Confocal Microscopy In Vivo in Corneas of Long-Term Contact Lens Wearers

Sanjay V. Patel,1 Jay W. McLaren,1 David O. Hodge,2 and William M. Bourne1

PURPOSE. To compare keratocyte density, stromal backscatter, epithelial thickness, and corneal sensitivity between corneas of long-term contact lens wearers and those of non-contact lens wearers.

METHODS. Twenty corneas of 20 daily contact lens wearers (>10 years' duration) and 20 corneas of 20 age-matched (±5 years) control subjects who had never worn contact lenses, were examined by confocal microscopy in vivo. The contact lens wearers removed their lenses 12 to 24 hours before the examination. Full-thickness images were recorded from the central and temporal cornea, and bright objects (keratocyte nuclei) in images were manually counted to calculate keratocyte density. Stromal intensity (backscatter) was measured by calculating the mean grayscale value (corrected for camera and light source variations) from the center of stromal images. Epithelial thickness was determined from the distance between images of the surface epithelium and subbasal nerve plexus. Central corneal sensitivity was measured by Cochet-Bonnet esthesiometry and correlated with the number of nerve fiber bundles in the subbasal nerve plexus.

RESULTS. Full-thickness central and temporal keratocyte densities in contact lens wearers were 22,122 ± 2,676 cells/mm3 (mean ± SD) and 20,731 ± 2,627 cells/mm3, respectively, and were not significantly different from central and temporal keratocyte densities in control subjects (P = 0.29). The minimum detectable difference in cell density was 11% (2346 cells/mm3 and 2235 cells/mm3 in central and temporal stroma, respectively). Temporal epithelial thickness was 46.3 ± 4.7 μm in contact lens wearers and 50.9 ± 4.7 μm in control subjects (P = 0.02). Central epithelial thickness and stromal backscatter did not differ between contact lens wearers and control subjects (P > 0.05). Corneal sensitivity was lower in contact lens wearers than it was in control subjects (P = 0.05) and did not correlate with the number of nerve fiber bundles in the subbasal nerve plexus.

CONCLUSIONS. Long-term daily contact lens wear and its associated stromal hypoxia and acidosis have no demonstrable effect on keratocyte density. The temporal epithelium is thinner in corneas of long-term contact lens wearers than in control subjects. Decreased corneal sensitivity in contact lens wearers is not accompanied by decreased nerve fiber bundle density.

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The effects of long-term contact lens wear on the corneal epithelium, stroma, and endothelium have been studied extensively. However, specific changes in keratocytes after long-term contact lens wear have received little attention. Investigators have shown that keratocytes degenerate and may die after acute (24 hour) polymethylmethacrylate (PMMA) contact lens wear in primates, but chronic changes in human corneas after long-term contact lens wear have been associated with only a 2% decrease in stromal thickness. Contact lens wear causes both stromal acidosis and hypoxia, and the effect of these chronic conditions on keratocytes is largely unknown. Because keratocytes maintain the structure of the corneal stroma, the stromal thinning may originate from changes in keratocyte function or density, if these changes are associated with acidosis or hypoxia.

The availability of clinical confocal microscopy provides an opportunity to study how the cornea may change after long-term contact lens wear. Methods for measuring keratocyte density by using confocal microscopy in human and rabbit corneas in vivo have been described and validated by comparing keratocyte density measured by confocal microscopy with density estimated from histologic sections of the same tissues. Clinical confocal microscopy was repeatable and acceptable to both subject and observer. We recently demonstrated, with this technique, a gradual decrease in keratocyte density with age in normal human corneas, confirming earlier work.

Confocal microscopy also enables investigators to study structures other than keratocyte nuclei. For example, the morphology and number of nerve fiber bundles in the subbasal plexus can easily be determined from confocal images. Corneal sensitivity is known to decrease after both short- and long-term contact lens wear, and although this loss has been related to corneal hypoxia, it is not known whether it is accompanied by morphologic changes in nerves.

