

Tissue Processing for Ultra-Thin Descemet Stripping Automated Endothelial Keratoplasty

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ABSTRACT

PURPOSE: To optimize tissue preparation for ultra-thin Descemet stripping automated endothelial keratoplasty (DSAEK) by evaluating outcomes of double-cut corneal tissue processing on an artificial anterior chamber.

METHODS: Thirty-two corneas were analyzed. Donor corneas underwent microkeratome (MK) double-cut tissue processing. The corneal tissue was first cut at the thickest peripheral point and then 180 degrees away from the first pass. The tissue was measured by ultrasound (US) and optical coherence tomography (AS-OCT) for corneal thickness analysis and by specular microscopy for endothelial cell density.

RESULTS: Utilizing a double-pass technique, investigators successfully processed 24 of 32 corneas for ultra-thin DSAEK. Eight corneas perforated during tissue processing. The perforated tissues had no difference in tissue thickness prior to MK cuts but had a statistically significant lower mean central ($P=0.034$) and thinnest peripheral thickness ($P=0.019$) between MK cuts compared to tissues that did not perforate. Perforated tissues were more asymmetric ($P=0.0092$). Of the successfully processed tissues, 70.8% of tissues achieved a thickness of $\leq 100 \mu\text{m}$; however, 9 had significant endothelial cell damage that did not correlate with tissue thickness. Regarding tissue cutting, a strong correlation existed between the amount of tissue cut and MK head size used for the first pass ($r=0.82$) but not for the second pass ($r=0.22$).

CONCLUSIONS: The double-pass technique can create ultra-thin DSAEK tissue; however, certain tissue characteristics, processing techniques, and MK head size play a role in successful donor corneal tissue processing.

KEYWORDS: corneal transplantation, Descemet stripping endothelial keratoplasty, eye banks

Since its introduction by Dr. Melles in 1998 and refinement with microkeratome (MK) dissection in 2006 by Dr. Gorovoy,^{1,2} endothelial keratoplasty (EK) has become the principle method of surgical treatment for corneal endothelial disorders including Fuchs dystrophy. Accounting for 49% of all corneal transplants performed in United States in 2012, EK has been found to provide earlier visual recovery, less induced astigmatism, and better maintenance of globe integrity than its predecessor, penetrating keratoplasty (PK).^{3,4}

Endothelial keratoplasty techniques have become more refined over time, transitioning from manual dissection: deep lamellar endothelial keratoplasty (DLEK) to Descemet stripping (automated) endothelial keratoplasty (DSEK or DSAEK) to Descemet membrane endothelial keratoplasty (DMEK). The transition from DLEK to DSAEK resulted in significant improvement in patients' final visual acuity and in the rapidity of visual recovery.⁵ Interface architecture between the host tissue and the donor tissue impacts visual outcomes after DLEK and also, but less so, in DSAEK.⁶ In addition to interface architecture, graft shape and interface haze affect visual outcomes; however, the impact of graft thickness is debated.⁷⁻¹¹

DSAEK currently remains the most commonly performed endothelial keratoplasty procedure.¹² However, there is growing interest in DMEK because of reported improved visual outcomes and lower rates of graft rejection.^{13,14} Adoption of DMEK is limited, partly to the technical difficulty in tissue processing and difficulty of surgical implantation. Ultra-thin DSAEK emerged as a compromise between traditional DSAEK and DMEK. Ultra-thin DSAEK is variably defined in the literature as tissue less than 100 or 130 microns. For the purposes of our study, we defined ultra-thin DSAEK tissue as thinner than 100 microns.

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Tissue processing for ultra-thin DSAEK is different than standard DSAEK. Three methods to process tissue have been reported: (1) a double-cut MK technique, (2) a femtosecond laser technique, and (3) a high-pressure single-cut MK technique. We performed a series of experiments in an effort to standardize the protocol for a double-cut MK technique.


METHODS

Thirty-two human corneas were procured uniformly according to eye bank procedures by an *in situ* excision technique and were placed into Optisol GS storage medium. All corneas were deemed surgically suitable by eye banking standards¹⁵ with the caveat that death-to-processing time was extended to 10 days.

