A Systematic Review of Emerging Therapeutic Strategies in the Management of Chemical Injuries of the Ocular Surface

Running short title:

Emerging Therapies for Ocular Surface Injuries

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Abstract

Objectives: To evaluate recent in vivo studies on emerging therapies for managing corneal epithelial injuries.

Methods: The search was conducted on PubMed for articles published between January 2015 and September 2019 and in English language.

Results: 30 studies were identified for evaluation, including those on mesenchymal stem cells, amniotic membrane-derived therapies, endogenous peptides and their inhibitors, as well as hydrogel therapies. Intermediate to strong levels of evidence are presented regarding the use of these strategies on chemically injured cornea, including their effects on healing of corneal epithelial defect, anti-inflammatory properties, prevention of corneal neovascularisation, as well as restoration of anatomy and functions of the anterior eye, although clinical trials are needed to determine the safety and efficacy of these strategies on humans.

Conclusion: Recent advances and understanding in various novel therapeutic methods for corneal epithelial chemical injuries should provide potential alternatives to current standard treatment regimens and help reduce risks of complications, hence improve patient outcomes.

Keywords: cornea, eye, epithelium, management, therapy, vision, wound healing

Introduction

Prevalence of corneal wound injury

The cornea is the most anterior structure of the human eye, serving importantly as both immune and mechanical barriers, a refractor of light for clear vision, as well as a carrier of nutrients and oxygen from tears to deeper structures of the eye [1]. The epithelial layer forms the outermost part of the cornea and is in direct contact with the outside world, hence is more prone to injuries. When there is loss of such epithelium, opportunistic infections may result, causing further damage to the eye and eventually blindness owing to inflammatory responses that lead to stromal scarring [2]. The World Health Organisation estimates that corneal blindness accounts for 5.1% of the global blind population, rendering it the fourth most common etiology of blindness, just falling behind cataract, glaucoma, and age-related macular degeneration (AMD) [3].

Common mechanisms of corneal injuries include foreign bodies, ultraviolet burns and chemical splashes [4]. Of all etiologies, chemical injury alone makes up between 11.5% and 22.1% of unilateral blindness [5], where visual outcome is guarded by the extent of involvement of intraocular components [6]. The highest incidence of such occurs in working age men of 20 – 30 years, with the most common setting being at work (67%), followed by assault incidents (33%) [7].

Pathophysiology of corneal epithelial chemical injuries

Alkali causes more debilitating injuries when compared to acidic agents, as it causes tissue injury by liquefactive necrosis, resulting in deep tissue penetration [8]. Damage of the ocular trabecular meshwork may result in high intraocular pressure, leading to complications such as glaucoma and corneal edema and subsequently reduced visual acuity. On the other hand,

acidic components tend to coagulate cellular proteins, hence limiting the extent of their penetration through ocular compartments.

When the corneal epithelial layer is damaged, it is imperative for reparation of the wound to take place, so as to prevent continuous and prolonged exposure of collagens and internal structures to the outer world. This is achieved via corneal re-epithelialisation, which is performed by limbal stem cells located at the periphery of the cornea. However, damage to these cells following a severe chemical injury may prevent this process from occurring. Although re-epithelialisation of the wound can alternatively be done with surrounding conjunctival-derived tissues to prevent stromal ulceration, this will compromise the vision of patients due to opacity of the tissue [9].

Once the cornea experiences damage to its structures, inflammation ensues and the site of injury attracts and induces a number of inflammatory cytokines to be secreted from corneal cells. These include interleukins (IL-1β, IL-6, IL-10) and tumour necrosis factor alpha (TNF-α) [10]. In addition, matrix metalloproteinases (MMPs) expression within the corneal tissue alters upon wounding of the cornea, playing a role in repair and organisation of stromal and epithelial tissue [11]. Injury and healing process of the eye is dependent on the interplay between these various molecules as well as their regulation.