Changes in the amount of light scattered by the cornea can also be measured from the intensity of the confocal image, because the image is formed from scattered light. The effect of age on intensity of backward scatter has been determined in corneas of non-contact lens wearers by other methods, but differences in backward scatter have not been compared between corneas of contact lens wearers and those of non-contact lens wearers.

In this study, we used confocal microscopy to examine several properties of the cornea in long-term contact lens wearers. We compared keratocyte density, epithelial thickness, the appearance of nerve fibers, and the intensity of backscattered light (mean intensity of images) in subjects who had worn contact lenses for at least 10 years, with the same variables in a group of age-matched subjects who had never worn contact lenses. We also compared corneal sensitivity (to light touch) in the two groups and attempted to relate sensitivity to the number of nerve fibers in the subbasal plexus.

METHODS

Subjects

We recruited 20 subjects (10 men, 10 women) from patients attending Mayo Clinic and from staff and their families who had worn any type...
Japan). In all cases, contact lenses were not worn on the night before specular microscope (Noncon ROBO SP-8000; Konan, Inc., Hyogo, Japan). The endothelium was examined and photographed by using a noncontact pachometry (Pachette; DGH Technology Inc., Exton, PA). The corneal epithelium to posterior to the endothelium (continuous through-focus imaging). Each image represented a coronal section approximately 475 μm thick. A series of confocal images (constituting one scan) was recorded as the optical section was advanced from anterior to the posterior movement of the focal plane. When this lens scans linearly, the focal plane advances as a cubic polynomial from the surface of the objective, according to specifications given by the manufacturer. Distance from the objective surface to an object in the cornea can be calculated from the number of video frames between the frame that contains an image of the objective surface and the frame that contains the object. However, in 51% of our scans, an image of the objective surface was not recorded. In these scans we calculated distances relative to the first image of the scan instead of the objective surface. This could have resulted in a maximum uncertainty of 1% in our estimate of thickness.

**Corneal Intensity, or Backscatter**

The light source of the confocal microscope was allowed to stabilize for at least 15 minutes before acquiring images. We standardized our instrument by adjusting intensities to intensity of a fluorescent glass lens that we scanned before scanning each subject. The intensity profile of the glass lens consisted of a surface reflection peak, followed by a progressive decline in intensity with increasing stromal depth. We

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (PMMA)</th>
<th>Rigid Gas-Permeable</th>
<th>Daily-Wear Soft</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53</td>
<td>10</td>
<td>25</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>25</td>
<td>5</td>
<td>—</td>
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<tr>
<td>3</td>
<td>40</td>
<td>18</td>
<td>8</td>
<td>—</td>
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<tr>
<td>4</td>
<td>40</td>
<td>—</td>
<td>9</td>
<td>13*</td>
</tr>
<tr>
<td>5</td>
<td>56</td>
<td>17</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>8</td>
<td>12</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are wearing time in years.

* Soft lenses worn 5 years before and 8 years after rigid gas-permeable lenses.
calculated the mean intensity of five depths at 120-μm intervals from the surface reflection peak, and used this as the intensity standard value (I_s) for that subject. Corneal intensities were multiplied by a factor C to adjust for variation in the light source and sensitivity of the camera with time. The correction factor C was defined as

\[ C = \frac{I_r}{I_s} \]  

(1)

where I_s was the intensity standard value of a reference session and was the same in every subject.

We measured intensity from images acquired with the camera operating in the fixed-gain mode. We defined stromal intensity as the mean intensity of images between the most anterior keratocytes and the last image of the stroma. The maximum intensity associated with the endothelium served as our measure of scatter by the endothelium.