For ultra-thin tissue processing, corneas were mounted on an artificial anterior chamber (Moria, Doylestown, Pennsylvania) filled with balanced salt solution. We used a modified version of the single-pass Moria device designed for double pass prior to the existence of disposable kits. The anterior chamber cap comes in two pieces; the upper piece fits on top and can rotate for the second pass. Ultrasound (US) measurements were performed centrally (CT) and at four peripheral points (approximately 3 mm from central point). The thickest peripheral point was marked and labeled as '0' for US and AS-OCT (Visante Model 1000, Zeiss, Germany) measurements.

A sterile MK was used at the thickest peripheral point for the first pass to create the superficial free anterior cap. Selection of MK head size, with cutting head depths of 200 μm , 250 μm , and 300 μm , was based on the nomogram provided by Moria (Lamellar Keratoplasty System, Moria, Doylestown, Pennsylvania). US measurements were performed CT and at four peripheral cornea locations (approximately 3 mm from central point) between cuts. The central thickness value was used to calculate the appropriate MK blade (50 μm , 100 μm , 150 μm , 200 μm) for the second pass. The second MK pass was performed 180 degrees away from the first pass. US measurements were performed CT and at the four peripheral locations of the residual bed (RB).

The tissue removed by the second MK pass was discarded, the superficial anterior cap replaced, and the cornea placed into a viewing chamber in Optisol GS storage media. Endothelial cell density (ECD) counts were performed by specular microscopy (Konan Medical, Irvine, California). The central and four peripheral thicknesses were measured by AS-OCT at the same marked axis as the US measurements with two perpendicular AS-OCT cuts. Tissue symmetry



$$\text{Symmetry of tissues} = [\text{Mean peripheral thickness}] - [\text{Mean thinnest quadrant}]$$

Fig. 1. To assess tissue symmetry after the microkeratome passes, we calculated the difference between the mean peripheral thickness and the thinnest-point peripheral thickness.

was calculated by the difference between the mean peripheral thickness and the thinnest point peripheral thickness (Fig. 1).

Statistical analysis was performed using SAS 9.3 (Cary, North Carolina). Comparisons were made using Chi square, Fisher's exact test, T-tests, and Pearson correlation coefficients. A P value < 0.05 was considered statistically significant.

RESULTS

Utilizing a double-pass technique, investigators successfully processed 24 of 32 corneas for ultra-thin DSAEK. Eight corneas (25%) perforated during tissue processing. There were no differences in initial central thickness, initial mean peripheral thickness, or initial thinnest-point peripheral thickness between completed cuts and perforated tissues (Table 1). The perforated tissues had a statistically significant lower mean central (P=0.034) and thinnest peripheral thickness (P=0.019) following the first MK pass, and there was also a trend toward a difference in the post-cut mean peripheral thickness (P=0.059). Perforated tissues were also significantly more asymmetric. In perforated tissues, the difference between the mean peripheral thickness and the thinnest-point peripheral thickness was significantly higher compared to non-perforated tissues (35.8 μm vs 23.3 μm , P=0.0092). In tissues that perforated, the stated size of the second MK head was more similar to the amount of remaining tissue (thinnest peripheral thickness measurement) than in tissues that did not perforate (P=0.048).

Of the tissues that did not perforate, the mean RB thickness was 92.4 μm ; 70.8% of tissues achieved a thickness of $\leq 100 \mu\text{m}$. Of the 24 successfully cut tissues, 9 (37.5%) had significant endothelial cell damage (as determined by the inability to measure the endothelium by specular microscopy) that did not correlate with any parameter of tissue thickness (Table 2). The remaining 15 tissues had no significant change in ECD from processing (mean

Table 1. Corneal Thickness in Tissues with Completed Versus Perforated Processings

	Initial Measurements		P value	Measurements After 1st Microkeratome Pass		P value
	Complete	Perforated		Complete	Perforated	
Central thickness (μm)	562 ± 45	570 ± 33	0.60	244 ± 42	205 ± 40	0.034
Thinnest peripheral (μm)	596 ± 36	596 ± 30	0.97	249 ± 36	194 ± 49	0.019
Mean peripheral (μm)	619 ± 37	628 ± 36	0.55	272 ± 38	232 ± 49	0.059

pre-ECD=2646, mean post-ECD=2784; $P=0.248$). The mean death-to-preservation time was 8 hr, 40 min ± 2 hr, 50 min. The mean death-to-processing time was 5.47 days ± 1.76 days for our sample.

