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 is 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 (Guthy Weiss Scientific Glass, Portland, OR), and a closed IOL injection system, the Viscoject 2.2 (Medicel, Wolfhalden, Switzerland).

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 preparation of 22.5%±6.5% 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 Heaslev 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 Menelcor casting, and reanimated with Trypan blue for 5 minutes to mimic standard surgical technique. The Trypan blue was then removed and the graft re-Roated in Optisol and allowed to form the Descemet corneal configuration.

Injection
Because the surgeons 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 syringe 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 removed 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 (Viscoel, Alcon, Ft. Worth, TX) mixed with Coatsen AM vital dye and carefully unfurled using viscoelastic. The grafts were allowed to sit for 20 minutes, and then imaged.

Cell Counts
Live cell counts were performed using Weck's image segmentation and the Fiji (manufacturer, location) software. A TSTA (TSTA Corp., College Park, TX) was used for analysis. A Mann–Whitney U test was performed to compare injectors.

Results

Figure 1. Grafts prepared with Viscoject Injector
Overall grafts prepared with the Viscoject had 32% ± 8%, cell loss (range 21% to 47%).

Table 1. Demographics and Cell Loss

<table>
<thead>
<tr>
<th>Donor Age</th>
<th>Jones Tube</th>
<th>Viscoject</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>53 years</td>
<td>61.5% ± 4.5%</td>
<td>63.5% ± 4.3%</td>
<td>0.23</td>
</tr>
<tr>
<td>62 years</td>
<td>56.0% ± 4.3%</td>
<td>58.5% ± 4.1%</td>
<td>0.17</td>
</tr>
</tbody>
</table>

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

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.