### Contrast between Cells and Background

In the central region of each frame that was used to estimate cell density, the mean intensity of bright objects and the background (area excluding bright objects) was determined by using an image-processing algorithm. Contrast was expressed as a percentage of the background intensity

\[ \text{Contrast} = 100 \times \frac{I_r - I_b}{I_b} \]  

(2)

where I_r and I_b are the mean intensities of the bright objects (cells) and background, respectively. Contrast was calculated from the same area of the frame that was used to determine cell density.

### Appearance of Nerve Fiber Bundles

We reviewed all through-focus confocal scans (fixed-gain and automatic-gain modes) from each subject and noted the appearance of the subbasal nerve plexus. We recorded the number of long nerve fiber bundles in the clearest image of subbasal nerves from the scan with the greatest number of visible nerve fiber bundles.

### Endothelial Morphologic Analysis

Apices of 100 central corneal endothelial cells were traced on digital images acquired by specular microscopy. The mean, SD, coefficient of variation (SD/mean), skewness of cell area, and percentage of hexagonal cells were determined by use of a commercial algorithm (Bambic system; Bio-Optics, Arlington, MA). The endothelial cell density (cells per square millimeter) was expressed as the reciprocal of the mean cell area.

### Data Analysis

We measured keratocyte density in the most anterior countable image (most anterior image of stroma with minimal motion blur), and in five anteroposterior layers through the depth of the stroma. We also calculated the number of keratocytes in a full-thickness column of stroma that had a cross-sectional area of 1 mm², as described previously. The mean density in the full-thickness stroma was estimated by dividing this number by stromal thickness. Differences between keratocyte density and number of keratocytes in contact lens wearers and control subjects were investigated by using the repeated-measures analysis of variance. Differences in keratocyte density between central and temporal locations and through the depth of the stroma were investigated by using a three-factor repeated-measures analysis with repeated-measures analysis of variance with repeated-measures on two factors (location and depth). Significant differences between locations (central and temporal) were investigated by using paired Student’s t-tests, adjusted for five comparisons (five layers) by using the Bonferroni technique. For differences between full-thickness keratocyte density, keratocyte density in the most anterior countable image, and number of keratocytes, we used a two-factor repeated-measures analysis of variance with repeated-measures on one factor (location).

We assessed differences in the thicknesses of the cornea, epithelium, Bowman layer, and stroma; differences in the intensity in the stroma and endothelium; and differences in contrast between cells and background between contact lens wearers and control subjects for the central and temporal cornea. We compared the same parameters between the central and temporal cornea for each group. Corneal thickness measured by ultrasonic pachometry was compared to corneal thickness measured from confocal microscopy recorded in fixed-gain mode. Correlations between keratocyte density and number and stromal intensity were assessed.

Differences in keratocyte density, epithelial thickness, and stromal intensity were also assessed between individual contact lens groups (soft lens, n = 11; rigid lens, n = 2; mixed lenses, n = 6) and control subjects. No comparisons were made between the one PMMA contact lens wearer and the control group. Minimum detectable differences were calculated for nonsignificant results (unpaired analyses, α = 0.05 or adjusted for multiple comparisons for keratocyte density, β = 0.80).

We compared central corneal sensitivity and the number of nerve fiber bundles in the central subbasal nerve plexus in contact lens wearers with the same variables in control subjects, and assessed correlations between these parameters. We also assessed the correlations between central corneal sensitivity and central epithelial thick-
TABLE 4.

