, altered epithelial cell metabolism increasing its susceptibility to trauma, infection, and loss of trophic factors supplied by corneal nerves.[84,220] Most cases of neurotrophic keratopathy are probably contributed by multiple causative factors in addition to the reduction of sensory innervation, with prior corneal herpetic infection being the most common etiology.[50,150]

The trophic effect of sensory nerves on corneal wound healing is mediated by neuropeptides, mainly substance P (SP) and calcitonin gene-related peptide (CGRP). SP is present in the tear film,[227] with its neurokinin receptors (NK-1) abundantly expressed on native and cultured cornea epithelial cells.[43,102,148] Upon binding of SP to NK-1 receptor, downstream signaling pathways such as EGFR-Akt and Sirt1 are activated,[132,229] which stimulates corneal epithelial cell proliferation,[63,174] migration,[152] and adhesion essential for wound healing to take place.[145–147] CGRP is often found co-localized with SP in most ocular nerve fibers, and is also thought to be important in corneal wound repair, as suggested by studies that have demonstrated an abundant expression of CGRP receptors on the corneal and limbal epithelial cells,[85,207] and an increased CGRP excretion into tears following corneal injury.[142] Nevertheless, the importance of CGRP on the rate of corneal wound healing is still controversial.[145]

While neurons release neuropeptides that support epithelial cell turnover and repair, healthy epithelia could also support corneal nerve function,[145] with corneal epithelial cells secreting soluble factors such as NGF and glial-derived nerve factor (GDNF) that promote neurite growth and survival. NGF in particular has been shown to be able to completely rescue a cornea from non-healing epithelial defects and stromal ulceration leading to impaired corneal innervation.[115,116] Moreover, a decreased availability of NGF results in impaired nerve and cornea functions.[31,44,50,159]

Therefore, conditions leading to impaired corneal innervation, such as herpetic keratitis and diabetes mellitus, deprive the cornea of sufficient neurotrophic support. As a consequence of increased epithelial cell loss, NGF and other trophic factors de-

rived from corneal epithelial cells for neuron growth and survival is reduced, causing further impairment in nerve function, forming a vicious cycle.[50]

2.5. Case discussion

In the case presented, all of the above mechanisms appear to be present. Because of the persistent corneal epitheliopathy over years due to severe dry eye and GVHD, there is a degree of limbal stem cell deficiency. This dry eye will also be associated with inflammation and inflammatory damage to the corneal nerves. The CMV infection causes loss of endothelial function and stromal hydration, resulting in epithelial bullae and some sub-epithelial inflammation. Rupture of the bullae can contribute to the formation of corneal epithelial defect. Chronic use of corticosteroids may also impact poor epithelial healing and possibly play a role in the reactivation of latent CMV in the endothelium,

although it has the beneficial effect of reducing inflammation. After corneal transplantation, the nerve trunks supplying the central cornea would have been cut. The afferent nerves may take a long while to fully reinnervate the cornea graft.

3. Current management and directions

Medical management of PED generally starts with aggressive lubrication in the form of high frequency preservative-free artificial tears or sterile ophthalmic ointments,[99] but in some cases healing cannot occur simply because the eye is too dry. Occasionally, discontinuing topical ophthalmic medications that contain preservatives such as benzalkonium chloride may also be helpful or even curative,[10,15,25] although the decision to stop or change the drug must be considered together with the clinical context. In more advanced or refractory cases of PED, the following therapeutic options may be considered:

3.1. Amniotic membrane transplantation

Amniotic membrane (AM) is the semitransparent innermost layer of the placenta located adjacent to the fetus. It consists of a single layer of metabolically active epithelium attached to a basement membrane and an underlying avascular stroma. Since the mid-to-late 1990s, AM has been successfully used to treat PEDs[11,61,73,112,122] and remains a popular management option till now. Some proposed mechanisms have been put forward to explain how AM is effective in promoting re-epithelialization and healing. These refer to its physical structure and molecular constituents:

The composition of the basement membrane of AM is largely similar to that of the cornea, with all of them sharing the same collagen types, fibronectin and laminin.[60] This allows the AM to

incorporate onto the ocular surface as a “scaffold” which allows corneal epithelial cells to anchor onto and subsequently proliferate.

AM stimulates proliferation and differentiation of the epithelium by producing a number of growth factors, including EGF, HGF and KG.[75,141,209] It is also able to reinforce the adhesion of basal epithelial cells to its underlying basement membrane [100] and suppress epithelial cell apoptosis.[21] In addition, AM is anti-inflammatory by inhibiting the expression of pro-inflammatory cytokines such as IL-1α, IL-2, IL-8, IL-10, interferon-gamma (IFN-γ), FGF and tumor necrosis factor-beta (TNF-β) from the area of defect.[194] The stroma of AM has been found to trap inflammatory cells as well where they eventually undergo apoptosis.

Furthermore, AM is antifibrotic by modulating the function of TGF-β, the key regulator of fibrosis. It suppresses TGF-β signaling, and decreases the expression of TGF-β and its receptor, thus inhibiting the proliferation and differentiation of corneal fibroblasts.[121,210] AM prevents neovascularization by the producing a number of anti-angiogenic substances including thrombospindin-1, endostatin and tissue inhibitors of metalloproteinases (TIMP).[77]

AM serves as an effective physical barrier by protecting the eye against mechanical trauma from lid closure, and preventing surface desiccation by maintaining a physiological moist microenvironment, thus promoting wound healing.[122] It also reduces the entry of pathogens.[89,108] Apart from physical protection, AM confers antimicrobial protection to the ocular surface as well via the various antimicrobial substances present in amniotic fluid,

including bactricidin, beta-lysin, lysozyme, transferrin and 7-S immunoglobulin.[62,76]

Other important advantages that make AM a suitable candidate for treating ocular surface disorders are described below: AM has a largely transparent structure and thus will not affect the recipient's vision. The source of AM, the placenta, is relatively available. Processing and preparation of AM for grafting is easy, and grafting can be readily performed.[176] With the availability of commercially prepared cryopreserved AM, it is now more accessible, convenient, and affordable, while satisfying regulatory requirements. There is virtually no risk of rejection in AMT due to the limited expression of major histocompatibility antigens by AM epithelial and mesenchymal cells,[87] but it usually disintegrates after a week or so.

