

Evaluation and Quality Assessment of Prestripped, Preloaded Descemet Membrane Endothelial Keratoplasty Grafts

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Purpose: To determine graft quality and feasibility of Descemet membrane endothelial keratoplasty (DMEK) grafts that are prestripped and preloaded into injectors by eye bank technicians before shipping to surgeons.

Methods: DMEK grafts ($n = 31$) were prepared from donor corneas and preloaded into Straiko Modified Jones tubes and set inside viewing chambers filled with 20 mL of Optisol-GS. Preloaded grafts were evaluated using specular microscopy and slit-lamp biomicroscopy. Endothelial cell loss (ECL) was captured by vital dye staining and quantified using FIJI. A subset of preloaded tissues was subjected to a shipping validation and 5-day storage assay. Fourteen additional DMEK grafts (not preloaded) were examined to quantify damage resulting from prestripping alone.

Results: Specular microscopy was able to be performed for all preloaded tissues. Average ECL for preloaded tissues quantified by vital dye staining and FIJI after overnight storage was $16.8\% \pm 5.9\%$, and differed from slit-lamp ECL estimation by an average of $5.3\% \pm 3.6\%$. The average damage caused by prestripping alone was $9.3\% \pm 5.9\%$, and it was significantly less than that of preloaded tissues ($P < 0.01$). Average ECL for preloaded tissues subjected to round-trip shipping events was $18.5\% \pm 12.4\%$, and ECL for tissues stored at 4°C for 5 days after preloading was $13.1\% \pm 9.5\%$.

Conclusions: It is possible to prepare, evaluate, and ship DMEK grafts loaded inside a glass carrier and viewing chamber. The ability to evaluate tissues after processing allows for adherence to the Eye Bank Association of America Medical Standards, and for surgeons to receive the most accurate tissue information.

Key Words: endothelial keratoplasty, Descemet membrane endothelial keratoplasty, corneal transplant, preloaded DMEK, corneal endothelium, specular microscopy

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Descemet membrane endothelial keratoplasty (DMEK) is a corneal transplantation procedure that enables exact anatomic replacement of the diseased Descemet membrane and endothelium complex.^{1–3} Several reports have proposed that DMEK provides improved postoperative visual outcomes, faster recovery times, and reduced rates of rejection compared with other endothelial keratoplasty procedures such as Descemet stripping automated endothelial keratoplasty and penetrating keratoplasty.^{4–9} Although Descemet stripping automated endothelial keratoplasty and penetrating keratoplasty remain the most widely performed corneal transplant procedures worldwide, DMEK is exponentially increasing its share of endothelial procedures in the United States.¹⁰

Many eye banks have developed internal processing programs to assist surgeons in preparing DMEK grafts.^{10–13} Eye bank-prepared prestripped tissues help reduce time in the operating room (OR) and potential complications that may arise if tissue preparation fails during surgery. Furthermore, prestripped tissues provide an additional level of quality assurance as eye banks can perform postprocessing evaluation of grafts using specular microscopy and slit-lamp biomicroscopy, which is not normally performed in the OR. More recently, the idea of eye banks providing preloaded DMEK grafts¹⁴ has sparked many conversations about the possibility of further reducing time in the OR and the overall costs of surgical centers. Concurrently, practitioners have raised concerns about how preloaded tissue for DMEK can be evaluated to ensure that surgeons are getting the most accurate information about the tissue their patients are receiving.

In the United States, the current Eye Bank Association of America (EBAA) Medical Standards require that all eye bank-prepared grafts be evaluated by specular microscopy and slit-lamp biomicroscopy.¹⁵ Beyond the requirements by governing bodies, eye banks also strive to release high-quality tissue for transplants. To this end, we have explored the possibility of preloading DMEK grafts in a manner that would allow for postprocessing evaluation. To the best of our

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knowledge, this is the first example of practical postprocessing evaluation of preloaded tissue for DMEK.

