# Comparison of the Modified Jones Tube Technique and the DMEK EndoGlide Technique With and Without Viscoelastic Material for DMEK Tissue Preparation

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**Purpose:** The aim of this study was to compare endothelial cell loss for DMEK (Descemet membrane endothelial keratoplasty) tissue preparation techniques using the modified Jones tube and the DMEK EndoGlide with and without viscoelastic material to protect the endothelium.

**Methods:** This ex vivo study included 10 DMEK grafts prepared using each of the 3 abovementioned techniques. After tissue preparation, transport conditions were simulated for a minimum of 45 hours before deployment of the DMEK tissue and quantification of endothelial cell loss. Comparisons between preparation technique groups were made using the Wilcoxon rank-sum test.

**Results:** The Jones tube group had a mean endothelial cell loss of 11.0 ± 4.8% compared with the EndoGlide group with 12.9 ± 6.7% and the EndoGlide with viscoelastic group with 25.7 ± 15.0%. The differences between the EndoGlide with viscoelastic group and the other 2 were statistically significant both before ($P < 0.01$ and $P = 0.01$) and after ($P = 0.01$ and $P = 0.02$) adjusting for baseline characteristics. The difference between the EndoGlide and Jones tube groups was not significant ($P = 0.73$ and $P = 0.53$ after adjustment). Microscopy revealed endothelial cell loss in the area of viscoelastic use for the EndoGlide with viscoelastic group.

**Conclusions:** Both the Jones tube and DMEK EndoGlide resulted in similar low rates of endothelial cell loss after tissue preparation, transport, and deployment. However, use of viscoelastic material to protect the endothelium using the DMEK technique actually resulted in increased cell loss in the area of its application resulting in overall higher rates of cell loss across the DMEK tissue.

**Key Words:** DMEK tissue preparation, EndoGlide, Jones tube, viscoelastic

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Descemet membrane endothelial keratoplasty (DMEK) has modernized the surgical treatment of Fuchs endothelial dystrophy and corneal endothelial failure. One challenge of the procedure is preparing and transporting the corneal tissue in a way that minimizes endothelial cell loss and maximizes ease of tissue deployment into the eye. One of the early DMEK tissue implantation techniques used a modified Jones tube (Gunther Weiss Scientific Glass, Portland, OR) into which the tissue was suctioned and suspended in fluid for storage, transport, and deployment. When using the Jones tube technique, the DMEK tissue scrolls endothelium-outward, which puts the cells at risk when coming in contact with the walls of the tube. In addition, lack of control of graft orientation when inserting into the eye can lead to inadvertent unscrolling with the endothelium toward the host stroma, forcing surgeons to reorient the tissue in the eye to allow for graft apposition.[1]

The use of the EndoGlide Ultrathin (AngioTech, Reading, PA/Network Medical Products, North Yorkshire, United Kingdom) for insertion of DMEK tissue was first described in 2016 with use of a stromal scaffold to assist folding of the DMEK tissue endothelial surface inward within the EndoGlide tube.[1] The DMEK tissue only is then pulled directly into the eye using forceps. The benefit of this technique is the protection of the endothelium and control of the graft orientation given the manual gripping of the graft with forceps. Later, this technique evolved with the use of the EndoGlide Ultrathin without scaffold[2] and the CORONET DMEK EndoGlide (Network Medical Products, Ripon, United Kingdom).[3]

The purpose of this project was to fill an existing gap in the literature by directly comparing the DMEK EndoGlide technique with the Jones tube technique for DMEK tissue preparation, as well as evaluate the effect of using viscoelastic material to protect the endothelial cells from mechanical disruption while using the DMEK EndoGlide loading technique.

## METHODS

This study was conducted in compliance with the tenants of the Declaration of Helsinki. Institutional review board approval was not required because it was not a human subject research.

