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Measurements of Microkeratome Cuts in Donor Corneas with Ultrasound and Optical Coherence Tomography

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Abstract

Purpose—To evaluate optical coherence tomography (OCT) in the measurement of donor corneas in preparation for endothelial keratoplasty.

Methods—Donor corneas were imaged by OCT while immersed in preservation medium. Central corneal thickness (CCT) was measured by OCT from Bowman's layer to the endothelium. The corneas were then mounted on an artificial anterior chamber and the epithelium removed. Ultrasound pachymetry (USP) was used to measure CCT just prior to sectioning with a microkeratome. The central endothelial graft thickness (CGT) was measured by USP. The graft was then return to the medium and imaged by OCT.

Results—The study included 154 donor corneas. The average CCT by OCT ($550 \pm 63 \mu\text{m}$) was thicker ($p < 0.001$) than that measured by USP ($507 \pm 54 \mu\text{m}$). Similarly, the CGT by OCT ($158 \pm 41 \mu\text{m}$) was thicker ($p < 0.001$) than that measured by USP ($153 \pm 38 \mu\text{m}$). The predictability of cut depth, as assessed by pooled standard deviation (SD), was better ($p = 0.023$) for USP ($41 \mu\text{m}$) compared to OCT ($48 \mu\text{m}$). The graft was thicker ($p < 0.001$) peripherally than centrally in OCT images. The predictability of cut depth by OCT was better ($p < 0.001$) for corneas thinner than $600 \mu\text{m}$ ($\text{SD} = 45.6 \mu\text{m}$) compared to those thicker than $600 \mu\text{m}$ ($\text{SD} = 86.9 \mu\text{m}$).

Conclusion—The donor corneal measurements by OCT were not as predictable as USP. The predictability of graft thickness, however, could be optimized by using OCT to select for corneas thinner than $600 \mu\text{m}$, and then using immediate pre-cut USP to set the microkeratome depth. Graft thickness profile measured by OCT could be useful to the surgeon.

Keywords

Eye bank; donor cornea; endothelial keratoplasty; DSAEK; optical coherence tomography; ultrasound pachymetry

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Introduction

Endothelial keratoplasty has emerged as an attractive alternative to full-thickness penetrating keratoplasty for endothelial dysfunction.^{1, 2} The latest technique for endothelial keratoplasty, Descemet's stripping automated endothelial keratoplasty (DSAEK), allows faster visual recovery, lower risk of wound complications, and more predictable refractive outcome.³ More recently, many surgeons in the United States have been using pre-sectioned posterior lamellar tissue from eye banks to simplify the procedure.⁴ During eye bank preparation, the donor cornea is positioned on an artificial anterior chamber and sectioned by a trained eye bank technician using a microkeratome. Thickness measurements are necessary before and after sectioning. Before sectioning, the overall corneal thickness is measured to choose a proper microkeratome depth setting. After sectioning, the endothelial graft thickness is measured to confirm if it falls within the range specified by the surgeon. Ultrasound pachymetry (USP) is the standard instrument for these measurements. It, however, requires contact with the tissue and thus may be a contamination risk. Optical coherence tomography (OCT) is an alternative instrument to measure corneal thickness in vitro and has the advantage of being non-contact. In this study, we evaluated whether or not OCT can be a suitable alternative to USP in measuring corneal and graft thickness in an eye bank setting.

Materials and Methods

Donor corneas were obtained by the National Eye Bank Center - Tissue Banks International (TBI; Memphis, TN, USA). All of the corneas were stored in Optisol GS (Bausch and Lomb, Irvine, CA, USA) at 4°C and were sectioned for DSAEK in TBI according to the surgeons' requests. The tissues were handled in TBI facilities by designated trained technicians who performed the lamellar dissections.

Instruments

An ultrasonic pachymeter (DGH Technology, Exton, PA, USA) and a Visante OCT system (Carl Zeiss Meditec, Inc., Dublin, CA, USA) were used in this study. The ultrasonic pachymeter has a transducer frequency of 20 MHz and a calibrated velocity of 1640 m/s in normal human cornea. The Visante is a commercial time-domain OCT system with a scanning speed of 2000 axial scans per second. It operates at a wavelength of 1310 nm and has an axial resolution of 17 µm full-width-half-maximum in normal human corneas. Both devices were pre-calibrated by manufacturer technicians according to their specifications.

