

# Microkeratome-Assisted Preparation of Ultrathin Grafts for Descemet Stripping Automated Endothelial Keratoplasty

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**PURPOSE.** To compare three microkeratome-assisted techniques for the preparation of ultrathin (UT) grafts for Descemet stripping automated endothelial keratoplasty.

**METHODS.** After dissection with a 300- $\mu\text{m}$  microkeratome head in 40 donor tissues, a second cut was performed with a 130- $\mu\text{m}$  head either after manual stromal hydration (group A,  $n = 10$ ) or osmotic hydration at the eye bank (group B,  $n = 10$ ) or with a 50- or 90- $\mu\text{m}$  head, depending on residual bed thickness (group C,  $n = 10$ ); no further dissection was performed in the control group (group D,  $n = 10$ ). Corneal thickness and endothelial cell (EC) count were determined at all appropriate stages. Statistical analysis was performed using a Fisher exact test.

**RESULTS.** Final graft thicknesses in groups A ( $89.1 \pm 34.1 \mu\text{m}$ ), B ( $84.1 \pm 18.6 \mu\text{m}$ ), and C ( $72.1 \pm 10.1 \mu\text{m}$ ) were significantly lower than in group D ( $201.9 \pm 25.3 \mu\text{m}$ ) ( $P < 0.001$ ). EC loss did not differ significantly among the groups. Multiple areas of Descemet detachment were seen in 4 of 10 corneas of group A.

**CONCLUSIONS.** All methods proved equally efficient in producing UT grafts, but stromal hydration induced tissue structural changes. EC loss was unaffected by the additional manipulation required to prepare UT grafts. (*Invest Ophthalmol Vis Sci.* 2012;53:521-524) DOI:10.1167/iovs.11-7753

Although Descemet stripping automated endothelial keratoplasty (DSAEK) has established itself in recent years as the surgical treatment of choice for endothelial dysfunction, visual outcomes may vary in terms of both speed of visual recovery and final visual acuity. Recent reports have shown that grafts consisting of only Descemet membrane and endothelium produce 20/25 or better vision in a significantly higher percentage of patients than grafts that also include the posterior stromal tissue.<sup>1-3</sup> In addition, the time required to achieve this level of vision is shorter for the former type of donor grafts. However, Descemet membrane endothelial keratoplasty (DMEK) is technically very difficult and, for this reason, has not gained widespread popularity to date.

Controversial evidence has been presented linking the thickness of a DSAEK graft to visual outcome. Neff et al.<sup>4</sup>

recently showed that grafts thinner than 131  $\mu\text{m}$  achieve better final visual acuity than do thicker ones. To date, neither surgeons nor eye banks have addressed the issue of final DSAEK graft thickness when preparing tissue to be transplanted, and no standardized method has been developed to consistently obtain ultrathin (UT) donor tissue. Consequently, it is impossible to prospectively evaluate a possible correlation between DSAEK graft thickness and visual outcome.

We therefore investigated the feasibility of three different microkeratome-assisted methods to obtain UT-DSAEK grafts in a simple, standardized, and reproducible manner.

## METHODS

The possibility of dissecting UT grafts for DSAEK surgery was investigated in the laboratory setting of Fondazione Banca degli Occhi in Venice (FBOV), Italy; the research was approved by our institutional review board. Forty human donor corneas preserved in tissue culture medium were used. All tissues were free of corneal pathology and were unsuitable for transplantation because of donor medical contraindications discovered after donation and retrieval.

Consent for the use of tissue for research was obtained from the donor's relatives at the time of donation in the event that the tissue might not have been suitable for transplantation. Human corneas were cultured according to conventional eye bank techniques.<sup>5,6</sup> Briefly, corneas were transferred from a short hypothermic storage (<12 hours between recovery and arrival at the eye bank) to an organ culture medium containing 2% (wt/vol) newborn calf serum. The basic medium was composed of MEM-Earle containing 25 mM HEPES, 26 mM bicarbonate, 1 mM pyruvate, 2 mM glutamine, 250 ng/mL amphotericin B, 100 IU/mL penicillin G, and 100  $\mu\text{g}/\text{mL}$  streptomycin. The mean storage time was 336.4 hours (minimum, 144 hours; maximum, 692.5 hours). To allow deturgescence, all corneas were transferred to transport medium (i.e., culture medium + 6% dextran) 24 hours before dissection.

