Original Article

Comparing the effect of three Descemet membrane endothelial keratoplasty injectors on endothelial damage of grafts

Elizabeth Shen, Adam Fox[1], Brian Johnson[1], Marjan Farid

Purpose: Various injectors are commercially available for Descemet membrane endothelial keratoplasty (DMEK) but not all injectors have been studied for endothelial damage of grafts. The aim of the study was to compare endothelial damage in pre-stripped DMEK tissue from three clinically used injector devices: the modified Jones tube, the STAAR intraocular (IOL) injector, and the Geuder glass cannula in a laboratory setting. **Methods:** Twenty-four human donor corneas were used for this study, eight for each study arm. Each endothelial graft was pre-stripped, trephined to 8.0 mm diameter, then loaded into either the modified Jones tube, the STAAR IOL injector, or the Geuder glass cannula by an eye bank technician who had no prior experience with any of the injectors. Grafts were then ejected, stained with Calcein acetoxymethyl (AM), and quantitatively analyzed using FIJI image software. The primary outcome was the percent of endothelial damage from injector loading and injection. Donor demographics were analyzed using Fisher's exact test. The percentage of endothelial cell loss was compared across groups using the Kruskal–Wallis test. **Results:** The mean percent of endothelial damage from after injection of the graft was 37.8% (±SD 12.2%) for the modified Jones tube, 37.0% (±SD 13.9%) for the STAAR IOL injector, and 23.5% (±SD 5.1%) for the Geuder cannula ($P = 0.008$). **Conclusion:** DMEK injectors contribute to intraoperative endothelial damage of transplanted grafts. The Geuder glass cannula may offer increased ease of use and less endothelial damage compared to the modified Jones tube or STAAR IOL injector for the novice user in early cases.

Key words: Descemet membrane endothelial keratoplasty, Descemet membrane endothelial keratoplasty injector, Geuder glass cannula, graft preparation, modified Jones tube

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Descemet membrane endothelial keratoplasty (DMEK) has increased in popularity in recent years, largely due to rapid postoperative visual rehabilitation and excellent visual outcomes[1-6] since its advent, numerous techniques and injection methods have been described.[7-12] Commonly used injectors include the modified Jones tube (Gunther Weiss Scientific Glass, Portland, Oregon) and the STAAR intraocular lens (IOL) injectors, which are The Food and Drug Administration (FDA)-approved in the United States and used off-label for DMEK.

One of the primary goals in DMEK is the preservation of endothelial cells in the graft by minimizing trauma during manipulation of the issue. Endothelial cell loss may occur at all stages of the procedure, from eye bank preparation of the graft and loading of the tissue into the injector to insertion into the anterior chamber and even unfolding and centering the graft. The use of a closed-system injection system, such as the modified Jones tube or an IOL injector, allows the graft to be suspended in fluid and injected into the anterior chamber without direct contact with instrumentation. Prior studies comparing and quantifying endothelial cell loss between different injector methods have evaluated the modified Jones tube and IOL cartridges.[13,14] The Geuder glass injector (Geuder AC, Heidelberg, Germany) was recently introduced for the purpose of DMEK insertion but there have been no studies to date on its effect on endothelial cells. In this study, we compare the effect of three different injectors—the modified Jones tube, the STAAR IOL injector (STAAR Surgical, Monrovia, California), and the Geuder glass cannula—on endothelial cell loss in DMEK grafts.

Methods

Tissue preparation

Twenty-four corneas were prepared using the eye bank's DMEK protocol by one eye bank technician. Grafts were trephined to 8.0 mm, stained with Trypan blue (Invitrogen, Eugene, Oregon) for 30 s to visualize the edge of the tissue, and then suspended in a balanced salt solution (BSS) (Alcon Laboratories Inc., Fort Worth, Texas) until ready for aspiration into the injector. A prespecified number of eight grafts were used in testing for each injector [Fig. 1].

