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Corneal Epithelial "Neuromas": A Case of Mistaken Identity?

Mary Ann Stepp, PhD,* Sonali Pal-Ghosh, MS,* Laura E. Downie, PhD,†
Alexis Ceecee Zhang, BOptom (Hons),† Holly R. Chinnery, PhD,† Joshua Machet, BMedSc,‡ and
Nick Di Girolamo, PhD‡

Abstract: Laser scanning in vivo confocal microscopy is a useful clinical tool to assess the corneal nerves in human and laboratory animals. With this new technology, the use of terms such as "neuromas" and "microneuromas" is becoming popular to describe nerve structures seen in humans. Here, we point out that the sites where stromal nerves enter the corneal epithelium are often hyper-reflective and can appear dysmorphic when imaged using in vivo confocal microscopy. Furthermore, we clarify what is known anatomically about how the nerves enter the corneal epithelium from the stroma, and we urge colleagues to differentiate between hyper-reflective foci at the corneal stromal–epithelial nerve penetration sites and alterations in nerve morphology secondary to injury or disease.

Key Words: neuroma, intraepithelial corneal basal nerves, in vivo confocal microscopy

(Cornea 2020;39:930–934)

The confocal scanning microscope was invented in 1955 by Professor Marvin Minsky.[1] In 1988, it provided the first images of the epithelial cells, keratocytes, and endothelial cells in the living human cornea.[2] In the 1990s, in vivo confocal microscopy (IVCM) was being used to characterize the human corneal nerves, which were referred to as the subbasal nerves.

IVCM can be used to diagnose disease in patients presenting with various ocular and nonocular symptoms.[3–9] Figure 1 shows examples of the corneal nerves from healthy human (Figs. 1A–C) and mouse corneas (Fig. 1D); these images emphasize the sites where stromal nerves enter the epithelium. Although a powerful clinical tool, IVCM is not without limitations, as described by Tervo et al[10] and De Silva et al.[11] Imaging the corneal epithelial basal axons is relatively straight-forward, but the apical extensions cannot be readily visualized. The curved surface of the cornea can also limit resolution, particularly in the periphery. IVCM uses reflected light to obtain images; backscatter from disrupted extracellular matrix can affect the quality of the images obtained. In addition, the cost of the IVCM device is substantial and, unlike optical coherence tomography, it is still not readily available in many outpatient ophthalmology clinics or practices.

To make full use of IVCM as a clinical tool requires an appreciation of the knowledge gained from human and animal studies using fixed tissues stained to reveal the corneal nerves at a high resolution.[12–14] In 1983, Rozsa et al[15] described reinnervation of the sensory nerves in the rabbit cornea after different types of corneal injury, including incisions and keratectomies. The method used to study the morphology of the nerves was state of the art at that time: gold chloride staining using bright-field microscopy. In images shown of rabbit corneas 60 days after radial keratectomy, Rozsa et al[15] referred to disorganized epithelial nerves as "neuromas," as shown in Figure 2A. Figures 2B–E show the IVCM images we acquired from people with diabetes. Since that time, several authors have used the term "microneuroma" or "neuroma" to describe anomalous nerve features typically associated with corneal injury and disease.[6,16,17] Here, we propose that, in some contexts, the terms neuroma and microneuroma may be misleading, given that similar nerve morphologies are evident in healthy corneas, with no reported pathology. In addition, we believe there is a need for a standardized terminology to accurately describe corneal nerve anatomy in healthy corneas and anomalies seen in patients (Table 1).

