Review Article

Neuronal Changes in the Diabetic Cornea: Perspectives for Neuroprotection

Guzel Bikbova, Toshiyuki Oshitari, Takayuki Baba, and Shuichi Yamamoto

Department of Ophthalmology and Visual Science, Chiba University Graduate School of Medicine, Inohana 1-8-1, Chuou-ku, Chiba, Chiba 260-8670, Japan

Correspondence should be addressed to Toshiyuki Oshitari; tarii@aol.com

Received 23 August 2016; Revised 14 October 2016; Accepted 23 October 2016

Academic Editor: Nick DiGirolamo

Copyright © 2016 Guzel Bikbova et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Diabetic neuropathy is associated with neurotrophic ulcerations of the skin and cornea. Decreased corneal sensitivity and impaired innervation lead to weakened epithelial wound healing predisposing patients to ocular complications such as corneal infections, stromal opacification, and surface irregularity. This review presents recent findings on impaired corneal innervation in diabetic individuals, and the findings suggest that corneal neuropathy might be an early indicator of diabetic neuropathy. Additionally, the recent findings for neuroprotective and regenerative therapy for diabetic keratopathy are presented.

1. Introduction

Diabetes mellitus is a major disease worldwide, and the incidence of diabetes has risen markedly in the past several decades. The complications associated with diabetes are the leading cause of blindness in the working age adults. Diabetic neuropathies characterized by a progressive loss of nerve fibers are common complications affecting about 50% of patients with diabetes [1].

It was recently demonstrated that retinal neuronal components were associated with the pathogenesis of diabetic retinopathy [2, 3], and the neuronal degeneration in the retina may be dependent on the mitochondrial- and caspase-dependent cell-death pathway [4]. It is known that neuronal abnormalities directly affect visual function in diabetic retinopathy, and those neuronal changes are probably also the reason for diabetic keratopathy, as cornea is one of the most highly innervated tissues [5]. However, there are limited numbers of studies available that focused on evaluation of corneal innervation changes in diabetic patients. Early diagnosis of neuropathy is very important for evaluation of risks and therapeutic management. Thus in this review we aim to summarize the most recent findings on impaired corneal innervation in diabetic patients and findings on neuroprotective and regenerative therapy approach for diabetic keratopathy.

2. Corneal Innervation

The density of corneal epithelial nerves is 300–600 times higher than that of the skin with approximately 7000 nociceptors/mm² [6]. The sensory nerve fibers in the peripheral cornea have a myelinated shell and can be seen by slit-lamp biomicroscopy. The central cornea is acutely sensitive especially along the horizontal meridian and less sensitive along the vertical meridian [7].

The ophthalmic nerve and occasionally the maxillary branch of the trigeminal nerve innervate the cornea [8, 9], and the superior cervical ganglion supplies sympathetic innervation to the limbus and peripheral cornea [10]. Light [10–12] and electron microscopic [13, 14] studies and more recently confocal microscopic studies [15–18] have provided detailed information on the distribution of the nerves in the human cornea.

There are about 70 to 80 large diameter myelinated nerves that enter the cornea at the posterior to mid-stromal level, and they run radially and anteriorly toward the center of the cornea. The anterior stromal layers are innervated by multiple branches of these nerves that have no perineurium and myelin. They penetrate the cornea approximately 1 mm from the limbus, pass through Bowman's membrane, and turn in a clockwise direction forming the subbasal nerve plexus that lies between Bowman's layer and the epithelium.
forming the subbasal nerve vortex. Its geographic center is located between 2.18 and 2.92 mm from the corneal apex. In some cases, the subbasal nerves do not form a prominent spiral but end on opposite sides of an imaginary interface [13].

The functioning of the corneal nerve is assessed by corneal sensitivity tests. There are three main groups of receptors in the cornea: mechanical or mechanonociceptors, chemical or polymodal nociceptors, and thermal or cold receptors [19, 20].

3. Mechanisms of Impaired Corneal Innervation and Neurotrophic Role of Corneal Nerves

Recent studies have provided strong evidence that glycation plays an important role in the pathogenesis of retinal diabetic neuropathy with the triggering of different mechanisms that result in neuronal dysfunction [21, 22]. Obrosova and Julius reported that oxidative stress and poly(ADP-ribose) polymerase activation were fundamental mechanisms that play a role in the pathogenesis of diabetic neuropathy [23]. Byun et al. confirmed that poly(ADP-ribose) polymerase inhibition prevented the loss of epithelial innervation and promoted epithelial wound healing. They concluded that poly(ADP-ribose) polymerase activation played a role in the pathogenesis of diabetic neuropathy [24].

