**ABSTRACT**

**PURPOSE:** To assess corneal microarchitecture and regional epithelial thickness profile in eyes with keratoconus, postoperative corneal ectasia (ectasia), and normal unoperated eyes (controls) using spectral-domain optical coherence tomography (SD-OCT).

**METHODS:** Regional corneal epithelial thickness profiles were measured with anterior segment SD-OCT (Optovue RTVue-100, Optovue Inc., Fremont, CA). Epithelial thickness was assessed at 21 points, 0.5 mm apart, across the central 6-mm of the corneal apex in the horizontal and vertical meridians.

**RESULTS:** One hundred twenty eyes were evaluated, including 49 eyes from 29 patients with keratoconus, 32 eyes from 16 patients with ectasia, and 39 eyes from 21 control patients. Average epithelial thickness at the corneal apex was 41.18 ± 6.47 µm (range: 30 to 51 µm) for keratoconus, 46.5 ± 6.72 µm for ectasia (range: 34 to 60 µm), and 50.45 ± 3.92 µm for controls (range: 42 to 55 µm). Apical epithelial thickness was significantly thinner in eyes with keratoconus (P < .0001) and ectasia (P = .0007) than in controls. Epithelial thickness ranges in all other areas varied widely for keratoconus (range: 21 to 101 µm) and ectasia (range: 30 to 82 µm) compared to controls (range: 43 to 64) (P = .0063).

**CONCLUSION:** SD-OCT demonstrated significant central and regional epithelial thickness profile differences between keratoconus, ectasia, and control eyes, with significant variability and unpredictability in ectatic eyes. This regional irregularity may necessitate direct epithelial thickness measurement for treatments where underlying stromal variations may be clinically relevant, including corneal collagen cross-linking or topography-guided ablations.

to determine the relative thickness consistency and predictability for each condition.

**PATIENTS AND METHODS**

Patients with progressive keratoconus and postoperative corneal ectasia presenting for the U.S. Food and Drug Administration CXL clinical trial (Clinical Trials.gov identifier #NCT00567671, physician-sponsored IND with approval from the Emory University Institutional Review Board) from May 2009 to June 2010 were evaluated in this study. Keratoconus cases were classified for severity using the grading scheme proposed by McMahon et al.\(^6\) These eyes by definition had no clinical evidence of scarring because that was a specific exclusion criterion for the CXL study. Normal eyes of candidates for refractive surgery were used as the control group and evaluated under separate Emory Institutional Review Board approval.

**SD-OCT Analysis of Corneal Architecture**

An SD-OCT device (Optovue RTVue-100; Optovue Inc, Fremont, CA) with a scan rate of 26,000 axial scans per second, axial resolution of 5 µm, transverse resolution of 15 µm, and an add-on lens (CAM-L mode: 6.0 to 2.0 mm) was used to assess the regional corneal architecture and epithelial thickness profile in eyes with keratoconus, eyes with ectasia, and normal eyes. OCT mean corneal power was measured by the SD-OCT Pachymetry map with the additional corneal power software (Cpwr) and corneal tomography was obtained by Scheimpflug imaging (Pentacam; Oculus Optikgeräte GmbH, Wetzlar, Germany).

The specific imaging capture technique for this study has been previously described.\(^17\) Briefly, patients were asked to fixate on the target light source and consecutive images were acquired with the patient’s forehead and chin stabilized by a headrest. Images were obtained in duplicate to confirm thickness measurement reproducibility. Measurements with deviation greater than 3 µm in central corneal thickness were repeated.

Anatomical landmarks for the epithelium and Bowman layer were identified by direct visualization of the area of increased reflectivity corresponding to the epithelium–Bowman interface. The high-resolution cross-line scan OCT tool of the add-on lens was used to measure the epithelial thickness. The flap tool and manual measurement tool were used in this study. The SD-OCT measurements include the tear film. The flap tool measurement setting is perpendicular to either the front or back surfaces of the cornea and the minimum thickness difference between mouse clicks is 4 µm. The aspect ratio of the window display should be adjusted to 1:1 scale for perpendicular appearance of the flap tool. The actual measurement values do not change because the measurements are calculated independently to the display. In eyes with high aberration (severe keratoconus cases), the flap tool did not reliably find the front and back surfaces of the cornea in the peripheral regions, so the manual measurement tool was used for these cases. We manually adjusted the aspect ratio of the display window to 1:1 scale to acquire perpendicular measurements.

