Evaluation of a new method for the measurement of corneal thickness in eye bank posterior corneal lenticules using Anterior Segment Optical Coherence Tomography

Domenico Amato,1 Marco Lombardo,1 Francesco Oddone,1 Mario Nubile,2 Rossella A M Colabelli Gisoldi,3 Carlo M Villani,3 Sonia Yoo,4 Jean-Marie Parel,4 Augusto Pocobelli3

ABSTRACT

Background/aims To preliminarily evaluate the repeatability of central corneal thickness (CCT) measurements performed with Anterior Segment Optical Coherence Tomography (AS-OCT) on eye bank posterior corneal lenticules.

Methods Six donor lenticules were created with a 350 µm head microkeratome (Moria, Antony, France). All donor tissues were stored at 4°C in Eusol-C solution (Alchimia S.r.l, Ponte S. Nicolò, Italy), without the anterior cornea lamella. The CCT of each lenticule, maintained in the glass phial, was measured using a commercial AS-OCT instrument (Visante, Carl Zeiss Meditec, Dublin, California, USA) and a specially designed adaptor immediately and 4, 24 and 48 hours after dissection. Immediately after AS-OCT, CCT values were measured with the ultrasound pachymetry method used at the Eye Bank.

Results The mean donor cornea central thickness was 647±36 µm and 660±38 µm (p=0.001) as measured by AS-OCT and ultrasound, respectively; immediately after dissection, CCT values of posterior lenticules were 235±43 µm and 248±44 µm, respectively (p=0.001). No statistically significant changes in CCT values of donor lenticules were assessed over the 48 h period with both methods. There was a high level of agreement, evidenced by Bland–Altman analysis, between the two methods of pachymetry.

Conclusion AS-OCT, with the corneal tissue in the vial, was revealed to be a repeatable and reliable method for measuring posterior donor lenticule central thickness. Lenticule CCT values measured with the investigational AS-OCT method were on average 10 µm thinner than those measured with the established ultrasound method.

INTRODUCTION

Descemet's stripping automated endothelial keratoplasty (DSEAK)1 2 is a surgical technique for the treatment of corneal endothelial diseases. The preparation of DSEAK donor tissue was originally done in the operating room by the surgeon1 3 4 but currently an increasing number of surgeons prefer to obtain donor tissue directly from the eye bank just before tissue shipment.5 6 Precut donor tissue for DSEAK offers the advantage of saving surgery time with precise information regarding central thickness, providing at the same time visual and refractive outcomes and rate of postoperative complications comparable to surgeon-dissected donor tissue.7–11

Contact ultrasound pachymetry is in general the preferred method for measuring the corneal thickness in eye banks worldwide.5 7 Anterior Segment Optical Coherence Tomography (AS-OCT) has been also recently used for characterising eye-bank donor tissues.12 AS-OCT is a non-contact method that can give central and peripheral thickness measurements of the entire cornea and it is widely used in the clinical environment for the examination of DSEAK donor tissue during follow-up of patients. On the other hand, the knowledge of either central or peripheral thickness values before surgery may be valuable for DSEAK surgeons, since it has been widely demonstrated how either the central thickness or a non-uniform thickness profile of the donor tissue may influence the definite refractive outcome after surgery.13–16 The purpose of this study was to preliminarily evaluate a new method for measuring the central thickness of posterior corneal lenticules using a commercial AS-OCT instrument. A custom adaptor was used in order to maintain the tissue in the glass phial, thus avoiding tissue manipulation during measurement.

METHODS

Six donor corneas obtained from the eye bank of Rome (Italy) were used for the study. All corneas were used within 4 days post-mortem and were stored at 4°C in corneal storage medium Eusol-C (Alchimia S.r.l, Ponte S. Nicolò, Italy). The donors had a negative ophthalmic history but positive serology, which made the tissue unsuitable for transplantation.

All tissues were prepared by an expert eye bank operator (DA) using the standardised method for obtaining DSEAK precut tissues at the eye bank of Rome.17 Each corneal lenticule was prepared from the donor cornea by using a 350 µm head microkeratome (Moria One, Moria S.A., Antony, France) and an artificial anterior chamber (AAC, Moria S.A., Antony, France) cushioned with Eusol-C. Each lenticule was created by a full pass of the microkeratome blade which resulted in a posterior lamellar donor and a free cap. The free anterior cap was then eliminated and the posterior lamellar tissue was stored over a period of 48 h at 4°C in Eusol-C between measurements.
Donor corneal central thickness (CCT) before dissection and lenticule CCT immediately and after 4, 24 and 48 hours post-cut were first measured by AS-OCT (Visante, Carl Zeiss Meditec, Dublin, California, USA) and then by ultrasound pachymetry using a 20 MHz probe (Hiscan, Optikon 2000 S.p.A., Rome, Italy), capable of measuring thickness values lower than 100 µm and calibrated by the manufacturer. Measurements were performed five times by the same operator to assess repeatability of both methods.

