INTRODUCTION

Overnight orthokeratology (OK), also known as corneal refractive therapy (CRT) or corneal reshaping, is a clinical technique designed to transiently reduce or eliminate myopia. The main benefit of OK for myopic subjects is an independency from optical aids. Recent research has examined the technique of orthokeratology in humans, evaluating its effects on corneal topography, visual acuity, contrast sensitivity, the optical quality of the eye, and its biomechanical properties. However, few studies have addressed how this technique affects the cell density of the corneal layers and their morphological characteristics in the long term. Most histological assessments of changes in the thickness of the epithelium and stroma have been performed on animals and human studies seeking to determine the histological and morphological changes induced by OK have been retrospective in design or of short duration. There is therefore a need for prospective studies in which these factors can be monitored over longer time spans in individual subjects, because little is known about the cellular basis for the changes induced by OK. Several studies...
have shown that stromal keratocyte density decreases after refractive surgery. These changes have been demonstrated prospectively and similar changes could occur in the OK lens wearer. In a study by Efron, basal epithelial cell size at the limbus was found to be greater in lens wearers than non-lens wearers. Recently, Zhong et al. reported reduced keratocyte density throughout the entire corneal thickness in two groups of subjects who wore OK lenses for 5 years or only 1 night. In this retrospective human study, epithelial morphology and density changes along with increased stromal levels of activated keratocytes were reported. However, as far as we know, no study has monitored the same variables in the same subjects in the long term.

This study was designed to prospectively analyze the changes produced both in cell density and cell morphology in the different corneal layers by in vivo confocal microscopy in subjects undergoing OK. Corneal thickness measurements were also examined. Our working hypothesis was that some rapidly induced changes might persist over time and even continue after discontinuation of contact lens wear.

MATERIALS AND METHODS

We designed a prospective, longitudinal, single-center study of 13 months’ duration. The study was approved by the Ethics Committee of the Hospital Carlos III and the study protocol adhered to the tenets of the Declaration of Helsinki. The participants were students from the Complutense University of Madrid and informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study.

Subjects

Subjects were selected according to a set of inclusion criteria as well as an interest in trying the treatment under study. Our main interest was to evaluate changes in stromal cell density so calculations were based on the mean and SD of anterior keratocyte density (cell/mm²). A sample size of 18 subjects (Granmo 7.2 software) would have an 80% power to detect a size effect, taking a clinically significant difference in keratocyte density as 100 and a typical SD for this measurement of ± 150, using a paired t-test with a 5% two-tailed level. At the study outset, we started off with 21 subjects, but were left with a final population size of 15– three men (20%) and 12 women (80%) of mean age 25.5 ± 2.5 years. One subject refused to stop wearing the contact lenses at the pre-established time. Before the study, 10 of these subjects occasionally wore disposable contact lenses and two wore conventional hydrophilic contact lenses.

The baseline refractive state of each participant was recorded as manifest refraction determined through the phoropter at the study outset. Mean (± SD) manifest refraction was −2.10 ± 0.95 D, mean cylinder was −0.4 ± 0.3 D and mean spherical equivalent (SE) was −2.30 ± 0.95 D. Distribution by sphere magnitude was: 2 eyes (6.67%) −0.50 to −0.75 D, 18 eyes (60%) −1 to −1.75 D, 5 eyes (16%) −2 to −2.75 D, 3 eyes (10%) −3 to −3.75 D, and 2 eyes (6.67%) −4.00 to −4.75 D.

Inclusion/Exclusion Criteria

The inclusion criteria were: men or women aged 18–30 years, ocular refraction of −0.50 to −4.5 D of myopia with astigmatism no greater than 1.00 D, and a best-corrected visual acuity of at least 0.04 logMAR in both eyes. Subjects were excluded if they used or had in the past used gas permeable contact lenses. Hydrophilic lens wearers were instructed to stop wearing their contact lenses 4 weeks prior to the start of the study. Subjects were also required to be available for the follow-up visits on the established dates: 15 days, 1 month, 3, 6 and 12 months. At this last time point, subjects had to stop wearing their contact lenses to monitor the recovery of the parameters examined 15 days and 1 month after the cessation of OK. Subjects were excluded if they: were pregnant or intended to have children over the next two years, had any systemic or eye disease, a history of eye surgery, blepharitis, recurrent erosion, dry eye syndrome, neovascularization, evidence of keratoconus, corneal irregularity, pupils larger than 5.5 mm as measured with the Colvard pupillometer (Oasis, Glendova, California, USA) in photopic conditions, or were participating in another clinical trial.