The principle of an initial "debulking" cut followed by a second "refinement" cut was used in preparing the UT grafts. At the beginning of each dissection, the epithelium was removed, thus eliminating possible differences among the corneas induced by variations in epithelial swelling. After measuring central corneal thickness (CCT) by ultrasound (US) pachymetry (Alcon, Fort Worth, TX), the debulking of the donor graft using a Carriazo-Barraquer (CB) microkeratome with a 300- $\mu\text{m}$  head was performed on all donor corneas mounted on the artificial anterior chamber (AAC) of the ALTK system (Moria, Antony, France), as per conventional DSAEK surgery. A second microkeratome-assisted refinement dissection was carried out in three different ways on 10 corneas each (groups A, B, and C). In these groups, pressure in the system was standardized by raising the infusion bottle to a height of 120 cm above the level of the AAC and then clamping the tube at 50 cm from the entrance to the AAC. In addition, maximal care was taken to maintain a uniform, slow movement of the hand-driven microkeratome, requiring a time between 4 and 6 seconds for each of the two dissections in all cases. In addition, the dovetail of the AAC lid was

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rotated 180° before the refinement cut was started, which was performed from a direction opposite that of the debulking cut. Previous experiments at FBOV had clearly shown that the level of microkeratome dissection is deepest at the beginning of the cut, when tissue is engaged by the instrument. Performing the two cuts from opposite directions was instrumental not only in avoiding perforation but also in making the final thickness of the residual lamella even.

The remaining 10 corneas (group D) were not subjected to further manipulation and served as a control group. Corneas were matched for donor age, preoperative cell count, and death to preservation time in all four groups. Donor age varied between 60 and 74 years, endothelial cell count was between 2300 cells/mm<sup>2</sup> and 2800 cells/mm<sup>2</sup>, and time between death and preservation ranged between 15 and 18 hours.

In group A, the residual bed was artificially thickened by the injection of balanced salt solution (BSS) intrastromally with a 27-gauge needle through four peripheral sites (Fig. 1), one for each quadrant. BSS injection was stopped when hydration was completed, as shown by uniform bleaching of the tissue. This required an amount of fluid between 2 and 3 mL in all cases. Then CCT was measured by US pachymetry, and a commercially available 130- $\mu$ m microkeratome head was used to perform the second cut.

In group B, thickening of the residual bed was achieved at the eye bank by immersing the tissue in hypo-osmotic tissue culture medium solution for 24 hours before performing US pachymetry and the second cut, as in group A.

In group C, the residual bed was not artificially thickened. US pachymetry of the central cornea was measured, and dedicated microkeratome heads were used for the second cut. A 90- $\mu$ m microkeratome head was chosen for CCT values higher than 150  $\mu$ m ( $n = 8$ ), whereas a 50- $\mu$ m microkeratome head was selected for CCT values of 150  $\mu$ m or less ( $n = 2$ ).

To assess the final central thickness of the residual bed, all corneas were submitted for histologic examination at the end of the experiment. US pachymetry was not used because artificial hydration would affect the values recorded in groups A and B. In addition, thickness below 100  $\mu$ m cannot be measured with this method, and endothelial damage could be caused by the mechanical trauma on very thin tissue. Instead, direct measurement of the specimens under light microscopy was performed. Although moderate thinning of corneal tissue is induced by dehydration occurring after fixation, the use of light microscopy to determine final thickness was instrumental to even out the artifacts induced by artificial hydration of the tissue in groups A and B. The values obtained were arbitrarily increased by 15%, a corrective factor determined in previous experiments conducted in our laboratory (unpublished data on file, 2006) necessary to obtain the equivalent value of normally hydrated stromal tissue.

