Corneal Health during Three Months of Scleral Lens Wear

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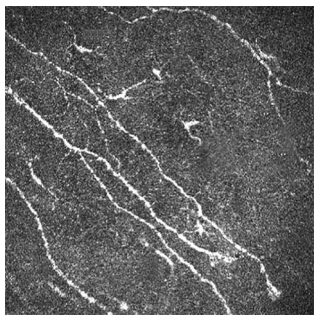
SIGNIFICANCE: This study evaluated the effects scleral lens wear has on corneal health using fluorometry and *in vivo* confocal microscopy. No subclinical changes on healthy corneas of young subjects were observed during 3 months of scleral lens wear.

PURPOSE: This study aimed to evaluate the effects 3 months of scleral lens wear has on the corneal epithelial barrier function, dendritic cell density, and nerve fiber morphology.

METHODS: Twenty-seven neophytes (mean [standard deviation] age, 21.4 [3.9] years) wore scleral lenses of a fluorosilicone acrylate material bilaterally (97 Dk, 15.6 to 16.0-mm diameter) for 3 months without overnight wear. Subjects were randomized to use either Addipak (n = 12) or PuriLens Plus (n = 15) during lens insertion. Measurements of corneal epithelial permeability to fluorescein were performed with automated scanning fluorophotometer (Fluorotron Master; Ocumetrics, Mountain View, CA) on the central cornea of the right eye and the temporal corneal periphery of the left eye. Images of the distributions of corneal nerve fibers and dendritic cells and nerve fibers were captured *in vivo* with a confocal laser scanning microscope (Heidelberg Retina Tomograph, Rostock Cornea Module; Heidelberg Engineering, Heidelberg, Germany) on the central and inferior peripheral cornea of the left eye. Corneal measurements and imaging were performed at baseline and after 1 and 3 months of lens wear.

RESULTS: The corneal permeability values in natural log, dendritic cell densities, and nerve fiber morphology did not significantly change from baseline to 1 and 3 months of lens wear, for both central and peripheral corneal regions ($P > .05$). Dendritic cell density at the inferior cornea was higher than the central cornea throughout the study ($P < .001$). No relationships were observed between each outcome measurements and the saline solution groups ($P > .05$).

CONCLUSIONS: Scleral lens wear for 3 months on healthy cornea of young subjects did not affect corneal epithelial barrier function, nerve fiber, and dendritic cell densities. Buffered and nonbuffered saline solutions impacted the corneal health in similar ways.



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The cornea is an innate defense system of the eye and acts as a protective barrier from the outside environment. Previous *in vivo* studies used fluorometry and confocal microscopy to effectively monitor subclinical changes of the cornea induced by contact lens wear and care solutions.[1–8] Lin and Polse[1] and Lin et al.[3] have shown with fluorometry that overnight eye-closure hypoxia alone without contact lenses and open-eye conditions with contact lenses do not alter corneal epithelial permeability; however, continuous-wear modality with soft contact lenses had a greater impact on increased corneal epithelial permeability than did corneal gas-permeable lenses, even with a high oxygen-transmissible lens material. In addition, higher corneal epithelial permeability was associated with increased risk of contact lens–induced inflammatory adverse events.[4] Clinical studies using confocal microscopy for corneal imaging observed a higher density of dendritic cells at the central cornea with contact lens wearers than with non–contact lens wearers.[6] There was no difference in dendritic cell densities between soft contact lens wearers and corneal gas-permeable contact lens wearers,[7] but a greater density of dendritic cells was observed with subjects who wore silicone hydrogel lenses compared with traditional hydrogel lenses.[8]