Contact Lens

The orthokeratology lens selected for the study was the HDS 100 Paragon CRT design (Paragon Vision Sciences). The adaptation procedure for the lenses was performed according to the manufacturer’s protocol as follows: (1) the specifications for the lens were determined using the calculation rule provided by the manufacturer, (2) adequate fitting was assessed with fluorescein, and (3) in a corneal topography acquired after an overnight trial, a satisfactory fit was confirmed by the typical bull’s eye pattern.

Procedures/Instrumentation

The study followed a controlled protocol. The methods used were performed according to the literature or the manufacturers of the instruments. All measurements were performed in the same office and at the same time of day within a 2-hour margin. In each follow-up session, the same clinical procedures were conducted in the same order by two clinicians.
At each follow-up visit, best-unaided visual acuity and objective refraction (as ocular refraction) were determined. High and low contrast visual acuity (HCVA and LCVA) were assessed using ETDRS LogMAR charts without optical correction. Using the Atlas Mastervue topographer (Humphrey Instruments, Zeiss), the flattest and steepest corneal meridians and corneal eccentricity were recorded as the average of two measurements. The instrument was calibrated before data acquisition according to the manufacturer’s recommendations. Biomicroscopy was also performed in all the follow-up visits.

In vivo confocal microscopy (CM) was performed using a ConfoScan 4 instrument (CS4, Nidek Technologies, Italy). The microscope was calibrated by the manufacturer prior to the study. This microscope uses a 40X Zeiss Acroplan objective with a 0.75 numerical aperture. Observations were made on the central cornea of both eyes of each subject using a sensor called a “z-ring encoder”, which provides the exact depth of the scan. Fixation was controlled using the eye tracker, which is included in the CS4 microscope’s software. The procedure followed has been described in previous studies. Images were taken of all corneal layers and stored in the instrument’s software. Morphological analysis was performed by one examiner in a masked and randomized manner. Images of the endothelium were used to assess cell density, the coefficient of variation in cell area (polymegethism) and percentage of hexagonal cells (pleomorphism). Previous studies have shown that, rather than cell density, these factors are the best indicators of corneal endothelial stress in contact lens wearers. The image analysis software (Confocommander 2.7.1, Nidek Technologies, Italy) of the CS4 allows automated or semiautomated and manual endothelial image analysis. We used the semiautomated method which uses correction tools to correct the mistakes made by the automated analysis. When possible, this method was also used to count cells in the three layers of the corneal stroma. For cell counts in the stroma, this layer was divided into three: anterior (AKD), middle (MKD) and posterior (PKD). Images of the anterior stroma were taken immediately posterior to the Bowman’s layer. Images of the middle stroma were taken in the middle of the stroma depth. Finally, images of the posterior stroma were taken immediately anterior to Descemet’s membrane, or endothelium if the Descemet layer was not visible. Three images of each corneal layer 16 microns apart were analyzed to obtain an average reading. This value (16μ) was chosen to ensure that the cells visible on the frame were not present in the other two frames. Two types of cell counts were conducted to assess keratocyte density: (1) for measurements over time, counts were performed using the CM software (Confocommander 2.7.1) in a semiautomated fashion, and (2) to verify the counts obtained, the ImageJ analysis software (http: www.rsbio.nih.gov/ij) designed for morphology assessment was used at the following time points: baseline, after one year of OK treatment and one month after the cessation of OK. This analysis was performed in the three layers of the corneal stroma and the steps taken by the software are: (1) transform into 8 bit image, (2) adjust local brightness to uniform field, (3) convert into a binary image, (4) remove noise, and (5) count cells.

Due to the difficulty in counting cells of the epithelium, we selected 15 images of the basal and superficial cell layer, respectively. Images were selected on the basis of good visibility of the cells. In each of these images, we selected two cells for height and width measurements. These measurements are conducted in a plane parallel to the cornea as the CM records the image rather than in a perpendicular plane as observed in a histological section. In order to evaluate the repeatability of these measures, two examiners reviewed the images. The comparisons between the values obtained (mean and SD) by each examiner were within 95% limits of agreement. These measurements were made at the baseline exam, at 12 months after the start of OK treatment, and 1 month after OK cessation. Further variables were also defined and assessed at each follow-up session: the number of nerve fibers observed in the subbasal nerve plexus and the number of activated keratocytes observed throughout the corneal stroma. The latter were defined as keratocytes with highly reflective nuclei.