MATERIALS AND METHODS

Donor Characteristics

All donor corneas used in this study were deemed unsuitable for transplantation because of reasons other than endothelial pathology, and consent for research use of all donor tissue was obtained. Donor age ranged from 46 to 75 years (median: 65 years). Of the donors, 51% were male, 9% were pseudophakic, and 20% had a history of diabetes. Death-to-recovery time for all tissues was between 3 and 24 hours. Endothelial cell densities (ECD) ranged from 1751 to 3125 cells/mm² (median: 2660 cells/mm²).

Tissue Preparation

Prestripped DMEK grafts (with S-stamps) were prepared according to previously described protocols^{11,16} by trained eye bank technicians at Lions VisionGift (Portland, OR). Preloaded DMEK graft preparations are described in detail below. All prepared grafts were stored at 4°C in 20 mL of Optisol-GS (Bausch and Lomb, St. Louis, MO) after preparation.

To prepare a preloaded DMEK graft, prestripped tissue was punched using an 8.0-mm Barron Hessburg trephine (Barron Precision Instruments, Grand Blanc, MI). Excess endothelium–Descemet membrane surrounding the graft zone was removed. Grafts were stained with trypan blue (C-Blue; Stephens Instruments, Lexington, KY) for 30 seconds and washed gently with balanced salt solution (BSS) (Alcon, Ft. Worth, TX) to visualize the graft edge. Grafts were submerged in Optisol-GS, lifted using a Moria Micro-dissector (Moria, Antony, France), and allowed to scroll for 2 minutes before loading. Loading of the injector was performed with minor modifications to a previously described technique.¹³ The whole injector apparatus was filled with Optisol-GS, and DMEK scrolls were drawn into the Straiko Modified Jones tube. The tube containing the tissue scroll was removed and placed inside a Krolman viewing chamber (Krolman, Boston, MA) filled with 20

mL of Optisol-GS. No caps were placed on either end of the tube so that the preservation media could freely contact the preloaded graft.

Measurements of Straiko Modified Jones Tubes

Digital calipers were used to measure the glass Straiko Modified Jones tubes (Gunther Weiss Scientific Glassblowing, Portland, OR). The average tube length was 34.9 ± 2.8 mm (range: 32.00–37.6 mm), and the average tube width (at widest point) was 5.8 ± 0.2 mm (range: 5.6–6.0 mm). The average distal tip-beveled internal opening lengths and widths were 1.8 ± 0.3 (range: 1.5–2.1 mm) and 1.4 ± 0.2 (range: 1.2–1.5 mm), respectively.

Postprocessing Evaluation

Postprocessing evaluation was performed according to the current standard operation procedures of our eye bank that are compliant with EBAA Medical Standards.¹⁵ All postprocessing evaluations were performed within 2 hours of processing. Slit-lamp evaluations were performed on the entire DMEK scroll, and the transparent nature of the scrolls allowed for examination of the inner scroll layers. Slit-lamp images were acquired on a Haag-Streit BX900 slit-lamp system (Haag-Streit USA, Mason, OH) equipped with a Canon digital SLR camera (Canon USA, Melville, NY), and specular images were acquired on a Konan Kerato Analyzer EKA-10 with the EB10 software package (Konan Medical, Irvine, CA). For standard prepeeled DMEK grafts, 3 central images were acquired and approximately 100 cells were used to determine ECD. For preloaded grafts, 3 to 4 images were acquired and an average of 97 cells were measured to calculate ECD. For loosely scrolled grafts, the central cornea could be readily identified and imaged. For tightly scrolled grafts with overlapping layers, technicians acquired specular images midway along the length of the scroll and may have, in some cases, image a peripheral region of the 8.0-mm graft. Although this is a limitation of the current system, we did not observe a significant increase in ECD for preloaded tissues (Table 1).

