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REVIEW

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Corneal Innervation and Sensation: The Eye and Beyond

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The cornea is one of the most densely innervated and sensitive tissues in the body. In addition to their important sensory functions, corneal nerves induce reflex tear production, blinking, and the release of trophic factors – all of which combined help to maintain the structural and functional integrity of the surface of the eye. Consequently, damage to corneal nerves as a result of disease, surgery, or trauma can lead to diminished corneal sensitivity, epithelial defects, and possible blindness. In this review, we describe commonly used tools that have provided considerable new information on corneal architecture and sensation in healthy and diseased corneas, with special emphasis on changes seen in herpes zoster ophthalmicus, corneal and other therapeutic ocular procedures, antiglaucoma medical therapy, aging, and diabetes. With its potential applications ranging from managing ocular-specific to systemic diseases, the study of corneal innervation has implications for future therapies extending beyond just the eye itself.

INTRODUCTION

The cornea, the transparent tissue covering the front portion of the eye, is the most densely innervated tissue in the body [1]. It is comprised of five layers: the epithelium, Bowman's layer, the stroma, Descemet's membrane, and the endothelium. In 1957, Kitano demonstrated that innervation of the corneal epithelium first occurs at 5 months of gestation [2]. Corneal sensory nerves originate from the ophthalmic division of the trigeminal ganglion [3], traveling in the nasociliary nerve and its long ciliary nerve branches, and ultimately branching into nerve fibers that penetrate the cornea. These branches divide and run parallel to the superficial surface of the cornea between the basal epithelium and Bowman's layer, forming the subbasal nerve plexus (SBNP†) that supplies the overlying corneal epithelium (Figure 1) [4,5]. Extensive branching of the corneal nerve fibers produces large receptive fields for each sensory axon. This organization results in poor stimuli localization or acuity as a consequence of overlapping receptive fields, but produces an extraordinary level of sensitivity to external stimuli [6]. With a central corneal nerve density of approximately 7,000 nociceptors per square millimeter [4,7], the cornea is 300 to 600 times more sensitive than skin [8].

CORNEAL NERVE FUNCTION

Its abundant sensory nerve supply allows the cornea to transduce various thermal, mechanical, and chemical stimuli into the conscious perception of ocular dryness,

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†Abbreviations: HZO, Herpes zoster ophthalmicus; IOP, Intraocular pressure; IVCM, *in vivo* confocal microscopy; LASIK, Laser-assisted *in situ* keratomileusis; PKP, penetrating keratoplasty; PRK, photorefractive keratectomy; PRP, panretinal photocoagulation; SBNP, subbasal nerve plexus.

Keywords: corneal sensation, corneal innervation, ophthalmology, subbasal nerve plexus, ophthalmic disease

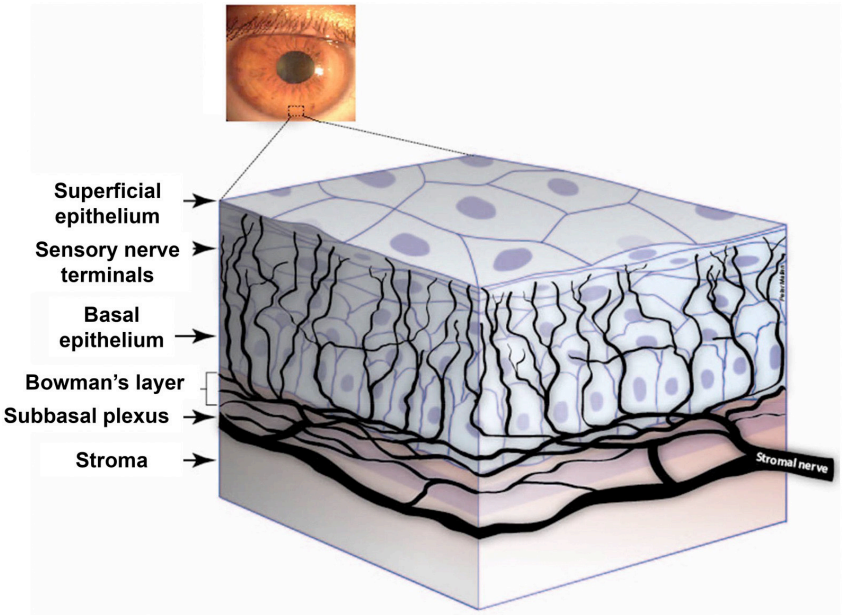

Figure 1. Diagrammatic representation of human corneal nerves. Reprinted from [5], with permission from Elsevier®.

