In Vivo Confocal Microscopy of Corneal Nerves: Analysis and Clinical Correlation

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Abstract

Corneal confocal microscopy is a growing technique for the study of the cornea at the cellular level, providing images comparable to ex vivo histochemical methods. In vivo confocal microscopy (IVCM) has an enormous potential, being a noninvasive procedure that images the living cornea, to study both its physiological and pathological states. Corneal nerves are of great interest to clinicians and scientists due to their important roles in regulating corneal sensation, epithelial integrity, proliferation, wound healing, and for their protective functions. IVCM enables the noninvasive examination of corneal nerves, allowing the study of nerve alterations in different ocular diseases, after corneal surgery, and in systemic diseases. To date, the correlation of sub-basal corneal nerves and their function has been studied in normal eyes, keratoconus, dry eye, contact lens wearers, and in neurotrophic keratopathy, among others. Further, the effect of corneal surgery on nerves has been studied, demonstrating the regenerative capacity of corneal nerves and the recovery of sensation. Moreover, IVCM has been applied in the diagnosis of peripheral diabetic neuropathy and the assessment of progression in this systemic disease. The purpose of this review is to describe the principles, applications, and clinical correlation of IVCM in the study of corneal nerves in different ocular and systemic diseases.

Keywords

cornea; nerves; innervation; esthesiometry; sensation; confocal microscopy

INTRODUCTION

The cornea is the most densely innervated tissue in the human body, supplied by the terminal branches of the ophthalmic division of the trigeminal nerve as ciliary nerves. Nerve bundles enter the peripheral mid-stroma in a radial pattern, course anteriorly, and give multiple branches innervating the anterior and mid-stroma.[1] Branches from the nerves in the anterior stroma penetrate Bowman's layer throughout the central and peripheral cornea. These branches then divide and run parallel to the superficial corneal surface, between Bowman's layer and the basal epithelium, configuring the sub-basal nerve plexus that supplies the overlying corneal epithelium. In addition to these sensory fibers, the cornea contains autonomic sympathetic nerve fibers that may have specific physiologic roles.

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The high density of sensitive neural structures within the corneal epithelium is important to allow the detection of stimuli of small magnitude. Thus, corneal sensation elicits a very sensitive defense reflex, crucial for the protection of the cornea and for the eye as a whole. However, corneal innervation provides not only corneal sensation and a protective function, but also has trophic functions, exerting an influence on the regulation of epithelial integrity, proliferation, and wound healing.[1,2,3] The blinking and tearing reflexes are elicited by a reflex arch that includes the ocular surface, intact corneal innervation, interconnecting nerves from the functional unit, and lacrimal glands. Compromised function in any part of this reflex arch results in impaired ocular health.[4]

Studies of the cellular and molecular mechanisms underlying the pathogenesis of corneal epithelial disorders in neurotrophic keratopathy have led to the understanding of the important role the trigeminal nerve plays in maintenance of corneal health and function. Histochemical studies have revealed the presence of various neurotransmitters, including substance P, calcitonin gene-related peptide, neuropeptide Y, vasoactive intestinal peptide, galanin, methionine-enkephalin, catecholamines, and acetylcholine in the cornea.[5]

Human corneal nerves have also been studied extensively by both light and electron microscopy.[6] Studies using these techniques may be unreliable because human corneal nerves are known to degenerate within fourteen hours of death.[7] The sub-basal corneal nerve plexus is not visible by conventional slit-lamp biomicroscopy. However, sub-basal corneal nerves are clearly visible by in vivo confocal microscopy, which allows the examination of the living human cornea at the cellular level (Figure 1A). Studies of corneal nerves by IVCM have contributed to the understanding of the human cornea in health, disease, and following surgery. This method is rapid, noninvasive and precise, with good interobserver variability.[8]

Goldman described a confocal slit system with line illumination in 1940, and demonstrated 3D imaging of the eye. However, the confocal microscope was invented and patented by Minsky in 1957.[9] In 1968, the tandem scanning confocal microscope (TSCM) and later the slit scanning confocal microscope (SSCM) were developed, but it was not until 1985 that confocal microscopy was used by Lemp et al. to examine the cornea.[10] A more recent improvement in this technology was the use of coherent light to produce laser scanning confocal microscopes (LSCM). All confocal microscopes use the same basic principle to obtain a high-resolution image, in the context of the confocality of the examined object with the light source and the detector plane, hence the term "confocal."[11]

The exponential use of IVCM over the last years has led to significant strides in understanding the role of corneal nerves in health, ocular, and systemic diseases. The purpose of this article is to review the literature assessing the sub-basal corneal nerve plexus by IVCM in different pathological states, and their clinical correlation.

