PURPOSE: To examine the effects of 1 year of overnight orthokeratology (OK) treatment on the sub-basal nerve plexus (SBNP) and corneal sensitivity and to assess the reversibility of these effects 1 month after treatment interruption.

METHODS: 32 subjects with low-moderate myopia underwent OK treatment for one year. 15 non-contact lens wearers served as controls. At the time points baseline, 1 year of treatment, and 1 month after removing the OK lenses, two tests were conducted: corneal sensitivity (Cochet-Bonnet esthesiometer) and SBNP imaging by in vivo confocal microscopy.

RESULTS: In participants wearing OK lenses, significant reductions over the year were produced in SBNP nerve density (p=0.001 and p=0.006) and number of nerves (p<0.001 and p=0.001) in the central and mid-peripheral cornea respectively. Differences over the year were also detected in central objective tortuosity (p=0.002). Following lens removal, baseline values of nerve density (p=0.024 and p=0.001) and number of nerves (p=0.021 and p<0.001) for the central and mid-peripheral cornea respectively were not recovered. At 1 month post-treatment, a difference was observed from 1 year values in central corneal sensitivity (p=0.045) and mid-peripheral Langerhans cell density (p=0.033), and from baseline in mid-peripheral objective tortuosity (p=0.049). Direct correlation was detected at 1 year between nerve density and tortuosity both in the central (p<0.01; r= 0.69) and mid-peripheral cornea (p<0.01; r= 0.76).

CONCLUSIONS: Long term OK treatment led to reduced SBNP nerve density and this was directly correlated with corneal tortuosity. After 1 month of treatment interruption, nerve density was still reduced.
Long-term impacts of OK treatment on sub-basal nerve plexus and corneal sensitivity responses and their reversibility

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NUMBER OF FIGURES AND TABLES

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DATE OF SUBMISSION
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None

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KEYWORDS: sub-basal nerve plexus, corneal sensitivity, overnight orthokeratology, density,
tortuosity.
INTRODUCTION

The cornea is the most densely innervated tissue in the human body. Its morphological changes in response to factors such as ageing, disease or surgery have been widely studied ex-vivo\textsuperscript{1-5} and in-vivo\textsuperscript{6-10}. The methods used to detect these changes have been traditionally electron and light microscopy. However, the more recently introduced in vivo confocal microscopy (IVCM) has proved especially useful for this purpose\textsuperscript{11} due to its non-invasive nature. This has resulted in significant progress in the in vivo study of corneal morphological changes, with special emphasis placed on the epithelium and sub-basal nerve plexus (SBNP).

Using this technology, it has been possible to assess these changes in the corneas of healthy individuals\textsuperscript{10}, soft contact lens wearers\textsuperscript{7,8}, subjects undergoing corneal surgery\textsuperscript{12,13}, patients with a systemic disease\textsuperscript{14,15} and those undergoing overnight orthokeratology (OK) treatment\textsuperscript{9,16,17}.

In a clinical trial conducted in 2011 in patients who wore OK lenses daily for a whole year\textsuperscript{9}, Nieto-Bona et al noted structural changes in the cornea, though warned that their data needed confirming in a larger study. More recently, another research group conducted a study\textsuperscript{16} which revealed that wearing OK lenses modifies the normal pattern of SBNP nerve distribution. Additionally, OK lenses have been reported to compromise corneal nerve function\textsuperscript{18}. This latter effect can be assessed through corneal sensitivity testing. Several authors have addressed correlation between corneal architecture and corneal sensitivity\textsuperscript{12-15,19}.

Patel et al\textsuperscript{8} correlated SBNP nerve density, SBNP nerve tortuosity and corneal sensitivity in a large population of non-contact lens wearers. More recently, Lum et al reported a reduction in corneal sensitivity and SBNP nerve density over both short-term\textsuperscript{20} and long-term\textsuperscript{21} periods of OK lens wear. However, correlations between SBNP nerve density, nerve tortuosity and corneal sensitivity have not yet been detected in the healthy corneas of OK users in the long term.