The USP measurements were not performed if a surgeon requested not to touch the graft surfaces. Neither USP nor OCT measurements were performed if a cornea were perforated after microkeratome cut.

Tissue Preparation

Central corneal thickness (CCT) was first measured by OCT while the corneoscleral disc was in a vial filled with Optisol GS under room temperature (20°C to 22°C). The vial was placed in an apparatus mounted on the chinrest using two padded clamps. OCT scanned through the bottom of the vial. The OCT images were captured and the pachymetry was measured using a computer caliper. The OCT pachymetry was measured between Bowman's layer and the endothelium using computer calipers (Fig. 1A). The disc was then placed onto an artificial anterior chamber (Moria Inc., Doylestone, PA, USA) on a cushion of sodium hyaluronate (Healon, Advanced Medical Optics, Santa Ana, CA, USA) and Optisol GS. The pressure within the artificial anterior chamber was adjusted by infusion of balanced salt solution (BSS) to physiologic levels determined by finger touch palpation. The

epithelium was then wiped off with a cellulose sponge. The CCT was measured by USP immediately after epithelium removal. The eye bank technicians noted that the CCT gradually decreased while mounted on the artificial anterior chamber, therefore the microkeratome cut was performed immediately after USP. Microkeratomes (LSK or CBM, Moria Inc, Doylestown, PA, USA) were set to 200, 250, 300, or 350 μm , according to the USP-determined CCT and the graft thickness range specified by the surgeon. The microkeratome head passed over the tissue and created a cap. The cap was lifted immediately after sectioning, and USP of the central graft thickness (CGT) was performed. The microkeratome cut depth was calculated by subtracting the CGT from the CCT. The cap was then replaced and the tissue was returned to the Optisol GS vial. The graft thickness was measured on the OCT image using computer calipers at 5 locations (Fig. 1B): central, two pericentral (3.21 mm diameter), and two peripheral (6.29 mm diameter). The central OCT measurements were compared with the USP measurements. Because the Visante OCT system corrected for the refraction between air and cornea but in the eye bank measurements, the Optisol GS media replaced air, this refractive compensation should have been much smaller. We applied a correction factor to reduce this error by assuming an average anterior corneal radius of curvature of 7.7 mm. This correction was -2.2% for graft thickness measured at pericentral locations and -8.7% at peripheral locations. Central thickness measurement was not affected because the OCT beam had perpendicular incidence there and did not change direction due to refraction.

Statistical Analysis

Because the measurements from both eyes of the same donor were included in data analysis, the generalized estimating equation (GEE)^{5,6} was used to account for the correlation between eyes of the same donors. Regression analysis of USP on OCT measurements and Bland-Altman plots^{7,8} were used to evaluate the agreement between USP and OCT measurements.

The predictability of USP (X) and OCT (Y) measurement was compared by determining if the standard deviation (SD) of OCT and USP measurements were equal. For this analysis,

$$\text{Covariance (X+Y, X - Y)} = \text{Variance (X)} - \text{Variance (Y)},$$

and therefore the testing for equal variances was equivalent to testing the correlation between the sum and difference of the two variables. The latter could be directly obtained from the two axes of the Bland-Altman plots.

The level of significance was 0.05. All data were analyzed using SAS Institute Inc., Cary, North Carolina, USA) and MATLAB 7.0 (Mathworks Inc., Natick, Massachusetts, USA).

Results

Two hundred eighty-seven corneoscleral discs (139 right eyes and 148 left eyes) from 221 donors were included in the study. The mean age of the donor corneas was 50.5 ± 15.2 years (mean $\pm$ standard deviation, range: 2.7 – 74.7), and the gender distribution was 69.6% male and 30.4% female. One hundred fifty-four eyes had a complete set of OCT and USP measurements and were used for comparative analysis.