The issue of thickness regularity of the dissected tissue in the different groups was not addressed in this study. In fact, though CCT would be the same in any section passing through the center of the dissected area (such as the one measured in each specimen of our study), peripheral measurements could vary according to the orienta-

tion of the section examined, thus making proper evaluation impossible.

Central endothelial cell density and viability (trypan blue staining) were measured before and after dissection according to the method previously described.<sup>5,7</sup> Before assessment with light microscopy, the endothelium was exposed to 0.25% (weight/volume) trypan blue to count the nuclei of nonviable cells and to a hypotonic sucrose solution to visualize swelling of the intercellular borders, possibly indicating metabolic impairment and cell degeneration. The endothelial cell count was estimated using the same method before and after the experiments (i.e., at a magnification of 100 $\times$ ) with the help of a 10  $\times$  10 calibrated graticule mounted in the ocular of the microscope (fixed-frame technique). Endothelial density was expressed as the mean ( $\times$ 100) of five different counts, each performed in a different region of the corneal central area, avoiding the overlapping of cell counting. The spread of the five counts (coefficient of variation) ranged from 5% to 12%.<sup>5,7</sup>

The Fisher exact test was used to assess the statistical significance of differences in corneal thickness and endothelial cell density among the groups.  $P = 0.05$  was considered significant. Statistical analysis was performed using statistical analysis software (Advanced Statistical 11.0 software; SPSS Inc., Chicago, IL).

## RESULTS

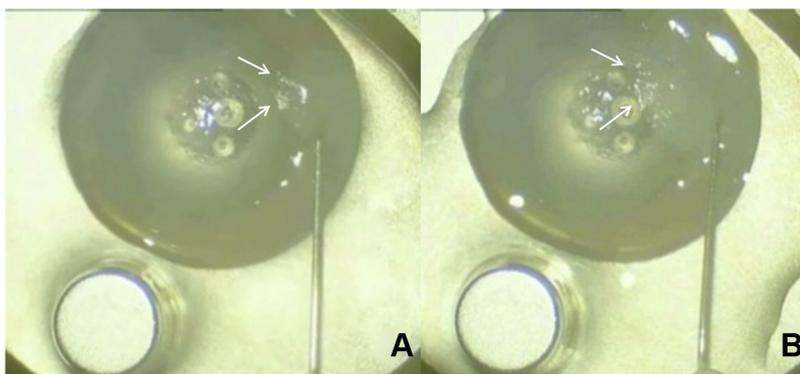
All corneas could be prepared successfully in each group, without any perforation. However, multiple areas of Descemet detachment were seen in 4 of 10 corneas in group A (Fig. 2).

Table 1 summarizes the CCT data for all groups at every stage of the experiment. There was no significant difference among the groups either in the initial thickness or after the debulking step. The average final CCT was significantly thinner in groups A, B, and C compared with the control group D ( $P < 0.001$ ). In addition, there was no significant difference in final CCT among the three groups undergoing the refinement step with different techniques, although the SD in group C was approximately half that in groups A and B. Statistical analysis showed  $P$  values of 0.14 when comparing group A with group C and 0.09 when comparing group B with group C.

Average endothelial cell counts did not differ significantly among any of the groups at the beginning and at the end of the experiment. Endothelial cell loss averaged 3.5% in group A, 4.2% in group B, 3.1% in group C, and 3.9% in group D.

## DISCUSSION

Descemet stripping endothelial keratoplasty (DSEK) was first described in 2005 by Price.<sup>8</sup> With this technique, donor grafts were prepared by manual dissection from donor corneas mounted on an artificial chamber. Although some authors have reported very satisfactory visual outcome after DSEK, manual



**FIGURE 1.** Bleaching of the stroma (white arrows) results from hydration by means of multiple intrastromal injections of BSS with a 27-gauge needle (A) and spreads across the tissue (B).