Of note, the eye bank technician did not have any prior experience with any one injector and received basic training on loading the grafts into each injector by the surgeon (M.F.) prior to the study. For the Jones tube, grafts were aspirated

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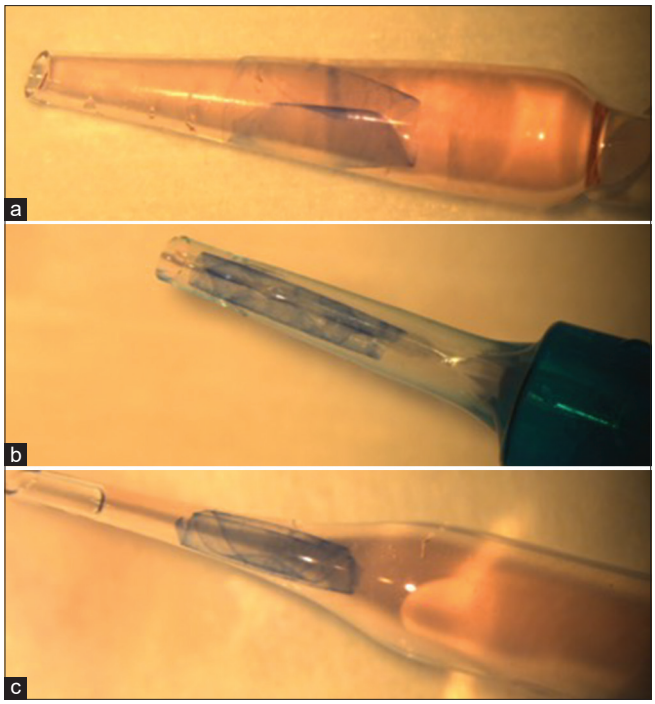

Figure 1: Descemet membrane endothelial keratoplasty injector systems. (a) Modified Jones tube, (b) STAAR intraocular lens injector, (c) Geuder glass cannula

through the injection tip of the tube. For the STAAR IOL injector, grafts were loaded into the IOL cartridge under BSS, which was then placed into the injector. For the Geuder cannula, grafts were aspirated through the larger entry port of the cannula. For each injector, grafts were ejected into a 50 µg Calcein AM (Invitrogen, Eugene, Oregon)/100 µL dimethyl sulfoxide (Sigma-Aldrich, St Louis, Missouri)/20 mL BSS solution and were allowed to stain for 15 min. Grafts were then transferred to a flattened bed of Healon 5 (Johnson & Johnson Vision, Santa Ana, California) in a petri dish and carefully unscrolled using a few drops of BSS, toothless forceps, and a Sinskey hook. Once unscrolled, the grafts were covered with a layer of Healon 5 for endothelium protection and for holding the graft in place.

Endothelial cell viability analysis

The stained grafts were then imaged using an inverted light microscope using blue light (495–515 nm) filter. Images were digitally stitched together using Microsoft Paint into a composite image. Viable cells appear as hyperfluorescent or bright green, whereas areas of cell death appear hypofluorescent or dark [Fig. 2].

FIJI open software,[15] previously described as a reliable method for performing cell counts, was used to estimate endothelial cell viability.[16] The individual performing the analysis of endothelial cell viability was not the technician who performed the experiment and was blinded to the injector used.

Statistical methods

Our study was powered to detect a 10% difference in endothelial cell loss between three injector groups with a confidence level of 90% and an α of 0.05. This resulted in eight grafts per group. Donor demographics were analyzed using Fisher's exact test. The amount of endothelial cell loss was estimated as a percentage and compared across groups

