Swelling of the Human Cornea Revealed by High-Speed, Ultrahigh-Resolution Optical Coherence Tomography

Natalie Hutchings,¹ Trefford L. Simpson,¹ Chulbo Hyun,² Alireza A. Moayed,² Sepideh Hariri,² Luigina Sorbara,¹ and Kostadinka Bizheva²,³

PURPOSE. To evaluate the change in thickness of the anterior, stromal, and posterior corneal laminae in response to hypoxia-induced corneal swelling, by means of ultrahigh-resolution optical coherence tomography (UHR-OCT).

METHODS. A UHR-OCT system, operating in the 1060-nm range, was used to acquire in vivo cross-sectional images of human cornea with a 3.2 × 10-μm (axial × lateral) resolution in corneal tissue. Corneal edema was induced by inserting a thick, positive-powered, soft contact lens, over which the eye was closed and patched for 3 hours. Tomograms were acquired from eight non–contact-lens wearers. Baseline images were obtained before inducing corneal edema, immediately after removal of the patch and the lens, and then every 15 minutes for ~2 hours. All images were postprocessed with a segmentation algorithm to identify the laminae visible in the image. The apical thickness of the laminae (epithelium [EPI], epithelial-Bowman’s membrane [Ep-BM] complex, stroma, and endothelial-Descemet’s membrane [En-DM] complex) were determined at each time interval.

RESULTS. There was an interaction between time after removal of the hypoxic stimulus and deswelling of the layers (RM-ANOVA; P < 0.001). The epithelial and stromal thickness reduced significantly with time (P = 0.001; P < 0.001, respectively), whereas the Ep-BM and En-DM complexes did not (P > 0.50). All layers except the En-DM complex exhibited a biphasic pattern of recovery.

CONCLUSIONS. UHR-OCT showed regional differences in swelling due to hypoxic provocation. On removal of the hypoxic stimulus, the rate of recovery varied between layers, and all layers except the En-DM complex exhibited a biphasic recovery. (Invest Ophthalmol Vis Sci. 2010;51:4579–4584) DOI:10.1167/iovs.09-4676

Corneal metabolic function is reflected in corneal thickness. Since corneal deturgescence is tightly controlled, primarily for optical reasons, and as the hydration state changes, so does corneal thickness. This thickness (and corneal epithelial thickness), in addition to normal (diurnal) physiological variation, varies with the environment, in systemic and ocular disease, after surgery, and with contact lens wear, among other influences.

The cornea swells in hypoxic conditions and recovers when normoxia returns. Edema of the cornea is a result of the reduced availability of oxygen (primarily sourced at the epithelial surface), and the barrier function of the epithelium has been shown to be disrupted with 1% hypoxia and due to deprivation of oxygen after 1 hour of eye closure with a low-Dk/t lens. The endothelial barrier is the primary gatekeeper of edema, as the extent of edema is greater when the endothelial barrier is removed than with removal of the epithelial barrier. The rate of recovery of hypoxia-induced edema is thought to represent the endothelial pump function. In humans, extended eye closure during contact lens wear is an effective model of hypoxia and has been used often to model the normal physiological response of the cornea as well as in altered conditions in postsurgical and lens-wearing patients.

It has been shown that the cornea swells regionally under these conditions, with the greatest swelling occurring in the anterior and posterior stroma. These measures were made with clinical optical coherence tomography (OCT) systems with limited axial resolution but having been previously proposed to be particularly useful in monitoring corneal physiological changes, particularly thickness and scatter. We therefore examined hypoxia-related corneal swelling and deswelling by using ultrahigh-resolution optical coherence tomography (UHR-OCT). UHR-OCT has considerably higher spatial resolution than OCTs that have been used to examine corneal swelling and deswelling, and thus enabled us to segment the cornea into detailed, identifiable laminae.

The objectives of the study were to quantify regional lamellar swelling after the hypoxic provocation of eye closure and contact lens wear and to quantify deswelling after eye opening and lens removal, to infer metabolic differences in these layers using an UHR-OCT.