Though generally safe, there is a small risk of transmitting bacterial, viral, or fungal infections, particularly if microbiological screening of the donor was not adequately performed or if sterile conditions and aseptic techniques were not adopted during any stages of preparation and processing of the AM.[135] Other surgical complications include early graft detachment or dislocation despite rigorous suturing,[169] hemorrhage under the membrane and early disintegration of membrane.[169]

Inter and intradonor variation, including donor age, race, health status, diet, fetal sex, gestational age and specifics of labor, may affect the effectiveness of AM to some degree.[169] There exist many different methods of handling and processing the AM tissue, and studies have shown that the preservation and processing

techniques can have significant effects on the biochemical composition of the membrane, which could directly affect the therapeutic effect of AM.[67,86]

Some cytokines and growth factors contained within the AM may not be beneficial for wound healing.[169,223] For example, AM contains pro-inflammatory prostaglandins, and cytokines such as IL-6 and IL-8. Perhaps the interplay of these substances are more important than their mere presence, and whether they are available in their active form after grafting is still largely understudied.[49]

Clinical outcomes from studies should be interpreted with caution, as the definition of treatment success or failure vary across different studies. Moreover, the underlying pathology giving rise to PED may be intrinsically different among different patients,

and the techniques utilized in preparation and laying down of the graft may also contribute to variations in outcome.[126] Randomized controlled studies is the gold standard for evaluating the efficacy of AMT, but such experiments are still lacking.

A study suggested that AM transplantation may need to be combined with stem cell transplantation and other procedures if the PED is related to severe LSCD. This study conducted over a 12-year timeframe concluded that the effectiveness of AMT was largely dependent on the type of pathological condition being treated. AMT was able to promote re-epithelialization and restore a stable corneal epithelium when performed for PED without major LSCD. However, if there was severe LCSD to begin with, AMT showed less satisfactory results in promoting epithelial regeneration. Nonetheless, AMT was still able to help relieve pain and inflammation in this group of patients, and serves

as the preliminary treatment prior to limbal transplantation surgery.[157]

In another small study of 7 patients with PED, the use of AMT achieved rapid corneal healing and pain reduction in 1 to 2 weeks, as well as significant improvement in visual acuity. However, most cases required further surgery for visual and ocular surface rehabilitation, with 3 patients experiencing epithelial defect recurrence.[28] In an older study that performed AMT for 30 eyes with PED,[126] corneal healing was successfully achieved in 21 eyes (70%) within an average of 25.5 days after surgery, but there was recurrence in 6 eyes (29%) a rather high recurrence rate.

3.2. Bandage soft contact lenses and scleral lenses

The role of contact lenses is to protect the cornea and provide a reservoir of constant lubrication, hence optimizing the ocular surface environment for the healing of PEDs to occur.[90] Bandage soft contact lenses are frequently used early in the treatment of PEDs to aid reepithelialization of the cornea, often as a first line. They protect the advancing, healing epithelial cells from being sloughed off during blinking and provide pain relief.[17,99] They must, however, be used in conjunction with preservative-free artificial tears frequently every 1 to 2 hours to prevent desiccation as well as adhesion of the lens to the ocular surface. Antimicrobial eye drops are necessary to prevent infection, as the same lens may be worn continuously sometimes up to 2 weeks.

Scleral lenses are another type of contact lenses used in the treatment of PEDs and have been gaining popularity in recent

years. They are described as “large-diameter, rigid, gas-permeable devices that are completely support the sclera, that measurably vault the cornea and limbus, and that maintain a fluid reservoir in the space between the posterior surface of the lens and the anterior surface of the cornea”.[185] Due to these unique fitting characteristics, they are able not only to shield the cornea from the eyelid or environmental insults, but also to provide added benefits of continuous hydration and oxygenation.[178] Moreover, by establishing a transparent and smooth optical surface over the irregular, diseased or damaged cornea, the visual acuity of patients are often improved.[74,90,185,197] Several studies have demonstrated the effectiveness of scleral lenses in achieving epithelialization, improving vision and providing comfort in patients with PED.[129,179,185,202]

Nevertheless, scleral lenses are not without limitations. Firstly, challenges in the fitting of lenses to avoid problems such as conjunctival compression, lens seal-off, conjunctival prolapse, limbal bearing, etc. are yet to be overcome, and the rather time-consuming training performed for the patient or caretaker in daily insertion or removal also limits the use of scleral lenses as a short term "bandage lens".[90] Furthermore, the lenses are expensive and nondisposable.

There is an inevitable risk of microbial keratitis, as with any other forms of contact lens use, that has been shown to occur with scleral lens wear.[55,179,185,238] This is particularly important among PED patients as the ocular surface disease in itself is already a risk factor for infection [155] by allowing invading microorganisms to enter via the breached ocular surface.[218] In addi-

tion, most PED patients are receiving oral and/or topical corticosteroids for their underlying conditions,[218] which puts them in an immunosuppressed state. Therefore, careful monitoring via frequent follow-up is essential in reducing the incidence of microbial keratitis.

It is important for patients to be educated on the symptoms of infective keratitis, although they may not notice them if the PEDs are neurotrophic. The use of prophylactic antibiotics – usually topical fourth generation fluoroquinolones and polymyxin-B/trimethoprim – may be useful, but do not entirely prevent bacterial keratitis.[17] The potential benefits of topical prophylactic antibiotics should be balanced against the risk of ocular surface toxicity since certain antibiotics, especially aminoglycosides and higher strength fluoroquinolones (levofloxacin), may delay epithelial wound healing.[6,163,191]

Proper storage and cleaning of the lenses should also be emphasized.[189,238] Patients on overnight wear of scleral lens should be converted to daily wear as soon as possible to avoid wearing the lenses for extended periods.[129,184] Finally, other complications associated with the use of scleral lenses include sterile melts, corneal neovascularization [90] and inflammatory-related responses;[24,218] although these complications may be related to the underlying ocular disease that required lens wear and not the lenses *per se*.

3.3. Recombinant human nerve growth factor eye drops

NGF has become prototypical of a class of proteins – neurotrophins – characterized by their involvement in neuronal protection and growth. The proenzyme, pro-NGF, remains latent following secretion until its activation by cleavage where it can

bind to cell-surface receptors.[96] The neurotrophic effects are mainly mediated via tropomyosin kinase receptor A ($TrkA^{NGFR}$),[97,109] with secondary effects mediated by the p75 neurotrophin receptor ($p75^{NTR}$).[92] Regulation of ocular surface homeostasis is achieved by multiple factors such as corneal epithelial health, limbal stem cell proliferation, immune modulation, and tear production.[118] The potential of NGF as a therapy for various ocular surface disease has been supported by various studies. One such study noted increased corneal NGF expression and decreased epithelial defect closure times among rabbits with iatrogenic corneal epithelial defects following topical NGF administration.[117] Another study documented the effects of topical NGF with docosahexaenoic acid (DHA) in enhancing the regrowth of epithelial and subbasal nerve bundles, as well as corneal wound healing, in albino rabbits after photorefractive

keratectomy.[53] Oral administration of an ergoline derivative, nic-ergoline, has been shown to increase NGF secretions in rat tears and lacrimal glands, therefore enhancing the rate of corneal healing,[105] with Lee and colleagues reporting an 85% rate of complete epithelial defect healing, improved BCVA, increased corneal sensitivity, and increased tear NGF levels among 27 eyes.[123]