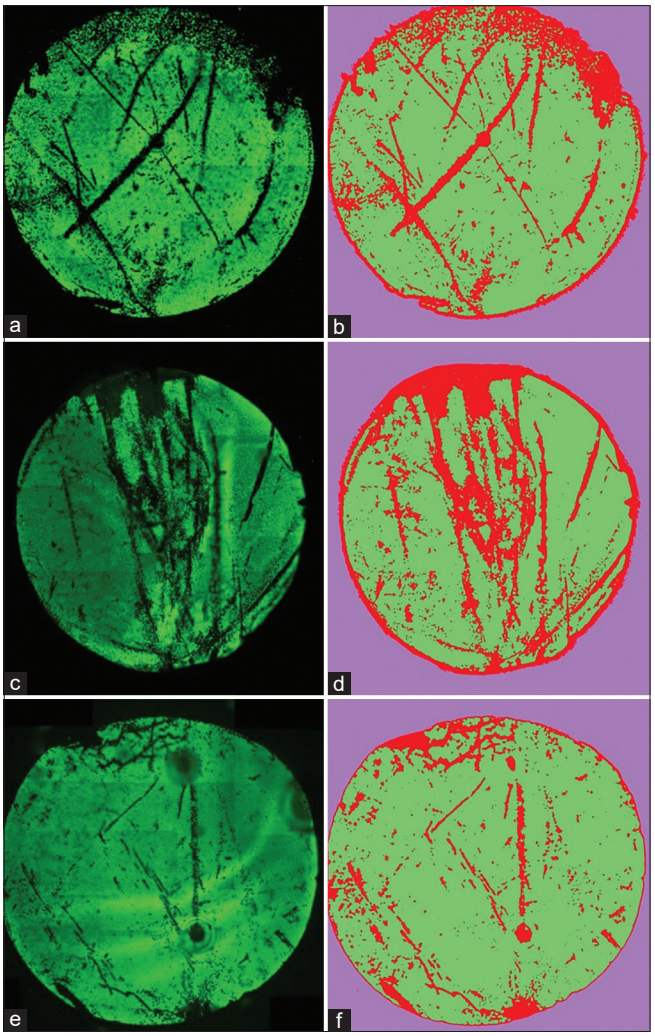

Figure 2: After injection of a graft with either (a) modified Jones tube, (c) STAAR intraocular lens injector, or (e) Geuder glass cannula, the tissue was stained with Calcein-acetoxymethyl, imaged using an inverted light microscope and stitched to create a composite image. Images were then segmented using FIJI software (b, d and f)

using the Kruskal–Wallis test. Analyses were performed using STATA (Version 13.0).

Results

Twenty-four grafts were prepared for this study, eight in each arm. There were no significant differences in donor age or death-to-preservation time between groups, though endothelial cell counts were lower in the Geuder glass cannula group ($P = 0.02$) [Table 1].

Grafts injected with the modified Jones tube had a mean percentage of endothelial cell loss of 37.8% (±SD 12.2%). Grafts injected with the STAAR IOL injector had a mean percentage cell loss of 37.0% (±SD 13.9%). Grafts injected with the Geuder glass cannula had a mean percentage cell loss of 23.5% (±SD 5.1%), which was a statistically significant difference compared to the other two injector methods ($P = 0.008$, Kruskal–Wallis test) [Table 2 and Fig. 3]. Both the modified Jones tube group and STAAR IOL injector group had one outlier each: 64.9% and 65.3% endothelial cell loss, respectively, due to stiff tissue that did not scroll. The mean

Table 1: DMEK* graft demographics

	Modified Jones tube	STAAR IOL†	Geuder glass cannula	*P*
Donor age (±SD) (year)	67±4	62±11	70±15	0.86
Death-to-preservation (±SD) (h)	14.2±6	13.4±9	13.1±6	0.89
Endothelial cell count (±SD)	2549±664	2890±317	2162±457	0.02

*DMEK=Descemet membrane endothelial keratoplasty. †IOL=intraocular lens

percentage endothelial cell loss when these two outliers were excluded was 34.9% (±SD 7.4%) in the modified Jones tube group and 32.9% (±SD 8.6%) in the STAAR IOL injector group. The difference in mean percentage endothelial cell loss across the three groups remained statistically significant when the outliers were excluded from the analysis ($P = 0.01$).

Discussion

In this study, we compared the amount of endothelial cell damage induced by three clinically used DMEK injectors. We found the Geuder glass cannula caused less damage than either the modified Jones tube or the STAAR IOL injector in the early learning curve of adopting these injectors.

Since the advent of DMEK, there has been continued refinement in technique. Different studies have proposed various injector techniques and expanded injector options.[2,7-12] One of the earliest injectors employed was the glass Pasteur pipette. In the USA today, commonly used injectors include the modified Jones tube, IOL injectors, and most recently, the Geuder glass cannula. The DORC injector (DORC, Zuidland, the Netherlands) is another option that is currently available only in Europe. The DORC and Geuder injectors are double port injectors specifically designed for DMEK use, such that there is a wide entry port for aspiration and a narrow exit port (sized for the corneal incision) for injection.

The primary differences in injectors reside in their material (i.e., glass or plastic) and the diameters of their openings. Prior studies have postulated that glass may be less toxic to endothelium compared to plastic, which may accidentally adhere to the endothelium.[8,10,17]

The diameter of the injector opening would intuitively appear to play a role, as smaller openings are more likely to traumatize the scrolled tissue during aspiration. However, existing studies have not supported this hypothesis. One study compared glass injectors with various openings (0.5 mm, 0.9 mm, and 1.4 mm) and did not find a statistically significant difference in cell viability.[17] Using the same technique as described in this study, Schallhorn *et al*. compared the modified Jones tube, a glass injector with a 2.4 mm opening, to the Viscoject IOL injector (Viscoject 2.2, Medicel), a plastic injector with a 1.9 mm opening, and found no statistically significant difference in cell viability.[14] More recently, Downes *et al*. compared the DORC injector with a 1.4 mm opening to the modified Jones tube and found no difference in cell loss.[18]

This is the first study to directly compare the Geuder glass cannula with two commercially available injectors in clinical use. We found a difference of 14.3% and 13.5% less endothelial damage caused by the Geuder glass cannula compared to the modified Jones tube and STAAR IOL injector, respectively, in a novice user.

