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Eye-bank Preparation of Endothelial Tissue

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Abstract

Purpose of review—Eyebank preparation of endothelial tissue for keratoplasty continues to evolve. While eye bank personnel have become comfortable and competent at Descemet Stripping Automated Endothelial Keratoplasty (DSAEK) tissue preparation and tissue transport, optimization of preparation methods continues. Surgeons and eye bank personnel should be up to date on the research in the field. As surgeons transition to Descemet Membrane Endothelial Keratoplasty (DMEK), eye banks have risen to the challenge of preparing tissue. Eye banks are refining their DMEK preparation and transport techniques

Recent findings—This article covers refinements to DSAEK tissue preparation, innovations to prepare DMEK tissue, and nuances to improve donor cornea tissue quality.

Summary—As eye bank supplied corneal tissue is the main source of tissue for many corneal surgeons, it is critical to stay informed about tissue handling and preparation. Ultimately the surgeon is responsible for the transplantation, so involvement of clinicians in eye banking practices and advocacy for pursuing meaningful research in this area will benefit clinical patient outcomes.

Keywords

Corneal transplantation; Descemet Stripping Endothelial Keratoplasty; Eye Bank

INTRODUCTION

In 1998, Dr. Melles re-introduced a technique to selectively replace the posterior cornea via endothelial keratoplasty (EK) [1] resulting in great enthusiasm for EK. In 2006, when eye banks began supplying pre-cut corneal tissues for EK, the number of corneas prepared for penetrating keratoplasty (PKP) began to decline, and corneas utilized for EK dramatically increased. In 2012, the Eye Bank Association of America reported that 68,681 corneas were distributed for keratoplasty [2] using U.S. supplied tissue, of which 24,277 (35%) were for EK, representing a 4.2% increase from 2011 and a 38.9% increase from 2008 [2].

EK has become the treatment of choice for corneal endothelial dysfunction. Donor corneas for EK can be prepared by surgeons or pre-dissected by eye-bank personnel. Studies examining eye-bank preparation report low tissue-processing failure rates and excellent quality [3] with comparable endothelial cell loss, visual outcomes, and detachment rates

between eye-bank and surgeon-prepared tissue [4]. Fungal and bacterial contamination rates and clinical infection rates [5] appear no higher for eye-bank prepared EK tissue than for PKP [5,6] and for anterior lamellar keratoplasty [6]. Eye-bank preparation of tissues increases operating room efficiency, minimizes tissue wastage, and allows for the preoperative measurement of graft thickness and endothelial cell density (ECD). The current and evolving techniques for eye-bank tissue preparation for EK are reviewed here.

Descemet Stripping Endothelial Keratoplasty (DSAEK)

DSAEK is the most commonly performed type of EK providing reduced visual recovery time and minimizing astigmatism compared to PKP. However, many patients fail to achieve 20/20 vision. Many factors contribute to visual outcomes following DSAEK, including presence of a stromal interface, and surgeons debate techniques to prepare optimal donor lenticules.

DSAEK tissue is most often prepared with a microkeratome. The donor corneoscleral rim is mounted on an artificial anterior chamber (ACC), and microkeratome cutting depth is adjusted to control the thickness of the resulting posterior lenticule [7]. Graft thickness asymmetry and irregularity can lead to a postoperative hyperopic refractive shift [8–10] and studies have demonstrated that microkeratome-prepared DSAEK corneas are nonuniform, non-concentric, and non-circular [11*]. Thinner lenticules can be obtained by using slower microkeratome passes, but graft asymmetry is more difficult to control [12]. Researchers investigated smoothing tissue with an excimer laser after the microkeratome pass. Cleary et al. demonstrated reduced stromal roughness, improved contour, and reduced thickness asymmetry without endothelial cell damage after excimer laser smoothing passes [13]. However, the clinical significance of these findings has not yet been established.

Femtosecond preparation of DSAEK tissue has been explored in hopes of making lenticules more precise and uniform. Compared to microkeratome-prepared tissues there is greater irregularity of the posterior corneal surface, rougher stromal beds, and increased thickness irregularity in femto-prepared tissues [14, 15]. No difference was noted in endothelial cell density and viability between the two techniques [15]. Both Vetter et al. and Mootha et al. suggest that irregular stromal dissections may occur because the femtosecond laser appplanation cone compresses and deforms the donor cornea [16]. One study suggested that optimized laser settings could improve the surface quality of femtosecond-prepared tissues making them equitable to microkeratome-prepared grafts [17].