A phase I and a subsequent double-masked phase II clinical trial (NGF0212/REPARO phase I/II) in 2018 helped reaffirm the safety of topical recombinant human NGF (rh-NGF) among patients with moderate to severe neurotrophic keratopathy, having demonstrated a significant reduction in epithelial defect healing time as well as reduced recurrence rate compared to the control group.[18,19] As such, Cenegermin, a rh-NGF, represents the first United States Food and Drug Administration approved treatment

modality directed at the underlying pathophysiology of moderate to severe neurotrophic keratopathy.[96] The effectiveness of topical rh-NGF in promoting corneal recovery is further supported by a study conducted by Meduri and coworkers. In this study, Cenegermin was administered 1 drop 6 times a day for a total of 8 weeks, with complete recovery of all corneal defects noted in all 11 patients with moderate and severe neurotrophic keratopathy due to underlying systemic diseases.[139] Although clinical studies have supported the use of topical rh-NGF, as well as its potential benefits in treating other ocular conditions, topical rh-NGF remains not widely accessible due to the high cost of the current formulation, making long-term use being cost-prohibitive.[96]

3.4. Serum-derived eye drops

Serum-derived eye drops for the treatment of PED have become increasingly popular. Apart from lubricating the ocular surface,

they are able to promote epithelial cell proliferation and migration by providing a high concentration of growth factors and cytokines that are normally present in natural tears, including vitamin A, vitamin E, EGF, TGF-β, PDGF, FGF, fibronectin, substance P, IGF, and NGF.[66,91,211,212] The effects of serum-derived eye drops on the survival, proliferation and migration of corneal epithelial cells have been supported by multiple *in vitro* and *in vivo* experimental studies.[5,58,166] Apart from using autologous serum or plasma, similar products can be obtained from the blood of a healthy donor (allogeneic serum) or umbilical cord blood collected at the time of delivery.

Among all the serum-derived eye drops, autologous serum is the oldest and most commonly used. Its efficacy in promoting corneal healing is well-documented in a number of prospective interventional studies,[32,125,137,237] and it has also been shown to be

effective in closing PEDs resistant to conventional therapies;[91,153,166,186,212,235] however, not all patients are suitable candidates for autologous serum. These include individuals suffering from an active infection, systemic diseases such as anemia, hemodynamic instability or having poor venous access.[66,99] In those with blood disorders, GVHD or Sjo:gren syndrome, there may be pro-inflammatory cytokines in the serum which reduced its efficacy.[199,217] Reduced healing properties have also been reported in chronic renal failure and rheumatoid arthritis.[80,95]

Banked allogenic eye drops have the advantage of being prepared and ready for patients' use without delay. One of the sources of banked blood product is the umbilical cord blood serum that is collected during vaginal or caesarean delivery, especially in cord blood banks. Such products may accelerate the healing of PED refractory to medical management at a much

faster rate than autologous serum.[52,214,232] In one study, allogeneic serum eye drops was shown to achieve complete healing in 23 out of 36 patients with PED resistant to conventional treatment;[33] however, another study documented the lack of significant improvement observed in either subjective symptoms or objective signs among 14 PED patients treated with allogeneic serum eye drops.[81] Nevertheless, allogeneic serum eye drops still present as an attractive alternative as it is possible to evaluate the content of cytokines and growth factors, making it possible to preselect or modify eye drops with certain compositions.[66]

Banked cord plasma or serum has additional advantages over blood donated by donors. Umbilical cord blood serum is shown to contain more growth factors compared to other blood-derived eye drops, namely EGF, TGF-β, NGF and VEGF.[217] It is also

bacteriostatic due to the presence of higher levels of antibacterial agents such as IgG, complement and lysozyme, and therefore does not require preservatives which are toxic to the ocular surface.[231] Despite containing a lower amount of vitamin A and IGF-1 compared to peripheral blood serum, such an amount is still greater than that contained in natural tears.[216]

More recently, platelet-derived eye drops, namely platelet-rich plasma, plasma rich in growth factors (PRGF), and platelet lysate, are being introduced at some centers as further alternatives for treating PED. PRGF comprises of autologous platelet protein extracts containing numerous growth factors, with the wound healing effects of these preparations mainly mediated through growth factors and cytokines contained within their alpha granules.[66] Success in the use of PRGF for treating ocular surface disease, including neurotrophic keratopathy, has been confirmed

by Sabater and coworkers.[180] A study by Rao and coworkers found noted improvement in BCVA, decrease in fluorescein staining, increased corneal sensitivity, and increased number, length, width, and density of subepithelial nerves among 11 eyes of 6 patients following autologous plasma treatments.[172] Another study of 28 eyes with PED found that the healing rate of corneal epithelium was significantly faster when treated with platelet-rich plasma compared to autologous serum.[104] Plasma rich in PDGF eye drops and platelet lysate preparations have also been used with success to treat the corneal epitheliopathy in ocular GVHD.[164,215]

One major limitation of serum-derived eye drops is the lack of standardized protocols in their preparation from whole blood. An international survey found that the methods of processing serum eye drops differ largely across centers.[136] The lack of a standardized protocol not only results in variation of the quality

and properties of the final product,[64] but also poses difficulties for routine clinical use. Because serum is not considered a traditional blood product and not administered via blood transfusion, there is not yet a consensus on how to classify this therapeutic product by regulatory bodies.[66,140] Furthermore, the extraction of blood followed by aliquoting or plasmapheresis is rather expensive and involve both time- and resource intensive procedures, hence this may not be the most cost-effective option.

There is also a risk of transmitting blood-borne infectious diseases if banked products are used, although this can be minimized by maintaining strict aseptic conditions throughout processing and performing adequate donor screening. If the products require application 6 to 10 times per day, it would be rather

inconvenient for patients, and the fluctuation in tear environment throughout the day as induced by the drops may not be physiologically ideal for wound healing.[192,223]

3.5. Surgery

Surgical options for patients with PED include tarsorrhaphy, botulinum toxin injection, and conjunctival flaps. These are generally not the first line of treatment. Recent advances in the surgical techniques and understanding of corneal neurotization also provided us with a new approach for addressing nerve loss, potentially serving as a permanent cure to neurotropic keratopathy that has traditionally been managed conservatively with limited success.[130]

Tarsorrhaphy, temporary or permanent, is a less commonly implemented, but highly effective, method in the management of

PED. It decreases tear evaporation by reducing palpebral fissure width [98] and minimizes the traumatic effect of lid opening and closure over the healing corneal epithelium.[40] The healing of corneal epithelium is thus optimized in such a favourable environment.