METHODS

The imaging probe of the state-of-the-art, high-speed UHR-OCT system was modified to enable in vivo acquisition of two-dimensional, cross-sectional images of the human cornea. Briefly, the device utilizes a low coherence light source (Superlum, Ltd., Carrigtwohill, Ireland) with a spectrum centered at 1020 nm and a spectral bandwidth of 110 nm used in combination with a linear array CCD camera (47kHz line rate; InGaAs; SU1 Goodrich, Princeton, NJ), a detector array with optimal efficiency for the source used. In corneal tissue, the system provides 3.2-μm (axial) and 10-μm (lateral) resolution and a Rayleigh range of ~300 μm. The image-acquisition rate of 47,000 A-scans/s corresponds to 38 frames/s. The optical power of the imaging beam incident on the cornea was limited to 1.3 mW, which is well below the maximum permissible exposure, as defined by the ANSI standard. At this imaging power, the measured sensitivity of the UHR-OCT system.
was 102 dB. In the absence of literature describing swelling of the Bowman’s endothelial and Descemet’s layers, study sample size was determined on the basis of power calculations of effect size, to show corneal and epithelial swelling. Images were obtained from one eye of eight non-contact-lens wearers (age range, 22−56 years). During image acquisition, fixation was controlled by means of an external LED derived from the absolute pixel distance, corrected for resolution, with a tissue refractive index of 1.376.

A series of two-dimensional tomograms (1000 × 512 pixels corresponding to −5 × 1-mm physical distance) were acquired from approximately the same location in the cornea in each volunteer. Initially, the condensing lens of the imaging probe was positioned approximately level with the corneal apex. The central specular reflection was avoided just barely by shifting the position of the imaging probe inferiorly by the smallest increment at which the optimum reflection was avoided just barely by shifting the position of the imaging probe inferiorly by the smallest increment at which the optimum reflection was avoided just barely by shifting the position of the imaging probe.

Tissue boundaries were identified with a semiautomated segmentation algorithm. In the first step, the region of interest was manually defined by identifying the front and back surface of the cornea. In the second step, the segmentation algorithm sought four layers within this region (Fig. 1). The definition was achieved by maximizing the fitting criteria of a fourth-order polynomial function (based, initially, on the characteristics of the curvature of the manually defined surfaces) across the central 200 pixels of each two-dimensional scan. The search criteria for the maxima followed a path that was perpendicular to the surface and moved in a posterior direction from the front surface for the epithelium/Bowman’s membrane (Ep-BM) complex and (Ep-BM)/stroma interfaces and moved in an anterior direction from the back surface for the endothelial/Descemet’s membrane (En-DM) complex/stroma interface.

Segmented images were individually reviewed, and in cases in which the segmentation algorithm had failed, the images were discarded. The apical thickness of the epithelium, Ep-BM complex, stroma, and posterior En-DM complex were estimated as the average difference between each segmented layer across the region of interest. Repeatability of the thickness measures for each layer and for the total corneal thickness was assessed by using the intraclass correlation coefficient (ICC).

was derived from the absolute pixel distance, corrected for resolution, with a tissue refractive index of 1.376.

Table 1. Demographic and Refractive Data of the Study Sample

<table>
<thead>
<tr>
<th>ID</th>
<th>Age</th>
<th>Sex</th>
<th>Refractive Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>F</td>
<td>OD emmetropic</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>F</td>
<td>OD emmetropic</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>M</td>
<td>OD −8.25 DS</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>F</td>
<td>OD −3.00 DS</td>
</tr>
<tr>
<td>5</td>
<td>41</td>
<td>F</td>
<td>OD −2.25/−0.50 × 135</td>
</tr>
<tr>
<td>6</td>
<td>46</td>
<td>F</td>
<td>OD −4.75/−1.25 × 013</td>
</tr>
<tr>
<td>7</td>
<td>53</td>
<td>M</td>
<td>OD +0.75/−0.50 × 180</td>
</tr>
<tr>
<td>8</td>
<td>56</td>
<td>F</td>
<td>OD +0.25/−0.75 × 035</td>
</tr>
</tbody>
</table>

TABLE 2. Mean Thickness of the Layers

<table>
<thead>
<tr>
<th>Layer</th>
<th>Before Hypoxia</th>
<th>After Hypoxia</th>
<th>Average Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelium</td>
<td>58.40 ± 1.04</td>
<td>59.14 ± 1.26</td>
<td>0.74 ± 0.83</td>
</tr>
<tr>
<td>Ep-BM</td>
<td>18.33 ± 0.46</td>
<td>19.37 ± 1.22</td>
<td>1.04 ± 0.36</td>
</tr>
<tr>
<td>Stroma</td>
<td>519.22 ± 7.51</td>
<td>568.53 ± 8.13</td>
<td>49.31 ± 6.97</td>
</tr>
<tr>
<td>En-DM</td>
<td>18.52 ± 0.95</td>
<td>18.60 ± 0.84</td>
<td>0.08 ± 0.63</td>
</tr>
<tr>
<td>Total</td>
<td>613.91 ± 7.24</td>
<td>658.40 ± 7.71</td>
<td>51.20 ± 6.61</td>
</tr>
</tbody>
</table>

