In Vivo Imaging and Morphometry of the Human Pre-Descemet’s Layer and Endothelium With Ultrahigh-Resolution Optical Coherence Tomography

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PURPOSE. To visualize in vivo and quantify the thickness of the posterior corneal layers: the acellular pre-Descemet’s layer (PDL), Descemet’s membrane (DM), and endothelium (END) in healthy subjects, using ultrahigh-resolution optical coherence tomography (UHR-OCT).

METHODS. A research-grade, 800-nm UHR-OCT system with 0.95-μm axial resolution in corneal tissue was used to image in vivo the posterior cornea in healthy subjects. The system offers approximately 98 dB sensitivity for 680 μW optical power incident on the cornea and 34,000 A-scans/s image acquisition rate. This study comprised 20 healthy subjects, aged 20 to 60 years. The thickness of the PDL, DM, and END layers was measured both with a custom, automatic segmentation algorithm and manually.

RESULTS. The boundaries and structure of the posterior corneal layers were clearly visible in the UHR-OCT images. The average thickness was measured to be 6.6 ± 1.4 μm (PDL), 10.4 ± 2.9 μm (DM), and 4.8 ± 0.4 μm (END), which agrees well with published data from ex vivo studies. Both the END and DM thickness showed minor spatial variations, whereas the PDL showed up to 2× thickness change for different locations on the same cross-sectional corneal image or over the entire imaged region of the cornea.

CONCLUSIONS. Our data indicate that all three layers of the posterior cornea can be clearly visualized in vivo and their thicknesses measured precisely with UHR-OCT. Although the PDL thickness showed large spatial variations, the thickness of the DM and END layers was consistent over the entire imaged region of the cornea.

Keywords: optical coherence tomography, cornea, Descemet’s membrane, endothelium, pre-Descemet’s layer, Dua’s layer

New treatment options for advanced corneal degenerations and dystrophies include lamellar keratoplasties, such as deep anterior lamellar keratoplasty (DALK), Descemet’s membrane (DM) endothelial keratoplasty (DMEK), and Descemet’s stripping automated endothelial keratoplasty (DSEK). These precise surgical techniques require knowledge of the exact locations of the corneal sublayer boundaries, and could benefit greatly from knowledge regarding the morphology and biomechanical properties of the posterior corneal layers.

There have been recent advancements in the knowledge of corneal anatomy, specifically regarding the pre-Descemet’s layer (PDL) in the posterior cornea. Although the existence of this posterior stromal layer has been known for a while, its unique properties and role as a distinct layer of the corneal stroma have not been investigated until very recently. Work by Dua et al.2 has contributed to the biomechanical and morphologic understanding of this layer that was briefly termed “Dua’s Layer.” To demonstrate the uniqueness of the PDL, Dua et al.2,3 have shown that this layer is impermeable to air, contains little or no keratocytes, has a different collagen composition than the rest of the stroma, and is continuous with the collagen framework of the trabecular meshwork beyond the corneal borders.

The PDL has been shown to play an important role in corneal surgery, particularly for DALK and endothelial keratoplasties.3–5 Specifically, it appears that the role of this relatively thin and strong layer is to aid in the big-bubble technique that is used in DALK to separate the stroma from the DM. It has been found that the big-bubble technique does not bare the DM as previously thought; instead, a strong PDL separates the stroma from the DM. The PDL in the central 8.5-mm zone can be easily separated from the rest of the stroma and therefore limits the zone size of the big bubble when inserted. The purpose of leaving behind this layer is to add strength to the wound with minimal residual stromal tissue that may lead to better success with this technique due to the improved shape, clarity, and biomechanical properties of the host cornea after surgery.2–4 It is important to know the exact location of this layer, because it has been shown that if the big bubble is injected too superficially, then the stroma will become cloudy, obstructing the surgeon’s view of the posterior cornea and anterior chamber and if injected too deeply then perforation may occur.5 More recently, it was shown that the
PDL may also play a role in corneal pathology when considering the mechanism behind the development of corneal hydrops. Therefore, fast, noninvasive in vivo imaging and precise thickness measurement of the posterior corneal layers, PDL, DM, and endothelium (END), would be a valuable procedure for lamellar keratoplasties.