The tissue was analysed by AS-OCT through the glass phial containing Eusol-C. OCT measurements were performed using a specially designed adaptor; the device consists of a holder accepting standard glass phials or plastic viewing chambers and a mirror placed in the optical path of the AS-OCT instrument allowing measurements through the phial’s or chamber’s optically clear bottom (figure 1). The adaptor (Abeamed Inc, Miami, Florida, USA) does not require modification of the AS-OCT instrument and can be plugged in/removed without compromising tissue sterility.

In the AS-OCT image, the corneal apex was identified from the peak of the reflectivity profile on the horizontal axis and the automated flip tool of the instrument was then used for thickness measurement. Ultrasound pachymetry was obtained in a perpendicular direction from the corneal surface to the central cornea by direct contact of the probe with the corneal tissue mounted on the AAC cushioned with Eusol-C and pressurised to approximately 40 mm Hg, as measured with hand-held applation tonometry (Tono-pen, Mentor Ophthalmics, Norwell, Massachusetts, USA). The tip of the probe was moistened with Eusol-C.

Statistical analysis
Repeatability was determined with the coefficient of variation (CV) value, expressed in a percentage that was calculated from the intrasession standard deviations for the five independent consecutive measurements. Bland–Altman plots were used to assess the degree of agreement between AS-OCT and ultrasound pachymetry and their 95% CI. Differences in measurements between AS-OCT and ultrasound were evaluated by paired t-test. A p value of 0.05 or less was considered statistically significant. Statistical data analysis was carried out using SPSS for Windows (version 10.0, SPSS Inc, Chicago, IL, USA).

RESULTS
The mean central corneal thickness before dissection was 647±35 µm and 660±38 µm (p=0.001), as measured by AS-OCT and ultrasound, respectively. Immediately after dissection, posterior lenticule CCT values were 235±43 µm and 248±44 µm, as measured by AS-OCT and ultrasound, respectively (p<0.001). No statistically significant changes in lenticule CCT values (1-way ANOVA, p>0.05) were assessed during the post-cut time course, as measured by both devices (table 1).

The mean AS-OCT thickness values were statistically significantly lower than ultrasound measurements before dissection and at all time points after dissection: mean differences ranged from 14 µm at 4 h post-cut to 7 µm at 48 h post-cut. The mean CCT differences between the two methods of pachymetry before and immediately after cut were approximately 13 µm. The limits of agreement (LOA) between the two methods ranged from –6.1 µm to –18.9 µm immediately after dissection and from 2.1 µm to –16.4 µm at 48 h after cut. Full details with 95% LOA are given in figure 2. An example of the AS-OCT imaging of a corneal tissue during the time course of examinations is shown in figure 3.

DISCUSSION
AS-OCT is a valuable imaging tool for providing information on donor posterior lamella apposition after DSAEK and further in the management of surgical complications after the procedure, such as dislocation, primary graft failure, and anterior chamber crowding with consequent chamber angle encroachment and...
pupillary block. On the other hand, AS-OCT may provide useful information prior to surgery, with detailed description of central thickness and profile of donor tissue. Recently, a custom built AS-OCT has been demonstrated to be a potential instrument for the preoperative eye bank routine analysis of donor lamellar tissues for transplantation. In this work we aimed at preliminarily estimating the repeatability of a new method for measuring donor corneal thickness of eye bank-prepared tissues for DSAEK using a commercial AS-OCT device. A specially designed adaptor was used to maintain the cornea in the glass phial during AS-OCT measurements. CCT values obtained with the investigational AS-OCT method were compared to those performed with the established ultrasound method of pachymetry at our eye bank. There was a high level of agreement between methods with mean differences of approximately 10 μm before and after dissection. A high repeatability of the investigational AS-OCT method of central pachymetry, comparable to ultrasound pachymetry, was calculated and further confirmed previous findings. In our study, mean AS-OCT central pachymetry revealed lower values, ranging from 7 to 14 μm, than ultrasound pachymetry, both before and after dissection. The results from the present work are in accordance

Table 1 Central corneal thickness values and coefficient of variation (CV, %) calculated with the investigational AS-OCT and ultrasound methods of pachymetry during the time course