Data are expressed as mean micrometers ± SE. Thickness was determined on the basis of power calculations of effect size, to show corneal and epithelial swelling. Images were obtained from one eye of eight non-contact-lens wearers (age range, 22−56 years). During image acquisition, fixation was controlled by means of an external LED derived from the absolute pixel distance, corrected for resolution, with a tissue refractive index of 1.376.

FIGURE 1. A representative two-dimensional UHR-OCT scan of the human cornea obtained in the prehypoxic condition before (A) and after (B) segmentation in a 27-year-old female participant. Four distinct layers were defined: epithelium (EPI), Ep-BM, stroma (STR), and En-DM.

FIGURE 2. The overall change in thickness for the sample as a whole from the time of corneal thickness was assessed by using the intraclass correlation coefficient (ICC).
coefficient (ICC) and correlation coefficient of concordance (CCC) between eight images obtained at two different acquisitions. ICCs for all layers were /H11022 0.912 for images of the baseline and for images of the hypoxic conditions. The corresponding CCCs ranged between 0.770 and 0.940 for the baseline condition and /H11022 0.933 for the hypoxic condition.

Repeated-measures ANOVAs were used to examine the main effects of time and position on the outcome variable percentage of swelling. In addition, post hoc tests (Tukey HSD) were used to determine pairwise differences between swelling measures after eye opening, and one-sample t-tests were used to examine whether swelling differed from zero. For the En-DM measurements, there were three outliers (each from different subjects) that were not used in the analysis or reported in the figures.

RESULTS

The mean thicknesses of the identified layers are shown in Table 2. Thicknesses were obtained by dividing the optical thickness of each layer by the average refractive index of corneal tissue: 1.376.

The overall corneal swelling and deswelling are shown in Figure 2. Time was a statistically significant predictor, and the interaction between time and position was also significant (both \( P < 0.001 \)). The RM-ANOVA results are shown in Table 3. Post hoc comparisons generally showed that there were significant differences in swelling at time points separated by two steps, except for the first (different from the next consecutive step) and the last three (no difference across steps). One-sample t-test showed that swelling was not significantly different from 0 after an interval of 100 minutes (as is also illustrated by the 95% confidence intervals).

Regional Deswelling

The interaction indicated that the effect of time needed to be determined for each layer. Significant effects of time on swelling were found for the epithelium (\( P = 0.011 \)) and the stroma (\( P < 0.001 \)), but not for the Ep-BM complex and the En-DM complex (\( P = 0.512, P = 0.658 \), respectively).

The decline in thickness after removal of the lens showed a two-phase recovery; an initial rapid phase of thickness change followed by a phase with stabilized thickness.

Epithelial thickness had increased from baseline at the first measure after lens removal and then decreased beyond baseline values in recovery. The stroma and total corneal thickness increased from baseline and then decreased to baseline in recovery. Figure 3 shows the group mean change in thickness for the Ep-BM complex, stroma, and En-DM complex.

Rate of Deswelling

A bilinear function was fitted to the data to investigate the rate of recovery for each structure and the time interval at which stabilization of the change in thickness occurred. The function was fitted to the group average thickness over 15-minute intervals. Table 4 shows the regression parameters. The thickness change from baseline was different between structures, but the magnitude of change from the first measure to stabilization was similar (~10%). The rate of thinning before stabilization was different between structures, with the anterior lamellae structures showing thickness decreasing at a greater rate than the stroma. Although the Ep-BM complex did not show a significant difference between

![Figure 3](image-url)
measures (RM ANOVA; F = 0.27, df = 10; P = 0.623), it too exhibited a biphase pattern of recovery.

**Discussion**

In this study, we identified separate layers of the cornea, including fine layers anterior and posterior to the stroma. These layers were nominally labeled the Ep-BM complex and the En-DM complex. The thicknesses obtained in prehypoxia conditions (Table 1) corresponded approximately to histology, reported measures of the epithelium, total corneal thickness, obtained with a low-resolution OCT, and epithelial thickness obtained with the confocal Rostock laser scanning microscope and a VHF ultrasound system (Artemis; Ultralink, St. Petersburg, FL). Therefore, even though the device (with its current sagittal scanning design) does not unambiguously image individual cells, the physical thickness of the layers derived by the backscattered light are very similar to these histologically defined structural regions. The total thickness of the cornea was found to be thicker than those obtained with the confocal Rostock laser scanning microscope. This difference may have arisen as a function of the values used for the refractive index. In this study, physical thickness of the cornea was computed by using an average refractive index of 1.376, although the layers undoubtedly have different refractive indices that are not known for the individual layers.