Corneal tissue used in this study was deemed appropriate for research purposes due to donor-related health criteria. However, all tissue used in this study was found otherwise

suitable for transplantation after undergoing standard slit-lamp evaluations and graded as having mild or less endothelial cell damage, stress lines, or cell drop out. DMEK grafts were prepared by 1 experienced eye bank technician using a modified scuba technique according to standard Rocky Mountain Lion's Eye Bank (RMLEB) procedures leaving a small peripheral attachment. Descemet membrane (DM) disks were peeled using the "heel" portion of curved tying forceps. Fluid and capillary action were used to return the peeled DM disk to the original position on the donor stroma, and the disks were punched to 8.0-mm diameter surgical graft size. Ten grafts received 2 minutes of trypan blue 0.06% (C-Blue; Stephens, Lexington, KY) and were then trifolded endothelium-in and pulled into the DMEK EndoGlide using microforceps (EndoGlide group). Ten additional grafts received a bead of viscoelastic (Amvisc Plus; Bausch & Lomb, Rochester, NY) covering the central third of the graft after the 2-minute trypan application and then were trifolded and pulled into the EndoGlide using microforceps (EndoGlide with viscoelastic group). Ten additional grafts received 2 minutes of trypan blue 0.06% application and were then scrolled endothelium-out and were suctioned into a modified Jones tube (Jones tube group). All DMEK grafts and devices were then sealed in a vial of standard corneal storage media (Life4C; Numedis, Isanti, MN).

Transport conditions were simulated for a minimum of 45 hours by packaging the grafts according to RMLEB standard packaging and shipping procedures. After shipping, the EndoGlide-loaded grafts were deployed using microforceps from the inserter (and the Jones tube–loaded grafts using fluidics) onto a bed of viscoelastic and calcein AM (Invitrogen; Thermo Fisher Scientific, Eugene, OR).[4] Calcein AM-stained grafts were imaged with a Leica DMIL inverted fluorescence microscope after 20 to 40 minutes of calcein AM exposure. Multiple images over the entire graft area were captured and then stitched together. The image was then analyzed with Fiji ImageJ with trainable segmentation to determine endothelial cell loss (ECL) using living versus damaged endothelial percentages.

Donor age, death to preservation time, initial cell count, and percent damaged endothelial cells after loading and unloading were collected for each graft. Means and standard deviations for each variable were presented and compared using analysis of variance testing, and comparisons between groups were made using the Wilcoxon rank-sum test before and after adjusting for donor age, death to preservation time, and initial cell count. Analyses were performed using SAS software, version 3.8, SAS Institute Inc., Cary, NC.

## RESULTS

The average donor age, death to preservation time, and initial endothelial cell count are displayed in Table 1. After the process of graft preparation and simulated transport, the preparation using the EndoGlide and viscoelastic resulted in an average cell loss of 25.7% ± 15.0%, compared with the EndoGlide with 12.9% ± 6.7% cell loss and the Jones tube with 11.0 ± 4.8% cell loss (Fig. 1). The difference in cell loss between Jones tube and EndoGlide with viscoelastic as well as between the EndoGlide and the EndoGlide with viscoelastic both reached statistical significance ($P < 0.01$ and $P = 0.01$) while the difference between the Jones tube and the EndoGlide was not significant ($P = 0.73$). After adjusting for donor age, death to preservation time, and initial cell density, these differences maintained statistical significance ($P = 0.01$ and $P = 0.02$) while the difference between the EndoGlide and the Jones tube remained insignificant ($P = 0.53$).

Photographs of the calcein AM staining fluorescent microscopy and postsegmentation in Fiji are displayed in Figure 2. The EndoGlide group displayed a typical pattern after tissue preparation of patchy endothelial cells loss, while several grafts in the EndoGlide with viscoelastic group tissue demonstrated a linear area of endothelial cell loss in the area of viscoelastic application, some had more diffuse cell loss and some displayed a pattern more similar to the EndoGlide without viscoelastic group (Fig. 3).