SBNP nerve redistribution has been linked to increased levels of pro-inflammatory molecules\textsuperscript{22}. Consequently, and bearing in mind that inflammation due to the use of contact lenses induces the migration of Langerhans cells (LC) to the cornea\textsuperscript{23,24}, we recently performed a study\textsuperscript{17} in which it was observed that the use of OK lenses over a short period
increases LC density in the cornea. However, little is still known about the course of these changes in the longer term and their possible recovery after interrupting OK lens wear. The present study was designed to objectively and prospectively analyze the changes produced in LC density and SBNP morphology and their correlation with corneal sensitivity in a large population of subjects undergoing OK treatment for 1 year. The reversibility of these responses after discontinuing treatment was also assessed. Based on the recent finding of different short term responses to different OK lenses, two different OK lens designs were fitted in our participants. SBNP thickness changes were also examined. Our working hypothesis was that over a long term period, OK lenses will produce morphological changes in the corneal SBNP layer with repercussions on central and mid-peripheral corneal sensitivity.

**METHODOLOGY**

This was a prospective, longitudinal, single-center study of 13 months’ duration. The study protocol was approved by the Ethics Committee of the Hospital Clinico San Carlos and adhered to the tenets of the Declaration of Helsinki. The follow up times established were baseline, 1 year and 13 months, when corneal morphology and corneal sensitivity were determined in patients undergoing 1 year of OK treatment with two different types of OK lenses. As a control group, non contact lens wearers were also examined at the three time points.

**Sample size calculation**

Based on the mean SBNP nerve densities observed in prior work (n=71, 10687 ± 5046 µm/mm²), we calculated the sample size required for this study using Granmo 7.12 software. For an alpha risk of 0.05 and a beta risk of 0.2 in a 2-tailed test, it was calculated that 28 subjects would be needed in the treatment group and 14 in the control group to detect a significant difference equal or greater than 3200 µm/mm² and a correlation coefficient between the initial and final measurement of 0.8. The dropout rate anticipated was of 15%.

**Subjects**

Participants were subjects from our previous study who were offered the possibility of continuing OK treatment for this stage of the study. Written consent was obtained from participants after the nature and possible consequences of the study had been explained to
them. Subjects contacted the research group via the free access website 
http://www.ucm.es/accion-social.

In a preliminary interview and tests, it was determined whether subjects met the following 
inclusion criteria: age 18 to 30 years old, refractive status -0.50 to -5.00 DS and -0.25 to -1.25 
DC, no systemic or eye disease, no history of eye surgery, and no evidence of keratoconus or 
corneal irregularity. Rigid gas permeable contact lens (CL) wearers were excluded from the 
study. Hydrophilic CL users were instructed to remove their lenses four weeks before starting 
the study. Pregnant women and those planning to become pregnant during the study were 
also excluded.

The subjects enrolled were randomly assigned to 2 groups: a control group comprised of 20 
individuals who wore no contact lenses, and a treatment group comprised of 20 subjects 
(subgroup CRT) who wore HDS 100 Paragon CRT lenses (Paragon Vision Sciences; 
Interlenco, Madrid, Spain) and 20 subjects (subgroup SF) who wore Seefree® lenses made 
of Boston XO2 material (Conóptica, Barcelona, Spain) in both eyes. After enrolment, 13 
participants withdrew from the study: three in the CRT subgroup, two in the SF subgroup and 
three in the control group because they did not keep all the follow-up appointments and three 
in the SF subgroup and two in the control group because they moved away from Madrid. This 
left a study population of 47 subjects: 15 in the control group and 32 in the treatment group 
(17 in CRT and 15 in SF).

Tests

At each follow-up time (baseline, 1 year, 13 months), corneal sensitivity was measured using 
a Cochet-Bonnet esthesiometer (Luneau, Paris, France) and corneal morphology was 
examined by IVCM using a corneal module (Heidelberg Retina Tomograph II [HRT], Rostock 
Corneal Module [RCM]; Heidelberg Engineering GmbH, Heidelberg, Germany). Image 
acquisition and image analysis of the SBNP was conducted followed the procedure outlined 
in a previous study\textsuperscript{17}. This involved a masked-fashion analysis of the best-focused image of 
each set of images with three different software packages: ImageJ (http: www. 
rsb.info.nih.gov/ij), NeuronJ (http: imagescience.org/meijering/software/neuronj/) and Matlab 
(MatLab; The Mathworks, Natick, MA). Each package was used for different SBNP variables: 
ImageJ for SBNP thickness, global and local reflectivity, width, orientation, length and density
of nerves as well as the number of Langerhans cells; NeuronJ for nerve reflectivity and
number of nerves; and Matlab for nerve tortuosity. All tests were conducted in the right eye
by a single experienced clinician within 2 to 4 h of OK lens removal. Besides the three
planned visits, patients were required to attend all the standard visits for OK lens fitting and
fluorescein staining to assess ocular surface health at each visit. These clinical procedures
have been formerly described elsewhere.