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-cut*</th>
<th>Post-cut (p-c)*</th>
<th>4 h p-c*</th>
<th>24 h p-c*</th>
<th>48 h p-c*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS-OCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (±SD, μm)</td>
<td>647±35</td>
<td>235±43</td>
<td>224±37</td>
<td>239±42</td>
<td>233±39</td>
</tr>
<tr>
<td>Min (μm)</td>
<td>594</td>
<td>173</td>
<td>181</td>
<td>187</td>
<td>185</td>
</tr>
<tr>
<td>Max (μm)</td>
<td>680</td>
<td>280</td>
<td>271</td>
<td>281</td>
<td>277</td>
</tr>
<tr>
<td>CV (%)</td>
<td>5%</td>
<td>18%</td>
<td>16%</td>
<td>18%</td>
<td>17%</td>
</tr>
<tr>
<td>Ultrasound</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (±SD, μm)</td>
<td>660±38</td>
<td>246±44</td>
<td>238±42</td>
<td>247±41</td>
<td>240±40</td>
</tr>
<tr>
<td>Min (μm)</td>
<td>605</td>
<td>185</td>
<td>188</td>
<td>195</td>
<td>189</td>
</tr>
<tr>
<td>Max (μm)</td>
<td>705</td>
<td>296</td>
<td>283</td>
<td>289</td>
<td>282</td>
</tr>
<tr>
<td>CV (%)</td>
<td>6%</td>
<td>18%</td>
<td>17%</td>
<td>17%</td>
<td>17%</td>
</tr>
</tbody>
</table>

*p<0.05: t-test between methods of pachymetry.
AS-OCT, Anterior Segment Optical Coherence Tomography.

Figure 2 Bland–Altman plots analysing the agreement between Anterior Segment Optical Coherence Tomography (AS-OCT) and Ultrasound thickness measurements. Average and difference central corneal thickness (CCT) values between methods of pachymetry are plotted in the x- and y-axes, respectively. (A) measurements before the microkeratome dissection; (B) measurements immediately after dissection; (C) at 4 h post-cut; (D) at 24 h and (E) at 48 h post-cut. All measurements were within 95% limits of agreement (LOA).
with several clinical studies that have been conducted with the aim to compare corneal thickness measurements obtained with different devices, including ultrasound and AS-OCT.\textsuperscript{21–27} In general, all the authors have reported a reproducible systematic difference between CCT measurements taken with ultrasound and OCT, with the latter method providing an average underestimation of approximately 15–30\textmu m of central pachymetry readings.

Possible bias in the measurement of CCT values between AS-OCT and ultrasound methods of pachymetry could be introduced by the fact that, during AS-OCT measurements, the corneal tissue was completely immersed in liquid and it was not pressurised into the AAC as done during ultrasound measurements. A potential source of error in the conversion from OCT distance to geometric thickness is therefore the assumption of a constant corneal refractive index, as in general discussed for the OCT technique.\textsuperscript{28,29} Although changes of the refractive index were considered unlikely, intra-individual and local variations of the refractive index, due to increasing hydration and thickening of corneal tissue, could influence the propagation of light through different corneal layers.\textsuperscript{30,31} A constant corneal thickness, however, has been measured during the time course of examinations in all specimens. On the other hand, one should bear in mind how the sound velocity in corneal tissue has been a subject of long debate and the speed of sound is likely to vary between different layers of the cornea.\textsuperscript{32} In addition, central thickness measurement cannot be precisely determined at the corneal apex with ultrasound pachymetry.

Despite the limited number of cases, the investigational AS-OCT system provided a non-contact and repeatable method in the evaluation of corneal thickness of posterior donor cornea lenticules. The measurement of corneal thickness directly inside the phial can reduce the mounting time of the cornea on the artificial anterior chamber, thereby minimising the stress placed upon the endothelium and the risks of contamination; in addition, the method can quantify the tissue swelling rate during prolonged storage at the eye bank and has the potential to provide information about regional variations in the donor lenticule profile that could be used to correlate with postoperative DSAEK outcomes.\textsuperscript{16} Notwithstanding these advantages, the cost of an AS-OCT instrument is clearly a hindering factor when compared to that of an ultrasound pachymeter and could limit widespread use by eye banks.

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Competing interests None.

Patient consent Obtained.

Ethics approval This study was conducted with the approval of the Ethics Committee of S. Giovanni-Addolorata-Britannico Hospital, Rome, Italy.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES


Figure 3 Anterior Segment Optical Coherence Tomography (AS-OCT) images of a corneal sample obtained during tissue storage. The corneal central thickness was 670 \textmu m before dissection (A). The posterior lenticule central corneal thickness was 240 \textmu m immediately after dissection (B); 250 microns at 4 h (C); 230 \textmu m at 24 h (D) and 220 \textmu m at 48 h post-cut (E). Thickness measurement was done using the automated \textit{flap tool} of the instrument.


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