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Endothelial Cell Loss Due to Injector Method in the Preparation of Descemet's Membrane Endothelial Keratoplasty Grafts

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Purpose

Descemet's membrane endothelial keratoplasty (DMEK), is a new method of lamellar corneal transplantation where the graft consists of only Descemet's membrane and the attached endothelial cells. Due to the extreme thinness of the graft, cautious and deliberate surgical manipulation are necessary to prevent endothelial loss during transplantation. Several methods for injection of the tissue into the anterior chamber have been described, but there is currently no data available in the literature on cell loss due to injector method. In this study, we aim to evaluate endothelial cell loss due to injector method with two popular injectors, the modified Jones tube (Guther Weiss Scientific Glass, Portland, OR)[1], and a closed IOL injection system, the Viscoject 2.2 (Medicel, Wolfhalden, Switzerland)[2].

Methods

Sample Size

The study was powered to detect a 10% difference in cell loss between injector groups with a confidence level of 90% with an α of 0.05. A prior reported cell loss during prepration of 22.5%±6.5%[3] was used in the calculation. This yielded 9 grafts per injector arm.

Graft Preparation

All grafts were prepared by eye bank technicians skilled in DMEK graft preparation and stored in Optisol GS. Grafts were partially separated from the underlying stroma using manual peeling and laid back down. All grafts then received an S stamp for orientation. The grafts were then stained with Trypan blue for 30 second and punched with a new 8.0mm Barron Hessburg trephine without suction. The graft was then fully separated from the underlying stroma while in Optisol using gentle manipulation with smooth forceps. The Optisol was then drained using MeroCel wicking, and restained with Trypan blue for 5 minutes to mimick standard surgical technique. The Trypan blue was then removed and the graft re-floated in Optisol and allowed to attain the Dead Sea Scroll configuration.

Injection

Because the authors routinely use the modified Jones tube injector, six practice grafts were performed using the Viscoject prior to the study grafts. The modified Jones tube was attached to a 3cc syring and filled with BSS. The injector tip was then placed in the well of Optisol containing the graft, and very gentle suction applied to draw the graft into the tube. The Viscoject was prepared by removing the spring from the injector handpiece. The cartridge was submerged in a shallow dish containing BSS and all air bubbles were removed. The graft was then grasped at one end with a smooth forceps and placed in the groove in the cartridge. The wings of the cartridge were closed, and it was loaded into the injector handpiece. With both injectors, the grafts were allowed to sit in the injector for 1 minute to mimic surgical technique.

The grafts were then injected onto a bed of dispersive viscoelastic (VisCoat, Alcon, Ft. Worth, TX) mixed with Calcein AM vital dye and carefully unfurled using viscoelastic. The grafts were allowed to stain for 20 minutes, and then imaged.

Cell Counts

Live cell counts were performed using Wecka image segmentation and the FIJI (manufacturer, location) software.[3] STATA (STATA Corp., College Park, TX) was used for analysis. A Mann Whitney test was performed to compare injectors.

References

1. Straiko, MD Descemet Membrane Endothelial Keratoplasty: Enhanced Results With a Standardized Technique. Poster at the 2014 American Acadamey of Ophthalmology Meeting, Chicago, Il
2. Price FW Jr. DMEK in a Pseudophakic Eye, AAO Master Course in Corneal Transplantation. Available at http://aao.org/master-class-video/dmek-in-pseudophakic-eye, accessed 4/23/15
3. Jardine, G et al, Imaging and quantification of endothelial cell loss in eye bank prepared DMEK grafts using trainable segmentation software. Curr Eye Res 2014 39 (9): 894-901

Results

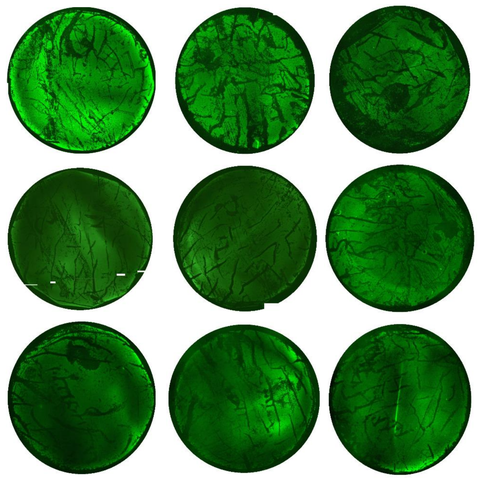
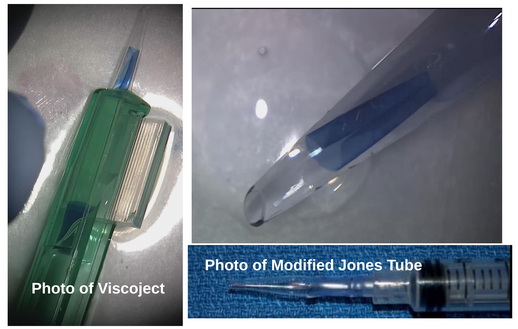


Figure 1. Grafts prepared with Viscoject Injector

Overall grafts prepared with the ViscoJect had 32% ± 8%, cell loss (range 21% to 47%).

