Cornea

Mapping the Corneal Sub-Basal Nerve Plexus in Orthokeratology Lens Wear Using in vivo Laser Scanning Confocal Microscopy

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Purpose. This study was designed to map the sub-basal nerve plexus (SBNP) in the cornea of orthokeratology (OK) lens wearers.

Methods. Laser scanning confocal microscopy (LSCM) was performed in vivo on three subjects: a non–lens wearer and two OK lens wearers. Scans were performed on the right eye while the left eye fixated a moving target. A total of 575, 430, and 676 contiguous images of the SBNP were taken from the non–lens wearing and the OK lens wearing subjects, respectively, and used to construct maps of the central to mid-peripheral SBNP.

Results. In the non–lens wearing eye, nerves radiated towards a whorl-like complex centered nasally and inferiorly in an overall pattern consistent with previously reported studies. In the OK lens wearing eyes, this whorl pattern was absent, replaced by a tortuous network of nerve fibers centrally, and thicker curvilinear fibers mid-peripherally, particularly in the nasal, inferior, and temporal regions.

Conclusions. This study maps the corneal SBNP in OK lens wearers and provides compelling evidence that OK lens wear alters the normal SBNP distribution observed in healthy, non–lens wearing eyes. (*Invest Ophthalmol Vis Sci.* 2012;53:1803–1809) DOI:10.1167/iovs.11-8706

The human cornea is the most densely innervated surface tissue in the human body.[1–2] The rich supply of nerve fibers controlling the sensory and trophic functions is located mainly in the anterior layers of the cornea.[3] The arrangement of these nerves is highly complex[1] and not yet completely known. However, it is important from a clinical perspective to understand and further investigate the architecture of these nerves because of recent studies showing a relationship between nerve morphology and ocular and systemic disease,[4–15] as well as nerve damage following refractive surgery.[16–17]

The nerve fibers located between the basal epithelium and Bowman's layer are known as the sub-basal nerve plexus (SBNP).[18] They are detected relatively easily using contemporary in vivo laser scanning confocal microscopy (LSCM) and consequently are a main focus for evaluation of corneal nerve morphology.[19–20] Patel and McGhee were the first to map a significant portion of the SBNP in normal subjects, describing the neural architecture of the central to mid-peripheral cornea as having a whorl-like pattern.[21] In a follow-up study,[22] they perform repeated scans on the same subject one week apart and found dramatic changes in the SBNP, concluding that it is a highly dynamic structure.

Orthokeratology (OK) is a clinical technique that uses specially designed rigid lenses to reshape the corneal contour. Lenses are worn during sleep and removed on waking to temporarily correct refractive error, thereby providing clear, unassisted vision throughout the day.[23] Many studies have shown that OK-induced changes to the corneal contour are the result of changes in the epithelial layer[24–27]; however, no studies have investigated the effects of OK treatment on the SBNP. Furthermore, several researchers have suggested that sub-basal nerves and epithelial cells migrate in tandem,[21,28–29] so an investigation of SBNP changes during OK treatment may increase our understanding of epithelial layer changes during this modality of contact lens wear. In addition, two previous case reports[29–30] suggest that the appearance of fibrillary lines at the onset of OK treatment may represent altered nerve fibers in the SBNP,[31] but this has yet to be proven. Therefore, the purpose of this study was to investigate the corneal nerves in healthy human eyes by mapping the SBNP in OK lens wear using in vivo LSCM and thereby better understand epithelial layer changes as well as the origins of fibrillary lines during OK treatment.

Methods

The research described in this paper followed the tenets of the Declaration of Helsinki 1975, as revised in 2000. After approval for the study had been obtained from the University of New South Wales Human Research Ethics Committee, three subjects were recruited for this study, a short-term OK lens wearer (STOK) of approximately 1-year experience, a long-term OK lens wearer (LTOK) of approximately 9-years experience, and a non–contact lens wearer (NC). The STOK, LTOK, and NC subjects were all healthy Asian females aged 21, 29, and 28 years, respectively.

