Histomorphometric Profile of the Corneal Response to Short-term Reverse-geometry Orthokeratology Lens Wear in Primate Corneas

A Pilot Study

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Purpose: To investigate the histological changes in primate cornea induced by short-term overnight orthokeratology (OK).

Methods: Nine young adult primates were used. One animal served as negative control. The remaining 8 animals wore reverse-geometry OK lenses for periods of 4, 8, 16, and 24 hours on 1 eye with the other eye as control. Central and midperipheral corneal thickness, as well as ultrastructural changes in corneal epithelium, stroma and endothelium in response to OK lenses, were evaluated.

Results: OK significantly reduced the thickness of the central cornea in all treatment groups. The central corneal thinning was both stromal and epithelial in origin. Substantial midperipheral corneal thickening was seen in 16-hour and 24-hour lens-wear groups and this effect was both stromal and epithelial in origin as well. Histology evidence indicated the primary epithelial response in the central cornea was compression of cells that resulted in wing cells becoming shorter and basal cells being squatted rather than lost or migration of cell layers. These pronounced cell shape changes occurred without compromising the structural integrity of the desmosomes. The thickened corneal epithelium has normal cell layers. The squamous cells have larger surface sizes and are composed of oval instead of flattened nuclei. This implied delayed surface cell exfoliation at the thickened midperipheral epithelium. Physical presence of OK lens over the cornea did not influence the microstructures of microvilli and microplicae, endothelium, and collagen distribution.

Conclusions: The primate cornea, particularly the corneal epithelium, responds rapidly to the application of reverse-geometry OK lenses with significant epithelial cell shape alterations with short-term OK lens wear. This finding suggests that the corneal epithelium is moldable in response to the physical forces generated by the OK lenses.

Key Words: overnight orthokeratology, cornea, histological changes, primate

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Orthokeratology [OK; also known as corneal refractive therapy (CRT)] is a clinical method that uses specially designed rigid reverse-geometry OK lenses (RGLs) to reshape the corneal contour to temporarily reduce or eliminate refractive error.[1] Several studies have reported the clinical effectiveness of OK, and many topographic studies have shown OK lens–induced flattening at the central cornea.[2–5]

Proposed theories to explain the refractive effect of OK included the alteration of the anterior corneal surface (curvatures and thickness) and the overall bending of the cornea (modulation of the anterior and posterior corneal curvatures). Swarbrick et al[2] proposed that the changes in corneal thickness involved central thinning and were possibly accompanied by thickening toward the periphery. Later, Alharbi and Swarbrick[3] concluded that overnight OK causes rapid central corneal epithelial thinning and midperipheral stromal thickening. The consequent change in corneal sagittal height is the primary factor that contributes to the refractive effect of OK.

Although extensive clinical studies, including evaluations of topographic changes in corneal thickness, have been carried out, the effect of OK lenses on the underlying corneal tissue at the microscopic level is still uncertain. It is impossible to obtain human corneal samples for microscopic studies to reveal the histologic changes during corneal reshaping induced by OK lenses; therefore, an animal model is the best option in this particular study.

There is an increasing number of studies on corneas by using animal models (rabbits and cats)[6,7] to investigate the mechanism that contributes to corneal reshaping. Various hypotheses have been proposed to explain the corneal reshaping

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Supplementary Figures 1–3 are available online at http://www.corneajrnl.com.

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exerted by OK lenses. These include epithelial cell redistribution (overall loss or gain of cell layer), epithelial cell compression with intercellular fluid transfer, modification of basal cell proliferation, increased cell retention, alteration of surface cell exfoliation, and stromal remodeling.[8,9]

Structurally, the primate's cornea consists of 3 main layers (epithelium, stroma, and endothelium) and 2 auxiliary layers (the Bowman membrane and the Descemet membrane). Comparative anatomical studies have shown that the primate cornea is most similar anatomically and histologically to the human cornea, especially because of the presence of the Bowman membrane,[10,11] which is not present in some species of mammals such as rabbits.[12] In this study, we acquired primate corneas (cynomolgus monkeys [*Macaca fascicularis*]) instead of rabbit corneas because it is the most relevant model to study the OK lens–induced histologic changes in the cornea.

Clinically, partial reduction in refractive power and central corneal flattening can actually be achieved within the first few hours of wearing the OK lens.[13–15] This study investigated the subsequent histologic changes in primate corneas that occur in short-term OK lens wear (at selected OK lens wear intervals over a 24-hour study period) in a closed-eye manner.

MATERIALS AND METHODS

Animals

Before starting this study, we obtained ethics approval from the Animal Ethics Committee of the Department of Wildlife and National Parks, Malaysia, and the Animal Ethics Committee of the National University of Malaysia. The welfare of primates (*M. fascicularis*) used in this biomedical research was given serious consideration by these 2 animal ethics committees. Both committees recommended reducing the number of primates used in this study, and thus the sample size in this study is small and limited. This pilot study acquired 9 young adult cynomolgus monkeys of both sexes, weighing 3.0–4.5 kg. The experimental subjects were supplied by the Conservation of Biodiversity Division, Department of Wildlife and National Parks, Malaysia. The animals were treated according to the Animal Ethics Statement for the Use of Animals of the National University of Malaysia. The animal procedures were conducted in accordance with the principles of the Declaration of Helsinki. One subject was assigned to a negative control group (0 hours of OK lens wear). Another 8 subjects were randomly assigned to 4 selected lens wear durations over a 24-hour study period, with 2 animals in each group: (1) short time frame, 4 hours; (2) typical time frame, 8 hours; (3) extended time frame, 16 and 24 hours. One eye of each monkey was randomly chosen to be the experimental eye by coin toss, whereas the other eye served as the positive control.

