BMJ Open Diabetes Research & Care

# Experimental modeling of cornea wound healing in diabetes: clinical applications and beyond

Yashan Bu,[1] Kendrick Co Shih ,[1] Sum Sum Kwok,[1] Yau Kei Chan,[1] Amy Cheuk-Yin Lo,[1] Tommy Chung Yan Chan,[2] Vishal Jhanji,[3] Louis Tong[4]

**To cite:** Bu Y, Shih KC, Kwok SS, *et al*. Experimental modeling of cornea wound healing in diabetes: clinical applications and beyond. *BMJ Open Diab Res Care* 2019;**7**:e000779. doi:10.1136/bmjdrc-2019-000779

Received 25 July 2019
Revised 6 October 2019
Accepted 3 November 2019



[1]Department of Ophthalmology, University of Hong Kong, Hong Kong
[2]Department of Ophthalmology, Hong Kong Sanatorium and Hospital, Hong Kong
[3]Department of Ophthalmology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA
[4]Department of Ophthalmology, Singapore National Eye Centre, Singapore

**Correspondence to**
Dr Kendrick Co Shih;
kcshih@hku.hk

## ABSTRACT

Diabetes mellitus is the most common cause of blindness in working age populations worldwide. While much of the focus for public health has been on secondary prevention in sight-threatening diabetic retinopathy, the cornea, including its epithelium and nerves, represents a major site of damage by chronic hyperglycemia. On injury, the diabetic cornea exhibits a delayed wound-healing response, as well as an altered ocular surface immune response. This suggests a potential association between the dysfunctional wound healing response and altered inflammation on the ocular surface. However, the presence of potential confounders makes this association difficult to investigate in human epidemiological studies. Thus, we turn to animal diabetic models for a better understanding. In this review, 20 original studies, published between 2008 and 2018, describe in vivo and in vitro models of diabetic cornea disease. We compared different models of diabetic cornea wound healing and discussed the relative strengths and drawbacks of each model. A number of molecular and cellular components involved in the corneal wound healing response that are altered in the presence of diabetes have been identified in the reviewed studies. Particularly, altered corneal epithelial protein concentrations of lumician and occludin were detected in diabetic eyes compared with controls. Additionally, the importance of IL-1β in modulating the inflammatory response after corneal injury in patients with diabetes and controls was further elucidated. Meanwhile, abnormal P2×7 receptor localization and decreased corneal sub-basal nerve density in diabetic eyes were shown to contribute to altered corneal nerve signaling after injury and thus affecting the wound healing response. Finally, the discovery of the therapeutic effects of topically administered aloe vera, Serpine 1, Resolvin D1 (RvD1), pigment epithelium-derived factor (PEDF) and Pro-His-Ser-Arg-Asn in diabetic animal models of cornea epithelial and nerve injury provide encouraging evidence for the future availability of effective treatment for diabetic keratopathy.

## INTRODUCTION

Diabetes mellitus (DM) is an important chronic disease, affecting more than 342 million people worldwide, with a trend of increasing prevalence in the last decade. Patients with DM are at a higher risk of developing ocular complications and account for the major cause of acquired blindness in working-age populations.[1–3] In recent years, much of the focus for public health system worldwide has been on secondary prevention of sight-threatening diabetic retinopathy. While in contrast, there is relatively less attention to diabetic keratopathy, it is a common complication, affecting more than half of patients with DM worldwide.

Diabetes keratopathy is characterized by ocular surface disease and impaired corneal epithelial wound healing. Previous studies have indicated molecular changes occurred on the ocular surface, including higher tear levels of insulin-like growth factor-binding protein 1, accumulation of advanced glycation end products (AGE) and loss of disruption of tight junctions on the corneal epithelium. It is also known that diabetic status is associated with alterations in the systemic cellular adaptive immune system. This in turn results in high serum levels of tumor necrosis factor-α, interleukin (IL)-6 and IL-8 in patients with diabetes compared with controls.[4] Currently, the impact of this altered immune response in patients with diabetes on ocular surface wound healing remains unclear.

We performed a systematic review of recent published literature, focusing on the association between corneal injury and re-epithelialization in the context of animal models of sustained hyperglycemia. We highlighted the different diabetic animal models used and methods of corneal injury. The molecular changes involved in corneal injury caused by hyperglycemia and those involved in the recovery of corneal epithelium and corneal nerves are discussed. Potential drugs or drug targets discovered from the reviewed studies are also introduced.

## MATERIALS AND METHODS

An Entrez PubMed search was conducted on 1 January 2019 using the search terms

Table 1 Summary of animal models used in studies of diabetic corneal epithelial complications

| Model | Authors, year | Phenotype | Advantages | Drawbacks |
|---|---|---|---|---|
| STZ mice/rats | Yamamoto *et al*, 2018[1]<br>Huang *et al*, 2016[3]<br>Zhang *et al*, 2018[23]<br>Atiba *et al*, 2015[28]<br>Yang *et al*, 2014[16]<br>He *et al*, 2017[57]<br>Li *et al*, 2017[50]<br>Morishige *et al*, 2017[51]<br>Sun *et al*, 2015[58] | Type I diabetes | Rapid onset of hyperglycemia.<br>Cost-effective and time efficient.<br>Development of significant diabetic cornea neuropathy, even in the absence of an external insult. | Prone to adverse events, including significant weight loss, bladder dysfunction, poor general health and respiratory distress, resulting in high mortality rates.<br>The cornea re-epithelialization response after injury altered by pre-existing diabetic cornea neuropathy and cornea inflammation. |
| DIO mice | Kneer *et al*, 2018[2]<br>Yan *et al*, 2016[2 43] | Type II diabetes | Established model for studies on obesity-related complications. | Later onset and milder sustained hyperglycemia as compared with STZ mice. |
| db/db mice | Dai *et al*, 2015[11] | Type II diabetes.<br>Hyperphagia with obesity. | Phenotype similar to humans with sustained severe hyperglycemia and diabetic neuropathy. | The phenotype does not model the clinical manifestations of diabetic retinopathy completely. |
| OLETF rats | Nagai *et al*, 2010[13] | Type II diabetes with mild obesity. | A long-established model in translational research for diabetes. | Later onset of hyperglycemia compared with STZ mice.<br>Augmented immune response, which may affect studies on altered immunity in patients with diabetes. |

DIO, diet-induced obesity; OLETF, Otsuka Long-Evans Tokushima Fatty; STZ, streptozocin.