Laser scanning in vivo confocal microscopy was performed with the use of a corneal module (Heidelberg Retina Tomograph II [HRT], Rostock Corneal Module [RCM]; Heidelberg Engineering GmbH, Heidelberg, Germany) on the right eye of each subject. The instrument was set up using standard techniques described previously.[20–21] The mapping of the SBNP was based on the technique developed at the laboratory of Professor Nathan Efron (Queensland University of Technology, Brisbane, Australia).[32] During the examination, all subjects

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were asked to fixate on an LCD screen that displayed a meandering white spot on a black background. This spot followed a motion path tracing out a line pattern as illustrated in Figure 1a. The overall dimensions of the line pattern on the screen were 11 cm × 13 cm (height [h] × width [w]). This screen was positioned at 70 cm perpendicular to the contralateral eye and corresponded to a scan area of approximately 2.5 mm × 3 mm (h × w) on the corneal surface (Fig. 1b). All subjects were emmetropic during the examinations and hence could clearly see the fixation spot with the contralateral eye. The HRT RCM was set on "sequence mode" to scan several sets of 100 high-quality contiguous images of the SBNP, taking approximately 40 seconds to capture each set of images. The position of the RCM probe was adjusted between each set of scans in order to target different areas of the cornea. Nine, 18, and 22 sets of scans were taken over 3, 5, and 7 visits for the NC, STOK, and LTOK subjects, respectively. Each visit lasted approximately 20 minutes, with a total confocal exposure time of less than 10 minutes. Hence, the subjects and operator did not exceed the manufacturer's imposed limit of 50 minutes of total exposure to the HRT laser at any single visit.

The scanned images for each subject were arranged into a wide-field montage using Photoshop Elements 9 (Adobe Systems Inc., San Jose, CA). Some images required brightness correction before inclusion into the montage. Any blurred or duplicate images were discarded. A total of 575, 430, and 676 images were acquired for the NC, STOK, and LTOK subjects, respectively. All nerves on the montage were then manually traced on a Wacom Graphics Tablet (Wacom Co., Saitama, Japan) using a grip pen to outline the overall pattern of the nerve map. The overall dimensions (h and w) of the final montage were calculated using Image-Pro Analyser 7.0 (Media Cybernetics Inc., Bethesda, MD).

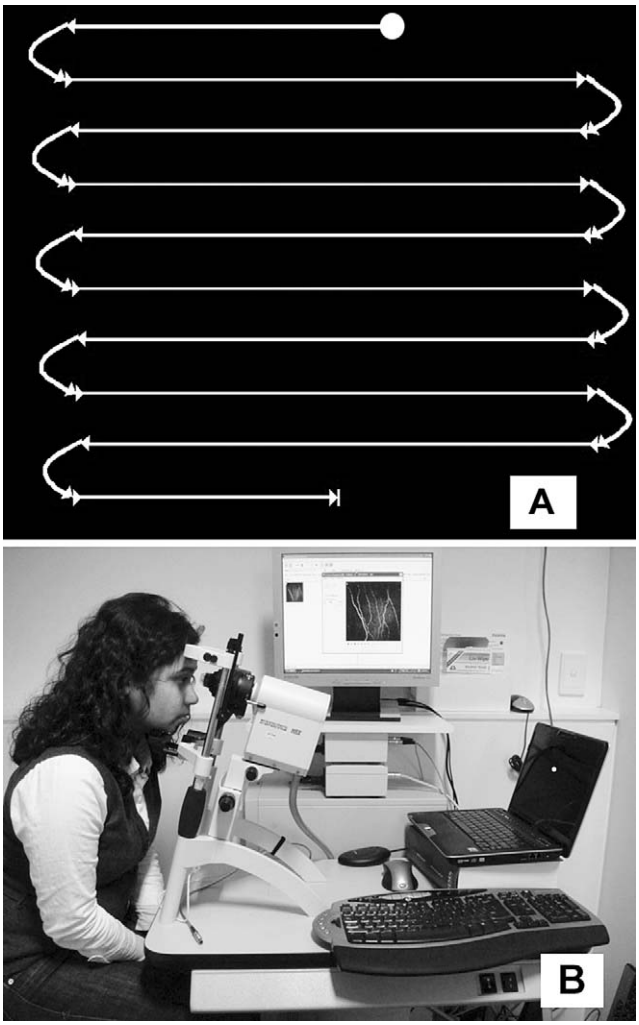

Figure 1. Fixation target (meandering white dot) and line pattern followed by contralateral eye during a single "sequence mode" scan of the sub-basal nerve plexus (**A**). Setup of subject and LCD screen at 70 cm perpendicular to fixating eye (**B**). The LCD screen displayed the moving fixation target for the contralateral eye.