## DISCUSSION

Attempts to minimize endothelial cell loss during donor preparation and insertion while maximizing endothelial cell counts long term have resulted in a multitude of graft preparation and insertion techniques. The goal of this study was to compare the Jones tube and the DMEK EndoGlide preparation techniques, as well as determine the effect of viscoelastic material used to protect against mechanical damage to the endothelium in the DMEK EndoGlide technique. We found that the Jones tube and EndoGlide techniques had comparable profiles of endothelial cell loss after graft loading, transport, and unloading. However, using viscoelastic material to protect the endothelium resulted in a significant increase in cell loss, specifically in the area of viscoelastic application.

**TABLE 1.** Initial Characteristics of Endothelial Graft Tissue

| Preparation Technique | Donor Age (years±Standard Deviation) | Death to Preservation Time (hours±Standard Deviation) | Initial Cell Count (Cells/hpf±Standard Deviation) | Initial Nonviable Cells (% ± Standard Deviation) |
|---|---|---|---|---|
| EndoGlide (n = 10) | 60.5 ± 12.6 | 10.8 ± 4.5 | 2456 ± 514 | 12.9 ± 6.7 |
| Jones Tube (n = 10) | 53.9 ± 9.2 | 6.08 ± 2.1 | 2252 ± 622 | 11.0 ± 4.8 |
| EndoGlide with viscoelastic (n = 10) | 57.4 ± 10.8 | 11.7 ± 5.6 | 2874 ± 453 | 25.7 ± 15.0 |
| *P*-value (ANOVA) | 0.415 | 0.016 | 0.049 | 0.0047 |

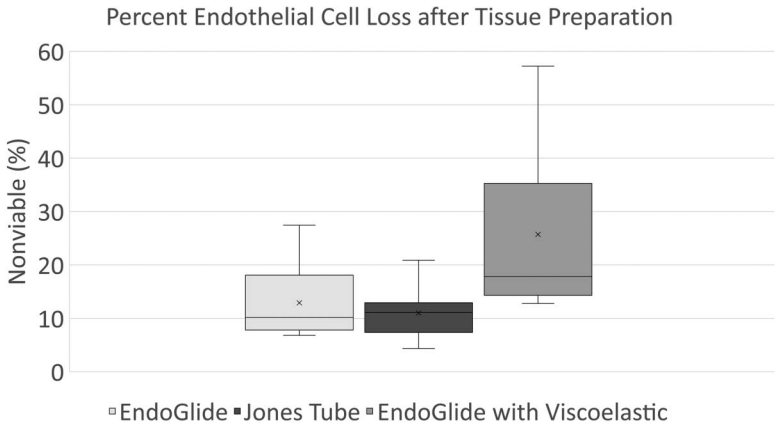


**FIGURE 1.** Percent endothelial cells loss for each technique. The "x" marks the mean, and the horizontal line represents the median for each preparation technique. The box marks the range encompassing the 25% to 75% quartiles, and the whiskers are the minimum and maximum value. The EndoGlide with viscoelastic technique resulted in a significantly higher percentage of endothelial cells lost than either the EndoGlide or Jones Tube techniques ($P = 0.01$ and $P < 0.01$ respectively).

Although our study characterized only endothelial cell loss ex vivo posttissue processing, several studies have described cell loss in the months that follow surgical implantation. Early research using the EndoGlide Ultrathin with a scaffold demonstrated an endothelial cell loss of 48% ± 11% at 6 months after transplantation in the after learning curve[1] in comparison to 19% to 40% at the 6 to 12 months follow-up in the DMEK literature of various endothelium-out techniques.[5] Later, studies using the EndoGlide Ultrathin with stromal carrier resulted in 24.2% ± 17.5% endothelial cell loss at 6 months in the after learning curve.[6] A direct comparison of endothelial cell loss using the endothelium-in EndoGlide Ultrathin technique without a stromal scaffold versus the endothelium-outward Geuder cartridge technique (Geuder AG, Heidelberg, Germany) resulted in 41.5% ± 8.9% endothelial cell loss versus 63.1% ± 24.9% ($P = 0.013$) in an ex vivo study.[7]