<u>Current management of ocular chemical burns</u>

Urgent and acute management of corneal chemical burns should include immediate and copious irrigation with saline. A subsequent ophthalmic examination should be carried out in order to determine the extent of injury to the eye, hence come up with a treatment plan based on the grade of injury [12]. The standard treatment regime for corneal epithelial defects

resulting from chemical burns include artificial tears application, bandage contact lens, alongside topical steroids or nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics and anti-glaucoma medications [13]. Unfortunately, with more severe cases of chemical injuries, such therapeutic strategies may be insufficient in achieving re-epithelialisation, in particular in patients with limbal stem cell deficiency, or other etiologies including Stevens-Johnson syndrome and diabetic keratopathy that may result in persistent epithelial defects. Therefore, surgical techniques including keratoplasties and amniotic membrane transplantation (Prokera), or autologous serum are adopted in these situations [14]. However, in light of the various morbidities and economic burden brought about by current treatments including glaucoma, persistent corneal defects and immunorejection, we hence conduct this systematic review to evaluate potential therapies which may act as alternatives or adjuncts to present first-line strategies. Here, we review studies on therapies that have demonstrated success in animal models with ocular chemical injury. These are usually created with an alkali-soaked filter paper disc, which is subsequently applied onto the ocular surface of the anaesthesised animal to produce a corneal wound.

Method

Literature search

We performed the literature search on the PubMed database (All fields) in September 2019 using the following keywords: (Cornea* OR “ocular”) AND (Injur* OR “chemical” OR burn* OR alkali*) AND (therap* OR “management” OR treat*) AND (epitheli*). We limited our search to full-text articles written in English and those that were published in the last 5 years. There was no minimal sample size requirement. The articles which were included documented in vivo studies on therapies which have not been known to translate to clinical

trials yet. The studies also had to assess the efficacy of treatments in treating corneal chemical injuries, with at least one of the parameters of efficacy related to cornea epithelial repair. All literature search was performed by SHLP, review of abstracts and full texts were completed by SHLP and KCS. Figure 1 describes the selection process for identified studies. The review was not registered in any database, and no librarians were involved in the literature search process. Where there was disagreement over inclusion of articles, KCS, the corresponding author, made the final decision.

Results

Table 1 summarises the 30 *in vivo* studies which we reviewed, including types of animal models used, sample size, parameters of efficacy of the therapeutic strategies, as well as main results and a brief commentary on each study.

1. Alterations in Amniotic Membrane

The current front-line method for treatment of chemical ocular burns includes the application of one or more pieces of human amniotic membrane (AM) onto the site of injury, hence creating a scaffold and protective layer to promote corneal re-epithelialisation. Available studies have identified alternations to such technique to enhance its effectiveness. Recently, one team has constructed a composite membrane with decellularized AM (dAM) layered using a poly(e-caprolactone) (PCL) nanofiber mesh, thereby increasing the toughness of the membrane [15]. This proved more effective with respect to retention time of the sutured AM layer on the surface of the alkali-injured limbal stem cell deficient-cornea rabbit model. In addition, comparison between the group of Limbal Stem Cells (LSCs) with dAM, and LSCs with composite membrane (CM) revealed that the use of CM-LSC led to improved repair of

morphological and hierarchical characteristics of the corneal epithelium, with respect to the shape of basal cells, pterygium, and superficial flat cells based on tissue section staining. On the contrary, the use of dAM-LSC resulted in bulky, fusiform and polygonal shaped corneal epithelial cells, which did not resemble normal morphology. A higher rate of recovery of epithelial layer was also observed in the CM-LSC group in contrast with dAM-LSC, dAM-only, and CM-only groups.

In addition, with the incorporation of retinoic acid (atRA) into AM, one study was able to demonstrate a significant reduction in ulcer depth ($p=0.008$) and area (decreased by 6%) when compared with normal AM transplantation *in vivo*. With the atRA-treated homogenate form of AM, the wound area was reduced by 20%, coupled with a decrease in ulcer depth ($p=0.067$) as opposed to a normal AM homogenate [16]. To explain this, further *in vitro* tests were conducted, which identified the inhibitory effect of atRA on matrix metalloproteinase-9 (MMP-9), a proinflammatory molecule participating in the breakdown extracellular matrix and delaying corneal epithelial healing when present in excess amounts, as well as disintegrating the epithelial barrier [17 18].

