# Abstracts from 2017 Scientific Symposium

## PRACTICE PATTERNS FOR SELECTING DONOR CORNEAS FOR TRANSPLANTATION

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**Purpose:** In 2015, more than 6000 corneas released for transplantation ended up expiring because of inability to place them. This represents a waste of time and money for eye banks, a disappointment for donor families, and a loss of opportunity for international locations needing tissue. The purpose of our study is to help eye banks tailor their recovery process and management of tissues and a better knowledge of US surgeons' criteria for ordering corneas for transplantation.

**Methods:** An anonymous survey of 17 items was developed on the SurveyMonkey website. An email was sent out on kera-net, the official forum of the Cornea Society, to EBAA Paton Society members, and through eye banks to invite cornea surgeons to participate in the online survey. Results were analyzed to identify practice trends.

**Results:** Eighty ophthalmologists participated in the survey performing an average of 66 corneal transplants a year. Sixty percent worked in a private practice. Concerning corneal tissue for penetrating keratoplasty, the main criteria accepted by surgeons were, on average: maximum donor age 74.8 y ± 12.9; minimum cell count 2219 cells/mm2 ± 259.8; death to preservation time 19.0h ± 10.8; time in corneal storage solution 8.7d ± 3.3.These preferences did not differ statistically for endothelial keratoplasty tissue except for the minimum endothelial cell count that was higher for both DSEK and DMEK (2303 ± 290.2 and 2339 ± 284.3 ($p<0.05$)) and storage time that was a little shorter for DMEK (8.1 ± 3.4 ($p<0.05$)). There was no association with a surgeon's years of experience or surgical volume as determined by regression analysis.

**Conclusions:** There are multiple studies in the literature trying to identify donor-related factors associated with graft failure with varying conclusions. This study shows that surgeons end up developing their own preferences regarding donor corneas they use. A better knowledge of these preferences can help eye banks meet surgeons' expectations and improve their efficiency.

## ENDOTHELIAL CELL LOSS DUE TO EYE BANK PREPARATION AND INJECTOR METHOD IN DESCEMET MEMBRANE ENDOTHELIAL KERATOPLASTY TISSUES

*Kenneth Downes, MD; Khoa D. Tran, PhD; Chris Stoeger, CEBT; and Winston Chamberlain, MD PhD*

**Purpose:** To evaluate the endothelial cell damage caused by two different injectors for Descemet's membrane endothelial keratoplasty (DMEK) grafts.

**Methods:** Eighteen DMEK grafts with 'S' stamps were prepared by trained eye bank technicians. Nine grafts were assigned to injection with a glass modified Jones tube injector with the 2.4 mm opening and nine were assigned to injection with the DORC glass pipette injector (<1.5 mm opening). The grafts were prepared and loaded into the injectors using standard surgical technique, injected onto a bed of viscoelastic on a glass slide, and unscrolled using further viscoelastic. The grafts were stained with the vital dye Calcein-AM, digitally imaged and analyzed using the FIJI. The percentage of cell loss was calculated by measuring the area of non-fluorescent pixels and dividing it by the total graft area pixels. Statistical comparison was performed using a two tailed unpaired T-test.

**Results:** Grafts injected using the Jones tube injector had overall cell loss of 22.95% +/- 5.05% (95% CI). Grafts injected using the DORC injector overall cell loss of 29.16% +/- 8.50% (95% CI). This difference was not statistically significant ($p=0.17017$), however the patterns of cell loss on the grafts was different between injectors.

**Conclusion:** Graft injection with the either glass injection system seems to cause approximately 20-30% overall cell loss in DMEK grafts. There were characteristic differences in patterns of cell loss seen between injectors, which may be clinically relevant, but indicates more the types of stresses that grafts are put under during passage through the injector.

## A SIMPLE 60-SECOND SWELLING TECHNIQUE FOR MORE CONSISTENT ULTRATHIN DSAEK GRAFT PREPARATION

*Neil Farbman, MD, JD; Jennifer Li, MD; Jennifer Ling, MD; Chris Conwell, CEBT; and Tiffany Ramirez, CEBT*

**Purpose:** The purpose of this study is to describe a simple but novel sixty-second swelling technique for ultrathin DSAEK graft preparation. In addition, we aim to demonstrate the effectiveness of this technique in obtaining thinner DSAEK grafts more consistently and without compromising graft quality.

**Methods:** We performed a retrospective case-control study comparing standard DSAEK preparation using an ML7 Microkeratome Donor Cornea System (Med-logics Inc., Athens, TX) with an additional 60 seconds of stromal swelling with balanced salt solution after removal of the epithelium but prior to the microkeratome pass. Thirty cases using this novel swelling technique were compared with controls matched by age, sex, and pre-cut corneal thickness. Donor characteristics and both pre-cut and post-cut graft characteristics were analyzed.