Although these two instruments are widely accepted for evaluating *in vivo* corneal changes induced by soft and corneal gas-permeable lenses, it remains unclear if similar changes occur during scleral lens wear. A scleral lens is a large-diameter gas-permeable lens that maintains a tear reservoir between the lens and the cornea, and the reservoir is filled with different types of preservative-free saline solutions before lens insertion. Depending on how the lens and tear reservoir interact with the cornea, scleral lens wear may have the potential to either rehabilitate the corneal health or cause adverse effects. In recent years, the numbers of scleral lens wearers have increased for individuals with healthy and diseased ocular surfaces; however, there is scarce evidence regarding how a scleral gas-permeable lens together with the saline solution in the post-lens tear film alters the corneal health compared with other contact lens types. The primary aim of this study was to assess the effects of 3-month scleral lens wear on the corneal epithelial barrier function, corneal dendritic cell, nerve fiber, and endothelial cell densities on the cornea. The secondary aims were to monitor subjective ratings (e.g., lens wear comfort, ocular dryness, vision quality, and fogginess) during 3-month scleral lens wear and to determine whether different lens care solutions altered the relationship between scleral lens wear and the corneal health.

METHODS

Study Design

This was a prospective, double-masked, randomized, bilateral, single-center (University of California, Berkeley, Clinical Research

Center) study involving five visits. Subjects wore commercially available scleral lenses bilaterally with no overnight wear for a minimum of 8 hours a day and 5 days a week for 3 months. Each subject was assigned to use one type of preservative-free saline solutions according to a pre-determined randomization scheme. This research project adhered to the tenets of the Declaration of Helsinki; it was approved by the institutional review board (Committee for Protection of Human Subjects, University of California, Berkeley) and was compliant with the Health Insurance Portability and Accountability Act.

Subjects

Neophytes, defined as individuals with no history of contact lens wear or no contact lens wear for at least 1 year before enrollment, were recruited from the University of California, Berkeley campus and the surrounding community. Eligibility criteria included age older than 18 years, a self-reported eye examination in the last 2 years, spectacle spherical prescription between –0.25 and –8.00 D, and corrected visual acuity of 20/30 or better in each eye with habitual spectacles. Subjects with a history of systemic or ocular disease or surgery or currently taking systemic or ocular medications that affect ocular health were excluded from the study.

Instrumentation

An automated scanning fluorometer (Fluorotron Master; Ocumetrics, Mountain View, CA) was used to measure stromal fluorescence and the rate of sodium fluorescein penetration into the cornea to determine corneal epithelial permeability (P_{dc}). After baseline stromal fluorescence measurements of both eyes were taken, 2 μL of 0.35% sodium fluorescein was instilled on the superior bulbar conjunctiva of each eye, followed by alternate scans of both eyes during a 20-minute period. Thereafter, eyes were irrigated with preservative-free saline solution to remove residual tear-film fluorescein to obtain final stromal fluorescence readings. The eye to be measured first with the fluorometer was block randomized. P_{dc} measurements were performed on the central cornea of the right eye and on the temporal corneal periphery of the left eye. After measurements, slit-lamp examination with sodium fluorescein was conducted to screen for the presence of corneal staining according to the Mandel grading system.[9] If more than five punctate corneal staining spots were observed in the central cornea or temporal periphery, the measurement was excluded from the analysis because excessive staining could potentially bias the estimates of corneal epithelial permeability.[10] Details of the fluorometry procedure and estimation of P_{dc} from the fluorescence decay readings had been reported previously.[11]

A confocal laser scanning microscope (Heidelberg Retina Tomograph, Rostock Cornea Module; Heidelberg Engineering, Heidelberg, Germany) was used to capture images of the cornea on the left eye only in two separate areas: center and periphery at 6 o'clock. The tip of the objective lens of the Rostock Corneal Module was filled with GenTeal Tears Gel (Alcon: A Novartis Division, Fort Worth, TX) before the attachment of a new disposable Perspex cap (Tomocap) to facilitate optical coupling between the objective lens and the Tomocap.[6] After an anesthetic drop (Proparacaine Hydrochloride Ophthalmic Solution USP 0.5%; Bausch & Lomb Inc., Tampa, FL) and a drop of GenTeal Tears Gel were instilled into each eye, the subject was advised to fixate on a white light located in front of the right eye to capture images of the left eye. Images were focused at the levels of the subbasal nerve plexus and the endothelium layer with a field of view of 300 × 300 μm^2 and produced a digital image size of 384 × 384 pixels (Fig. 1). A minimum of three volume scans and two sequence scans per central and inferior