Corneal thicknesses were also determined using the CM instrument’s software. The use of the z-ring adapter with the CS4 for measuring central corneal thickness (CCT) has been validated elsewhere. The software provides a profile in which the X and Y axes represent the depth of the cornea (μm) and image intensity, respectively. Intensity peaks correspond to the superficial epithelium, the subbasal nerve plexus, the most anterior keratocytes and the endothelium. Each layer’s thickness was taken as the distance between the above peaks as suggested by Patel et al. If the intensity peak corresponding to the subbasal nerve plexus or the most anterior keratocytes did not appear, the first focused images obtained of each was manually defined and used to determine thickness. In addition to the previous layers, we defined the thickness of the subbasal nerve plexus as the distance between this plexus and the first focused image of basal cells.

Validating Thickness Measurements

A Visante optical coherence tomographer (OCT, Carl Zeiss, Germany) was used to obtain pachymetry measurements in the last follow-up visit. The details of the use of OCT for corneal pachymetry have been described previously and OCT has also been
approved to monitor epithelial changes. OCT thickness values were compared with the measurements obtained by CM to establish agreement between measurement methods. CCT, stromal thickness and central epithelium thickness were recorded in the right eye of each subject \((n=14)\) and they were compared with the corresponding values obtained by CM. It should be highlighted that the OCT measurement of stromal thickness includes Bowman’s layer thickness; the measurement of epithelial thickness includes the subbasal nerve plexus thickness. For this reason, the five corneal layers defined by CM were divided into 3 to match the measurements obtained by the scanned image as captured by OCT and its axial reflectivity profile.

**Data Analysis**

Data were recorded in Excel (2003, Microsoft) and analyzed using the SAS 9.3 package. In general, statistical analysis was performed only for the right eye of each subject but measurements were made in both eyes.

We checked the distribution of data was normal using the Kolmogorov-Smirnov normality test. To analyze changes over time, we used 1-factor repeated measures analysis of variance and the Kruskal-Wallis test when the data were not normally distributed. Paired t-tests were conducted whenever significant differences were detected in the ANOVA and \(p\) values adjusted for multiple comparisons. Also, the Bland Altman method was used to establish agreement between the thickness of each subject so the following results correspond only to the right eye of each subject.

FIGURE 1 Changes in polymegethism percentages during the 13-month trial. Error bars represent the means and standard deviation at the 95% confidence level. M = month, wo/L = without contact lens (after interruption OK wear).

The central cornea was significantly flattened \((p<0.01)\) along the steep and flat meridians at all the follow-up times after the onset of OK lens wear. The flattening recorded at 12 months was 1.20 ± 0.34 D and 1.16 ± 0.46 D for the steepest and flattest meridians, respectively. No significant differences were detected in both uncorrected HCVA and LCVA after OK compared to baseline values with optical correction. All these variables had returned to baseline 15 days after cessation of OK.

Table 1 shows the baseline morphological data obtained. Reliable confocal microscopy data after 15 days of OK cessation could only be obtained in seven subjects so the following results correspond only to the time point 1 month post OK cessation. Analysis of variance revealed significant changes in endothelial polymegethism \((p=0.0129, \text{Figure 1})\). Post-hoc pairwise analysis indicated statistically significant differences in this variable between baseline and 12 months \((p=0.008)\) and between baseline and 13 months (after 1 month of lens discontinuation, \(p=0.03)\). In addition, ANOVA detected a significant difference in activated keratocyte numbers \((p=0.0017, \text{Figure 2})\). Thus, the paired t-test indicated significant differences between baseline and 1 month \((p=0.04)\), between baseline and 3 months \((p=0.04)\) and finally between baseline and 6 months \((p=0.04)\). In contrast, activated keratocyte numbers were significantly lower after lens wear cessation than baseline values \((p<0.01)\). In response to OK, ANOVA revealed a significant decline in basal cell density.