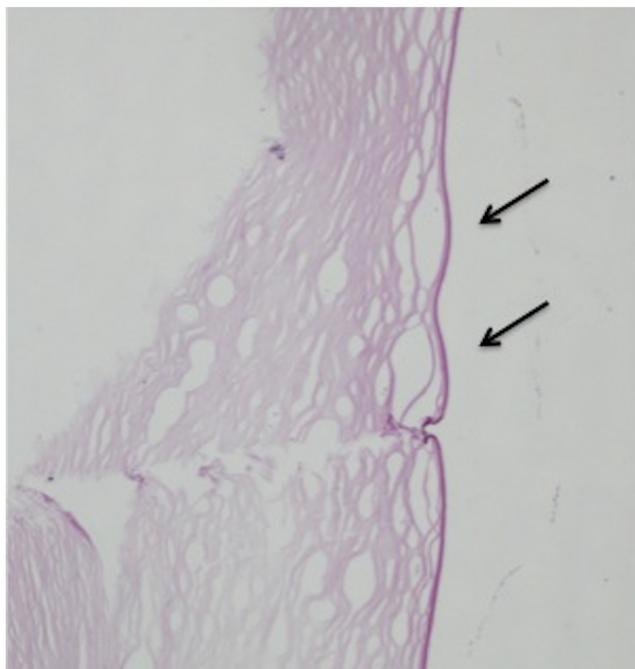


FIGURE 2. Histology of the residual bed of a cornea of group A, showing focal detachments of Descemet membrane (black arrows).

dissection of donor tissue is a time-consuming, painstaking procedure that may also result in a high rate of undesirable waste.<sup>9-11</sup> With the introduction of DSAEK, microkeratome-assisted dissection of donor corneas has become the gold standard for the preparation of grafts for endothelial keratoplasty, primarily because of the ease of this technique, the elimination of tissue waste, and the excellent quality of the stromal surfaces obtained in this way.<sup>12,13</sup> More recently, femtosecond laser technology has been used by several authors in limited series of patients but has not yet gained popularity because of the high costs involved and the suboptimal quality of the surfaces obtained with this type of dissection, which is strongly influenced by the characteristics of the donor tissue (hydration, thickness, clarity).<sup>14,15</sup>

The only major limitation of microkeratome dissection is its poor accuracy in determining the final thickness of the dissected tissue. In particular, microkeratome heads with wider slits produce lamellae with a greater variability in thickness.<sup>16</sup> To prevent perforation, tissue for DSAEK is cut with heads no wider than 300  $\mu\text{m}$  or 350  $\mu\text{m}$ , often resulting in a donor button with a significant amount of residual deep stroma.

To date, because no standardization of graft thickness has been introduced, the importance of this variable in determining the visual outcome of DSAEK could not be evaluated prospectively. In particular, it remains unknown whether only thinner grafts have the potential for faster visual recovery, 20/20 vision, or both. The reason for obtaining donor grafts with a very thin layer of stroma (UT grafts) is based on evi-

dence that in deep anterior lamellar keratoplasty, the visual outcome is better if the residual stromal layer in the recipient bed is limited to approximately 100  $\mu\text{m}$ <sup>17</sup> and on findings of the DSAEK study presented by Neff et al.<sup>4</sup>

In addition, thin grafts may result in a smaller or negligible hyperopic shift.<sup>18</sup> Finally, though not crucial to the final visual outcome, a very thin layer of residual deep stroma in the endothelial graft would allow donor tissue to be handled as it is in thicker DSAEK grafts, thus maintaining the same ease of delivery and the very low postoperative detachment rate. UT-DSAEK could share with DMEK the same excellent visual outcome but could represent a decisive improvement over a complex procedure requiring very high surgical skills but prone to a high postoperative graft detachment rate.<sup>2,19</sup>

To standardize microkeratome dissection of donor tissue, we arbitrarily set the pressure in the ALTK system at a fixed level by clamping the infusion tubing at 50 cm from its entrance to the AAC. The "closed system" condition obtained in this way guarantees homogeneous pressure in the system throughout the dissection and is easily reproducible. In addition, to minimize the risk for perforation, we used a double-cut type of preparation. If a single cut is performed using microkeratome heads with slits wider than 350  $\mu\text{m}$ , the actual thickness of the excised lamellae can vary from the intended thickness of 100  $\mu\text{m}$  or more.<sup>16</sup> Instead, after bringing down the thickness of donor tissue to <200  $\mu\text{m}$  with the first debulking dissection, a second cut can be carried out safely using microkeratome heads with narrow slits (130  $\mu\text{m}$  or less), which allow for more limited variations in the thickness of the excised lamella. Finally, based on previous experience collected at the eye bank, we realized that the risk for perforation is increased if both dissections are started at the same site, probably because the microkeratome blade reaches the deepest point in the tissue at the beginning of the dissection. By rotating the dovetail by 180° and therefore starting the second dissection from the end of the first one, we carried out the intended procedure successfully in all corneas tested, independently of the technique used.