The results of a recent study have shown that one of the functions of corneal nerve fibers is in maintaining a healthy cornea and promoting wound healing after eye injuries [5]. The results of in vitro studies suggested that there is a trophic support between corneal epithelial cells and neurons. For example, trigeminal neurons release neurotransmitters and neuropeptides to provide for corneal epithelial cell growth, proliferation, differentiation, and type VII collagen production [25, 26]. Accordingly, corneal epithelial cells release soluble factors such as NGF and GDNF that stimulate neurite survival and extension [27–29]. Lambiase et al. reported that stromal keratocytes also produce neurotrophins, for example, neurotrophins 3 and 4 [30–32]; however their trophic influences on corneal nerve fibers still remain undetermined. BDNF is found in the corneal epithelial cells and stromal keratocytes, and it is believed to be also contained in corneal sensory neurons [24, 32].

Nerve-derived trophic factors regulate the biochemistry of the corneal epithelium and control the normal and renewal processes of maintaining the corneal epithelial cells. Thus, patients with impaired corneal innervation, for example, after herpetic keratitis, diabetes, prolonged contact lens wear, advanced age, and refractive surgery, are at increased risk of corneal damage because of diminished trophic support [24, 33]. Ferrari et al. reported that nerve-secreted neuropeptides influence corneal cell proliferation in vitro, and the rate of corneal epithelial cell mitosis is altered in denervated corneas of rats [34]. Ciliary neurotrophic factor (CNTF) has been detected in corneal endothelial cells [35]. Zhou et al. discovered that the mRNA of CNTF was more significantly downregulated in diabetic mice than in normal mice [36]. A subconjunctival injection of CNTF significantly reduced the size of the corneal epithelial defect of 67.89 ± 12.27% to 30.10% ± 10.13% after 48 hours. These results suggest that impaired corneal epithelial wound healing in diabetic mice can be caused by reduced levels of CNTF, and CNTF supplementation can promote corneal epithelial wound healing by activating corneal epithelial stem/progenitor cells [36].

In a very recent study, Gao et al. 2016 demonstrated that dendritic cells mediate sensory nerve innervation and regeneration through CNTF, and diabetes reduces the dendritic cells populations in both normal and injured corneas. This then results in decreased CNTF and impaired sensory nerve innervation and regeneration [37]. They also suggested that diabetes disrupted the neural communications of dendritic cells resulting in diabetic neuropathy and impairs sensory nerve regeneration in the cornea. Thus, dendritic cell-based therapy should be explored for diabetic neuropathy [37].

A detailed listing of the neurotrophic factors present in the cornea is shown in Table 1.

4. Corneal Sensitivity in Diabetes

Patients with diabetes have decreased corneal sensitivity and thus are very vulnerable to trauma. In the study by Nielsen it was demonstrated that corneal sensitivity (determined using Cochet and Bonnet's aesthesiometer) in 83% of diabetic patients was reduced below 60 mm against 38% of the controls alongside with reduced perception of vibrations (vibratory perception of the left index finger and great toe by biothesiometer) [55]. A decrease in corneal sensitivity may cause a delay in epithelial wound healing and be the cause of recurrent erosions. This is because the corneal nerves release epitheliotropic substances that promote the maintenance of the integrity of corneal surface [56]. Alterations of the corneal nerves decrease the corneal sensitivity resulting in corneal hypoesthesia that disrupts the epithelial architecture and function. These changes would further delay the reepithelization of the cornea.

Confocal microscopy has shown promise as a noninvasive method for quantifying the damage and repair of corneal sensory nerves that can serve as markers for diabetic neuropathy. Thus, Rosenberg et al. using confocal microscopy found decreased corneal sensitivity together with a decreased number of long nerve fiber bundles in the subbasal nerve plexus. In addition, patients with diabetes had fewer nerve fiber bundles than healthy control subjects possibly due to the presence of polyneuropathy. In all patients with diabetes with neuropathy, the subbasal nerve densities were significantly reduced [57]. Additionally, the authors found that even if most patients with diabetes had nerve fiber bundles with a normal morphology, patients under dialysis with mild to moderate neuropathy had abnormally tortuous nerve fiber bundles in the subbasal nerve plexus. This confirmed the presence of an impairment of corneal sensitivity, and the
Table 1: Neurotrophic factors in the cornea.