2. Mesenchymal stem cell-related methods

Mesenchymal stem cells (MSCs) possess potential for multilineage differentiation, with the ability to express corneal epithelial markers cytokeratins 3/12 once differentiated. MSCs may act as a better alternative to limbal stem cells as cell-sourcing candidates for corneal cell therapy, since the latter needs to be obtained from the healthy contralateral eye and consequently such procedure poses a risk to the patient. One way to induce MSC differentiation into corneal epithelial cells is to culture them in medium that is conditioned with corneal limbal explants. Via this method, one team successfully made use of adipose

mesenchymal stem cells (ASCs) from rabbits for construction of corneal epithelial cell sheets [19]. The treatment group yielded better recovery outcomes compared with the sham control, as observed from the clear corneas and minimal vascularisation, with integration of stratified corneal epithelia into stroma and absence of corneal epithelial defects. In another study, MSCs mixed in a gel comprised of hyaluronic acid (HA) and chondroitin sulfate (CS) was shown to improve corneal epithelial wound closure in a rat model, reaching 90.5% $\pm$ 5.9% closure 24 hours post-injury, compared to 66% $\pm$ 21.8% for HA/CS alone, 62.4% $\pm$ 25.9% for MSCs alone, and 63.6% $\pm$ 11.12% for sham control with saline irrigation only ($p<0.05$). In addition, MSC-HA/CS treatment yielded a smaller wound compared to sham control ($p<0.05$), and was the quickest in reaching full recovery amongst other groups [20]. Similar application of bone marrow MSCs on a polysaccharide hydrogel led to a significant decrease in wound area after 7 days, reaching only 0 - 10% of defect area. On the contrary, a 20 – 30% defect area remained in groups with polysaccharide alone ($p<0.05$) and MSCs alone ($p<0.01$), and 50% defect area for the control group ($p<0.001$). In addition, the combined treatment group (MSC-P group) was the only group that retained a low level of neovascularisation [21]. With human uterine cervical stem cells (hUCESC) grown in conditioned culture medium, a significantly higher rate of corneal epithelium regeneration was seen in both low and high concentrations of hUCESC topical treatment groups. Moreover, PCR assays demonstrated much reduced levels of inflammatory markers including TNF-α, MCP-1, and IL-6 in corneas treated with high concentrations of hUCESC, thereby reflecting its efficacy in promoting corneal healing [22].

3. Antifibrotic agents

Pirfenidone is a novel drug that was recently licensed to treat idiopathic pulmonary fibrosis, owing to its anti-fibrotic and anti-inflammatory properties. [23] Aside from its therapeutic effect

on the lung, liver, kidney, and the nervous system, studies have suggested potential benefits in treating ocular chemical injuries. A rat corneal alkali injury model illustrated earlier complete reepithelialisation after 7 days of treatment, a statistically significant positive result when compared to the control. The treatment group also demonstrated lower collagen IV content and lower integrated optical density ($p<0.05$), as well as decreased expression of pro-fibrotic substances (α-SMA), pro-angiogenic molecules (VEGF and PEDF), and pro-inflammatory molecules (CD34, CD31, NF-kB), concurring with previous studies on its anti-fibrotic and anti-inflammatory characteristics on other organs [24]. Due to its short half-life, a liquid crystal nanoparticle drug delivery system was developed in a separate study, and its efficacy was investigated in rabbit corneal injury models. All groups treated with pirfenidone exhibited 6 to 7 layers of stratified epithelia with more parallelly aligned collagen fibre compared to the group which only received the vehicle alone. Furthermore, the incorporation of the vehicle enhanced bioavailability of pirfenidone with respect to drug perfusion and retention. [25]

4. Anti-inflammatory agents

An anti-inflammatory plant extract widely used in cosmetic or dermatological products is aloe vera, which also possesses properties in wound healing and skin re-epithelialisation [26]. Histological data from one study revealed that albeit appearance of fibroblastic changes to the stromal keratocytes, inflammatory cells were much lower in count for the aloe vera-treated groups compared to controlled groups in both wild type and diabetic rat models. In both wild type and diabetic treatment groups, the wound area was significantly reduced ($p<0.05$) after two days of treatment [27]. The authors hypothesised the involvement of growth factors, likely encompassing TGF and bFGF, in the re-epithelialisation process of the cornea, although exact molecules directly causing such events to have yet to be determined.