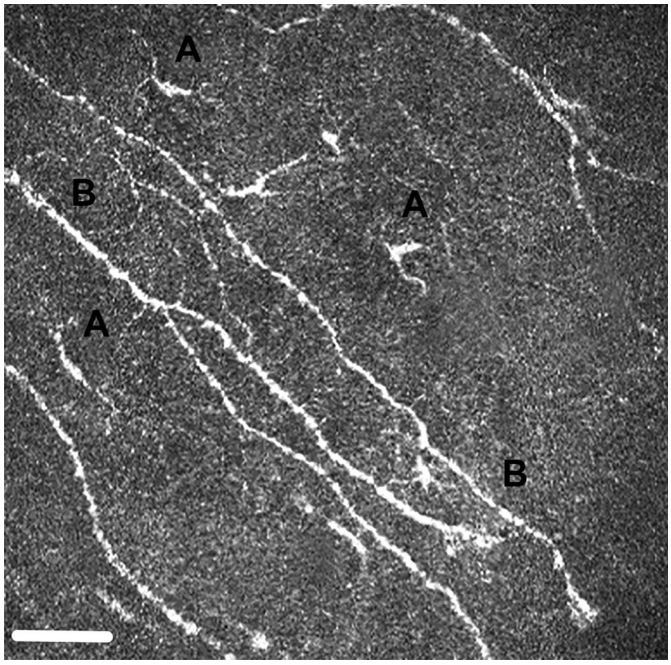

FIGURE 1. Dendritic cell (A) and corneal nerve (B) distributions of the central corneal region by confocal microscopy. Dendritic cells are located on the subbasal nerve plexus layer. Scale bar, 50 μm.

cornea for dendritic cell and nerve fiber morphology analyses were performed, whereas it was at least two volume scans of the central cornea for endothelial cell count. Each volume scan captured a series of 40 images, and each sequence scan captured a series of 100 images. A subset of images was selected from the volume and sequence scans for analysis. Images were excluded if they were overlapping with each other or a cross section of different layers of the cornea appeared in the same image. Means (standard deviations) of 10 (3) images of the central cornea and 9 (4) images of the inferior corneal periphery were selected for each subject at each visit. A subset of four images was randomly selected for each subject at each visit for dendritic cell count analysis. The number of dendritic cells was counted manually by two investigators in each image per epithelial section and was given as cells per millimeter squared. ACCMetrics, an image analysis software developed at the University of Manchester (Manchester, United Kingdom), was used for corneal nerve analysis, and the nerve fiber morphology was based on three parameters: nerve fiber density, nerve branch density, and nerve fiber length.[12–16] Nerve fiber density was the number of fibers per millimeter squared, nerve branch density was the number of branch points on the main fibers per millimeter squared, and nerve fiber length was the total length of nerves in millimeters per millimeter squared. Endothelial cell count was obtained by selecting two images per subject for analysis using the Heidelberg IVCM image capture cell count software.

Study Protocol

At the first visit, subjects read and signed an informed consent form, followed by a screening examination of the ocular surface and scleral lens fitting. Baseline visual acuity was measured, corneal topography was taken (Medmont E300 Medmont International Pty Ltd., Vermont, Australia), and anterior ocular surface health was assessed using a slit-lamp biomicroscopy (SL120; Carl Zeiss

Meditec Inc., Jena, Germany) with sodium fluorescein (BioGlo Fluorescein Strips; HUB Pharmaceuticals, LLC, Rancho Cucamonga, CA). Based on corneal sagittal height, keratometry readings, and elevation maps generated by Medmont topography, all qualified subjects were fitted with Essilor Jupiter Scleral Lens (Essilor of America, Inc., Dallas, TX) with a standard design to determine appropriate lens parameters for acceptable lens fits. After 20 to 30 minutes of lens settling, lens assessment and overrefraction were performed, and central post-lens tear thickness was measured using high-resolution spectral domain optical coherence tomography (ENVSISU 2300; Bioptigen Inc., Durham, NC). Based on these measurements, a pair of scleral lenses was ordered for each subject. At the end of this visit, lenses were removed, and anterior ocular surface health was assessed with slit-lamp biomicroscopy using sodium fluorescein, followed by exit visual acuity.