Later, the Coronet DMEK EndoGlide was developed and resulted in ex vivo endothelial cell loss of 9.2% ± 7.0% versus 11.3% ± 3.0% using the Geuder technique ($P = 0.07$)[3] and an endothelial cell loss of 33.6% after transplantation at 6 to 11 months follow-up.[8] Our DMEK preparation techniques resulted in overall similar ex vivo endothelial cell loss percentages as the previous DMEK EndoGlide study versus the Geuder study,[3] indicating that both endothelium-out Geuder and modified Jones tube techniques remain comparable to the endothelium-in DMEK EndoGlide for endothelial cell loss attributable to the tissue preparation, storage, and deployment processes. In addition, previous studies by our group using a different type of endothelial-in plastic cartridge for DMEK delivery compared with the Jones tube resulted in comparable ex vivo ECL to one another, as well as similar numbers to those in the present study, which underscores the repeatability of processing techniques at our eye bank and comparability of the endothelium-in and endothelium-out techniques.[9] The DMEK EndoGlide technique maintains promise for decreased endothelial cell loss after surgical implantation compared with the Jones tube and Geuder tube techniques due to less expected need for tissue manipulation during surgery given control over tissue orientation during implantation.

Viscoelastic is routinely used to protect the corneal endothelium during cataract surgery. It has also been used as a surface on which to fold the endothelial side of the DMEK tissue for purposes of marking on the stromal side of the folded tissue (negating the need for a paracentral punch in the stroma)[10]; however, this technique involves folding the tissue back on to the stromal side of the graft and rinsing off all remnant of viscoelastic material from the endothelial side before loading. Furthermore, viscoelastic has also been used to protect the endothelium when using femtosecond laser to prepare grafts, but again contact time between the endothelium and viscoelastic was limited.[11] In the case of DMEK tissue preparation and storage in the present study, the viscoelastic likely smothered the endothelial cells, limiting

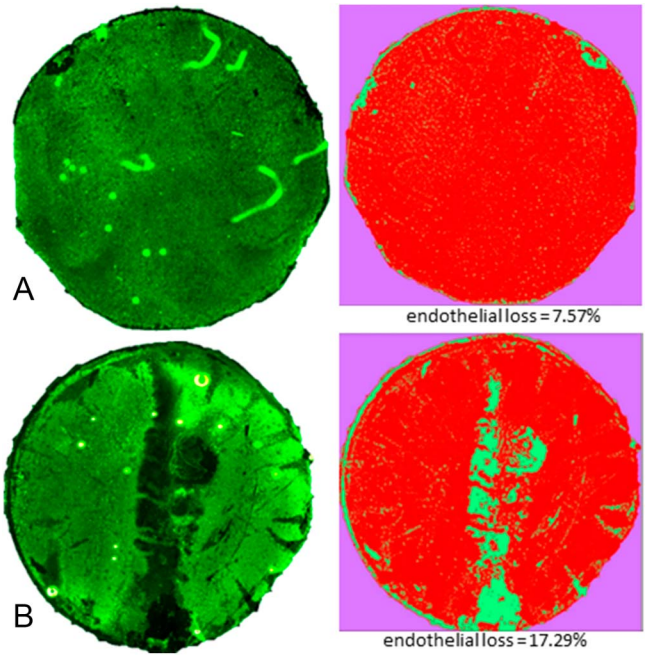


**FIGURE 2.** Photographs of endothelial keratoplasty tissue prepared using the EndoGlide technique (A) and EndoGlide with viscoelastic technique (B). 1) Calcein AM staining of Descemet membrane endothelial keratoplasty tissue demonstrating cell loss in the area of viscoelastic application. 2) Photograph of segmented image in Fiji of the same tissue disk demonstrating area of endothelial cell loss. (The full color version of this figure is available at www.corneajrnl.com.)