The cytosolic protein, nucleotide-binding oligomerisation domain 1 (NOD1), serves an essential function in recognising bacteria at the cornea [28 29]. However, its activation may also bring about numerous inflammatory cytokines, hence contributing to corneal neovascularisation (CNV) [30]. Topical Nodinhibit-1 can lessen the extent of alkali-induced corneal neovascularisation by blocking the activity of NOD1, hence limits its destruction towards immune privilege and organisation of the corneal epithelium. An investigation into the effect of Nodinhibit-1 eye drops on rats demonstrated an earlier completion of epithelial wound healing (7 days) compared to control (non-healing upon observation at day 14), with a reduction in the number of apoptotic cells in the cornea [31].

Adiponectin is a peptide originating from adipose tissue and exists in prevalence within the blood circulation. Whilst having anti-inflammatory properties including inhibitive effects on TGF-β and NF-kB pathways [32 33], it also has the potential to regenerate tissues including bone, muscle and skin [34]. The time to complete re-epithelialisation in 0.01% and 0.001% adiponectin-treated mice were 7.33 ± 1.55 days and 6.87 ± 1.00 days respectively, which were significantly shorter than hyaluronic acid-treated (8.50 ± 1.37 days, both $p=0.03$) and the NaOH sham control (9.33 ± 1.65 days, $p=0.02$ and $p=0.03$ respectively). Histological examination of eyes treated with 0.01% and 0.001% adiponectin revealed multi-layered epithelia with reduced inflammatory cell numbers [35]. The authors speculated the involvement of AdipoR1 and AdipoR2 corneal surface receptors on activating the mitogen activated protein kinase/extracellular-signal regulated kinases 1/2 (MAPK/ERK) pathway for wound healing [36], where ERK 1/2 molecules are responsible for cell proliferation [37].

The telomeric protein RAPI is believed to upregulate the NFκB pathway for inflammatory and angiogenic responses. In one study, RAPI knockout mice demonstrated complete healing of epithelium on day 7, in contrast to wild type RAPI positive mice in which 67.69% $\pm$ 3.39% defect areas were retained ($p<0.01$) and complete recovery by day 14. Moreover, in vitro tests revealed an increase in migration of corneal epithelial cells. Suggested mechanism of action relates to the downregulation of inflammatory cytokine production leading to improved corneal surface healing [38].

In the human corneal epithelium, neprilysin (NEP), an inflammatory regulatory enzyme, is believed to break down neuropeptides including substance P and bradykinin [39]. Using NEP knockout mice, a study was able to show a 42.72 $\pm$ 4.91% and 52.08 $\pm$ 7.19% reduction in area of corneal defect on day 3 and 7 respectively after chemical injury ($p<0.0001$). High dose administration of the NEP inhibitor thiorphan on wildtype mice also did not result in corneal perforation, unlike groups treated with low dose NEP and vehicle only. However, no statistical significance was demonstrated between high dose or low dose thiorphan, or vehicle-only groups with respect to corneal epithelial wound healing [40].

An oxidised species of deoxyguanosine, known as topical 8-oxo-2'-deoxyguanosine (8-oxo-dG), is hypothesised to be able to suppress the formation of reactive oxygen species as well as inflammatory cytokines, and therefore may replace corticosteroids as an anti-inflammatory medication and avert its associated complications [41]. The treatment groups using 8-oxo-dG presented with more optimal epithelial integrity scores, of which a dose-responsive effect was observed ($p<0.0001$). Higher dosage of 8-oxo-dG was also associated with hastened healing of wounds [42]. Since nucleic acids are naturally derived molecules, and coupled with promising results from above studies, it could potentially replace current medications for the

treatment of corneal chemical injuries whilst sparing patients a considerable number of side effects.