**Results:** DSAEK grafts prepared with our simple swelling method were approximately 15 microns thinner on average than those prepared with our conventional ultrathin DSAEK preparation technique ($p<0.05$).The frequency of grafts under 100 microns was much greater with swelling (>90% versus <70% with conventional technique).There were no significant differences in post-cut cell counts, decrease in cell counts, or post-cut endothelial characteristics between groups.

**Conclusions:** A simple 60-second swelling technique can yield significantly thinner DSAEK tissue in a more consistent range and without significant impact on cell-count or post-cut endothelial characteristics.

## TECHNIQUE REFINEMENTS FOR REDUCING S-STAMP ASSOCIATED CELL-LOSS ON DMEK GRAFTS

*Joshua Galloway, CEBT; Philip K. Dye, CEBT; Kelly Odell; Mark A. Terry, MD; and Khoa D. Tran, PhD*

**Purpose:** To examine S-stamp boldness in relation to ink retention and endothelial cell loss (ECL) at 2 days post-processing.

**Methods:** Nineteen DMEK grafts with S-stamps were prepared by trained eye bank technicians. Images of the S-stamps were acquired and rated as 'thin' or 'thick' after preparation. DMEK grafts were stored in cold storage for 2 days. Bright field images of the corneas were captured to document visibility of the S-stamps 2 days post-preparation. ECL in the 2-mm diameter region of the S-stamp was quantified by vital-dye staining and FIJI segmentation. An additional 5 DMEK grafts were prepared with a larger, but thinner S-stamp. Two S-stamps were applied per DMEK graft. The first S-stamp was applied and the stromal plug was immediately closed. For the second S-stamp the technician allowed for 30 seconds of air-dry time prior to closure of the stromal plug. The differences in ECL caused by these two S-stamps were examined.

**Results:** All S-stamps were readily observable after 2 days in cold storage. Nine 'thin' S-stamps contributed to an average of 0.80% ECL of the whole DMEK graft (range: 0.55-0.94%), while another 5 'thin' S-stamps showed averaged ECL of 1.5% (range: 1.32-1.71%).

Five S-stamps were rated as 'thick' and contributed significantly more ECL (average: 2.58%, range: 2.16-3.99%, P=0.005). Revised- shaped S-stamps (10 of 10) were all rated as 'thin' and caused an average of 0.68% ECL (range: 0.37 – 1.11%). S-stamps applied and allowed to air-dry showed a slight but insignificant reduction in ECL.

**Conclusions:** Thinner S-stamps cause less ECL without compromising visibility. Additional studies are required to determine if dry time before and after S-stamp application further reduces ECL.

## COMPARISON BETWEEN CENTRAL CORNEAL THICKNESS MEASUREMENTS BY ENHANCED SPECULAR MODALITIES AND OPTICAL COHERENCE TOMOGRAPHY

*Kayla Gray; Kayla Jones, CEBT; and Gregory H. Grossman, PhD, CEBT*

**Purpose:** The accuracy of the pachymetry function of the Konan CellChek D+ has not yet been independently assessed. This study sought to compare central corneal measurements taken by the CellChek D+ against the standard method in eye banking, optical coherence tomography (OCT).

**Methods:** The central thickness of 28 corneas in corneal viewing chambers was measured by two separate groups of technicians using the Optovue RT100 OCT and the Donor Enhanced mode of the Konan CellChek D+. A paired t-test was used to test significance between groups.

**Results:** The mean thickness of the 28 corneas measured by OCT was 563 µm (SD +/- 49.8), as compared to 556 µm (SD +/- 61.8) recorded by the CellChek D+. A t-test revealed no significance between the two groups (p= 0.3702). When corneas noted with severe folds were taken out, the remaining 21 corneal thickness t-test revealed a high significance between the two groups (p=0.0008).

**Conclusions:** While there are no statistical significant differences between OCT and CCD measurements detected, the differences noted in thickness could prove to be problematic because of the tight parameters of surgeon preference on the thickness of their keratoplasty grafts and the advent of ultrathin cuts and DMEK. Within the data set, there was higher availability noted in corneas rated for severe folds, which may be signaling that one technology may be more effective at collecting data from corneas noted to have folds.

## EFFECT OF DEATH-TO-PRESERVATION TIME ON POSTOPERATIVE ENDOTHELIAL CELL MORTALITY

*Matthew M. Habib, BS; Bryan Abessi, MD; Robert A. Eden, MD; Jonathon Stone, BS, CEBT; and Robert L. Schultze, MD*

**Purpose:** Elucidation of the impact of death-to-preservation (D to P) interval on donor endothelial cell mortality 3 months postoperatively.