MATERIALS AND METHODS

Articles assessed for inclusion in this review were identified by an electronic search of Medline databases in November 2009 using individual and combinations of keywords "confocal," "microscopy," "in vivo," "corneal," "nerves," "sensation" and "sensitivity," and by review of the reference section of the identified publications. Review and synthesis of the selected literature that has studied the correlation of the subbasal corneal nerve density with corneal sensation was included.

RESULTS

Normal Subjects

Few studies have quantitatively analyzed the sub-basal nerve plexus by IVCM and its relationship with corneal sensitivity in normal human corneas.[1,12–14] Studies performed in normal subjects demonstrated that corneal sensation decreases with age.[15,16] However, the data regarding the correlation of aging to reduction in sub-basal nerve density are variable.[12,14,17] Further, a recent study performed by Niederer et al. showed a corresponding linear decline in sub-basal nerve density of 0.9% per year.[18]

Contact Lens Wear

Long-term contact lens wear has been associated with a considerable reduction in corneal sensitivity,[19] although it does not appear to affect corneal nerve density, distribution, or morphology.[20,21] Corneal sensitivity alteration has been shown to vary between different types of contact lenses, with rigid gas permeable lenses associated with a lower corneal sensitivity than PMMA lenses.[20] Further, cessation of contact lens wear is associated with a recovery of corneal sensitivity.[22] The lack of correlation between nerve function and structural changes may be due to a sensory adaptation to the permanent mechanical stimulus by contact lenses.[23] Another possibility favored by Murphy et al. is the attribution of the loss of sensation in soft contact lens wearers to metabolic causes.[19]

Keratoconus

The pathophysiology of keratoconus has yet not been completely elucidated, though there appear to be some environmental and genetically predisposing factors.[24] Sub-basal nerve density has been shown to be lower in corneas with keratoconus, and qualitatively appeared more tortuous in keratoconic corneas as compared to controls, with abnormal architecture in the region of the cone.[25–28] Diminishment of nerve density has been significantly correlated with loss of corneal sensation in keratoconus patients, this correlation being stronger in keratoconic patients who wore contact lenses.[26] A positive correlation between nerve density with the severity of the disease has been demonstrated as well.[27]

Regarding the density of the basal epithelium, various studies have reported decreased epithelial cell density,[26, 27, 29, 30] with a correspondent increase in cell area. Corneal sub-basal nerve alteration may lead to changes in basal epithelial cell density, and may be involved in the pathogenesis of the disease.[26] Brookes et al. have shown that the destructive process in keratoconus involves the nerves, or their associated Schwann cells, which express proteolytic enzymes (cathepsin B and G) more extensively in keratoconus as compared to normal corneas.[31]

Dry Eye Syndrome

Decreased corneal sensation has been demonstrated in dry eye patients,[32–34] although other studies have shown a hypersensitivity.[35,36] Similarly, studies present conflicting results regarding the effect of dry eye on sub-basal nerve density. Several studies have observed a significantly reduced sub-basal nerve density in both Sjögren's syndrome and non-Sjögren's syndrome dry eye compared to controls, which correlated to corneal sensation in these patients.[34,36,37] In contrast, other studies have noted no difference in sub-basal nerve density,[33,38] but rather demonstrated increased corneal nerve density in a subgroup with Sjögren's syndrome.[39] The variability of results in regard to the correlation of corneal sensitivity and sub-basal nerve density may be attributed to different stages and severity of dry eye in patients enrolled in these studies. However, there is agreement among the studies that sub-basal corneal nerve tortuosity is significantly increased. An increased number of beadlike formations has been noted and interpreted as metabolically active transmitter-

containing nerve fibers, which attempt to improve the abnormal epithelial trophism.[38, 40] Alternatively, the beadlike formations are thought to represent nerve damage, followed by secretion of nerve growth factors due to inflammatory processes.[37]

Clinical correlation to slit-lamp biomicroscopy findings has been shown in several studies. Benitez et al. demonstrated that the number of sub-basal nerves and the level of corneal sensation correlates with Schirmer's test results.[34] Further, Zhang et al. have shown that corneal Rose Bengal staining correlates positively with nerve density, but is inversely related to beading of nerves.[39] The results of IVCM studies in dry eye patients strongly suggest a role of corneal nerve density and morphology, as well as function, in the pathogenesis of this disease. The discrepancy between signs and symptoms, as well as the increase in patient symptoms in the face of corneal sensation loss, could be explained by injury of corneal nerve endings due to inflammatory processes, followed by altered excitability in regenerated nerves.