**Statistical analysis**

Data were recorded in Excel (2003, Microsoft) and analyzed using SigmaPlot version 12
(Systat Software Inc.). Statistical analysis was performed on the data obtained for the right
eye of each subject. We checked the distribution of data using the Kolmogorov-Smirnov
normality test. To analyze changes over time in each group, we used one-way repeated
measures analysis of variance (ANOVA) and the post-hoc paired t-test according to the
distribution of data with Bonferroni correction for multiple comparisons. For variables that
changed significantly over time, we searched for correlations among them through simple
regression analyses and also compared the two OK treatment subgroups for these variables
by 2-way analysis of variance (group x time) and conducted post-hoc paired tests with
Bonferroni correction. All data are reported as the mean ± standard deviation (SD).
Significance was set at p<0.05.

**RESULTS**

The final study sample was made up of 47 subjects, 25 male and 22 female, of mean age ±
SD 25.1 ± 3.7 years. The treatment group (subgroups CRT and SF) included 32 healthy right
eyes. None of the OK lens wearers who completed the treatment protocol reported adverse
effects related to lens wear, and no eye abnormalities were detected on slit-lamp microscopy.
As may be seen in Table 1, the treatment and control groups were well matched in terms of
subject numbers, age and refractive and keratometry measurements.
Baseline values for SBNP variables measured in the central cornea in the control group are
shown in Table 4. As may be seen, none of these variables showed a significant difference
over time. Mid-peripheral data could not be obtained in the control group since best-focused
SBNP images were insufficiently clear. Thus, only corneal sensitivity (4.98 ± 0.52 cm) and
SBNP thickness data (11.41 ± 1.27 μm) were available for the mid-peripheral cornea in the
control group. Data for the control group at the 1-month post-treatment visit are not shown in
the tables because almost 75% of the subjects did not attend this visit such that we had
insufficient data for this time point.
Post-hoc comparisons of means indicated no differences over time in neither the central nor
mid-peripheral cornea for almost all variables as $p > 0.05$ for each pair of data (baseline vs 1
year). The central and mid-peripheral corneal variables recorded in the treatment group are
provided in Tables 2 and 3 respectively. Statistical analysis revealed almost no differences
over time for many of the variables considered in the treatment group. However, some
significant changes over the study period were produced in the factors central and mid-
peripheral number of nerves, nerve density and objective tortuosity, central corneal sensitivity
and mid-peripheral number of Langerhans cells. Number of nerves fell significantly in the
central ($p<0.001$) and mid-peripheral cornea ($p=0.001$) from baseline to 1 year and thereafter
remained stable after 1 month of lens removal ($p=0.18$ central cornea, $p=0.11$ mid-peripheral
cornea). Nerve density also fell over the year in the central ($p=0.001$) and mid-peripheral
($p=0.006$) cornea and remained stable after lens removal ($p=0.51$ central cornea, $p=0.43$ mid-
peripheral cornea). Central objective tortuosity also fell significantly over the year ($p=0.002$)
while mid-peripheral objective tortuosity was reduced 1 month after lens removal ($p=0.049$).
Central corneal sensitivity improved significantly from 1 year to 1 month post treatment
($p=0.045$) and number of Langerhans cells in the mid-peripheral cornea decreased between
these two time points ($p=0.033$).
No correlation was detected between nerve density and corneal sensitivity ($p= 0.24; r= -0.24$)
in the central cornea and between nerve density and LC density ($p= 0.34; r =0.19$) in the mid-
peripheral cornea. However, we did find direct correlation between nerve density and
tortuosity both in the central ($p<0.01; r= 0.69$) and mid-peripheral ($p<0.01; r= 0.76$) cornea
after 1 year of treatment.
Graphs were prepared to compare the trends produced in the variables varying significantly
over time in the CRT and SF subgroups. These variables showed similar trends over time
(Fig 1a-1f). By 2-way ANOVA, we calculated the statistical power of any differences detected
between the two OK treatment subgroups. With the exception of central corneal sensitivity,
power values revealed similar trends in the variables measured over the year: central corneal
sensitivity $\beta=0.27$, central nerve density $\beta=0.17$, central objective tortuosity $\beta=0.04$, mid-
219 peripheral Langerhans cell density $\beta=0.05$, mid-peripheral nerve density $\beta=0.12$ and mid-
220 peripheral objective tortuosity $\beta=0.10$.