The online *Merriam Webster's Dictionary* lists the first use of the term neuroma in 1829 to describe "a tumor growing from a nerve and consisting of nerve fibers." [18] In medicine, the term is no longer limited to tumors. Although the online medical dictionary[19] states that neuroma is a "general term for any neoplasm derived from cells of the nervous system," it then describes several types of noncancerous neuromas,
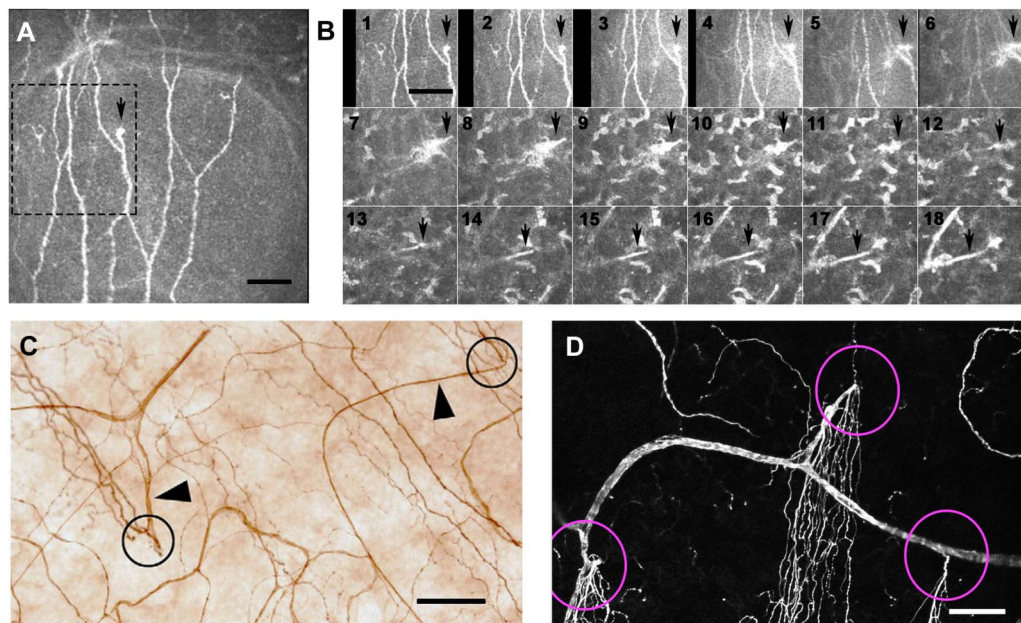
FIGURE 1. Corneal stromal-epithelial nerve penetration sites. A, An IVCM image from a human cornea highlighting a feature (black arrow) that has similarities to previous reports of "stumps of severed nerves/abrupt endings of a nerve fiber." Bar = 50 μm. B, A montage of serial images from a depth volume scan of inset in (A). These images show that the apparently "abrupt" nerve ending is actually continuous with a stromal nerve trunk. Bar = 50 μm. C, An en face image from Marfurt et al[30] of an unwounded human cornea shown after staining the tissue with an antibody against βIII-tubulin and visualized by 3,3′-diaminobenzidine. Black circles highlight the sites where stromal nerves penetrate the epithelium (CSENPS). Bar = 150 μm. D, An en face image of an unwounded mouse cornea stained with an antibody against βIII-tubulin and visualized using a fluorescent secondary antibody. The magenta circles highlight the CSENPS, that is, the sites where stromal nerves enter the epithelium. Bar = 50 μm.

including acoustic neuromas, amputation neuromas, neuroma cutis, plexiform neuromas, and traumatic neuromas. Traumatic neuromas[19] arise from trauma to the nerves and consist of tangles of nerves, Schwann cells, and perineural fibroblasts and are found in the dermis or other interstitial tissues where they can persist and cause pain and discomfort.

In 1982, corneal sensory nerve fibers were believed to be located beneath the corneal epithelial basement membrane.[20] They were called the subbasal nerves; the network the nerves assume was referred to as the subbasal nerve plexus. Nerve terminals branched apically from the subbasal nerves.[20] Since then, improved imaging procedures have shown that the subbasal nerves actually localize within the basal cell layer and are enclosed by the cell membranes of the corneal epithelial basal cells.[13,21] To minimize confusion regarding where these sensory nerves localize within the cornea, in 2017, the term intraepithelial corneal nerves (ICNs) was proposed[21] to include the subbasal nerves and nerve terminals. This terminology has yet to be broadly adopted in the literature. Here, we propose that once the sensory nerves enter the epithelium and travel within the basal epithelial cell layer above the basement membrane, they be referred to not as the subbasal nerves but as the intraepithelial corneal basal nerves (ICBNs); the ICBNs branch and extend between the epithelial cells toward the apical squames forming the intra-epithelial corneal nerve terminals (ICNTs). Together, the ICBNs and ICNTs comprise the ICNs, as indicated in Table 1.