The subsequent visits required subjects to arrive at least 2 hours after awakening for baseline measurements with fluorometry and *in vivo* confocal microscopy on 2 separate days with a minimum of 24 hours apart. Before each instrument use, visual acuity was measured, and anterior ocular health was assessed with slit-lamp biomicroscopy and white light. After baseline P_{dc} measurements and *in vivo* corneal imaging were taken, subjects were then dispensed ordered scleral lenses for daily wear adaptation and returned for a 1-week progress check with central post-lens tear thickness measurement and ocular health examination with sodium fluorescein. Once an acceptable lens fit with good comfort, vision, and ocular surface health was achieved, subjects were instructed to wear scleral lenses on both eyes for at least 8 hours a day and 5 days a week for 3 months with no overnight wear. Subjects were instructed on proper lens insertion and removal techniques and were randomized to fill their scleral lenses before lens insertion with either PuriLens Plus (The LifeStyle Company, Inc., Freehold, NJ) or Addipak (Hudson RCI; Teleflex Medical, Morrisville, NC) preservative-free saline solutions. PuriLens Plus saline solution is a sterile 0.9% sodium chloride solution, buffered with boric acid and sodium borate, compared with Addipak saline solution, which is an unbuffered, sterile 0.9% sodium chloride solution. Three samples each of PuriLens Plus and Addipak saline solutions were tested for their pH values with a standard Ag/KCl glass electrode (Cole Parmer, Vernon Hills, IL) and a digital pH-meter ionalyzer (model 501; Orion Research Inc., Cambridge, MA). Subjects were instructed to use Boston Simplus Multi-Action Solution (Bausch & Lomb Inc., Rochester, NY) for lens cleaning and storing of their scleral lenses. P_{dc} measurements and *in vivo* corneal imaging were repeated after 1 and 3 months of scleral lens wear. All appointment times were kept approximately the same (±30 minutes), with each subject arriving at least 2 hours after awakening[17] and with discontinuation of eye drops or allergy medications for 1 full day before the visit. For 1-week, 1-month, and 3-month visits, subjects had worn scleral lenses for a minimum of 2 hours before the visit to control for scleral lens settling and corneal ocular health.[18] Upon arrival of 1- and 3-month visits, questionnaires were administered to rate their comfort, visual quality, fogginess, and dryness of each eye on 100-point visual analog scales. Comfort, visual quality, fogginess, and dryness were rated on a 0- to 100-point scale, where 0 was defined as "cannot be worn, causes pain," "extremely poor vision," "no fogging ever," and "no dryness felt," whereas 100 was defined as "cannot be felt," "excellent vision," "extreme fogging," and "extremely dry," respectively. At the end of every visit, anterior ocular surface health was assessed with biomicroscopy and sodium fluorescein, followed by exit visual acuity.

Statistical Analysis

The study featured a nested hierarchy design with observations from two eyes and repeated measurements of each subject. Mixed-effects model was applied to examine variation of P_{dc}, dendritic cells, endothelial cell count, and nerve fiber during the 3-month study period. The statistical analysis was programmed with SAS PROC MIXED procedure (SAS Institute Inc., Cary, NY). Potential contributing factors such as demographics, lens solution, ocular surface health, and so on were specified as fixed effects, and the correlation between paired eyes and repeated measurements was specified as random effects. The component of random effects produced a more accurate variance-covariance structure, taking into consideration the relative kinship. Variables with 0.05 significance level or less were kept in the model. Model selection was based on Akaike information criterion and Schwarz's Bayesian information criterion.