discomfort, or pain [9]. Approximately 20 percent of corneal nociceptors are mechanoreceptors that convey acute sharp pain in response to mechanical contact with the corneal surface through thin myelinated Aδ type corneal nerves [10]. Approximately 70 percent are polymodal nociceptors, which through slow-conducting unmyelinated C type nerves, convey sharp and sustained pain in response to chemical stimuli like acetylcholine, prostaglandins, and bradykinin, as well as heat and mechanical irritants to the cornea [11]. The remaining 10 percent are Aδ and C fiber cold receptors, which fire in response to tear film evaporation and exposure of the cornea to cold solutions or air [12,13].

In response to external threats and stimuli, such as dust, pathogens, or xenobiotics, corneal nerves not only induce tear production, but also stimulate the blinking reflex through an elaborate interplay between the corneal surface and lacrimal glands [14]. Maintaining a well-lubricated and smooth ocular surface with an intact epithelium helps to minimize visual distortion and dry eye symptoms, while protecting the ocular surface. In addition, corneal nerves release numerous trophic substances such as neuropeptides, neurotrophins, and growth factors that are crucial to regulating proliferation of corneal epithelium, epithelial integrity, and wound healing in the cornea [15,16]. Loss of sensory innervation of the cornea can result in a vision-threatening clinical condition known as neurotrophic keratopathy, which is characterized by reduced corneal sensation, tear film abnormalities and, in the most severe cases, persistent corneal epithelial defects, ulceration, and perforation of the stroma. Many other diseases are associated with decreased corneal sensitivity in humans, including herpetic keratitis, keratoconjunctivitis sicca, and keratoconus [4,17].

Various neurotransmitters, including substance P (SP), calcitonin gene-related peptide (CGRP), neuropeptide Y, vasoactive intestinal peptide, catecholamines, and acetylcholine are present in the cornea [15]. SP is released directly from corneal fibers following inflammation, and has received increasing attention given its effects on promoting epithelial proliferation and corneal wound healing in synergism with insulin-like growth factor-1 (IGF-1) and epidermal growth factor (EGF) [6,18]. On the basis of this research, investigators have formulated eye drops containing peptide sequences derived from SP and IGF-1 for the treatment of persistent corneal epithelial defects associated with neurotrophic keratopathy [19]. CGRP, among other neurotransmitters, is also involved in neurogenic inflammation and has been shown to enhance corneal epithelial healing *in vitro* [20].

Neurotrophic factors are another family of biomolecules thought to maintain homeostasis and repair of the cornea. They include nerve growth factor (NGF), neurotrophin 3 (NT-3), neurotrophin 4/5 (NT-4), brain-derived neurotrophic factor (BDNF), ciliary neurotropic factor (CNTF), and glial cell-derived neurotrophic factor (GDNF) as well as their corresponding tyrosine receptor kinase (TrK) receptors, many of which are expressed in the human cornea [21]. Mice with wounded corneas treated with CNTF-containing eye drops showed an increase in nerve fiber density 8 weeks after wounding [22]. NGF is

expressed in the cornea during re-innervation after nerve surgical transection [21]. Both NGF and GDNF promote epithelial colony formation and proliferation [23]. This has translated into human case studies and clinical trials where NGF has been shown to improve non-healing epithelial defects such as corneal neurotrophic ulcers, with return of corneal sensation [24,25]. BDNF is capable of inducing both central and peripheral nerve growth and regeneration [26], and stimulating other neurotrophic factors such as NT-4/5 and Gap-43 to achieve neurite re-growth [27]. On the other hand, loss of neurotrophic signaling negatively impacts corneal nerve function. For example, inactivation of TrkA, results in loss of nociceptive neurons, stromal nerves, and corneal epithelium, as well as reduced response to noxious stimuli [28].