The sites where the corneal stromal nerves exit the stroma, penetrate the basement membrane, and enter the basal epithelial cell layer have complex morphologies. The stromal nerves, like other nerves of the peripheral nervous system, have 2 types of neural crest–derived cells, namely the Schwann cells and endoneurial fibroblasts, associated with them. The Schwann cells can be of 2 types: myelinating and nonmyelinating. Studies have shown that the myelinating Schwann cells in the cornea are restricted to the periphery[12,14]; in the central cornea, the nonmyelinating Schwann cells wrap their plasma membranes around each axon. The endoneurial fibroblasts secrete collagen around the axons to insulate individual axons from one another and increase their ability to resist mechanical forces without severing. As the stromal nerves penetrate the epithelial basement membrane, they shed associated Schwann cells, endoneurial fibroblasts, and the matrix proteins they secrete.

Al-Aqaba et al[14] have used scanning electron microscopy and transmission electron microscopy to demonstrate the unique anatomical features of the junction between the stromal nerves and ICBNs in human corneas. They observed bulb-like structures; a stromal nerve entered the stromal side of the bulb and a thinner ICBN exited the epithelium. The bulbs did not stain with antibodies against βIII-tubulin or contain the nuclei but were positive for the neuronal enzyme acetylcholinesterase. Based on these observations, the authors concluded that the bulbs contained the cytoplasm and/or matrix surrounding the Schwann cells and/or endoneurial
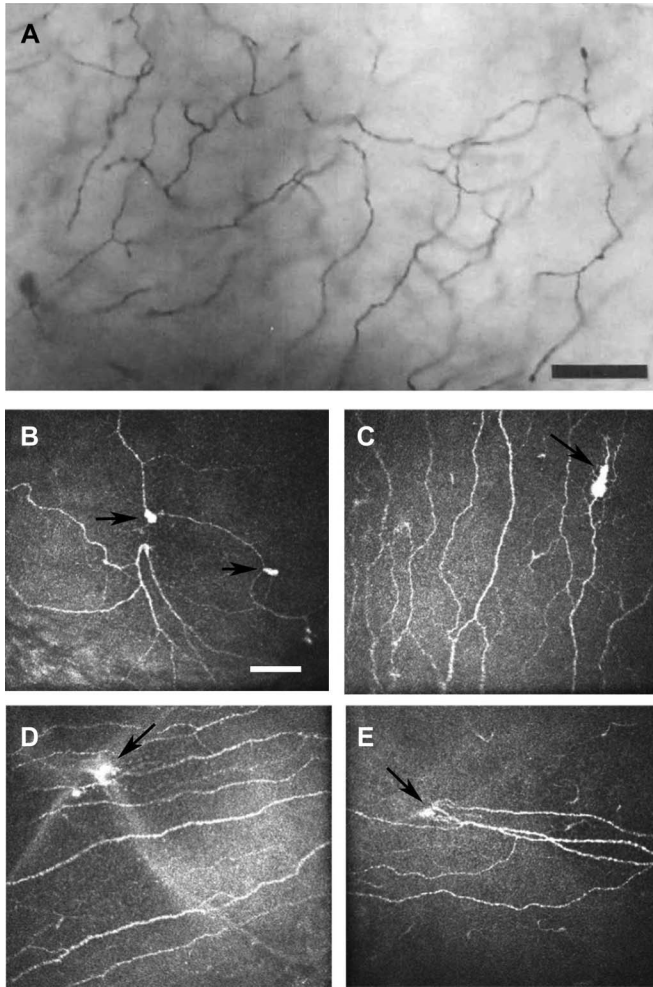
FIGURE 2. Altered nerve morphology after injury in animals and patients with diabetes. A, Rabbit corneal nerves stained with gold chloride 60 days after radial keratectomy. Image from Rosza et al,[15] who stated that "numerous, fine, disorganized axon terminals appear to form a neuroma." Bar = 25 μm. B–E, Laser scanning IVCM images providing examples of nerve anomalies (arrows) present in the corneal epithelium of patients with diabetes. Enlarged hyperreflective bulges or axonal distensions (B, C) present in the basal epithelium. Hyperreflective diffuse patterns associated with branch points (D, E) seem similar to the CSENPS described in the literature.[4,30,31] B–E, All at the same magnification. Bar (B) = 50 μm.

