Cornea

Evaluation of Contact Lens–Induced Changes in Keratoconic Corneas Using In Vivo Confocal Microscopy

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PURPOSE. To quantitatively analyze laser scanning in vivo confocal microscopy (IVCM) images of all corneal layers in contact lens–wearing and noncontact lens–wearing keratoconus patients.

METHODS. The study population included rigid gas permeable (RGP) contact lens–wearing keratoconus patients (group 1; N = 29), keratoconus patients who did not wear contact lenses (group 2; N = 30), and subjects who neither had keratoconus nor wore contact lenses (group 3; N = 30), with groups 2 and 3 matched to group 1 by age and sex. The central cornea was examined with IVCM in all subjects. The mean duration of contact lens wear was 5.50 ± 3.68 years (range, 2–15 years).

RESULTS. Eyes with keratoconus showed significantly lower basal epithelial cell and anterior and posterior stromal keratocyte densities, as well as subbasal nerve fiber density, nerve branch density, and nerve fiber length compared with healthy control subjects. Furthermore, compared with group 2, group 1 had significantly lower basal epithelial cell density (4920 ± 476 cells/mm² vs. 4503 ± 461 cells/mm², P = 0.001) and anterior stromal keratocyte density (561 ± 91 cells/mm² vs. 464 ± 55 cells/mm², P < 0.001), but there was no significant difference for posterior stromal keratocyte density (P = 0.808), endothelial cell density (P = 0.699), or subbasal nerve fiber density (P = 0.142), nerve branch density (P = 0.614), and nerve fiber length (P = 0.850).

CONCLUSIONS. Significant corneal microstructural abnormalities were observed in eyes with keratoconus. RGP contact lens wear was associated with a further reduction in the basal epithelial cell and anterior stromal keratocyte densities, but with no effect on posterior stromal keratocyte density, endothelial cell density, or corneal nerve morphology.

Keywords: keratoconus, confocal microscopy, contact lens, cornea

Keratoconus is a progressive, noninflammatory corneal ectasia, which is characterized by stromal thinning and apical protrusion leading to irregular astigmatism and loss of vision.1 The exact pathophysiologic process of keratoconus remains obscure. It is thought that the earliest structural change begins at the basal epithelial layer of the cornea.2 Subsequently, release of cytokines and catabolic enzymes from the damaged epithelial cells causes breaks in Bowman’s layer, and induces apoptotic cell death and keratocyte loss in the stroma, which is believed to be associated with corneal thinning and degeneration of normal corneal architecture.3,4 Management depends on the disease severity. Contact lens wear is the most common and successful treatment option which provides good visual acuity and keratoconus progression control.5,6 In vivo confocal microscopy (IVCM) enables real-time, in vivo, high resolution microscopic imaging of the cornea and has been increasingly used to evaluate the corneal changes in keratoconus,5,7–12 as well as the effect of contact lenses on the cornea.13–16 In studies performed with IVCM, numerous corneal microstructural changes, such as decreased basal epithelial cell, keratocyte and endothelial cell densities, increased basal epithelial cell area, pleomorphism and polymegathism in the endothelial cell layer, and decreased nerve fiber density have been reported in keratoconic corneas.8,12 Patients with keratoconus generally use contact lenses, which in itself may result in structural changes in the cornea.15,17 Previous IVCM studies have shown a range of abnormalities in healthy subjects wearing contact lenses, which include an increased epithelial cell size, reduced keratocyte density, increased highly reflective stromal microdot deposits, and bleb formation in the endothelial cell layer.18–22 The purpose of this study was to quantitatively analyze laser scanning IVCM images of all corneal layers in contact lens–wearing and noncontact lens–wearing keratoconus patients to establish if it was associated with added pathology.

MATERIALS AND METHODS

Fifty-nine eyes of 59 patients with the diagnosis of keratoconus, and 30 eyes of 30 healthy subjects were enrolled in this study undertaken at a single university hospital. The keratoconus patients were assessed in two groups: a group of rigid gas permeable (RGP) contact lens wearers (29 patients; 18 female, 11 male), and a group of noncontact lens wearers (30 patients; 19 female, 11 male). The contact lens–wearing keratoconus group (group 1) was chosen first. A random sample of keratoconus patients who did not wear contact lenses (group 2) was chosen next, with this group matched to group 1 by age (±2 years), sex, and disease severity. Disease severity was established using Pentacam topography in group 1 and then
patients from group 2 were matched for a similar distribution of disease severity. Finally, a random sample of control subjects who did not have keratoconus or wear contact lenses (group 3) were chosen, and matched to group 1 for age and sex. Patients (group 1 and group 2) and control subjects were selected over a 6-month period and had been referred from other ophthalmologists in the region. Patients in group 1 and 2 were randomly selected from the registers of our contact lens unit and cornea and ocular surface disorders unit, respectively. Subjects in the control group were selected at random from visitors to Meram Medical Faculty Hospital (Konya, Turkey).