**Method:** A retrospective study of fourteen recipient eyes with D to P of greater than 18 hours (range, 18:03 to 19:37) was carried out. Donor records were retrospectively reviewed for age, D to P, eye/body-refrigeration time, death-to-surgery time, endothelial cell count and condition of endothelium prior to surgery. Three month post-operative endothelial cell counts were reviewed. A matched control group (patients who received grafts with minimal D to P < 18 hours) was gathered of 14 patients. Student's t-test was utilized to compare parameters.

**Results:** The average D to P of the 14 donor corneas in the variable group was 18 hours and 54 minutes. The average D to P of the 14 donor cornea of the matched control group was 5 hours and 21 minutes. Averages of donor age, death-to-surgery time, preoperative endothelial cell count and the condition of the tissue showed no statistically significant difference between the variable group and the control group. The mean post-operative endothelial cell count showed no statistically significant difference between groups (p= 0.63).

**Conclusion:** Donor tissue with D to P averaging greater than 18 hours demonstrated no increase in endothelial cell mortality seen three months postoperatively in comparison to donor tissue with a D to P averaging less than 18 hours. Maximal acceptable D to P of the donors used for corneal transplants has yet to be elucidated. More research in the future should be directed determine the impact of longer death to preservation times.

## EVALUATION OF CORNEAL DONOR TISSUE USING OPTICAL COHERENCE TOMOGRAPHY COMPARED TO SLIT LAMP EXAMINATION

*S. Tammy Hsu; Christine Shieh, MD; Isaac Perry, BS, MBA, CEBT; Sandra Stinnett, MS, DrPH; Anthony Kuo, MD*

**Purpose:** Transplant suitability of donor corneas are evaluated using slit lamp examinations (SLE); however, SLE estimations of the depth of corneal scars or "lesions" are subject to interpretation. More precise measurement of lesion depth by optical coherence tomography (OCT) could provide useful information for lamellar use of the tissue. In this study, we compared the estimations of lesion depth in donor corneas using OCT vs. SLE.

**Methods**: Ninety-two donor corneas from Miracles in Sight Eye Bank were identified on SLE to have cornea lesions, defined as scars or pathologic findings affecting stroma. Technicians estimated lesion depths as a percentage of total cornea thickness using SLE. OCT scans were then taken of these same corneas. A masked grader identified and measured lesion depths on OCT images. Estimations of lesion depth using OCT vs. SLE were assessed for agreement.

**Results**: Estimation of lesion depth was significantly different between SLE and OCT ($p < 0.0001$) test of symmetry, percent agreement=44%, kappa=0.1372). Lesions identified and graded by SLE were deeper than those identified and graded on OCT (48% vs. 7%, respectively).

**Conclusions**: Compared to OCT, slit lamp examination estimated lesions as extending deeper into the cornea. As OCT has higher depth resolution, the use of OCT may allow more tissue to be used for endothelial keratoplasty if identified corneal lesions are not truly as deep as the SLE assessment. However, as the individual lesions identified on SLE were not directly matched with those identified on OCT, further studies are needed to ensure 1:1 comparison of the same lesions using SLE and OCT.

## USE OF OPTICAL COHERENCE TOMOGRAPHY TO SCREEN DONOR CORNEAS

*Narae Ko, MD; Anthony Kuo, MD, PhD; Isaac Perry, MBA, CEBT; and Michael Tramber, MBA, CEBT, CTBS*

**Purpose:** A severe imbalance exists in supply and demand of corneas world-wide. Given such a shortage, it is critical to maximize the utilization of procured corneas. Optical coherence tomography (OCT) has been used to screen for laser in situ keratomileusis in donor corneas. We applied a commercially available, micrometer resolution OCT to characterize 22 donor corneas with subtle opacities that are assumed to be infectious and deemed unsuitable for transplantation.

**Methods:** Donor corneas at Miracles In Sight underwent slit lamp exam and were scanned while sealed within the sterile container immersed in Optisol GS. OCT scanning was performed within 10mm diameter using 100 radial scans. The images were automatically mapped using our processing software. The depth and thickness of corneas were assessed using ImageJ.

**Results:** All 22 corneas had opacities near the inferior limbus on slit lamp exam. All opacities were easily identified by increased reflectivity. Seventeen (77.3%) corneas had epithelial defect over the area of opacities. The mean depth of lesion from the surface was 56.9% (range 34-100%), with just two corneas with full thickness involvement. Twenty corneas had an increased thickness in the area of opacity (range 2.4-36.8%). Four corneas had hyper reflective deposits on the endothelium.