### RESULTS

In the final sample (15 right eyes), mean \((± SD)\) manifest refraction was \(-1.9 ± 0.9\) D, mean cylinder was \(-0.2 ± 0.2\) D and mean spherical equivalent (SE) was \(-1.95 ± 0.90\) D. Distribution by sphere magnitude was: 10 eyes (66.7%) \(-1\) to \(-1.75\) D, 3 eyes (20%) \(-2\) to \(-2.75\) D, 1 eye (6.7%) \(-3\) to \(-3.75\) D, and 1 eye (6.7%) \(-4.00\) to \(-4.75\) D.

After 15 days, 1 month, 3 months, 6 months and one year of orthokeratology treatment, the mean sphere magnitude and SE had decreased significantly from baseline with \(p\)-values \(<0.0001\) and \(0.0001\), respectively.

<table>
<thead>
<tr>
<th>Endothelium cell density (cell/mm²)</th>
<th>Polymegethism (%)</th>
<th>Pleomorphism</th>
<th>Posterior keratocyte density (cell/mm²)</th>
<th>Middle keratocyte density (cell/mm²)</th>
<th>Anterior keratocyte density (cell/mm²)</th>
<th>Activated keratocyte number (units)</th>
<th>Nerve fibre number (units)</th>
<th>Epithelial basal density (cell/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M ± SD 2831 ± 367</td>
<td>29.7 ± 6.2</td>
<td>59.3 ± 8.3</td>
<td>657 ± 75</td>
<td>630 ± 81</td>
<td>1027 ± 196</td>
<td>4.3 ± 2.8</td>
<td>4.2 ± 1.6</td>
<td>4036 ± 327</td>
</tr>
</tbody>
</table>

M, mean; SD, standard deviation.

**TABLE 1** Morphological baseline values obtained in the study.
These significant changes were recorded at 15 days, 1 month, 3 months, 6 months and one year (paired t-test \( p < 0.01 \) for the 5 follow-up times) compared to baseline. Basal cell densities returned to baseline 1 month after OK discontinuation.

Changes in the cell densities of the stromal layers were not shown to be significant by ANOVA (AKD \( p = 0.23 \), MKD \( p = 0.91 \), and PKD \( p = 0.208 \)). However, a clinical drop in the density of AKD was observed with significance reached after 6 and 12 months of OK lens wear \( (p < 0.05 \) paired t-test). In the anterior stroma, the cell counts performed using the Confocommander software and automated counts obtained by ImageJ analysis differed significantly \( (p < 0.01) \) with higher counts obtained using the former method. When counts were obtained in the medium and posterior stromal layers over time, no differences were detected using either method but the same decreasing trend was observed in the AKD. Nerve fibers were unaffected by OK treatment.

Since it was difficult to distinguish between the wing and superficial cells of the epithelium, we assessed these cells in terms of their visibility at each time point. The percentages obtained (Figure 4) revealed a significant increase \( (p < 0.05) \) in the visibility of both wing and superficial cells over time. Height and width measurements could only be made in the basal and superficial epithelial cells. Basal cell heights and widths failed to vary significantly from baseline to 1 year of OK treatment. However, we did note a more irregular distribution of the cell network and reduced visibility at the 1-year time point. This perception improved after cessation of contact lens wear. Although limits between adjacent wing cells were insufficiently clear, we did observe an increase in nuclear size. Finally, superficial cells showed increased heights and widths over time (Table 2), but the difference was only significant for width measurements \( (p < 0.01) \).

Analysis of variance revealed no changes in the thicknesses of the central cornea, stroma, Bowman’s layer (Figure 5) or subbasal nerve plexus (Figure 6). Although post-hoc analysis identified significant changes for the Bowman’s layer and subbasal nerve plexus at some time points (Table 3), significant differences emerged in the thickness of the epithelium \( (p < 0.01 \), Figure 7A) and these persisted when subbasal nerve plexus thickness was included in the measurement \( (p < 0.01 \) Figure 7B).

![FIGURE 2](image2.png) Changes in activated keratocyte numbers produced during the 13-month trial. Error bars represent the means and standard deviation at the 95% confidence level. M = month, wo/L = without contact lens (after interruption OK wear). *Denotes a statistically significant difference \( (p < 0.01) \).

![FIGURE 3](image3.png) Changes in basal cell densities produced during the 13-month trial. Error bars represent the means and standard deviation at the 95% confidence level. M = month, wo/L = without contact lens (after interruption OK wear). *Denotes a statistically significant difference \( (p < 0.05) \).