**DISCUSSION**

This study was designed to examine the SBNP in detail and to assess the changes produced
in several of its parameters during the prolonged use of OK lenses and after one month of
their discontinued use. Baseline data for SBNP thickness and the remaining variables
examined were consistent with the ranges reported for normal corneas$^{7-9,19}$. All the nerve
morphology variables that showed some variation over the year of treatment returned to
baseline after treatment was interrupted with the exception of central and mid-peripheral
SBNP nerve density and number of nerves.

Corneal sensitivity values were within expected normality values in each study visit. However,
they were slightly lower than those reported for hydrophilic CL wearers$^{19}$ and normal
corneas$^{18,26}$. No control-treatment group differences were observed at baseline. Surprisingly,
our findings showed stable corneal sensitivity values from baseline to 1 year of OK treatment.
This is in conflict with the reduction in corneal sensitivity observed after 1 month$^{17}$ and 3
months$^{20}$ of OK lens wear. A possible explanation for the stabilization of the corneal
sensitivity after 1 year of OK treatment could be neural adaptation to the pressure exerted by
the lens described by Draeger$^{27}$. We propose that this neural adaptation could have occurred
somehow between three months and one year of treatment. Further study would be needed
to confirm this theory. In line with this idea, other authors$^{20}$ have noted the recovery of corneal
sensitivity 3 months after the removal of OK lenses. This finding is consistent with our
observations, given the significant increase in central corneal sensitivity observed between 1
year of treatment and 1 month post-treatment ($p=0.045$). In the CRT subgroup we observed a
similar decreasing trend in this variable followed by an increasing trend one month after lens
removal. In contrast, no mid-peripheral changes were observed. This finding is in line with
previous results in OK wearers$^{20,28}$ in studies in which it was speculated that changes in
corneal sensitivity would be limited to the central cornea while the peripheral cornea would be
unaffected.
The variables SBNP nerve density and number of nerves are closely linked. As expected both
experienced changes during the course of OK treatment. Our nerve density values are lower
than those reported for subjects with healthy corneas\(^8\) and OK lens wearers\(^{21}\). This difference
may be attributed to the fact that we only considered the main nerves detected in the best-
focused image while others have included all nerve branches. It should also be considered
that the present study participants were healthy subjects tested after 1 year of OK lens wear.
Recently Lum et al\(^{21}\) noted a reduction in nerve fiber density in response to 3 months of OK;
with respect to controls, this reduction was of around 31%. In 2010, Nieto-Bona observed
following 3 months of OK lens wear changes including effects on the SBNP\(^{29}\). In the present
study, a 42% reduction in nerve density was produced from baseline to 1 year post OK
treatment. In the central cornea, both nerve density and nerve fiber numbers significantly fell
over the year (\(p=0.001\) and \(p<0.001\) respectively), consistent with the central nerve loss
reported by Lum et al\(^{16,21}\). Nieto-Bona et al\(^8\) also noted a decreasing trend in nerve density
using the same procedure to assess this variable, though we also used a semi-automated
tool (NeuronJ) for this purpose. In our study, one month after discontinuing the use of OK
lenses, nerve density values were still diminished compared to baseline (\(p=0.024\) and
\(p=0.021\) respectively). In line with this result and given previous descriptions of the
redistribution of nerve fibers towards the periphery\(^{16}\), the decrease in nerve density (\(p=0.006\))
and nerve number (\(p=0.001\)) detected here in the corneal periphery after one year of
treatment is surprising. A possible explanation is that we took these measurements in a more
peripheral zone possibly explaining the discrepancy with other observations\(^{16}\). In effect, our
target location for nerve measurements was 4 mm infero-temporal and thus it is possible that
our mid-peripheral data are representative of the corneal region beneath the landing zone
rather than the optic or return zone area of the OK lens. This could possibly explain the
reduced nerve density observed mid-peripherally following treatment. The fact that both
variables did not return to baseline values (\(p=0.001\) and \(p<0.001\) respectively) is consistent
with the non-significant trend reported by Nieto-Bona\(^{29}\). Our comparison of nerve density
between the two treatment subgroups revealed the similar behavior of this factor at both
locations (Fig.1b and Fig.1e). In addition, the fact that nerve density did not increase in the
mid-periphery (Fig.1e), as expected according to prior findings\(^{16}\), could be caused by the
proximity of the measurement zone to the return zone. In future work, it would be interesting to assess a more peripheral zone of the cornea at a later time point following OK lens discontinuation.