So far, the PDL has been imaged and morphometrically quantified only ex vivo in the human and animal cornea. In vivo corneal imaging modalities, such as slit scan imaging or videokeratography, do not provide sufficient axial resolution to allow for in vivo visualization of the PDL boundaries with the stroma and the DM. Although confocal microscopy (CM) offers sufficient high axial resolution to image the PDL, the DM, and endothelium (END), it is not possible to measure the PDL thickness with high precision. In vivo CM imaging of the human cornea requires contact of the imaging probe with the corneal tissue to generate images with sufficient lateral resolution to visualize individual cells. Confocal microscopy generates enface images of the imaged biological tissue and its sectional ability in depth (axial resolution) are determined by the Rayleigh range of the imaging optics. Therefore, for three-dimensional imaging, CM requires clear morphologic features that can be identified and used as markers to determine the exact depth location from which an enface CM image was acquired. In case of the cornea, the fairly uniform structure of the stroma causes large ambiguity regarding the exact depth location from which an enface CM image is acquired. Furthermore, because the PDL is almost optically clear and does not contain large enough or easily identifiable morphologic features, and has thickness larger than the axial resolution of the CM system, it is not possible to measure the PDL thickness in vivo and precisely with CM.

Optical coherence tomography (OCT) is a noninvasive optical imaging method capable of generating cross-sectional and volumetric images of biological tissue with cellular-level resolution without ambiguity of the depth. Over the past 25 years, OCT has found a wide range of biomedical applications, including imaging of the human cornea in health and disease. Recent developments of broad-bandwidth light sources and high-speed, large pixel-number cameras resulted in development of spectral-domain (SD) and full-field ultrahigh-resolution (UHR)-OCT technology with axial resolution close to or below 1 μm, suitable for imaging the cellular and subcellular structure of biological tissue. When applied to the cornea, UHR-OCT technology was able to image in vivo some of the major corneal layers, such as the epithelium (EPI), Bowman’s membrane (BM), the stroma, and the Descemet’s–endothelial complex; visualize and count keratocyte cells in the corneal stroma; measure in vivo physiological changes in the cornea; and quantify the tear film thickness. Optical coherence tomography imaging technology is well suited to meet the challenges of in vivo imaging of the posterior corneal layers. The resolution achieved by these instruments could potentially visualize the PDL, DM, and END, and allow more detailed assessments of corneal structure and precise thickness measurement of the individual corneal layers. These capabilities can be used to optimize anterior segment ophthalmic surgeries, particularly for lamellar keratoplasties, and to advance scientific knowledge of the natural history of both healthy and diseased corneas. Optical coherence tomography image acquisition is fast and noninvasive, which is a practical advantage for patients and clinicians.

In this article, we present for the first time, in vivo cross-sectional images of the PDL, the DM, and the corneal END, acquired in healthy human subjects with a sub-1-μm axial resolution OCT system. We also report results from morphometric analysis of all three posterior corneal layers.

**Materials and Methods**

**Ultrahigh-Resolution OCT System**

The research-grade UHR-OCT system used for this study was based on a fiber-optic Michelson interferometer, powered with a supercontinuum laser (SuperK; NKT Photonics, Birkerød, Denmark). The output of the laser was filtered to generate a spectrum centered at 785 nm with full width at half maximum spectral bandwidth of approximately 250 nm. The corneal imaging probe of the UHR-OCT system consisted of an achromat doublet collimator (f = 10 mm), a beam expander composed of 2 achromat doublets (f = 40 mm and f = 80 mm), and a 5× near infrared radiation microscope objective (Mitutoyo Sakado, Takatsuku, Kawasaki, Kanagawa, Japan). The imaging probe was mounted on a modified slit-lamp platform. The detection end of the UHR-OCT system was composed of a customized commercial spectrometer (Wasatch Photonics, Durham, NC, USA), interfaced with a 8192-pixel, 34-kHz complementary metal-oxide semiconductor (CMOS) camera (Piranha NH-800-08K-10; Teledyne Dalsa, Waterloo, ON, Canada). With this design, the UHR-OCT system provided approximately 0.95-μm axial and approximately 5-μm lateral resolution in corneal tissue. To ensure safety of the imaged subjects, the optical power of the imaging beam incident on the corneal surface was limited to approximately 680 μW, which is below the maximum permissible exposure as specified by the American National Standards Institute. The sensitivity of the system near the zero delay line was measured to be 98 dB with approximately 10-dB roll-off over 1-mm scanning range.

**Subjects**

This study was conducted under a protocol approved by the Research Ethics Committee at the University of Waterloo and was carried out in compliance with the tenets of the Declaration of Helsinki. Twenty healthy female and male subjects, with ages varying from 20 to 60 years, were recruited for this project. All subjects passed a slit-lamp bio-microscopy screening and provided written consent for participation in the study.

**Imaging Procedure**

In this study, the left eye of all participants was imaged. Volumetric images (512 × 512 × 8192) were acquired from approximately 1 × 1-mm area in the central part of the cornea, located slightly inferior of the corneal apex, to avoid the back-reflection imaging artifact at the apex. Because acquisition of the volumetric images took approximately 8 seconds, the subjects were instructed to fixate on a target, blink normally during the alignment part of the imaging procedure, and keep the eye open during the image acquisition. When necessary, the imaging procedure was repeated after a brief resting period.