OK Lenses

Five primates (3 males and 2 females) were randomly selected, and corneal measurements were taken before lens ordering. The keratometric data (K readings) were measured by using an autorefractokeratometer (Retinomax Kplus-2; Nikon, Tokyo, Japan). Five trial multicurve RGLs were manufactured by BE, UltraVision Pty Ltd (Brisbane, Queensland, Australia) from these K readings. Trial fitting was done by experienced optometrists who practiced clinical OK in human subjects. The best fit lens with good fluorescein pattern and good centration that met with the criteria of optimum fit as specified by BE, UltraVision Pty Ltd, was ordered from the company. The lenses were supplied in Boston XO material [oxygen permeability (Dk) = 100×10^{-11} ($cm^2 \cdot L\ O_2$)/($s \cdot mL \cdot hPa$)], and the lens center thickness measured 0.22 mm. The total diameter measured 9.0–10 mm, and the back optic zone diameter measured 6.0–6.5 mm. The lenses were designed to flatten the cornea −3.00 D.

Anesthesia and Euthanasia

Experimental subjects were preanesthetized with a dose of 0.1 mL/kg of 100 mg/mL ketamine (Mavlab, Queensland, Australia) intramuscularly. The subjects were removed from the cages and transferred immediately to the research theater. Subsequent induction and prolongation of anesthesia were carried out as described by Cheah et al.[16] Throughout the experimental period, the centration of the lens was assured by retracting the eyelid at 2-hour intervals. At the end of the experiment, the animals were euthanized by intravenous injection of sodium pentobarbital (Dolethal; Vetoquinol, Madrid, Spain) at a dose of 1 mL/kg. The eyes were enucleated for subsequent tissue processing protocols.

Tissue Processing for Transmission Electron Microscopy

Four milliliters of phosphate-buffered 2.5% glutaraldehyde (pH 7.4) was injected into the eyeball at 4 aspects (superior, inferior, nasal, and temporal) with 1 mL of fixative in each region.[17,18] The eyes were enucleated carefully without damaging any ocular tissues, especially the cornea. The specimens were washed with 0.9% physiologic saline thoroughly and repeatedly for 10 seconds, followed by a 2.5% glutaraldehyde fixative bath. The intact eyeballs were further fixed with glutaraldehyde for 4 hours at 4°C. The cornea with a 1- to 2-mm-wide sclera rim was excised carefully and subjected to overnight fixation at 4°C. The next day (minimal tissue fixation duration was 12 hours), rectangular samples (1 mm × 1.5 mm) were cut from the central and midperipheral (3 mm from center) corneal regions of the fixed cornea by using a single-edge razor. The samples were further processed according to the rapid and modified protocol for transmission electron microscopy (TEM) visualization of the cornea.[17,18]

Quantitative Histologic Examination

Corneal Thickness

In our unpublished procedure, we suggest that the resin-embedded cornea serves as a better target for histomorphometric study under the light microscope than the paraffin-embedded cornea (unpublished data). The thick resin-fixed cornea was sectioned perpendicular to the corneal thickness at 300–400 nm (semithin section). Ten sections were selected, and each section was mounted on a slide, stained with toluidine blue, and made ready for quantitative histologic measurement. The measurement was carried out by using a light microscope (Olympus BX 51) and image analysis software

(Soft Imaging System Analysis Version 3.1). Total corneal thickness and stromal thickness were measured directly from the representative sections. The epithelial thickness was calculated by subtraction (total − stromal thicknesses). Fifty to 80 readings were recorded for statistical analysis (3–5 readings from each corneal section). All data are reported as mean ± SD.

Corneal Epithelial Cell Measurements

Ten light micrographs of the epithelial layer at the central and midperipheral corneal regions were taken at a magnification of ×400. Five to 10 distinguishable epithelial cells (basal and wing cells) of each micrograph were labeled, and the height of each labeled cell was measured. All data are reported as mean ± SD.

Statistical Analyses

For each parameter of corneal measurement such as total corneal thickness, stromal thickness, and epithelial thickness, >1 measurement was made on each corneal sample. Because the measurements of corneal parameters within the same cornea are not independent of each other, these measurements were analyzed as within-subject (within cornea) dependent variables by using the repeated-measures multivariate general linear model in testing parameter differences between lens wear corneas (treatment) and non–lens wear corneas (control). Individual experimental subjects (monkeys) were assigned as the between-subject factor to control for differences across experimental subjects. The duration of OK lens wear (hours) was specified as the covariate. The α level was established at the 0.05 level a priori.

Qualitative Histologic Examination

Appropriate ultrathin sections (90–120 nm) were prepared and mounted perpendicular to the 200-mesh thin bar copper grids. These sections were stained singly with 2% Reynold lead citrate for 5 minutes at room temperature. The morphology of epithelial cells and corneal ultrastructures such as the surface features (microvilli and microplicae), desmosomes, collagen fibril distribution, and endothelial cells were observed carefully with a TEM (TEM Technai G^2; Philips, The Netherlands).

RESULTS

Quantitative Study

Total Corneal Thickness

Table 1 shows the means and SDs of the total corneal thickness of all lens wear groups and the negative control group at the central and midperipheral regions. At baseline (0 hours of OK lens wear), there was no statistically significant difference in total central corneal thickness between right and left eyes ($P = 0.962$). Within the 4 durations of lens wear groups (4-, 8-, 16-, and 24-hour lens wear groups), the total central corneal thickness was reduced significantly in the lens wear eyes (treatment) compared with the non–lens wear eyes (0-hour lens wear; $P < 0.001$, repeated-measures multivariate general linear model) after controlling for experimental subjects (between-subjects factor) and duration of lens wear (covariate). The reduction of the total corneal thickness for each duration of lens wear group was as follows: 4 hours = −25.35 ± 7.88 μm; 8 hours = −21.37 ± 3.80 μm; 16 hours = −27.87 ± 14.27 μm; 24 hours = −22.26 ± 13.05 μm.

At baseline, there was no statistically significant difference in the total midperipheral corneal thickness between right and left eyes ($P = 0.694$). Total midperipheral corneal thickness was significantly thinner in the lens wear eyes than in the non–lens wear eyes in both the 4- and 8-hour lens wear groups (4 hours = −3.72 ± 2.46 μm; 8 hours = −2.00 ± 7.27 μm; $P < 0.001$). In contrast, total midperipheral corneal thickness of the lens wear eyes was significantly thicker than the non–lens wear eyes in both the 16- and 24-hour lens wear groups (16 hours = +10.35 ± 12.58 μm; 24 hours = +16.87 ± 12.73 μm; $P < 0.001$).