To date, it has been established that nerve tortuosity increases with the severity of a neuropathy and with age. Notwithstanding, in the present study, objective tortuosity was assessed in healthy young OK wearers. Despite baseline values being in agreement with reported values, after one year of study, a significant reduction in objective tortuosity was produced (p=0.002). In the mid-peripheral cornea, reduced tortuosity was observed in the 1 month post treatment visit (p=0.049). This finding is consistent with the reduced nerve density observed at this recovery time point. Further, our linear regression values indicate direct correlation between nerve density and tortuosity in both the central (p<0.01; r= 0.69) and peripheral (p<0.01; r= 0.76) cornea. This correlation has been addressed by others in healthy corneas, and weak correlation was observed in the inferior and nasal cornea regions at a single time point. Lum et al, in contrast, observed a tortuous network of less dense nerve fibers, though these authors examined only two long term OK lens wearers and assessed a much larger area than in our study.

The immunological functions of dendritic cells and their role in corneal inflammation and dry eye have been well defined. Accordingly, it may be assumed that the cornea will respond to contact lenses as it would to a foreign body by increasing the density of Langerhans cells. This response is triggered by the mechanical irritation and hypoxia associated with lens wear. We observed a clear trend for LC density to increase in the mid-peripheral cornea following one year of OK lens wear. Notwithstanding, the only significant change noted was the decrease of this variable (p=0.033), a behavior that has not been detected in prior work.

In conclusion, our findings indicate a significant reduction in the nerve density of the sub-basal plexus in response to one year of OK treatment that was not associated with changes in corneal sensitivity but was linked to changes in nerve tortuosity both in the central and peripheral cornea. This effect was not reversed one month after treatment interruption neither in the central nor mid-peripheral cornea, suggesting a longer period of non OK lens wear would be needed to recover baseline values. In contrast, in this same 1 month period, corneal sensitivity was higher after lens removal. This determines that 1 month after OK lens
discontinuation, there is a reduction in nerve density 4 mm from the corneal apex that do not correlate with our corneal sensitivity findings. According to our 2-way ANOVA and post-hoc statistical power calculations, the two OK lenses used showed similar effects on SBNP morphology. Although the corneal zone observed by IVCM was smaller than that examined by others\cite{8,16}, our large sample size provides sufficient evidence of these changes. In future studies, these findings should be confirmed by examining a larger area over a longer recovery period.

ACKNOWLEDGMENTS

The authors acknowledge the laboratories Interlenco (Madrid, Spain) and Conóptica (Barcelona, Spain) for their support.

REFERENCES


Table 1. Refractive and corneal variables recorded at baseline prior to OK treatment. n number of subjects, m mean, SD standard deviation, Sim Kflat and Sim Ksteep simulated keratometry readings along the flatter and steeper meridians.

Table 2. Variables recorded at baseline prior to OK treatment and changes produced in the central cornea after 1 year of continuous OK treatment and 1 month after removing lenses. n number of right eyes of subjects/number of items, m mean, SD standard deviation, cm centimeters, _m microns, mm2 squared millimeters, ND nerve density, LCD Langerhans cells density, STC subjective tortuosity coefficient, OTC objective tortuosity coefficient. (†) indicates significant differences between values at baseline and 1 year at p<0.01; (*) indicates significant differences between values at baseline and 1 month post-treatment at p<0.05. (_) indicates significant differences between values at 1 year of treatment and at 1 month post-treatment at p<0.05.