Most patients, however, are only willing to consider tarsorrhaphy after failing all conservative measures for cosmetic reasons. Tarsorrhaphy can be combined with other procedures such as AMT. Even a partial tarsorrhaphy will reduce the visual field of that eye, therefore tarsorrhaphy can only be recommended for one eye at a time. Moreover, this procedure poses difficulties to viewing the cornea during eye examinations, and patients may also find it difficult to administer eye drops.[51]

Potential complications include premature opening, pyogenic granuloma of eyelid, keloid formation,[40] focal cellulitis, cheese wiring of sutures and skin breakdown.[34] In rare instances, persistent adhesion between upper and lower lids may remain after attempted reversal of tarsorrhaphy. Other complications that have been reported include permanently scarred lid margins, cicatricial entropion, trichiasis, and distichiasis, but these may be due to the underlying ocular disease.[51] In addition, eye movements may generate friction between the cornea and tarsal conjunctival surfaces, particularly during sleep [68] and in neurotrophic corneas where there is a tear-deficient state.[84] Any active infections should be excluded prior to tarsorrhaphy.

An alternative to tarsorrhaphy is the injection of botulinum toxin (Botox®) into the levator palpebrae muscle to induce chemically a temporary complete ptosis.[99] Corneal protection is achieved

through similar mechanisms as tarsorrhaphy. Complete ptosis usually is achieved 3 days to 12 days post-injection, with the effects lasting from 6.5 weeks to 12 weeks.[51,98] Compared to tarsorrhaphy requiring surgical suturing of the lids, the Botox-injected upper lid can be easily lifted up using a cotton swab, hence permitting frequent ocular examinations and convenient application of topical drops.[98] Complications reported include preseptal haemorrhage,[120] superior rectus underaction and diplopia, although decreasing the depth of injection may help to prevent underaction of the superior rectus.[98] Despite being more expensive, this procedure is considered safer than surgical tarsorrhaphy with fewer side effects and may perhaps be a more acceptable option from the patients' point of view.

In more severe cases of PED, a conjunctival flap (CF) may be placed over the cornea to provide a stable ocular surface for

wound repair to take place.[204,223] Gundersen CF are commonly performed amongst the different types of CF, Gundersen CF involves a 360 degrees peritomy and mobilization of the conjunctiva at the fornix to cover the entire corneal surface.[190] CF promote wound healing via the following 2 mechanisms. First, CF are richly vascularized, thus supplying nutrients and growth factors to the corneal surface essential for wound healing, as well as suppressing inflammation by reducing pro-inflammatory mediators and proteases in the microenvironment. Second, its lymphatics can increase the resistance to infection.[190] Moreover, such grafts relieve pain, and subsequently reduces the need for topical anti-inflammatory eye drops.[37] Nevertheless, iatrogenic LCSD may occur during the peritomy and mobilisation of conjunctiva, and long-standing CF may result in corneal opacification and vascularization,[190] thus more ocular surface damage. Furthermore, as a Gundersen CF covers the entire surface of the

cornea, vision will be further compromised and monitoring of the disease progression may be difficult.

Corneal neurotization is a promising surgical procedure that aims to re-innervate the pathological cornea via the use of a healthy donor nerve graft transplanted to the perilimbal region, thus directing the growth and repair of the cornea.[173] The procedure was first described in 1972 involving the use of a sural nerve as an interpositional graft between the greater occipital nerve and the transected ophthalmic nerve, but was not readily incorporated into routine clinical practice due to its complexity and prolonged duration.[130,182,183] Terzis and coworkers.[203] reported the first successful direct corneal neurotization in 2009 and, over the years, refinements made to the surgical technique have led to promising results in restoring the corneal function,

making it a potentially effective treatment modality for consideration among suitable candidates with neurotropic keratopathy. Corneal neurotization can either be performed via a direct approach involving the direct transfer of donor nerve to the corneoscleral limbus, or an indirect approach utilizing an intermediary nerve graft connecting the donor sensory nerve to the diseased cornea.[173] Successful reinnervation of the cornea is confirmed with the use of confocal microscopy, and is estimated to take around 6 months or longer.[150] While both approaches reported high rates of recovery in corneal defects, Rathi and coworkers noted reduced surgical morbidity among cases where the indirect approach involving sural nerve graft was employed.[173]

4. Preclinical development of new therapies

Recently, there were reviews concerning some treatment modalities such as the use of stem cells or peptide therapies.[223] We have

reviewed some of the important research studies published and indexed in the NCBI Pubmed database over the last 10 years.

4.1. Cultivated epithelial cells

The transplantation of cultivated epithelial cells, more specifically limbal epithelial stem cells, has now become the main definitive treatment for patients suffering from PED caused by LSCD. A common technique called cultivated limbal epithelial transplantation (CLET) involves the expansion of epithelial cells from limbal explants onto a human amniotic membrane. After a few days, a confluent sheet of epithelium will spread across the membrane, which can be used for transplantation.[170]

The outcomes of ocular surface reconstruction using CLET is highly successful in patients with LSCD resulting in PED. It has

been reported that these patients showed significant improvement in their corneal condition 1 to 2 years after CLET.[54,171] Nevertheless, certain factors that may compromise the success of CLET have been reported. In cases with not only PED but also fibrovascular pannus, dissection may result in inadvertent microperforation. Bleeding under the graft, post-operative inflammation and the use of chronic corticosteroids are also complications that prolong epithelial healing and delay successful outcome.[54]

In recent years, research on CLET mainly focuses on the optimization of the method and evaluation of the factors that determine clinical success. A study investigated the cellular profile of the cultured epithelium in different carriers and culture media. Human amniotic membrane (HAM) was compared with tissue culture coated plastic (PL), and human serum (HS) media were

compared with more complex medium (COM). Epithelial cells showed more well-developed adhesion complexes when cultivated on HAM compared to PL. This suggests that HAM is superior to PL, as cultivation in HAM is more effective with the subsequent graft being more stable. Gene expression in the growing epithelial cells was affected by the culture media: HS and COM. The molecules matrix metalloproteinase (MMP)13 and NADPH oxidase (NOX)4 were found to be significantly up-regulated in cells cultured in HS compared to COM. MMP13 is important as it degrades collagen in the matrix during the process of wound healing, whereas NOX4 catalyses the conversion of oxygen molecules to reactive oxygen species (ROS), thus contributing to the oxidative stress pathways. However, clinical outcomes may not be associated with gene expression patterns. Further studies need to be done to understand the significance of these findings.[161]

For some patients, allogeneic stem cell transplantation using cadaveric limbal tissue may be the only option as they may not have a human leukocyte antigen-matching relative and no healthy autologous tissue. Excessive delay between harvesting the limbal tissue and epithelial expansion may affect the epithelial growth. Prior to the cell culture, storage of the tissue in organ culture media may not be optimal in sustaining the regenerative capacity of living cells. Prolonged epithelial cultures are also not desirable as the cells may have undergone a longer lag phase before proliferation. Interestingly, the age of the donor was not correlated to the subsequent epithelial outgrowth. This is a promising finding as cadaveric limbal tissue transplantation can be used where autologous grafts are not feasible.