The Rho-kinase (ROCK) inhibitor fasudil hydrochloride was found to reduce corneal epithelial defect areas in alkali-burned corneas, as well as corneal neovascularisation and inflammation thereby limiting delay of wound healing of the epithelium [43]. Inhibition of the negative regulatory effects of ROCK on corneal epithelial proliferation and cell adhesion may have also contributed to such outcomes [44].

It is postulated from results of several studies that activity of TNF-α, an inflammatory cytokine, may induce apoptotic cell death of corneal epithelial cells, hence corneal damage [45] [46]. As a macrolide, azithromycin possesses anti-TNF-α properties [47] [48]. Comparison between alkali-injured corneas revealed a lower count of apoptotic cells in the corneal epithelium in 1.5% azithromycin-treated group (75.8 ± 15.7) as opposed to the group treated with an artificial tear gel (117.1 ± 23.8) ($p < 0.05$). Moreover, with respect to the mean density of TNF- α, levels of such parameter were reduced in the azithromycin treatment group (1.65 ± 1.1) when compared to the artificial tear gel group (2.65 ± 1.3) ($p < 0.05$) [49].

5. Antioxidant

Melatonin, a neuropeptide commonly known to participate in the circadian rhythm, is demonstrated to also be secreted in tear fluid. Its receptors, notably the MT2 receptor, are distributed along the corneal epithelium. Coupled with the circadian pattern of epithelial renewal in the cornea as well as the published effects on melatonin's potential in repairing dermal wounds, many have hypothesised its potential role in regulating corneal homeostasis [50] [51]. Postulated therapeutic mechanism of this compound may be related to its ability to

reduce reactive oxygen species at the eye, thereby offering increased protection towards the epithelium [52]. A study reported an increased epithelial migration rate by 35 μm/hour compared to control rate of 75 ± 5 μm/hour ($p<0.001$), as well as a reduction in epithelial healing time of 9.4 hours in comparison to the control value of 29.8 ± 1.9 hours, although no p-values were reported in the findings. Interestingly, addition of a non-selective antagonist for melatonin receptor and one which was MT2-selective prolonged epithelial healing times in both instances, whilst MT3-receptor antagonism failed to conform to such findings. This suggests that the MT2 receptor could play a role in the corneal epithelial wound healing process [53]. Nevertheless, it should be noted that different concentrations of melatonin can induce opposite effects on epithelial regeneration in organs including the breast, which necessitates further investigations on melatonin's therapeutic effects specifically on the cornea [54].

6. Hydrogels

Hydrogels are crosslinked polymeric matrices, the primary functions of which include high-capacity water absorption, yet is also utilised as a scaffold for limbal stem cell transplantation [55]. Chitosan is classified as a natural polysaccharide-based hydrogel and serves as a popular biomaterial-of-choice for hydrogel-related therapies. One *in vivo* rabbit study showing the efficacy of a carboxymethyl chitosan and sodium alginate-derived hydrogel revealed a near-complete healing of corneal epithelium post-treatment. Corneal thickness 28 days post-treatment was closely matched with the normal cornea, results of which were supported by statistical inference. Moreover, expression of cytokeratin 3/12 as well as vimentin in the stroma of the treatment group intimately correlated with the normal unburned group, indicating a high efficacy of the hydrogel in treating corneal alkali injuries [56]. Another study from the same team investigated a blend of chitosan, gelatin and hyaluronic acid for the same

purpose, illustrating a reduction in perforated area to 20 – 40%, compared with a 50% wounded area which remained in the control model. More importantly, when the hydrogel was used as a cell carrier of rabbit corneal epithelial cells for transplantation, such combination therapy yielded a promising improvement in managing corneal opacity and restoring corneal thickness, with a significant reduction in perforation area to 10% [57]. With chitosan-based hydrogels, it may also serve as a delivery system for drugs such as ferulic acid, as shown in a rabbit model a significantly reduced wound area in the first 3 hours of treatment, and minimal corneal hyperplasia after 24 hours. However, long term effects of the treatment were not reported in this study [58]. In addition, it was not certain whether the positive effects generated from the *in vivo* study were a result of ferulic acid or the hydrogel as there was no investigation of the effects of using the hydrogel alone.