TABLE 1. ECL by Experimental Groups

	N	ECL (% Cell Death)			Average ECD (cells/mm ²)		P
		Average ± SD	Median	Range	Pre	Post	
Total preloaded tissues	31	15.6 ± 6.4	14.1	6.9–50.4	2607	2724	0.1
Processing and evaluation	16	16.8 ± 5.9*	16.7	7.0–25.9	2528	2560	0.34
Shipping study	10	18.5 ± 12.4	14.0	8.5–50.4†	2697	2854	0.08
5-day graft storage	5	13.1 ± 9.5	10.6	6.9–29.8‡	2634	2714	0.67
Prestripped only	14	9.3 ± 5.9*	7.2	3.7–26.0	2701	2553	0.12

The row labeled “Total preloaded tissues” contains combined results from the processing and evaluation, shipping, and storage studies.

P values in the table are specific to preprocessing and postprocessing ECD measurements.

*ECL is significantly different ($P < 0.01$). The donor age range is not significantly different between both groups ($P = 0.37$).

†One tissue fell out of the glass tube during a shipping event and incurred extensive damage (50.4%).

‡Tissue with 29.8% final ECL had approximately 17% ECL before processing based on trypan blue staining and FIJI analysis.

Shipping of Preloaded DMEK Grafts

A total of 10 preloaded DMEK grafts were shipped over 3 separate occasions. On each occasion, tissues were packed using our standard eye bank protocol and shipped overnight to the University of Texas Southwestern Medical Center at Dallas—Transplant Services Center (UT-Southwestern). At UT-Southwestern, tissues were repacked with fresh wet ice and returned overnight to our eye bank for analysis, resulting in a total of 2 shipping events per shipping experiment.

Endothelial Cell Viability Analysis

Cell loss due to prestripping alone was determined by vital dye staining after prestripped tissues were stored at 4°C overnight. Prestripped grafts were stained with Calcein-AM (2.5 µg/mL; ThermoFisher, Grand Island, NY) for 40 minutes at room-temperature. Grafts were gently rinsed with BSS before trephination using a 9.5-mm Barron Hessburg trephine. The graft and underlying stroma were transferred onto a glass slide containing a bed of dispersive viscoelastic (Occulon; Stephens Instruments) for image acquisition. Only the 8.0-mm central region of the graft was analyzed to exclude trephination damage that is not part of the prestripping process.

Cell loss of preloaded DMEK grafts was also analyzed after overnight storage at 4°C, unless otherwise noted for the shipping and 5-day storage experiments. Grafts were injected on to a bed of Calcein-AM-infused viscoelastic on a microscope slide. Calcein-AM at 12.5 µg/mL was mixed with Occulon at a ratio of 4:1 to make a final cocktail of 2.5 µg/mL Calcein-AM + 80% viscoelastic. Grafts were unfurled in this mixture and left to continue staining for 40 minutes before image acquisition.

All grafts were imaged using an XDY-1 inverted fluorescent microscope (Alltion, Wuzhou, China). For each graft, approximately 20 to 30 images were acquired at 20× magnification and stitched together using Adobe Photoshop Elements 7.0 (Adobe Systems, San Jose, CA). Cell viability analysis was performed using Trainable Weka Segmentation in FIJI¹⁷ as previously described.^{18,19}

Statistics

Descriptive values are shown as mean ± SD. Non-parametric Wilcoxon tests²⁰ were used to determine statistical significance, which was defined as $P < 0.05$. Statistical analysis was performed using R Statistical Software²¹ (version 3.2.4).

RESULTS

Preloaded DMEK Grafts Stored in a Viewing Chamber

After separation from the stroma, all DMEK grafts (31/31) submerged in Optisol-GS scrolled into their natural conformations with the endothelium facing outward. All grafts remained in scrolled conformation after being drawn

into a Straiko Modified Jones tube. Each tube containing a graft was placed in a Krolman viewing chamber (Fig. 1).

Specular Microscopy of Preloaded DMEK Grafts

Specular images and ECD of all preloaded grafts used in this study were successfully obtained (Fig. 2A, Table 1). Averaged ECD for all tissues before processing was 2607 ± 312 cell/mm² and was not significantly different from the postprocessing average of 2724 ± 339 cell/mm² ($P = 0.1$, Table 1). The average number of cells used to calculate ECD for preloaded grafts was 97 ± 17 cells and ranged from 71 to 130 cells. The number of cells that can be measured was determined by DMEK scroll tightness (Fig. 2A, see Discussion).