'diabetes', 'cornea' and 'injury'. Only articles published within 10 years and written in English language were included. With this criteria, 37 articles were identified. The resulting articles were then manually curated for relevance by YB and KCS. For example, papers concerning diabetes (both type 1 and 2), cornea, injury and recovery, corneal re-epithelialization were considered as relevant. Furthermore, only original articles were included, while review papers and meta-analyses were excluded. This produced a list of 14 of relevant articles. Additionally, we identified six studies in the references of our finalized results to give a total of 20 papers for our review.

## RESULTS

In total, 20 papers were reviewed after manual curation. For analysis, we grouped our discussion of the finalized papers into the following headings: (1) models of the diabetic cornea; (2) models of corneal injury; (3) mechanisms of impaired wound healing investigated; and (4) treatments investigated to improve wound healing outcomes.

### Modeling the diabetic cornea

#### Animal models

Established animal models of type 1 (insulin deficiency) and type 2 (insulin resistance) diabetes enable a better characterization of diabetic cornea complications (see table 1). Among the articles detailing animal models, the majority either performed intraperitoneal injection of streptozocin (STZ) in mice/rats, modeling type I diabetes or administered high-fat diet in mice, causing diet-induced obesity (DIO) and increased insulin resistance, modeling type 2 diabetes. For other models, two papers used db/db mice, and one paper used Otsuka Long-Evans Tokushima Fatty (OLETF) rats, both which are models of type 2 diabetes.

#### *STZ injection*

STZ is a compound that has preferential toxicity toward pancreatic β islet cells. STZ injection was first reported to have diabetogenic properties in 1963 and has since become a chemical widely used to model diabetes in experiments. Briefly for this model, rats are given daily injections of 160–240 mg/kg STZ for 2 days in order to mimic type I diabetes.[1 5] The onset of hyperglycemia is rapid. The majority of animals have significantly increased blood glucose level 48 hours after STZ injection, which stabilizes 6–10 days after injection.[6] It is an efficient and cost-effective method for experimental use. Evidence from published studies demonstrate that STZ-treated mice have significant diabetic cornea neuropathy, with signs of progressive sub-basal nerve plexus damage, even in the absence of an external insult. While these findings would make STZ-treated rodents excellent models for studying cornea neurotrophic ulcers, these features may also potentially alter epithelial wound-healing outcomes after cornea injury.[7] Documented adverse effects of STZ injections include potentially lethal hepatotoxicity and nephrotoxicity. Furthermore, STZ-treated mice have been reported to suffer bladder dysfunction and excessive

urination.[8] More importantly, STZ-induced diabetic mice are more likely to suffer respiratory distress, significant weight loss and poorer generalized health, resulting in high mortality rates compared with other animal models of diabetes. These factors need to be taken into consideration when using STZ-treated rodents for modeling of diabetes.

### DIO model

This is a model for studying type 2 diabetes. Briefly, mice are fed for 4–30 weeks with chow containing high fat and sugar (mostly sucrose and occasionally fructose). DIO mice are known to develop obesity, dyslipidemia and impaired glucose tolerance.[2] DIO mice are primarily used in studies modeling obesity rather than diabetes, and the induction of sustained hyperglycemia is only achieved at 16–20 weeks after initiation of the diet.[9] It is also important to note that the hyperglycemia achieved is mild compared with that seen in STZ injections. Depending on the goals and hypothesis of a study, special experimental procedures such as glucose tolerance test, insulin level measurement, insulin tolerance test and body composition measurement are required when DIO mice are used.

### Db/db mouse

Two groups investigating diabetic neuropathy used db/db mice for in vivo experiments.[10 11] The db/db mouse is an experimental model for type 2 diabetes with the development of sustained severe hyperglycemia. Db/db mice are genetically obese leptin receptor-deficient mice.[10] They develop hyperphagic obesity and non-ketotic diabetes similar to type 2 DM in humans.[12] This model has been previously used to study diabetic neuropathy with reported altered nerve and ocular properties compared with controls.[10] However, it is important to note that both studies showed that while peripheral neuropathy is present in db/db mice, not all of the structural changes observed in human diabetic retinopathy can be detected using this model.

### OLETF rats

Nagai *et al* used OLETF rats to investigate corneal epithelial wound healing over time. Compared with controls, OLETF rats had significantly slower wound healing rates.[13] The OLETF rat is an established model for human type 2 DM where the rats develop sustained hyperglycemia and clinical signs of diabetes starting from 25 weeks of age.[13] OLETF rats have been used in diabetes research since the 1990s, modeling mild obesity and late onset of hyperglycemia (defined as after 18 weeks of age). It has been reported that the diabetic phenotype more often manifests in male rats compared with their female counterparts.[14] The experiments by Nagai *et al* demonstrated a strong relationship between the cornea wound healing rate constant and plasma glucose level. Aside from glucose level, the rate of wound healing decreased with age in OLETF rats. It is important to note however that unlike diabetes in humans, OLETF rats have an augmented immune response, resulting in an overproduction of proinflammatory cytokines in response to insults compared with controls.[15] This would potentially adversely impact diabetic studies investigating changes in immunity.

## In vitro models

Cultured human epithelial cells have been used to study corneal epithelial wound healing in health and disease, allowing for an improved understanding in differential gene expression, cell differentiation and various biochemical properties between epithelial cells in glucose-rich media and controls (see table 2). After scratch wound injury, cell proliferation and migration assays are employed to quantify differences between groups.[16 17] Cell culture studies allow for improved understanding of disease pathogenesis and molecular mechanism of potential therapeutic agents while avoiding ethical concerns with animal welfare.[18–20] However, while the local effects of high glucose levels can be studied using in vitro models, the lack of a blood supply and innervation

**Table 2** Summary of in vitro models used in studies of diabetic corneal epithelial complications

| Model | Authors | Type | Major application | Advantages | Drawbacks |
|---|---|---|---|---|---|
| Cultured human epithelial cells | Yang *et al*[16] | Primary | Diabetic keratopathy | An established cell culture system for investigating epithelial wound healing in high glucose medium. | Limited lifespan of cells. The lack of blood supply and innervation lowers clinical translatability of results. |
| Mouse corneal epithelial cell line | Yang *et al*[16] | Cell line | Diabetic keratopathy | Allows for genetic manipulation to study mechanisms of pathogenesis. | Cells may have genetic and phenotypical differences as well as altered morphology when compared with normal corneal epithelial cells. |
| Trigeminal ganglia neuronal cells | Dai *et al*[11] | Primary | Diabetic corneal neuropathy | A tight control of the extracellular environment ensuring a precise neuronal environment for studies. | The lack of corneal epithelial cells prevents investigation into the impact of trigeminal nerve health on wound healing. |

prevents a more well-rounded and clinically translatable approach to investigating the effect of diabetes on injured corneal epithelial cells.