RESULTS

Subject Demographics and Lens Parameters

A total of 75 subjects were screened for eligibility. Of these, 31 subjects met the inclusion criteria and were able to adapt successfully to scleral lens wear. Four subjects discontinued from the study after 1 month because three subjects were unable to return for their 3-month visits and one subject misplaced the scleral lenses. A total of 27 neophytes (19 female and 8 male subjects; 14 Asians and 13 non-Asians) with a mean (standard deviation) age of 21.5 (3.9) years completed the study. Fifteen subjects (11 female and 4 male) used PuriLens Plus saline solution with their scleral lenses, and 12 subjects (8 female and 4 male) used Addipak saline solution. PuriLens Plus saline solution was tested to have a mean (standard deviation) pH level of 7.50 (0.03), and Addipak saline solution was tested to have a pH level of 5.48 (0.01). Table 1 reports the values of mean (standard deviation) of ocular features and lens parameters of completed subjects. A majority of the subjects wore bilaterally the Jupiter Scleral Lens with a standard design, and three subjects wore the Jupiter Scleral lens with a reverse geometry design. One subject wore scleral lenses designed with toric peripheral curves, and the rest of the lenses maintained spherical peripheral curves.

Corneal Epithelial Permeability

The overall descriptive statistics for $\ln(P_{dc})$ values on the central and temporal corneal regions for each visit are shown in Table 2. P_{dc} values were transformed to natural logarithm to approximate normal distribution for analysis. A smaller negative value of $\ln(P_{dc})$ indicated higher corneal epithelial permeability. Eight P_{dc} measurements were excluded from analysis because there were more than five corneal punctate staining spots at the observed areas. The inclusion of these data points could have resulted in an overestimate of the corneal permeability. In addition, six P_{dc} measurements were excluded from analysis because of negative P_{dc} values, which were physiologically impossible and were likely caused by differences in the corneal alignment between the background and post-rinse fluorescence readings. The mean $\ln(P_{dc})$ values from baseline did not change significantly at 1 and 3 months of lens wear, for both central and temporal corneal regions ($P > .05$). Age, sex, ethnicity, and saline solution groups had no association with the mean $\ln(P_{dc})$ values at both corneal regions in the multivariate analysis ($P > .05$).

TABLE 1. Descriptive statistics of ocular and lens parameters

Variable	Total, Mean (95% CI)	PuriLens Plus, Mean (95% CI)	Addipak, Mean (95% CI)
No. subjects	27	15	12
Lens base curve (mm)	7.55 (7.47 to 7.63)	7.58 (7.47 to 7.70)	7.51 (7.41 to 7.62)
Lens power (D)	–5.40 (–5.99 to –4.82)	–5.11 (–5.94 to –4.27)	–5.77 (–6.57 to –4.97)
Lens thickness (μm)	420.1 (408.4 to 431.7)	415.6 (399.6 to 431.7)	425.2 (408.1 to 442.3)
Lens diameter (mm)	15.61 (15.58 to 15.63)	15.59 (15.56 to 15.62)	15.63 (15.59 to 15.68)
Post-lens tear thickness at 1-wk visit (μm)	220.0 (199.0 to 240.9)	216.2 (191.5 to 240.9)	222.8 (190.7 to 254.9)
Ocular sagittal height at a chord of 10 mm (μm) degree 0	1.69 (1.68 to 1.70)	1.69 (1.67 to 1.70)	1.70 (1.68 to 1.72)
Ocular sagittal height at a chord of 10 mm (μm) degree 180	1.70 (1.69 to 1.71)	1.69 (1.68 to 1.71)	1.71 (1.70 to 1.73)
Horizontal visible iris diameter (mm)	11.5 (11.4 to 11.6)	11.4 (11.3 to 11.6)	11.6 (11.4 to 11.8)

CI = confidence interval.

Dendritic Cell Density

The overall descriptive statistics for dendritic cell density on the central cornea and inferior corneal periphery are shown in Table 3. There were no significant changes in dendritic cell densities from baseline to 1 and 3 months of lens wear, for both central and inferior peripheral regions ($P > .05$). The mean dendritic cell density at the inferior corneal periphery was significantly higher than that at the central cornea throughout the study ($P < .001$). Age had no association with the dendritic cell densities at both corneal regions; however, the dendritic cell density at the central cornea was significantly lower in Asians than in non-Asians ($P = .03$), and the dendritic cell density at the inferior cornea was significantly lower for female than male subjects ($P = .04$). There was no significant association between the dendritic cell densities at different corneal regions and the saline solution groups ($P > .05$).