Double-pass microkeratome techniques to yield ‘ultrathin’ DSAEK lenticules are another area of active research. The definition of ‘ultrathin’ tissue is variable, with most studies using 100µm but some studies using 130µm in thickness. The double-pass technique produces thinner grafts [18–20], but some studies report high perforation rates [20] and increased endothelial cell damage [18]. Sikder et al. observed that larger microkeratome head sizes led to greater variation in final graft thickness [20]. To decrease corneal perforation risk, Busin et al. hydrated grafts after the initial microkeratome cut, by intrastromal injections of balanced salt solution (BSS) or by immersing tissue in hypo-osmotic tissue culture medium for 24 hours [19]. Both strategies thickened the residual tissue bed and prevented perforations during the second microkeratome pass. However, the

BSS injections resulted in multiple areas of Descemet detachment, making this hydration technique impractical for clinical use.

Ultrathin tissue can be prepared using a low-pulse energy, high-frequency femtosecond laser [21]. There was no increased endothelial cell damage with this technique, but the resulting stromal surface appeared irregular. The authors of the study suggest that stromal bed quality can be improved with modified laser settings.

Descemet Membrane Endothelial Keratoplasty (DMEK)

In DMEK, the transplanted lenticule includes only Descemet membrane (DM) and the corneal endothelium. In contrast to DSAEK, no stroma-to-stroma interface exists with DMEK. Retrospective studies have shown that DMEK provides quicker visual recovery and improved visual acuity compared to DSAEK [22]. DMEK has not been widely accepted because of the challenges associated with preparing and handling the delicate graft tissue. DMEK graft preparation strategies are not as standardized as DSAEK graft preparation techniques.

Several harvesting strategies for DMEK donor preparation have been described. Melles et al. described a manual peeling method where a donor corneoscleral rim is immersed in BSS and the DM is peeled with 1-point fine non-toothed forceps [23–25]. A 4–7% endothelial cell loss rate was reported using this technique [23]. Giebel and Price subsequently described the SCUBA (submerged corneas using backgrounds away) technique, where a cornea is submerged in Optisol or BSS during harvesting to minimize surface tension; this allows the DM to settle back onto the stroma [26, 27]. Studies using the SCUBA technique had a 4.2% [28] to 8% [26] rate of unsuccessful graft preparation. The methods described by Melles and Lie and by Giebel and Price are similar, but have some specific differences reviewed in a previous article [29]. To better distribute tension, Kruse et al. used a second pair of forceps during peeling with a reported 1% graft loss rate [30]. Using the same technique, Schlötzer-Schrehardt et al. obtained uncomplicated and complete peeling in 96% of corneas and successful grafts with isolated tears in 2% of grafts [31*]. They found that 2% of corneas had extremely strong adhesions, created by ultrastructural peg-like interlockings or increased adhesive glycoproteins along the DM-stromal interface, preventing successful graft preparation [31*]. Yoeruek et al. proposed using a curvilinear forceps with a half-moon shaped non-toothed anterior segment to equally distribute the force needed for DM separation [32]. This technique decreased preparation time and resulted in lower endothelial cell death. Sikder et al. evaluated using a microkeratome to remove a majority of the donor stroma, followed by a Barraquer sweep to dissect the residual stroma from the DM [33]. There was minimal endothelial cell loss, but anterior segment optical coherence tomography (AS-OCT) revealed residual corneal stroma following preparation.

Pneumatic dissection is another technique utilized for DMEK graft preparation. First described by Anwar and Teichmann for anterior lamellar keratoplasty, air is injected into the cornea to create a dissection plane between the donor stroma and DM [34]; this technique has since been applied for DMEK graft preparation. Venzano et al. described the use of an artificial anterior chamber (AAC) in the Anwar air-bubble technique to harvest the

Descemet-endothelium complex [35]. They recommended trypan blue-staining of the endothelium to visualize needle positioning and pressure reduction in the AAC prior to air injection to facilitate big bubble formation with a success rate of 89%. Additionally, they reported a low endothelial cell loss (15%) when the bubble was immediately deflated after DM separation, but high endothelial cell loss (83%) when the bubble remained inflated. Zarei-Ghanavati et al. used a reverse big-bubble technique and reported that pneumatic dissection had greater success in older donors with high endothelial cell counts [36]. Busin et al. modified the pneumatic dissection technique to include a superficial keratectomy prior to air injection and reported a 5% preparation failure rate with 4% endothelial cell loss [37]. To add structural support and facilitate tissue handling with the pneumatic dissection technique, Studeny et al. described “DMEK with a stromal rim” (DMEK-S) [38]. In this technique, the central graft consists of only DM and endothelium, while an additional layer of posterior stroma is maintained in the periphery. The tissue loss rate fell to 5% with experience performing the technique. However, other studies evaluating the DMEK-S technique report a 23% tissue loss rate due to big bubble rupture and failure of big bubble formation [39].