Table 1. Measured Lens Parameters of OK Lenses Worn by Subjects

Parameter	Short-Term Wearer	Long-Term Wearer
Lens diameter (mm)	11.0	10.6
Optic zone diameter (mm)	6.00	5.75
Base curve (mm)	8.97	8.30
Back vertex power (D)	+0.75	+0.50

There were no adverse effects following any examination session other than slight distance vision blur lasting approximately 5 minutes in the NC and LTOK subjects. This was due to temporary distortion of the corneal surface by the instrument probe. A Fischer-Schweitzer mosaic was also seen in the same subjects following the scan and persisted for approximately the same period as the blurred vision.

The OK lens wearing subjects wore standard reverse geometry lenses. The parameters of the lenses were measured using standard lens metrology techniques, and these details are described in Table 1. A computerized corneal topography map was taken of each OK subjects' right eye using a Medmont E-300 corneal topographer (Medmont Pty. Ltd., Melbourne, Australia) to describe the profile of the cornea. Standard digital slit-lamp photography was used to record the presence of fibrillary lines in the corneal mid-periphery.

Results

Two-dimensional maps of the SBNP were reconstructed from the scanned images (Figs. 2a–c) and were approximately 6.4 mm × 6.7 mm, 5.8 mm × 7.2 mm, and 6.8 mm × 8.5 mm (h × w) in dimension for the NC, STOK, and LTOK eyes, respectively. In the non–lens wearing eye, nerves converged radially from the outer edges of the map towards a clockwise whorl-like complex, centered inferior-nasally approximately 1 mm below the corneal center. The tracing of the nerves in Figure 3a shows this pattern more clearly. In the OK lens wearing eyes, the whorl-like complex was absent, replaced by a tortuous network of less dense and fewer interconnected nerve fibers centrally, and curvilinear fibers mid-peripherally, particularly in the nasal, inferior, and temporal regions, shown more clearly in the nerve tracing in Figures 3b and 3c. These curvilinear nerves in the mid-periphery were located approximately 2.5 to 3 mm from the center of the cornea and were thicker than the surrounding nerves in the LTOK eye. There appeared to be a reduction in the density of nerve fibers in the central region of the map and an increase in density in the mid-periphery in both OK subjects compared with the non–lens wearing eye.

Figure 4 shows the nerve tracings of the SBNP from the OK subjects superimposed (to scale) upon computerized corneal topography maps of the same eye. The areas of reduced nerve fiber density over the central cornea corresponded with the area of relative corneal flattening on the topography maps. The curvilinear nerves in the mid-periphery arcing beneath the central corneal zone appeared to coincide with the outer edges of the area of relative corneal flattening, as well as the mid-peripheral areas of relatively no change and slight steepening in corneal curvature.

Fibrillary lines following a curvilinear pattern were observed in the mid-periphery of the nasal, inferior, and temporal cornea of the LTOK eye using slit-lamp biomicroscopy.