In order to investigate diabetic corneal neuropathy in vitro, primary trigeminal ganglia (TG) neuronal cells were used for by Dai *et al*.[11] They studied the role of the neuropeptide FF (NPFF) in corneal nerve injury. TG consist of neuronal cells and two types of glial cells: satellite cells and Schwann cells,[21] both of which are involved in the pathogenesis of diabetic neuropathy. The protocol for producing dissociated primary sensory neurons is as previously described by Malin *et al*.[22] Neuronal cultures guarantees a tight control of the extracellular environment, which ensures a precise neuronal environment. This is particularly advantageous in the study of the intrinsic electrical properties of neurons and sensory transduction.[22]

## Modeling epithelial injury

### Mechanical Injury

Twelve published animal studies induced direct mechanical injury either via a scratch on the corneal epithelium or via corneal epithelial debridement.[3 23] Briefly, a corneal epithelial wound, about 2–4 mm diameter, is fashioned using an Algerbrush remover or a blunt scalpel blade under local or general anesthesia. Corneal epithelium debridement has been used as a model to study delayed wound healing[24 24 24 24] and recurrent corneal epithelial erosion syndrome.[24 25]

### Chemical injury

Two papers have applied alkali burn to the mouse cornea in order to induce injury and inflammation. Compared with cornea epithelial debridement, the recovery time for re-epithelialization after burn is significantly longer,[26] with associated ocular surface inflammation.[27] Briefly, alkali injury is induced by applying a filter paper, soaked in NaOH solution cut to 2–4 mm diameter for set time period, on the cornea epithelium.[28]

For both mechanical and chemical injury, antibiotics, such as rapamycin or aureomycin eye ointment, is usually applied daily or regularly after the injury to avoid superimposed cornea infection. For monitoring over time, the epithelial defect size can be visualized by fluorescein strip staining, and the total surface area can be quantified with imaging software, such as the National Institute of Health Image J software. Another method of quantifying corneal injury is corneal opacity scoring[29 30] in which the degree of cornea opacity is determined clinically on a numerical scale of 0–4: with 0: clear cornea; 1: mild stromal opacity; 2: moderate stromal opacity; 3: severe corneal opacity with visible iris; and 4: opaque cornea with iris not visible.[31]

## Molecular mechanisms of impaired corneal wound healing in patients with diabetes

### Altered protein concentrations

Delayed corneal epithelial wound healing may lead to sight-threatening complications, including ocular surface irregularities, microbial keratitis and corneal scarring.[32] The treatment of delayed wound healing in diabetic corneas remains a therapeutic challenge.[33]

A number of published studies have reported delayed corneal wound healing in the presence of sustained hyperglycemia.[34] Various proteinases, growth factors and cytokines that have regulatory effects of inflammation and wound healing process are also shown to be altered locally in DM eyes compared with controls. The proteins thought to be involved in the wound healing process include epidermal growth factor,[35] transforming growth factor beta 3, ciliary neurotrophic factor, insulin-like growth factor-1[36] and matrix metalloproteinase 3 and 10 (MMP-3 and MMP-10).[37 38] Furthermore, recent studies have elucidated novel factors involved in delayed corneal epithelial wound healing in diabetes (see table 3).

Yamamoto *et al*'s study used shotgun liquid chromatography mass spectrometry to detect differentially expressed proteins in the cornea of STZ rats compared with controls.[1] The study identified 188 proteins that were expressed at least twofold higher in STZ rats compared with controls and were serving as candidate proteins in the pathogenesis of delayed wound healing in diabetes. By gene ontology analysis, the roles of these proteins identified were further examined. A total of 15 proteins were noted to be differentially expressed in the cornea of STZ rats compared with controls and were determined to be extracellular matrix proteins. In particular, the level of lumican expression in the cornea of STZ rats was significantly higher than that of the normal rats. Yamamoto *et al* noted that in the cornea of the control rat, the expression level of lumican was elevated during the wound healing process, and it returned to the baseline expression level after the wound was healed completely. However, a high expression level of lumican in the cornea of STZ rats was still maintained even after the wound was healed completely. The authors noted that in experiments, STZ rats had poorer adhesion between basal cells of the cornea epithelium and the underlying Bowman's membrane during the healing response and thus postulated a role for lumican in its pathogenesis.

Huang *et al* identified that abnormal occludin expression may contribute to delayed epithelial wound healing in diabetic corneas.[3] Occludin is an important component of tight junctions (TJs) in the cornea epithelium. It has been identified as a target for signaling events and involved in regulating paracellular permeability.[39] Huang *et al* used 100 Sprague-Dawley male rats for experiments and induced diabetes in half of them using a combination of HFD and STZ injection. Would healing after epithelial debridement was documented and compared between groups using fluorescein staining, H&E staining and scanning electron microscopy. Immunofluorescence and Western blot analysis demonstrated that occludin expression in the corneal epithelium of the diabetic group was weaker compared with the normal group at the 16 and 48 hours time-points after debridement. This corresponded with a slower wound healing rate in diabetic mice compared with controls.

Table 3 Summary of recent investigations on molecular mechanisms of altered diabetic corneal wound healing after injury

| Author | Model | Comparison | Findings |
|---|---|---|---|
| Yamamoto *et al*[1] | STZ-treated diabetic rat. | Comparison of cornea epithelial lumican levels at baseline and at 0 hour, 14 hours and 25 hours after the corneal abrasion between STZ-treated rats and controls. | Differential lumican expression levels in the cornea after injury in STZ-treated mice compared with controls. |
| Huang *et al*[3] | STZ-treated diabetic mice. | Comparison of occludin expression in the corneal epithelium of the STZ-treated mice versus controls 16 and 48 hours after cornea epithelial debridement. | Weaker occludin expression levels in corneal epithelium of STZ-treated mice compared with controls. |
| Royer *et al*[40] | Rag2 knockout (OT-II) T-cell receptor transgenic mice. | Comparison of corneas harvested from mice 6 days after scratch injury, ragweed pollen–induced allergy or herpes simplex virus type 1 infection versus healthy tissue controls. | Corneal epithelial cells exhibited myeloid cell lineage characteristics. |
| Yan *et al*[43] | DIO diabetic mice. | Comparison of immune cell count and level of secretory interleukin-1 receptor antagonist (sIL-1Ra) after cornea epithelial injury in diabetic corneas versus controls. | A reduction in neutrophil and natural killer (NK) cell infiltration during wound closure response in diabetic corneas is associated with lower levels of IL-1Ra. |
| Kneer *et al*[2] | DIO diabetic mice | Comparison of P2×7 expression and localization at baseline and 12 hours after injury in diabetic mice versus controls. | A significant reduction in cornea sub-basal nerve density is noted in diabetic mice compared with controls.<br>P2×7 was significantly overexpressed in the cornea epithelium of diabetic mice compared with controls. |
| Dai *et al*[11] | db/db mice (in vivo) and trigeminal ganglion (TG) neuronal cells (in vitro). | In vivo – comparison of NPFF expression in db/db mice compared with controls.<br>In vitro – dendrite length of TG sensory neurons in hyperglycemic conditions versus controls, with or without exogenous NPFF. | NPFF expression was significantly lower in the TG tissues in diabetic mice compared with controls. |