![FIGURE 4](image4.png) Percentages of right eyes in which different epithelial cells were visualized at each follow-up time. M = month, wo/L = without contact lens (after interruption OK wear).

### TABLE 2. Epithelium cell widths and heights (μm) measured parallel to the cornea.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Eyes</th>
<th>Baseline</th>
<th>After 1 year</th>
<th>1 month after OK cessation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 15</td>
<td>M ± SD Width</td>
<td>Height</td>
<td>n = 14</td>
</tr>
<tr>
<td>Basal cell (μm)</td>
<td>M ± SD</td>
<td>14.7 ± 1.9</td>
<td>14.4 ± 2.1</td>
<td>16.1 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>9.6–18.6</td>
<td>10.4–18.6</td>
<td>13.0–17.4</td>
</tr>
<tr>
<td>Superficial cell (μm)</td>
<td>M ± SD</td>
<td>26.8 ± 4.2</td>
<td>30.1 ± 5.1</td>
<td>42.1 ± 8.2*</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>21.8–35.0</td>
<td>20.7–39.2</td>
<td>38.8–57.2</td>
</tr>
</tbody>
</table>

M, mean; SD, standard deviation. *Denotes a significant difference \( (p < 0.01) \).
After cessation of OK, baseline epithelial thickness values were recovered but Bowman layer and subbasal nerve plexus thicknesses were still reduced.

All the OCT thickness measurements were higher than the CM measurements yet were in good agreement. The factors determined were the mean difference the standard deviation (SD) and the limits of agreement (estimated by the mean difference ± 2[standard deviation of the differences]) at the 95% level. Also, the distribution of the differences was checked and they were normal in the three corneal thickness: CCT (1.3 ± 36.2, p = 0.83), epithelium thickness (5.4 ± 16.2, p = 0.08) and stromal thickness (5.1 ± 33.4, p = 0.36).

**DISCUSSION**

Our study was designed to examine the changes produced in corneal morphology in eyes wearing OK lenses overnight for 1 year and to determine whether these changes were reversible 1 month after treatment was discontinued. The cell density values and thicknesses of the different layers of the cornea observed here are within the ranges reported previously for the normal cornea.28–30 No significant differences were detected in endothelial cell density, although densities were higher at each time point than baseline values. The instruments used to make corneal measurements take images of the center of the cornea. However, since the area imaged is small, the region of interest may vary from image to image within a given instrument, as indicated by Sheng et al.31 Moreover, this area is also likely to depend on subject cooperation during the test such that ocular movements could determine a more peripheral area suggesting some variation in the results obtained. Notwithstanding this limitation, we were able to detect a significant increase in endothelial cell polymegethism which improved significantly on cessation of OK contact lens wear, despite values not returning to the levels observed at baseline. Endothelial pleomorphism and polymegethism have been reported in long-term contact lens wearers although some authors claim that these abnormalities can be reduced by increasing the gas permeability of the lenses.32,33 Odenthal et al.32 claim that endothelial polymegethism and pleomorphism caused by PMMA or HEMA contact lens wear is partly reversible, as noted by us for the overnight use of these gas permeable contact lenses.

The cell densities of the three stromal layers remained unchanged throughout the study. However, we did observe the increased presence of activated keratocytes, particularly in the anterior stroma. Clinically, mean anterior keratocyte density had fallen by 7% after 1

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**TABLE 3**. The p-values in the biometric measurements using analysis of variance.

<table>
<thead>
<tr>
<th>Central thickness</th>
<th>ANOVA</th>
<th>½ month</th>
<th>1 month</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
<th>1 month wo/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M ± SD, n = 15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cornea</td>
<td>0.98</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Stromal</td>
<td>0.99</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Bowman layer</td>
<td>0.237</td>
<td>0.039</td>
<td>0.043</td>
<td>ns</td>
<td>0.041</td>
<td>0.0035</td>
<td></td>
</tr>
<tr>
<td>Subbasal nerve plexus</td>
<td>0.11</td>
<td>ns</td>
<td>0.048</td>
<td>0.031</td>
<td>ns</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Epithelium</td>
<td>0.0073</td>
<td>0.07</td>
<td>0.002</td>
<td>ns</td>
<td>0.029</td>
<td>ns</td>
<td>0.014</td>
</tr>
<tr>
<td>Subbasal nerve plexus + Epithelium</td>
<td>0.0064</td>
<td>0.002</td>
<td>0.001</td>
<td>0.029</td>
<td>ns</td>
<td>0.001</td>
<td>0.014</td>
</tr>
</tbody>
</table>