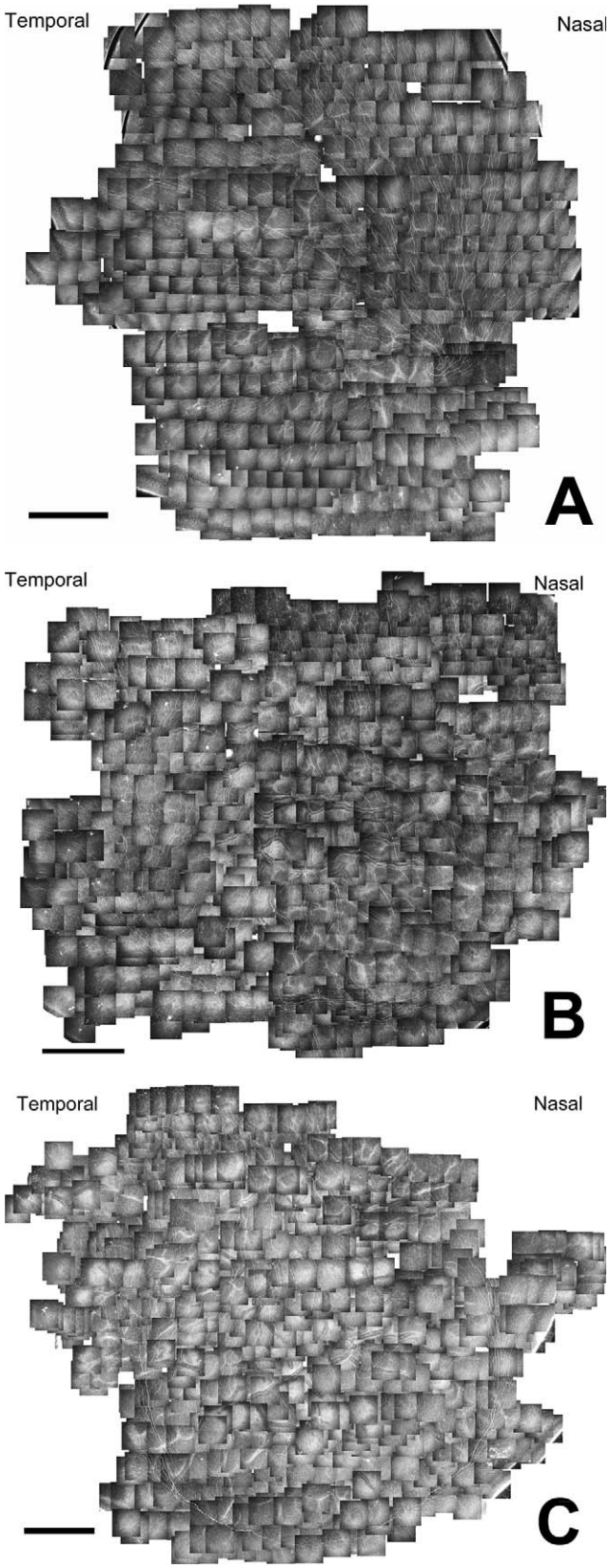

Figure 2. Two-dimensional maps of the sub-basal nerve plexus reconstructed from 575 scanned images of the non–contact lens wearing eye (**A**), 430 scanned images of a short-term OK lens wearing eye (**B**), and 676 scanned images of a long-term OK lens wearing eye (**C**). Bar = 1 mm.