Corneal Nerve Fiber Morphology

The overall descriptive statistics for the three parameters of corneal nerve fiber morphology on the central cornea and inferior corneal periphery are shown in Table 4. There were no significant changes in corneal nerve fiber densities, branch densities, and fiber lengths at both corneal regions from baseline to 1 and 3 months of lens wear ($P > .05$). Age, sex, ethnicity, and saline solution groups were not associated with the three parameters of the corneal nerve fiber morphology at both corneal regions in the multivariate analysis ($P > .05$).

TABLE 2. Descriptive statistics of natural logarithm-transformed corneal epithelial permeability measurements

Location	Visit	ln(P_{dc}), Mean (95% CI; nm/s)
Center	Baseline	–3.6 (–3.9 to –3.3)
	1 mo	–3.4 (–3.6 to –3.2)
	3 mo	–3.6 (–3.9 to –3.3)
Temporal periphery	Baseline	–3.1 (–3.4 to –2.7)
	1 mo	–3.3 (–3.6 to –3.0)
	3 mo	–3.0 (–3.2 to –2.8)

CI = confidence interval; ln = natural logarithm-transformed; P_{dc} = corneal epithelial permeability.

Endothelial Cell Density

The overall mean (95% confidence interval) endothelial cell densities at the central cornea were 3010 (2885 to 3135) cells/mm^2 at baseline, 3032 (2898 to 3166) cells/mm^2 at 1 month, and 3072 (2931 to 3214) cells/mm^2 at 3 months. The mean endothelial cell densities did not change significantly from baseline to 1 and 3 months after lens wear ($P < .05$). Age, sex, ethnicity, and saline solution groups were not associated with the endothelial cell densities at each time point.

Subjective Ratings

The overall descriptive statistics of subjective rating scores on ocular comfort, visual quality, fogginess, and dryness during scleral lens wear are shown in Table 5. All subjective rating scores on the questionnaire were not significantly different between 1 and 3 months of lens wear ($P > .05$). All subjective responses on comfort, visual quality, fogginess, and dryness at 1- and 3-month visits were not associated with the P_{dc} values, dendritic cell densities, corneal nerve fiber morphology parameters, endothelial cell densities, saline solution groups, age, sex, and wearing time. Asians reported significantly higher subjective rating scores of dryness compared with non-Asians ($P = .01$); however, the other subjective responses were similar between Asians and non-Asians. Two low comfort scores (15 and 21) were reported by one subject because of poor lens surface wettability on the anterior surface of the scleral lenses during two visits.

TABLE 3. Descriptive statistics of dendritic cell density

Location	Visit	Mean (95% CI; cells/mm^2)
Center	Baseline	36 (16–57)
	1 mo	27 (10–45)
	3 mo	31 (11–50)
Inferior periphery	Baseline	81 (53–109)
	1 mo	79 (52–106)
	3 mo	71 (49–94)

CI = confidence interval.

DISCUSSION

To evaluate subclinical changes of the cornea during scleral lens wear, fluorometry and *in vivo* confocal microscopy were performed on 27 subjects at baseline and at 1 and 3 months after lens wear. The corneal health was monitored by investigating the corneal epithelial barrier function, nerve fiber morphology, and densities of dendritic and endothelial cells. The results of this study suggested that 3 months of scleral lens wear had no subclinical effects on the healthy corneas of young subjects. In addition, there was no significant difference in the effects on the corneal health between the saline solutions (PuriLens Plus vs. Addipak).

The impact scleral lens had on the corneal epithelial barrier function did not change significantly at different corneal regions after 3 months of full-time scleral lens wear with no overnight use. This finding is similar to what have been observed during open-eye condition with other contact lens types.[1–3] Interestingly, there was also no significant change in dendritic cell densities after 3 months of scleral lens wear. This finding is unlike the reported increased of dendritic cell densities at the central cornea with habitual soft and corneal gas-permeable contact lens wearers compared with non–contact lens wearers.[7,8] When comparing the dendritic cell density based on location, the cell density was expectedly higher in the corneal periphery than in the central cornea for all visits, similar to what was found on healthy corneas[6] and corneas with soft and corneal gas-permeable contact lens wearers.[7] With no changes in dendritic cell densities after 3 months, scleral lens wear seems to not induce significant subclinical inflammation and does not change the distribution of the dendritic cells throughout the cornea. These null findings may be in part due to the limited duration of lens wear because the present study required only 3 months of scleral lens wear, whereas other studies examined habitual contact lens wearers with a minimum of 1 year of wearing experience. Studies with a longer wearing time may be warranted.