Cultivated oral mucosal epithelial transplantation (COMET), a newer method of transplantation, has shown very promising results.[201] The oral mucosal epithelium is ideal as cells from the oral mucosa are relatively un-differentiated and do not keratinise quickly, allowing easy cultivation.[82] Moreover, they express differentiation markers like the K3 protein which is an indicator of corneal epithelial differentiation.[103,133]

In a clinical trial of LSCD resulting from chemical or thermal injury, COMET has been associated with 75% success rates in restoring a stable ocular surface, although not all of the severe cases had PED. In this study, the oral mucosal epithelial cells were cultivated on a human amniotic membrane, and medium supplemented by HS, corticosteroids, antibiotics and growth factors. The resulting cell sheet was then transplanted onto the cornea and bare sclera 3 to 4 mm from the limbus. In cases of PED

where there were coexisting adhesions between eyelid and conjunctiva or symblepharon, further dissection was necessary. In such cases, the transplanted cells sheet has to be large enough for further extension toward the fornix. A successful outcome in this study was defined as complete healing of the epithelium without corneal vascularization, fibrovascular tissue invasion over the pupillary zone, symblepharon, and at most little conjunctival inflammation. This was achieved with postoperative corticosteroids and antibiotics.[167]

The efficacy of COMET in more challenging contexts has also been investigated. The COMETs have been evaluated in rabbit recipients where the oral mucosa was obtained from patients with Stevens-Johnson syndrome (SJS) or human controls. 5 days after transplantation, the animals were sacrificed and the proliferation of donor cells was evaluated by immunohistochemical

staining. The cells from SJS or controls showed similar proliferation, as well as rates of re-epithelialization.[106] However, the sample size for this study is low, and a larger study will be required to support these findings.[106]

There was decreased secretion of EGF in SJS patients as compared to non-SJS patients, possibly due to severe inflammation in the eye that resulted in a reduced ability of the mucosal epithelial cells to secrete EGFs. As EGFs play a significant role in wound healing,[78] administration of EGFs can better facilitate the reconstruction of the cornea when using COMET with SJS donors. For treatment of aniridia, an inherited cause of LSCD, COMET has shown promise in the treatment of PED. Compared to pre-transplantation corneas, 76.4% of the 17 eyes displayed a more transparent, regular epithelium and 88.2% of the 13 patients had improved visual acuity.[46]

4.2. Cell replacement based on differentiation of stem cells

The principles of multipotent stem cell differentiation can be exploited in the treatment of PEDs. The lack of cell replacement in LSCD is potentially addressed effectively by multipotent stem cells after they are committed to the corneal epithelial cell lineage. Regenerative therapy involving mesenchymal stem cells (MSCs), often derived from the bone marrow, appears to be the most promising form of such replacement.[233] In addition to their tissue regenerative capabilities, MSCs have immunosuppressive properties. This is particularly relevant for the cornea as its structural integrity depends on the absence of inflammatory response.[236]

A study induced the differentiation of human bone marrow-derived MSCs into the corneal lineage by culturing them in a special limbal medium for 10 days. Eventually, the expanded cells expressed various biomarkers characteristic of corneal stem cells such as beta1-integrin, ABCG2, and p63. The cells were then transferred onto HAM carrier and cultured. Transplantation of the cells to a LSCD animal model was subsequently performed and slit lamp examinations were done after 8 weeks. The ocular surface was significantly improved with reduced vascularization and opacity.[177]

A different study used an alkali injury-induced rabbit model and treatment was administered in the form of intrastromal and subconjunctival injections containing adipose-derived MSCs or only phosphate buffer solutions (PBS). The use of MSCs in this study reduced angiogenesis and increased wound healing. The

study also reported reduced levels of vascular endothelial growth factor (VEGF) and serum glutamic-pyruvic transaminase (SGPT) in the cornea. Reduced VEGF expression would thus explain the reduced angiogenesis, but the significance of corneal SGPT levels remain unknown.[7]

Umbilical cord-derived stem cells are another type of stem cell potentially effective in the treatment of PEDs.[128] The fact that they are less immunogenic, nontumorigenic, highly proliferative, and widely available makes them a suitable source of stem cell for transplantation.[181] A recent study used such cells cultured and differentiated on HAM, and eventually transplanted onto a LSCD deficient rabbit model. These transplanted mucin-secreting epithelial cells were able to re-epithelialize the ocular surface successfully with minimal neovascularization, inflammation and graft rejection.[175] The bioengineered cell sheet that

was transplanted onto the ocular surface also showed phenotypic characteristics of a normal cornea epithelial surface. There was no expression of CK4 and CK19, while there was strong expression of CK3 and CK12, which are all phenotypic indicators of a healthy corneal surface.[29,113]

4.3. Tissue engineering to make artificial scaffolds

Another treatment option to encourage the healing of serious corneal defects would be through the use of tissue-engineered scaffolds that can be sutured onto eyes with PEDs. The various forms of cell expansion reported in COMET produce single layers of cells without a stromal support. It is highly likely that having a transplanted stromal environment or support enhances the survival of transplanted stem cells, as stem cell survival may depend on communication with surrounding niche.[65] Tissue-engineered scaffolds are highly customizable. An optimal cornea

substitute is made of one or more layers of specific polymers that are organized in fibers with particular orientation and thicknesses. Various studies mentioned below have reported the use and properties of different materials and matrices for the treatment of PEDs.