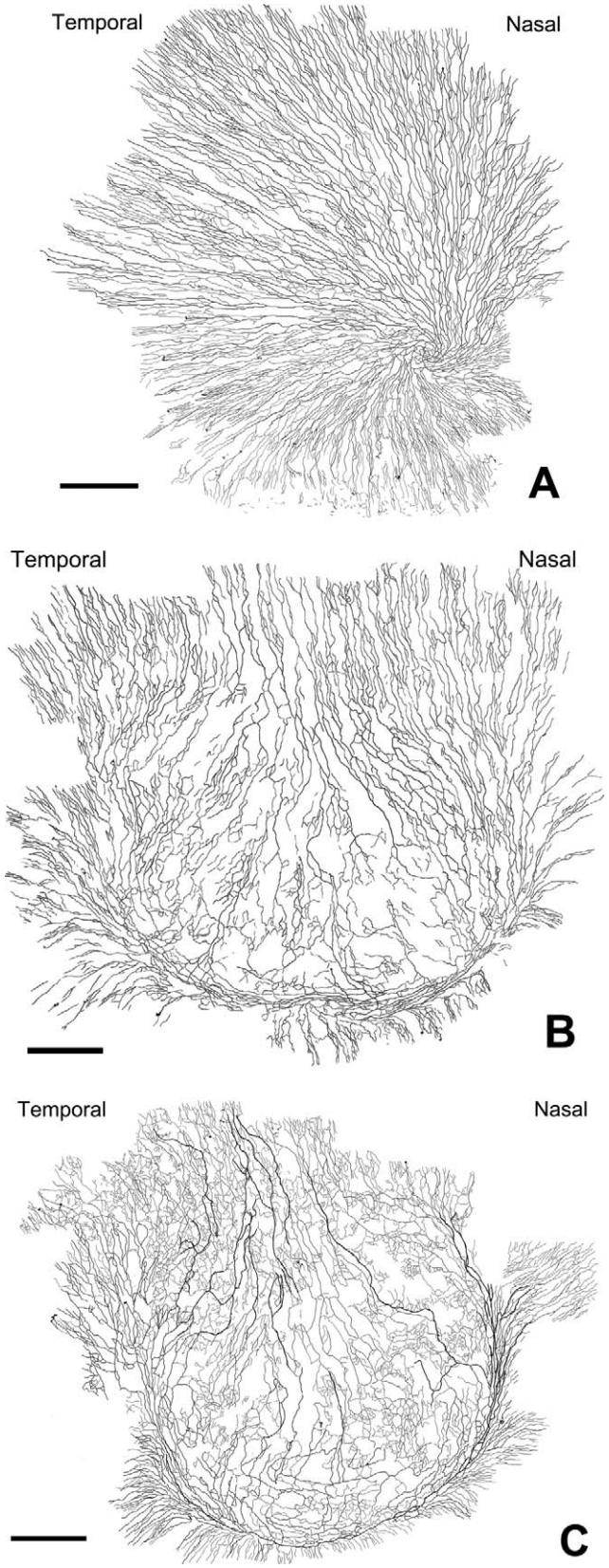

Figure 3. Nerve tracings of the sub-basal nerve plexus map from a non–contact lens wearing eye (**A**), short-term (**B**) and long-term (**C**) OK lens wearing eye. Bar = 1 mm.

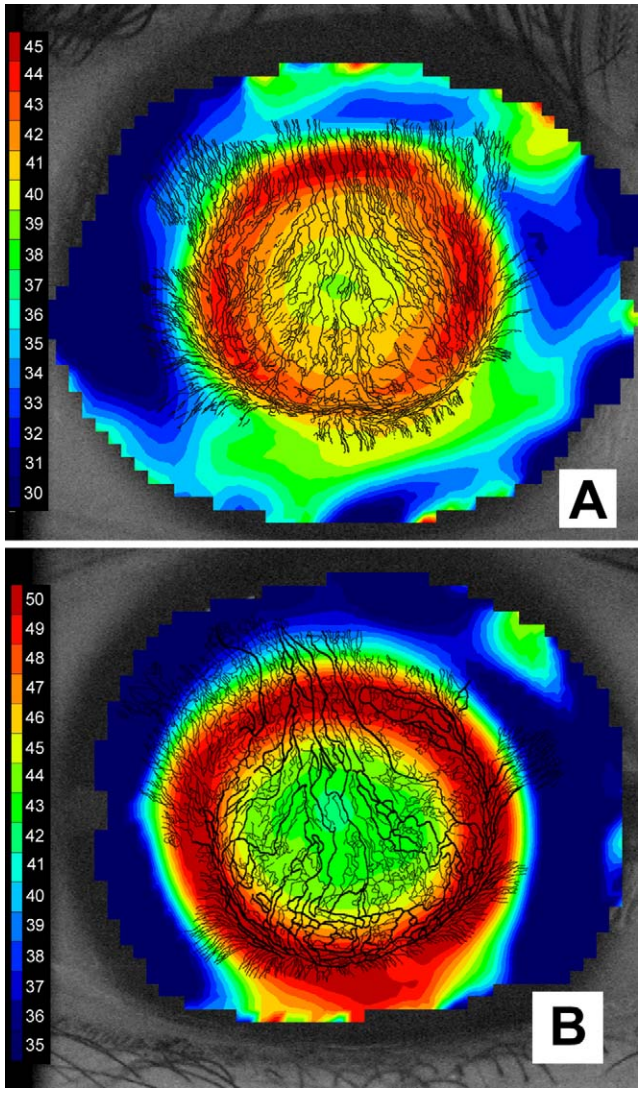

Figure 4. Nerve tracing of the SBNP superimposed upon a computerized corneal topography map (tangential power) in the same eye of a short-term (**A**) and long-term (**B**) OK lens wearing subject.