	Jones Tube	Viscoject	p value
Donor Age			
Donor Eye			
Death-to-Preservation (hours)			
Storage time (days)			
Pre-peel Cell Count			
Post-peel Cell Count			
% Cell loss from Injection	27% ± 5% (21% to 35%)	32% ± 8% (21% to 47%)	0.3

Table 1. Demographics and Cell Loss



A B

A: Graft preparation with Modified Jones Tube. https://www.youtube.com/watch?v=mCuSz12dBzE

B: Graft preparation with Modified Jones Tube. https://www.youtube.com/watch?v=slgXmFvTZko

Video – Preparation and Injection of a Graft

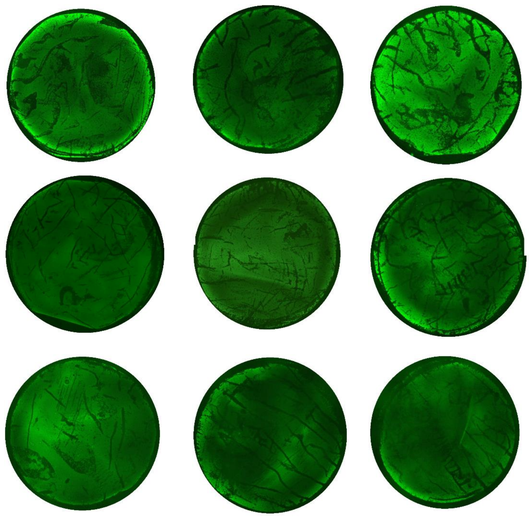
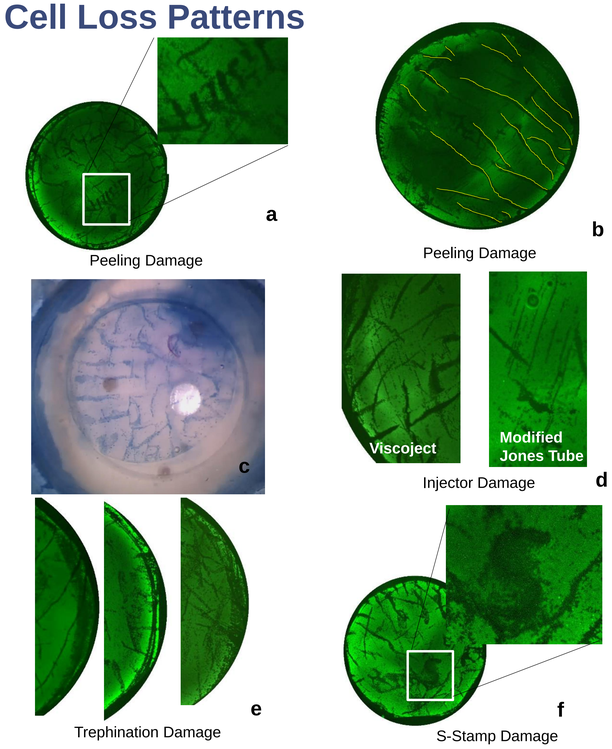


Figure 2: Grafts prepared using Modified Jones Tube injector

Overall grafts prepared with the Modified Jones Tube had 27% ± 5% (range 21% to 35%).

Cell Loss Patterns



Graft peeling sometimes caused short, broad parallel zones of cell loss (a) that were visible on Trypan staining of the graft prior to punching. Peeling also caused longer wavy zones of cell loss, marked in yellow, (b). These patterns can be seen in a graft prior to injection (c). Both the Modified Jones Tube and Viscoject Injectors had fine, linear zones of cell loss (d). Trephination caused a circular zone of cell loss in some grafts, due to the pinching of the graft between the corneal button and the rim (e) The S-stamp, placed for graft orientation, was visible on one graft (f).

Discussion

- There was no significant difference in cell loss after injection with the Viscoject or modified Jones tube.
- Grafts lost, on average, a little less than 1/3 of their cells from the preparation and injection process.
- There are identifiable cell loss patterns from stripping, S stamp placement, trephination and injection.
- Further study of graft preparation and injection may yield ways to decrease cell loss and possibly improve long-term graft survival.