7. Extracellular matrix components

Hyaluronic acid (HA), a ubiquitous extracellular matrix substance, is known to be expressed at wound margins to help with tissue repair and re-epithelialisation [59]. Based on this knowledge, cross-linked thiolated hyaluronic acid films were investigated for its efficacy in treating corneal wounds in rabbits. For this method, although an initial phase of complete re-epithelialisation at 48 hours failed to sustain by 96 hours, two weeks post-therapy presented a significant improvement in re-epithelialisation of 83% in hyaluronic acid film-treated group, compared to 63% in non-treated controls ($p < 0.01$) [60]. Similar results were rendered from a study on sodium hyaluronate, which has been a popular substance for investigation of its wound healing characteristics including cell migration and inflammatory suppression. The study identified a significant decrease in wound healing time in the experimental group treated with 1% sodium hyaluronate (11 days) compared with the saline control group (15

348 days) ($p < 0.05$), although other aspects of wound healing such as corneal epithelial
349 morphology, stratification and inflammation were not evaluated [61].
350
351 Topically administered chondrocyte-derived ECM is hypothesised that inhibiting IL-8
352 production may deregulate the recruitment of neutrophils expressing metalloproteinase-9
353 (MMP-9), thereby increasing the rate of re-epithelialisation. MMP-9 is an enzyme which acts
354 to degrade Type IV collagen present in corneal tissue, increased levels of which are
355 demonstrated to delay wound healing of the epithelium [62]. Rabbit corneas exhibited recovery
356 in the epithelial layer and reduction in inflammatory cells, as opposed to the control group in
357 which corneas became thicker with abundance of inflammatory cells, as well as a
358 disintegrated corneal epithelium [63].
359
360 The neuropeptide netrin-4 has been shown to promote epithelial adhesion via integrin
361 interaction, as well as to maintain the structure of the basement membrane [64 65]. In one study
362 investigating the effects of topical netrin-4 application on corneal chemical burns, although
363 the primary outcome was to determine its effect on angiogenesis, the results nevertheless
364 indicated that the netrin-4-treated group had comparatively lower numbers of apoptotic cells
365 in the epithelium, suggesting its function in regulating apoptosis [66].
366
367 8. <u>Cell proliferation and repair modulators</u>
368 A recent advancement in DNA nanotechnology, the tetrahedral framework nucleic acids
369 (tFNA), were shown to hasten the reduction of corneal epithelial defect area, as well as
370 reducing corneal opacity when they were administered in rabbits [67]. It was postulated via *in*
371 *vitro* studies that tFNA induced such positive effect on the epithelium as a result of the

upregulated phosphorylation of ERK1/2 and p38, which are molecules responsible for cell proliferation and migration respectively [37 68].

Nitric oxide administered in low concentration may also act via the same signalling cascade, the MAPK/ERK pathway, to improve corneal epithelial cell proliferation. It is important to regulate its concentration as a higher amount may generate undesirable effects including cell cycle arrest and reduced proliferation [69]. A study conducted on mice demonstrated a significantly enhanced corneal epithelial wound healing with reduced corneal opacity when administering 10uM dose of the nitric oxide compound $NaNO_2$ [70].

(-)-Epigallocatechin-3-gallate (EGCG) is a phenol-containing metabolite of numerous traditional Chinese medicinal herbs, and is believed to be a viable treatment option for proliferative and inflammatory pathologies. From histological analysis of mice eyes, the EGCG groups displayed promoted cell differentiation and reduced corneal epithelial thickness with close matching of normal morphology. This was most likely due to anti-proliferative effects of EGCG, allowing for more controlled regrowth of the cornea. These results were confirmed via in vitro studies, which exhibited that the catechin component of EGCG aided in a 50% reduction of apoptotic cell numbers. [71]

Neuregulin-1 (NR-1) is typically involved in multiple neural-related physiological functions, including growth and differentiation of neurons [72]. *in vitro* studies revealed that the neuronal growth factor neuregulin-1 (NR-1) increased phosphorylation of ERK 1/2, p38, and STAT3 resulting in cell proliferation of corneal epithelium, whilst simultaneously activating corneal epithelial stem cells. From *in vivo* studies, mice treated with NR-1 eye drops were able to significantly reduce the corneal epithelial wound area to 10.09% and 1.89% at 16 hours and

24 hours post-injury, whereas in controls these respective values were 22.13% and 4.78% ($p < 0.01$) [73].

