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Viability of Descemet membrane endothelial keratoplasty grafts folded in the eye bank

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Abstract

Purpose—Preloaded, tri-folded grafts in Descemet Membrane Endothelial Keratoplasty require transfer of the tri-folding process from the cornea transplant surgeon to the eye bank technician. We sought to assess if tri-folding may be safely conducted by an eye bank technician with cell loss comparable to standard peeling and lifting.

Methods—A total of ten grafts were stained, peeled and transferred directly onto a bed of Calcein-AM and Amvisc Plus by an eye bank technician. Five grafts were removed and stained as a scroll and five grafts tri-folded with the endothelium in before transfer. Photographs were acquired with an inverted fluorescence microscope and image segmentation was performed. A t-test was conducted to compare for difference in endothelial cell loss across groups.

Results—Mean cell loss in the scroll group was 18.5% [95% confidence interval (CI): 15.2–21.9%] compared to 7.6% the tri-folded group (95% CI: 1.7–13.5%). A two-tailed t-test indicated decreased cell loss in the tri-folded group ($p=0.013$).

Conclusion—Despite additional manipulation of the graft, tri-folding of Descemet membrane and endothelium may be performed by an eye bank technician without significantly increased cell loss relative to graft preparation as a scroll.

Keywords

Descemet membrane endothelial keratoplasty; cornea transplantation; eye banking

INTRODUCTION

Descemet membrane endothelial keratoplasty (DMEK) provides optimal visual acuity, decreased healing time, smaller incisions, and lower rates of rejection relative to Descemet

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stripping automated endothelial keratoplasty (DSAEK).^{1,2} However, intraoperative tissue handling and unrolling a scrolled graft within the anterior chamber remains challenging for many surgeons.

Numerous techniques and devices have been described to simplify this procedure. Grafts can be fully peeled and loaded into cartridges in the eye bank and sent preloaded to the surgeon.³⁻⁵ The tri-fold technique, which folds the DMEK graft endothelium-side-in, thereby protecting the endothelium during entry, reduces the need to unscroll the tissue within the anterior chamber.^{6,7} While it is known that the DMEK graft can be tri-folded and pre-loaded in a cartridge,^{8, 9, 10} published methods for folding the graft have focused on the perioperative setting, in which tissue manipulation is conducted by the surgeon. Preloading requires a transfer in the process and performance of tri-folding from the surgeon to a technician. To assess feasibility of this transfer, we assess viability of tri-folding a DMEK graft by a technician within the setting of the eye bank, using a standard cell loss measurement technique.

MATERIALS AND METHODS

Graft procurement

Corneas recovered by Rocky Mountain Lions Eye Bank (RMLEB, Aurora, CO) from donors with research consent and found non-eligible for transplant use were identified. Corneas with full thickness wounds or scars as well as anterior infiltrates were excluded. Suitable study corneas had no worse than mild localized endothelial cell loss or damage as rated by routine eye bank technician slit lamp evaluation.

The endothelium for each cornea was verified via slit lamp to be reflective and have clearly visible cell borders. Study corneas were from donors 35 to 71 years of age (median of 58 years), had a death to preservation duration ranging from 3:59 to 14:56 hours (median of 5:46 hours) and death to DMEK prep duration from 3 to 14 days (median of 6 days). As a confirmation immediately prior to DMEK prep, each cornea was removed from the corneal storage media (Optisol-GS, Bausch and Lomb, St Louis, MO) and stained with Trypan Blue (VisionBlue, Dutch Ophthalmic) for 2 minutes in order to identify if there was any unnoticed cell loss or damage that would preclude use for the study.

The eye bank was selected as a third-party tester as the principal investigator (AE) has no financial relationship or tissue orders associated with this organization.

Peeling of grafts

DMEK grafts were prepared according to standard RMLEB procedures without an “S” (stromal side of membrane) stamp. The cornea Descemet membrane (DM) was punched using a 9.5 mm diameter guarded corneal punch (Moria, Antony, France). A short (30 second to 1 minute) trypan blue stain was conducted for wound visualization of the DM punch. The peripheral apron of DM was removed, leaving the central 9.5mm of DM intact (DM disk). Within a pool of corneal storage media, a Sinskey hook was used to separate the peripheral edge of the 9.5mm DM disk from stroma at the punch wound for approximately 350 degrees, leaving a small peripheral attachment. The peripheral attachment was oriented

to 6'oclock and the 12'oclock edge of the DM disk grasped with the “heel” portion of curved tying forceps and carefully peeled from stroma by pulling towards the peripheral attachment until approximately 90% of the DM disk was separated. The peeled DM was carefully laid down in the pool and sponges and corneal storage media used to return the disk to its original orientation on the stroma by use of fluid movement and capillary action, by drawing the fluid towards one side using the sponge. Excess Optisol-GS was removed to ensure a flat, unscrolled position. The disk was then punched to 8.0mm diameter surgical graft size using a standard corneal punch (Moria, Antony, France), followed by trypan blue application ensuring stromal side exposure.

Graft Transfer

Method 1: Unsupported Forceps Method (Scroll Method)—Using forceps, the DMEK graft was gently lifted off the stromal bed to completely separate the peripheral attachment and held briefly unsupported and contracted over the corneal bed. Trypan was introduced to the well formed by the cornea and the contracted DMEK graft placed back into the stain pool for 2 minutes. The graft was rinsed with BSS (Alcon, Ft. Worth, TX) then lifted unsupported with forceps and transferred directly onto a bed of Calcein-AM and Amvisc Plus. This method of lifting the graft unsupported, or out of solution and contracted, is commonly used by surgeons to trypan stain and then move the graft into a dish of BSS for inserter loading.