The OCT measurements were thicker than USP measurements for both CCT ($p < 0.0001$, Table 1) and CGT ($p = 0.0005$, Table 1). The OCT and USP measurements were strongly correlated for both CGT ($R^2 = 0.77$) and CCT ($R^2 = 0.41$). On the Bland-Altman plot of

CCT (Fig. 2), the difference between OCT and USP measurement was significantly correlated with the average (slope = 0.17, $p = 0.04$). The 95% confidence interval of the difference was between -41 and $127 \mu\text{m}$. There were four outliers where the OCT-USP difference was greater than 3 SDs above the mean difference. All of the outliers occurred when the average CCT was above $610 \mu\text{m}$. On the Bland-Altman plot of CGT (Fig. 3), the difference between OCT and USP measurement was not significantly correlated with the average ($p = 0.11$). The 95% confidence interval of the difference was between -34 and $44 \mu\text{m}$.

We did not perform statistical analysis for the $200 \mu\text{m}$ microkeratome setting because only five eyes had complete measurements. For both microkeratomes, both OCT and USP measured cut depths that were significantly greater than the nominal settings ($p \leq 0.005$ for all settings, Table 2). The OCT measured cut depth was significantly larger than the USP measurement ($p < 0.001$ for GEE-adjusted paired t-test). The pooled standard deviation (SD) for OCT measured cut depth, $48 \mu\text{m}$, was significantly greater than the USP measurement, $41 \mu\text{m}$ ($p = 0.023$ for test of equal variance), which indicates better predictability for USP measurements. For corneas thicker than $600 \mu\text{m}$ measured by OCT (85th percentile), the predictability of cut depth using OCT or USP was significantly worse than that for thinner corneas ($p < 0.001$, Table 2).

The peripheral graft thickness measured by OCT was significantly greater than CGT ($p = 0.03$ for CBM, $p < 0.001$ for LSK, Table 3). The pericentral graft thickness was not significantly different from CGT ($p = 0.12$ for CBM, $p = 0.11$ for LSK, Table 3).

Discussion

Ultrasound pachymetry is the standard clinical method for central corneal thickness measurements due to its simplicity, low cost, and long history. However, it requires corneal contact and only provides a point measurement where the probe is manually placed. Optical coherence tomography is a newer technique that does not require contact and can provide corneal thickness profiles and maps.⁹ Clinical studies have shown that USP and OCT pachymetry measurements are both highly reproducible and correlated with one another.^{9, 10} Li et al. showed that though corneal thickness measured by Visante OCT was $14.7 \mu\text{m}$ thinner than USP, the two measurements were highly correlated.¹¹ In eye bank settings, noncontact OCT measurement theoretically poses lower contamination risk compared to USP. This study was performed to test whether or not OCT could be a suitable replacement for USP in guiding the selection of microkeratome setting for the preparation of DSAEK grafts in the eye bank.

Our data showed that the predictability of cut depth was significantly worse with OCT, when compared with USP. The most likely explanation for this difference was the different settings for the measurements. The USP measurements were taken while the donor corneas were mounted on the artificial anterior chamber, just before the microkeratome cut. Thus they take into account the corneal drying due to air exposure and corneal stretching due to the pressure in the chamber. On the other hand, OCT measurements were taken when the donor corneas were in a relaxed state in Optisol-filled vials. In Optisol, donor corneas swell with increased storage time due to the gradual decrease in the endothelial pump function.¹² These swollen corneas could undergo the greatest deturgescence while mounted on the artificial anterior chamber. This hypothesis was supported by our data, which showed that OCT overestimates corneal thickness relative to USP in the thickest corneas. This was also supported by the eye bank technicians' experience that serial USP of corneas mounted on the artificial anterior chamber showed gradual thinning. A slight off center OCT scan could also contribute to the overestimation. However our results do not rule out the eventual use of

OCT in guiding the microkeratome cut. If the OCT system could be designed to image the cornea while mounted on the artificial anterior chamber, we believe it could provide reliable guidance for cut depth selection.

For corneas measured by OCT to be thicker than 600 μm , which represented 15 percent of the total corneas, the predictability of cut depth became worse. Therefore, if these corneas were used in endothelial keratoplasty, the graft thickness after the microkeratome cut would become harder to control. Using OCT may be helpful to screen out very thick corneas when they are still in vial, before precutting them for endothelial keratoplasty.