In a previous study, we used high-resolution cross-line scans in the vertical and horizontal meridians and SD-OCT pachymetry maps to evaluate eyes with keratoconus before and after epithelium removal on the day of the cross-linking treatment.\(^18\) SD-OCT anatomical landmarks for epithelium and Bowman layer were confirmed from this analysis. The area of increased reflectivity corresponding to Bowman layer interface was intact after epithelium removal (Figure A, available as supplemental material in the PDF version of this article). An intact solid white line was observed in all cases. Therefore, our intended target for epithelial measurement was the anterior most portion of the solid white line, and we avoided including Bowman layer and the anterior stroma because the Bowman layer itself can overestimate the epithelial thickness measurements by 8 to 19 µm.\(^19,20\) SD-OCT anatomical landmarks for the epithelium and Bowman layer are demonstrated in Figure 1.

The OCT scans were centered on the apex of the cornea, and both horizontal and vertical high-resolution cross-sectional images were acquired. Measurements exactly at the corneal apex were avoided because both internal and interface reflectivity is high and contrast is poor. The flap tool coordinate “0.00” was used to mark the highest point in the meridian. Epithelial thickness was assessed at 21 points 0.5 mm apart across the central 6-mm of the cornea in the horizontal and vertical meridians. Regularity and morphology of the corneal epithelial thickness were assessed.

**Statistical Analysis**

JMP 9.0 software (SAS Institute Inc., Cary, NC) was used for statistical analysis. The Student’s t test was
used to evaluate independent samples, and Pearson correlation coefficient and analysis of variance (ANOVA) were used to compare peripheral to central measurements across the cornea. *P* values less than .05 were considered statistically significant.

**RESULTS**

One hundred twenty eyes from 66 patients were evaluated, including 49 eyes from 29 patients with keratoconus, 32 eyes from 16 patients with ectasia, and 39 eyes from 21 control patients. Patient demographics are shown in Table A (available as supplemental material in the PDF version of this article). There were significant differences in age, spherical equivalent, mean Sim-Ks measured by Scheimpflug tomography (Pentacam), OCT mean corneal power (Cpwr), and thinnest corneal point (SD-OCT).

Table 1 shows the epithelial thickness profiles at various regions throughout the cornea for each group. Average epithelial thickness at the highest point in the meridian was significantly thinner in eyes with keratoconus (*P* < .0001) and ectasia (*P* = .0007) than in normal eyes. There were also statistically significant differences at 0.5, 1.0, and 1.5 mm above and at 0.5, 1.0, 1.5, and 2.0 mm below the highest point in the meridian (*P* < .05). The thickest point in all groups was found 2.5 mm below the corneal apex and nasally displaced. There were no statistically significant differences between groups at this thickest point.

The corneal epithelium was statistically significantly thinner in both the vertical (Figure 2A) and horizontal (Figure 2B) meridians in multiple locations in eyes with keratoconus and ectasia compared to normal eyes (*P* < .05). Epithelial thickness was not, on average, thicker in eyes with keratoconus or ectasia at any location compared to normal eyes (Table 1). Table 2 demonstrates the wide range of standard deviations for eyes with keratoconus and ectasia compared to a relatively tight range for normal eyes. Focal areas of significantly thickened epithelium were noted in eyes with keratoconus and ectasia at any location compared to normal eyes (Table 1). Table 2 demonstrates the wide range of standard deviations for eyes with keratoconus and ectasia compared to a relatively tight range for normal eyes. Focal areas of significantly thickened epithelium were noted in eyes with keratoconus and ectasia at any location compared to normal eyes (Table 1). 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DISCUSSION

In this study, anterior segment SD-OCT demonstrated significant irregularity, variability, and alterations in regional epithelial and total corneal thickness profiles in eyes with keratoconus and ectasia compared to the epithelial thickness profiles of normal eyes.