Photographic images of these lines from the nasal region are shown in Figures 5a and 5b. The pattern of these lines closely matches the scanned nerves from the SBNP in the same region as shown in Figure 5c. There were no such lines observed in the NC or STOK eyes.

Discussion

Although this study only reported the appearance of the SBNP in two OK subjects, the obvious differences between the nerve map for a non–lens wearer compared with the OK lens wearers provide compelling evidence that overnight OK lens wear alters the normal nerve pattern observed in healthy, non–lens wearing eyes.

This study also supports previous suggestions[29–31] that the appearance of fibrillary lines during OK treatment are altered nerve fibers in the SBNP. This is seen in the matched patterns between the fibrillary lines observed using slit-lamp biomicroscopy with those of the thick, curvilinear nerves in the mid-peripheral SBNP using LSCM in the LTOK subject (Fig. 5). Alterations in the thickness of these nerves may have made visible the fibers that are normally invisible using slit-lamp biomicroscopy. This suggestion is further supported by the absence of fibrillary lines in the STOK subject, where the thickness of mid-peripheral, curvilinear nerves appeared equal to surrounding nerves.

The mechanism underlying these OK-induced alterations in the nerve map is unknown. We speculate that the forces exerted by the OK lens during eye closure to produce corneal profile change[33] are somehow responsible for the reorganization of the SBNP. This is supported by our nerve/topography map overlays (Fig. 4), which illustrated how the apparent reduction in nerve fiber density centrally coincided with the area of relative corneal flattening, thereby suggesting that the positive force or pressure exerted by the OK lens on the corneal surface may have been responsible for the reduction in nerve density. In addition, the apparent increase in nerve fiber density and thickness mid-peripherally occurred in an area of no profile change or slight corneal steepening, suggesting this region of neutral or negative pressure under the OK lens, otherwise known as the return zone area, may have played a role in the increase in nerve density. Furthermore, the curvilinear mid-peripheral nerves appear to follow a similar curvature as the return zone area, suggesting that pressure from the lens has manipulated the orientation of these nerves. Therefore, the unique balance of positive, neutral, and negative pressures exerted by an OK lens during overnight wear is possibly the cause for the distinctive pattern seen in the SBNP of the OK lens wearers.

The cause of the apparent increase in nerve fiber thickness in the mid-periphery of the LTOK subject is unknown. Many studies have reported the enlargement of corneal nerves in eye diseases, with some investigators suggesting that increases in the myelination of nerves contributes to their enlargement and consequently thickening.[34] However, Patel and McGhee reported that illumination intensity of the HRT confocal microscope affects the apparent thickness of corneal nerves, particularly as they approach the limits of resolution.[35] In this study, the illumination intensity was constantly set on automatic mode. Consequently, if the background of a single scan appeared underexposed to the instrument, an automatic increase in the illumination intensity occurred, thereby potentially increasing the apparent thickness of the nerve fibers. Hence, the thickness change may be an artifact created by the LSCM. Alternatively, these thicker curvilinear nerves may represent compressed bundles of separate nerve fibers running closely together rather than an actual thickening of a single fiber. This is suggested in Figures 3c and 5c, where nerve fibers from the periphery appear to converge to create a single line, which becomes large enough to be resolved using slit-lamp biomicroscopy. Thus, fibrillary lines may well be a compressed bundle of separate nerves running very closely together.