<table>
<thead>
<tr>
<th>Stromal Depth (%) Thickness</th>
<th>Contact Lens Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference*</td>
<td>( P^\dagger )</td>
</tr>
<tr>
<td>Full-thickness</td>
<td>1,392 ± 2,525</td>
<td>0.02</td>
</tr>
<tr>
<td>Most anterior countable image†</td>
<td>3,770 ± 9,056</td>
<td>0.08</td>
</tr>
<tr>
<td>0%-10% (anterior)</td>
<td>1,669 ± 6,731</td>
<td>1.0</td>
</tr>
<tr>
<td>11%-33%</td>
<td>14 ± 5,272</td>
<td>1.0</td>
</tr>
<tr>
<td>34%-66%</td>
<td>360 ± 3,401</td>
<td>1.0</td>
</tr>
<tr>
<td>67%-90%</td>
<td>2,417 ± 3,210</td>
<td>0.02</td>
</tr>
<tr>
<td>91%-100% (posterior)</td>
<td>6,028 ± 3,887</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Differences (mean ± SD, cells/mm\(^3\)) between central and temporal keratocyte densities (central − temporal). \( n = 20 \) in both groups.
† Paired Student’s \( t \)-tests (\( n = 20 \)). Probabilities in layers were Bonferroni-adjusted for five comparisons.
‡ Minimum detectable differences (cells/mm\(^3\)) between central and temporal keratocyte densities (paired analyses, \( a = 0.05 \), \( \beta = 0.20 \); MDDs in layers were Bonferroni-adjusted for five comparisons, \( a = 0.05/5 = 0.01 \), \( \beta = 0.20 \)).
§ Most anterior image of stroma that was least affected by motion blur to enable manual counting of keratocyte nuclei.

**RESULTS**

**Keratocyte Density**

Overall, there was no difference in keratocyte density in corresponding layers between contact lens wearers and control subjects (\( P = 0.29 \), repeated-measures analysis of variance). However, in each group there were differences in density between anteroposterior layers and between central and temporal locations (\( P < 0.001 \), repeated-measures analysis of variance; Table 3, Fig. 1). In the central cornea of both groups, keratocyte density was highest in the anterior 10% of the stroma. In the temporal cornea, keratocyte density progressively decreased with stromal depth in both study groups.

There were no differences in full-thickness keratocyte density (\( P = 0.54 \)) or keratocyte density in the most anterior countable image (\( P = 0.57 \)) between corneas of contact lens wearers and control subjects. However, the parameters differed between the central and temporal cornea (\( P < 0.001 \) in each case).

Keratocyte density in any specific region did not differ between soft contact lens wearers and non-soft contact lens wearers (\( P = 0.30 \)), but it differed within each group across anteroposterior layers and between central and temporal corneas. There were no differences in full-thickness keratocyte density (\( P = 0.75 \)) or keratocyte density in the most anterior countable image (\( P = 0.76 \)) between corneas of soft contact lens wearers and non-soft contact lens wearers.

There was no overall difference in keratocyte density between individual contact lens groups and control subjects (\( P = 0.69 \)). Minimum detectable differences between individual lens groups and control subjects for the full-thickness stroma ranged from 2598 cells/mm\(^3\) to 5391 cells/mm\(^3\). Minimum detectable differences between individual lens groups and control subjects in regional keratocyte densities ranged from 3,265 cells/mm\(^3\) to 16,099 cells/mm\(^3\).

Within the contact lens group, central and temporal full-thickness keratocyte densities were different (\( P = 0.02 \)); posterior stromal keratocyte density was lower in the temporal cornea than in the central cornea (Table 4). Within the control group, temporal full-thickness keratocyte density was lower than central full-thickness keratocyte density (\( P < 0.001 \)); both anterior and posterior stromal keratocyte densities were lower in the temporal cornea (Table 4).
Numbers of Keratocytes
The number of keratocytes in a full-thickness column of stroma that had a cross-sectional area of 1 mm² was 10,247 ± 1,350 (central) and 11,631 ± 1,374 cells (temporal) in the contact lens group, and 10,275 ± 1,412 (central) and 10,962 ± 1,200 cells (temporal) cells in the control group. Overall, there was no difference between the two groups (*P = 0.40), although the difference between central and temporal cornea within each group was significant (*P < 0.001). The number of keratocytes did not differ between corneas of soft contact lens wearers and those of non-soft contact lens wearers (*P = 0.68).