Stromal Thickness

Table 1 also presents the means and SDs of the stromal thickness of all experimental groups. At baseline, there was no statistically significant difference in the central stromal

TABLE 1. Means and SDs of the Total Corneal Thickness (Central and Midperipheral Regions) in All Groups in the Short-term OK Study

	Control			Treatment		
Corneal Thickness (μm)	Total	Stromal	Epithelial	Total	Stromal	Epithelial
Central corneal region						
0 h (n = 1)	422.76 ± 31.94	383.15 ± 28.08	39.61 ± 5.56	422.25 ± 31.61	382.00 ± 28.03	40.05 ± 5.07
4 h (n = 2)	411.52 ± 13.07	373.06 ± 12.42	38.45 ± 3.97	386.17 ± 19.85	351.46 ± 19.87	34.71 ± 3.62
8 h (n = 2)	404.51 ± 25.88	364.99 ± 25.23	39.52 ± 5.18	383.14 ± 23.15	345.81 ± 22.22	37.32 ± 5.88
16 h (n = 2)	409.26 ± 25.53	369.54 ± 25.79	39.71 ± 4.52	381.39 ± 12.32	343.79 ± 10.96	37.60 ± 5.00
24 h (n = 2)	441.09 ± 13.77	396.07 ± 15.53	45.03 ± 7.72	418.83 ± 26.28	376.36 ± 23.54	42.48 ± 8.51
Midperipheral corneal region						
0 h (n = 1)	420.55 ± 27.20	381.53 ± 23.80	39.03 ± 5.60	423.07 ± 26.86	382.18 ± 24.03	40.89 ± 4.04
4 h (n = 2)	421.19 ± 17.96	382.55 ± 17.73	38.64 ± 4.51	417.47 ± 18.09	379.27 ± 17.50	38.20 ± 4.93
8 h (n = 2)	439.66 ± 28.53	400.00 ± 26.80	39.66 ± 6.89	437.66 ± 27.06	398.86 ± 26.66	38.80 ± 5.45
16 h (n = 2)	436.44 ± 13.85	397.29 ± 15.61	39.15 ± 5.68	446.80 ± 23.15	401.33 ± 23.11	45.47 ± 6.26
24 h (n = 2)	451.17 ± 19.89	410.00 ± 17.20	41.17 ± 7.29	468.04 ± 30.88	419.88 ± 29.59	48.16 ± 9.46

thickness between right and left eyes ($P = 0.941$). Within the 4 durations of lens wear groups, central stromal thickness was reduced significantly in the lens wear eyes compared with the non–lens wear eyes (4 hours = -21.61 ± 10.54 μm; 8 hours = -19.18 ± 8.26 μm; 16 hours = -25.75 ± 17.52 μm; 24 hours = -19.71 ± 14.42 μm; $P < 0.001$).

At baseline, midperipheral stromal thickness showed no statistically significant difference between right and left eyes ($P = 0.906$). Midperipheral stromal thickness was reduced significantly in the lens wear eyes compared with the non–lens wear eyes in both the 4- and 8-hour lens wear groups (4 hours = -3.285 ± 6.89 μm; 8 hours = -1.14 ± 10.87 μm; $P < 0.01$). However, the lens wear eyes in the 16- and 24-hour lens wear groups had thicker midperipheral stromal layers than the non–lens wear eyes (16 hours = $+4.04 \pm 15.50$ μm; 24 hours = $+9.88 \pm 17.16$ μm; $P < 0.01$).

Epithelial Thickness

Both the means and SDs of the epithelial thickness of all experimental groups are presented in Table 1. At baseline, there was no statistically significant difference in the central epithelial thickness between the right and left eyes ($P = 0.468$). Central epithelium was significantly thinner in the lens wear eyes than in the non–lens wear eyes in the 4 durations of lens wear groups (4 hours = -3.74 ± 5.17 μm; 8 hours = -2.19 ± 7.42 μm; 16 hours = -2.12 ± 6.75 μm; 24 hours = -2.55 ± 11.27 μm; $P < 0.001$).

At baseline, there was no statistically significant difference in the midperipheral epithelial thickness between right and left eyes ($P = 0.097$). In both the 4- and 8-hour lens wear groups, the midperipheral epithelial thickness was reduced significantly in the lens wear eyes compared with the non–lens wear eyes (4 hours = -0.44 ± 6.36 μm; 8 hours = -0.86 ± 8.57 μm; $P < 0.001$). In contrast, the midperipheral epithelial layers of the lens wear eyes were thickened significantly compared with the non–lens wear eyes after 16- and 24-hour lens wear treatments (16 hours = $+6.31 \pm 9.00$ μm; 24 hours = $+6.99 \pm 10.38$ μm; $P < 0.001$).

Profile of the Basal Cells

Table 2 presents the means and SDs of the height of basal cells in both the central and midperipheral epithelia of all experimental groups. In the central epithelium, the height of the basal cells showed no statistically significant difference between the right and left eyes at baseline ($P = 0.348$). The height of the basal cells was reduced significantly in the lens wear eyes compared with the non–lens wear eyes in all durations of lens wear groups (4 hours = -1.55 ± 0.29 μm; 8 hours = -0.70 ± 0.62 μm; 16 hours = -2.91 ± 0.46 μm; 24 hours = -1.85 ± 1.21 μm; $P < 0.001$). In the midperipheral epithelium, there was no statistically significant change in the height of the basal cells between the right and left eyes at baseline ($P = 0.631$). There were also no significant changes in the height of the midperipheral located basal cells in all durations of lens wear groups ($P = 0.162$).

Profile of the Wing Cells

The means and SDs of the height of the wing cells in both the central and midperipheral epithelia are presented in Table 2. At baseline, there was no statistically significant difference in the height of the wing cells between right and left eyes ($P = 0.319$). In the central epithelium, there was also no significant change in the height of the wing cells in all durations of lens wear groups ($P = 0.371$). In the midperipheral epithelium, the height of the wing cells showed no statistically significant difference between the right and left eyes at baseline ($P = 0.667$). Similarly, the height of the wing cells showed no statistically significant difference between the non–lens wear and lens wear eyes in all durations of lens wear groups ($P = 0.280$).