In this study, a clockwise whorl-like nerve pattern was observed in the non–lens wearer's map, which is consistent with the findings from previous reports using a similar in vivo mapping technique in normal subjects,[21] and ex vivo microphotography techniques on normal cadaver eyes.[36–37] Given that several researchers have suggested nerves and cells migrate in tandem in healthy human corneas,[21,28–29] this nerve pattern suggests that in a normal eye cells migrate centripetally towards the inferior-central cornea. The whorl-like pattern, however, was absent in the OK lens wearers' maps, replaced by a tortuous network of nerves centrally, and a curvilinear pattern of nerves mid-peripherally, suggesting a disruption to the normal nerve and epithelial cell migration pattern. Hence, we speculate that the corneal contour changes occurring during OK treatment may not only be the result of epithelial thickness change but also a redistribution of epithelial cells.

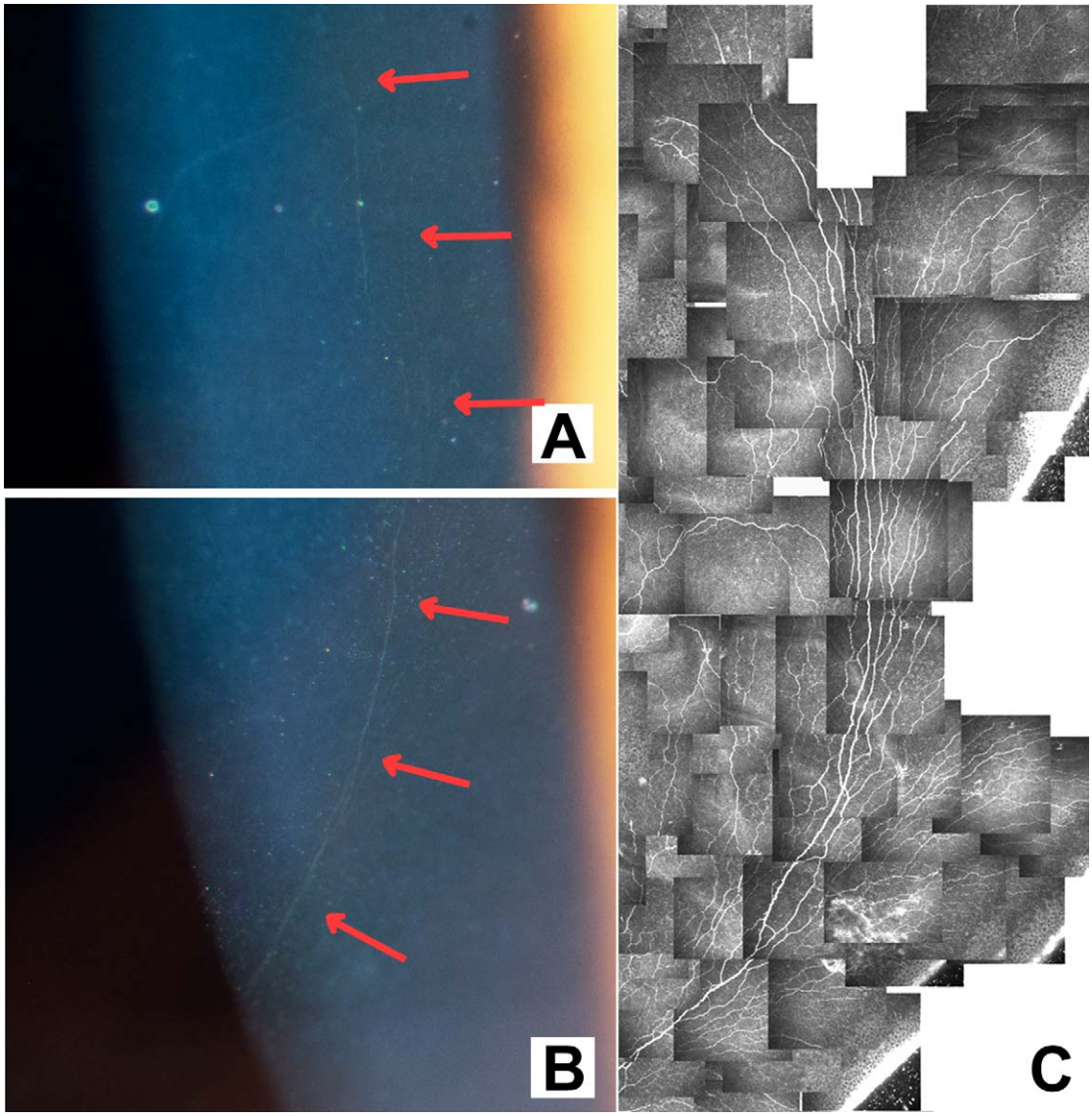

Figure 5. Fibrillary lines (arrows) in the nasal mid-periphery of a long-term OK lens wearer (**A**, **B**), and the corresponding map of the SBNP from the same subject (**C**).