| Growth factor                               | Healthy cornea                                                                 | Injured cornea                                                                 | Topical application                                                                 |
|---------------------------------------------|-------------------------------------------------------------------------------|--------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| Nerve growth factor (NGF)                   | (i) Found in corneal epithelium and stromal keratocytes                       | Upregulated during reinnervation after nerve surgical transection [41], and in dry eye syndrome [42], in inflamed conjunctiva of patients with vernal keratoconjunctivitis [43] | (i) Augments corneal wound healing and provides recovery of corneal sensitivity and photophobia [44], (ii) Has potent antiviral properties (restrict herpes simplex virus-1 [45]) |
|                                            | (ii) Critical for corneal nerve survival and maintenance, axonal branching, elongation, neuronal sprouting, and regeneration following nerve damage [40] |                                                                                  |                                                                                   |
| Brain-derived neurotrophic factor (BDNF)    | (i) Found in corneal epithelium and stromal keratocytes, originate from corneal sensory neurons [32] | Expressed after experimental flap surgery in putative corneal stromal and/or inflammatory cells in a positive association with neurite extension [40] | Produces complete epithelial healing in a patient with a progressive neurotrophic ulcer [46] |
|                                            | (ii) Exact role related to corneal nerves is unclear                           |                                                                                  |                                                                                   |
| Glial cell-derived neurotrophic factor (GDNF) | Expressed in human corneal stromal keratocytes and may operate similarly to or synergistically with NGF by triggering gene transcription governing epithelial cell migration and wound healing [32] | Possibly plays an important role in corneal regeneration and wound healing [46] |                                                                                   |
| Neurotrophins 3, 4/5 (NT-3, NT-4/5)         | (i) NT-3 transcribed in epithelial cells and stromal keratocytes [32]         | Minimal changes in NT-3 gene expression following surgical transection of corneal nerves [41] |                                                                                   |
|                                            | (ii) NT-4 is present in corneal epithelium and is a neurotrophic factor that may be involved in the regulation of stromal keratocytes by epithelial cells [32] |                                                                                  |                                                                                   |
| Ciliary neurotrophic factor (CNTF)          | Promotes corneal epithelial wound healing by activating corneal epithelial stem/progenitor cells [47] | (i) Upregulated in corneal epithelium after injury in mice [47]                  | VEGF supplementation promotes trigeminal nerve repair, and abrogation of VEGF signaling reduces corneal nerve growth [48, 49] |
| Vascular endothelial growth factor (VEGF)    | Minimally present [48]                                                       | (i) Upregulated in the injured cornea [48]                                      |                                                                                   |
|                                            |                                                                                 | (ii) Required for efficient corneal nerve regeneration                          |                                                                                   |
| Hepatocyte growth factor (HGF)              | Expressed in stromal keratocytes, stimulates corneal epithelial proliferation [50] | Upregulated after corneal epithelial wounding and probably contributes to the epithelial wound healing process [49] |                                                                                   |
| Keratocyte growth factor (KGF)              | (i) Expressed in stromal keratocytes [32], fibroblasts [51]                   |                                                                                  |                                                                                   |
|                                            | (ii) Stimulates corneal epithelial proliferation, acts specifically on cells of epithelial origin as a paracrine mediator [51] |                                                                                  |                                                                                   |
| Transforming growth factor-α (TGF-α), interleukin-1β (IL-1β), and platelet-derived growth factor-B (PDGF-B) | (i) Exclusively expressed in the corneal stroma [53] |                                                                                  |                                                                                   |
|                                            | (ii) TGF-α and IL-1β can upregulate the transcription of neurotrophins, such as NGF in 3T3 mouse fibroblasts [54] |                                                                                  |                                                                                   |
duration of diabetes was significantly and directly correlated with the degree of polyneuropathy.

In the subbasal nerve plexus, a decrease in the nerve density, number of branches, single nerve fiber length, and increased tortuosity have been found to be significantly correlated with established electromyography and nerve conduction parameters and with the results of the skin biopsy [58, 59].

Tavakoli et al. suggested using confocal microscopy in longitudinal studies to assess progression of diabetic neuropathy [60]. In their recent study it was shown that patients with diabetic autonomic neuropathy had a progressive and significant reduction of corneal nerve fiber density, branch density, and length compared to healthy control subjects [61]. Misra et al. also described a clinical application of in vivo confocal microscopy in patients with diabetes mellitus type 1 [62]. They measured the corneal nerve parameters in type 1 diabetics and controls and confirmed a decrease in the subbasal nerve density and corneal sensitivity in diabetic patients. They also found a significant relationship between corneal neuropathy and systemic neuropathy. They concluded that corneal neuropathy might be an early indicator of diabetic neuropathy because it preceded other clinical and electrophysiology tests of neuropathy [62]. Also Pritchard et al. reported application of confocal microscopy in assessment of diabetic polyneuropathy [63] by measuring the corneal nerve fiber length, ability of confocal microscopy to predict the development of diabetic polyneuropathy with 63% sensitivity and 74% specificity, for a corneal nerve fiber length threshold cutoff of 14.1 mm/mm² was demonstrated [63].

Several studies have confirmed that the cornea innervations were altered in animal models of diabetes. Davidson et al. reported a 50% loss of corneal nerve fibers after 12 weeks of high fat diet in low-dose streptozotocin-induced diabetic rats [64]. Yin et al. described corneal changes in streptozotocin-induced type 1 diabetic rats. A 50% decrease in tear secretion was found after eight weeks of diabetes induction in SD rats, corneal sensitivity was decreased, and the corneal nerves had fewer branches and were thinner and shorter by 75% [65].

Ueno et al. found a decreased density of the corneal subbasal nerve plexus and corneal epithelial branches in leptin receptor mutant mice which are an accepted animal model of type 2 diabetes. The corneal subbasal nerves were more tortuous in these mutant mice than in normal mice [66].

