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

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33 **Abstract**

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

37 **Methods:** Six donor lenticules were created with a 350 μm head microkeratome (Moria,
38 Antony, France). All donor tissues were stored at 4°C in Eusol-C solution (Alchimia S.r.l,
39 Ponte S. Nicolò, Italy), without the anterior cornea lamella. The CCT of each lenticule,
40 maintained in the glass vial, was measured using a commercial AS-OCT instrument (Visante,
41 Carl Zeiss Meditec, Dublin, CA, USA) and a special designed adaptor immediately and 4-,
42 24- and 48-hours after dissection. Immediately after AS-OCT, CCT values were measured
43 with the ultrasound (US) pachymetry method used at the Eye Bank.

44 **Results:** The mean donor cornea central thickness was $647 \pm 36 \mu\text{m}$ and $660 \pm 38 \mu\text{m}$
45 ($P=0.001$) as measured by AS-OCT and US respectively; immediately after dissection, CCT
46 values of posterior lenticules were $235 \pm 43 \mu\text{m}$ and $248 \pm 44 \mu\text{m}$ respectively ($P=0.001$). No
47 statistically significant changes in CCT values of donor lenticules were assessed over the 48
48 hours period with both methods. There was a high level of agreement, evidenced by Bland-
49 Altman analysis, between the two methods of pachymetry.

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

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57 **Introduction**

58 Descemet's stripping automated endothelial keratoplasty (DSAEK)^{1,2} is a surgical technique
59 for the treatment of corneal endothelial diseases. The preparation of DSAEK donor tissue
60 was originally done in the operating room by the surgeon^{3,4} but currently an increasing
61 number of surgeons prefers to obtain donor tissue directly from the eye bank just before
62 tissue shipment^{5,6}. Precut donor tissue for DSAEK offers the advantage of saving surgery
63 time with precise information regarding central thickness, providing at the same time visual
64 and refractive outcomes and rate of postoperative complications comparable to surgeon-
65 dissected donor tissue^{7,8,9,10,11}.

66 Contact ultrasound (US) pachymetry is in general the preferred method for measuring the
67 corneal thickness in eye bank worldwide^{5,7}. Anterior Segment Optical Coherence
68 Tomography (AS-OCT) has been also recently used for characterizing eye-bank donor
69 tissues¹². AS-OCT is a non-contact method that can give central and peripheral thickness
70 measurements of the entire cornea and it is widely used in the clinical environment for the
71 examination of DSAEK donor tissue during follow-up of patients. On the other hand, the
72 knowledge of either central or peripheral thickness values before surgery may be valuable
73 for DSAEK surgeons, since it has been widely demonstrated how either the central thickness
74 or a non-uniform thickness profile of the donor tissue may influence the definite refractive
75 outcome after surgery^{13,14,15,16}. The purpose of this study was to preliminary evaluate a new
76 method for measuring the central thickness of posterior corneal lenticules using a
77 commercial AS-OCT instrument. A custom adaptor was used in order to maintain the tissue
78 in the glass vial, thus avoiding tissue manipulation during measurements.

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81 **Methods**

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

87 All tissues were prepared by an expert eye bank operator (DA) using the standardized
88 method for obtaining DSAEK precut tissues at the eye bank of Rome¹⁷. Each corneal
89 lenticule was prepared from the donor cornea by using a 350 µm head microkeratome (Moria
90 One, Moria S.A., Antony, France) and an artificial anterior chamber (AAC, Moria S.A.,
91 Antony, France) cushioned with Eusol-C. Each lenticule was created by a full pass of the
92 microkeratome blade which resulted in a posterior lamellar donor and a free cap. The free
93 anterior cap was then eliminated and the posterior lamellar tissue was stored over a period of
94 48 hours at 4° C in Eusol-C between measurements.

95 Donor corneal central thickness (CCT) before dissection and lenticule CCT immediately and
96 after 4-, 24- and 48-hours post-cut were first measured by AS-OCT (Visante, Carl Zeiss
97 Meditec, Dublin, CA, US) and then by US pachymetry using a 20 MHz probe (Hiscan,
98 Optikon 2000 S.p.A., Rome, Italy), capable of measuring thickness values lower than 100 µm
99 and calibrated by the manufacturer. Measurements were performed 5 times by the same
100 operator to assess repeatability of both methods.