TABLE 2. Means and SDs of the Heights of Basal and Wing Cells at Central and Midperipheral Corneal Regions in All Lens Wear Groups in the Short-term OK Study

Duration of OK Lens Wear		Central Epithelium		Midperipheral Epithelium	
		Control	Treatment	Control	Treatment
Basal cells (μm)					
0 h (n = 1)	Mean	16.43	16.20	16.03	15.93
	SD	1.63	2.14	1.6	1.98
4 h (n = 2)	Mean	15.14	13.60	14.30	14.51
	SD	1.33	1.66	1.09	1.30
8 h (n = 2)	Mean	15.85	15.14	15.48	14.84
	SD	1.82	1.5	1.48	1.20
16 h (n = 2)	Mean	16.91	14.00	16.35	16.03
	SD	1.39	1.17	1.31	1.48
24 h (n = 2)	Mean	17.78	15.96	16.81	17.40
	SD	2.15	0.97	1.97	2.25
Wing cells (μm)					
0 h (n = 1)	Mean	10.05	10.24	9.38	9.31
	SD	1.33	1.33	1.16	1.24
4 h (n = 2)	Mean	9.59	8.98	9.07	9.29
	SD	0.81	0.79	1.34	1.54
8 h (n = 2)	Mean	10.17	9.17	8.43	8.31
	SD	1.25	1.23	0.69	0.96
16 h (n = 2)	Mean	9.61	8.76	9.64	9.56
	SD	0.96	0.73	1.07	1.00
24 h (n = 2)	Mean	11.82	10.28	9.94	10.90
	SD	1.28	0.90	1.13	1.20

Corneal Response at the Microscopic Level

Effect of OK on Corneal Epithelium

The control central epithelium of the primate cornea is made up of 7–8 regularly arranged layers of differentiating epithelial cells (single layer of basal cells, 2–3 layers of wing cells, and 3–4 layers of squamous cells; Fig. 1A). Although the central corneal epithelia in all treated corneas were flattened during corneal reshaping, the total layers of epithelial cells remained in a range of 7–8 (Fig. 1B). Similarly, the thickened midperipheral corneal epithelia of treated corneas in the 16- and 24-hour lens wear groups also remained in a range of 7–8 epithelial cell layers (Fig. 1C). No evidence of a gain or loss of epithelial layers was detected over single overnight OK treatment during corneal reshaping.

Microscopic evidence showed that the central corneal epithelial thinning was accompanied by morphologic alterations

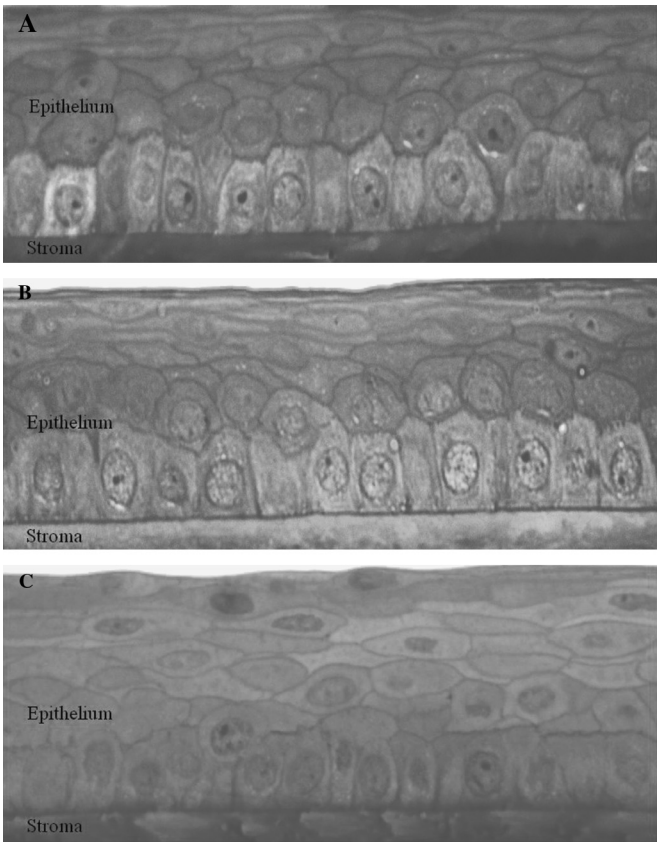

FIGURE 1. Representative LM of the control (A), thinned (B), and thickened (C) corneal epithelia of primate corneas. All epithelia are made up of 7–8 cell layers (×400).

in epithelial cells; for example, the single-layer basal cells appeared rounded and became squat instead of normal columnar shape (Fig. 2). Electron microscopic study showed that desmosomes among adjacent epithelial cells of both the treated and control corneas had normal morphologic appearance. In all treated corneas, the desmosomes remained separated and visible as a distinct feature regardless of duration of lens wear (Fig. 3). No fusion or fission of desmosomes was observed.

Histologic evidence showed that the midperipheral corneal epithelial thickening was also accompanied by morphologic alteration in epithelial cells, particularly superficial squamous cells. In this region, the superficial squamous cells had an enlarged surface area compared with those in the control corneal epithelium. The squamous cells in the thickened epithelium either had oval (Fig. 4) or larger (Fig. 5) nuclei instead of flattened nuclei as seen in the control cornea. Also, the basal cells at the thickened epithelium were elongated vertically and appeared thinner but were still arranged regularly and had a perfect alignment with the basement membrane (Fig. 4).

At the anterior portion of the corneas, normal surface fingerlike projections such as microvilli covered the apical surface of the superficial squamous cells. Regardless of the duration of OK lens wear, both the microvilli and microplicae maintained their normal appearance over the thinned and thickened corneal epithelia compared with the control epithelium (Supplementary Data, Fig. 1).