Table 3. Variables recorded at baseline prior to OK treatment and changes produced in the mid-peripheral cornea after 1 year of continuous OK treatment and 1 month after removing lenses. n number of right eyes of subjects/number of items, m mean, SD standard deviation, cm centimeters, _m microns, mm2 squared millimeters, ND nerve density, LCD Langerhans cells density, STC subjective tortuosity coefficient, OTC objective tortuosity coefficient. (†) indicates significant differences between values at baseline and 1 year, p<0.01; (*) indicates significant differences between values at baseline and 1 month post-treatment at p<0.05; (‡) indicates significant differences between values at baseline and 1 month post-treatment at p<0.01, (_) indicates significant differences between values at 1 year of treatment and at 1 month post-treatment at p<0.05.

Table 4. Parameter values for the control group and changes produced over 1 year. Positive values shown in the ∆ column correspond to a rise in the parameter value. n number of subjects/items, m mean, SD standard deviation, _ difference between values at baseline and in the 1 year visit, cm centimeters, _m microns, mm2 squared millimeters.

Figure 1. Two-way ANOVA (subgroup x time). Graphs a to f display the means (shown on y-axis) of the variables quantified objectively in the subgroups CRT and SF (0 = baseline, 1 = after 1 year of OK treatment and 2 = 1 month post-treatment) in the central (a-c) and mid-peripheral (d-f) cornea. pv is the value of p for the variable time point; pg is the value of p for the variable subgroup; pvxg is the value of p for the product of the variables time point and subgroup.
<table>
<thead>
<tr>
<th>Male/Female (n)</th>
<th>Control group (n=15)</th>
<th>Treatment group (n=32)</th>
<th>Unpaired t-test</th>
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<tbody>
<tr>
<td></td>
<td>m ± SD</td>
<td>m ± SD</td>
<td></td>
</tr>
<tr>
<td>9/6</td>
<td>25.6 ± 3.3</td>
<td>24.86 ± 3.92</td>
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<td>16/16</td>
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<td>43.03 ± 1.95</td>
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<td>43.63 ± 1.76</td>
<td>44.02 ± 1.49</td>
<td>p=0.43</td>
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</table>

Table 1. Refractive and corneal variables recorded at baseline prior to OK treatment. n number of subjects, m mean, SD standard deviation, Sim Kflat and Sim Ksteep simulated keratometry readings along the flatter and steeper meridians.
<table>
<thead>
<tr>
<th>TABLE 2 CENTRAL (n = 32)</th>
<th>BASELINE (m ± SD)</th>
<th>TREATMENT 1 YEAR (m ± SD)</th>
<th>1 MONTH POST-TREATMENT (m ± SD)</th>
<th>ANOVA p-value</th>
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<tr>
<td>Corneal sensitivity (cm)</td>
<td>5.28 ± 0.66</td>
<td>5.27 ± 0.54</td>
<td>5.67 ± 0.29†</td>
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<td>SBNP thickness (µm)</td>
<td>10.05 ± 1.56</td>
<td>10.25 ± 2.98</td>
<td>11.08 ± 2.67</td>
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<td>Global reflectivity</td>
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<td>0.28 ± 0.04</td>
<td>0.29 ± 0.04</td>
<td>0.87</td>
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<td>Local reflectivity</td>
<td>0.32 ± 0.05</td>
<td>0.31 ± 0.06</td>
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<td>0.73</td>
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<td>Nerve reflectivity</td>
<td>0.75 ± 0.13</td>
<td>0.72 ± 0.12</td>
<td>0.77 ± 0.11</td>
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<td>Length (µm)</td>
<td>325.00 ± 87.12</td>
<td>308.54 ± 100.01</td>
<td>316.93 ± 84.59</td>
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<td>Width (µm)</td>
<td>3.64 ± 0.49</td>
<td>3.51 ± 0.77</td>
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<td>6442.60 ± 3713.64†</td>
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<tr>
<td>LCD (µm/mm²)</td>
<td>61.60 ± 36.32</td>
<td>64.48 ± 42.88</td>
<td>57.44 ± 33.76</td>
<td>0.24</td>
</tr>
<tr>
<td>STC</td>
<td>1.44 ± 0.75</td>
<td>1.75 ± 0.65</td>
<td>1.59 ± 0.78</td>
<td>0.35</td>
</tr>
<tr>
<td>OTC</td>
<td>19.74 ± 9.90</td>
<td>14.27 ± 6.66†</td>
<td>15.62 ± 9.23</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Table 2. Variables recorded at baseline prior to OK treatment and changes produced in the central cornea after 1 year of continuous OK treatment and 1 month after removing lenses. n number of right eyes of subjects/ number of items, m mean, SD standard deviation, cm centimeters, µm microns, mm² squared millimeters, ND nerve density, LCD Langerhans cells density, STC subjective tortuosity coefficient, OTC objective tortuosity coefficient. (†) indicates significant differences between values at baseline and 1 year at p<0.01; (*) indicates significant differences between baseline and 1 month post-treatment at p<0.05; (φ) indicates significant differences between 1 year and 1 month post-treatment at p<0.05.
### Table 3