Regarding tissue cutting, there was a strong correlation between the amount of corneal tissue removed and MK head size for the first pass ($r=0.82$) (Fig. 2a), but only a weak correlation between tissue thickness and MK head size for the second pass ($r=0.22$) (Fig. 2b). After the first MK pass, the thinnest peripheral point between cuts was found at any of the four peripheral points. Despite the fact that the first MK pass always started at the thickest peripheral point, the distribution of the location of the thinnest point at the intermediate measurements were: 12% at 0 degrees, 47% at 90 degrees, 16% at 180 degrees, and 25% at 270 degrees. So although the tissue was cut at the thickest peripheral point, the thinnest peripheral point (which influenced perforation rates) could be at any location.

Table 2. Corneal Thickness in Tissues Processed Successfully With or Without Endothelial Damage

Parameter	Thickness (microns)	Thickness (microns)	P value
Mean pre-cut thickness	568 ± 46	552 ± 46	0.43
Mean post-cut #1 thickness	247 ± 44	239 ± 40	0.67
Mean post-cut #2 thickness (US)	131 ± 25	126 ± 27	0.71
Mean post-cut #2 thickness (AS-OCT)	98 ± 34	83 ± 25	0.22

DISCUSSION

DSAEK tissue processing protocols were developed using the same MK technology used for creating laser *in situ* keratomileusis (LASIK) flaps.^{8,16} However, actual protocols used in eye banks were developed *ad hoc* and have not been rigorously studied. As eye banks began performing tissue processing, they developed internal protocols to process donor tissue by lamellar dissection and ship it to the surgeon.^{17,18} Perhaps the reason for the lack of evidence-based practices is that current *ad hoc* processing techniques

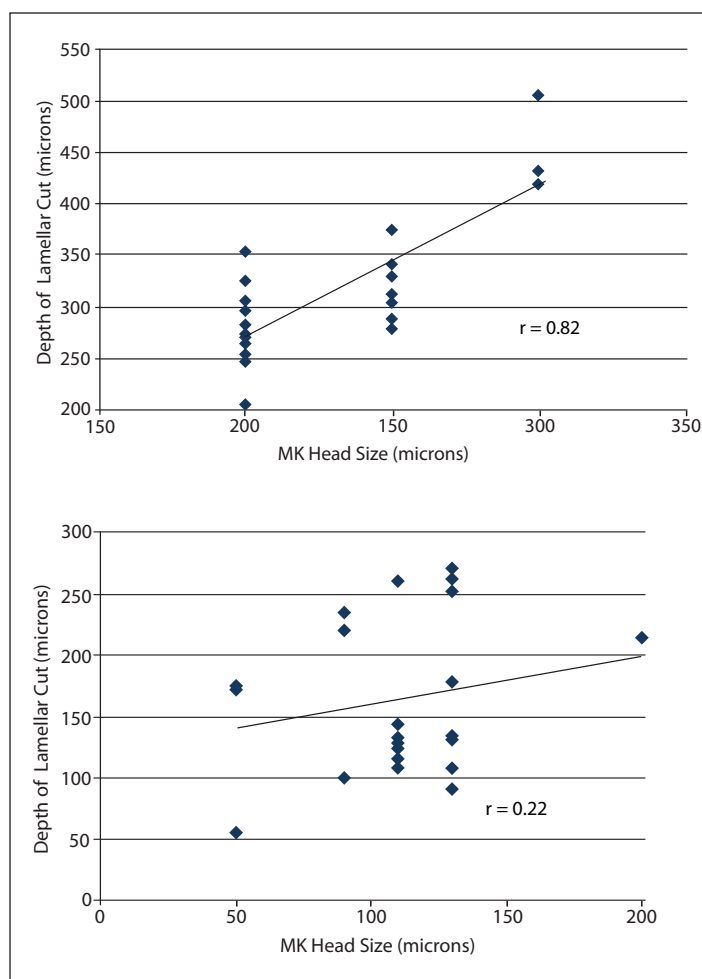


Fig. 2. Correlation assessments of the amount of corneal tissue removed compared to the labeled microkeratome head size are shown for the first microkeratome pass (Fig. 2a, top) and for the second microkeratome pass (Fig. 2b).

provide tissue that is effective for surgical use whether it is from an eye bank or provided by the surgeon.¹⁹ In an effort to improve the methods of tissue processing to generate ultra-thin DSAEK tissue, we studied ultra-thin DSAEK processing outcomes. The goal was to create double-cut tissue that maintained a healthy endothelium and that used a technique that could be reproduced consistently.