Yoeuruk et al. compared DMEK preparation using air dissection and single-forceps direct peeling and found that air dissection reduced the time to prepare the graft [40]. There were no significant differences between the two techniques in terms of endothelial cell loss or rate of apoptosis, and they reported that both techniques produced stroma-free grafts. However, other studies evaluated the pneumatic dissection technique and observed, through histological analysis, that there was residual stroma in all grafts [41, 42].

In 2013, Muraine et al. [43**] reported a technique in which the cornea is mounted on an AAC and a 330° superficial trephination is performed. A cannula is inserted under the resulting flap and BSS is injected to detach the DM. They reported an endothelial cell loss of 4% after three days in storage. All tissue preparations for research were successful and grafts were stroma-free. Among corneas prepared for clinical use, there was a 4% failure rate.

Tissue Quality

Tissue quality can be assessed through the impact of donor tissue characteristics on endothelial keratoplasty complications such as graft failure and dislocation. In particular, recent research has evaluated tissue storage times, methods for assessing ECD and tissue thickness, tissue transportation, and strategies for storing and marking tissue.

ECD is an important consideration in determining donor tissue quality because it influences long-term corneal graft survival. A preoperative donor ECD of 2000 cell/mm² was determined as the lower limit for adequate PKP graft survival [44, 45], but a safe minimum ECD value for EK has not been validated in the literature. Using specular microscopy, no associations have been found between preoperative donor ECD and donor dislocation or 1-year postoperative ECD after DSAEK [46]. In fact, standard eye-bank methods for determining ECD, either with specular microscopy for cold stored tissues or with transmitted light microscopy for organ cultured tissues, overestimate the actual pool of viable endothelial cells [47]. Pipparelli et al. presented a method to determine the viable endothelial cell pool by triple labeling with Hoeschst/ethidium homodimer/calcein-AM and

performing image analysis of the whole graft surface [47]. By comparing the viable endothelial cell pool with the ECD determined by standard counting methods, they found that current protocols overestimate the viable endothelial cell pool in predissected EK grafts by 20%. Saad et al. also described the limitations of specular microscopy in determining ECD and developed an alternative method for ECD estimation [48]. Their method combines vital dye staining of endothelial cells with quantitative image analysis using Adobe Photoshop software. Future studies that assess viable ECD may be helpful in determining a safe minimum preoperative donor ECD for EK.

The safety of extending graft storage times is being investigated, as longer storage times would allow for an expansion of the corneal donor pool. Terry et al. performed a retrospective analysis on 362 eyes undergoing DSAEK and found no correlation between death-to-surgery time and endothelial cell loss at any postoperative time point [49]. In 2013, Ruzza et al. demonstrated that DSAEK donor tissue could be precut, trephined, and then stored in organ culture as a ready-to-use lenticule for up to 14 days without causing endothelial damage or tissue thickening [50]. A prospective multicenter National Eye Institute study, the Cornea Preservation Time Study is currently underway [51]. This study will track 3-year graft failure rates following DSAEK and compare outcomes of tissues stored 8–14 days with those stored 7 days or fewer.