Eight patients in group 2 were excluded from the analysis due to the corneal scarring (N = 4), vernal keratoconjunctivitis (N = 3), and a history of prior corneal transplantation (N = 1). In the first group, the subjects were wearing RGP lenses on a daily-wear basis and the mean duration of contact lens wear was 5.50 ± 3.68 years (range, 2–15 years). Reasons for loss of contact lens wear in group 2 included recent diagnosis (N = 9), poor adherence (N = 6), and patient preference (N = 15). For subjects in whom both eyes were suitable for the study, one eye was randomly selected. Exclusion criteria were as follows: any previous ocular trauma or ocular surgery, any coexisting corneal pathology, and clinical evidence of corneal scarring.

This study followed the tenets of the Declaration of Helsinki and was approved by the institutional review board of Necmettin Erbakan University. Written informed consent was obtained from all subjects after a detailed explanation of the nature of the study.

All patients underwent complete ophthalmologic evaluation, including retinoscopy, slit-lamp examination (SLE), and computerized topography (Pentacam; Oculus Optikgerate GMbH, Wetzlar, Germany). An eye was diagnosed as having keratoconus if there was scissoring reflex on retinoscopy, with central or paracentral thinning, anterior bulging or concuity, hemosiderin deposition (Fleischer ring), stromal striae (Vogt striae), on SLE, and central or paracentral steepening of the cornea on computerized topography. Corneal curvature readings were classified using the criteria adopted in the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) study.23 According to this system, disease severity is classified with respect to the curvature of the steepest corneal meridian as follows: mild: less than 45 diopters (D); moderate: 45 to 52 D; severe: greater than 52 D.

The subjects in group 3 (17 female, 13 male) had no history of ocular surgery, no previous or active ocular disease, other than refractive error, no prior contact lens wear, and no systemic disease that might affect the cornea.

Laser scanning IVCAM was performed on all subjects using the Rostock Corneal Module/Heidelberg Retina Tomograph III (RCM/HRT III; Heidelberg Engineering GmbH, Dossenheim, Germany). This microscope was equipped with a 63x objective water immersion lens with a numerical aperture of 0.95 (Zeiss, Oberkochen, Germany), and utilizes a 670-nm red wavelength diode laser source-a class 1 laser system that by definition, does not pose any ocular safety hazard. As specified by the manufacturer, the acquired two-dimensional images are defined by 384 × 384 pixels covering an area of 400 μm × 400 μm with a lateral digital resolution of 1 μm/pixel and a digital depth resolution of 2 μm/pixel. The module is associated with a manual z-axis drive to move the focal plane, which enables imaging of the corneal layers at any depth. The RCM uses an entirely digital image capture system.

During the IVCAM examination, the patient was asked to fixate on a distance target aligned to enable examination of the central cornea. Two frames per location that contained the clearest images were selected from each of the following levels: basal epithelium, anterior stroma, posterior stroma, and endothelium. Anterior stroma was defined as the first two clear images (without motion blur or compression lines) immediately posterior to Bowman’s layer, and the posterior stroma was defined as the first two clear images immediately anterior to Descemet’s membrane. A standard central counting frame size of 200 μm × 200 μm was used for all epithelial and endothelial images and a frame size of 300 μm × 300 μm was used for the stromal images. Cells that overlapped the counting frame were counted at only the superior and the left half of the frame. Two frames were analyzed for each corneal layer and an average was taken. The number of cells per millimeter squared was calculated by the proprietary software within the RCM/HRT III. Three to five high-quality images of the subbasal nerve plexus from the center of the cornea were assessed from each subject. For all subbasal nerve plexus images, the full 400 μm × 400 μm frame was used. Automatic CCMMetrics software, version 1.0 (University of Manchester, Manchester, UK) was used for the quantitative analysis of the nerve fibers.24 Three parameters were quantified: corneal nerve fiber density (NFD), the total number of major nerves per square millimeter; nerve fiber length (NFL), the total length of all nerve fibers and branches (millimeters per square millimeter); and nerve branch density (NBD), the number of branches emanating from major nerve trunks per square millimeter. Long nerve fibers traversing at least 75% of the image frame and with a larger diameter were selected to be the major nerves and were selected for NFD, while the nerve fibers rising from major nerves were selected to be nerve branches. A nerve branching index (number of branches per main nerve fibers) was derived from the NBD/NFD ratio.