The nerve map pattern observed in the OK lens wearing subjects shows some similarity to the maps described by Patel and McGhee[8] in keratoconic corneas using the same in vivo LSCM technique. In their study, a similar reduction in nerve density is observed centrally. Patel and McGhee also observe nerve fiber bundles following a curvilinear pattern at the base of the cone, similar to what is seen in the mid-periphery of our OK subjects. However, keratoconus is a degenerative eye disease that results in an irregular corneal profile and is accompanied by other nerve morphology changes, which were not observed in the current study. In addition, changes due to keratoconus are permanent, whereas the corneal profile changes in OK are known to be reversible. Since the corneal profile returns to its original shape on cessation of lens wear,[38] we speculate that changes to the SBNP during OK treatment may likewise revert to the normal pattern when treatment ceases. This requires further investigation.

There are three possible mechanisms underlying the apparent reduction in central nerve density and interconnections in the OK lens wearers: diversion of nerves from their usual trajectory, increased distance between nerves, or a loss of nerves, such as occurs in ocular disease.[4–14] First, from the nerve map, it appears that the paths of the nerves that originate from the outer limits were diverted from their normal trajectory, towards a new path within the mid-periphery. Hence, the nerves normally seen in the center appear to have been redistributed towards the mid-periphery. Second, the reshaping of the corneal profile causes compression of the epithelial cells centrally,[24,27] thereby increasing the en face surface area of each cell. Since fibers in the SBNP are thought to mainly run between neighboring epithelial cells,[18] the flattening of cells may increase the separation of nerves, thereby giving the impression of reduced nerve density. Third, the reduction in the nerve density centrally may be the result of nerve loss, which should have implications for the sensitivity of the cornea, particularly in relation to its regional variations. Hiraoka et al.[39] found an overall reduction in corneal sensitivity after 3 months of OK treatment. In that study, measurements are taken at five locations: at the corneal apex, and at 2 mm from the limbus in the superior, inferior, nasal, and temporal corneal locations, and no regional variations in sensitivity were found at baseline or at the 3-month visit. However, sensitivity

was measured using the Cochet-Bonnet aesthesiometer with the thicker 0.12-mm diameter nylon monofilament, which has been demonstrated to have a truncated stimulus intensity range.[40] Therefore subtle differences across the cornea may not have been detected. A change in sensitivity would be expected with such marked changes in morphology as have been observed in this present study, as many studies involving subjects with eye[4–5,7,11–12,14] and systemic disease,[12–13] as well as following corneal surgery,[16–17] show a relationship between changes in nerve morphology and corneal sensitivity. In addition, a reduction in nerve density may also have implications on corneal wound healing[41–42] and the immune response to corneal infections.[15] Hence, the clinical implications of restructuring the SBNP on corneal sensitivity as well as the long-term health of human eyes treated with OK require further investigation.

In summary, this study reports the mapping of the corneal SBNP in OK lens wear. The apparent changes in these nerve maps compared with the map of a non–lens wearer appeared to coincide with the contour changes on the computerized topography maps. The nerve maps also provided evidence to suggest that the migration pattern of epithelial cells was altered during OK treatment, thereby adding to our understanding of the complex changes that occur to the epithelial layer during OK treatment. This study also confirmed previous suggestions that fibrillary lines observed during OK treatment are altered nerve fibers in the SBNP. Further studies are required to determine the onset and possible recovery of these changes in the SBNP, as well as whether functional changes such as altered corneal sensitivity reflect the morphologic changes seen in this study.

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