Neurotrophic Keratopathy and Infectious Keratitis

Several recent studies by our group have demonstrated the role of corneal nerves and the correlation with corneal sensation in patients with neurotrophic keratopathy, in patients with herpes simplex keratitis (HSK)[41] and herpes zoster ophthalmicus (HZO).[42] These studies demonstrated a significant decrease in total number of sub-basal nerve fibers, as well as nerve density in both HZO and HSK eyes, strongly correlating with the decrease in corneal sensation (Figure 1 A–D). Further, IVCM revealed that the loss of the sub-basal nerve plexus started within days in acute HSK. Interestingly, the contralateral unaffected eyes also presented with a loss of sub-basal nerve plexus as compared with normal subjects.[41, 42] Moreover, profound HZO- and HSK-induced changes were observed in the superficial epithelium, which showed an increase in cell size, a decrease in cell density, and squamous metaplasia in both HSK and HZO, strongly correlating with decreased corneal sensation and nerve density.[43, 44] Similarly, a profound diminishment of the sub-basal corneal nerve plexus was also observed in patients with fungal and *Acanthamoeba* keratitis.[45] More recent studies demonstrated that the decrease in sub-basal corneal nerve density is associated with increased density and morphological changes of central epithelial dendritic cells in HSK, bacterial, fungal and *Acanthamoeba* keratitis, suggesting a direct interaction between the immune and nervous system in the cornea (Figure 2A–B).[46]

Corneal Surgery

In addition to corneal disease, IVCM enables us to morphologically and functionally correlate corneal reinnervation after surgery. It has been demonstrated that the corneal sub-basal nerve plexus is not a static, but a dynamic, structure.[47] Corneal reinnervation is affected by several factors, including the time elapsed after surgery, the patient's age, the preoperative diagnosis, and the surgical procedure.

Corneal Transplantation

Full-thickness penetrating keratoplasty involves transection of all corneal nerves in both the host and donor cornea. The nerves slowly regenerate over time, although nerve morphology remains abnormal. The study with the longest follow-up has been performed by Richter et al.,[48] reporting that none of the patients after penetrating keratoplasty recovered normal nerve morphology or sensitivity during the follow-up period of up to 3 years. Reinnervation of the central cornea with sub-basal nerves occurred at 2 years, and with stromal nerves, at 7 months postoperatively.[48] In another study by Darwish et al.,[49] although no sub-basal nerves were detected at 12 months, corneal sensation improved over the 12-months period to near normal levels, suggesting that IVCM may not be able to detect some of the finer regenerating sub-basal nerve fibers.[49] Comparison of nerve morphology between eyes with

nonsurgical trauma and corneal grafts indicated that surgically induced scar formation may limit nerve regeneration in grafts. Two cross-sectional studies demonstrated reduced sub-basal nerve density and increased tortuosity, even 40 years after surgery.[50,51]

Corneal Refractive Surgery

Photorefractive Keratectomy (PRK)—The sub-basal corneal nerve plexus is undetectable in the treatment area of post-PRK corneas.[52] These nerves have been shown to slowly regenerate, returning to normal nerve density within 2 years.[53] Nevertheless, morphological alterations can still be observed up to 5 years following surgery.[52, 54] Recovery of corneal sensitivity after PRK, in contrast, has been reported to start at 4–6 weeks after surgery, and appears to be completed within 6–12 months of surgery.[55, 56]

Laser In Situ Keratomileusis (LASIK)—The recovery of corneal sensation after LASIK has been estimated to be approximately 6 months.[57,58] Within the first month following surgery, the number of subbasal and stromal nerve fiber bundles decreases by 90% as compared to the preoperative values.[59] During the first year after LASIK, reinnervation occurs, with corneal nerves being detected in the central cornea by 6 months.[60] However, the nerve density after LASIK remains less than a half of the pre-operative values even at 12 months.[59,61] In addition, decreased sub-basal nerve density is observed at 2 and 3 years after LASIK,[60] and even at 5 years following surgery, nerve regeneration appears to remain incomplete.[53] A strong correlation has been observed between corneal sensation and sub-basal nerve morphology and density after LASIK.[57,61] Interestingly, a study comparing corneal wound healing and nerve regeneration showed no difference between flaps created with femtosecond laser as compared to mechanical microkeratome.[62]

Laser-Assisted Sub-epithelial Keratectomy (LASEK)—Corneal sensation has been shown to return to preoperative levels at 3 months after LASEK surgery, although sub-basal nerve density was still at half of pre-operative values 6 months following surgery.[63] However, in a separate study, no difference was observed in nerve recovery between LASIK and LASEK.[64]