Wang et al. reported delayed corneal wound healing in the Akita diabetic mice, a model of chronic complications of type 1 DM [67]. In a longitudinal study of corneal nerve density in a rat model of type 1 diabetes, Chen et al. found that density of nerves was initially increased in the subbasal plexus after 8 and 16 weeks of diabetes. However, the density remained unchanged in the stromal layer [68]. An increase could be explained by increased nerve tortuosity or collateral sprouting as reported in patients with impaired glucose tolerance [69].

It is difficult to translate the results from the streptozotocin-induced animals to type 1 human diabetes and that from the db/db mouse to type 2 human diabetes due to recessive homozygous mutation in the leptin receptor (fa/fa) [70, 71]. Yorek et al., 2015, studied C57Bl/6J mice fed a high fat diet that caused an elevated level of glucose in the fasting blood, and they demonstrated that the diet-induced obesity led to the development and progression of peripheral neuropathy and nerve structural damage in the cornea with or without hyperglycemia [72].

Diabetic neurotrophic keratopathy, a manifestation of diabetic polyneuropathy, also plays a significant role in limiting epithelial wound healing. Neurotrophic keratopathy according to Mackie [73] has three stages: Stage 1, positive Bengal staining of the inferior palpebral conjunctival surface, punctate keratopathy, and a decrease in tear breakup time; Stage 2, epithelial breakdown with epithelial deficits surrounded by loose epithelium that becomes hazy, edematous, and poorly adherent to Bowman's layer; and Stage 3, corneal ulceration that can lead to melting and perforation of the cornea.

5. Therapeutical Management

A diabetic corneal ulcer is a challenging clinical condition. The development of persistent corneal epithelial defects is often associated with severe peripheral neuropathy. The success in the management of neurotrophic keratopathy is generally based on achieving epithelial healing and preventing a progression of the corneal damage. Pathogenetic-oriented pharmacological treatment for neurotrophic keratopathy is still not available. Preservative-free artificial tears, topical antibiotics, bandage contact lenses, amniotic membrane transplantation, tarsorrhaphy, and a conjunctival flap are useful methods that have been used to treat refractory neurotrophic corneal ulcers [74]. Therefore, the establishment of a pathogenetic treatment that can lead to neuroprotection and neuroregeneration is extremely important.

Different nerve-secreted factors, such as NGF and BDNF, are important factors necessary for epithelial regeneration [75–77]. The application of exogenous NGF was able to reverse the damage of peripheral nerves and completely heal corneal epithelial defects in patients with neurotrophic keratitis [44]. According to Ueno, corneal stem/progenitor cells are closely associated with corneal wound healing and insulin-like growth factor-I administration may be a promising agent that can be used to prevent persistent corneal ulcers in patients with type 2 diabetes. Tavakoli et al. examined patients with type 1 diabetes by confocal microscopy after simultaneous pancreas-kidney transplantation, and they obtained evidence of an early regeneration of corneal nerves [78]. Their following study showed that continuous subcutaneous insulin infusion showed an improvement in corneal nerve morphology, consistent with small fiber regeneration (assessed by confocal microscopy), most probably due to more stable blood glucose control [79].

By accelerating neuronal cell death, diabetes can lead to inhibiting neurite regeneration. Dai et al., 2015, reported that the neurites were significantly longer in the neuropeptide FF-treated diabetic neurons compared with the nontreated controls in primary cultured diabetic trigeminal sensory neurons [80]. They concluded that neuropeptide FF provided nerve growth-promoting effects to the db/db mice through...
the ERK1/2 pathway which is known to play a central role in controlling Schwann cell plasticity and peripheral nerve regeneration [81].

Recent laboratory investigations have shown that new corneal nerve modulators, for example, pigment epithelial-derived factor (PEDF), with DHA enhance the regeneration of corneal nerves and the recovery of corneal sensitivity following corneal nerve damage [82]. Ishibashi et al. reported that a PEDF-derived synthetic modified peptide inhibits tubular cell damage through its antioxidative properties under diabetes-like conditions. This suggested that supplementation of modified PS-3 peptide may be a therapeutic strategy for diabetic nephropathy [83]. Oswald et al., 2012, suggested intranasal delivery of nanomolecule curcumin solution promoted corneal epithelial/nerve healing in diabetic mice. This was because the pharmacologically active ingredient was delivered to the trigeminal ganglion neuron and thus helps in the diabetic corneal epithelial/nerve wound healing [38].

There are other reports that miRNAs, including miR-21 and miR-29b, can be used as therapeutic agents to stimulate peripheral nerve regeneration [84, 85]. Wang et al. reported that miR-182 can protect trigeminal neurons from peripheral nerve damage in an experimental mouse model of diabetes. They suggested that this was accomplished by its ability to enhance neurite outgrowth in isolated trigeminal sensory neurons. This overcame the detrimental effects of hyperglycemia by stimulating corneal nerve regeneration by decreasing the expression of one of its target genes, NOX4 [86].

Other approaches include gene therapy [87] and use of α-Lipoic Acid in Soluplus readily renders nanomicelles as alternative ocular treatment of diabetes-associated corneal diseases had been reported recently [88].