Method 2: Tri-fold and Drag Method—The DMEK graft peripheral attachment was separated with a Sinskey hook by sliding gently under the membrane. Two or three drops of trypan were introduced and the graft edges were freed from the punch wound by slightly lifting edges with a Sinskey hook allowing trypan to flow into the interface between DM and stroma. After approximately 1 minute the graft was carefully tri-folded⁶ using forceps so that endothelium was on the inside of the overlap fold as shown in Figure 1. The tri-folded graft was then transferred next to the bed of Calcein-AM and Amvisc Plus by use of the anterior corneoscleral rim for graft support. The graft was then carefully dragged off the cornea and onto the Calcein/Amvisc bed.

Staining and Imaging

Once transferred, fluorescence staining was performed with a solution of Calcein-AM (Invitrogen, Thermo Fisher Scientific) for cell viability. Amvisc Plus (Bausch & Lomb, Rochester, NY) was used to protect the cells and unscroll the graft without direct instrument manipulation for viability assessment and imaging.¹⁰ Cell loss assessment was performed using the same solutions and techniques between both transfer methods. After approximately 20 - 40 minutes of Calcein-AM exposure, the entire graft endothelium was imaged. Several images over the entire graft area were captured through a Leica DMIL inverted fluorescence microscope using an attached Amscope model MT5000(IFR) CCD. Images from each graft were stitched into one complete whole image using Adobe Photoshop. The whole image was then analyzed using Fiji ImageJ¹¹ with trainable segmentation by an individual not involved with the DMEK graft preparations.

Statistical Analysis

We utilized a t-test to compare endothelial cell loss between groups as measured by Fiji segmentation analysis. Based on control data from previous studies,¹³ with 5 grafts in each group and alpha of 0.05, we would be powered at 0.98 to identify a 10-percentage point absolute difference in cell loss between the two methods. The null hypothesis tested indicated that the tri-fold group and scroll group resulted in comparable endothelial cell loss.

RESULTS

A total of five grafts were prepared using each technique and randomly distributed across groups. Mean donor age was 54.4 in the scroll group and 63 years old in the tri-folded group ($p=0.23$). There was no difference in death-to-preservation time across groups, with mean of 7 hours, 40 minutes in the scroll group and 8 hours, 26 minutes in the tri-fold group, respectively ($p=0.78$). Preoperative endothelial cell density was 2686 and 2161 cells/mm² in the two groups, respectively ($p=0.25$).

Neither approach resulted in tears or loss of tissue. Representative images of stained and segmented grafts are shown in Figure 2 and measured endothelial cell loss is shown in Table 1.

Mean cell loss in the scroll group measured 18.5% (95% CI, 15.2-21.9%) compared to 7.6% cell loss in the tri-fold group (95% CI, 1.7-13.5%). Two-tailed t-test demonstrated significantly less cell loss in the tri-fold and drag method ($p=0.013$).

DISCUSSION

We demonstrate the ability of an experienced technician from an eye bank to tri-fold tissue with associated cell loss that is not inferior to the lifting technique commonly utilized by clinicians in the operating room.

The rate of cell loss in the samples peeled and removed as a scroll are consistent with the 22.0% and 19.2% rates of cell loss in groups measured by Wolle and colleagues using a comparable methodology of assessment.⁴ It is notable that tri-folding resulted in significantly less cell loss in this study, which we believe may be due to the protection of an endothelium-in morphology during manipulation and staining. The data suggest that at the minimum, the additional manipulation of the periphery of the graft required for tri-folding does not appear to contribute to increased cell loss in the tri-folding group.

This study is limited by its sample size, and additional studies are necessary to determine if our observation of non-inferior cell loss in tri-folding extends to a larger and more diverse test set of eye banks, technicians, and grafts. A post-hoc analysis of power based on the distribution of results suggests 88.4% power to identify the difference found between groups.

As DMEK continues to gain adoption, an increasing proportion of the tissue preparation process is moving from the surgeon to the eye bank. The results of this study suggest that tri-

folding may be conducted by an experienced technician in an eye bank setting without compromising graft viability.

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FIGURE 1.

Tri-folding of the DMEK graft. (A) The first flap of tri-fold is lifted with forceps. (B) This flap, approximately one-third of the DMEK graft in width, is laid onto the central, posterior corneal surface. (C) The corneal bed is rotated 180 degrees to facilitate folding and the second flap of tri-fold is lifted with forceps. (D) The second flap completely overlaps the first flap, creating a tri-fold configuration.

FIGURE 2.

(A) Sample image from DMEK graft pulled up using unsupported forceps method by a certified, experienced eye bank technician. A “V sign” of endothelial damage extending from the point of fixation was commonly seen using this method. (B) Sample image from DMEK graft tri-folded by a certified, experienced eye bank technician. Linear streaks are consistent with iatrogenic folds applied to the graft. (C, D) Segmented image in Fiji demonstrates focal areas of cell loss.

TABLE 1

Percentage cell loss as measured by Fiji segmentation analysis of DMEK grafts stained, peeled, and removed in a standard lifting or tri-folding approach by an eye bank technician. Tri-folding resulted in significantly decreased cell loss relative to the standard scrolling method ($p=0.01$).

Unsupported Forceps	Tri-fold
19.2	3.3
15.9	7.9
15.8	18.6
16.8	1.3
24.9	7.0