fibroblasts and stromal axons that terminate at the epithelial basement membrane. Other cell types that could contribute to the bulbs are dendritic cells, which have been shown to be present at the sites where stromal nerves enter the epithelium.[22] A recent study by Courson et al[23] also demonstrated bundles at the sites of stromal nerve penetration into the epithelium in the mouse cornea using serial block-face scanning electron microscopy. The morphology of these sites suggests to the authors that some stromal nerves fuse with the corneal epithelial cells. Before accepting this view, it is necessary to determine the contributions of the other cell types present at these sites, including the nonmyelinating

Schwann cells, endoneurial cells, corneal epithelial cells, and immune cells, and the potential involvement of collagen produced by the endoneurial fibroblasts and extracellular matrix in the epithelial basement membrane. Reports from studies of the human[4,11,24] cornea indicated more of these sites within the periphery; Al-Aqaba et al[24] reported an average of 185 per cornea. To promote consistency in the reporting of corneal anatomical observations in both clinical and basic science literature, we propose a new terminology to describe these nonpathological entry points as the corneal stromal–epithelial nerve penetration sites (CSENPS) (Table 1).

The stromal nerves navigate past the epithelial basement membrane in mice and the epithelial basement membrane and Bowman layer in human and primate corneas. Figure 3A, B shows en face confocal microscopy images of whole flat-mounted mouse corneas immunostained to colocalize βIII-tubulin (axons), sdc3 (Schwann cells), and LN332 (basement membrane) to highlight these CSENPS. The hemidesmosomes that maintain epithelial adhesion to the stroma are displaced. Hemidesmosomes located in the basal cell membrane link $\alpha_6\beta_4$ integrin to LN332 in the basement membrane and type VII collagen in the anterior stroma within the adhesion complexes[25]; when the stromal nerves push the adhesion complexes aside at the CSENPS, mechanical strain is transferred to the anterior basement membrane and stromal matrix. Syndecan-3 (sdc3) is a heparan sulfate proteoglycan expressed by the glial cells in both central and peripheral nervous systems.[26] The mouse stromal nerves have been shown previously to express sdc3.[27] Some stromal nerve axons may terminate at the CSENPS, whereas others enter the epithelium. We propose that these factors contribute to the complex morphologies seen at the stromal nerve exit sites and give rise to hyperreflective structures in the IVCM images. A complete understanding of the contribution of stromal matrix deformation and each cell type contributing to the morphology of the CSENPS is not yet possible. It will require additional high-resolution transmission electron microscopy imaging and the ability to define each cell type involved.

Once the axons enter the epithelium and branch, they extend parallel to the basement membrane. These long, extended axon bundles have been called leashes.[28–30] There are examples in the literature where the so-called microneuromas[8,17] appear similar to the sites of stromal nerve

TABLE 1. Recommended Changes to Corneal Nerve Terminology

Canonical Terminology	Proposed Amended Terminology
Subbasal nerves	ICBNs
Nerve terminals	ICNTs
Corneal sensory nerves	ICNs = ICBNs + ICNTs
Stromal nerve exit sites	CSENPS
Neuroma and microneuroma	Axon (or ICBN) bulges, varicosities, tangles, and/or hyperreflective sites