Slit-Lamp Biomicroscopy and Cell Viability of Preloaded DMEK Grafts

Sixteen preloaded grafts were prepared by 3 trained eye bank technicians, and each graft was evaluated using the slit-lamp by a technician who did not prepare that graft (Fig. 2B). Various patterns of graft damage due to prestripping, injector

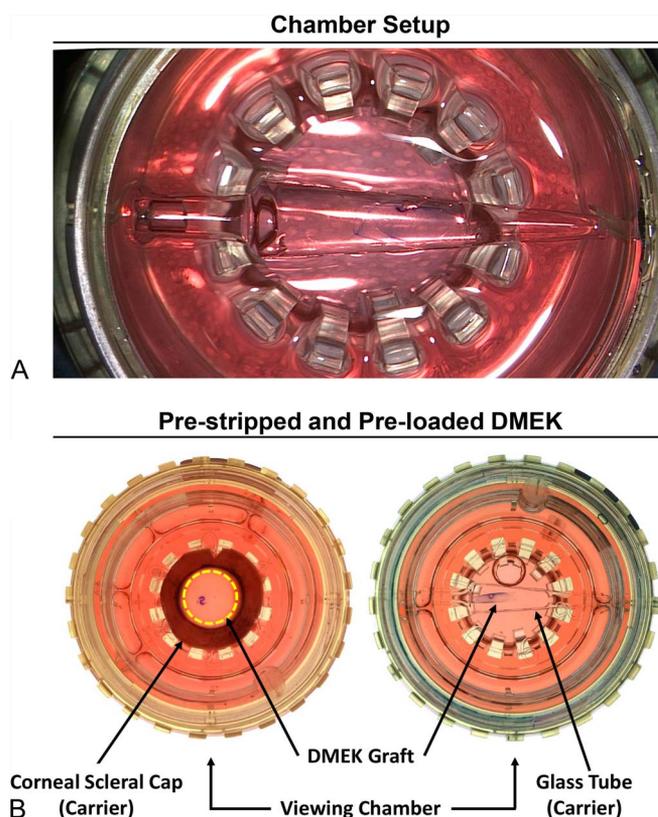
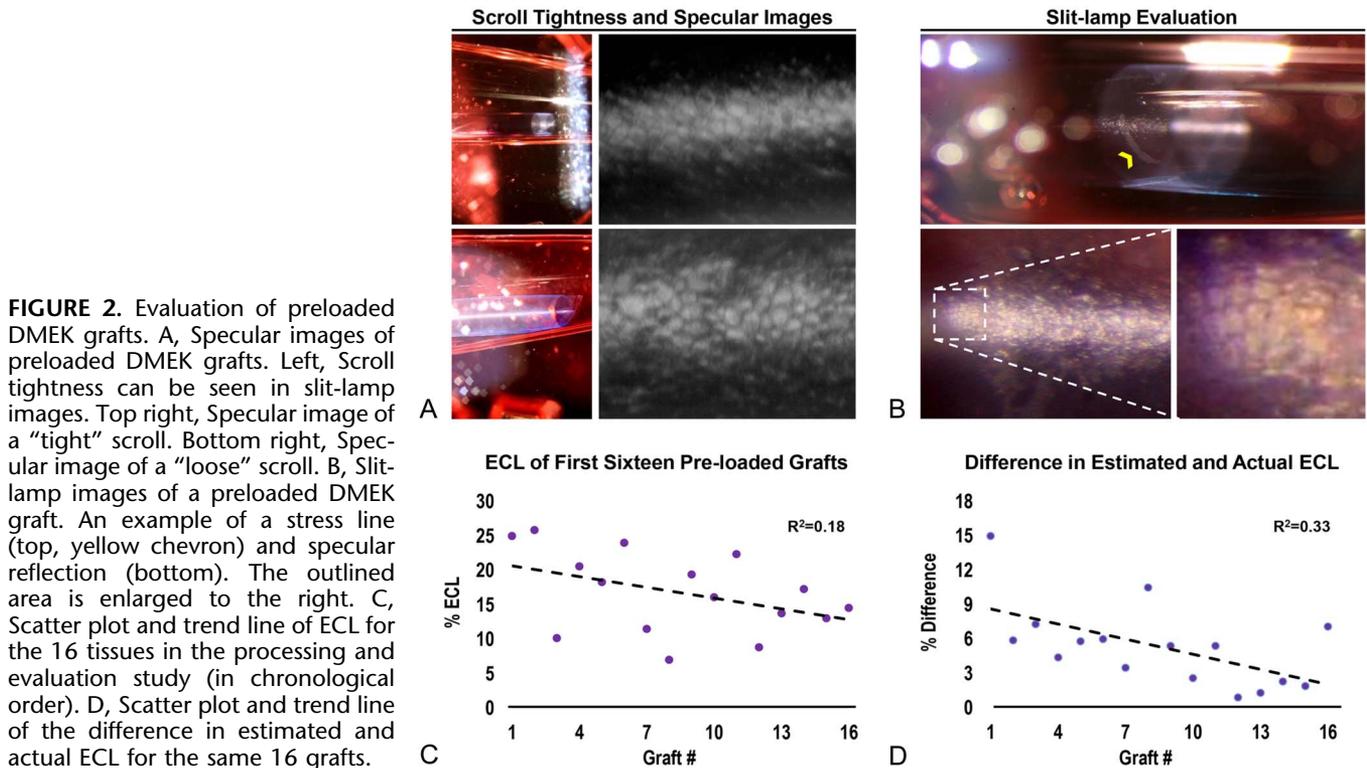