6. Conclusions

Impaired corneal innervation in diabetes is an important early indicator of diabetic neuropathy. The decrease in corneal sensitivity with a longer duration of diabetes is correlated with the severity of neuropathy. Further investigations on mechanisms of corneal nerve damage and establishment of pathogenetic-oriented therapy for diabetic neuropathy should be the main focus of future research.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This paper is supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of the Japanese Government. The authors thank Professor Duco Hamasaki of the Bascom Palmer Eye Institute of the University of Miami for editing the manuscript.

References

[1] S. Tesfaye, A. J. M. Boulton, P. J. Dyck et al., "Diabetic neuro-ropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments," Diabetes Care, vol. 33, pp. 2285–2293, 2010.
[2] T. Oshitari, M. Dezawa, S. Okada et al., "The role of c-fos in cell death and regeneration of retinal ganglion cells," Investigative Ophthalmology and Visual Science, vol. 43, no. 7, pp. 2442–2449, 2002.
[3] T. Oshitari, G. Bikkova, and S. Yamamoto, "Increased expression of phosphorylated c-Jun and phosphorylated c-Jun N-terminal kinase associated with neuronal cell death in diabetic and high glucose exposed rat retinas," Brain Research Bulletin, vol. 101, pp. 18–25, 2014.
[4] T. Oshitari, S. Yamamoto, N. Hata, and S. Roy, "Mitochondria- and caspase-dependent cell death pathway involved in neuronal degeneration in diabetic retinopathy," British Journal of Oph-thalmology, vol. 92, no. 4, pp. 552–556, 2008.
[5] L. J. Müller, C. F. Marfurt, F. Kruse, and T. M. T. Tervo, "Corneal nerves: structure, contents and function," Experimental Eye Research, vol. 76, no. 5, pp. 521–542, 2003.
[6] E. Zander and G. Weddel, "Observations on the innervation of the cornea," Journal of Anatomy, vol. 85, no. 1, pp. 68–99, 1951.
[7] C. F. Marfurt, "Corneal nerves: anatomy," in Encyclopedia of the Eye, D. A. DarT, Ed., pp. 485–492, Elsevier, Oxford, UK, 2010.
[8] A. R. Vonderheide, "Corneal and scleral anesthesia of the lower hale of the eye in a case of trauma of the superior maxillary nerve," Archives of Neurology & Psychiatry, vol. 20, no. 4, pp. 836–837, 1928.
[9] G. L. Russell, "Ocular fibres of the maxillary nerve in monkeys," Journal of Anatomy, vol. 118, part 2, pp. 195–203, 1974.
[10] C. F. Marfurt, C. J. Murphy, and J. L. Florczak, "Morphology and neurochemistry of canine corneal innervation," Investigative Ophthalmology & Visual Science, vol. 42, no. 10, pp. 2242–2251, 2001.
[11] L. J. Müller, L. Pels, and G. F. J. M. Vrensen, "Ultrastructural organization of human corneal nerves," Investigative Ophthalmology & Visual Science, vol. 37, no. 4, pp. 476–488, 1996.
[12] M. A. Jones and C. F. Marfurt, "Peptidergic innervation of the rat cornea," Experimental Eye Research, vol. 66, no. 4, pp. 421–435, 1998.
[13] C. F. Marfurt, J. Cox, S. Deek, and L. Dvorscak, "Anatomy of the human corneal innervation," Experimental Eye Research, vol. 90, no. 4, pp. 478–492, 2010.
[14] H. Beckers, J. Klooster, G. Vrensen, and W. Lamers, "Sympathetic innervation of the rat’s eye and peripheral ganglia: an electron microscopic autoradiographic tracing study," Graefes Archive for Clinical and Experimental Ophthalmology, vol. 232, no. 1, pp. 57–65, 1994.
[15] D. V. Patel and C. N. J. McGhee, "Mapping of the normal human corneal sub-basal nerve plexus by in vivo laser scanning confocal microscopy," Investigative Ophthalmology & Visual Science, vol. 46, no. 12, pp. 4485–4488, 2005.
[16] D. V. Patel and C. N. J. McGhee, "Mapping the corneal sub-basal nerve plexus in keratoconus by in vivo laser scanning confocal microscopy," Investigative Ophthalmology & Visual Science, vol. 47, no. 4, pp. 1348–1351, 2006.
[17] T. Møller-Pedersen, H. F. Li, W. M. Pettor, H. D. Cavanagh, and J. V. Jester, "Confocal microscopic characterization of wound repair after photorefractive keratectomy," Investigative
H. Yokogawa, A. Kobayashi, and K. Sugiyama, “Mapping of normal corneal anterior collagen fiber bundles by in vivo laser scanning confocal microscopy,” Investigative Ophthalmology & Visual Science, vol. 48, no. 13, article 3853, 2007.

L. Henderson, D. Bond, and T. Simpson, “The association between eye color and corneal sensitivity measured using a belmonte esthesiometer,” Optometry and Vision Science, vol. 82, no. 7, pp. 629–632, 2005.