Corneal and Regional Thickness
The difference between corneal thickness measured by ultrasonic pachometry and confocal microscopy was –3.4 ± 10.9 μm (ultrasonic pacheterm – confocal; mean ± SD), and this did not significantly differ from zero (*P = 0.06, paired Student’s t-test, *n = 40). The central subbasal nerve plexus was visible in 95% (19/20 in both groups) of scans of contact lens wearers and control subjects, whereas the temporal subbasal nerve plexus was visible in only 55% (11/20) of scans of contact lens wearers and 45% (9/20) of scans of control subjects.

The temporal epithelium was thinner in corneas of contact lens wearers (46.5 ± 4.7 μm) than in those of control subjects (50.9 ± 4.7 μm, *P = 0.02, Wilcoxon rank sum test). There were no other significant differences in thickness measurements between contact lens wearers and control subjects (Table 5). Comparison of these parameters between corneas of soft and non-soft contact lens wearers also resulted in no significant differences.

Central and temporal epithelial thicknesses did not differ between individual contact lens groups and control groups. Minimum detectable differences ranged from 5.0 to 10.9 μm.
There were no significant differences between central and temporal epithelial and Bowman layer thicknesses in either the contact lens (*P = 0.77, epithelium; *P = 1.0, Bowman layer) or the control group (*P = 0.72, epithelium; *P = 0.99, Bowman layer).

Corneal Intensity
There were no significant differences between corresponding intensity (backscatter) measured in corneas of contact lens wearers or control subjects (Table 5, Fig. 2). There were also no significant differences between corneas of soft and non-soft contact lens wearers.

No differences in stromal intensities were noted between individual contact lens groups and control subjects, except for the central stromal intensity in soft contact lens wearers (96.8 ± 16.3, *n = 11), which was less than that in control subjects (111.3 ± 14.9, *n = 20, *P = 0.02). The minimum detectable differences for the nonsignificant results ranged from 19.9 to 30.1 in the central and 29.0 to 57.8 in the temporal stroma.

In the contact lens wearers, stromal intensity was higher and endothelial intensity was lower in the temporal than in the central cornea. Within the control group, stromal intensity was also higher in the temporal cornea than in the central cornea, whereas there was no difference between endothelial intensities in the central and temporal cornea (Fig. 2). Full-thickness keratocyte density did not correlate with stromal density in the central and temporal corneas of either contact lens wearers or control subjects (Table 6). There were no significant differences between corneas of soft and non-soft contact lens wearers.

![Figure 2](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933593/) Intensities (mean ± SD) of central and temporal corneal stroma and endothelium of 20 long-term contact lens wearers and 20 control subjects. There were no significant differences in intensity between either group (*P > 0.05), although differences between central and temporal intensities within groups were noted.
no significant correlations between central or temporal stromal intensity and age in contact lens wearers or control subjects.

**Contrast**

Contrast between cells and background was greatest in the anterior stroma and decreased at midstroma (Table 7). In the central cornea, contrast increased in the deepest layer of stroma compared with midstroma, whereas in the temporal cornea, contrast continued to decrease in the deepest layers. In each stromal layer there were no differences in contrast between contact lens wearers and control subjects. In the deepest layer of stroma (91%-100%) contrast was lower temporally than centrally, by 21% in contact lens wearers (P = 0.04) and by 24% in control subjects (P < 0.001).

**Corneal Sensitivity and Nerve Fibers**

Corneal sensitivity measured by Cochet-Bonnet esthesiometry was lower in contact lens wearers (57.0 ± 5.0 mm) than in control subjects (59.5 ± 1.5 mm, P = 0.05, Wilcoxon rank sum test). The number of nerve fiber bundles in the subbasal plexus in corneas of contact lens wearers (5.3 ± 1.7) and control subjects (5.3 ± 1.6) did not differ (P = 1.0, unpaired Student’s t-test). The minimum detectable difference in the number of nerve fiber bundles in the subbasal plexus between the two groups was 1.5 (unpaired analysis, α = 0.05, β = 0.20). Corneal sensitivity did not correlate with the number of visible nerve fiber bundles in the subbasal plexus in either contact lens wearers (r = 0.08, P = 0.74, n = 20) or control subjects (r = 0.10, P = 0.67, n = 20). Comparison of these same parameters between corneas of soft and non-soft contact lens wearers showed no significant differences. There was no correlation between central corneal sensitivity and central epithelial thickness in either contact lens wearers (r = −0.06, P = 0.81, n = 19) or control subjects (r = −0.21, P = 0.39, n = 19).