FIGURE 1. Packaging of preloaded DMEK grafts. A, Close-up of a preloaded graft after preparation inside the Straiko modified Jones tube and Krolman viewing chamber. B, Standard eye bank prestripped DMEK graft still attached to the stroma (left) and preloaded DMEK graft (right) where the corneal scleral cap has been replaced by the glass tube.



loading/unloading, and touch defects were observed during slit-lamp evaluation and revealed by Calcein-AM staining (Fig. 3). However, no specific defects caused by the tissue settling on to the glass tube were identified.

Average estimated endothelial cell loss (ECL) (by slit-lamp biomicroscopy) for all grafts was $15.6\% \pm 5.8\%$ (range: 7.5%–30.0%). Quantified ECL by vital dye staining and analysis using FIJI Weka Segmentation revealed an average ECL of $16.8\% \pm 5.9\%$ (range: 7.0%–25.9%). Total ECL of preloaded grafts was underestimated 62% of the time, and the average difference between estimated and actual ECL was $5.3\% \pm 3.6\%$ (range: 0.9%–15%). The amount of quantified ECL and the differences in estimated and actual ECL decreased over the course of this study (Figs. 2C, D).

ECL Due to Eye Bank Prestripping Alone

Average ECL due to prestripping alone was $9.3\% \pm 5.9\%$ (median: 7.2%, range: 3.7%–26.0%, $n = 14$), and was significantly lower than that of preloaded tissues ($P < 0.01$, Table 1). Donor age range and preprocessing ECD of both groups were not significantly different ($P = 0.37$, $P = 0.19$, respectively).

Shipping of Preloaded DMEK Grafts

The average amount of ECL of the 10 shipped tissues was $18.5\% \pm 12.4\%$ (median: 14.0%, range 8.5%–50.4%). One of the 10 shipped tissues fell out of the Straiko Modified Jones tube and incurred 50.4% ECL. When the

graft that was dislodged from the carrier tube is excluded, average ECL from this shipping study decreased to $15.0\% \pm 5.7\%$ (median: 13.5%, range: 8.5%–26.4%) and was not significantly different from preloaded tissues that were not shipped ($P = 0.48$).