The corneal ECM is composed of a meshwork of fibrous macromolecules and non-fibrous molecules like proteoglycans and other soluble factors.[57,138] It is essential for polymers to be cross-linked and provide an environment for residing cells to be viable, thus allowing the cells to produce more ECM. In the context of the cornea, the stromal layer ECM is composed mainly of collagen I, with some presence of collagen V and proteoglycans.[151]

Electrospinning is a process where a liquefied polymer becomes charged upon application of an electrical field, migrates to the

oppositely-charged electrode, and deforms to become long continuous nano-fibers.[3] This process is often used during tissue engineering in the ocular surface. Since collagen is naturally occurring, it is not surprising that many tissue-engineered constructs are based on collagen. In one study, the stroma layer of the cornea is constituted by N-ethyl-N-(3-dimethyl aminopropyl)carbodiimide/N-hydroxysuccinimide (EDC/NHS)-cross-linked collagen-chondroitin sulfate foam, seeded with human keratocyte (HK) cells. To simulate the Bowman layer of the cornea, collagen type I is crosslinked with fibrin and electrospun, as an overlay on top of the stroma.[1] When recovering from corneal wounds, proper restoration of the Bowman layer is very important in maintaining transparency.[114] In the absence of cross-linkages, collagen would be susceptible to degradation and this would compromise the mechanical integrity of the reconstructed Bowman layer.[158] Epithelial cells were then grown on top of the

artificial ‘Bowman layer’. In this study, retinal pigment epithelial (RPE) cells were used to serve as the epithelial layer instead of corneal epithelial cells (HCEp). The reason for this was unclear but may possibly be attributed to previous success or efficiency of RPE cultures in their laboratory.[1]

In the system described above, a high level of ECM secretion has been observed, suggesting that the scaffold was structurally stable and may facilitate ocular surface reconstruction when transplanted on corneas with PED. The findings also suggest that the fibrous mat was behaving like a functional ‘Bowman layer’. Since this study was conducted *in vitro*, it is unclear whether rejection of the artificial construct may occur after transplantation in an animal or human.[1] The properties of the reconstituted stroma have been further investigated in another study. This construct is superior to a simple collagen film in

terms of moisture retention and stability, and may be beneficial in recipients with dry eye or those predisposed to dry eye.[131]

Apart from collagen, other synthetic polymers can be used in corneal replacements. Recently, polycaprolactone (PCL) combined with either poly glycerol sebacate (PGS) or chitosan (CHI) have been suggested for this purpose. The nano-fibers used in this study have widths ranging from 108 nm to 536 nm, depending on the type of polymers. Combination of polymers was achieved via electrospinning. The study showed that both PCL-PGS and PCL-CHI matrices were able to facilitate the adherence and proliferation of the seeded HCEp and HK cells. The metabolic activity and organisation of the cells were studied via colorimetric cell counting kit-8 assay and phalloidin staining. By day 11 after seeding, both PGL-PGS and PCL-CHI matrices were observed to have similarly confluent cell sheets, with

proper orientation of F-actin.[196] The group also evaluated the effect of fibers with random orientation compared to an aligned orientation. Cells showed similar growth in either of these conditions, although the random orientation of fibers was associated with slightly better growth of cells. There may be increased pores sizes between the randomly-oriented compared to aligned fibers, and the increased space favored infiltrating cells. Overall, the study suggested that the synthesized fibers have very similar properties to the natural corneal ECM;[196] however, for firm conclusions, *in vivo* studies should be conducted.

An *in vivo* study investigated the use of poly(ethylene-glycol) (PEG) modified- silk fibroin (SF) membrane as a carrier for limbal epithelial stem cells (LESCs). In this technique, LESCs were obtained from the rabbit limbus and together with a carrier, transplanted to a rabbit with LSCD.[127] SF is a fibrous protein

mixture secreted by insects and spiders, belonging to the classes *Insecta* and *Arachnida* respectively. The use of SF is desirable as it is widely available at a low cost and it facilitates corneal cell proliferation.[79] After 4 months, all the LESC/SF graft groups had confirmed re-epithelialization. At all post-operative evaluation times (20, 30, or 60 days), the LESC/SF graft groups had significantly less corneal neovascularization and clearer cornea compared to the control SF-only group, proving that LESCs were responsible for the beneficial effect of the procedure.[127]

Nevertheless, it is worth pointing out that the use of SF has certain disadvantages. SF tends to exist as beta-sheets, which are hydrophobic and very stable. As a result of the stability, there may be more foreign body reaction after a prolonged period.

The inflammatory damage associated with a foreign body reaction may prolong the ocular surface recovery and even worsen the PED.[127]

While all the above biopolymers show promise, naturally derived components of the cornea may still be preferred in theory as these may be more biocompatible. A group of investigators constructed a cornea stromal equivalent with small-incision lenticule extraction (SMILE)-derived lenticules in conjunction with fibrin glue. To have a reasonably thick construct, two SMILE lenticules could be adhered together via the fibrin glue, with the superior aspect facing upwards. Epithelial cells were seeded on the construct and subsequent evaluation showed the formation of a continuous epithelium, consisting of a basal layer of cuboid-shaped cells and 3 to 4 suprabasal layers of elongated cells. This

multilayer structure of the epithelium is similar to that of the native human cornea.[230]

For transplantation of corneal lenticules *in vivo*, they must first be decellularized (removal of any donor cells like keratocytes) before implantation through an epithelial wound. In an experiment involving rabbits, within 2 weeks after transplantation there was corneal reepithelialization from the surrounding recipient cells and the grafts were noted to have similar degrees of transparency compared to the neighboring cornea. None of the rabbits developed corneal neovascularization, graft degradation nor graft rejection over 3 months. The use of lenticules for medical purposes is a good idea because these are otherwise likely discarded after SMILE. Given that SMILE is increasingly popular for the treatment of myopia,[228] we will expect lenticules to become more readily available; however, it remains unclear if

such a strategy will be effective for a recipient with significant corneal inflammation and disease rather than just a mechanical epithelial wound such as in the experiments involving rabbits. Furthermore, in human disease where the recipient has existing LSCD, it is questionable if sufficient cells will migrate over the lenticules.[230]

4.4. Modifying matrix factors

Proteoglycans found in the ECM may play an active role in corneal epithelial wound healing. In most cases of PED and corneal injury, the level of proteoglycans is abnormal, thus delaying wound healing. Matrix factors such as proteoglycans should therefore be included in other forms of treatment such as artificial scaffolds and cell replacement, as this should potentially accelerate wound healing by enhancing the ECM environment for cell migration and adhesion.