In this study, both the CBM and LSK microkeratomes cut much deeper than the nominal settings. The technician would get much more accurate graft thicknesses by using the USP measured cut depth rather than the nominal setting. Our specific results may not be generalizable to all microkeratomes, however, as other studies have shown that Moria microkeratomes cut thinner than the nominal setting.¹³ It is advisable that each microkeratome be calibrated using USP measurements to provide a nomogram for depth setting selection.

The systematic bias of CGT measurements between the USP and OCT was small relative to the overall graft thickness. Thus OCT may be useful for imaging grafts to provide surgeons with an assessment of their average thickness and uniformity. The graft thickness profile measured by OCT showed that the periphery of the graft was thicker than the center (Table 3).

In summary, immediate precut pachymetry by USP is best to select the depth setting because it takes into account deturgescence for corneas mounted on artificial anterior chambers. An OCT system designed to scan corneas that have been conveniently mounted on artificial anterior chamber might be a good option in the future, with an advantage of avoiding possible contact contamination. Currently, OCT could be used to select corneas thinner than 600 μm before precutting them for endothelial keratoplasty. Corneas that are thicker in the periphery would theoretically lead to greater myopic shift. Further studies are needed to link OCT graft thickness profiles with refractive shifts.

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Figure 1.

(A) Horizontal OCT cross-sectional image of a donor corneoscleral disc with pachymetry measurements taken between Bowman's layer and the endothelium. Central corneal thickness (CCT) was circled. (B) Graft thickness measurements were taken after the microkeratome cut. The cap was replaced after sectioning. Less than 1% cases of cap detachment were observed after placing the tissue into the Optisol-GS. The graft thickness was measured at the vertex center, pericentral (Position 1), and peripheral (Position 2) locations. The radii at the measurement locations are shown under the positions.

Figure 2. Bland-Altman plot of agreement on central corneal thickness (CCT) measured by OCT and USP. The mean difference of CCT between OCT and USP measurements was $43 \pm 43 \mu\text{m}$ (mean $\pm$ standard deviation).

Figure 3. Bland-Altman plot of the agreement on central graft thickness (CGT) measured by OCT and USP. The mean difference of CGT between OCT and USP measurements was $5.1 \pm 20 \mu\text{m}$ (mean $\pm$ standard deviation).

Table 1

Central Corneal and Graft Thickness Measurements by Optical Coherence Tomography and Ultrasound Pachymetry.

	OCT	USP	P-value	R ²
CCT (μm)	550±63 [427 – 673]	507± 54 [401 – 612]	<0.0001	0.41
CGT (μm)	158± 41 [77 – 240]	153± 38 [79 – 227]	0.0005	0.77

CCT = central corneal thickness; CGT = central graft thickness; OCT = optical coherence tomography; USP = ultrasound pachymetry. P-value = p-value of paired t-test.

Parameters were displayed as mean ± standard deviation [range].

Table 2

Predictability of Cut Depth Measurement by Optical Coherence Tomography and Ultrasound Pachymetry for Corneas with Different Thickness

Microkeratome		OCT			Ultrasound		
Type	Depth Setting (μm)	# eyes	Less than 600 μm	# eyes	More than 600 μm	# eyes	More than 600 μm
CBM	250	16	325 ± 52	-	-	22	297 ± 29
	300	37	375 ± 53	2	495 ± 64	54	365 ± 31
	350	4	447 ± 38	4	495 ± 72	4	487 ± 60
LSK	250	23	334 ± 53	-	-	53	301 ± 29
	300	53	379 ± 31	9	442 ± 94	70	361 ± 32
	350	16	443 ± 51	8	476 ± 87	17	447 ± 42
Overall pooled SD *			45.6		86.9		32.2
							72.7

* p < 0.001 for the comparison of the standard deviation of thick cornea group (> 600 μm) and thinner cornea group (< 600 μm) for both OCT and ultrasound measurements. Parameters are displayed in the format: mean ± standard deviation. CBM and LSK are two microkeratome models made by Moria Inc.

Table 3

Graft Thickness Profile Measured by Optical Coherence Tomography

Measurement Diameter	Graft thickness (μm)	
	CBM	LSK
Center	165 ± 35	151 ± 34
3.21 mm	169 ± 37	153 ± 35
6.29 mm	175 ± 41	168 ± 35

Graft thicknesses are displayed in the format: mean $\pm$ standard deviation. CBM and LSK are two microkeratome models made by Moria Inc.