Five-Day Storage of Preloaded DMEK Grafts

Five preloaded grafts were prepared and stored at 4°C for 5 days before analysis by vital dye staining and FIJI. Average ECL at the end of the 5-day study was $13.1\% \pm 9.5\%$ (median: 10.6%, range: 6.9%–29.8%). One tissue showed 29.8% ECL after 5 days in storage; however, trypan blue staining of this tissue immediately before processing revealed approximately 17% cell loss. In contrast, trypan blue staining of the other 4 tissues before processing showed less than 2% cell loss on average. When the tissue with 29.8% ECL was excluded, average ECL at the end of the 5-day study for the remaining 4 tissues was $8.9\% \pm 2.1\%$ (median: 8.9%, range: 6.9%–10.7%).

DISCUSSION

We have demonstrated one example of preloaded DMEK grafts prepared in a manner that allows for post-processing evaluation. Our results suggest that it is feasible for eye banks to provide this novel service while continuing to provide surgeons with accurate tissue information without compromising tissue quality. In this study, we discuss several issues that warrant consideration before implementation of preloaded DMEK grafts for clinical use.

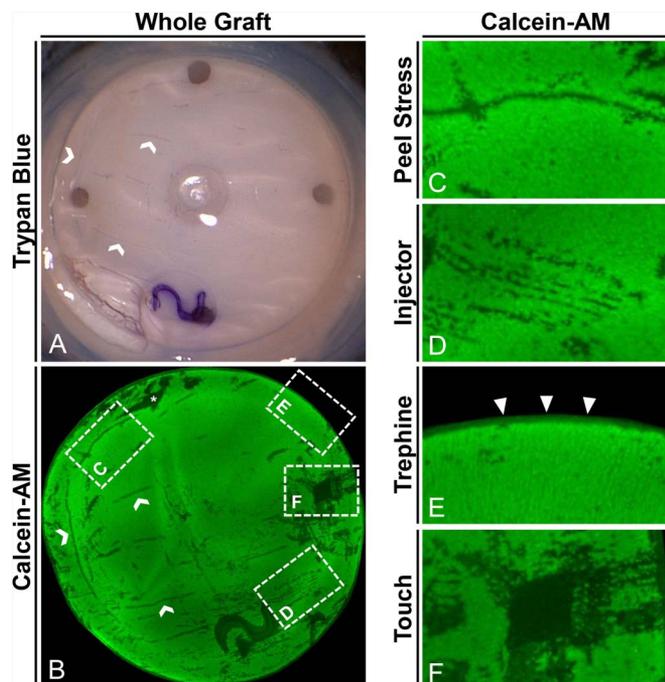


FIGURE 3. Examples of different types of graft damage. White chevrons in A and B indicate stress lines due to prestripping. Labeled regions in B are enlarged to the right. A, Graft after prestripping and trephination, before loading into the injector. B, The same graft as in A with examples of several types of damages as revealed by Calcein-AM staining. * indicates possible touch damage during tissue manipulation. C, Close-up of damage caused by prestripping. D, Close-up of damage caused by scraping against the glass injector. E, Close-up of graft edge damage due to trephination. White arrowheads in E indicate damage caused by trephine. F, Close-up of touch defect possibly caused during tissue loading or unfurling for analysis.

Postprocessing Evaluation

Postprocessing evaluation of eye bank-prepared tissues is required by the EBAA Medical Standards¹⁵ and is an additional quality control step before tissue transplantation. Although specular microscopy alone cannot be used to examine overall tissue quality (eg, tissue damage caused by processing), it can provide important information such as endothelial cell morphology and ECD. The number of endothelial cells that can be examined per specular image is affected by the tightness of the DMEK scroll. Fewer cells can be counted on a graft that scrolls tightly (Fig. 2), and these grafts may require up to 4 specular images to obtain the desired number of cells for accurate cell measurements.