Thus, corneal sensation is critical to the structural and functional integrity of the ocular surface. Surgical procedures, trauma, and disease in the eye can all potentially damage corneal nerves and diminish sensation, resulting in transient or long-lasting ocular complications.

ASSESSING THE SUBBASAL CORNEAL NERVE PLEXUS

Corneal nerves of the adult human eye have been extensively studied both *ex vivo* and *in vivo*, with the aim of capturing changes in morphology via imaging or qualitative assessment, in addition to quantitative assessment of corneal sensitivity and pain [29,30]. However, *ex vivo* studies by light and electron microscopy may generate unreliable results, since human corneal nerves are known to degenerate within the first 14 hours of death [31]. As an *in vivo* exam of the cornea, slit-lamp biomicroscopy is a vital and routinely used tool in the ophthalmologist's office. Combined with use of various dyes, such as fluorescein and lissamine green, it can help reveal the general health of the ocular surface. However, a major restriction of the slit-lamp biomicroscopy is limited magnification, offering only up to 40x or 100x, and precluding visualization of the corneal cellular architecture and the SBNP.

With a growing interest in noninvasive techniques to study corneal tissue physiology with greater resolution, the invention and application of *in vivo* confocal microscopy (IVCM) enables ophthalmologists to image the cornea at the cellular level. The first scanning confocal microscope was developed by Minsky in 1988, which was followed by the invention of the tandem-, slit-, and more recently the laser-scanning confocal microscope, which allows for magnification up to 800x [32,33]. As a rapid and noninvasive technique, IVCM has many advantages and can provide images nearly comparable with *in vitro* histochemical methods [34]. IVCM has proven useful in the assessment of corneal cellular and nerve morphology in normal health, post-operative conditions, and a variety of diseases, including dry eye disease, contact lens wear, neurotrophic keratopathy, post-refractive surgery, among others [35-38]. Alterations in corneal nerve morphology in the SBNP, including nerve sprouting and thickening, reduced nerve fiber density, increased tortuosity, branching, reflectivity, neuromas, and beading, have been observed in patients with corneal pathology as compared to controls [39,40].

IVCM itself is not without limitations, however. Depending on the area and volume of the cornea sampled, and therefore the area of SBNP, findings cannot necessarily be extrapolated to the entire cornea [41]. The lack of consensus regarding quantifying or even defining SBNP density, tortuosity, beading, or branches can make it difficult to compare the results of different studies [42]. Manual analysis of these parameters is laborious, slow, and partial to human variability and bias. While research groups have developed automated analysis of IVCM images, to date, there is no commercially available software to standardize analysis [43,44].

Consequently, direct and quantitative measurement of corneal sensation in response to different stimuli has emerged as an increasingly popular method for assessing corneal nerve function. The Cochet-Bonnet esthesiometer, where a mechanical stimulus is applied to the corneal surface using nylon filaments of variable diameter and length [45], is widely used due to its portability and ease of use. The stimulus is applied successively with step-wise shortening of the filament until the patient reports a sensation. The longer the length of the filament that elicits sensation, the more sensitive the cornea. However, the Cochet-Bonnet requires contact with the eye and has a limited range of testing values, as it permits assessment only of corneal mechanoreceptors. The Belmonte's gas esthesiometer addresses some of these limitations. Investigators can adjust the flow, composition, and temperature of a fine stream of gas to more precisely apply and assess three different modalities of stimuli to the ocular surface: mechanical sensitivity to air flow, chemical sensitivity to carbon dioxide, and thermal sensitivity to different temperatures [46]. Therefore, whereas Cochet-Bonnet esthesiometers are thought to quantify only Aδ fiber function, the Belmonte esthesiometer is potentially informative of the state and function of both Aδ and C fibers.