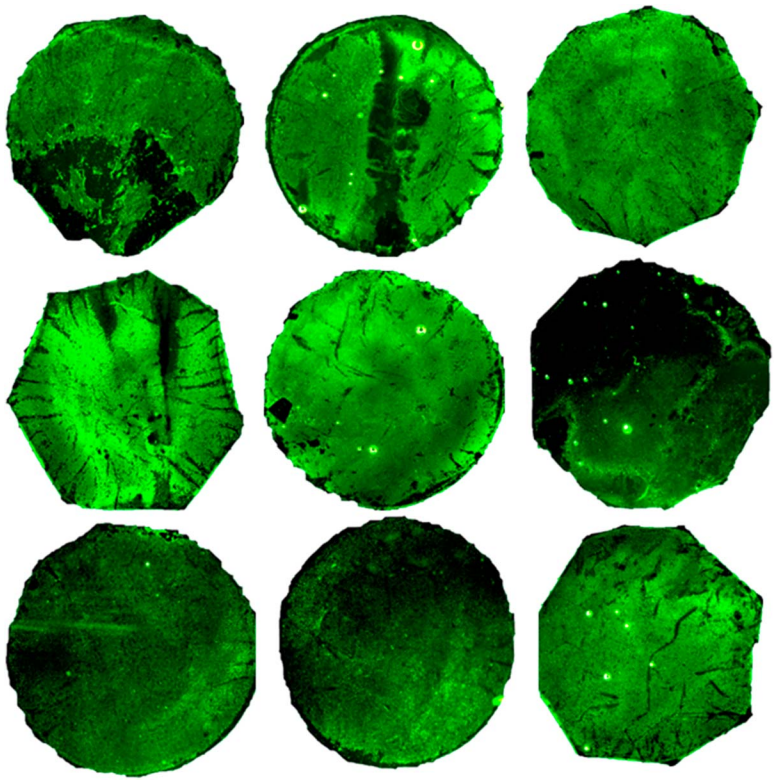

**FIGURE 3.** Montage of photographs of calcein AM staining from the EndoGlide with viscoelastic technique showing some tissues with a linear area of cell loss, some with more diffuse loss, and some with minimal cell loss more similar to the EndoGlide without viscoelastic technique. (The full color version of this figure is available at www.corneajrnl.com.)

their ability to absorb nutrients from the tissue media while in storage and resulting in their demise.

Differences in endothelial cell loss patterns in the viscoelastic group (some with linear areas of cell loss along the area of viscoelastic application, some more with diffuse cell loss, and some with minimal cell loss more similar to the DMEK EndoGlide without viscoelastic tissues) are likely due to differences in actual viscoelastic distribution on the tissue while in the EndoGlide. Tissues with more protracted loading maneuvers may have had more viscoelastic washed away, and tissues with more balanced salt solution on the tissue surface when viscoelastic was applied may have allowed for viscoelastic dissipation over larger areas of endothelial cells. Regardless, presence of unrinsed viscoelastic on the corneal tissue had a damaging effect on endothelial cell health. This effect had not previously been reported, and judicious irrigation and removal of viscoelastic material from the endothelium in tissue preparation is imperative for endothelial cell survival. Retained viscoelastic has also been reported to be present on the stromal side of a graft and remains in the interface after endothelial keratoplasty surgery, which has resulted in visually significant haze and sometimes donor/recipient separation in Descemet stripping endothelial keratoplasty surgeries.[12]

The Jones tube and DMEK EndoGlide preparation techniques resulted in comparable and nominal endothelial cell loss. Using the DMEK EndoGlide device holds promise for decreased endothelial cell loss after implantation of the DMEK tissue into the eye due to better control of graft orientation during implantation into the eye, allowing for potential of less manipulation. Use of viscoelastic devices to protect against mechanical damage to the endothelium should be avoided unless used transiently due to the prevention of adequate endothelial cell nutrition.

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