We observed a slight but insignificant increase in ECD of preloaded tissues compared with ECD before tissue preparation ($P = 0.1$). The slight increase may be due to the scrolled shape of the preloaded grafts causing the cells to appear closer together. These results are consistent with a previous report comparing prepreparation and postpreparation cell counts for eye bank-prepared prestripped DMEK tissues.²²

Because of their transparent nature, all preloaded DMEK grafts (tightly or loosely scrolled) can be examined in their entirety by slit-lamp biomicroscopy. Technicians were able to focus on inner and outer layers of the tissue scrolls to visualize and estimate total graft damage. We found that technician-estimated cell loss based on slit-lamp examination was lower than actual cell loss for 62% of the grafts. This underestimation is likely due to a combination of limited experience in examining DMEK grafts in scrolled conformation and further manipulation required to open the grafts before analysis (further discussed below).

We further found that graft damage estimations improved over time. The largest differences between estimated and actual ECL occurred in the first 6 grafts of the validation study (range: 4.4%–15%), whereas the smallest differences were found in the last 6 grafts of the series (range: 0.9%–7.1%) (Fig. 2D). This trend continues for grafts prepared for the shipping and storage studies. Thus, our results suggest that postprocessing slit-lamp evaluations can be done with reasonable accuracy by trained eye bank technicians, and that evaluations will improve as technicians become more familiar with evaluating DMEK grafts in scrolled conformation.

Tissue Preparation and Graft Quality

The Straiko Modified Jones tube is made of clear glass, and it fits into the Krolman viewing chamber that many eye banks currently use (Fig. 1). One limitation of this study is that only the glass Straiko Modified Jones tube was used. Surgeons who currently use other methods to deliver DMEK grafts may find the proposed preloaded method to be incompatible with their current practice.

A notable difference in ECL of prestripped and preloaded grafts was observed in this study (Table 1). We attribute the higher ECL of preloaded grafts to the additional manipulation required to process preloaded tissues such as graft trephination, lifting of the graft, and injector loading (Fig. 3). Furthermore, preloaded grafts must be unfurled on a bed of viscoelastic for analysis, a procedure that can cause additional tissue damage.¹⁹ Average ECL of preloaded tissue in our study was similar to ECL previously reported by Schallhorn et al, who analyzed prestripped DMEK grafts using similar methods after graft injection through a modified Jones tube.¹⁹ Thus, we believe that some of the observed increase in ECL may be due to tissue injection and the method of analysis rather than preloading. The similar outcomes of these 2 studies support further clinical studies to examine the safety of using preloaded grafts for human transplantation.

We experienced a steep learning curve for processing and evaluating preloaded DMEK grafts (Figs. 2C, 4). Analysis of tissues processed earlier in this study revealed higher than desired amounts of ECL (>25% ECL). The cell loss occurred because of several technical reasons that included partial trephination of the desired graft zone, scraping of the graft against the opening of the injector while loading, and unwanted touch defects while unfurling the tissue for analysis (Figs. 3, 4). We observed a weak correlation in the improvement in graft quality as technicians prepared the first 16 grafts in the