DIO, diet-induced obesity; IL, interleukin; NPFF, neuropeptide FF; P2×7, P2X purinoceptor 7; sIL-1Ra, secreted form of IL-1 receptor antagonist; STZ, streptozocin.

## Altered inflammatory response

Royer *et al*[40] first reported that corneal epithelial cells exhibited myeloid characteristics. They were found to express lymphocyte antigen 6 complex locus G6D, C-C chemokine receptor type 2 and CX3C chemokine receptor 1. Particularly, $Ly6C^{+}$ cells are found to function as antigen presenting cells. This means that the cornea epithelium serves as the first line of immunity in the ocular surface after insult.

Neutrophils are early responders in the inflammatory process and play an important role in the cornea wound healing process after epithelial debridement.[41] Natural killer cells are part of the innate immune system, and its main role is to kill aberrant cells and to assist in the function of cytotoxic T lymphocytes.[42] Yan *et al* reported that both neutrophil and natural killer (NK) cell infiltration were reduced during wound closure in diabetic corneas compared with controls.[43] This was associated with a lower level of the secreted form of IL-1 receptor antagonist (sIL-1Ra) in diabetic corneas compared with controls. IL-1β and its receptor IL-1a are important mediators of the wound healing response. It has been shown to enhance infiltration of neutrophils and NK cells after injury and also promotes corneal regeneration. In the normal cornea, both IL-1β and sIL-1Ra are increased in response to wounding. The findings of this study suggest an important role for reduced IL-1Ra secretion in the pathogenesis of diabetic cornea wound healing.

## Altered nerve signaling

Diabetes is a major cause of peripheral nerve damage. Diabetes alters corneal nerve structure[44] and down-regulate the release of neuropeptides, neurotrophins and growth factors.[45] Kneer *et al*[2] reported a significant decrease in cornea sub-basal nerve density in DIO mice compared with controls, demonstrating similar

established phenotypes to that of human diabetics. In this study, a DIO mouse model of type 2 diabetes was used to characterize changes in sensory nerves and P2X purinoceptor 7 (P2×7). This is a trimeric ATP-gated cation channel expressed in a number of tissues. It serves as a pain receptor. Kneer *et al*[2] previously reported that P2×7 mRNA was significantly overexpressed in diabetic human corneas compared with controls. Therefore, the authors hypothesized that the P2×7 receptor acts to sense changes at the leading edge following an epithelial abrasion, with this form of regulation lost in diabetic eyes.

The NPFF is an octapeptide that has been implicated in a wide variety of physiological functions in the brain.[46–48] Dai *et al*[11] found using real-time polymerase chain reaction (RT-PCR) that NPFF expression was significantly lower in the trigeminal ganglion (TG) tissues of type 2 diabetic db/db mice compared with controls. The team further demonstrated that NPFF enhanced neurite elongation in diabetic TG neurons in vitro, thereby implicating its deficiency in the pathogenesis of diabetic corneal neuropathy. When delivered via subconjunctival route, NPFF promoted corneal nerve injury recovery and epithelial wound healing in db/db mice. This effect could be completely abolished with the addition of RF9, a selective NPFF receptor antagonist. NPFF is thus a potential neuroregenerative factor in the treatment of diabetic corneal neuropathy.

Substance P (SP) is a nociceptive neurotransmitter, primarily released from sensory nerve fibers and also immune cells, including macrophages, eosinophils, lymphocytes and dendritic cells, in response to injury.[49] SP exhibits potent proinflammatory effects in immune and epithelial cells and participates in the pathogenesis of various inflammatory diseases. It is known to have positive effect on corneal epithelial wound healing. Yang *et al* investigated the potential therapeutic effects of SP on hyperglycemia-induced delayed corneal wound healing in vitro and in vivo. SP application promoted epithelial wound healing, recovery of corneal sensation, improvement of mitochondrial function and increased reactive oxygen species (ROS) scavenging capacity in vivo and in vitro.[16] The promotion of SP on diabetic corneal epithelial healing was completely abolished by a neurokinin-1 (NK-1) receptor antagonist. Moreover, the subconjunctival injection of NK-1 receptor antagonist also caused diabetic corneal pathological changes in normal mice suggesting a role for SP deficiency in the pathogenesis of diabetic cornea neuropathy.

## Potential treatments for delayed corneal wound healing in patients with diabetes

A number of studies investigated the potential efficacy of novel therapeutic agents for diabetic keratopathy. The treatments include Resolvin D1 (RvD1), safflower extract and aceglutamide (SA) injection, the synergy peptide Pro-His-Ser-Arg-Asn (PHSRN), pigment epithelium-derived factor (PEDF), Serpine 1 and aloe vera (AV) (see table 4).

Li *et al*[50] explored the effects of SA in treating diabetic cornea neuropathy. A physical injury was induced on the central part of the cornea of STZ mice and controls. Following injury both groups were given SA intraperitoneally for 21 days. Compared with controls, the diabetic cornea had a lower density of cornea nerve fibers at baseline. Treatment with SA promoted cornea secretion of vascular endothelial growth factor-B (VEGF-B), nerve growth factor (NGF) and Glial cell line-derived neurotrophic factor (GDNF) and increased cornea nerve fiber density. Furthermore, the beneficial effects of SA were shown to be completely abolished by inhibition of the VEGF-B receptor.