MG53 is a molecule that is believed to be involved in corneal homeostasis due to its ubiquitous nature in corneal epithelium and tear film. Its function in the repair process of the cell membrane is explained by its contribution towards recruitment of intracellular vesicles to migrate to the site of injury and form a reparative patch. Using mg53 knockout rats, one study was able to restore epithelialisation in alkali-injured corneas via exogenous administration of recombinant human MG53, as opposed to non-healing outcomes in those treated with saline as controls [74].

Histatin-1, a histidine-rich peptide present in saliva, has been demonstrated in cell studies to enhance epithelial growth in tissues including the oral mucosa, as well as endothelial proliferation [75 76]. From previous in vitro testing, it seems that histatin-1 acts as a promoter for epithelial cell migration via the MAPK/ERK pathway, as well as a demoter for the release of MMP-9 which is shown to complicate tight junction formation in epithelial cells [77 78]. An *in vivo* investigation using a rabbit model confirmed a time-dependent healing process of the corneal alkali wound on all experimental groups with topically administered histatin-1. Treatment groups were free from neovascularisation and inflammation [79].

Discussion

We have evaluated and described a range of relatively newer therapeutic strategies for chemically-induced ocular surface injuries. Amongst them, alterations to current therapies including the use of nanofiber-reinforced amniotic membranes, or retinoic acid-improved

homogenate of amniotic membranes, seems to be a viable option in that it might be relatively easier to incorporate the discussed therapeutic entities into existing and clinically-proven therapies. In addition, another promising technique is mesenchymal stem cell differentiation for cell therapy, since this is useful in treating a wider range of corneal diseases such as limbal stem cell deficiency. However, its reliability regarding differentiation potential into corneal epithelial cells needs to be better confirmed via replication of results in future studies. Moreover, some studies have pointed out the influence of the tumour protein p63 expression on the viability of transplanted cells once on the ocular surface, an issue that calls for strict evaluation and regulation of the cells produced via this method, and on the other hand requires more time and money to cultivate quality-controlled cells [80]. Recently, a similar idea was employed in the use of tissue-engineering to construct a cultivated oral mucosal epithelial cell-derived cell sheet for transplantation (COMET) to corneal wounds, which have demonstrated success in a small population of patients [81]. More clinical trials are yet required to prove feasibility of the application of this method, and to evaluate the severity of potential complications involved.

It is worth noting the challenges faced by current ophthalmologist when treating chemically-injured corneal epithelial defects. For instance, topical corticosteroids, a first-line treatment, are known to lower inflammatory cell counts and moderate the release of MMPs [82]. However, they are also found to hinder corneal re-epithelialisation in the long term, and risk corneal perforation [83]. This also highlights the significance in carrying out investigations on newer alternative medication such as topical melatonin and pirfenidone mentioned above, to act as an adjunct therapy and lessen such burden. On the other hand, keratoplasty, also known as cadaveric corneal transplantation, is one of the techniques commonly sought for management of severe corneal diseases. Yet, severe complications may arise including allograft rejection