The epithelium was statistically significantly thinner over the corneal apex in eyes with keratoconus and ectasia. Focal central epithelial thinning is suggestive but not pathognomonic for keratoconus. Epithelial thickening in keratoconus has been associated with breaks in Bowman layer and the stromal thinning has been related to the number of breaks in Descemet’s membrane, which may account for the cases we found with thickened epithelium overlying stromal thinning (Figure 3), in contrast to what has been reported in other series. In some regions, high-resolution cross-sectional scans revealed areas of epithelial thickening “filling space” in a way that partially compensates for stromal irregularities. Specifically, we observed the epithelium to be thin over areas in which the anterior stromal curvature was steep and the surface elevated, whereas the epithelium tended to be thicker over areas in which the curvature was flat (or concave). These observations lead to the conclusion that the overall corneal thickness does not readily predict the underlying stromal thickness in eyes with keratoconus and ectasia, and thicker epithelium can occur in regions of thinner stroma when those stromal regions do not correlate with the steepest area of the cornea. Further, this epithelial hypertrophy associated with stromal thinning occurred in mild, moderate, and severe keratoconus cases; thus, severity level cannot predict this epithelial irregularity. It would be interesting to see what these corneas would look like with “head-on” images generated by very high-frequency (50-MHz) digital ultrasound compared to the cross-sectional views currently available with the SD-OCT. A new pattern software may soon be widely available for the Optovue system that will allow this “head-on” view; in initial studies, Li et al. have reported averaged epithelial thickness patterns that significantly differ from normal eyes, with significantly lower central and minimum thicknesses and a higher deviation across the cornea similar to the averaged findings in our study.

It seems logical that thinned epithelium overlies thin, protruding stroma and thickened epithelium overlies thin, concave stroma, and that this may occur with reasonable predictability. However, regardless of specific anatomic remodeling patterns, localized epithelial remodeling patterns cannot currently be predicted based on any technique other than direct measurement. Neither corneal curvature nor whole corneal thickness can predict localized regions of stromal concavity or epithelial thickening. Therefore, the epithelial thickness distribution is functionally, clinically unpredictable, resulting in the need to directly measure regional epithelial thickness profiles in situations where significant difference from
expected patterns could have clinical significance. Specific situations where this variability would be important include CXL treatments, ICRS placement in thinner corneas, and topography-guided excimer laser ablations.

Reinstein et al. characterized the epithelial thickness profile of 56 normal eyes using a very high-frequency digital ultrasound. The mean epithelial thickness at the corneal apex was 53.4 ± 4.6 µm and the epithelium was 5.9 µm thicker inferiorly and 1.3 µm thicker nasally at the 3-mm radius. In our study, the corneal epithelium in normal eyes was slightly thicker inferiorly and nasally (Figure 2) but no statistically significant differences were found. Reinstein et al. also analyzed the epithelial thickness profile of 54 eyes with keratoconus using the Artemis very high-frequency digital ultrasound system. The mean epithelial thickness at the corneal apex was 45.7 ± 5.9 µm. The authors reported a localized zone of epithelial thinning surrounded by an annulus of thickened epithelium over the region of the cone (“donut shape”) in all cases. Kanellopoulos et al. reported a larger variation in epithelial thickness in patients with keratoconus and overall thickening of the corneal epithelium in the center of the pupil, with the epithelium being 3.1 µm thicker, using the Artemis-II. The authors also reported a variation of ± 3 to 4 µm in both corneal and epithelial thickness measurements, compared to the values (0.58 µm) reported by Reinstein et al. In our case series, we observed localized areas of thickened epithelium overlying a thinner stroma, occurring particularly in moderate and severe keratoconus cases with thinner epithelium across the corneal apex.

Reinstein et al. reported the efficacy of using these epithelial findings to distinguish true ectatic disease from pseudokeratoconus with epithelial thickening in an otherwise normal cornea. If the findings of regional epithelial thickness variability found in this study prove to be applicable to less advanced ectatic disease presentations, this could be relevant to refractive surgical screening and partially explain the occurrence of postoperative ectasia in patients seemingly without risk factors and with normal topographic findings.
In SD-OCT, a Fourier transformation is used to extract the frequency spectrum of the signal, which is used to generate the high-resolution scans. With SD-OCT it is possible to measure all echoes of light from different delays simultaneously, allowing for significant increases in speed and sensitivity.\(^{26-28}\) Faster acquisition times minimize artifacts from eye motion. There are several implications of the increased speed and sensitivity offered by SD-OCT, including images with a lower signal-to-noise ratio compared to time-domain OCT\(^ {26-28}\) and excellent axial resolution images (axial resolution of 5 \(\mu m\)). Three-dimensional epithelial and total corneal maps can be generated by interpolating thickness profiles from different meridians.\(^ {29}\) However, the resolution of the cross-sectional scans is superior to the pachymetry map scans. In addition, OCT-based corneal topography and pachymetry maps seem promising for evaluating highly irregular corneas despite corneal opacities, as demonstrated by Li et al.\(^ {30}\) and Nakagawa et al.\(^ {31}\)