TABLE 4. Descriptive statistics of corneal nerve fiber morphology

Parameter	Location	Visit	Mean (95% CI)
CNFD (no. fibers/mm^2)	Center	Baseline	39.9 (36.2–43.7)
		1 mo	42.0 (39.3–44.7)
		3 mo	42.5 (39.4–45.5)
CNBD (no. branch points on the main fibers/mm^2)		Baseline	46.3 (36.3–56.2)
		1 mo	47.8 (38.3–57.3)
		3 mo	48.9 (39.8–58.0)
CNFL (total length of nerves in millimeter/mm^2)		Baseline	17.1 (15.7–18.5)
		1 mo	17.7 (16.4–19.0)
		3 mo	17.9 (16.7–19.0)
CNFD (no. fibers/mm^2)	Inferior periphery	Baseline	29.7 (24.2–35.2)
		1 mo	27.5 (22.3–32.7)
		3 mo	27.4 (23.1–31.7)
CNBD (no. branch points on the main fibers/mm^2)		Baseline	31.4 (22.4–40.4)
		1 mo	27.0 (20.3–33.7)
		3 mo	28.4 (19.6–37.2)
CNFL (total length of nerves in millimeter/mm^2)		Baseline	14.7 (12.8–16.5)
		1 mo	13.9 (12.2–15.7)
		3 mo	14.0 (12.5–15.5)

CI = confidence interval; CNBD = corneal nerve branch density; CNFD = corneal nerve fiber density; CNFL = corneal nerve fiber length.

TABLE 5. Descriptive statistics of overall subjective ratings

Visit	Variable	Mean (95% CI)	Min	Max
1 mo	Comfort	85 (75–94)	21	100
	Visual quality	83 (72–93)	27	100
	Dryness	11 (6–16)	0	33
	Fogginess	22 (9–36)	0	87
3 mo	Comfort	88 (79–97)	15	100
	Visual quality	84 (76–92)	39	100
	Dryness	13 (6–19)	0	51
	Fogginess	16 (6–26)	0	91

CI = confidence interval; Max = maximum value; Min = minimum value.

Previous reports have shown the suitability of confocal microscopy to image corneal nerves in healthy,[19,20] compromised, and diseased corneas.[21,22] Therefore, corneal nerve fiber morphology was also investigated in this study. Three months of scleral lens wear on healthy corneas did not significantly alter the nerve fiber density, nerve branch density, and nerve fiber length of the subbasal epithelial nerve plexus at the central and inferior corneal periphery. These findings agreed with another clinical study using a fluid-filled prosthetic device for PROSE treatment (BostonSight®, Needham, MA), which was a gas-permeable lens similar to a scleral lens. Two to 6 months of wearing the PROSE device did not alter the corneal nerve morphology of distorted corneas and ocular surface diseased subjects.[23] Of interest, studies have also shown that 12-month extended wear and one-night overnight wear of soft contact lenses had no effect on corneal nerve fibers as well.[24,25] Although contact lenses have not been shown to significantly affect the integrity of corneal nerves, it is conceivable that their impact on corneal nerves may require a longer lens-wear duration (e.g., years), as well to become measurable by the same instrumentation.

Furthermore, it has been reported by López-De La Rosa et al.[26] that there was no significant association between the corneal nerve morphology imaged with confocal microscopy and its subjective comfort ratings. Similarly, none of the outcome measurements obtained from this study had any influence on the subjective responses on comfort, visual quality, fogginess, and dryness during 3 months of scleral lens wear. Factors other than the ones assessed in this study, such as corneal edema, must be considered for further understanding the interplay between subjective symptoms and objective clinical assessments during scleral lens wear.