C. Belmonte, M. C. Acosta, and J. Gallar, “Neural basis of sensation in intact and injured corneas,” Experimental Eye Research, vol. 78, no. 3, pp. 513–525, 2004.

G. Bikbova, T. Oshitari, T. Baba, and S. Yamamoto, “Altered expression of NF-κB and SPI after exposure to advanced glycation end-products and effects of neurotrophic factors in AGE-exposed rat retinas,” Journal of Diabetes Research, vol. 2015, Article ID 543818, 11 pages, 2015.

G. Bikbova, T. Oshitari, T. Baba, and S. Yamamoto, “Mechanisms of neuronal cell death in AGE-exposed retinas—research and literature review,” Current Diabetes Reviews, in press.

I. G. Obrosova and U. A. Julius, “Role for poly(ADP-ribose) polymerase activation in diabetic nephropathy, neuropathy and retinopathy,” Current Vascular Pharmacology, vol. 3, no. 3, pp. 267–283, 2005.

Y.-S. Byun, B. Kang, Y.-S. Yoo, and C.-K. Joo, “Poly(ADP-ribose) polymerase inhibition improves corneal epithelial innervation and wound healing in diabetic rats,” Investigative Ophthalmology & Visual Science, vol. 56, no. 3, pp. 1948–1955, 2015.

K. S. Baker, S. C. Anderson, E. G. Romanowski, R. A. Thoft, and N. SundarRaj, “Trigeminal ganglion neurons affect corneal epithelial phenotype. Influence on type VII collagen expression and Nerve Growth Factor as a pharmacological tool for human corneal and skin ulcers,” Pharmacological Research, vol. 57, no. 4, pp. 253–258, 2008.

A. Lambiase, M. Coassini, N. Costa et al., “Topical treatment with nerve growth factor in an animal model of herpetic keratitis,” Graefes Archive for Clinical and Experimental Ophthalmology, vol. 246, no. 1, pp. 121–127, 2008.

L. You, S. Ebner, and F. E. Kruse, “Glia cell-derived neurotrophic factor (GDNF)-induced migration and signal transduction in corneal epithelial cells,” Investigative Ophthalmology & Visual Science, vol. 42, no. 11, pp. 2496–2504, 2001.

L. You, E. F. Kruse, and H. E. Völcker, “Neurotrophic factors in the human cornea,” Investigative Ophthalmology & Visual Science, vol. 41, no. 3, pp. 692–702, 2000.

M. Yamada, M. Ogawa, M. Kawai, and Y. Mashima, “Decreased substance P concentrations in tears from patients with corneal hypesthesia,” American Journal of Ophthalmology, vol. 129, no. 5, pp. 671–672, 2000.

G. Ferrari, S. K. Chauhan, H. Ueno et al., “A novel mouse model for neurotrophic keratopathy: trigeminal nerve stereotactic electrolysis through the brain,” Investigative Ophthalmology & Visual Science, vol. 52, no. 5, pp. 2532–2539, 2011.

Z. Li, A. R. Burns, L. Han, R. E. Rumbaut, and C. W. Smith, “IL-17 and VEGF are necessary for efficient corneal nerve
regeneration,” American Journal of Pathology, vol. 178, no. 3, pp. 1106–1116, 2011.

[49] C. Q. Yu, M. Zhang, K. I. Matis, C. Kim, and M. I. Rosenblatt, “Vascular endothelial growth factor mediates corneal nerve repair,” Investigative Ophthalmology & Visual Science, vol. 49, no. 9, pp. 3870–3878, 2008.

[50] Q. Li, J. Weng, R. R. Mohan et al., “Hepatocyte growth factor and hepatocyte growth factor receptor in the lacrimal gland, tears, and cornea,” Investigative Ophthalmology & Visual Science, vol. 37, no. 5, pp. 727–739, 1996.

[51] C. Sotozono, S. Kinoshita, M. Kita, and J. Imanishi, “Paracrine role of keratinocyte growth factor in rabbit corneal epithelial cell growth,” Experimental Eye Research, vol. 59, no. 4, pp. 385–392, 1994.

[52] S. E. Wilson, L. Chen, R. R. Mohan, Q. Liang, and J. Liu, “Expression of HGF, KGF, EGF and receptor messenger RNAs following corneal epithelial wounding,” Experimental Eye Research, vol. 68, no. 4, pp. 377–397, 1999.

[53] D.-Q. Li and S. C. G. Tseng, “Three patterns of cytokine expression potentially involved in epithelial-fibroblast interactions of human ocular surface,” Journal of Cellular Physiology, vol. 163, no. 1, pp. 61–79, 1995.

[54] A. Hattori, S. Iwasaki, K. Murase et al., “Tumor necrosis factor is markedly synergistic with interleukin 1 and interferon-y in stimulating the production of nerve growth factor in fibroblasts,” FEBS Letters, vol. 340, no. 3, pp. 177–180, 1994.

[55] N. V. Nielsen, “Corneal sensitivity and vibratory perception in diabetes mellitus,” Acta Ophthalmologica, vol. 56, no. 3, pp. 406–411, 1978.