M, mean; SD, standard deviation; ns, not significant; wo/L = without contact lens
Activated keratocytes (AK) were defined as keratocytes with highly reflective nuclei. This greater activity in the nucleus could be the outcome of several cell processes: apoptosis, proliferation, necrosis, etc. These processes consecutively take place triggering the repair response to a corneal lesion. Since the epithelium, stroma and tear film contribute to maintaining ocular surface integrity, it is possible that the increased presence of AK in our subjects may have been influenced by the stromal thickening observed and particularly by the epithelial cell changes. These changes are analyzed below. Further, enhanced AK could be related to additional hypoxic stress during OK lens wear reflected by changes in tear components as suggested by Choy et al. The changes produced in response to OK lens wear may be of a physiological nature (edema), mechanical (lens forces) or biochemical (cell-mediated).

Wilson et al. reported that epithelial cells release a variety of cell factors such as cytokines in response to changes in their environment. According to Wilson and co-workers, any source of epithelial stress, such as epithelial pressure from contact lens wear, could trigger stromal keratocyte apoptosis. Stromal apoptosis occurs immediately following corneal injury and, depending on the type and extent of injury, may persist in the tissue for months or even years. Research into the wound healing response of the stroma has revealed the presence of activated keratocytes and a more intense response results in the cell bodies becoming visible. Transmission electron microscopy has shown that these hyper-reflective cells represent keratocytes activated to a repair phenotype. These effects over time could lead to the decrease in keratocyte density reported by Zong et al. over 5 years of OK lens wear.

Epithelial basal cell densities fell in response to OK treatment and remained unchanged at each time point except the last, when baseline values were recovered. This reduction was 12–15% and is consistent with the 11% loss recorded by us in a larger study sample and with the results of Zong et al. who noted a significant decrease in basal cell density. We speculate that this loss may be due to compression of the epithelial layers resulting in thinning, which impairs the visibility of these cells. In a histological study, Ren et al. established for the first time that extended wear of rigid contact lenses significantly decreases central corneal epithelial proliferation in the basal layer. Simultaneously, increases in corneal limbal and conjunctival epithelial cell number were observed, suggesting that activation of limbal stem cells may constitute an attempt to counteract ongoing suppression of central basal cell proliferation. On the other hand, Matsubara et al. found more mitotic cells in the central area where basal cells appear flattened. This indicates epithelial proliferation in the central zone and these authors hypothesized that this was a response to the mechanical stress induced by the lens. Hence, OK lens wear seems mainly to affect the state of the epithelial cell layer and this could explain our observation of increased wing cell and, especially, superficial cell densities. The morphological changes noted in cell width and nucleus size may also be related to this response. In effect, Ladage et al. demonstrated a significant increase in superficial cell size...
in daily rigid lens wearers and in overnight contact lens wearers and informed that overnight contact lens wear suppresses central basal cell proliferation, delays the time basal cells leave the basal cell layer, decreases surface cell death while also suppressing cell exfoliation, increases cell surface size and thins the central epithelium, as we were able to observe here. This would indicate a change in surface exfoliation at the epithelium as suggested by Cheah et al., who reported a reduction in the sagittal height of the basal cell. Such compression could result in the parallel expansion of the cells as observed in our subjects. Interestingly, Efron reported that the surface cell layer is difficult to image by CM and is only occasionally seen in some subjects or when the epithelium has been disturbed, as we also noted. Our results are in line with those of Choo et al., who reported that epithelial cell compression and deformation appear to be the dominant factors of the OK response. Since epithelial thickness was still reduced after 1 year of treatment, it seems that these epithelial cell changes were more persistent than the other changes observed.