The corneal swelling response with lens wear and eye closure averaged (across the group) −3.5% and −10% for the epithelium and total cornea, respectively. This magnitude of swelling is within the range found previously and deswelling rates are also similar to those reported for the epithelium and total corneal thickness with in vivo optical methods. As with the prehypoxia condition, the boundaries of the layers in the posthypoxia condition were identified with a polynomial model that sought the position of minimum intensity and maximum gradient in intensity. Three assumptions were made: First, the optical interfaces occur at or near the physical (cellular) interfaces and correspond with the position of minimum intensity and maximum gradient; second, a polynomial approximation is a reasonable model to determine the position of the interface across the examined area, and local differences across the region are a minor factor in the overall swelling response; and third, the optical and/or physical characteristics of the boundaries are similar in the prehypoxia and posthypoxia conditions. As a first approximation, these appear to be reasonable assumptions because, as stated previously, the thickness of the identified layers in the prehypoxia condition corresponded reasonably with values obtained from histology (which themselves have an inherent error due to the preparation required for visualization of ex vivo samples) and, over the relatively small portion of the central cornea examined, adjacent structures may be more likely to behave similarly than different.

In normal configuration, light-scattering in the stroma is thought to be primarily due to the nuclei of keratocytes and contributes ~1% of the total scattering observed, most being observed at the anterior surface of the epithelium and the posterior endothelium. With edema, the extrafibrillar matrix in vitro appears to increase in volume, resulting in a reduction of refractive index of the stroma, but this does not seem to represent the total extent of increased scattering observed with swelling. If the swelling is accompanied by injury, decreased crystallin expression as a function of the healing mechanism also appears to contribute to increased light scattering. It is clear that the dispersion of the source as it traverses the corneal structure can be affected by several factors, even in the absence of injury. One advantage of the UHR-OCT system used in this study is the illumination source used. Water dispersion has a null at 1060 nm and changes slowly over the spectral range of the source and, as a result, the axial resolution is preserved throughout the entire thickness of the cornea. This advantage is not inherent to OCT systems using illumination sources of ~800 nm. It could be argued that the imaging procedure adopted in the study by translation of the imaging probe inferiorly to avoid the strongest central specular reflection represents a shortcoming of the imaging method. The specular reflex is an intrinsic complication of imaging the cornea and represents an artifact in the image that must be managed. If the image is acquired with the strongest specular reflection present, the contrast of the boundaries posterior to the air–epithelium interface is reduced, and the data from the central portion of the image become unusable. Reducing the power of the illuminating beam to reduce the strength of the reflection concurrently reduces the contrast of lateral portions of the image and the deeper layers of the cornea to an unusable level. For these reasons, a mechanical translation away from the position orthogonal to the central apex, while maintaining orthogonal alignment with the apical region of the cornea, was selected as the preferable option. Although this may inevitably have introduced some error into the measurement of thickness, averaging across the central 200 pixels and across images would minimize the error to some extent. In addition, the thicknesses obtained were in the expected range and were repeatable. Thus, we conclude that the technique, while perhaps not ideal, is valid.

The response to hypoxia was different among the various layers. The En-DM layer did not show a significant difference in pre- and posthypoxia conditions. The epithelium showed a ~3.5% increase in thickness from the prehypoxia condition and a deswelling phase that stabilized to a thickness below baseline values. This relative epithelial thinning has been reported in corneas recovering from hypoxia, and central epithelial thinning has also been reported in a cohort of long-term soft contact lens wearers. The finding may occur as a result of an autoregulation effect leading to continuous extraction of water beyond the baseline level, or it may be a mechanical effect associated with lens wear. The stroma and total corneal thickness showed an increase of ~10% in thickness from the prehypoxia condition. Although our experiment demonstrates that the proportional swelling response is different between layers we assume, but do not know, that the relationship between the backscattered light in the pre- and posthypoxia conditions, in terms of the parameters used in our model (minimum intensity and maximum gradient) may alter in absolute terms, but remain consistent in relative terms.