**Image and Data Analysis**

All images were numerically dispersion compensated up to the ninth order with a custom Matlab-based algorithm (Mathworks, Natick, MA, USA). Next, a custom automatic algorithm developed by our group was used to automatically segment and determine the thickness of the END. Because the boundary between the DM and the acellular stromal layer appears diffuse...
on the UHR-OCT images, and the PDL boundary to the stroma is defined as the last layer of keratocyte cells, which appear as a string of interspaced highly reflective dots in the UHR-OCT images, the existing automatic segmentation algorithms failed to generate reproducible results for the thickness of the of the DM and PDL. Therefore, in our study, multiple locations were identified along DM/PDL and the PDL/stroma boundaries in each cross-sectional corneal image and the shortest distance between the selected points was calculated. The total thickness of the DM and the PDL were computed as an average from all measurement locations, whereas the END layer thickness was computed from the automatic segmentation data.

RESULTS

Figure 1A shows a representative cross-sectional image of the healthy human cornea acquired with the UHR-OCT system. All five major corneal layers (the EPI, BM, stroma, DM, and the END) are clearly visible on the tomogram. Because of the relatively large change in refractive index between extracellular space (n ≈ 1.35) and cell nucleus (n ≈ 1.4), keratocyte cells within the corneal stroma appear as highly reflective white dots in the UHR-OCT image. Figure 1B shows a typical hematoxylin-eosin (H&E)-stained histologic cross-section of the healthy human cornea. A 10X vertically magnified view of the posterior section of the cornea marked with the white dashed line rectangle in Figure 1A is presented in Figure 1C. It shows well-defined boundaries of the corneal END and hyperreflective dots inside that layer, such as those marked with the green arrows, which considering their location and reflectivity properties most likely correspond to nuclei of the endothelial cells. The boundary between the DM and the stroma is also very clearly visible, although of somewhat lower contrast compared with that of the END. The magnified image of the posterior cornea (Fig. 1C) also shows a layer of very low optical reflectivity located between the upper boundary of the DM and the last posterior layer of keratocyte cells (reflective dots in Fig. 1C, marked with red arrows). A magnified view of the posterior end of the histologic corneal image (Fig. 1D) shows the PDL as an acellular layer positioned between the DM and the stroma with distinctly visible keratocyte cells (marked with red arrows) located at the boundary between the PDL and the corneal stroma. Anatomically, the existence of an acellular layer at the boundary between the corneal stoma and the DM has been known for a long time, and more recently, the thickness of the PDL was measured ex vivo in human eyes.

To the best of our knowledge, our study presents the first in vivo images of the PDL and provides the first in vivo measurement of the PDL thickness.

Figure 2 shows representative cross-sectional corneal images acquired from two different subjects. The image from one subject (Fig. 2A) shows almost consistent thickness of all three layers of the posterior cornea. In contrast, the image acquired from the other subject (Fig. 2B) shows extreme spatial variations in the PDL thickness (thickness at the location marked with the red arrow is approximately two times the thickness measured at the location marked with the green arrow), whereas the thickness of the DM and the END for each subject varied only slightly with the spatial location.

Figure 3 shows statistical data for the thickness of each of the posterior corneal layers. The thickness of the END was found to be 4.76 ± 0.4 μm (range, 4.14–5.78), for the DM was 10.44 ± 2.9 μm (range, 6.5–14.6), and for the PDL was 6.5 ± 1.4 μm (range, 4.7–9.67).

DISCUSSION

Similar to previously published studies, the epithelial layer, the BM, and keratocyte cells in the stroma can be clearly visualized in the corneal images acquired with the UHR-OCT system (Fig. 1A). A former study conducted by our research groups has shown that hyperreflective spots in the corneal stroma observed in UHR-OCT images most likely correspond to keratocyte cells, by measuring the keratocyte cell density from the UHR-OCT tomograms and comparing the results with data acquired in vivo with CM, as well as data available from histology.

In the current study, the high axial OCT resolution also allowed for visualization of both the boundaries and the internal structure of the END, DM, and PDL. Highly reflective dots observed midway between the boundaries of the END layer (Fig. 1B, green arrows) most likely correspond to endothelial cell nuclei, considering their location and the difference in refractive indices between cytoplasm (n ≈ 1.36) and nucleus (n ≈ 1.4). However, we could not verify this hypothesis by selecting a transverse cross-section through the middle of the END from the volumetric corneal data, due to severe motion artifacts associated with the low speed of the camera relative to the fast eye motion. Although faster CMOS cameras with readout rates up to approximately 150 kHz are commercially available, their spectral response in the 850 to 950 nm range is not as...
**FIGURE 2.** Vertically magnified ($\times 10$) regions of the posterior stroma for two representative subjects in the study are shown. One of the subjects (A) has DM thickness comparable to the END and PDL thicknesses, and the PDL thickness is fairly constant throughout the entire scanned region of the cornea. The DM thickness for the other subject (B) is almost double the END thickness, whereas the PDL shows special thinning (green arrow) and thickening (red arrow).