**MID-PERIPHERY**  
*(n = 32)*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (m ± SD)</th>
<th>Treatment 1 Year (m ± SD)</th>
<th>1 Month Post-Treatment (m ± SD)</th>
<th>ANOVA p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corneal sensitivity (cm)</td>
<td>5.23 ± 0.66</td>
<td>5.36 ± 0.64</td>
<td>5.58 ± 0.36</td>
<td>0.07</td>
</tr>
<tr>
<td>SBNP thickness (μm)</td>
<td>11.76 ± 4.12</td>
<td>9.83 ± 2.05</td>
<td>10.24 ± 2.51</td>
<td>0.07</td>
</tr>
<tr>
<td>Global reflectivity</td>
<td>0.28 ± 0.03</td>
<td>0.29 ± 0.04</td>
<td>0.29 ± 0.03</td>
<td>0.71</td>
</tr>
<tr>
<td>Local reflectivity</td>
<td>0.31 ± 0.04</td>
<td>0.31 ± 0.05</td>
<td>0.32 ± 0.05</td>
<td>0.86</td>
</tr>
<tr>
<td>Nerve reflectivity</td>
<td>0.75 ± 0.11</td>
<td>0.73 ± 0.11</td>
<td>0.72 ± 0.13</td>
<td>0.32</td>
</tr>
<tr>
<td>Length (μm)</td>
<td>332.38 ± 93.93</td>
<td>327.51 ± 97.17</td>
<td>276.97 ± 92.78</td>
<td>0.40</td>
</tr>
<tr>
<td>Width (μm)</td>
<td>3.60 ± 0.42</td>
<td>3.48 ± 0.60</td>
<td>3.61 ± 0.63</td>
<td>0.68</td>
</tr>
<tr>
<td>Orientation (degrees)</td>
<td>95.32 ± 29.72</td>
<td>107.89 ± 42.95</td>
<td>102.02 ± 44.38</td>
<td>0.29</td>
</tr>
<tr>
<td>Nerves (n)</td>
<td>4.66 ± 1.84</td>
<td>2.93 ± 1.59†</td>
<td>3.00 ± 1.59†</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ND (μm/mm²)</td>
<td>11056.25 ± 5918.35</td>
<td>6257.53 ± 4213.78†</td>
<td>6547.09 ± 4423.58†</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LCD (μm/mm²)</td>
<td>55.68 ± 36.16</td>
<td>76.32 ± 47.84</td>
<td>52.32 ± 41.76*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>STC</td>
<td>1.38 ± 0.62</td>
<td>1.79 ± 0.96</td>
<td>1.53 ± 0.57</td>
<td>0.09</td>
</tr>
<tr>
<td>OTC</td>
<td>20.21 ± 10.47</td>
<td>15.87 ± 8.94</td>
<td>15.26 ± 9.74*</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 3. Variables recorded at baseline prior to OK treatment and changes produced in the mid-peripheral cornea after 1 year of continuous OK treatment and 1 month after removing lenses. n number of right eyes of subjects/number of items, m mean, SD standard deviation, cm centimeters, μm microns, mm² squared millimeters, ND nerve density, LCD Langerhans cells density, STC subjective tortuosity coefficient, OTC objective tortuosity coefficient. (†) indicates significant differences between values at baseline and 1 year, p<0.01; (*) indicates significant differences between baseline and 1 month post-treatment at p<0.05; (‡) indicates significant differences between baseline and 1 month post-treatment at p<0.01, (φ) indicates significant differences between 1 year and 1 month post-treatment at p<0.05.
<table>
<thead>
<tr>