Table 2: Comparison of endothelial cell loss after graft preparation and injection

	Modified Jones tube	STAAR IOL*	Geuder glass cannula	*P*
Mean % cell loss (±SD)	39.5±12.2	37.0±13.9	23.5±5.08	0.008

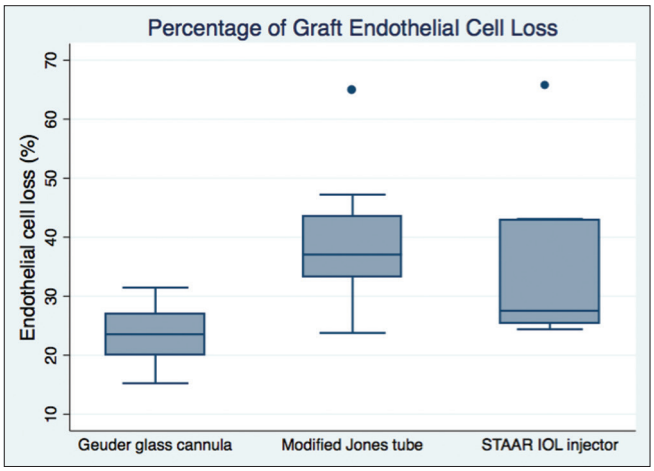

Figure 3: Box-plot comparison of endothelial cell loss after graft preparation and injection

Cell loss from graft preparation alone has been estimated to range from 12.4% to 28%, while tissue preparation and subsequent aspiration and injection can result in cell loss of 23% to 32%.[14,18-20] Our results for the Geuder glass cannula are at least comparable; for the modified Jones tube, our reported cell loss is slightly higher than the 19–32% of endothelial loss reported in the literature.[14,18,21-23]

One possible explanation for our results compared to prior studies is that familiarity with the DMEK injection technique has been shown to influence the amount of endothelial cell loss.[24] The abovementioned studies were performed by surgeons experienced with the injection technique; in our study, an eye bank technician who was naïve to the three injectors performed the stripping and injection of the grafts. The lack of prior experience likely resulted in a higher rate than expected of endothelial loss with the modified Jones tube compared with experienced users. However, this provides insight into the learning curve for each injector. It is plausible that the modified Jones tube and the STAAR IOL injector have a more difficult learning curve than the Geuder cannula, resulting in a higher endothelial loss in early cases. This suggests that the Geuder glass cannula may be easier to learn and be more forgiving of endothelial trauma.

One important limitation of this study is that our findings are limited to the laboratory setting and may not correlate

with clinical outcomes. Droutsas *et al*. compared three glass injectors (DORC injector, Geuder glass cannula, and Pasteur pipette) in patients who underwent DMEK and did not find a clinically significant difference in endothelial cell density at 12-month postoperatively.[13] Future studies are needed to determine whether differences in these commonly used injector systems translate to a difference in the longevity of DMEK grafts implanted in patients. Another limitation to consider is that, while the technician lacked prior experience with the injector at the beginning of the experiment, the experience was gained throughout the study. The increasing familiarity with the injectors may have an undetermined effect on our results. We hypothesize that increasing experience would most affect the outcome of endothelial loss on injectors with the easiest learning curves; however, our sample size was not sufficient to assess for this effect.

Conclusion

In conclusion, we found that the modified Jones tube, STAAR IOL injector, and Geuder glass cannula all offer acceptable tissue quality and cell loss for DMEK transplantation. We found statistically significant less cell loss induced by the Geuder glass cannula compared to the other two injectors, suggesting the Geuder glass cannula may have an easier learning curve for the novice user in early cases.

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Conflicts of interest

Elizabeth Shen, MD has no proprietary interests and no financial disclosures. Adam Fox, CEBT is a former employee of CorneaGen. Brian Johnson, CEBT is an employee of CorneaGen. Marjan Farid, MD is a consultant for CorneaGen. No authors have any proprietary interests.

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