**FIGURE 3.** Statistical data for the thickness of the corneal END (A), the DM (B), and the PDL (C).
efficient as the spectral response of the camera in our UHR-OCT system, which would compromise the axial OCT resolution. Furthermore, for most of the high-speed cameras, the readout rates greater than 70 kHz are available for reduced number of pixels (2048 or less), which would shorten the scanning range of the UHR-OCT system by two times. Another way to increase the image-acquisition rate and reduce motion artifacts is to reduce oversampling of the OCT images in the X and Y direction. In our study, we used 2.5 times oversampling in both directions to generate higher-quality images in which hyper-reflective spots in the corneal stroma (corresponding to keratocytes) and in the END (most likely corresponding to cell nuclei) are clearly visible. Reducing the oversampling by a factor of two times would allow for four times faster imaging of the same region of the cornea and reduce significantly the appearance of motion artifacts in the UHR-OCT images. However, it will also reduce significantly the visibility of fine morphologic details in the corneal UHR-OCT images.

In general, some motion artifacts can be suppressed or eliminated through the use of motion-correction algorithms. Both custom motion-correction algorithms developed by our group and algorithms published by other research groups were tested on the volumetric corneal data generated in this study; however, not all motion artifacts were eliminated, which prevented the generation of enface images of the endothelial cellular structure. Future improvement in the camera technology and motion-correction algorithms would potentially allow for visualization and counting of endothelial cell nuclei from enface projections generated with our UHR-OCT system.

The collagen fibers of the DM are oriented randomly; therefore, the appearance of the DM in the OCT images in terms of speckle pattern and contrast varied significantly between tomograms acquired from the same subject, depending in the orientation of the collagen fibers relative to the plane of the cross-sectional OCT image. Some tomograms showed a dot-like speckle pattern for the DM, whereas the DM appeared striated on other images. In most images, the PDL appeared of low reflectivity and almost devoid of structure, which is consistent with the acellular structure of that layer as reported from histologic studies. The magnified view of the histology image of the posterior cornea (Fig. 1D) shows keratocyte cells lined up along the boundary between the PDL and the corneal stroma in similar pattern to what is observed in the magnified view of the UHR-OCT tomogram (Fig. 1C). Similar to other publications, for the PDL thickness analysis, we considered the most posterior layer of keratocytes cells to mark the boundary between the PDL and the corneal stroma.

The thicknesses obtained for each of PDL and DM are in agreement with current literature using ex vivo images. The studies by Dua et al., examining donor eyes on a population ranging in age from 53 to 94 and average age of 77.7 years, which was older than our sample, found the PDL to be 10.15 ± 3.6 μm (range, 6.3–15.83) and DM to be 10.97 ± 2.4 μm (range, 7.8–13.98). There are several factors that could explain the difference in the PDL thickness reported by Dua et al. and our measurements. The study by Dua et al. was carried out on ex vivo donor corneas, where artificial thickening of the PDL may occur due to tissue processing. Furthermore, the PDL thicknesses with age, according to the analysis by Dua et al. of Marshall and Grindle’s work.

Endothelial thickness varies with age, becoming thinner with time as the endothelial cells flatten. According to DelMonte and Kim, it ranges from 10 μm at birth to approximately 4 μm in adulthood. The average endothelial thickness of 4.8 μm measured in vivo in our study agrees well with the results of DelMonte and Kim. Due to the limited scanning range of our UHR-OCT system (approximately 1 mm) and image-acquisition rate, as well as the curvature of the human cornea, our study was restricted to imaging a small region (approximately 1 × 1 mm) of the central cornea. Future advances in camera technology (faster linear array cameras with larger number of pixels) or tuneable lasers could allow for development of SD-OCT and swept-source OCT technology by two times, another way to increase the pre-Descemet’s layer (Dua’s layer).

CONCLUSIONS

This study demonstrated that UHR-OCT technology with approximately 1-μm axial resolution is able to visualize the boundaries and the internal structure of the corneal END and DM, as well as to measure precisely the layer thickness. It also allowed for the first time for in vivo visualization and morphometry of the PDL. These data, being the first in vivo measurement of the posterior corneal layers, will be valuable in further characterization of this unique division of the stroma primarily for surgeons and for a better understanding of the pathophysiology of the cornea. Further studies are needed to elucidate the exact role of the PDL in the mechanism and management of corneal disease.

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