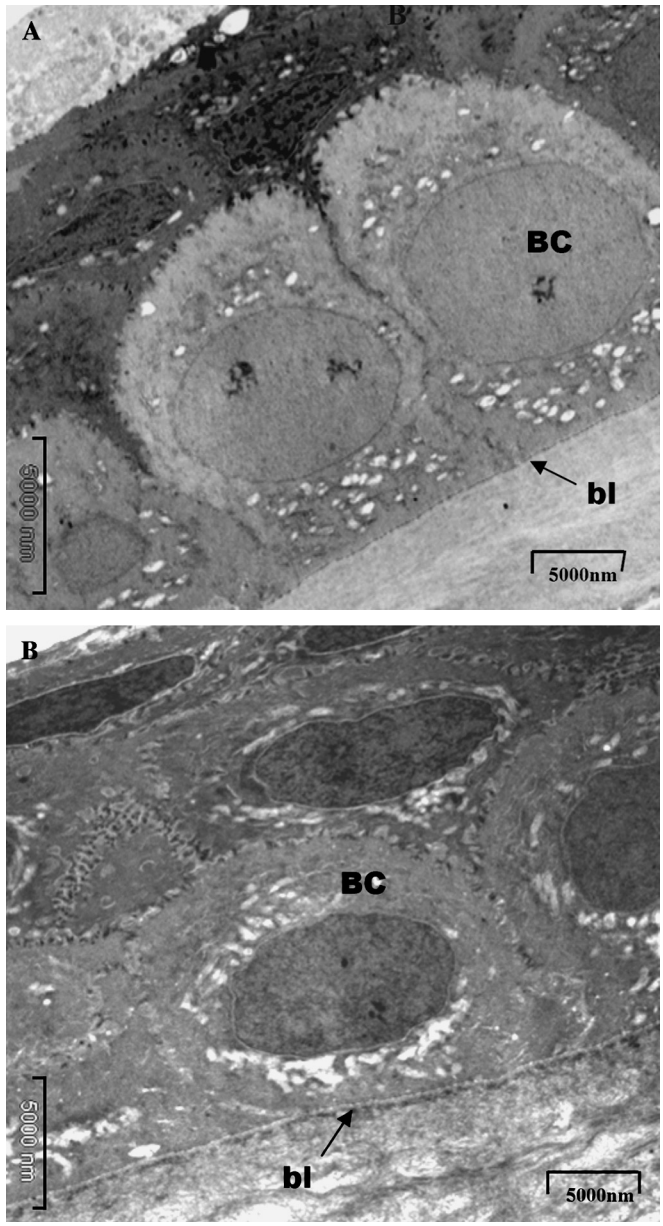

FIGURE 2. Representative TEM of the basal cells of the control (A) and central thinned corneal epithelia (B). The basal cells (BCs) of the primate cornea have perfect alignment on a basal lamina (bl). In the control cornea, the basal cells are columnar with rounded heads and flat bases. In the central thinned corneal epithelium, the basal cells became squat and appeared more cuboidal (×2700).

Effect of OK on Stroma

Microscopic investigation was carried out on the corneal stroma in both the thinned central and midperipheral thickened corneas. No histologic evidence of stromal edema was found. Morphologically, all collagen fibrils had equal diameters and were randomly distributed with even spacing, similar to those in the control cornea (Supplementary Data, Fig. 2).