Morishige *et al*[51] discovered that topical administration of fibronectin-derived peptide PHSRN greatly facilitated healing of corneal epithelial wounds in diabetic rats. The PHSRN sequence is one of the cell-binding sites for fibronectin and has been previously reported to promote corneal epithelial cell migration.[52] As an eye-drop, PHSRN was demonstrated to be effective at a minimum concentration of 2 mM and has the greatest efficacy at a concentration of 200 mM. The study also reported that PHSRN facilitated corneal wound healing in both diabetic mice and controls.[51]

AV is a perennial succulent belong to the Lily (Liliaceae) family. Reported therapeutic use of AV include promoting wound healing, attenuating inflammatory response, antibacterial, antioxidant, antiviral and antifungal actions, as well as antidiabetic activities.[53] Atiba *et al*[28] used a corneal alkali-burn injury model on STZ-treated rats to study the efficacy of topically applied AV on diabetic corneal wound healing. The investigators demonstrated the efficacy of topical AV in enhancing corneal re-epithelialization rate and reducing ocular surface inflammation after alkali burn in diabetic rats.

Known as a proresolving mediator, RvD1 has been widely investigated in studies of various diseases, such as arthritis, pulmonary disease and kidney injury.[54] Zhang *et al*[23] reported that topical administration of RvD1 significantly promoted corneal epithelial wound healing, reactivated epithelial regeneration-related signaling pathways and sped up resolution of corneal inflammation. Additionally, RvD1 treatment promoted regeneration of diabetic corneal nerves and restored corneal mechanical sensation in STZ-treated mice. Increased oxidative stress in the corneal epithelium has been identified as part of the diabetic complications exhibited on the ocular surface.[55] The presence of advanced glycation end products in diabetes increases the generation of intracellular ROS

in human cornea epithelial cells.[56] Zhang *et al*[23] further demonstrated that topical administration of RvD1 reduces AGE-mediated ROS accumulation and NADPH oxidase 2/4 overexpression in human cornea epithelial cells in vitro.

He *et al*[57] demonstrated that topical treatment with PEDF, in combination with docosahexaenoic acid (DHA), promotes corneal nerve regeneration and

Table 4 Summary of recent investigations on potential therapeutic agents for treating diabetic corneal complications

| Source | Country | Animal model | Methods of injury | Outcomes |
|---|---|---|---|---|
| Li *et al*[50] | China | STZ-treated diabetic mice. | Central cornea epithelium debrided via Alger brush. | ► Safflower extract and aceglutamide injection reduces ameliorates diabetic cornea neuropathy.<br>► Its protective effect is associated with the elevated cornea epithelial secretion of vascular endothelial growth factor-B (VEGF–B), nerve growth factor (NGF) and Glial cell line-derived neurotrophic factor (GDNF). |
| Morishige *et al*[51] | Japan | STZ-treated diabetic rats. | A portion of corneal epithelium is debrided by a scraper. | ► Administration of Pro-His-Ser-Arg-Asn significantly facilitated healing of corneal epithelial wounds in both diabetic rats and controls.<br>► The effect is postulated to be due to increased cornea epithelial cell migration response after injury. |
| Atiba *et al*[28] | Egypt | STZ-treated diabetes rats. | Alkali-burn injury with 0.01 M NaOH on it for 45 s. | ► Aloe vera enhances corneal re-epithelialization rate and attenuates the ocular surface inflammatory response after alkali burn in both diabetic rats. |
| Zhang *et al*[23] | China | STZ-treated diabetic mice. | Central cornea epithelium debrided by Alger brush. | ► Topical application of Resolvin D1 (RvD1) promoted corneal epithelial wound healing and reduced ocular surface inflammation after injury in diabetic eyes.<br>► Furthermore, RvD1 promoted regeneration of corneal nerves and significantly sped up restoration of normal corneal sensation after injury.<br>► The underlying mechanism is purported to be related to RvD1's ability in reducing AGE-mediated oxidative stress. |
| He *et al*[57] | USA | STZ-treated diabetic mice. | A 2 mm diameter cornea epithelial injury with corneal rust ring remover. | ► Topical treatment with pigment epithelium-derived factor+docosahexaenoic acid promoted corneal nerve regeneration and epithelial wound healing after injury in diabetic mice.<br>► Furthermore, it increased tear production and attenuated the ocular surface inflammatory response after injury. |
| Sun *et al*[58] | USA | STZ-treated diabetic mice | Corneal epithelial debridement. | ► Topical application of Serpine1 accelerated corneal wound healing after injury and reduced secretion of proteolytic enzymes like MMP-3. |

AGE, advanced glycation end products; MMP-3, matrix metalloproteinase 3; STZ, streptozocin.

wound healing in diabetic mice. After administration of pigment epithelium-derived factor and docosahexaenoic acid (PEDF+DHA), elevated corneal sensitivity and increased tear production was observed. Additionally, PEDF+DFA also selectively recruited type 2 macrophages and prevented neutrophil infiltration in diabetic wounded corneas. Meanwhile, the regenerated nerves were shown to be functional, as demonstrated by the restoration of corneal sensitivity, tear volume increase and upregulated expression of the sensory neuropeptide SP.

Serpine1 is a serine protease inhibitor that functions as the principal inhibitor of tissue and urokinase plasminogen activator (uPA). It was reported by Sun *et al* that Serpine1 expression is upregulated during cornea injury, and this response was found to be significantly

suppressed in diabetic corneas compared with controls. For diabetic corneas, the addition of exogenous Serpine1 significantly accelerated wound healing and modulated local proteolytic enzymatic activities, including that of MMP-3. Sun *et al*[58] suggested that early intervention with systemic and local therapies modulating Serpine1 and/or uPA proteolytic activities may provide hope for preventing sight-threatening complications in diabetic cornea disease.

## DISCUSSION

### Advantages and limitations of current models for diabetic corneal wound healing

The diversity of current models for diabetes allows for presentations of DM with more than one model, thus corresponds to the diversity of clinical manifestations of DM. However, certain disadvantages are also present. Injected chemicals that induced diabetes in rodent are suspected to have detrimental effects on other organs. Particularly, STZ was reported to cause lymphopenia and a relative increase in T-regulatory cells, which may interfere with studies regarding systemic immunity.[59] Despite the rapid onset of diabetes brought by STZ administration, this model of diabetes has reported detrimental effects on multiple organs[6]; in severe cases death can result from multiorgan failure. DIO mice and OLETF rats are more commonly used in studies of type 2 diabetes, obesity-induced metabolic disorders and diabetic complications.[60] Aside from those used in reported studies, other animal models could be used for future experiments in diabetic cornea wound healing, including mice heterozygous for the Akita spontaneous mutation ($Ins2^{Akita}$). This a model of type I diabetes, with heterozygous $Ins2^{Akita}$ mice developing hyperglycemia, hypoinsulinemia, polydipsia and polyuria by 3–4 weeks of age. As such this is an excellent model for studying diabetic microvascular and macrovascular complications.