Recent studies have reported on techniques for marking and storing donor lenticules. Gentian violet (GV) can be used to mark DSAEK donor stroma to facilitate appropriate tissue orientation in the anterior chamber. However, markings can persist at the graft-host interface [52], and there are case reports of GV causing significant corneal edema after DSAEK [53]. Using an in vitro model, Ide et al. demonstrated that GV markings on the donor stroma damage the corneal endothelium [54]. Accordingly, researchers developed marking strategies with smaller peripheral GV markings [55]. Stoeger et al. hypothesized that isopropyl alcohol in markers was causing endothelial damage [56]. They proposed applying GV ink to a Moria “S” stamp and then allowing the ink to dry before applying the stamp to the donor stroma. With this technique, there was no significant difference in endothelial damage between marked and unmarked corneas. Researchers have also evaluated the impact of culture conditions on visual outcomes in EK. Laaser et al. compared tissue storage in short-term culture (Optisol-GS; Bausch & Lomb) at 4C and organ culture (Dulbecco Modified Eagle Medium [Biocrom]; CorneaMax Medium [Eurobio]) at 34C and looked at outcomes following DMEK [57]. There were no differences in best-corrected visual acuity, postoperative ECD, or central corneal thickness between the two groups. However, more air injections were necessary in the short-term culture group to obtain graft adherence.

Methods for measuring EK donor tissue thickness have also been evaluated. Ultrasound pachymetry (USP) is the most commonly used method to assess donor tissue thickness, but AS-OCT has been investigated as it does not require corneal contact. Tang et al. found that predictability of microkeratome cut depth was significantly worse with AS-OCT measurements than with USP [58]. However, another study by Fante et al. found that AS-OCT measurements of central corneal thickness in precut EK donor tissues were not significantly different from USP measurements [59]. Tang et al. evaluated DSAEK graft

deturgescence following the microkeratome cut to determine the optimal time for graft thickness measurement [60]. Their study found that the thickness of precut tissue stabilizes around 2 hours after microkeratome cut.

Studies have also investigated tissue transportation. Ide et al. observed significant endothelial cell damage in DSAEK lenticules that were transported in Optisol GS without the anterior lamellar corneal tissue (ALCT) cap [61]. Interestingly, Jhanji et al. later observed that when DSAEK lenticules were stored in organ culture, there was no increase in endothelial cell damage in lenticules that had been stored without overlying ALCT cap [62]. Global shortages in the corneal donor pool have also prompted researchers to evaluate the safety of transporting precut DSAEK lenticules for use overseas. Yamazoe et al. retrospectively reviewed 124 DSAEK tissues that had been transported overseas to Japan and found that all received tissues used had an ECD >2000 cells/mm² before surgery [63*]. They argued that with overseas transport and precutting, one can maintain acceptable graft quality.

Conclusion

Surgical techniques for endothelial keratoplasty and tissue preparation methods evolve as we attempt to optimize clinical outcomes of transplant patients. The process of corneal tissue transplantation starts with a donor's gift of sight and a recipient's need for a new cornea. The numerous steps in the process to result in successful transplantation with excellent visual outcomes are influenced by the research done in the field of eye- banking. This article highlights current preparation techniques, refinement of each technique, and ongoing research in this area. Many corneal transplant recipients will directly benefit from the dedicated work devoted to optimizing endothelial keratoplasty.

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Key Points

- Endothelial keratoplasty represents over one third of the corneal transplantations performed in the United States.
- Descemet stripping endothelial keratoplasty (DSAEK) is the most common form of endothelial transplantation.
- The donor tissue preparation method for DSAEK has been modified using microkeratome and femtosecond lasers to optimize the characteristics of the transplanted tissue.
- Tissue preparation methods for Decemet membrane endothelial keratoplasty (DMEK) are being refined and now eye banks are preparing tissue for DMEK.
- Donor corneal tissue quality parameters must be used to evaluate new techniques including endothelial cell evaluations (cell density and vital dye staining), graft storage times, culture media, and tissue microscopic characteristics (smoothness, thickness, and symmetry).

Table 1

Summary of Endothelial Keratoplasty Techniques

Technique		Intervention	Targeted Graft Thickness	Graft Components
DSAEK	Microkeratome-prepared	A mechanical microkeratome is used for intrastromal cutting in donor corneal preparation.	200µm	• Posterior donor stroma
	Femtosecond-prepared	A femtosecond laser is used for intrastromal cutting in donor cornea preparation	100–200µm	• Donor Descemet's membrane
	Ultrathin	Donor corneas undergo two passes with a microkeratome, first with a thicker and second with a thinner pass.	100µm to 130µm	• Donor endothelium
DMEK		The DM-donor endothelial complex is isolated from adjacent stromal by peeling, pneumatic dissection, or superficial trephination with hydrodissection.	N/A	• Donor Descemet's membrane • Donor endothelium

DSAEK: Descemet's stripping automated endothelial keratoplasty; DMEK: Descemet's membrane endothelial keratoplasty; DM: Descemet's membrane