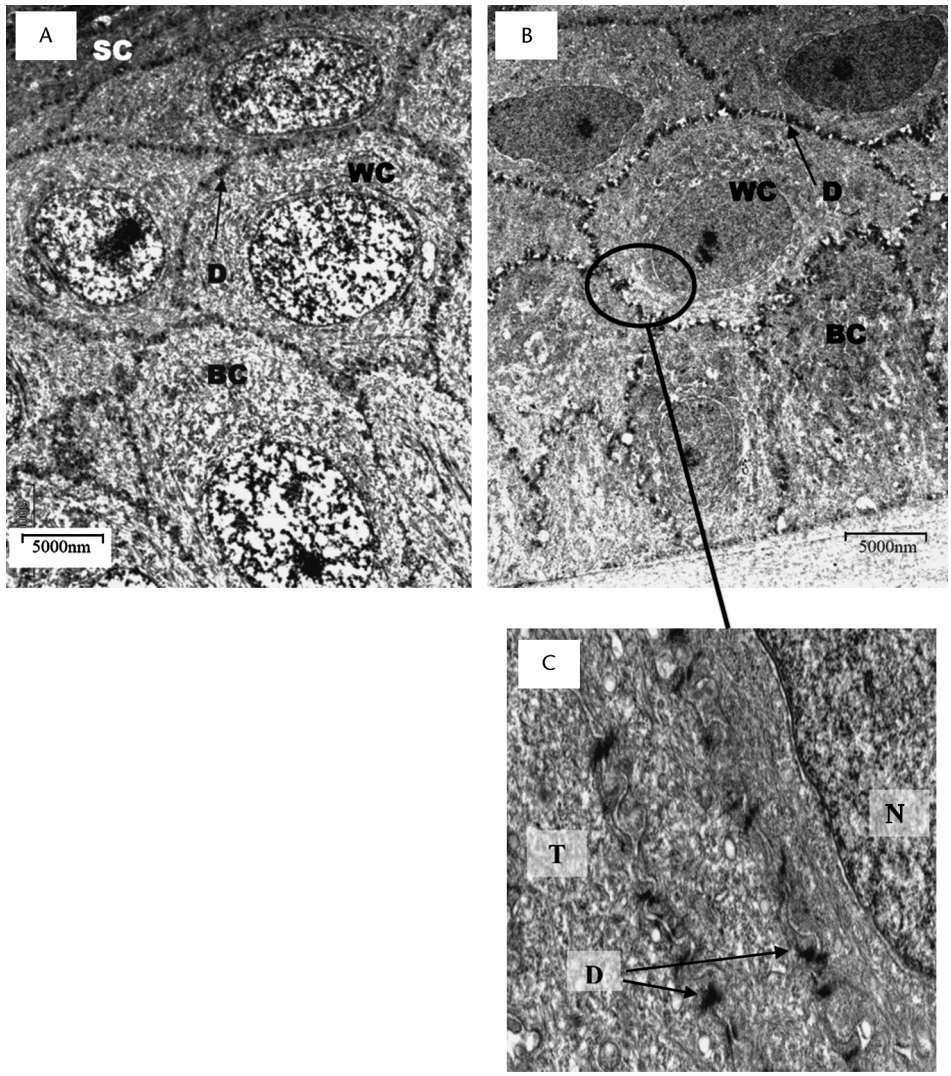

FIGURE 3. Representative TEM of primate central corneal epithelium made up of basal (BC), wing (WC), and squamous cell (SC) layers. The interdigitating cell membranes with electron-dense desmosomes (D) are prominent among the epithelial cells, and their structural integrity is well maintained in both the control (A) and the central thinned (B) corneal epithelia (×2700). (C) Normal ultrastructural appearance of desmosomes consists of 2 symmetrical opposing dense plaques on the neighboring cytoplasmic membranes with regular intercellular gap. These dense plaques served as anchoring sites for the electron-dense tonofilaments (T) (×11,000). N, nucleus.

Effect of OK on Endothelium

Morphologically, there was no apparent difference between the endothelial layers of the control and treated corneas. Ultrastructurally, all endothelial cells contained many cytoplasmic organelles such as mitochondria, smooth and rough endoplasmic reticula, and Golgi apparatus. Focal tight junctions or zonulae occludentes on the apical aspect of the lateral membranes were found in which the outer leaflets of the plasma membranes of adjacent cells appeared to have fused and obliterated the extracellular space. Gap junctions were also found formed between adjacent cells and were located at all levels of the lateral plasma membrane below tight junctional complexes (Supplementary Data, Fig. 3).

DISCUSSION

The corneal thickness changes on the primate cornea induced by RGLs in this study are similar to corneal responses previously found in human subjects after overnight OK lens wear (closed-eye manner).[20–23] The significant changes in central corneal thickness in this study started to occur in the 4-hour lens wear group. Thereafter, significant central corneal thinning was observed in other groups with longer lens wear durations (8, 16, and 24 hours). This finding indicates that the specially designed OK lenses used in this study have achieved the desired OK effect on primate corneas and are similar to those seen in clinical studies. Our observations are also in agreement with those of Choo et al,[6] who reported significant central epithelial thinning in eyes fitted with RGLs (closed-eye manner) for myopia correction in cat models. Similarly, Matsubara et al[7] showed significant central epithelial thinning in rabbit eyes fitted with RGLs (closed-eye manner).

Histologic evidence reveals that the single overnight OK in a closed-eye manner, ranging from a short time frame (4 hours) to a typical time frame (8 hours) and an extended time frame (16 and 24 hours), resulted in apparent corneal changes at the microscopic level. The primary epithelial response in the central thinned corneal epithelium is focal cell compression (morphologic changes) rather than cell migration or loss of cell layers. The primate corneal epithelium maintained 7–8 layers of regularly arranged differentiating

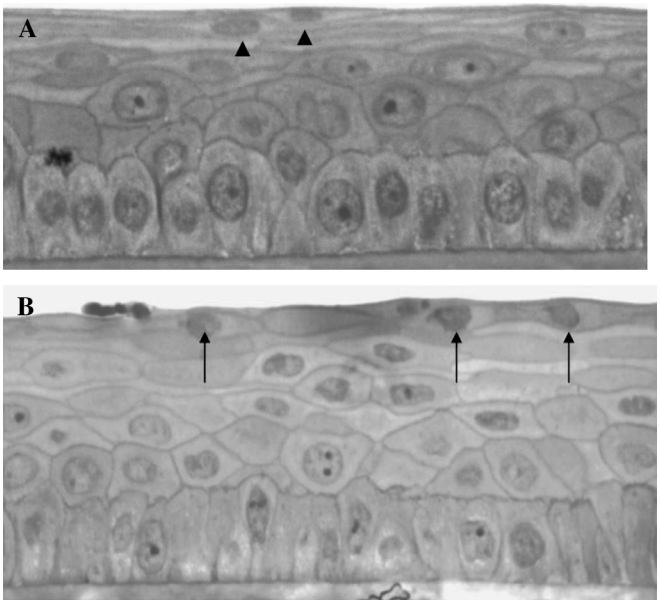

FIGURE 4. Light micrograph of cross-section through the corneal epithelium of the primate. The surface sizes of the apical squamous cells in the thickened epithelium (B) are wider than those in the control epithelium (A). The nuclei of the superficial squamous cells in control cornea are flattened (arrowheads), whereas the nuclei are slightly oval (arrows) in shape in thickened epithelium (×400).

epithelial cells in all thinned central epithelia, as was seen in control corneas. The nature of the cellular events underlying the central epithelial thinning involved the wing cells becoming shorter and the basal cells becoming squat. This finding is in agreement with the study of Choo et al,[6] who, by using the cat as a study model, showed that the basal cells became rounded compared with the normal elongated shape within 8 hours of OK lens wear in a closed-eye manner.

There was significant thickening in midperipheral corneal thickness occurring in the 16- and 24-hour lens wear groups. We found that the thickening effect in the midperipheral cornea originated from both epithelial and stromal components (discussed later). In this study, we showed that the midperipheral corneal thickening (16 hours = +2.4%; 24 hours = +3.7%) was associated with thickening of the stroma (16 hours = +1.02%; 24 hours = +2.4%) and epithelial (16 hours = +16.14%; 24 hours = +16.98%) components in primates fitted with OK lens for 16 and 24 hours. The area of thickening seemed to correspond with the midperipheral tear reservoir created under the steeper secondary curve of the RGL. Clinically, Alharbi and Swarbrick[3] reported midperipheral stromal thickening (2.5%) in 18 subjects who wore RGLs over 3 months in a closed-eye manner. Our finding is also in an agreement with that of Choo et al,[6] who reported a similar result in cat models. Matsubara et al[7] also reported a marked increment in midperipheral epithelial thickness of rabbit corneas that were fitted with RGLs (8 hours daily) for 28 days.