[56] P. G. Mylonas, P. T. Matsouka, E. V. Papandioniou, C. Vagianos, F. Kalfarentzos, and T. K. Alexandridis, “Growth hormone and insulin-like growth factor I protect intestinal cells from radiation induced apoptosis,” Molecular and Cellular Endocrinology, vol. 160, no. 1-2, pp. 115–122, 2000.

[57] M. E. Rosenberg, T. M. T. Tervo, I. J. Immonen, L. J. Muller, C. Gronhagen-Riska, and M. H. Vesaluoma, “Corneal structure and sensitivity in type 1 diabetes mellitus,” Investigative Ophthalmology & Visual Science, vol. 41, no. 10, pp. 2915–2921, 2000.

[58] J. Tavee and L. Zhou, “Small fiber neuropathy: a burning problem,” Cleveland Clinic Journal of Medicine, vol. 76, no. 5, pp. 297–305, 2009.

[59] N. Papanas and D. Ziegler, “Corneal confocal microscopy: a new technique for early detection of diabetic neuropathy,” Current Diabetes Reports, vol. 13, no. 4, pp. 488–499, 2013.

[60] M. Tavakoli, P. Kallinikos, A. Iqbal et al., “Corneal confocal microscopy detects improvement in corneal nerve morphology with an improvement in risk factors for diabetic neuropathy,” Diabetic Medicine, vol. 28, no. 10, pp. 1261–1267, 2011.

[61] M. Tavakoli, P. Begum, J. Mclaughlin, and R. A. Malik, “Corneal confocal microscopy for the diagnosis of diabetic autonomic neuropathy,” Muscle and Nerve, vol. 52, no. 3, pp. 363–370, 2015.

[62] S. L. Misra, J. P. Craig, D. V. Patel et al., “In vivo confocal microscopy of corneal nerves: an ocular biomarker for peripheral and cardiac autonomic neuropathy in type 1 diabetes mellitus,” Investigative Ophthalmology & Visual Science, vol. 56, no. 9, pp. 5060–5065, 2015.

[63] N. Pritchard, K. Edwards, A. W. Russell, B. A. Perkins, R. A. Malik, and N. Efro, “Corneal confocal microscopy predicts 4-year incident peripheral neuropathy in type 1 diabetes,” Diabetes Care, vol. 38, no. 4, pp. 671–675, 2015.

[64] E. P. Davidson, L. J. Coppey, and M. A. Yorek, “Early loss of innervation of cornea epithelium in streptozotocin-induced type 1 diabetic rats: improvement with ilepatril treatment,” Investigative Ophthalmology & Visual Science, vol. 53, no. 13, pp. 8067–8074, 2012.

[65] J. Yin, J. Huang, C. Chen, N. Gao, F. Wang, and X. Y. Fu-Shin, “Corneal Complications in streptozotocin-induced type I diabetic rats,” Investigative Ophthalmology and Visual Science, vol. 52, no. 9, pp. 6589–6596, 2011.

[66] H. Ueno, T. Hattori, Y. Kumagai, N. Suzuki, S. Ueno, and H. Takagi, “Alterations in the corneal nerve and stem/progenitor cells in diabetes: preventive effects of insulin-like growth factor-1 treatment,” International Journal of Endocrinology, vol. 2014, Article ID 312401, 8 pages, 2014.

[67] Y. Wang, X. Zhao, D. Shi et al., “Overexpression of SIRT1 promotes high glucose-attenuated corneal epithelial wound healing via p53 regulation of the IGFBP3/GF-IR/AKT pathway,” Investigative Ophthalmology & Visual Science, vol. 54, no. 5, pp. 3806–3814, 2013.

[68] D. K. Chen, K. E. Frizzi, L. S. Guernsey, K. Ladt, A. P. Mizisin, and N. A. Calcutt, “Repeate monitoring of corneal nerves by confocal microscopy as an index of peripheral neuropathy in type-1 diabetic rodents and the effects of topical insulin,” Journal of the Peripheral Nervous System, vol. 18, no. 4, pp. 306–315, 2013.

[69] M. Tavakoli, A. Marshall, R. Pitecaethey et al., “Corneal confocal microscopy: a novel means to detect nerve fibre damage in idiopathic small fibre neuropathy,” Experimental Neurology, vol. 223, no. 1, pp. 245–250, 2010.

[70] J. E. Davis, J. Cain, W. J. Banz, and R. G. Peterson, “Age-related differences in response to high-fat feeding on adipose tissue and metabolic profile in ZDSD rats,” ISRN Obesity, vol. 2013, Article ID 584547, 8 pages, 2013.

[71] S. Reinwald, R. G. Peterson, M. R. Allen, and D. B. Burr, “Skeletal changes associated with the onset of type 2 diabetes in the ZDF and ZDSD rodent models,” American Journal of Physiology—Endocrinology and Metabolism, vol. 296, no. 4, pp. E765–E774, 2009.