The epithelium and endothelium are barriers that prevent water from entering the stroma, to maintain the delicate balance of hydration and transparency of the corneal structure. The transparency of the cornea appears to be a function of the precise arrangement of the collagen fibrils in the stroma and the uniformity of the refractive index of the extracellular matrix, with the endothelial barrier acting as an active pump to maintain transpar-

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**Table 4. Parameters for Bilinear Fit of the Recovery of Corneal Swelling Response over Time to Follow-up for the Layers Studied**

<table>
<thead>
<tr>
<th></th>
<th>Epithelium</th>
<th>Ep-BM Complex</th>
<th>Stroma</th>
<th>En-DM Complex</th>
<th>Total Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept, %</td>
<td>+3.46</td>
<td>+10.70</td>
<td>+9.90</td>
<td>0</td>
<td>+8.70</td>
</tr>
<tr>
<td>Slope, %/min</td>
<td>−0.172</td>
<td>−0.187</td>
<td>−0.090</td>
<td>0</td>
<td>−0.085</td>
</tr>
<tr>
<td>Time to break, min</td>
<td>44.4</td>
<td>41.6</td>
<td>98.1</td>
<td>—</td>
<td>98.0</td>
</tr>
</tbody>
</table>

All fits, P < 0.05. The En-DM did not show a change in thickness.
cornea. These rates of recovery are similar to those found in the of recovery of 6.1%/h for the stroma and 5.3%/h for the total of time to reach a stabilized thickness (and total corneal thickness took approximately twice the length was similar at approximately 40 minutes. Conversely, the stroma complex 15.4%/h). The time to stabilization for both structures showed minimal change in thickness from the baseline thickness but showed different rates of recovery to a stabilized thickness between the posterior lamellae. In this study, we identified a different rate of recovery and return to a stabilized thickness for the posterior stroma and maintain stasis of the corneal water content. It would therefore be expected that the anterior lamellae may swell less and/or show a more rapid rate of recovery than the posterior lamellae. In this study, we identified a different rate of recovery and return to a stabilized thickness between the various layers identified. The epithelium and Ep-BM complex showed minimal change in thickness from the baseline thickness but showed different rates of recovery to a stabilized thickness after removal of the hypoxic stimulus (epithelium, 4.7%/h; Ep-BM complex 15.4%/h). The time to stabilization for both structures was similar at approximately 40 minutes. Conversely, the stroma and total corneal thickness took approximately twice the length of time to reach a stabilized thickness (~98 minutes) with a rate of recovery of 6.1%/h for the stroma and 5.3%/h for the total cornea. These rates of recovery are similar to those found in the epithelium and total cornea with a lower-resolution OCT.55

The En-DM complex did not exhibit a significant increase in thickness between the pre- and posthypoxia conditions. Examination of the data for the group as a whole (Fig. 3) and for individual subjects (Fig. 4) reveals a more variable response than for the other layers identified. There are several possible explanations for this observation. First, as this layer is very thin and close to the maximum resolution of the device, dispersion of the source as it traverses the anterior tissues would have the greatest impact at this layer. As a consequence, it may be expected that the identification of the boundaries of the inner layer would be more difficult to segment. The SD of the fit of the model within individual subjects was similar to that of other layers, suggesting that the high group variability did not derive simply from technical issues related to the device or algorithm. Second, the dissimilarities in the recovery profile between subjects could result from within- or between-subject variations. It has been reported that both the metabolic activity and the endothelial function are two factors that contribute to corneal swelling.53,57 The interaction of these and other factors in the recovery from edema may occur to different extents between subjects and at different time points after removal of the hypoxic stimulus for each subject, resulting in a lack of systematic behavior for the group over time. A third and related possibility is that the En-DM complex response is more dynamic than the biphasic changes observed in other layers. Last, central corneal thickness has been found to be related to the endothelial cell density in young, normal subjects,58 and there is a reduction in endothelial cell density with age.59 It may be that the between-subject variability is due to different endothelial cell densities across the various subjects’ ages in our sample, leading to a variation in the endothelial pump capacity across the sample. However, it has been reported that there is little relationship between endothelial cell density and the permeability of the endothelial barrier with age.54 In summary, using UHR-OCT and an automated segmentation algorithm, we found regional differences in swelling induced by hypoxia. On removal of the hypoxic stimulus, the rate of recovery varied among layers, and all layers except the En-DM exhibited a biphasic recovery.

Acknowledgments

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References