**Endothelial Cells**

Central endothelial cell density in the contact lens group was 2726 ± 513 cells/mm² (mean ± SD) and in the control group was 2735 ± 236 cells/mm², a nonsignificant difference (P = 0.41, Wilcoxon rank-sum test, Table 8). The coefficient of variation of cell area was greater in the contact lens group than in the control group (P = 0.007).

There were no significant correlations between central endothelial cell density and full-thickness keratocyte density in any stromal layer in contact lens wearers or control subjects. Endothelial cell density was not correlated with endothelial peak intensity in either contact lens wearers or control subjects.

**Morphology**

The size and shape of keratocytes in contact lens wearers appeared identical with those in control subjects. There were no gross morphologic differences in either the subbasal nerve plexus or stromal nerves between corneas of contact lens wearers and control subjects. Small (5-µm diameter), bright objects were noted in the central posterior stroma of one contact lens wearer.

**DISCUSSION**

In this study, no difference was found in mean central or temporal keratocyte density between long-term contact lens wearers and subjects who had never worn contact lenses. The study could not exclude a difference of 2346 cells/mm² (11%) or less, however. Full-thickness central keratocyte density in this study was similar to the central keratocyte density measured in our previous study of 70 non-contact lens-wearing subjects.7 There was also no difference between the groups in the mean number of keratocytes (10,250 cells) in a column of central stroma that had a cross-sectional area of 1 mm². This suggests that hypoxia and acidosis induced by contact lens wear have no net effect on keratocyte density or number. Keratocytes do not appear to redistribute from central to peripheral cornea in response to acidosis, as endothelial cells did in a previous study.22 In the present study we did not compare central and peripheral densities of endothelial cells. Our study is limited, however, by the small sample size and a minimum detectable difference of 11% to 31% of cell density in specific layers (Table 3) and by the inclusion of a mixture of contact lens types.

We found no difference in keratocyte density between wearers of soft hydrogel contact lenses and wearers of lenses other than soft, although our subject numbers were small. Jalbert and Stapleton23 used confocal microscopy in vivo to demonstrate that anterior and posterior keratocyte densities in corneas of nine long-term wearers of extended-wear hydrogel lenses were lower than in nine age-matched non-lens-wearing control subjects. Only one of the 11 soft contact lens wearers

<table>
<thead>
<tr>
<th>Stomal Layer (%) Thickness</th>
<th>Central Cornea</th>
<th>Temporal Cornea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contact Lens</td>
<td>Control</td>
</tr>
<tr>
<td>Most anterior</td>
<td>15.8</td>
<td>16.0</td>
</tr>
<tr>
<td>0%-10% (anterior)</td>
<td>14.9</td>
<td>14.6</td>
</tr>
<tr>
<td>11%-35%</td>
<td>11.8</td>
<td>11.5</td>
</tr>
<tr>
<td>34%-66%</td>
<td>10.2</td>
<td>10.7</td>
</tr>
<tr>
<td>67%-90%</td>
<td>10.8</td>
<td>10.7</td>
</tr>
<tr>
<td>91%-100% (posterior)</td>
<td>11.64</td>
<td>11.91</td>
</tr>
</tbody>
</table>

Data are mean percentage of contrast. n = 20, both groups.