101 The tissue was analyzed by AS-OCT through the glass vial containing Eusol-C. OCT
102 measurements were performed using a specially designed adaptor¹⁸: the device consists of a
103 holder accepting standard glass vials or plastic viewing chambers and a mirror placed in the
104 optical path of the AS-OCT instrument allowing to carry out measurements through the
105 vial'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 *flap tool* of the instrument was then used for thickness measurement. US 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 pressurized to approximately 40 mmHg, as measured with hand-held applanation tonometry (Tono-pen, Mentor Ophthalmics, Norwell, MA, US). The tip of the probe was wetted using Eusol-C.

Statistical analysis

Repeatability was determined with the coefficient of variation (CV) value, expressed in %, 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 US pachymetry and their 95% confidence interval. Differences in measurements between AS-OCT and US 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.).

Results

The mean central corneal thickness before dissection was $647 \pm 35 \mu\text{m}$ and $660 \pm 38 \mu\text{m}$ ($P=0.001$), as measured by AS-OCT and US respectively. Immediately after dissection, posterior lenticule CCT values were $235 \pm 43 \mu\text{m}$ and $248 \pm 44 \mu\text{m}$, as measured by AS-OCT and US 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).

Table 1 Central corneal thickness values and coefficient of variation (CV, %) calculated with the investigational AS-OCT and US methods of pachymetry during the time course						
	Variable	Pre-cut*	Post-cut (p-c)*	4 hours p-c*	24 hours p-c*	48 hours p-c*
AS-OCT	Mean ($\pm$ SD, μm)	647 $\pm$ 35	235 $\pm$ 43	224 $\pm$ 37	239 $\pm$ 42	233 $\pm$ 39
	Min (μm)	594	173	181	187	185
	Max (μm)	690	280	271	281	277
	CV (%)	5%	18%	16%	18%	17%
US	Mean ($\pm$ SD, μm)	660 $\pm$ 38	248 $\pm$ 44	238 $\pm$ 42	247 $\pm$ 41	240 $\pm$ 40
	Min (μm)	605	185	188	195	189
	Max (μm)	705	298	283	289	282
	CV (%)	6%	18%	17%	17%	17%
* $P<0.05$: t -test between methods of pachymetry						

The mean AS-OCT thickness values were statistically significant lower than US measurements before dissection and at all time points after dissection: mean differences ranged from 14 μm at 4 hours post-cut to 7 μm at 48 hours 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 hours

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 block¹⁹. 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 transplantation¹². In this work we aimed at preliminary 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 special-designed adaptor was used to maintain the cornea in the glass vial during AS-OCT measurements. CCT values obtained with the investigational AS-OCT method were compared to those performed with the established US 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 US pachymetry, was calculated and further confirmed previous findings^{20,21}. In our study, mean AS-OCT central pachymetry revealed lower values, ranging from 7 to 14 μm , than US pachymetry, both before and after dissection. The results from the present work are in accordance with several clinical studies that have been conducted with the aim to compare corneal thickness measurements obtained with different devices,

including US and AS-OCT^{21,22,23,24,25,26,27}. In general, all the authors have reported a reproducible systematic difference between CCT measurements taken with US and OCT, with the latter method providing an average underestimation of approximately 15-30 μm of central pachymetry readings.

Possible bias in the measurement of CCT values between AS-OCT and US 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 pressurized into the AAC as done during US 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^{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^{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 cornea³². Moreover, it is unlikely to take thickness measurements precisely at the corneal apex with US pachymetry.

Although 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 vial can reduce the mounting time of the cornea on the artificial anterior chamber, thereby minimizing 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 outcomes¹⁶. Notwithstanding these

advantages, the cost of an AS-OCT instrument is clearly a hindering factor when compared to that of an US pachymeter and could limit a widespread use by eye banks.

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Competing Interests

The authors have no financial or commercial interest in the materials described herein

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Figure Legends

Figure 1. The eye bank tissue adaptor does not require modification of the AS-OCT instrument and can be easily plugged in/removed without compromising sterility of the donor corneal tissue. A) The device consists of a holder accepting standard glass vials or plastic viewing chambers and a fixation mechanism fitting the AS-OCT. B) front and C): top views of the device.

Figure 2. Bland-Altman plots analyzing the agreement between AS-OCT and US thickness measurements. Average and difference 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 hours post-cut; D) at 24 hours and E) at 48 hours post-cut. All measurements were within 95% LOA.

Figure 3. AS-OCT images of a corneal sample obtained during tissue storage. The corneal central thickness was 670 μm before dissection (A). The posterior lenticule central corneal thickness was 240 μm immediately after dissection (B); 250 microns at 4 hours (C); 230 μm at 24 hours (D) and 220 μm at 48 hours post-cut (E). Thickness measurement was done by using the automated *flap tool* of the instrument.