ALTERATIONS OF CORNEAL INNERVATION AND SENSATION

Herpes Zoster Ophthalmicus

Herpes zoster ophthalmicus (HZO) or ocular zoster is

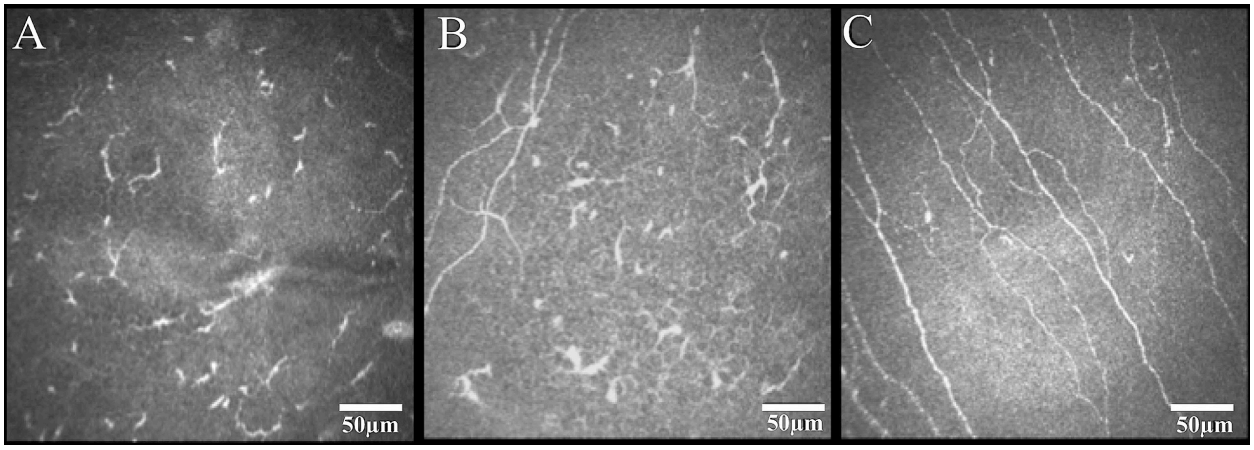

Figure 2. *In vivo* confocal microscopy images of the subbasal corneal nerve plexus in eyes with herpes zoster ophthalmicus (HZO) and controls. Both the eyes affected by HZO (**A**) and the contralateral clinically unaffected eyes (**B**) demonstrated a significant reduction in subbasal nerve plexus including number of nerves, branches, and total nerve length when compared to normal controls (**C**). Reprinted from [51], with permission from Elsevier®.

a painful and potentially devastating condition that occurs when latent varicella-zoster virus is reactivated in V1, the ophthalmic division of the trigeminal nerve. Ocular zoster can affect any part of the eye from the conjunctiva to the optic nerve, and is associated with a range of ocular and neurological complications including conjunctivitis, keratitis, uveitis, central retinal artery occlusion, nerve palsies, or neurotrophic keratopathy with ulceration and corneal perforation [47,48]. Permanent sequelae may include ocular inflammation and scarring, chronic severe pain and/or vision loss [49].

Several studies have examined the role of the structural destruction of corneal nerves in zoster infection, correlated with functional losses in sensation [50]. A scarcity of subbasal corneal nerves or significant decrease in SBNP density was shown in the eyes with unilateral HZO [36]. Surprisingly, patients with unilateral HZO exhibited a significant loss of the corneal nerve plexus even in the contralateral clinically unaffected eye as compared with normal subjects (Figure 2) [51]. Pathological alterations to the SBNP were strongly correlated with decreases in corneal sensation.

A loss of corneal sensation may progress to non-healing epithelial defects or acute corneal lysis and perforation; about 25 percent of all HZO patients will develop these clinical signs of neurotrophic keratopathy because of permanent corneal anesthesia [52]. Therefore, there is increasing interest both in IVCM as a clinical tool for monitoring corneal status, as well as in the possibility of restoring corneal innervation and sensation in severe or persistent HZO cases. Cruzat *et al*. described spontaneous corneal nerve regeneration of central subbasal nerves with partial recovery of corneal sensation in a single case with severe neurotrophic zoster keratopathy after 5.3 years of follow-up [53], confirmed by IVCM, corneal esthesiometry, and *ex vivo* immunohistochemistry. This study, among others that have also successfully treated neurotrophic keratopathy of different etiologies [54,55], suggest that it may be possible for some patients with HZO neurokeratopathy to regain corneal innervation and function with either extended observation or therapy.