* Unpaired t-test, control group vs. contact lens group.
† Significant difference between central and temporal cornea, paired t-test, P < 0.001 (Bonferroni-adjusted for five comparisons).
‡ Significant difference between central and temporal cornea, paired t-test, P = 0.04 (Bonferroni-adjusted for five comparisons).

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Table 6. Correlations between Full-Thickness Keratocyte Density and Stromal Intensity

<table>
<thead>
<tr>
<th>Stromal Layer</th>
<th>Contact Lens Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central</td>
<td>r_p = -0.09, P = 0.70</td>
<td>r_p = 0.13, P = 0.59</td>
</tr>
<tr>
<td>Temporal</td>
<td>r_p = -0.16, P = 0.49</td>
<td>r_p = -0.08, P = 0.75</td>
</tr>
</tbody>
</table>

r_p = Pearson’s correlation coefficient. n = 20, both groups.
In our study, we measured keratocyte density 2.5 mm from the limbus in the temporal cornea and found that full-thickness keratocyte density was lower than in the central cornea, despite the number of keratocytes in the full-thickness temporal stroma being greater than in the full-thickness central stroma. Although we found a correlation between the two groups, the results of both studies are limited to the peripheral cornea, keratocyte densities calculated by the two methods did not agree (41,000 cells/mm² by measuring DNA content and 15,000 cells/mm² by counting keratocyte nuclei if the stereologic principles described in our previous study were applied to the data). The limitations of these studies highlight the need for quantification of peripheral keratocyte density by using standard histologic methods.

We found no difference between central epithelial thickness in contact lens wearers and control subjects. With our sample size, however, we were able to detect only a 4.1-μm (8.5%) difference with 80% power. Holden et al.² found that the central epithelium of corneas of long-term extended-wear contact lens wearers was 5.6% thinner than the central epithelium of control corneas, a difference smaller than our minimum detectable difference. In their study, epithelial thickness was measured immediately after removal of contact lenses, whereas contact lenses were removed 12 to 24 hours before confocal microscopy in our study. The temporal epithelium was 9.0% thinner (P = 0.02) in corneas of contact lens wearers than in corneas of control subjects in our study. Reinstein et al.²⁰ demonstrated that epithelial thickness in the cornea of a contact lens wearer was thinner peripherally than centrally, by using high-frequency ultrasound. We found no significant differences in epithelial thickness between the central and temporal cornea for either contact lens wearers or control subjects.

We measured the intensity of backscattered light (image intensity) from different layers of the cornea in this study, referencing all data to a single standard for comparison. Although stromal intensity was slightly lower in corneas of contact lens wearers than control subjects, indicating greater clarity, this difference was not statistically significant. Central stromal intensity was lower than temporal stromal intensity in both groups, indicating greater central clarity, despite a higher keratocyte density centrally. Although there was no evidence in our study that long-term contact lens wear impairs the clarity of the cornea, Nagel et al.²¹ reported greater stromal intensity in contact lens wearers than in control subjects 1 hour after lens removal. Statistical results were not reported, however, so that the differences between their study and ours are difficult to evaluate. Although stromal intensity did not correlate with age in our study, we did not have a uniform age distribution to reliably assess this relationship. We did not measure intensity of the surface epithelium, because this appeared to vary according to the time of exposure of the epithelium to the hydroxypropyl methylcellulose optical coupling agent.

Corneal sensitivity of contact lens wearers was reduced compared with that in control subjects in our study. Corneal sensitivity was notably reduced in the two subjects who had worn only rigid, gas-permeable lenses, whereas the subject who had worn only PMMA lenses had normal corneal sensitivity. We did not observe any morphologic changes in nerve

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**Table 8. Central Endothelial Cell Data in Contact Lens Wearers and Control Subjects**

