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

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29
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32

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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80

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,

106 Florida, USA) does not require modification of the AS-OCT instrument and can be plugged
107 in/removed without compromising tissue sterility.

108 In the AS-OCT image, the corneal apex was identified from the peak of the reflectivity
109 profile on the horizontal axis and the automated *flap tool* of the instrument was then used for
110 thickness measurement. US pachymetry was obtained in a perpendicular direction from the
111 corneal surface to the central cornea by direct contact of the probe with the corneal tissue
112 mounted on the AAC cushioned with Eusol-C and pressurized to approximately 40 mmHg,
113 as measured with hand-held applanation tonometry (Tono-pen, Mentor Ophthalmics,
114 Norwell, MA, US). The tip of the probe was wetted using Eusol-C.

115 **Statistical analysis**

116 Repeatability was determined with the coefficient of variation (CV) value, expressed in %,
117 that was calculated from the intrasession standard deviations for the five independent
118 consecutive measurements. Bland-Altman plots were used to assess the degree of agreement
119 between AS-OCT and US pachymetry and their 95% confidence interval. Differences in
120 measurements between AS-OCT and US were evaluated by paired *t*-test. A *P* value of 0.05
121 or less was considered statistically significant. Statistical data analysis was carried out using
122 SPSS for Windows (version 10.0, SPSS Inc.).

123

124

125 **Results**

126 The mean central corneal thickness before dissection was $647 \pm 35 \mu\text{m}$ and $660 \pm 38 \mu\text{m}$
127 ($P=0.001$), as measured by AS-OCT and US respectively. Immediately after dissection,
128 posterior lenticule CCT values were $235 \pm 43 \mu\text{m}$ and $248 \pm 44 \mu\text{m}$, as measured by AS-
129 OCT and US respectively ($P<0.001$). No statistically significant changes in lenticule CCT

130 values (1-way ANOVA, $P>0.05$) were assessed during the post-cut time course, as measured
131 by both devices (table 1).

132

<p align="center">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</p>						
	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

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135 The mean AS-OCT thickness values were statistically significant lower than US
136 measurements before dissection and at all time points after dissection: mean differences
137 ranged from 14 μm at 4 hours post-cut to 7 μm at 48 hours post-cut. The mean CCT
138 differences between the two methods of pachymetry before and immediately after cut were
139 approximately 13 μm . The limits of agreement (LOA) between the two methods ranged from
140 -6.1 μm to -18.9 μm immediately after dissection and from 2.1 μm to -16.4 μm at 48 hours

141 after cut. Full details with 95% LOA are given in figure 2. An example of the AS-OCT
142 imaging of a corneal tissue during the time course of examinations is shown in figure 3.

143

144

145 **Discussion**

146 AS-OCT is a valuable imaging tool for providing information on donor posterior lamella
147 apposition after DSAEK and further in the management of surgical complications after the
148 procedure, such as dislocation, primary graft failure, and anterior chamber crowding with
149 consequent chamber angle encroachment and pupillary block¹⁹. On the other hand, AS-OCT
150 may provide useful information prior to surgery, with detailed description of central
151 thickness and profile of donor tissue. Recently, a custom built AS-OCT has been
152 demonstrated to be a potential instrument for the preoperative eye bank routine analysis of
153 donor lamellar tissues for transplantation¹². In this work we aimed at preliminary estimating
154 the repeatability of a new method for measuring donor corneal thickness of eye bank-
155 prepared tissues for DSAEK using a commercial AS-OCT device. A special-designed
156 adaptor was used to maintain the cornea in the glass vial during AS-OCT measurements.
157 CCT values obtained with the investigational AS-OCT method were compared to those
158 performed with the established US method of pachymetry at our eye bank. There was a high
159 level of agreement between methods with mean differences of approximately 10 μm before
160 and after dissection. A high repeatability of the investigational AS-OCT method of central
161 pachymetry, comparable to US pachymetry, was calculated and further confirmed previous
162 findings^{20,21}. In our study, mean AS-OCT central pachymetry revealed lower values, ranging
163 from 7 to 14 μm , than US pachymetry, both before and after dissection. The results from the
164 present work are in accordance with several clinical studies that have been conducted with
165 the aim to compare corneal thickness measurements obtained with different devices,

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

170 Possible bias in the measurement of CCT values between AS-OCT and US methods of
171 pachymetry could be introduced by the fact that, during AS-OCT measurements, the corneal
172 tissue was completely immersed in liquid and it was not pressurized into the AAC as done
173 during US measurements. A potential source of error in the conversion from OCT distance to
174 geometric thickness is therefore the assumption of a constant corneal refractive index, as in
175 general discussed for the OCT technique^{28,29}. Although changes of the refractive index were
176 considered unlikely, intra-individual and local variations of the refractive index, due to
177 increasing hydration and thickening of corneal tissue, could influence the propagation of light
178 through different corneal layers^{30,31}: a constant corneal thickness, however, has been
179 measured during the time course of examinations in all specimens. On the other hand, one
180 should bear in mind how the sound velocity in corneal tissue has been a subject of long debate
181 and the speed of sound is likely to vary between different layers of the cornea³². Moreover, it
182 is unlikely to take thickness measurements precisely at the corneal apex with US pachymetry.

183 Although the limited number of cases, the investigational AS-OCT system provided a non-
184 contact and repeatable method in the evaluation of corneal thickness of posterior donor cornea
185 lenticules. The measurement of corneal thickness directly inside the vial can reduce the
186 mounting time of the cornea on the artificial anterior chamber, thereby minimizing the stress
187 placed upon the endothelium and the risks of contamination; in addition, the method can
188 quantify the tissue swelling rate during prolonged storage at the eye bank and has the
189 potential to provide information about regional variations in the donor lenticule profile that
190 could be used to correlate with postoperative DSAEK outcomes¹⁶. Notwithstanding these

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

193

194

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203 experiments.

204

205 **Competing Interests**

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

207

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313 **Figure Legends**

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

318 **Figure 2.** Bland-Altman plots analyzing the agreement between AS-OCT and US thickness
319 measurements. Average and difference CCT values between methods of pachymetry are
320 plotted in the x - and y -axes respectively. A) measurements before the microkeratome
321 dissection; B) measurements immediately after dissection; C) at 4 hours post-cut; D) at 24
322 hours and E) at 48 hours post-cut. All measurements were within 95% LOA.

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