Systemic Diseases

IVCM is an accurate non-invasive method for diagnosis and assessment of the progression of systemic diseases with peripheral neuropathy, such as diabetes. Reduced corneal sub-basal nerve density and increased nerve fiber tortuosity in diabetes have been documented utilizing IVCM, and correlated with the stage of peripheral neuropathy.[65–67] Rosenberg et al. demonstrated a correlation between reduced corneal nerve bundles with loss of corneal sensation, and severity of somatic neuropathy in patients with type 1 diabetes.[66] In addition, IVCM allows detection of early peripheral neuropathy, as decreased nerve density has been shown to precede impairment of corneal sensitivity. Further, corneal nerves recover with improved glycemic control or within 6 months after pancreatic transplantation in patients with type 1 diabetes.[68]

A reduction in neurotrophic stimuli in severe neuropathy may induce a thin epithelium, potential leading to recurrent erosions.[66] This may explain the significantly higher risk of development of postoperative epithelial complications and relatively poor refractive results in diabetic patients who undergo LASIK.[69] Nerve fiber damage and repair in the corneal stroma has been shown to be partially regulated by collagen, fibronectin, and proteoglycans, as well as a number of growth factors including tumor growth factor-β, fibroblast growth factor, and nerve growth factor, many of which are upregulated in diabetes.[67]

DISCUSSION

The main function of the cornea is transparency, for which corneal innervation is key. Corneal sensation, epithelial metabolism, cell adhesion, and wound healing all depend on adequate corneal innervation. The mechanisms governing corneal nerve integrity and their structure are potentially complex. To date, the assessment of corneal innervation has only been possible through esthesiometry techniques. The advent of IVCM, together with the improving technological advances in this field, now allow for in vivo examinations of corneal nerve morphology and density in great detail. IVCM enables a direct comparison of corneal sensory innervation and clinical findings, such as sensitivity measured by esthesiometry, epithelial indemnity and transparency. Corneal dennervation has been shown to occur in local and systemic disease, as well as after surgical and pharmacological interventions.

The relationship between corneal innervation and sensation has been studied in normal human corneas and in several diseases, such as dry eye, primary Sjögren's syndrome, keratoconus, and diabetes. Further, the correlation between corneal reinnervation and esthesiometry has been observed after several surgical procedures, including penetrating keratoplasty and laser refractive surgery. However, conflicting results have been reported regarding the correlation of corneal sub-basal nerve density and sensation. The differences in some of the studies can be attributed to the different confocal microscopes used, which have different resolution, depth of field, and capacity to evaluate corneal nerves. A standardized method of analysis remains to be developed after rigorous validation. Furthermore, consistent image acquisition is necessary in order to use confocal microscopy in prospective and multicenter studies, allowing data to be compared between different microscopes. Additionally, some of these studies utilized different methods for the assessment of corneal sensitivity. Moreover, a consistent esthesiometry method is necessary to measure corneal sensation in a reproducible fashion.

In conclusion, the use of IVCM and esthesiometry allows the detection of corneal nerve morphology, density and function, opening the way for possible new lines of treatment for local and systemic diseases. Understanding the pathogenesis of nerve degeneration and regeneration both in health and in disease will potentially enable the development of novel strategies to prevent nerve destruction or to stimulate nerve regeneration, in order to restore corneal sensation and retain transparency.

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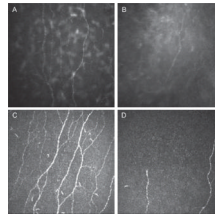
FIGURE 1.
Normal corneal sub-basal nerve plexus and after herpes simplex keratitis with two types of confocal microscopes. (A) Normal sub-basal nerve plexus. Slit scanning confocal microscopy (SSCM) Confoscan 4. (B) Herpes simplex keratitis with severe sensation loss. Note the decrease in total nerve count, length, and branching. SSCM. (C) Normal sub-basal nerve plexus. Laser scanning confocal microscopy (LSCM) HRT3/RCM. (D) Herpes simplex keratitis with severe sensation loss. Note the decrease in nerves density and branching. LSCM.
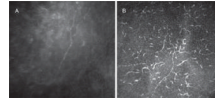
FIGURE 2.
Corneal sub-basal nerve plexus in Infectious keratitis. (A) Slit scanning confocal microscopy (Confoscan 4). Decrease in nerve density. (B) Laser scanning confocal microscopy (HRT3/RCM). Decrease in nerve density and increase in dendritic cells.