Despite outstanding axial resolution, the manual task of identifying Bowman layer based on a visual interpretation of the image may be limited by the resolution of the flap and manual tools. It is important to clarify that the epithelial thickness measurements using SD-OCT include the tear film, whereas very high-frequency digital ultrasound measurements do not because these are performed using an immersion technique. However, one of the advantages of SD-OCT compared to very high-frequency digital ultrasound includes its short time of image acquisition and ease of use, allowing clinicians to integrate this technology to their clinic workflow. This is opposite to very high-frequency digital ultrasound, which requires an immersion technique.

Using corneal epithelial thickness profile analysis in the clinical setting may aid in interpretation of corneal topography in clinical and subclinical corneal ectasia, corneal warpage in patients with contact lenses, and residual refractive errors after refractive surgery explained by epithelial remodeling. Furthermore, the direct visualization of areas of reactive epithelial thickening underlying a thin residual stromal bed may improve the safety and efficacy of several corneal procedures, including epithelium-off cross-linking treatments, corneal inlays, and lamellar corneal transplants, and pockets.

SD-OCT high-resolution cross-sectional scans demonstrated significant differences in regional corneal epithelial thickness profiles in eyes with keratoconus and postoperative corneal ectasia compared to normal eyes, with significant regional variability and unpredictability in these ectatic eyes.

**AUTHOR CONTRIBUTIONS**

Study concept and design (JBR, KMR, RDS); data collection (CEP-S, JBR, KMR); analysis and interpretation of data (JBR, KMR, RDS); drafting of the manuscript (JBR, KMR); critical revision of the manuscript (CEP-S, JBR, KMR, RDS); statistical expertise (JBR, KMR); administrative, technical, or material support (JBR); supervision (JBR, RDS)

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**TABLE A**

Demographics and Clinical Features of Eyes With Keratoconus, Eyes With Postoperative Corneal Ectasia, and Normal Eyes

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (SD)</th>
<th>SE (D)</th>
<th>Mean Sim-K (D)</th>
<th>OCT Cpwr (D)</th>
<th>Thinnest CT (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratoconus (n = 49)</td>
<td>36.01 ± 7.48</td>
<td>-4.20 ± 4.39</td>
<td>50.5 ± 5.96</td>
<td>50.12 ± 6.88</td>
<td>424.61 ± 62.08</td>
</tr>
<tr>
<td>Postoperative ectasia (n = 32)</td>
<td>45.63 ± 7.93</td>
<td>-5.05 ± 6.11</td>
<td>44.21 ± 5.06</td>
<td>43.36 ± 5.20</td>
<td>406.50 ± 47.63</td>
</tr>
<tr>
<td>Normal (control) (n = 39)</td>
<td>35.84 ± 6.38</td>
<td>-3.58 ± 3.62</td>
<td>43.40 ± 1.32</td>
<td>43.37 ± 1.21</td>
<td>524.29 ± 14.17</td>
</tr>
</tbody>
</table>

P (ANOVA)*

|                 | .0054       | .0421      | < .001        | < .001        | < .001 |

SD = standard deviation; SE = spherical equivalent; D = diopters; Sim-K = mean Sim-Ks measured by Scheimpflug tomography (Pentacam; Oculus Optikgerate GmbH, Wetzlar, Germany); OCT Cpwr = mean corneal power measured by the SD-OCT Pachymetry map with the additional corneal power software (Cpwr); thinnest CT = corneal thinnest point measured by SD-OCT; ANOVA = analysis of variance

*JMP 9.0 software, SAS Institute, Inc., Cary, NC.