All images were analyzed by one observer (GB) who was unmasked with regard to whether the images belonged to a patient with keratoconus or a control subject, but was masked with regard to contact lens-wearing status.

Statistical analyses were performed using SPSS version 17.0 (Chicago, IL) software. Basic descriptive statistics were calculated on all the data gathered and are reported as the mean ± SD, median (first quartile-third quartile) or n (%), as appropriate. The Pearson χ² test was used to compare categorical parameters. Normal distribution of continuous variables was confirmed with the Kolmogorov-Smirnov test. An independent samples t-test was used to compare the parameters between the keratoconus and control groups. Kruskal-Wallis test followed by Mann-Whitney U test with Bonferroni adjustment for nonnormally distributed data and ANOVA (one-way ANOVA) test followed by Tukey-HSD multiple comparison test for normally distributed data were used to compare the confocal microscopic parameters within the two groups of keratoconus patients and control subjects. The correlation between the duration of contact lens wear and IVCAM findings was measured by using Pearson’s correlation coefficient. For ANOVA tests, the significance level was adjusted since seven different ANOVAs were run with the Kolmogorov-Smirnov test.

For other statistical analysis, a P value of less than 0.0071 (0.05/7) was considered statistically significant. For other statistical analysis, a P value of less than 0.05 was considered statistically significant.

### Results

The mean age of the subjects was 26.7 ± 5.9 years (range, 20–40 years) in group 1, 26.3 ± 5.3 years (range, 18–38 years) in group 2, and 27.5 ± 5.0 years (range, 18–40 years) in group 3. There was no statistically significant difference within the three groups in terms of age (P = 0.672) and sex (P = 0.855).

On the basis of Pentacam topography, 13 (44.8%) eyes in group 1 had moderate, and 16 (55.2%) had severe keratoconus. In group 2, 13 (45.3%) eyes had moderate, and 17 (56.7%) had severe keratoconus with no statistically significant difference.
between the two groups for disease severity ($P = 0.908$). Mean central corneal thickness was significantly reduced in patients with keratoconus (447 ± 48 µm) compared with control subjects (548 ± 32 µm), ($P < 0.001$) and did not differ between group 1 (444 ± 45 µm) and group 2 (451 ± 50 µm), ($P = 0.576$).

The Table shows the comparison of quantitative IVCM findings in contact lens–wearing keratoconus patients (group 1), noncontact lens–wearing keratoconus patients (group 2), and healthy control subjects (group 3).

Qualitative analysis of the epithelial layer revealed structurally abnormal and elongated superficial epithelial cells, as well as enlarged basal epithelial cells (Fig. 1) in patients with keratoconus. The mean basal epithelial cell density was significantly lower in group 1 and group 2 compared with group 3 ($P < 0.001$ and $P < 0.001$, respectively) and there was a significant further reduction in group 1 compared with group 2 ($P = 0.001$).

Irregular arrangement of keratocytes and loss of keratocyte nuclei were observed in the stroma of the keratoconic corneas. Small, dense, highly reflective dots termed 'microdots' by Bohnke and Masters, were observed in the stromal images of 17 eyes (58.6%) in group 1, 6 eyes (20.0%) in group 2, and 2 eyes (6.7%) in group 3 (Fig. 2). There was a statistically

![Figure 1](http://iovs.arvojournals.org/)

**Figure 1.** Corneal confocal microscopic images of the basal epithelial cell layer. (A) Normal basal epithelial cell layer of a healthy subject. (B) Basal epithelium of a keratoconic cornea with large cells and faint cell borders.
significant difference for microdot deposits between group 1 and group 2 ($P = 0.002$).

The mean anterior and posterior stromal keratocyte densities were significantly reduced in both groups of patients with keratoconus compared with the control group ($P < 0.001$ and $P < 0.001$, respectively). There was a further significant reduction in anterior stromal keratocyte density between groups 1 and 2 ($P < 0.001$), but no difference in posterior stromal keratocyte density ($P = 0.808$).