CSENPS, corneal stromal–epithelial nerve penetration sites; ICBNs, intraepithelial corneal basal; nerves; ICNs, intraepithelial corneal nerves; ICNT, intraepithelial corneal nerve terminals.
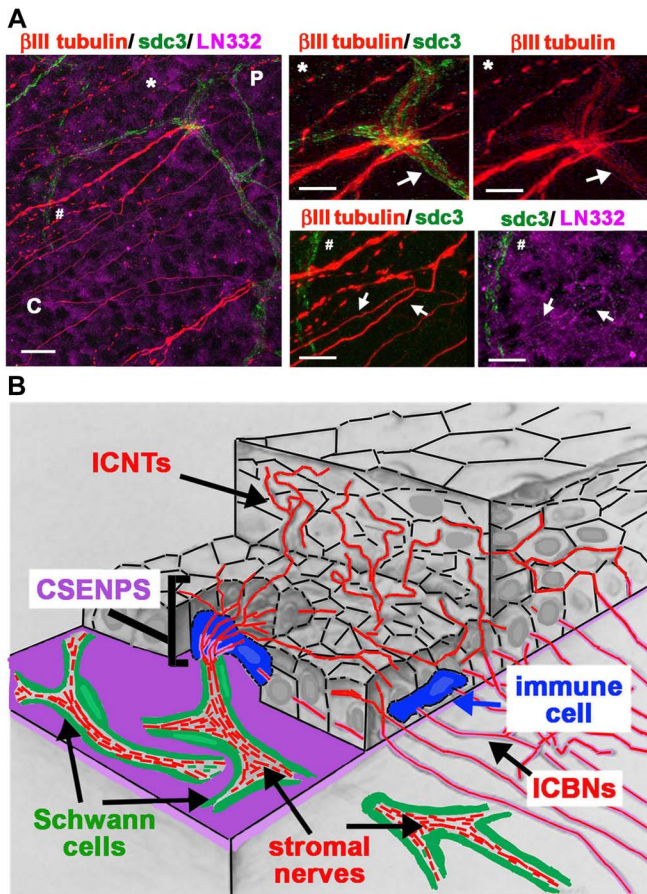
FIGURE 3. The stromal nerves shed sdc3+ Schwann cells in the epithelial basement membrane zone at the CSENPS. A, The image on the left shows an en face extended focus projection confocal image showing 16 μm of the corneal epithelium projected from the basal aspect of the tissue at the level of the corneal epithelial basement membrane. The corneal epithelium has been stained to show localization of βIII-tubulin (red), syndecan-3 (sdc3; green), and laminin 332 (magenta), an epithelial basement membrane protein. The image was acquired near the corneal periphery; the locations of the corneal periphery (P) and center (C) are indicated. The areas indicated by the * and # on the left have been magnified 3-fold and are presented on the right. The area highlighted by the * shows that although both ICNs and sdc3+ stromal nerves are positive for βIII-tubulin, the stromal nerves express less βIII-tubulin (arrows). After the stromal nerves enter the epithelium and become the ICBNs, sdc3 expression is lost and βIII-tubulin expression increases. The area highlighted by the # shows that some ICBNs have LN332 on their surface. Bars in the image on the left = 26 μm; bars in magnified images on the right = 8.7 μm. B, The schematic image shown is derived from that originally published by Rózsa and Beuerman[20] and has been updated to indicate the involvement of Schwann cells (green) at the CSENPS. Immune cells (blue) have been added based on the studies in the literature.[22] The CSENPS are present within the epithelial basement membrane, which is shown in magenta. Also included are proposed revisions to the names of these nerves. The ICNs (red) consist of the ICBNs and ICNTs, previously referred to as the subbasal nerves and nerve terminals.

penetration into the basal epithelium in healthy[31,32] and diabetic corneas, as shown in Figures 2D–E.

A schematic image showing the CSENPS is shown in Figure 3C. Once the nerve has penetrated the basement membrane, it aligns parallel to the basal surface of the corneal epithelial cells and branches into thinner nerve fibers, referred to as leashes, each of which consists of varying numbers of individual axons. Given the improvements in imaging hardware and platforms, it is anticipated that the CSENPS in the healthy and diseased mammalian corneas will soon be more accurately resolved. Complex as they may be, it is likely that some of the structures being reported in the clinical literature are not pathological microneuromas or neuromas but physiological phenomena, such as the CSENPS.

If, as a scientific community, we can agree to term these features as the CSENPS in healthy corneas, then this raises the question as to what to call the sites where we observe disordered nerves within the corneal epithelium, including those presented in Figure 2. Using terms such as axon tangles, clumps, bulges, varicosities, and hyperreflective sites more accurately describe these structures.[33] Standardization of the terminology used to classify corneal epithelial nerve features (Table 1) will lead to more consistent reporting and improved data collection to support an increased understanding of the etiology and potential functional significance of these neural phenomena. Epithelial cell injury and pathologies, including dystrophies, barrier defects, dry eye disease, ocular allergy, and exposure keratopathy, will impair the ability of the corneal epithelial cells to provide support for the ICNs. The relationship between corneal epithelial cells and sensory nerves that innervate them is similar to that between the Schwann cells and other peripheral nerves: they are interdependent. Pathology in one will lead to pathology in the other. When tangles persist over time in the corneal epithelium, but the stromal nerves seem normal, corneal epithelial cell pathology should be considered as a cause. We suggest that the terms microneuroma and neuroma should thus be strictly reserved for pathologies involving verifiable tumors or tangles of the stromal nerves with keratocytes and the Schwann cells.

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