In the thickened epithelium, the basal cells elongated vertically and the squamous cells also had a larger surface cell area. The morphologic changes in the basal cells may be

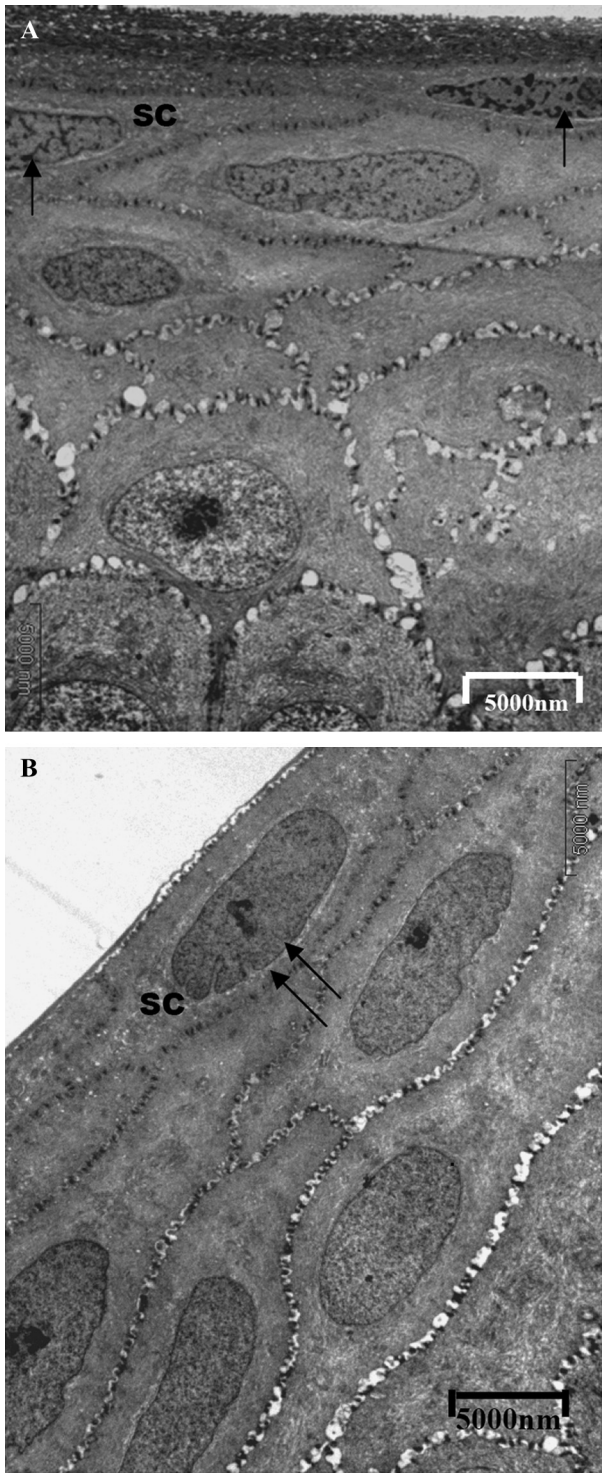

FIGURE 5. Representative TEM of the superficial squamous and wing cells of primate cornea. A, In the control cornea, both the bodies and nuclei of epithelial cells are increasingly flattened into thin superficial squamous cells (SCs) with elongated cell bodies and nuclei (single arrow). B, In the thickened epithelium, the surface squamous cell has larger surface area and larger nucleus (double arrows) than the control (×2700).

caused by the intercellular transferring of cytoplasm content from central to midperipheral regions, as suggested by Choo et al.[8] The epithelial cells are physically interconnected through gap junctions that serve as sites for intercellular communication and small-molecule transmission.[24] In this study, we noticed compressed and smaller cells in the center and vertically elongated cells in the midperiphery. Despite the drastic morphologic alteration, the vertically elongated basal cells were still arranged regularly and had perfect alignment with the basement membrane. In contrast, Matsubara et al[7] reported vertically elongated basal cells arranged irregularly in the thickened midperipheral epithelium in the rabbit model (8 hours of OK lens wear a day for 21 days). This histologic difference may be caused by the different durations of OK lens wear on the animal cornea.

The morphologic alteration of the superficial squamous cells at the thickened midperipheral epithelium of the primate model is an interesting finding. Every day, the corneal epithelium is continuously losing surface cells into the precorneal tear film through exfoliation that involves cell apoptosis in an orderly manner. As the squamous cells are lost, they are replaced by the underlying proliferating basal cell layer. As newly formed cells migrate first from the basal layer into the upper differentiating wing layers and then into the superficial layers, their cell bodies progressively flatten into thin superficial squamous cells with large surface areas.[25] Previous studies hypothesized that the increase in superficial cell size seen in contact lens wearers is the consequence of delayed epithelial exfoliation.[26,27] In this study, the squamous cells at the midperipheral thickened epithelium had increased surface sizes, and the nuclei were larger or oval-shaped instead of flattened compared with the normal squamous cells. The presence of larger, presumably older surface epithelial cells implies delayed surface cell desquamation. We suggest that there is a slowing of the corneal epithelial renewal rate that results in these morphologic changes where the cells remain resident on the surface, do not exfoliate at the usual surface epithelial exfoliation rate, and therefore continue to enlarge as they get older. The pooling of tear film underneath the steeper secondary or reverse curves of the OK lens may modify the microenvironment beneath the lens and thus delay the exfoliation of cells into the tear film. Further study is needed to determine the actual surface epithelial exfoliation rate (cells per minute) in the thickened epithelium.

The central cornea was significantly flattened throughout the study, showing that the treatment effect of OK was dramatic after 1 night of lens wear. This finding indicates a high degree of corneal plasticity in response to OK lens wear, which is designed to reshape the cornea. These immediate changes were most probably the result of pressure from the lens, the closed lid over the lens, and the overnight corneal edema. Particularly worrisome is the possibility that this thinning response may compromise the integrity of the epithelial barrier, the main defense against tearborne pathogens in the eye. Thus, examination was also carried out on the desmosomes (prominent cell adhesion structures), which are believed to be the major stabilizing elements among epithelial cells.[28] Choo et al[8] suggested that an appropriate stimulus, such as pressure (force originating from the tear film and the semirigid OK lens in the anterior and the stroma in posterior), can break down the cell junctions and thus promote the physical movement of the epithelial cells. Eventually, with the epithelial defect, physical displacement of the adjacent cells or epithelial migration to elsewhere may occur. Our finding shows that the desmosomes remain well defined and maintain a normal appearance during pronounced epithelial cell shape changes in the thinned corneal epithelium. This result suggests that desmosomes have extremely stable networks with inherent flexibility and elasticity in epithelial tissues. Therefore, this study suggests that the intrinsic elasticity of desmosomes permits the compression of cell borders in wing cells and basal cells in the thinned central corneal epithelium without disruption to their architecture.