Endothelial cells qualitatively showed pleomorphism and polymegathism in patients with keratoconus, both in contact lens wearers and noncontact lens wearers. The mean endothelial cell density did not differ significantly among the three groups ($P = 0.017$). None of the patients displayed endothelial blebs.

Patients with keratoconus exhibited abnormal subbasal nerve architecture compared with control subjects (Fig. 3). The mean NFD, NFL, and the median value of NBD were significantly lower in both groups of patients with keratoconus compared with control subjects ($P < 0.002$). There was no significant difference for NFD ($P = 0.142$), NBD ($P = 0.614$), or NFL ($P = 0.850$) between group 1 and group 2. The mean value of the nerve branching index did not differ significantly within the three groups ($P = 0.342$).

There was no significant correlation between the duration of contact lens wear and any of the IVCM parameters ($P > 0.05$).

**DISCUSSION**

Keratoconus is reported to occur with a variable frequency of 5 to 23 in 10,000 in the general population. Disease progression manifests itself with a loss of visual acuity, which cannot be compensated with spectacles. Although there are several types of contact lenses manufactured for keratoconus, RGP contact lenses remain the most commonly used lens type, as high levels of irregular astigmatism cannot normally be corrected with other contact lenses. IVCM has been used to establish a range of abnormalities in the corneas of patients with keratoconus. However, as contact lens wear has also been associated with significant corneal abnormalities, we have undertaken laser scanning IVCM to evaluate whether the patients with keratoconus wearing RGP contact lenses have differences compared with noncontact lens-wearing keratoconus patients and healthy control subjects.

The decrease in basal epithelial cell density of keratoconic corneas has been reported in several previous studies. This study confirmed these previous findings and showed a significant reduction in basal epithelial cell density in keratoconus patients, but additionally we showed a further significant reduction in the contact lens–wearing keratoconus group compared with noncontact lens–wearing keratoconus patients. Patel et al. have also reported a further reduction in contact lens–wearing keratoconus patients and, suggested that the basal epithelium is involved due to the pathophysiologic process of keratoconus, and that this is further exacerbated by contact lens wear.

A statistically significant reduction in anterior stromal keratocyte density in contact lens–wearing keratoconus patients compared with noncontact lens wearers, as well as in the two groups of patients with keratoconus compared with the healthy control group was observed in this study. Erie et al. have proposed that contact lens wear in keratoconus patients stimulates the epithelial injury-associated release of apoptotic cytokines and eventually decreases cell density in the anterior stroma by the process of regulated cell death. Keratocyte loss due to contact lens wear has been shown not only in keratoconic corneas but also in healthy corneas. The contact lens–induced keratocyte loss observed in these studies was attributed to three possible etiologies: hypoxia, cytokine-mediated, and mechanically-induced effects. These data are in contrast with those of Patel et al., who have shown that long term daily contact lens wear has no effect on keratocyte density.

Posterior stromal keratocyte density has been shown to be decreased in keratoconic corneas in previous IVCM studies. In this study, posterior stromal keratocyte density

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**Figure 2.** Corneal confocal microscopic images of the mid-stroma. (A) Normal stromal image of a healthy subject. (B) Microdot deposits (arrows) in a keratoconus patient wearing RGP contact lens for 6 years.
was significantly reduced in both groups of patients with keratoconus compared with controls, but there was no significant difference between the contact lens wearers and those who did not wear contact lenses. Compared with normal corneas, Jalbert and Stapleton28 and Efron et al.20 both found a reduction in posterior stromal keratocyte density in extended-wear soft contact lens wearers. Differences in the reported IVCM literature on keratocyte density in relation to contact lens wear may be attributed to the variations of contact lens types, the duration of contact lens wear, or even the type of confocal microscope used.

Stromal microdots were first described by Bohnke and Masters in 1997.21 These microdots are seen with the IVCM as small, discrete, brightly reflective spots that are scattered throughout the stroma. Trittibach et al.30 also demonstrated these microdots in their study, in which 36 myopic patients with a history of contact lens wear were evaluated. The authors suggested that microdot deposits may represent granules of lipofuscin-like material within the corneal stroma of long term contact lens wearers, formed as a result of chronic oxygen deprivation and chronic microtrauma to the cornea. Contrary to the findings of these studies, Efron and Mutalib31 reported that microdots are also seen in the stromal images of noncontact lens wearers. In a further study by Hollingsworth and Efron,16 microdot deposits were seen in both RGP contact lens–wearing subjects on a daily wear basis and noncontact lens wearers, however, the number of microdots were significantly higher in the contact lens–wearing group. In the current study, stromal microdot deposits were increased in contact lens–wearing keratoconus patients compared with noncontact lens–wearing keratoconus patients and noncontact lens–wearing healthy control subjects.