Conclusions: Optical coherence tomography is a useful technique to assess opacities in donor corneas. The lesions seldom involve posterior lamella, and hence the possibility of utilization for endothelial keratoplasty. Future work will include determining the true etiology of the opacities with histopathological studies and determining OCT imaging characteristics that appear to distinguish corneas which have infectious lesions versus non-infectious lesions.

## RISK FACTORS LEADING TO ENDOTHELIAL DAMAGE IN ULTRA-THIN DSAEK GRAFT PREPARATION

*Jennifer Ling, MD; Jennifer Li, MD; Neil Farbman, MD, JD; Chris Conwell, CEBT; and Tiffany Ramirez, CEBT*

**Purpose:** To describe a new pattern of endothelial damage that can occur when preparing ultra-thin (≤100 um) DSAEK grafts using the ML7 Microkeratome Donor Cornea System (Med-logics Inc., Athens, TX) and to identify factors that increase the risk of such damage.

**Methods:** A retrospective chart review was conducted on 233 consecutive ultra-thin DSAEK graft preparations from December 2015 to December 2017. Twenty grafts were noted to have a unique pattern of linear endothelial damage parallel to the orientation of the blade, which we have coined "chatter".

Donor characteristics, graft cutting parameters, and pre-and post-cut tissue measurements were analyzed. Primary outcome was the presence or absence of endothelial chatter. Secondary outcomes were the percentage endothelial cell density loss and severity of endothelial stress lines.

**Results:** Endothelial chatter occurred in 8.9% of ultra-thin DSAEK grafts. There were no donor or graft cutting characteristics that predicted the presence or absence of endothelial chatter. Thinner grafts were significantly correlated to increased severity of endothelial stress lines (Pearson 2-tailed test).

**Conclusions:** As ultra-thin DSAEK gains popularity, eye banks must meet the challenge of providing such tissue while maintaining endothelial integrity. We describe an uncommon pattern of damage termed endothelial chatter. Although the mechanism of such damage remains unclear, thinner grafts were related to more severe endothelial stress lines, a possible precursor to chatter. More importantly, there were no donor or graft cutting characteristics that led to an increased risk of endothelial chatter.

## A NEW "STAGE" FOR PRACTICING THE "DMEK DANCE" BEFORE VENTURING INTO THE OPERATING THEATRE

*Christopher Sales, MD, MPH; Ana Alzaga Fernandez, MD; Zachary Mayko, MS; and Khoa D. Tran, PhD*

**Purpose:** To evaluate a novel apparatus designed to replace whole eyes for DMEK wet labs.

**Methods:** The apparatus is assembled from a Barron artificial anterior chamber (AAC), a latex glove, two 3 cc syringes, and a donor cornea, which is temporarily mounted on the AAC with BSS to make the main and paracentesis incisions before it is dismounted for final assembly. A latex pupil-less iris measuring 15 mm in diameter is cut from the glove and placed on the backside of the corneoscleral cap, which is then remounted onto a dried AAC. The anterior chamber (AC) (i.e. the space between the latex and the cornea) is filled with BSS via one of the paracenteses. The AAC is then pressurized with air from a syringe, after which, the valves are closed. Releasing BSS from one of the paracenteses lowers the AC's pressure relative to the AAC, which causes the distensible latex iris diaphragm to bulge anteriorly. As a result, the AC shallows. The contrary occurs when the AC is filled with BSS.

**Results:** Tissue injection, collapsing the AC, the tap, the Dirisamer, the shuffle, the touch and release, and the paracentesis-flick could all be performed using the novel apparatus in the hands of a novice and experienced DMEK surgeon. The apparatus compared favorably to in-vivo surgery and offered at least three advantages over a whole globe in the wet lab: 1) cost, 2) clarity of the cornea, and 3) easier modulation of the AC depth without the corneal surface becoming concave.

**Conclusions:** This is the first description of a DMEK training tool that simulates in vitro the anterior chamber dynamics of in vivo DMEK surgery. Early experience suggests that the "DMEK Stage" may be a viable alternative to whole globes.

## COMPARING THE EFFECT OF DMEK INSERTERS ON ENDOTHELIAL DAMAGE OF GRAFTS

*Elizabeth Shen, MD; Adam Fox, CEBT; Kapil Mishra, MD; and Marjan Farid, MD*

**Purpose:** To compare endothelial damage in pre-stripped Descemet's membrane endothelial keratoplasty (DMEK) tissue from three different injector devices: the modified Jones tube, the STAAR intraocular (IOL) injector, and the Geuder glass cannula.