A cornea epithelial cell culture system is an excellent model for testing the local therapeutic effects of topical drugs as well as their pharmacokinetic properties.[61] Additionally, in vitro studies are particularly important for molecular studies into disease pathogenesis and resolution. Despite the added advantages of the sparing of animals for research work, in vitro studies are still limited by their inability to properly model the complex interactions between cells and tissues in the human eye, as well as the important roles the nerve and blood supply play in the disease. Furthermore, the cornea, being an exposed organ, is constantly interacting with the ocular adnexa, for example, during blinking, and the external environment. These factors remain difficult to model in vitro.[61] The engineering of multicellular coculture systems, the so-called cornea-on-a-chip, may provide a better platform for in vitro studies in the future.

Although in vivo model provides understanding of diabetic neuropathy on a physiological and cellular level, the availability of cell culture model is also imperative in presenting details of the molecular events that might be obscured in animal study. TG neuronal cells were mentioned earlier as one in vitro model in studying corneal nerve injury associated with hyperglycemia.[11] Other reported usage of primary cultures in studying diabetic neuropathy include dorsal root ganglia cells, Schwann cells and cortical neurons, yet they are likely to become heterogeneous due to presence of contaminating and highly proliferating fibroblasts and survive only limited passages.[62] These drawbacks can be circumvented by various experimentally-proved robust transformed cell lines.[62] In studies of impaired corneal innervation, the usage of in vitro model is relatively scarce. However, previous results of in vitro studies have indicated the functions of corneal nerve fibers in promoting corneal wound healing and maintaining normal physiology of the cornea.[63]

### Translatability to clinical practice

In this review, a number studies have reported potential treatment strategies of diabetic corneal diseases, such as AV, RvD1, SA injection and topical administration of PEDF or combined administration of PEDF+DHA.[23 28 50 51 58] Among them, most of the studied drugs can be administered topically as eye-drops. Particularly, AV is already widely accepted and used as a dietary supplement and in complementary medicine.

Aside from the mentioned therapeutic studies, a number of molecular alterations found in diabetic cornea indicates possible future drug targets in treating delayed corneal wound healing. They include targets involved in cell–cell adhesion, nerve regeneration and signaling and immunomodulation. As a link between altered immune response, cornea inflammatory cell invasion and cornea sub-basal nerve plexus damage have been demonstrated in studies on human diabetics, there is promise that these therapeutic targets are complementary and will be translatable to future clinical practice.[64] Previous studies have demonstrated the role of lumican in ocular diseases, immune response and wound healing.[65] Lumican is critical in maintaining the corneal collagen structure that ensures corneal transparency.[66] Given the multiple functions of lumican on the ocular surface, it is worthy to be considered as a target in treating diabetic corneal complications and restoring corneal clarity. Occludin is one component of the TJs present in the apical layer of the epithelium. It plays a crucial role in maintaining the barrier function of the corneal epithelium to protect the endothelium from the external environment. Novel findings of the effect of abnormally expressed occludin in diabetic corneas further suggests that it can potentially be a target of drug delivered at the ocular surface. The discoveries of NPFF as a potential neuroregenerative factor[11] and SP's role in promoting corneal wound healing[16] serve as promising areas for drug development. Neuropeptides possess broad spectrum of functions from neurohormone and neurotransmitter to growth factors and inflammatory mediators.[67]

Previous reports have highlighted the effects of neuropeptides on corneal epithelial cell growth, proliferation and differentiation.[63]

The main concern with making use of the described therapeutic targets in actual practice is the difficulty in developing topical treatments for the abnormal processes. This is especially a problem for proteins involved in cell–cell adhesion, as they are secreted by the diseased cornea epithelial cells and cannot be replaced externally. Treatments may thus require genetic modifications and gene silencing techniques. Thus, neuropeptides, including NPFF and SP, may provide a more readily translatable form of treatment in terms of direct topical application on cornea wounds.

## CONCLUSIONS

Keratopathy is a common and sight-threatening complication of diabetes, manifesting with delayed cornea wound healing, recurrent corneal erosion syndrome and neurotrophic ulcers. While there is significantly less attention to diabetic keratopathy compared with retinal complications, the cornea is the most superficial part of the eye and thus most accessible for research and treatment. The clinical manifestations of diabetic keratopathy involve a complex interplay between the cornea epithelial cell lining, ocular surface immunity and cornea sub-basal nerve plexus. Due to the recent availability of robust in vivo and in vitro models of diabetic cornea wound healing, we now have an improved understanding of the diabetic keratopathy pathogenesis and progression. These studies have provided novel targets for future preventive and therapeutic strategies. Furthermore, studies have used these models as a platform for testing novel therapeutic agents, with the close approximation between available in vivo models and human corneas allowing for early translation of results into clinical trials. This review highlights the continued importance of experimental modeling in solving sight-threatening complications of the eye.

**Contributors** All authors attest that they meet the current ICMJE criteria for authorship. YB and KCS were involved in study design, data collection, data analysis, manuscript writing and editing. SSK, YKC, AC-YL, TCYC, VJ and LT were involved in data collection, data analysis, manuscript writing and editing.

**Funding** The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

**Competing interests** None declared.

**Patient consent for publication** Not required.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** There are no data in this work.