Corneal Transplantations and Laser Refractive Surgeries

Corneal transplantation represents the oldest, most common, and most successful form of tissue transplantation worldwide [56]. Common procedures today range from full-thickness penetrating keratoplasty (PKP), a technique requiring a full-thickness 360-degrees corneal incision, to lamellar keratoplasty techniques that replace only the diseased layer of the cornea. After PKP, regeneration of the transected SBNP fiber bundles occurs at a far slower rate than after cataract or refractive surgery [57]. The nerve fiber density and branching in the SBNP can still be abnormal 40 years after PKP [58]. Several studies have observed corneal sensation to be markedly reduced, if not absent, for decades after transplantation [59-61]. Impaired sensory innervation after PKP is thought to contribute to the relatively high frequency of epithelial complications observed after the procedure [62]. The effect of different lamellar surgical techniques on corneal nerves is variable, ranging from endothelial keratoplasty (EK) in which corneal sensitivity is relatively preserved [63], to deep anterior lamellar keratectomy (DALK) in which there is a progressive recovery in corneal sensitivity, with no statistically significant difference from that of post-PKP recovery [64].

Laser-assisted *in situ* keratomileusis (LASIK) and photorefractive keratectomy (PRK) are the most common corneal refractive procedures. Both involve tissue removal by excimer photoablation, the depth of which can influence the extent of corneal hypoesthesia after corneal refractive surgery [65]. Corneal subbasal nerves become depleted after PRK and LASIK and slowly recover over several years [66]; the SBNP density is barely detectable for up to 6 months post-LASIK, and remains less than half of the preoperative values 1 year after [67,68]. However, corneal sensitivity progressively approaches preoperative LASIK values by 6 to 12 months when measured with Cochet-Bonnet esthesiometry, and by 2 years when measured by gas esthesiometry [69,70]. The alteration of corneal nerves after LASIK is hypothesized to be the most likely cause of subjective dry eye symptoms after refractive surgery [71].

Non-corneal Ocular Procedures

Corneal changes have been extensively described not only following corneal, but also other ocular procedures. Using the Cochet-Bonnet esthesiometer and IVCM, Bitirgen *et al*. were able to show that repeated intravitreal injections of anti-VEGF appear to have no harmful effects on central corneal sensation and innervation [72]. However, uneventful cataract surgery performed with phacoemulsification and intraocular lens implantation is associated with marked loss of SBNP density and corneal sensitivity, with a return to normal values within 4 to 8 months postoperatively [73-75].

SBNP density and corneal sensitivity has been found to be reduced after laser panretinal photocoagulation (PRP) in treating proliferative diabetic retinopathy (PDR) or central retinal vein occlusion, or after retinal surgeries such as 360-degree laser retinopexy, scleral buckles and encircling bands, and rectinectomy with endolaser, with neurotrophic corneal ulcerations sometimes developing as a consequence [76-79]. However, some studies have suggested that reduction in corneal sensitivity or nerve density after PRP treatment may be attributable to the effect of diabetes in PDR patients [80,81].

Glaucoma Medical Therapy

Decreased corneal sensation has been reported in glaucoma patients treated with medical therapy [82], particularly with topical beta-adrenergic antagonists [83,84]. Many of these adverse effects have been linked to benzalkonium chloride (BAC), the most commonly used preservative in topical antiglaucoma preparations [85,86]. Ocular surface disease has a prevalence of 59 percent in glaucoma cases, with higher prevalence in patients using BAC-containing antiglaucoma medications [87]. Patients may experience dryness, foreign body sensation, tearing, burning, and redness. Other side effects include chronic conjunctival inflammation, tear film alterations, medically resistant herpetic keratitis, corneal erosions, and impaired wound healing [88-91]. Of note, topical medication-related ocular surface disease results in worse symptoms, poorer compliance to treatment, and decreased quality of life in glaucoma patients [92,93].