**Methods:** Twenty-one human donor corneas were used for this study, 7 for each study arm. Each endothelial graft was pre-stripped, stained with trypan blue, and loaded into either the modified Jones tube, the STAAR IOL injector, or the Geuder glass cannula. Grafts were then ejected and stained again with trypan blue. Endothelial damage was quantitatively analyzed using Adobe Photoshop. The primary outcome was the percent of endothelial damage from injector loading and injection.

**Results:** The mean percent of endothelial damage from injector loading and injection was 6.0 ± 4.0% (95% CI) for the modified Jones tube, 5.4 ± 5.2% (95% CI) for the STAAR IOL injector, and 4.9 ± 3.2% (95% CI) for the Geuder cannula (p=0.88).

**Conclusion:** DMEK injectors induce minimal intraoperative endothelial damage. The modified Jones tube, STAAR IOL injector, as well as the Geuder cannula offer acceptable tissue quality and cell loss during loading and injection of DMEK grafts. While the variability in opening size may not have a significant impact on cell loss, it may affect ease of use.

## ASSESSING GENTIAN VIOLET TOXICITY DURING S-STAMP PROCEDURES FOR DMEK GRAFTS

*Jody Simon, MD; Gregory Grossman, PhD, CEBT; Kayla Gray; Kayla Jones, CEBT; and Jeffrey Goshe, MD*

**Purpose:** We have recently seen several patients with post-op graft edema and detachments after receiving DMEK grafts with micro S-stamps performed with gentian violet ink. These grafts specifically showed increased edema near the micro S-stamps. In this study we hope to elucidate the extent of endothelial damage during micro S-stamping DMEK grafts, and to define a safe, standardized micro S-stamp protocol for eyebanks.

**Methods:** Two mated pairs of corneas were prepared using the Eversight DMEK procedure. A 2mm punch and a P3 surgical marker were used to apply a micro S-stamp with 2 application techniques. Technique A applied the marker to the technician's glove, transferring the ink to the micro S-stamp and waiting 10 or 30 seconds prior to stamping. Technique B applied the ink marker directly to the micro S-stamp, after waiting 10 or 30 seconds for the ink to dry prior to stamping the graft. The prepared corneas were refrigerated for 18 hours. The immunofluorescent stain ethidium homodimer-1 was used as a marker for dead cells. Pan-corneal images were captured and photomicrographs were analyzed by a masked reader using a manual counting technique to compare endothelial counts between groups.

**Results:** There was greater cell death using Technique A with indirect marking of the graft. Technique B demonstrated less cell death than technique A at both dry times. There was no difference in cell death when comparing grafts prepared with a 10 or 30 second dry time.

**Conclusions:** Although a greater sample size is required to test significance, data suggests that gentian violet ink should be directly applied to DMEK S-stamps without an intermediate step. We believe that graft toxicity associated with endothelial cell death is most likely attributed to the alcohol vehicle.

# DROWNING VICTIMS: EYE BANKS PRACTICE PATTERNS AND TISSUE OUTCOMES

*Maria Woodward, MD, MS; Purak Parik, MD; Bradley Tennant, CEBT; Bob Albrecht, CEBT; Greg Grossman, PhD, CEBT; Leslie Niziol, MS; and Shahzad Mian, MD*

**Purpose:** To assess safety of donor corneal tissue from victims of water submersion (drowning or submersion secondary to death) by investigating eye banks' practice patterns and tissue outcomes.

**Methods:** All 79 Eye Bank Association of America accredited eye banks were contacted for a phone interview of practices regarding tissue from victims of water submersion. A retrospective review of all corneal tissues from 2014-2016 from a large eye bank network was performed to identify all donors submerged in water at time of death. Corneal epithelial integrity, endothelial cell count, donor rim cultures, and adverse events were analyzed for associations with water submersion characteristics.

**Results:** 49 eye banks (62% response) participated in the survey. 55% of these eye banks had specific, written protocol for tissue eligibility from donors submerged in water. With or without formal protocol in place, eye banks reported considering water type (84%) and the length of time submerged (92%) when determining eligibility. 22% of eye banks reported medical director involvement when eligibility determination was unclear. 79 tissues from 40 donors who were submerged were identified in 2014-2016 eye bank data. No donor tissues had pre-processing corneal infiltrates, positive donor rim cultures, or adverse events after keratoplasty. Corneal epithelial integrity and endothelial cell count were not associated with water type or length of time submerged.

**Conclusion:** Although universal protocol for tissue eligibility from victims of water submersion does not exist, data from a large eye bank network showed no adverse events or outcomes, indicating these tissues may be safe.