<th>TABLE 4 CONTROL GROUP (n = 15)</th>
<th>BASELINE (m ± SD)</th>
<th>∆ (1 YEAR) (m ± SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corneal sensitivity (cm)</td>
<td>4.90 ± 0.40</td>
<td>0.20 ± 0.27</td>
<td>0.18</td>
</tr>
<tr>
<td>SBNP thickness (μm)</td>
<td>9.20 ± 1.79</td>
<td>-3.00 ± 3.05</td>
<td>0.09</td>
</tr>
<tr>
<td>Global reflectivity</td>
<td>0.31 ± 0.02</td>
<td>0.01 ± 0.02</td>
<td>0.39</td>
</tr>
<tr>
<td>Local reflectivity</td>
<td>0.30 ± 0.03</td>
<td>-0.01 ± 0.03</td>
<td>0.61</td>
</tr>
<tr>
<td>Nerve reflectivity</td>
<td>0.69 ± 0.15</td>
<td>-0.03 ± 0.01</td>
<td>0.65</td>
</tr>
<tr>
<td>Length (μm)</td>
<td>347.80 ± 60.66</td>
<td>68.00 ± 116.81</td>
<td>0.42</td>
</tr>
<tr>
<td>Width (μm)</td>
<td>3.75 ± 0.28</td>
<td>-0.06 ± 0.21</td>
<td>0.55</td>
</tr>
<tr>
<td>Orientation (degrees)</td>
<td>87.85 ± 30.83</td>
<td>-0.80 ± 4.44</td>
<td>0.71</td>
</tr>
<tr>
<td>Nerves (n)</td>
<td>3.40 ± 0.64</td>
<td>-0.40 ± 1.52</td>
<td>0.59</td>
</tr>
<tr>
<td>ND (μm/mm²)</td>
<td>10778.80 ± 3593.14</td>
<td>458.20 ± 1327.98</td>
<td>0.48</td>
</tr>
<tr>
<td>LCD (μm/mm²)</td>
<td>19.60 ± 21.44</td>
<td>0.00 ± 0.71</td>
<td>0.91</td>
</tr>
<tr>
<td>STC</td>
<td>2.40 ± 0.55</td>
<td>-0.20 ± 0.45</td>
<td>0.37</td>
</tr>
<tr>
<td>OTC</td>
<td>17.24 ± 5.97</td>
<td>-2.60 ± 6.93</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Table 4. Parameter values for the control group and changes produced over 1 year. Positive values shown in the ∆ column correspond to a rise in the parameter value. n number of subjects/items, m mean, SD standard deviation, ∆ difference between baseline and visit at 1 year, cm centimeters, μm microns, mm² squared millimeters.
Central corneal sensitivity (cm)

Time point

- \( p_v = 0.047 \)
- \( p_g < 0.001 \)
- \( p_{vxg} = 0.646 \)
Central nerve density (μ/mm²)

Time point

0 1 2

p_v = 0.002
p_g < 0.001
p_vxg = 0.811
re-reviewed figure 1c

c

<table>
<thead>
<tr>
<th>Time point</th>
<th>Central Objective TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
</tr>
</tbody>
</table>

$p_v = 0.040$
$p_g = 0.001$
$p_{vxg} = 0.525$
Mid-peripheral LC density (μ/mm²)

Time point

p_v = 0.107
p_g = 0.103
p_vxg = 0.934
Mid-peripheral nerve density (µ/mm²)

<table>
<thead>
<tr>
<th>Time point</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18000</td>
</tr>
<tr>
<td>1</td>
<td>12000</td>
</tr>
<tr>
<td>2</td>
<td>9000</td>
</tr>
</tbody>
</table>

$p_v < 0.001$
$p_g = 0.779$
$p_{vxg} = 0.944$
Mid-Peripheral Objective TC

Time point

$p_v = 0.183$
$p_g = 0.873$
$p_{vxg} = 0.899$
re-reviewed figure 1g legend

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