<table>
<thead>
<tr>
<th></th>
<th>Contact Lens</th>
<th>Control</th>
<th>P*</th>
<th>MDD†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial cell density (cells/mm²)</td>
<td>2726 ± 513</td>
<td>2735 ± 236</td>
<td>0.41‡</td>
<td>354</td>
</tr>
<tr>
<td>Mean cell area (μm²)</td>
<td>384 ± 96</td>
<td>368 ± 32</td>
<td>0.41‡</td>
<td>64</td>
</tr>
<tr>
<td>Coefficient of variation of cell area</td>
<td>0.37 ± 0.10</td>
<td>0.30 ± 0.04</td>
<td>0.007 -</td>
<td>-</td>
</tr>
<tr>
<td>Skewness of cell area</td>
<td>0.86 ± 0.50</td>
<td>0.62 ± 0.22</td>
<td>0.06</td>
<td>0.34</td>
</tr>
<tr>
<td>Hexagonal cells (%)</td>
<td>53.6 ± 11.9</td>
<td>57.3 ± 7.6</td>
<td>0.24</td>
<td>8.9</td>
</tr>
</tbody>
</table>

Data are mean ± SD.

* Unpaired Student’s t-tests, unless stated.
† Minimum detectable differences between contact lens group and control group (unpaired analysis, α = 0.05, β = 0.20).
‡ Wilcoxon rank sum test.
fiber bundles of the subbasal plexus between contact lens wearers and control subjects in our study. The method we used to quantify the number of nerve fiber bundles in the subbasal nerve plexus is similar to that described by Rosenberg et al. We counted 5.3 nerve fiber bundles in the subbasal nerve plexus of contact lens wearers and control subjects, similar to the 4.9 nerve fiber bundles they counted in corneas of healthy nondiabetic subjects. Rosenberg et al. demonstrated fewer nerve fiber bundles in the subbasal nerve plexus of diabetic subjects than in nondiabetic subjects, and found that this preceded the reduction of mechanical sensitivity of corneas in diabetic subjects. We did not find a similar decrease in nerve fiber bundles in our study, which suggests that the decreased corneal sensitivity in contact lens wearers is due to a functional change in nerves and not to a change in nerve density. The recovery of corneal sensitivity after cessation of long-term contact lens wear also supports a functional change in corneal nerves, rather than a change in nerve density.

In the present study, neither endothelial cell density nor mean cell area differed between contact lens and control groups. The coefficient of variation (SD/mean) of cell area was significantly higher in the contact lens group than in the control group, indicating that contact lenses induce polymegathism. These findings confirm those of many investigations over the past 20 years. The skewness of cell area, another parameter of change in endothelial morphology, did not differ between the two groups.

Incidentally, we observed small, bright objects in one subject who had worn daily-wear soft contact lenses for 20 years. The significance of these objects is not known. Bohnke and Masters described the presence of highly reflective microdots, 0.3 to 0.6 μm in size, throughout the full-thickness stroma of all long-term soft contact lens wearers examined with a confocal microscope with a ×50 objective lens. We estimate the diameter of the objects that we observed with a ×24 objective lens to be approximately 5 μm and thus distinct from the microdots observed by Bohnke and Masters. The dimensions of pixels in our images were 0.78 × 0.78 μm, and thus we did not have the magnification to visualize the microdots described by Bohnke and Masters.

Our study was limited in that we combined all contact lens groups for comparison to control subjects. We assessed the differences in certain parameters between each contact lens group and the control group, except for the PMMA group, which consisted of a single subject. For clarity, we did not report the absolute values of each parameter in the individual groups, but we presented the range of minimum detectable differences based on the small sample sizes for each group, to demonstrate the limited power of these analyses, which found no significant differences.

In summary, we examined corneas 12 to 24 hours after contact lens removal and found no difference in keratocyte density and stromal intensity (light backscattered from the stroma) between corneas of long-term and non-contact lens wearers. Temporally, the epithelium was thinner in corneas of long-term contact lens wearers than in control subjects. The decreased sensitivity of corneas of long-term contact lens wearers compared with control corneas was not accompanied by a decrease in the density of nerve fiber bundles in the subbasal nerve plexus.

References