**ORCID iD**

Kendrick Co Shih http://orcid.org/0000-0001-6255-2941

## REFERENCES

1 Yamamoto T, Otake H, Hiramatsu N, *et al*. A proteomic approach for understanding the mechanisms of delayed corneal wound healing in diabetic keratopathy using diabetic model rat. *Int J Mol Sci* 2018;19:E3635.
2 Kneer K, Green MB, Meyer J, *et al*. High fat diet induces pre-type 2 diabetes with regional changes in corneal sensory nerves and altered P2X7 expression and localization. *Exp Eye Res* 2018;175:44–55.
3 Huang C, Liao R, Wang F, *et al*. Characteristics of reconstituted tight junctions after corneal epithelial wounds and ultrastructure alterations of corneas in type 2 diabetic rats. *Curr Eye Res* 2016;41:783–90.
4 Geerlings SE, Hoepelman AI. Immune dysfunction in patients with diabetes mellitus (DM). *FEMS Immunol Med Microbiol* 1999;26:259–65.
5 Graham ML, Janecek JL, Kittredge JA, *et al*. The streptozotocin-induced diabetic nude mouse model: differences between animals from different sources. *Comp Med* 2011;61:356–60.
6 Deeds MC, Anderson JM, Armstrong AS, *et al*. Single dose streptozotocin-induced diabetes: considerations for study design in islet transplantation models. *Lab Anim* 2011;45:131–40.
7 Leppin K, Behrendt A-K, Reichard M, *et al*. Diabetes mellitus leads to accumulation of dendritic cells and nerve fiber damage of the subbasal nerve plexus in the cornea. *Invest Ophthalmol Vis Sci* 2014;55:3603–15.
8 Leiria LOS, Mónica FZT, Carvalho FDGF, *et al*. Functional, morphological and molecular characterization of bladder dysfunction in streptozotocin-induced diabetic mice: evidence of a role for L-type voltage-operated Ca2+ channels. *Br J Pharmacol* 2011;163:1276–88.
9 Inui A. Obesity--a chronic health problem in cloned mice? *Trends Pharmacol Sci* 2003;24:77–80.
10 Pande M, Hur J, Hong Y, *et al*. Transcriptional profiling of diabetic neuropathy in the BKS db/db mouse: a model of type 2 diabetes. *Diabetes* 2011;60:1981–9.
11 Dai Y, Zhao X, Chen P, *et al*. Neuropeptide FF promotes recovery of corneal nerve injury associated with hyperglycemia. *Invest Ophthalmol Vis Sci* 2015;56:7754–65.
12 Orland MJ, Permutt MA. Quantitative analysis of pancreatic proinsulin mRNA in genetically diabetic (db/db) mice. *Diabetes* 1987;36:341–7.
13 Nagai N, Murao T, Okamoto N, *et al*. Kinetic analysis of the rate of corneal wound healing in Otsuka Long-Evans Tokushima fatty rats, a model of type 2 diabetes mellitus. *J Oleo Sci* 2010;59:441–9.
14 Kawano K, Hirashima T, Mori S, *et al*. OLETF (Otsuka Long-Evans Tokushima Fatty) rat: a new NIDDM rat strain. *Diabetes Res Clin Pract* 1994;24 Suppl:S317–20.
15 Nagai N, Murao T, Ito Y, *et al*. Involvement of interleukin 18 in lens opacification of Otsuka Long-Evans Tokushima fatty rats, a model of human type 2 diabetes. *Curr Eye Res* 2011;36:497–506.
16 Yang L, Di G, Qi X, *et al*. Substance P promotes diabetic corneal epithelial wound healing through molecular mechanisms mediated via the neurokinin-1 receptor. *Diabetes* 2014;63:4262–74.
17 Kenchegowda S, Bazan NG, Bazan HEP. EGF stimulates lipoxin A4 synthesis and modulates repair in corneal epithelial cells through ERK and p38 activation. *Invest Ophthalmol Vis Sci* 2011;52:2240–9.
18 Gruber FP, Hartung T. Alternatives to animal experimentation in basic research. *ALTEX* 2004;21 Suppl 1:3–31.
19 Rusche B. The 3Rs and animal welfare - conflict or the way forward? *ALTEX* 2003;20:63–76.
20 Furrer P, Plazonnet B, Mayer JM, *et al*. Application of in vivo confocal microscopy to the objective evaluation of ocular irritation induced by surfactants. *Int J Pharm* 2000;207:89–98.
21 Pannese E. Perikaryal surface specializations of neurons in sensory ganglia. *Int Rev Cytol* 2002;220:1–34.
22 Malin SA, Davis BM, Molliver DC. Production of dissociated sensory neuron cultures and considerations for their use in studying neuronal function and plasticity. *Nat Protoc* 2007;2:152–60.
23 Zhang Z, Hu X, Qi X, *et al*. Resolvin D1 promotes corneal epithelial wound healing and restoration of mechanical sensation in diabetic mice. *Mol Vis* 2018;24:274–85.
24 Ljubimov AV, Saghizadeh M. Progress in corneal wound healing. *Prog Retin Eye Res* 2015;49: :17–45.
25 Pal-Ghosh S, Pajoohesh-Ganji A, Brown M, *et al*. A mouse model for the study of recurrent corneal epithelial erosions: alpha9beta1 integrin implicated in progression of the disease. *Invest Ophthalmol Vis Sci* 2004;45:1775–88.
26 Cejková J, Lojda Z, Dropcová S, *et al*. The histochemical pattern of mechanically or chemically injured rabbit cornea after aprotinin