Current double-cut techniques use initial central corneal thickness to determine the appropriate MK head size. In this study, the MK head size for the first pass correlated well with the amount of corneal tissue removed, although the actual amount of tissue removed was consistently thicker than the labeled MK head size. However, unlike the correlation for the first pass, the second MK head size had a poor correlation with the amount of corneal tissue removed and was unpredictable.

We had a high perforation rate (25%) with double-cut MK tissue processing. If this rate persists in larger studies, it would have a large impact on processing costs to eye banks supplying tissue to surgeons and ultimately to corneal transplant tissue costs. All perforations occurred during the second MK pass. The technician performing all studies was highly experienced in single-pass DSAEK tissue processing; however, there were a limited number of total tissues included in the study. Perhaps the “learning curve” to acquire regular double-cuts and to minimize perforations requires more tissues than the total number in the study.

Several factors related to tissue thickness were significantly different between completed and perforated cuts. The between-cut central corneal thickness and thickness at the thinnest peripheral point were significantly lower in the perforated tissues. Higher residual thickness after the first cut likely plays a role in successful double-cut processing.

Tissue symmetry in the periphery also differed significantly between perforated and completed tissues. In tissues that perforated, the difference between mean peripheral corneal thickness and thickness at the thinnest peripheral point was significantly greater than in successfully processed tissues ($P=0.0092$).

MK blade selection affected perforation rates. In perforated tissue, the thickness at the thinnest peripheral point was closer in thickness to the selected MK head size. Technicians may want to select MK head size based on the thickness at the thinnest peripheral point between cuts rather than the central corneal thickness.

For the first MK pass, the tissue was cut at the thickest measured peripheral point. Our study showed that the between-cut thinnest peripheral location could be

found at any position in relation to where the cut was initiated. The location of initiating the cut does not predict where the thinnest point will be after the first pass. After the second MK pass, the thinnest peripheral point could again be found at any position on the tissue regardless of the between-cut thinnest peripheral point or the start location of the second MK pass.

Ultimately, 24 corneas successfully underwent double-cut processing with a mean RB thickness of 92.4 μm . Seventeen (70.8%) tissues were $\leq 100\mu\text{m}$ in central thickness as determined by AS-OCT. However, 9 corneas (37.5%) demonstrated significant endothelial damage. It remains unclear as to why these tissues had damage, given that all host corneas were determined to be of surgically suitable quality and were processed identically. There was no significant difference in mean corneal thickness in these 9 corneas at any stage of tissue processing. At the time of the study, we were unable to regulate the pressure in the artificial anterior chamber. Perhaps high intra-chamber pressure contributed to endothelial damage or low intra-chamber pressure led to contact between the corneal endothelium and the artificial anterior chamber (although this was not observed by the technician). To better assess tissue integrity in the future, we plan to perform vital dye staining on all tissues after tissue processing. Likewise, we will assess and standardize the pressure within the artificial anterior chamber during tissue processing to prevent inadvertent damage.²⁰

Although a relatively small number of tissues were used in this study, we believe these results confirm that double-cut MK tissue processing can be successful and can create healthy, thin tissue for endothelial transplantation. However, blade selection should be tailored to between-cut thickness measurements. More investigation is warranted for the causes of unexpected endothelial damage. We plan to modify our techniques to identify and optimize the best methods to process tissue to prevent perforations, minimize endothelial damage, and reproducibly create thin, symmetric tissue.

While a femtosecond laser technique to create ultra-thin tissue is appealing, there are financial and practical limitations to the use of a femtosecond laser. Certainly, the cost of tissue processing would rise with the use of a femtosecond laser. In addition, we have concerns that elevating the pressure in the artificial anterior chamber to create thin tissue with a single pass could damage the donor corneal endothelium. If all of the methods prove promising, vital dye studies and comparative studies of clinical outcomes would assist in validating the processing methods.

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