When comparing subjects who used PuriLens Plus and Addipak saline solutions during lens insertion, there were no significant differences between the saline solutions and their effects on the corneal health during 3 months of lens wear. PuriLens Plus saline

solution is buffered with boric acid and sodium borate, whereas Addipak solution is nonbuffered. Several studies demonstrated that the borate-based buffer in saline solutions and multipurpose disinfecting solutions compromised the corneal epithelial barrier function. Tanti et al.[27] conducted an *in vitro* study that exposed monolayers of corneal epithelial cells with buffered preservative-free saline solution and multipurpose disinfecting solution with borate acid buffer as well as phosphate buffer and found that the borate-based multipurpose disinfecting solution increased in cytotoxicity and significantly disrupted the tight junctions between the corneal epithelial cells. Lin et al.[4] also reported that daily irrigation with borate buffered preservative-free saline solution upon awakening over a course of 30-day continuous-wear soft contact lens wear did not diminish the increased corneal epithelial permeability and increased risk of inflammatory adverse events with continuous-wear soft contact lens, compared with the nonirrigation group. The effects of a soft contact lens and borate-based buffered saline solution had altered corneal epithelial barrier function and showed increased dendritic cell density.[7,8] Surprisingly, a scleral lens with either borate buffered or nonbuffered saline solutions did not alter the corneal health. This could be explained by different lens-cornea interaction between a soft contact lens and a scleral lens. The post-lens tear film between the lens and the cornea is significantly greater under a scleral lens than under a soft contact lens. The thicker post-lens tear film creates a protective fluid layer for the cornea and prevents the posterior scleral lens surface along with the accumulated debris in the tear reservoir from mechanically agitating against the corneal surface. In addition, this null finding suggested that other mechanisms may be more impactful in affecting how the ocular surface stays healthy during scleral lens wear.

On a healthy ocular surface, the pH level of tear films ranges from 7.2 to 7.6[28] and remains relatively stable throughout the day owing to its inherent buffering capacity,[28–31] tear turnover rate, and tear drainage.[28,31] Although some *in vitro* studies have demonstrated that decreases in extracellular pH directly can reduce intracellular pH[32,33] and cause alternations in cell morphology and corneal cellular functions,[32,34–36] Carney et al.[37] showed that tear pH ranging from 7.0 to 7.7 showed increased resistance to pH changes when challenged with nonbuffered water pH ranging from 3.5 to 8.0. These authors suggested that the tear buffering capacity was more effective and substantial in response to pH changes when it was more acidic than basic. These findings may in part explain why both saline solutions used in the current study affected the corneal health similarly despite the differences in pH levels. It is also conceivable that the difference in pH levels (e.g., ~2 pH unit on a log scale) between the two saline solutions may not be big enough to induce significant subclinical changes on the corneal health, as the natural tear buffering is able to stabilize the pH environment for the anterior ocular tissues.

Other saline solutions with different buffers may be considered to have a greater impact on the corneal health compared with borate-based buffer. Through clinical experiments, Bier[38] observed how buffered solutions in the tear reservoir under a scleral lens affected the corneal health such as corneal edema. Although corneal edema still occurred eventually for all types of buffers tested, Bier found that sodium bicarbonate buffer had the greatest efficacy in delaying the onset of corneal edema. Bier's observation is consistent with the recent findings of Kim et al.,[39] which showed that the limbal metabolic supply of bicarbonate ion has a significant effect on the pump-leak mechanism of the corneal endothelium to reduce corneal edema during contact lens wear. Likewise, the influx of bicarbonate ion from the buffer has a direct effect on the pump-leak mechanism of the cornea.[40] Direct influence of other buffers on the pump-leak mechanism is less known. Thus, a saline solution with sodium bicarbonate buffer has a positive effect on the corneal health and requires further investigation for scleral lens wear.

In conclusion, scleral lens wear for 3 months had no significant subclinical effect on the cornea of young and healthy subjects. Significant changes on the cornea health that were observed in previous studies with soft contact lens and borate-based saline solutions were not observed with scleral lens wear. This provided further evidence to support the increasing popularity of scleral lens wear on healthy subjects. Further research is necessary to explore the effects of long-term scleral lens wear and various buffers in saline solutions on the corneal health.

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