Vitronectin (VN) is one of the major cell adhesion proteins present in the ECM. A study showed that instillation of VN onto chemically and mechanically-wounded rabbit eyes hastened corneal epithelial wound healing.[94] Another group investigated the effect of VN *in vivo* in human cadavers. As contact lenses are able to absorb and release VN, they are used as a carrier for VN.[206] The contact lens soaked in VN was placed over debrided human cadaver corneas for 7 days before histological analysis and cell counting were performed. The number of cells present across the Bowman layer was higher in the VN group compared to control contact lens. In addition, the VN-containing lenses also produced a more continuous epithelial layer.[35]

Another type of matrix factor protein that has been investigated is galectin-3. Galectins are a family of proteins that bind to β-galactoside sugars. Galectin-3 is expressed in the cornea and has been shown to enhance the deposition of collagen IV in the basement membrane, thus accelerating corneal wound healing in rats.[225] In a more recent study, the effect of galectin-3 on monkey corneas has been investigated. Chemical injuries were induced in monkey corneas by placing a 7.5 mm diameter filter paper disk soaked in alkali sodium hydroxide over the central cornea for 60 seconds. The corneas were then excised and incubated. After a day, the wounds were observed under fluorescence, and microscopy showed that, upon the administration of 20 μg of galectin-3, there was more rapid wound closure compared to the untreated controls.[59]

The same study also isolated monkey corneal epithelial cells and seeded them into wells containing collagen I, IV, V, human fibronectin, human laminin-5 or human recombinant factors such as integrin α1β1, α3β1, or α5β1. These polymers and ligands are components of the ECM, and the differential adherence of cells to these culture wells with or without galectin-3 pretreatment was observed. After 19 hours, the non-adherent cells were washed away and the adherent cells were counted. Galectin-3 treatment was shown to produce higher corneal epithelial cell adherence to the wells compared to controls, suggesting that ECM components may facilitate cell adherence, and indirectly cell migration, during actual wound healing.[228] Galectin-3 may have therapeutic significance in the treatment of PED, however it only contributed 6% to 7% of the epithelial healing, and re-epithelialization mostly came from the migration and proliferation

of healthy epithelial cells. Furthermore, in cases of PED with ocular surface inflammation and poor healing such as dry eye, there was already increased expression of galectin-3 in the tears, and the benefit of further supplementation is unclear.[213]

4.5. Supplying growth factors and other peptides

As mentioned above, growth factors are a family of hormones and proteins that promote cell growth, proliferation and motility, which are critical steps in wound healing. The corneal epithelial layer not only plays a role for achieving physical coverage over the wounded area, but is also a major source of growth factors in the tear. Epithelial damage is very common in corneal injuries, and without sufficient growth factors to drive wound repair, delayed wound healing is likely as a result. Instillation of growth factors is therefore a potential strategy in the therapy of PED.

HEGF, secreted by platelets, fibroblasts and macrophages, is an example of a critical growth factor in wound healing. It facilitates wound healing in corneal PEDs, even in diabetes mellitus.[72,160] A study investigated the effectiveness of recombinant human epithelial growth factors (rHEGF) compared to protein-free calf blood extract (PFCBE) in wounded rabbit corneas. This form of treatment utilizing PFCBE is frequently practiced in China.[168] PFCBE contains free amino acids, small molecules and oligoes, and it has been reported to restore defective collagen to reduce scarring and hasten wound closure.[168] PFCBE and rHEGF were instilled 4 times a day for 21 days after injury was inflicted. The rHEGF group had the highest rate of epithelial healing, followed by the control (saline) and PFCBE groups. After 3 days, the rHEGF-treated rabbits had completely healed corneas; however, neovascularization increased for all 3 groups at 12 days and was similar between groups, suggesting that rHEGF

and PFCBE did not have effects on neovascularization. Nevertheless, this study showed that rHEGF was preferred to PFCBE for the treatment of PED.[224] As tissue wound healing is also delayed in diabetes mellitus, rHEGF may be particularly useful in these patients.[187]

HGF, as mentioned earlier, is another important growth factor expressed in cornea epithelial cells, endothelial cells and keratocytes. HGF is a mitogen that binds to a HGF receptor coded by the c-Met proto-oncogene, and like most other growth factors, it plays a role in cell proliferation, motility and even morphogenesis.[222] In one study, a mechanical wound was inflicted on C57BL/6 rat corneas before application of HGF or mouse serum albumin (MSA) twice a day for 7 days. The corneal epithelial defect completely healed after 5 days of HGF treatment but remained unhealed after 7 days with MSA. Some corneas were

harvested at day 3 and evaluated for the panleukocyte marker CD45 via immunostaining. Compared to MSA-treated eyes, HGF treatment decreased infiltration of CD45+ cells into the injured corneas. The expression of proinflammatory molecules interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) was also quantified via RT-PCR and found to be significantly lowered compared to MSA. Investigation on whether HGF treatment can enhance HCEp proliferation *in vitro* was performed in the same study as well. HCEps were cultured in different environments: basal keratinocyte serum-free medium (K-SFM), K-SFM with supplement (Invitrogen), and K-SFM/supplement with HGF treatment. Using the bromodeoxyuridine (BrdU) incorporation assay, it was found that with K-SFM supplement, there were increased HCEp proliferation, which was further enhanced upon HGF treatment.[143]

To study the effect of HCEp proliferation in inflammatory conditions, HCEp was cultured in K-SFM/supplement, with or without IL-1β. While IL-1β decreased HCEp cell proliferation, HGF treatment mitigated the anti-proliferative effect of IL-1β, resulting in cell expansion instead. The effect of HGF on the activation and function of CD11b+ cells was also studied. Murine CD11b+ cells were isolated from the spleen and cultured in IL-1β for stimulation, with or without HGF. The activation of CD11b+ cells was measured by their expression of MHC class II, via flow cytometry, and their expression of IL-1β and TNF-α, via RT-PCR. HGF treatment significantly decreased MHC-II, IL-1β and TNF-α expression. The findings showed that the activation and function of proinflammatory CD11b+ cells can be suppressed by HGF. This is unsurprising as it was shown in the study that HGF is capable of suppressing the effects of IL-1β *in*

vitro. Prolonged inflammation is a major concern in wound healing. In cases of PED where ocular surface inflammation is persistent, by stopping immune cell infiltration and cytokine activity, it can potentially lead to a positive effect on wound repair.[198] Other protein factors relevant in corneal disease include Keratinocyte growth factor-2 (KGF-2) and the fibronectin-derived peptide PHSRN. KGF-2 is secreted by fibroblasts and vascular endothelial cells, and promotes epithelial cell growth and differentiation. These effects on epithelial wound repair are well understood in rabbit models. In addition, a study has reported the efficacy of PHSRN in corneal wound healing.[144]

5. Conclusion

Current management of PED generally starts with the exclusion of an active infection, followed by aggressive lubrication and other methods to encourage healing of the corneal epithelium:

AMT, bandage lenses, scleral lenses, serum-derived eye drops and surgery. Such options may be successful to varying degrees; therefore, PED remains a challenge to eradicate as it is often contributed to by multiple underlying etiologies. The use of steroids in these patients should be balanced between the need to reduce inflammation and the risk of infection and of steroid-induced corneal melting. New therapies under development may be able to provide a more promising solution to these patients.