Another point of focus of our study was to check the effects of OK on the thickness of the different central corneal layers. We observed thinning of CCT, the Bowman’s layer, subbasal nerve plexus and epithelium along with thickening of the stroma over time. After cessation of OK, epithelial thicknesses returned to baseline. However, the thicknesses of Bowman’s membrane and the subbasal nerve plexus remained reduced. In our subjects, maximal CCT thinning was 3%. This percentage represents a mean of 13 microns difference with respect to its baseline value. The thickening observed in the central stroma was 3.3%, which is in agreement with the values obtained by Haque et al. whose OCT readings indicated 4.9% central corneal swelling immediately after lens removal, which rapidly fell to 0–1% after 3 hours. However, our results differ from those of Alharbi and Swarbrick, who detected no central stromal thickness changes. The data presented here were obtained between 10:00 and 15:00 hours, which could determine greater central stromal thickening induced by overnight edema, particularly in the subjects tested at 10 am. On the other hand, Alharbi’s studies used modified optical pachometry and not CM or OCT; so this might be a more direct measurement of stromal thickness change and might explain some of the differences observed.

Surprisingly, the Bowman’s membrane and sub-basal nerve plexus was reduced in thickness and these changes persisted 30 days after the cessation of OK. Bowman layer thickness has not been examined in OK human studies so we cannot compare results but CM measurements made in normal corneas (16.7 ± 4.4 μm) do not differ from our thickness values. Some of the reduction obtained could be accounted for more by the corneal epithelium than Bowman’s membrane proper. We speculate this finding could represent an image analysis artifact due to epithelial compression, since OK lens wear made it more difficult to differentiate the different corneal layers than when the epithelium had recovered its normal thickness after OK. This issue needs confirming in future studies.

Traditionally, the subbasal nerve plexus has been included in epithelium thickness measurements such that the reduction observed here in subbasal nerve plexus thickness corresponds to part of the reduction in epithelial thickness described by others. This was confirmed by our OCT validation of the CM readings. Hence, OCT-determined epithelial thickness did not differ from the thickness determined by CM as long as the subbasal nerve plexus was included in the measurements. These data need to be confirmed but the contribution of this study is that the reduction in epithelial thickness produced in response to overnight OK occurs at the Bowman’s membrane and at the two zones that form the corneal epithelium: the cell layers and zone of the nerve plexus. Our findings also indicate that while the reduction in thickness of the cell layers is reversible, that of the nerve plexus and Bowman’s membrane persists after treatment.

The central epithelium decreased in thickness by 17% to 32% and this thinning persisted at all points of time until thickening occurred after the interruption of treatment. These values are consistent with the 30% decrease in central epithelial thickness observed by Alharbi and Swarbrick, the 12% reported by Haque et al., or 13% to 25% recorded by ourselves in a similar study sample. According to the suggestions of Lu et al., differences in corneal thicknesses between patients or studies could depend on corneal malleability since our subject groups had similar refractive values to those examined by other authors, indicating that all corneas do not respond in the same way. The different lens designs used in the studies and different measurement techniques may also hinder comparisons.

Our study revealed obvious changes in several corneal structures in overnight OK lens wearers. Several recent studies have addressed the refractive effects of the histological and morphological changes induced by overnight orthokeratology. Most of these reports conclude that the epithelium appears to play a major role in the changes induced by orthokeratology and that this effect is dependent on treatment duration and lens design. Our results are in line with these reports since the subtle persistent physiological changes we were able to detect affected the epithelium, including both thickness and cell changes. However, the changes noted in the thickness of the stroma and particularly the Bowman layer and subbasal nerve plexus indicate that these layers may also influence the refractive changes produced.

In summary, as far as we know this is the first study to prospectively address the long-term physiological changes that occur in human eyes subjected to overnight
OK. Our findings indicate that most of these changes, both morphological and biometric, are reversible. More specifically, the morphological cell changes produced in the epithelial (increased cell areas and decreased cell densities) and stromal layers (increased keratocyte activation) in response to OK recovered after treatment was suspended, while the endothelial polymegathism detected did not. Among the thickness changes observed in the different corneal layers, the non-reversible thinning of the Bowman layer and subbasal nerve plexus warrents further investigation to assess possible repercussions on corneal physiology. The variable nature of the changes reported in the different studies suggests that future studies should be conducted in the same patients over longer time periods.

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Human Subjects and Informed Consent

The authors declare that this research was performed following the tenets of the Declaration of Helsinki and that informed consent was obtained from the subjects after the nature of the study had been explained to them in detail. The study protocol was approved by the Ethics Committee of the Hospital Carlos III.

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