The three methods chosen to attempt to thin out the residual bed of donor tissue to approximately 100  $\mu\text{m}$  proved equally effective.

To avoid perforation, artificial hydration is required to thicken the residual bed if the second cut is to be performed with a commercially available microkeratome head of 130  $\mu\text{m}$ . A different combination of microkeratome heads could also be used (i.e., a 200- $\mu\text{m}$  head twice), but, as mentioned, the smaller the slit of the microkeratome head used for the second dissection, the lesser the variation from the final intended thickness and the risk for perforation.

However, artificial hydration by means of direct injection of BSS into the corneal stroma (group A) was found to cause localized detachments of Descemet membrane in a high number of cases (4 of 10), making it impossible to apply this technique for clinical use.

The method of soaking donor tissue in hypo-osmotic solution (group B) could be easily used in the settings of any eye bank; it has the disadvantage of adding another step and of

TABLE 1. CCT Data for All Groups at Every Stage of the Experiment

Group	CCT Pre	CCT Deb	CCT Hyd	CCT Fin	ECD Pre	ECD Fin
A (stromal hydration)	558.3 $\pm$ 23.8	197.8 $\pm$ 33.2	301.2 $\pm$ 57.9	89.1 $\pm$ 34.1	2630 $\pm$ 176.6	2540 $\pm$ 164.6
B (hyposmotic solution)	552.9 $\pm$ 26.7	206.4 $\pm$ 34.2	257.2 $\pm$ 32.4	84.1 $\pm$ 18.6	2650 $\pm$ 217.3	2540 $\pm$ 195.5
C (dedicated heads)	554.8 $\pm$ 24.2	195.5 $\pm$ 34.5	N/A	72.1 $\pm$ 10.1	2600 $\pm$ 194.3	2520 $\pm$ 181.3
D (control)	559.5 $\pm$ 24.5	201.9 $\pm$ 25.3	N/A	201.9 $\pm$ 25.3	2580 $\pm$ 204.3	2480 $\pm$ 229.9

CCT, central corneal thickness; Pre, pre-cutting; Deb, post debulking; Hyd, post corneal hydration; Fin, final; ECD, endothelial cell density.

prolonging the time necessary for tissue preparation (to swell, the tissue must soak for at least several hours before the second cut), with a possibly increased risk for contamination. In addition, marking the site of the beginning of the first dissection may not last long enough to allow proper placement of the tissue in the AAC for the second dissection to be performed. UT grafts prepared in the eye bank would not have to be capped for shipment because the temporary increase in thickness caused by preservation after the second cut would facilitate handling at the time of surgery and would disappear after the recovery of endothelial function.

Finally, as in our series, dedicated microkeratome heads with slits narrower than 130  $\mu\text{m}$  can be used for the second dissection either at the eye bank or in surgery, immediately after the debulking dissection has been performed. This technique is simple and does not add a substantial amount of time to the procedure. In our series, variations from the final intended thickness of tissue cut with the 90- or 50- $\mu\text{m}$  heads were more limited than those obtained with the previous two techniques, probably because the variable of artificial tissue hydration was not present in this group. Marketing-dedicated microkeratome heads would probably be the ideal solution for eye banks and surgeons interested in evaluating possible advantages of UT grafts for DSAEK.

In conclusion, our series has shown that preparation of UT grafts for DSAEK can be easily standardized while avoiding waste of tissue. It does not require particular instrumentation unless dedicated heads of the ALTK system are used. Surgical use will show whether the theoretical advantages of UT grafts are confirmed by postoperative results.

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