treatment: relationships with the plasmin concentration of the tear fluid. *Histochem J* 1993;25:438–45.
27 Martin LFT, Rocha EM, Garcia SB, *et al*. Topical Brazilian propolis improves corneal wound healing and inflammation in rats following alkali burns. *BMC Complement Altern Med* 2013;13:337.
28 Atiba A, Wasfy T, Abdo W, *et al*. Aloe vera gel facilitates re-epithelialization of corneal alkali burn in normal and diabetic rats. *Clin Ophthalmol* 2015;9:2019–26.
29 Shin YJ, Hyon JY, Choi WS, *et al*. Chemical injury-induced corneal opacity and neovascularization reduced by rapamycin via TGF-β1/ERK pathways regulation. *Invest Ophthalmol Vis Sci* 2013;54:4452–8.
30 Bai J-Q, Qin H-F, Zhao S-H. Research on mouse model of grade II corneal alkali burn. *Int J Ophthalmol* 2016;9:487–90.
31 Yoon K-C, Heo H, Kang I-S, *et al*. Effect of topical cyclosporin A on herpetic stromal keratitis in a mouse model. *Cornea* 2008;27:454–60.
32 Yin J, Huang J, Chen C, *et al*. Corneal complications in streptozocin-induced type I diabetic rats. *Invest Ophthalmol Vis Sci* 2011;52:6589–96.
33 Schulze SD, Sekundo W, Kroll P. Autologous serum for the treatment of corneal epithelial abrasions in diabetic patients undergoing vitrectomy. *Am J Ophthalmol* 2006;142:207–11.
34 Chen W-L, Lin C-T, Ko P-S, *et al*. In vivo confocal microscopic findings of corneal wound healing after corneal epithelial debridement in diabetic vitrectomy. *Ophthalmology* 2009;116:1038–47.
35 Xu K, Yu F-SX. Impaired epithelial wound healing and EGFR signaling pathways in the corneas of diabetic rats. *Invest Ophthalmol Vis Sci* 2011;52:3301–8.
36 Shih KC, Lam KS-L, Tong L. A systematic review on the impact of diabetes mellitus on the ocular surface. *Nutr Diabetes* 2017;7:e251.
37 Takahashi H, Akiba K, Noguchi T, *et al*. Matrix metalloproteinase activity is enhanced during corneal wound repair in high glucose condition. *Curr Eye Res* 2000;21:608–15.
38 Saghizadeh M, Kramerov AA, Tajbakhsh J, *et al*. Proteinase and growth factor alterations revealed by gene microarray analysis of human diabetic corneas. *Invest Ophthalmol Vis Sci* 2005;46:3604–15.
39 Hirase T, Staddon JM, Saitou M, *et al*. Occludin as a possible determinant of tight junction permeability in endothelial cells. *J Cell Sci* 1997;110:1603–13.
40 Royer DJ, Elliott MH, Le YZ, *et al*. Corneal epithelial cells exhibit myeloid characteristics and present antigen via MHC class II. *Invest Ophthalmol Vis Sci* 2018;59:1512–22.
41 Gagen D, Laubinger S, Li Z, *et al*. ICAM-1 mediates surface contact between neutrophils and keratocytes following corneal epithelial abrasion in the mouse. *Exp Eye Res* 2010;91:676–84.
42 Topham NJ, Hewitt EW. Natural killer cell cytotoxicity: how do they pull the trigger? *Immunology* 2009;128:7–15.
43 Yan C, Gao N, Sun H, *et al*. Targeting imbalance between IL-1β and IL-1 receptor antagonist ameliorates delayed epithelium wound healing in diabetic mouse corneas. *Am J Pathol* 2016;186:1466–80.
44 Rosenberg ME, Tervo TM, Immonen IJ, *et al*. Corneal structure and sensitivity in type 1 diabetes mellitus. *Invest Ophthalmol Vis Sci* 2000;41:2915–21.
45 Bikbova G, Oshitari T, Tawada A, *et al*. Corneal changes in diabetes mellitus. *Curr Diabetes Rev* 2012;8:294–302.
46 Lozano I, Van der Werf R, Bietiger W, *et al*. High-fructose and high-fat diet-induced disorders in rats: impact on diabetes risk, hepatic and vascular complications. *Nutr Metab* 2016;13.
47 Mamikutty N, Thent ZC, Sapri SR, *et al*. The establishment of metabolic syndrome model by induction of fructose drinking water in male Wistar rats. *Biomed Res Int* 2014;2014:1–8.
48 Della Vedova MC, Muñoz MD, Santillan LD, *et al*. A mouse model of diet-induced obesity resembling most features of human metabolic syndrome. *Nutr Metab Insights* 2016;9:NMI.S32907–102.
49 O'Connor TM, O'Connell J, O'Brien DI, *et al*. The role of substance P in inflammatory disease. *J Cell Physiol* 2004;201:167–80.
50 Li D, Chen Y-G, Zhang C-J, *et al*. Safflower extract and Aceglutamide injection promoting recovery of peripheral Innervations via vascular endothelial growth factor-B signaling in diabetic mice. *Chin Med J* 2017;130:2829–35.
51 Morishige N, Uemura A, Morita Y, *et al*. Promotion of corneal epithelial wound healing in diabetic rats by the Fibronectin-Derived peptide PHSRN. *Cornea* 2017;36:1544–8.
52 Kimura K, Hattori A, Usui Y, *et al*. Stimulation of corneal epithelial migration by a synthetic peptide (PHSRN) corresponding to the second cell-binding site of fibronectin. *Invest Ophthalmol Vis Sci* 2007;48:1110–8.
53 Hamman JH. Composition and applications of Aloe vera leaf gel. *Molecules* 2008;13:1599–616.
54 Xu Z-Z, Ji R-R. Resolvins are potent analgesics for arthritic pain. *Br J Pharmacol* 2011;164:274–7.
55 Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res* 2010;107:1058–70.
56 Shi L, Chen H, Yu X, *et al*. Advanced glycation end products delay corneal epithelial wound healing through reactive oxygen species generation. *Mol Cell Biochem* 2013;383:253–9.
57 He J, Pham TL, Kakazu A, *et al*. Recovery of corneal sensitivity and increase in nerve density and wound healing in diabetic mice after PEDF plus DHA treatment. *Diabetes* 2017;66:2511–20.
58 Sun H, Mi X, Gao N, *et al*. Hyperglycemia-suppressed expression of SerpinE1 contributes to delayed epithelial wound healing in diabetic mouse corneas. *Invest Ophthalmol Vis Sci* 2015;56:3383–92.
59 Muller YD, Golshayan D, Ehirchiou D, *et al*. Immunosuppressive effects of streptozotocin-induced diabetes result in absolute lymphopenia and a relative increase of T regulatory cells. *Diabetes* 2011;60:2331–40.
60 Walley AJ, Asher JE, Froguel P. The genetic contribution to non-syndromic human obesity. *Nat Rev Genet* 2009;10:431–42.
61 Castro-Muñozledo F. Corneal epithelial cell cultures as a tool for research, drug screening and testing. *Exp Eye Res* 2008;86:459–69.
62 Hattangady NG, Rajadhyaksha MS. A brief review of in vitro models of diabetic neuropathy. *Int J Diabetes Dev Ctries* 2009;29:143–9.
63 Baker KS, Anderson SC, Romanowski EG, *et al*. Trigeminal ganglion neurons affect corneal epithelial phenotype. Influence on type VII collagen expression in vitro. *Invest Ophthalmol Vis Sci* 1993;34:137–44.
64 Lagali NS, Badian RA, Liu X, *et al*. Dendritic cell maturation in the corneal epithelium with onset of type 2 diabetes is associated with tumor necrosis factor receptor superfamily member 9. *Sci Rep* 2018;8:14248.
65 Amjadi S, Mai K, McCluskey P, *et al*. The role of lumican in ocular disease. *ISRN Ophthalmol* 2013;2013:1–7.
66 Chakravarti S, Petroll WM, Hassell JR, *et al*. Corneal opacity in lumican-null mice: defects in collagen fibril structure and packing in the posterior stroma. *Invest Ophthalmol Vis Sci* 2000;41:3365–73.
67 Hoyer D, Bartfai T. Neuropeptides and neuropeptide receptors: drug targets, and peptide and non-peptide ligands: a tribute to Prof. Dieter Seebach. *Chem Biodivers* 2012;9:2367–87.