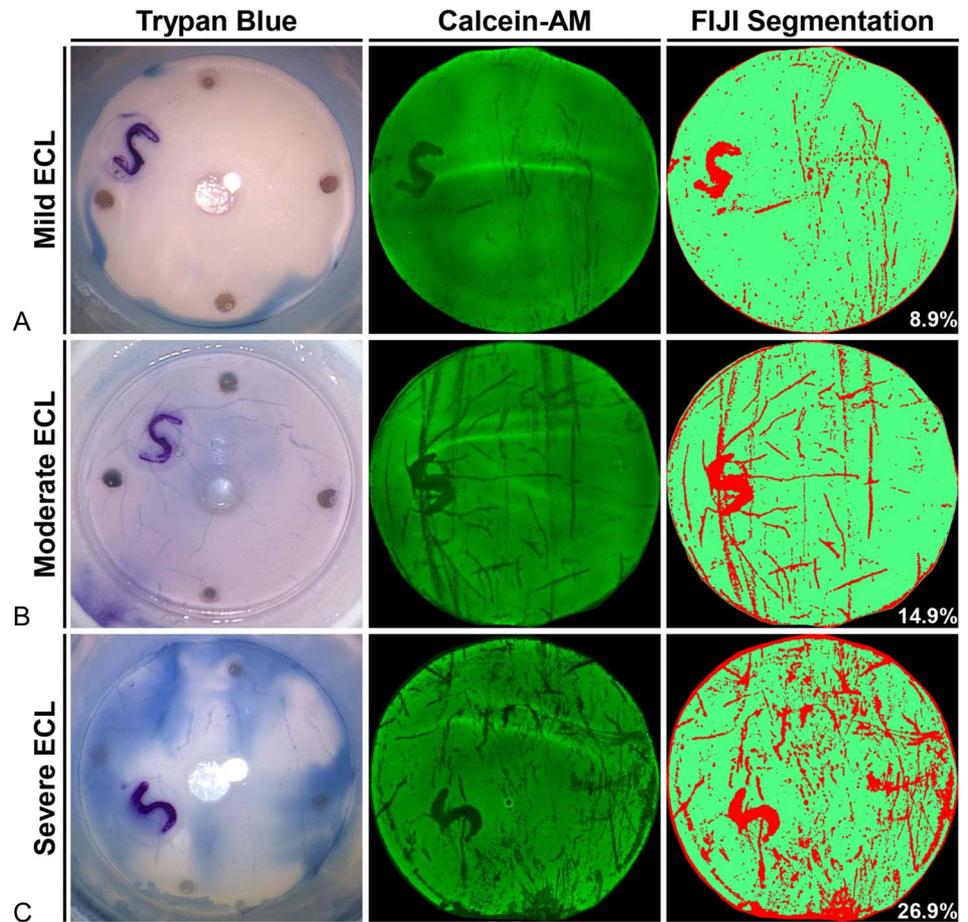


FIGURE 4. Patterns and example amounts of ECL on preloaded grafts. Left, Trypan blue-stained grafts just before loading. Middle, Calcein-AM stained grafts after removal from the injector and opening of the grafts on a bed of viscoelastic. Right, FIJI segmentation to quantify cell loss. Percent cell loss is shown in the right panels. A, Example of mild cell loss. B, Example of moderate cell loss. C, Severely damaged graft. The bottom edge of this graft was heavily damaged because of incomplete trephination and increased manipulation during injector loading.

validation experiments (Fig. 2C, $R^2 = 0.18$). However, continued improvement in graft quality can be seen throughout the remainder of the study, as the median ECL decreased slightly as technicians processed more tissues for the shipping and storage experiments (Table 1).

Cell viability of grafts subjected to 2 shipping events or stored for several days at 4°C was not dramatically different from that of grafts examined in the processing and evaluation study (Table 1). This suggests that shipping preloaded grafts inside a glass tube and viewing chamber does not cause additional graft damage (unless the graft falls out of the carrier tube). Our 90% shipping success rate has prompted us to consider future modifications to prevent grafts from dislodging from the carrier tube. The 5-day storage experiment was to ensure graft viability for surgeons who prefer to receive prepared grafts one day earlier than scheduled surgeries, as well as to account for possible shipping delays due to weather and returned grafts due to surgery cancellations. Altogether, our results suggest that endothelial cell viability does not seem to be dramatically influenced by shipping or short-term storage in Optisol-GS at 4°C.

Clinical Implications of Preloaded DMEK

Although our study provides evidence that preloaded DMEK tissues can be prepared successfully, it does not

definitively answer all the safety concerns of using preloaded tissue in a clinical setting. Additional clinical studies are warranted to determine the safety and efficacy of using preloaded tissue for transplantation in humans. Furthermore, a safe protocol for staining the graft in the OR, or for prestaining preloaded grafts, is necessary to aid in graft visualization during transplantation. These issues are subjects of ongoing research in our laboratory.

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