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Method of literature search

For the purpose of this research, a search was conducted using PubMed for studies published over the last 10 years that looked into the pathophysiology and causes of PED, as well as the present and novel therapeutic modalities. The following term: “persistent epithelial defect” and any of these terms: (“corneal dystrophy”, “ocular surface disease”, “dry eye”, “neurotrophic keratopathy”, “corneal wound healing”, “amniotic membrane transplantation”, scleral contact lens”, “bandage contact lens”, “limbal stem cell”, “serum-based therapy”, “tarsorrhaphy”, “botulinum toxin injection”, “conjunctival flap”, “corneal replacement”, “keratoplasty”, “matrix factor”, “growth factor”, “nerve growth factor”, “protein factor”, “inflammatory molecule”) were used to search for potential articles. Some of the articles published prior to the past decade are selectively included as well if

they contained additional relevant information such as the treatment modalities or the techniques involved. The articles found using this approach were manually curated to include clinical studies, including both animal and *in-vitro* studies.

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Table 1. Summary of some causes of persistent corneal epithelial defects

Type of disease	**Disease causing persistent corneal epithelial defects**
Iatrogenic	Laser or incisional ocular surgeries causing damage to corneal nerves: - Laser-assisted in-situ keratomileusis (LASIK) - Cataract surgery - Penetrating or lamellar keratoplasty Neurosurgeries causing damage to trigeminal ganglion
Injury	Exogenous injury: - Chemical or thermal burns - Ultraviolet light injury - Exposure keratopathy - Prolonged overuse of contact lenses External agents - Viral infection (most commonly herpetic keratitis) - Drug-induced e.g. Stevens Johnson syndrome
Autoimmune	Sjögren syndrome Ocular cicatricial pemphigoid (OCP) Ectodermal dysplasia
Allergic	Vernal keratoconjunctivitis Atopic keratoconjunctivitis
Other conditions	Vitamin A deficiency Severe dry eye disease Corneal dystrophies

Table 2. Summary of treatment modalities for persistent corneal epithelial defects

Treatment modality	
Conservative	Eye drops: - Lubricating eye drops - Recombinant human nerve growth factor eye drops - Serum-derived eye drops Therapeutic contact lenses: - Bandage contact lens - Scleral lens
Surgical	Amniotic membrane transplant Tarsorrhaphy Botulinum toxin injection Conjuctival flap Corneal neurotization
Novel treatment modalities under development	Cultivated epithelial cell transplant - Cultivated limbal epithelial transplant - Cultivated oral mucosal epithelial transplant Differentiated stem cell transplant - Mesenchymal stem cells - Umbilical cord-derived stem cells Tissue-engineered scaffolds Instillation of growth factors and other peptides

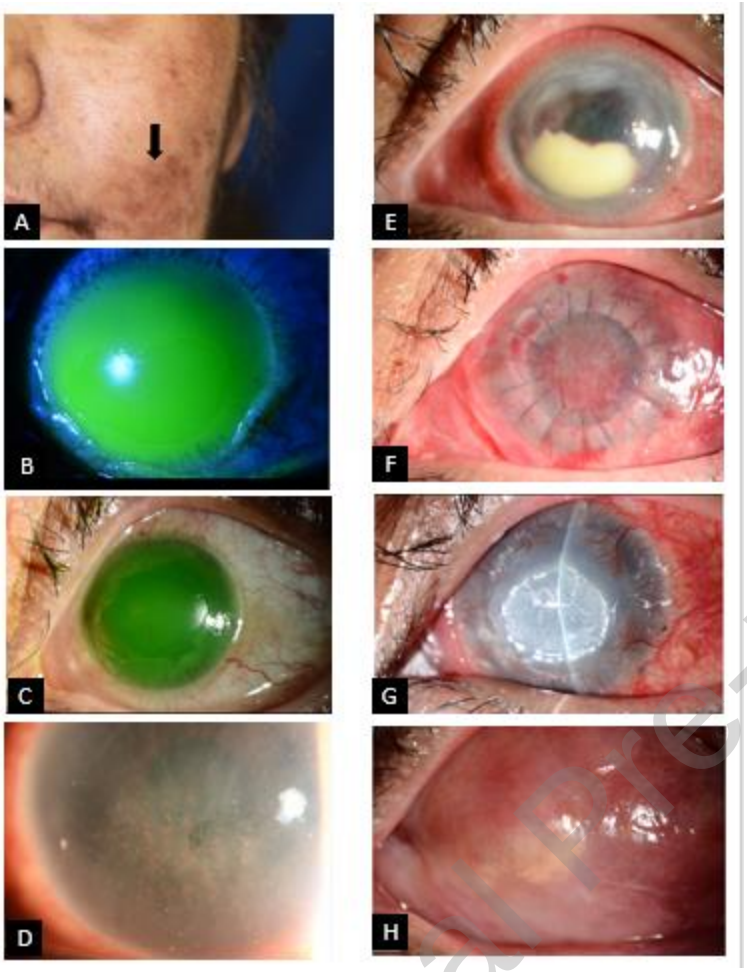

Figure 1. A Chinese-Burmese lady with chronic graft-versus-host disease presents with bilateral severe dry eye and skin papules (A). This developed into a right large inferior corneal defect and swelling of the corneal stroma (B, C). There were corneal keratic precipitates (D), later confirmed to be due to cytomegaloviral endotheliitis. A severe anterior chamber reaction ensued (E), forming a hypopyon. A Deep Anterior Lamellar Keratoplasty (DALK) followed by amniotic membrane transplant was performed (F). There was recurrence of the corneal epithelial defect after dissolution of the amniotic membrane, with increased peripheral corneal vascularization (G). A buccal mucosal graft was placed over the cornea (H).

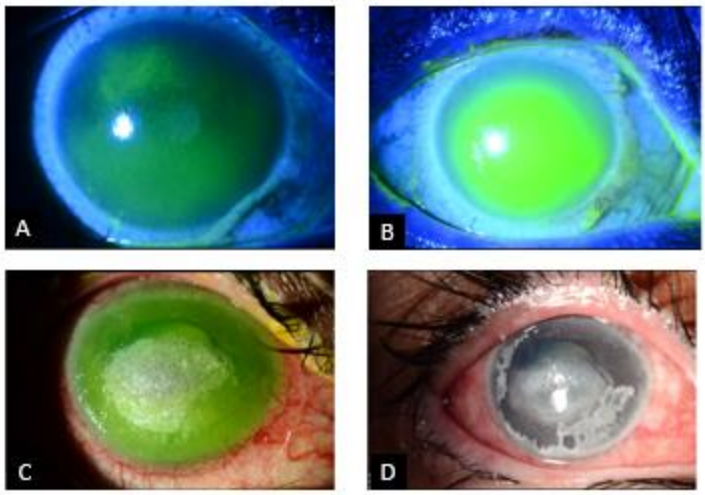

Figure 2. The right eye of the same patient in Figure 1 presented with severe corneal staining (A). This also broke down to a persistent corneal epithelial defect (B), which later calcified (C) to form a band keratopathy like appearance (D) with some scarring over the area of the epithelial defect.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: