AUTHOR QUERIES

DATE __10/1/2008__
JOB NAME __ICO__
ARTICLE __ICO201149__
QUERIES FOR AUTHORS __Zhong et al__

THIS QUERY FORM MUST BE RETURNED WITH ALL PROOFS FOR CORRECTIONS

AU1) Please clarify whether “Baseline: before lens wear; *Paired sample t test applied; †Unpaired sample t test applied” can be given as a footnote for “Table 2.”

AU2) Please note that academic degrees for authors in the authors field have been inserted from pdf. Please check and also please clarify whether the degree “Bachelor of Medicine” for author “Xiaolian Chen” can be changed to either “MB” or as “BM” as per AMA

AU3) Please note that affiliation “Zhongshan Ophthalmic Center and State Key Laboratory of Ophthalmology” has been treated as two different affiliations. Please check and provide the departments (if any) in all affiliations and provide the city for the affiliation “Vision Cooperative Research Centre.”

AU4) Please spell out “SRFDP” (if an acronym) in the footnote.

AU5) Please define “Dk” (if an acronym) in the sentence “The majority of the …”

AU6) Please spell out “LCD SVGA” in the sentence “This microscopic system …”

AU7) Please provide the software name for “version 3.12” in the sentence “The curvature, refractive …”

AU8) Please define "SimK" in the sentence "The SimK power …"

AU9) Please spell out “SRFDP” (if an acronym) in the footnote.

AU10) Please check if the edits made to the page range in Ref. 14 are correct.

AU11) Please note that figure captions 1–6 and Tables 1 and 2 have been inserted from pdf. Please check.
Differences Between Overnight and Long-term Wear of Orthokeratology Contact Lenses in Corneal Contour, Thickness, and Cell Density

Xingwu Zhong, MD, PhD,*‡ Xiaolian Chen, Bachelor of Medicine,*‡ Ru Zhong Xie, MD,‡ Jun Yang, MD,*‡ Saiqun Li, MD,*‡ Xiao Yang, MD, PhD,*‡ and Xiangming Gong, MD*‡

**Purpose:** To investigate changes in corneal topography and morphology in human eyes wearing orthokeratology (OK) lenses for overnight and over a 5-year period.

**Materials and Methods:** Fifty-six adults with moderate myopia were assigned to 2 groups based on age matching: 5-year lens wear (n = 26, 51 eyes) and 1-night lens wear (n = 30, 60 eyes). All subjects wore reverse-geometry OK lenses with the eyes before 1-night lens wear serving as a control to both the groups. Visual acuity, slit-lamp biomicroscopy, confocal microscopy, and corneal topography were assessed before and after lens wear.

**Results:** Visual acuity was improved satisfactorily with flattening of the central cornea in both lens wear groups. Corneal thickness increased extensively in the 1-night lens wear group but only paracentrally in the 5-year lens wear group. Central epithelial thinning with a decreased density of the basal cells was only observed in the 5-year group. Keratocyte density was reduced throughout the entire corneal thickness for the 2 groups. Endothelium was normal in density and morphology for the 2 groups.

**Conclusions:** Short-term and long-term OK lens wear can effectively correct myopia by flattening the cornea. The flattened cornea in the short-term lens wear is mainly because of the thickening of the midperipheral cornea. In the long-term lens wear, however, this change is associated with thickening of the midperipheral cornea and thinning of the central corneal epithelium.

**Key Words:** orthokeratology, cornea, cell density, thickness

(From 2008;00:000–000)
infection associated with OK lens wear is problematic because of its potentially poor outcome.

Corneal flattening with a reduction of central corneal power is observed within 8 hours of OK lens wear, which is mainly caused by central epithelial thinning and midperipheral thickening of the epithelium and stroma. In a 1-month schedule of OK lens wear, overnight stromal edema is found mainly in the area from middle to peripheral cornea; however, the severity of edema decreases with an increasing number of days in overnight lens wear. In a longer period of OK lens wear (3 months), there is a 33% reduction in central corneal epithelium, whereas only slight thinning occurs in the overall corneal thickness. These results indicate that overnight stromal edema in OK lens wearers plays a significant role in the thickening of the peripheral cornea and that the eyes seem to accept the lenses gradually with lens wear.

However, to the best of our knowledge, little is known in regard to microstructural changes of the cornea in response to short-term and long-term OK lens wear. In particular, studies on microstructural and topographical changes in long-term OK lens wear have not been reported in the literature. Therefore, this study investigated changes in corneal morphology, thickness, cell density, and topography in human myopes who had worn OK lenses overnight or over a 5-year period. Corneal changes responsible for the correction of myopia were also compared between a single night of OK wear and 5 years of OK lens wear.

MATERIALS AND METHODS

Subjects and OK Lens Wear
This study was approved by the Ethics Committee of Zhong shan Ophthalmic Center of Zhong Shan University, China, and adhered to the tenets of the Declaration of Helsinki. Twenty-six subjects (15 eyes from 10 males and 16 females) with an age of 19.4 ± 5.0 (mean ± SD) years and a refractive state of −3.15 ± 0.82 D wore OK lenses overnight for 5 years. Thirty subjects (60 eyes from 13 males and 17 females) with an age of 22.5 ± 4.1 years and the refraction of −3.06 ± 0.90 D wore OK lenses for 1 night (8 hours). There was no significant difference in the refraction between these 2 groups (P = 0.701). The eyes before 8-hour lens wear served as a control to both the groups. All the subjects had no history of ocular diseases, corneal trauma, or systemic diseases and were confirmed normal by slit-lamp biomicroscopy before lens wear.

The OK lenses used in this study were reverse-geometry rigid lenses (10.40–10.60 mm in overall diameter and 6.00 mm in optic zone diameter). All lenses were made of Boston XO material with a nominal central thickness of 0.22 mm and a Dk at 145 × 10−11 cm2 mL O2/s mL mm Hg (Macro Vision Corporation, Taiwan, China). The subjects were requested to insert the lenses into both eyes by themselves before going to sleep at night and to remove the lenses at 8 o’clock the next morning. In the morning at the end point of the study, all the subjects returned to the clinic with the lenses worn on the eyes and the lenses were removed by research staff. The cornea of these subjects was then examined with confocal microscopy and corneal topographer within 1 hour of removal of the lenses.

Confocal Microscopy
The corneas were examined with a scanning slit confocal microscope (ConfoScan 2.0 Model P3; Fortune Technologies Srl, Italy). This microscopic system consisted of a light source (halogen bulb), a scanning module containing a highly sensitive black-white camera (Proxicam HL 5), 3-axis stepper robot, a water immersion lens, a 15-inch monitor (LCD SVGA) attached to a personal computer, and an information system (Nidek Advanced Vision). The microscope has a magnification of ×1000, scanning slit width of 0.28 mm, depth resolution of 10 μm in optical sectioning, lateral optical resolution of 1 μm, and the final size of object area at 340 μm × 255 μm. The real-time images in each scanning can be recorded directly on the computer hard drive with a maximum memory for recording 350 images. The microscope was set for a scanning thickness between 650 and 700 μm with.

Two eye drops of 0.4% oxybuprocaine hydrochloride (Santen Pharmaceutical Co, Ltd, Japan) were instilled into the eye 5 minutes apart. The objective lens was immersed in 75% ethanol for 10 minutes and air-dried before each examination. A drop of viscous gel (Vidisic eye gel; Bausch & Lomb, Berlin) was applied onto the tip of the objective lens. The subject was guided to look forward with the forehead and chin resting on the support attached to the microscope. The objective lens was advanced toward the central cornea by controlling a joystick (ConfoScan 2.0, Operation manual) until images appeared on the monitor. The lens was aligned vertically to the cornea and was moved forward for a further 50 μm until images of the endothelium appeared. The images were recorded from this point.

The central cornea was measured for epithelial and stromal thickness (using Z-scan), basal cell density, keratocyte density in anterior stroma (501.5 ± 100.7 μm away from endothelium), and posterior stroma (100.8 ± 58.0 μm away from endothelium). The number of epithelial basal cells and keratocytes was manually estimated with the cell density provided. The density of the endothelial cells with the number of hexagon cells in proportion to total endothelial cells was also measured. Selected endothelial images were analyzed for the cell density using software (Confo Scan 2.0, NAVIS Cell Count User Guide). The result of each corneal parameter was recorded as the mean of 3 repeated measurements.

Corneal Topography
Corneal topography was measured with an Orbscan topographer (Orbscan II anterior segment analysis system, Utah). The subject rested his/her chin on the support in front of the topographer with both eyes fixating on a target light straight ahead. The topographer scanned the cornea with light slits at an angle of 45 degree to the cornea surface. The curvature, refractive power, and elevation of any point on anterior and posterior surface of the cornea were calculated by the software version 3.12. The corneal thickness was measured at central, nasal, temporal, superior, and inferior zones. The 4 noncentral zones were at least 3 mm away from central center, respectively. The distance between the anterior and the
posterior surfaces was used to determine the corneal thickness with the measuring deviation smaller than 1 μm.

**Statistical Analysis**

Data measured before and after the lens wear for same eyes were compared using paired-sample t test. Results from 5-year lens wear were compared with those from the control group (no lens wear) using unpaired-sample t test. The interocular and intergroup differences were defined as significant at $P < 0.05$ and highly significant at $P < 0.01$.

**RESULTS**

**Visual Acuity**

The visual acuity was improved significantly from $0.16 \pm 0.08$ to $0.24 \pm 0.11$ (decimal) ($P < 0.001$) after 8-hour lens wear and from $0.88 \pm 0.10$ to $1.20 \pm 0.16$ ($P < 0.001$) after 5-year lens wear. None of the subjects experienced adverse responses related to the lens wear, and there were no detectable abnormalities of the eyes under slit-lamp microscopy.

**Confocal Microscopy**

Before the lens wear, corneal epithelial cells demonstrated 3 types of reflectivity intensity: highly bright, bright, and dim (Fig. 1). The superficial epithelial cells were loosely arranged with a hexagon or pentagon cell profile. The nuclei of these cells are usually bright and visible. The basal cells were densely packed, had a clear cell profile, and were more homogeneous in cell size and reflectivity than the superficial cells (Fig. 2). However, the nuclei of the basal cells were invisible under confocal microscopy. Bowman layer was a noncellular homogeneous band with the presence of dendritic nerve fibers (Fig. 3). The corneal stroma showed average distribution of the keratocytes with the nuclei appearing to be oval or shuttle-shaped. The density and homogeneity of the keratocytes in the anterior stroma were higher than that in the posterior stroma (Table 1, Fig. 4). Descemet membrane could not be defined as clearly as Bowan layer. The endothelium was densely packed with hexagon-shaped cells, which were homogeneous in cell size and reflectivity (Fig. 5).

After an 8-hour lens wear, the cell morphology, thickness, and basal cell density in the central epithelium did not significantly differ from those before the lens wear (Fig. 1, Table 1). The central stromal thickness increased by 47 μm ($P = 0.012$ compared with same region before lens wear). The density of keratocytes decreased by 111/mm² in the anterior stroma and by 72/mm² in posterior stroma ($P < 0.001$ compared with the same region before lens wear). Furthermore, the keratocytes in the posterior stroma appeared to become spiky, elongated, and less homogeneous in cell size (Fig. 4). There was only 1-year difference in age between the eyes of 5-year lens wear and the control eyes at the end of the study (24 versus 23). The central corneal epithelium in this long-term lens wear group was 10 μm thinner, and the density of the basal cells decreased significantly by 1177/mm² compared with the control group ($P < 0.001$, Table 1). However, the cell morphology of the epithelium was similar between the long-term lens wear and the control groups (Figs. 1, 2). The number of keratocytes in eyes of the long-term lens wear decreased by 175/mm² in the anterior stroma and 137/mm² in the posterior stroma ($P < 0.001$ compared with the control eyes). However, the keratocyte morphology and stromal thickness in the central cornea did not change compared with the control eyes (Fig. 4). The Bowman layer was similar among the 8-hour lens wear, 5-year lens wear, and the control groups in morphology and nerve distribution. The endothelial cells were also similar among these 3 groups in cell morphology, cell density, and proportion of the hexagon cells ($P \geq 0.15$, Table 1, Fig. 5).

**Topography**

After 8 hours of lens wear, the cornea significantly flattened at the 3-mm central region ($P < 0.001$) but did not change at the 5-mm central region ($P = 0.86$, Table 2, Fig. 6) compared with the same regions before the lens wear. The SimK power was reduced in steep and flat meridians (prior
lens versus postlens wear: \( P < 0.001 \)). The corneal thickness increased significantly by 11–15 \( \mu \text{m} \) in the central region and other 4 quadrants (prior lens versus postlens wear: \( P = 0.038 \) in superior quadrant but \( <0.01 \) in all other regions). The minimum corneal thickness increased significantly by 10 \( \mu \text{m} \) with a decreased anterior chamber depth of 100 \( \mu \text{m} \) compared with the same eyes before lens wear (\( P < 0.01 \)).

Corneal power in eyes wearing lenses for 5 years did not differ significantly from the control eyes [at the 3-mm and 5-mm central regions (\( P \geq 0.434 \), Table 2)]. The SimK power in the flat meridian was significantly reduced (\( P = 0.006 \)), whereas this value in the steep meridian remained unchanged (\( P = 0.334 \)) compared with the control eyes. The corneal thickness did not change significantly in the central region (\( P = 0.804 \)) but increased significantly by 23–34 \( \mu \text{m} \) in the 4 paracentral regions after 5 years of lens wear (\( P \leq 0.04 \) compared with the control eyes, Table 2, Fig. 6). The minimum corneal thickness and anterior chamber depth remained unchanged over the 5-year period of lens wear compared with the control eyes (\( P \geq 0.402 \)). The anterior surface height did not change significantly in both the 8-hour and the 5-year lens wear groups (\( P \geq 0.196 \) compared with the control group). However, a mild but significant reduction of the posterior surface height was found only in the 8-hour lens wear group (\( P = 0.040 \) compared with the control group).

**DISCUSSION**

This study investigated changes in corneal morphology and topography in eyes wearing OK lenses for a single night and a 5-year period of overnight lens wear. The visual acuity is improved significantly in eyes from the 8-hour or the 5-year lens wear groups, and the amount of this improvement is similar between the 2 groups. Because the degree of myopia before the lens wear is similar for these 2 groups, it is

---

**FIGURE 2.** Basal cells of human corneal epithelium observed under confocal microscopy were similar among baseline, 8-hour, and 5-year lens wear in cell profile and homogeneity.

**FIGURE 3.** The Bowman layer of human corneas with the presence of dendritic nerve fibers was observed under confocal microscopy. The profile of basal cells was also visible on background of each image, indicating the site scanned was at a plane close to the basement membrane.
**TABLE 1. Central Cornea Measured by Confocal Microscopy in Eyes Wearing OK Lenses**

<table>
<thead>
<tr>
<th>Thickness (μm)</th>
<th>Baseline</th>
<th>8-hr Lens Wear</th>
<th><em>P</em> (8-hr Wear to Baseline)</th>
<th>5-yr Lens Wear</th>
<th>†P (5-yr Wear to Baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epithelium</strong></td>
<td>65.7 ± 17.2</td>
<td>65.9 ± 17.2</td>
<td>0.961</td>
<td>55.9 ± 10.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Stroma</strong></td>
<td>558.6 ± 72.5</td>
<td>605.2 ± 32.9</td>
<td>0.012</td>
<td>573.9 ± 59.8</td>
<td>0.150</td>
</tr>
<tr>
<td><strong>Cell density (per mm²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal cell</td>
<td>4419.0 ± 72.2</td>
<td>4253.0 ± 62.5</td>
<td>0.189</td>
<td>3242.0 ± 77.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Anterior stroma</td>
<td>1135.0 ± 113.1</td>
<td>1024.0 ± 73.0</td>
<td>&lt; 0.001</td>
<td>960.1 ± 156.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Posterior stroma</td>
<td>839.3 ± 59.3</td>
<td>767.2 ± 35.9</td>
<td>&lt; 0.001</td>
<td>702.0 ± 164.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Endothelial cell</td>
<td>2706.0 ± 289.1</td>
<td>2718.0 ± 206.8</td>
<td>0.859</td>
<td>2640.0 ± 294.4</td>
<td>0.172</td>
</tr>
<tr>
<td>% of hexagon endothelial cells</td>
<td>0.6 ± 0.07</td>
<td>0.6 ± 0.03</td>
<td>0.233</td>
<td>0.6 ± 0.1</td>
<td>0.150</td>
</tr>
</tbody>
</table>

Baseline: before lens wear.
*Paired- sample *t* test applied.
†Unpaired- sample *t* test applied.

**FIGURE 4.** Confocal microscopy showed an average distribution of keratocytes in the baseline group. The homogeneity of the keratocytes in the anterior stroma was higher than that in the posterior stroma in this group. The keratocytes in the posterior stroma appeared to become spiky, elongated, and less homogeneous in cell size after 8 hours of lens wear compared with the baseline. The keratocytes of the anterior and posterior stroma in the 5-year lens wear were normal in cell morphology and distribution compared with the baseline.
reasonable to suggest that a short-term lens wear is as effective as a long-term lens wear in OK refractive correction.

The cornea flattens significantly in the eyes of these 2 groups as indicated by a reduced corneal curvature on SimK (flat) (Table 2). After 8 hours of OK lens wear, the central stromal thickness increased significantly with a decreased density of keratocytes across the entire thickness of the stroma under confocal microscopy (Table 1). However, the proportion of the reduced numbers in keratocytes is similar for both the anterior and the posterior stroma (8.5%–10%). The cell activity in the posterior stroma appeared to increase as evidenced by the spiky and elongated keratocytes (Fig. 4). Furthermore, the minimum corneal thickness increases significantly. These results suggest that the thickening of the central stroma is because of a temporary tissue edema in response to mechanical pressure imposed by the OK lens and the relative hypoxia during the closed-eye condition. This speculation seems to be consistent with a similar degree of thickening among the 4 paracentral regions of the cornea (Table 2), which could have originated from the epithelium and the stroma.

It has been reported that a mild but significant epithelial thinning in central cornea (<10%) can be detected by optical coherence tomography and optical pachometry after overnight OK lens wear. However, significant changes in the central corneal epithelium are not detectable by confocal microscopy in the 8-hour wear group from this study. These results indicate that optical coherence tomography and optical pachometry may be more sensitive than confocal microscopy in the detection of minor biometric changes in corneal epithelium.

After 5 years of overnight lens wear, epithelial thickness in the central cornea decreases by 15%; however, the central stromal thickness does not change significantly compared with the control eyes (Table 1). The central epithelial thinning could be associated with the significantly reduced density of the

| TABLE 2. Corneal Topography and Anterior Chamber in Eyes Wearing OK Lenses |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|
| Corneal power (D)               | Baseline       | 8-hr Lens Wear| 5-yr Lens Wear| 5-yr Lens Wear|
| 3-mm anterior surface           | 43.3 ± 1.7     | 43.1 ± 1.7     | 43.2 ± 1.7     | 43.2 ± 1.7     | 0.711          |
| 5-mm anterior surface           | 43.3 ± 1.7     | 43.1 ± 1.7     | 43.2 ± 1.7     | 43.2 ± 1.7     | 0.434          |
| SimK (steep)                   | 43.8 ± 1.8     | 43.8 ± 1.7     | 43.2 ± 1.7     | 43.4 ± 2.0     | 0.334          |
| SimK (flat)                    | 42.8 ± 1.7     | 42.8 ± 1.6     | 42.0 ± 1.6     | 41.5 ± 1.7     | 0.006          |
| Anterior surface height        | 42.6 ± 1.6     | 42.5 ± 1.6     | 42.5 ± 1.6     | 42.7 ± 1.5     | 0.851          |
| Posterior surface height       | 52.4 ± 2.6     | 52.2 ± 2.6     | 52.2 ± 2.6     | 53.0 ± 2.0     | 0.347          |
| Central thickness (μm)         | 579.6 ± 35.1   | 590.7 ± 33.0   | 582.0 ± 31.8   | 582.0 ± 31.8   | 0.804          |
| Superior                       | 663.5 ± 37.9   | 674.4 ± 37.8   | 691.8 ± 43.7   | 691.8 ± 43.7   | 0.009          |
| Nasal                          | 685.0 ± 38.2   | 697.5 ± 36.7   | 719.8 ± 38.3   | 719.8 ± 38.3   | 0.001          |
| Inferior                       | 660.0 ± 43.9   | 674.1 ± 46.4   | 692.7 ± 33.9   | 692.7 ± 33.9   | 0.002          |
| Temporal                       | 659.3 ± 41.5   | 674.8 ± 38.2   | 682.0 ± 42.3   | 682.0 ± 42.3   | 0.040          |
| Minimum thickness              | 569.5 ± 36.1   | 579.4 ± 35.7   | 566.6 ± 38.6   | 566.6 ± 38.6   | 0.765          |
FIGURE 6. Corneal curvature and thickness evaluated by Orbscan II. Corneal topography in the 1-mm central zone appeared to become more regular with an increase in duration of OK lens wear. The central cornea flattened after 8 hours and 5 years of the lens wear (SimK1 and 2: 40.0 D and 39.5 D in 8-hour wear versus 41.2 D and 40.8 D in baseline; 40.0 D and 39.3 D in 5-year lens wear versus 41.2 D and 40.8 D in baseline). In contrast, the paracentral zone steepened after the short-term or long-term lens wear.
basal cells. The density of keratocytes in the 5-year lens wear decreases by 15%–16% in both anterior and posterior stroma. Furthermore, the keratocytes seem to be more reactive to 8-hour lens wear than to 5-year lens wear, suggesting that the cornea gradually adapts to OK lens wear with time. The entire thickness of the central cornea remains unchanged in eyes wearing OK lenses for 5 years. However, the thickness in the 4 paracentral regions of the cornea in these eyes significantly increases twice as much as in eyes wearing 8-hour lenses (Table 2). Unlike the 8-hour lens wear, the minimum corneal thickness remains unchanged in eyes with a 5-year lens wear. All these results indicate that eyes with a long-term wear of OK lenses undergo a rearrangement in corneal topography rather than a short-term edematous response.

In daily wearers of soft or other types of contact lenses over a period longer than 10 years, the thickness of central corneal epithelium does not change, whereas the peripheral epithelium thins compared with the age-matched control subjects. These results are opposite to the findings in the 5-year lens wear from this study, suggesting that the pressure exerted from the contact lenses is mainly imposed on the peripheral cornea, probably because of the geometric design of the lenses. Long-term daily wear of soft contact lenses does not significantly change keratocyte density of the entire cornea. However, long-term extended wear of the same lenses significantly reduces the keratocyte density in the anterior and posterior cornea. Endothelial pleomorphism and polymegethism are commonly found in long-term wearers of different contact lenses, although an increase in gas permeability of the lenses could reduce these abnormalities. However, both the short-term and the long-term wear of OK lenses in this study does not affect the morphology and density of the endothelium.

In summary, short-term and long-term wear of OK lenses can effectively improve visual acuity by flattening the central cornea of the myopic eyes. The flattened cornea in the short-term lens wear is mainly because of the thickening of the midperipheral cornea. The flattened cornea in the long-term lens wear, however, is associated not only with the thickening of the midperipheral cornea but also with the central epithelial thinning. Long-term OK lens wear does not cause similar morphological changes in the corneal endothelium to the long-term wear of contact lenses.

ACKNOWLEDGMENTS

This work was supported by a grant from SRFDP (20050558075), the National Natural Science Foundation of China (30572007, 30772389), and Fok Ying Tong Education Foundation (91043).

REFERENCES