and persistence of epithelial wounds [84]. Artificial methods to recreate such technique, the most popular one being Boston type 1 keratoprosthesis, have recently been made available in hospitals in attempts to lower the immunological rejection rates. Nonetheless, studies have identified certain post-operative morbidities including fungal infections and most prevalently glaucoma, hence calling for the need of surgical prophylaxis of glaucoma prior to carrying out the procedure [85] [86]. With amniotic membrane transplantation (Prokera, Bio-Tissue, Inc., Miami, FL), although it was proven in numerous clinical studies its benefits in re-epithelialisation of the corneal surface, acting as a scaffold for cell migration and proliferation due to the growth factors retained within the human-derived structure, some have raised concerns over the expensive preservation processes inevitably undergone, including dehydration and irradiation, during which alterations to the structures and functions of biomolecules may occur [87]. A similar novel AMT technique involves the use of a forniceal ring to aid in the application of a single sheet of cryopreserved amniotic membrane across the ocular surface [88]. This ensures adequate coverage of the entire mucosal surface, including the conjunctiva and fornices, thereby reduce the risk of fibrous tissue contraction. Retrospective review of patient outcomes found a tremendous drop in occurrence of temporal symblepharons, as well as a significant reduction in surgical time and cost.

Another potential therapeutic strategy that was recently developed is Simple Limbal Epithelial Transplantation (SLET), as first reported by Dr. Virender Sangwan. This surgical procedure entails placement of several small pieces of limbal fragments directly on the injured eye, thus minimising the amount of tissue biopsied, as well as the cost from *ex vivo* culturing of limbal cells. For autologous SLET, this surgical procedure is only reserved for unilateral limbal stem cell deficiencies (LSCD). A large prospective study reported a 71.4%

and 75% success rate in total LSCD and partial LSCD, which was defined as complete re-epithelialisation, clinical stability and absence of corneal neovascularisation [89]. Similarly, allogenic SLET involves transplantation of tissue from a donor, with efforts to reduce immune-rejection risk via distributing the limbal tissue fragments at the avascular midperipheral stroma. Limited studies are available at present, although it was report that allogenic SLET led to a 83.3% success rate in a population of 30 patients, alas with 16.7% LSCD recurrence [90].

With further development and determination of safety profiles of the discussed emerging therapeutic agents, it is likely that these will ultimately replace the need for keratoplasties and amniotic membrane transplantations, hence eliminating the requirement for matching donor tissues with that of recipients. Most of the discussed therapeutic strategies are administered via the topical route, which helps enhance patient adherence to treatment since the application of medication is simple and convenient. An exception is the hydrogel technology, which requires an intermediate level of surgical expertise during the transplantation process from the ophthalmologist. However, the surgery would likely be much less complex compared to current keratoplasty and amniotic membrane transplantation procedures, since it would only involve placement of a hydrogel film over the wounded ocular surface, and may not even require suturing.

There are some limitations to our study. Firstly, since all of the above studies were *in vivo* in nature, large-scale human clinical trials have yet to be conducted to determine the safety profile and efficacy of treatment methods specifically on humans. More importantly, long-term complications need to be thoroughly comprehended to provide patients with adequate pre-treatment evaluation and prophylaxis if necessary. Moreover, we included only

chemically-injured models, although many studies have made use of other wound models, for instance trephine-debrided or dry eye models, to demonstrate corneal epithelial healing effects of other potential medicinal candidates, which may in fact also be beneficial to managing corneal chemical injuries. Nevertheless, the inclusion criteria of the study were set as chemically injured *in vivo* models, since chemical burns, especially alkali burns, cause detrimental effects to other structures of the anterior chamber, and not merely to the epithelium. Hence, models investigated in this study would more likely evaluate other healing markers such as chemically-induced inflammation and scarring of the cornea, and offer a well-rounded representation of the effect of treatment strategies on the eye.

Conclusion

Corneal chemical injuries may result in detrimental effects to the structure and function of the eye, especially the anterior chamber. With adequate and timely management of resulting corneal epithelial damage, as well as meticulous control of pathological processes including inflammation and edema within the corneal stroma, the ultimate goal of the treatment regimen is to restore eye histology and functionality, thereby enhancing patient outcomes. Newer methods may help achieve such goals and lessen the burden that complications may bring on both healthcare providers and the patient, yet their safety profiles need to be determined prior to translation to humans.

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Legends

Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow chart illustrating selection process of articles.

Table 1. Summary of *in vivo* Studies on Strategies for Ocular Surface Chemical