[72] M. S. Yorek, A. Obrosov, H. Shevalye et al., “Effect of diet-induced obesity or type 1 or type 2 diabetes on corneal nerves and peripheral neuropathy in C57BL/6J mice,” Journal of the Peripheral Nervous System, vol. 20, no. 1, pp. 24–31, 2015.

[73] I. Mackie, “Neuroparalytic keratitis,” in Current Ocular Therapy, F. Fraunfelder and F. Roy, Eds., Philadelphia, PA, USA, pp. 506–508, WB Saunders, 4th edition, 1995.

[74] M. Sacchetti and A. Lambiase, “Diagnosis and management of neurotrophic keratitis,” Clinical Ophthalmology, vol. 8, pp. 571–579, 2014.

[75] K. Araki-Sasaki, S. Aizawa, M. Hiramoto et al., “Substance P-induced cadherin expression and its signal transduction in a cloned human corneal epithelial cell line,” Journal of Cellular Physiology, vol. 182, no. 2, pp. 189–195, 2000.

[76] A. Solomon, D. Meller, P. Prabhasawat et al., “Amniotic membrane grafts for nontraumatic corneal perforations, descemetoceles, and deep ulcers,” Ophthalmology, vol. 109, no. 4, pp. 694–703, 2002.

[77] H.-J. Chen, R. T. F. Pires, and S. C. G. Tseng, “Amniotic membrane transplantation for severe neurotrophic corneal ulcers,” British Journal of Ophthalmology, vol. 84, no. 8, pp. 826–833, 2000.

[78] M. Tavakoli, M. Mitu-Pretorian, I. N. Petropoulos et al., “Corneal confocal microscopy detects early nerve regeneration in diabetes,” Diabetes Reports, vol. 7, no. 1, pp. 58–64, 2014.
in diabetic neuropathy after simultaneous pancreas and kidney transplantation,” *Diabetes*, vol. 62, no. 1, pp. 254–260, 2013.

[79] S. Azmi, M. Ferdousi, I. N. Petropoulos et al., “Corneal confocal microscopy shows an improvement in small-fiber neuropathy in subjects with type 1 diabetes on continuous subcutaneous insulin infusion compared with multiple daily injection,” *Diabetes Care*, vol. 38, no. 1, pp. e3–e4, 2015.

[80] Y. Dai, X. Zhao, P. Chen, Y. Yu, Y. Wang, and L. Xie, “Neuropeptide FF promotes recovery of corneal nerve injury associated with hyperglycemia,” *Investigative Ophthalmology & Visual Science*, vol. 56, no. 13, pp. 7754–7765, 2015.

[81] I. Napoli, L. A. Noon, S. Ribeiro et al., “A central role for the ERK-signaling pathway in controlling Schwann cell plasticity and peripheral nerve regeneration in vivo,” *Neuron*, vol. 73, no. 4, pp. 729–742, 2012.

[82] M. S. Cortina, J. He, N. Li, N. G. Bazan, and H. E. P. Bazan, “Recovery of corneal sensitivity, calcitonin gene-related peptide-positive nerves, and increased wound healing induced by pigment epithelial-derived factor plus docosahexaenoic acid after experimental surgery,” *Archives of Ophthalmology*, vol. 130, no. 1, pp. 76–83, 2012.

[83] Y. Ishibashi, T. Matsui, J. Taira, Y. Higashimoto, and S. Yamagishi, “Protective role of PEDF-derived synthetic peptide against experimental diabetic nephropathy,” *Hormone and Metabolic Research*, vol. 48, no. 09, pp. 613–619, 2016.

[84] I. T. Strickland, L. Richards, F. E. Holmes, D. Wynick, J. B. Uney, and L.-F. Wong, “Axotomy-induced miR-21 promotes axon growth in adult dorsal root ganglion neurons,” *PLoS ONE*, vol. 6, no. 8, Article ID e23423, 2011.

[85] A. J. Kole, V. Swahari, S. M. Hammond, and M. Deshmukh, “miR-29b is activated during neuronal maturation and targets BH3-only genes to restrict apoptosis,” *Genes & Development*, vol. 25, no. 2, pp. 125–130, 2011.

[86] Y. Wang, X. Zhao, X. Wu, Y. Dai, P. Chen, and L. Xie, “MicroRNA-182 mediates Sirt1-induced diabetic corneal nerve regeneration,” *Diabetes*, vol. 65, no. 7, pp. 2020–2031, 2016.

[87] A. A. Kramerov, M. Saghizadeh, and A. V. Ljubimov, “Adenoviral gene therapy for diabetic keratopathy: effects on wound healing and stem cell marker expression in human organ-cultured corneas and limbal epithelial cells,” *Journal of Visualized Experiments*, no. 110, Article ID e54058, 2016.

[88] F. Alvarez-Rivera, D. Fernández-Villanueva, A. Concheiro, and C. Alvarez-Lorenzo, “α-lipoic acid in soluplus® polymeric nanomicelles for ocular treatment of diabetes-associated corneal diseases,” *Journal of Pharmaceutical Sciences*, vol. 105, no. 9, pp. 2855–2863, 2016.