Decreased SBNP density and increased number of nerve beadings and tortuosity may be associated with alteration of corneal sensation in patients treated with topical antiglaucoma medications [94-96]. Conversely, Rossi *et al*. revealed the potential benefits of using preservative-free drops, reporting that naïve glaucoma patients did not show significant changes when on a preservative-free formula, whereas previously treated patients had an improvement in number of corneal nerves, decreased number of bead-like formations, and nerve tortuosity after 1 year of treatment [97].

Aging

Corneal sensation appears to decrease with age [98,99], including thermal sensitivity to a cooling stimulus, regardless of gender or diabetic status [100,101]. However, current research yields conflicting results regarding the correlation of aging with changes in SBNP density, most likely due to differences in imaging techniques and analysis. Data from tandem-scanning and slit-scanning confocal microscopy suggest that SBNP density is maintained in an age-independent manner [102,103], whereas laser-scanning confocal microscopy reveal a pronounced loss of corneal epithelial nerve terminals and SBNP density with age [104], with a corresponding decline in SBNP density of 0.25 percent to 0.9 percent per year [105]. Increased nerve tortuosity has also been observed with advancing age [103,106].

Diabetes Mellitus

The examination of corneal nerves and sensation has also been pursued in the context of early detection and assessment of systemic diseases associated with peripheral neuropathies, such as diabetes. Currently, nerve electrophysiology, sural nerve, and skin punch biopsy are the gold standards for diagnosing and quantifying diabetic peripheral neuropathy [107]. However, these tests often detect diabetic peripheral neuropathy only when the neuropathy becomes well established, and have limited sensitivity for detecting early diabetic peripheral neuropathy [108]. Nerve biopsies are also invasive and expensive, limiting repeat testing and adoption as a routine diagnostic tool [109].

Unmyelinated C-class and Aδ small nerve fibers are adversely affected in diabetic peripheral neuropathy, contributing to paresthesias and loss of pain and

temperature sensation [110]. Small corneal nerve fibers also can be affected at this early stage, leading to changes that might be noninvasively and rapidly identified using IVCM [111]. Patients with type 1 or type 2 diabetes exhibit a marked reduction in corneal SBNP density, decreased nerve branching, and increased nerve tortuosity compared to healthy corneas [112,113]. While these abnormal corneal SBNP changes are associated with a reduction in corneal sensation [114], importantly, they can precede any clinical signs and symptoms of neuropathy, retinopathy, or microalbuminuria, or impairment of corneal sensitivity in patients with diabetes [115-117]. Interestingly, they are also seen in patients with impaired glucose tolerance, who do not yet meet clinical criteria for type 2 diabetes mellitus [118]. In addition to a moderate-to-high sensitivity and specificity of IVCM for diagnosis of diabetic neuropathy [119], a correlation between the loss of corneal nerve fibers and clinical and electrophysiological assessment of the severity of diabetes has been demonstrated; patients with worsened symptoms of peripheral neuropathy or diabetic retinopathy exhibit decreased SBNP density [119,120]. Finally, recovery of the corneal SBNP along with improving neuropathy has been shown in patients receiving simultaneous pancreas and kidney transplantation, suggesting the potential application of monitoring corneal nerves for therapeutic response and recovery in diabetes [121,122].

CONCLUSIONS AND OUTLOOK

Corneal nerves play a vital role in maintaining and protecting corneal integrity and sensation, and consequently, preserving ocular health and vision. Furthermore, the study of altered corneal innervation and sensation has implications beyond diseases of the eye; it can offer exciting insight into the clinical diagnosis or management of a wide variety of diseases that would otherwise require invasive testing or waiting for irreversible neuropathy to manifest. Additional investigation into the numerous factors essential for corneal nerve re-innervation and recovery of corneal sensation, beyond that in herpetic disease or ocular surgery, is needed. This, along with the continued development and validation of methods by which we analyze alterations in corneal nerve morphology and function, may someday lend significant therapeutic benefit to patients whom we may be currently limited in helping.

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