The corneal surface epithelial cells are covered by short projections, such as microvilli and microplicae, on which there is a filamentous cell coat, called glycocalyx.[29] Both the microvilli and microplicae provide a structural framework that supports and stabilizes a complex of related factors such as tears and mucus to protect the cornea. The interaction of various components (microprojections, glycocalyx, tears, and mucus) at the surface of the eye constitutes a complex protective barrier that must be breached by pathogens before infection can ensue. Dohlman[30] also proposed that these short projections play a mechanical role in preventing gravitational flow of the tear film over the cornea. Unlike conventional lenses, the RGL designs used for OK deliberately apply pressure through the tear film against the corneal surface. Thus, understanding of the corneal surface structures in response to these subtle pressures is also important. Our study shows that the physical presence of the OK lenses (especially the flat central base curve/primary curve) on the central corneal surface results in lens-induced corneal changes without affecting the morphology of these microstructures. There is no doubt that the data on density of microvilli (by using specular microscopy) under the influence of OK on corneal surface projections warrant further study.

This study also showed that the central stromal thinning contributed to the central corneal thinning in all treatment groups and thickening of the midperipheral stroma in the 16- and 24-hour groups. This outcome indicates that 1 night of OK lens wear may have altered the post–lens tear film pressure and thus contributed to the changes in the stromal components. The RGLs used in this study have a multicurve design with a relatively flatter central optic zone of 6.0–6.5 mm in diameter, surrounded by a steep secondary curve (the reverse curve). The more peripheral zones of the lens allow better alignment with the midperipheral and peripheral cornea. This lens design helps to maintain the centration of the lens and generates different post–lens tear film pressures across the cornea. When an RGL is placed on the surface of the cornea, the flatter central curve exerts positive force on the tear film. Conversely, the steeper secondary curve of the lens creates pooling of the tear film and thus generates negative force on the midperipheral cornea. Eventually, tear film is distributed unequally across the corneal surface. The uneven post–lens pressure may exert a "clamping" effect on the central cornea and thus lead to thinner central stromal components. Nonetheless, the TEM study showed an equal distribution of collagen fibrils in all

treated corneas, and there was no substantial difference in the overall arrangement of these fibrils at the anterior and posterior stroma. By comparison, Edelhauser et al[31] showed that the edematous stroma was made up of collagen fibrils that were unevenly distributed throughout the collagen lamellae along with intralamellar lakes of an amorphous material. This study shows that short-term (hour) OK lens wear does not induce corneal edema. Nevertheless, the exact mechanism causing the thinning and thickening of the corneal stroma in this study remains obscure.

The corneal endothelium is a monolayer of specialized, flattened, mitochondrion-rich cells that are located at the posterior surface of the eye.[32] This layer regulates the fluid and solute transport across the posterior corneal surface through the focal tight junctions (zonulae occludentes) and gap junctions to maintain the cornea in the slightly dehydrated state that is required for optical transparency. Focal tight junctions are located at the apical aspect of the lateral membranes and seem to fuse and obliterate the extracellular space.[33,34] However, these junctions do not wrap around the entire cell, and thus the endothelium allows fluid to leak from the anterior chamber into the relatively dehydrated corneal stroma. Gap junctions possess a characteristic pentilaminar structure and are located at all levels of the lateral plasma. They are important in narrowing the width between opposing cell membranes from the normal intercellular gap of 25–45 to ~3 nm.[35,36] Narrowed intercellular spaces produced by the formation of gap junctions force the fluid to move between the sinuous interdigitating lateral membranes. Therefore, they help to prevent bulk fluid flow across the endothelial monolayer and prevent stroma edema from occurring.

In this study, primates wore RGLs with high Dk in a closed-eye manner. The flat-fitting rigid contact lenses exert an OK effect mechanically by compressing the central region of the cornea. This mechanical stress may affect corneal metabolism and subsequently influence the integrity of endothelial cells. If the integrity of the endothelial layer is breached, corneal edema rapidly develops. Our electron microscopic studies revealed normal fine structures of the corneal endothelium, particularly the junctional complexes found at the apical side (zonulae occludens, zonulae adherens, and gap junctions). We found no loss of density of the zonulae occludentes at the apical zone between adjacent endothelial cells. Similarly, gap junctions were found at all levels of interdigitating lateral membranes among endothelial cells. There were narrowed and regular intercellular spaces between opposing cell membranes. This finding suggests that short-term overnight OK does not exert any effect on the integrity of different cell junctions between endothelial cells and thus does not challenge the nature of the permeable barrier in the cornea. This finding is in agreement with that of Hiraoka et al,[37] who showed that overnight OK (using RGLs with high Dk) for 1 year did not influence the density, size, and shape of the corneal endothelial cells. This outcome may be due to the high oxygen permeability of the lens used in the study.

In conclusion, this study showed that short-term RGL wear can induce substantial histomorphometric changes in primate corneas in a rapid manner. The primate cornea, particularly the corneal epithelium, responds rapidly with significant epithelial alterations. Profound morphologic changes observed in the corneal epithelial after OK lens wear included shortening of the basal and wing cells, which led to total central epithelial thinning. In addition, midperipheral epithelial thickening occurred and was accompanied by increased surface cell size. These results suggest that the corneal epithelium is highly flexible in response to the physical forces generated beneath the OK, leading to alterations at the microscopic level. This short-term primate study may be relevant to the clinical application of OK in human subjects. However, performing corneal thickness measurements from histologic sections remains the major limitation in this study because corneal thicknesses may vary dramatically with tissue processing, particularly sample sectioning procedures.

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