In this study, no significant difference in endothelial cell density was observed between contact lens–wearing and noncontact lens–wearing keratoconus patients, which is in agreement with the study by Mocan et al.15 Although increased apoptosis of endothelial cells has been reported in keratoconus,32 studies have shown either increased,4 decreased,8,9,12 or unchanged10 endothelial cell density in keratoconic corneas.

With regard to contact lens wear, long term rigid contact lenses did not alter the endothelial cell density in healthy subjects,16,33,34 whilst soft contact lens wear was shown to reduce endothelial cell density.55

Many studies have demonstrated the effect of contact lens wear on endothelial pleomorphism and polymegathism.13,16,36,57 We have observed a qualitative variation in the size and the shape of the endothelial cells in eyes with keratoconus, however, quantitative analysis of pleomorphism and polymegathism was not performed in the current study, limiting detailed analysis. Thus, it was not possible to investigate the exact effect of contact lens wear on endothelial cell morphology.

Endothelial bleb response is thought to occur as a result of contact lens–induced epithelial acidosis,58 and is described as irregularly shaped, round or oval, dark regions with a bright central spot within the endothelial mosaic in IVCM studies.22,31 It appears within minutes of inserting a contact lens, and diminishes with adaptation to contact lens wear.22 We did not observe endothelial blebs in lens–wearing lenses in keratoconus patients, likely because these patients were wearing contact lenses for a long enough period for adaptation.

In a study by Oliveira-Soto and Efron,14 corneal nerves of contact lens–wearing subjects and nonlens-wearing controls were examined and no quantitative difference in nerve morphology between the two groups was observed. Patel et al.13 also reported that the number of nerve fiber bundles in the subbasal nerve plexus remained unchanged in contact lens wearers, although corneal sensitivity was found to be lower. Additionally, previous studies have demonstrated abnormal corneal nerve morphology in keratoconic corneas.9,12,39,40 In the current study, NFD, NBD, and NFL were significantly lower in both groups of patients with keratoconus compared with healthy controls, but there was no difference in NFD, NBD, or NFL between contact lens–wearing and noncontact lens–wearing keratoconus patients.

Contact lens–associated stromal thinning was first described by Holden et al.41 when they demonstrated that the stroma had thinned an average of 11 μm in 5.2 years of soft
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Contact lens wear. Liu and Pflugfelder evaluated the effect of long term contact lens wear on corneal thickness in 35 subjects who had been wearing soft contact lenses for 13.5 ± 6 years and found that the mean central corneal thickness was significantly reduced in contact lens–wearing subjects compared with controls. Myrowitz et al. demonstrated a significant reduction in central corneal thickness in long term rigid contact lens–wearing subjects, however, they reported that soft contact lens wear was not associated with a decrease in the average central corneal thickness. In another study by rigid contact lens–wearing subjects, however, they reported significant reduction in central corneal thickness in long term contact lens–wearing subjects. In this study, a significant reduction was seen in central corneal thickness in patients with keratoconus compared with control group, whereas no significant difference was found between contact lens–wearing and noncontact lens–wearing keratoconus patients. We measured the central corneal thickness of subjects with Pentacam topography, but it may be preferable to evaluate epithelial and stromal thickness–es separately using IVCM.

Although there is a common belief that RGP contact lenses slow the progression of keratoconus, we have shown a reduction in basal epithelial cell and anterior stromal keratocyte densities. The pathophysiologic cause and consequence of this observation is not clear and only longitudinal studies may shed light on the long term clinical relevance of this observation.

The main limitation of this study is that the quantitative analyses of the IVCM images were performed by an observer who was unmasked with regard to whether the images belonged to a patient with keratoconus or a control subject, but the observer was masked with regard to contact lens–wearing status. A further limitation is that this is a retrospective observational study, and therefore subject to bias.

In conclusion, keratoconus adversely affects the corneal microstructure and RGP contact lens wear results in a further reduction in basal epithelial cell and anterior stromal keratocyte densities, with no effect on endothelial cell density or corneal nerve morphology.

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