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**DMEK SCROLL SCORING SCALE TO FACILITATE A PREFERRED CONFIGURATION**

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**Purpose:** Introduce an easy to use scoring scale to describe the tightness of DMEK scrolls. Using the scroll scale to decide the best configuration for pre-loaded DMEK grafts and to calculate scroll circumference and diameter.

**Methods:** Use DMEK processing photos and video to evaluate the tightness of DMEK scrolls and compare to OCT of DMEK grafts pre-loaded in a Straiko Modified Jones tube. Review recent DMEK prep data for scroll score (SS) and single versus double scroll configuration. SS is defined as the number of revolutions of DM that make up the scroll, free floating in solution. Grafts with SS=>2.0 can be manipulated into a double scroll prior to loading. Evaluate 3 client surgeon comments re ease of use.

**Results:** Pre-loaded DMEK grafts prepared from 9/2017 through 5/2018 N=106 or 40.8 % of all DMEK preps. Scroll score noted for 14 grafts. Range 1.3 to 3.4 with 8 grafts with SS=2.0 or more, loaded as double scrolls. Double scroll configuration was maintained in transit, insertion and noted as preferred by the surgeons. SS also applies to PDEK grafts. Graft size/SS=scroll circumference and divide by Pi=diameter, for a single and x2 if a double.

**Conclusions:** Using a scroll score (SS) to indicate degree of tightness can be easily applied to pre-loaded DMEK grafts. SS of 2.0 or more may guide formation of a double scroll as a preferred configuration. Pre-loaded DMEK grafts in a double scroll configuration may be easier to unfold. SS can be used to calculate scroll circumference and diameter to aid loading. More follow-up data is needed to further evaluate this technique.

**TITLE: PERFORMANCE OF FROZEN DMEK AND DSAEK GRAFTS IN A WET LAB SETTING**

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**Purpose:** Studies have shown that increased surgical experience leads to improved graft functionality and surgical outcomes in both Descemet membrane endothelial keratoplasty (DMEK) and Descemet stripping endothelial keratoplasty (DSAEK). This study focused on surgical training in a wet lab setting and the association between prior surgical experience and outcomes for frozen DMEK and DSAEK grafts.

**Methods:** DMEK and DSAEK grafts were processed and frozen at -80° Celsius. Grafts were shipped internationally to the wet lab. 5 instructors taught 2 separate wet labs for 27 DMEK participants and 15 DSAEK participants. Participants ranged from residents to fully licensed ophthalmologists. DMEK insertion was performed utilizing a Modified Straiko Jones tube, while DSAEK insertion was completed with the Tan Endoglide. After the wet lab, each participant completed a survey assessing: years of experience, prior DMEK/DSAEK practice, and numerical scales for tissue quality, maneuverability, orientation and successful simulated transplantation. Data was collected and compared between surgeons with less than 5 years vs more than 5 years of post-graduate surgical experience.

**Results:** 6 out of 27 participants in the DMEK wet lab had prior DMEK experience. 4 participants were either unable to unscroll or orient the graft properly, all of whom had less than 5 years of post-graduate surgical experience. 7 out of 15 DSAEK participants had prior DSAEK experience and all surgeons oriented the DSAEK grafts correctly, regardless of surgical experience. Numerical scales for graft quality, maneuverability, orientation and transplant success demonstrated no statistically significant findings between the two groups for both DMEK and DSAEK.

**Conclusion:** Frozen DMEK and DSAEK grafts remain a viable option for large volume wet labs and yield excellent success rates with complications associated with skill level rather than tissue quality.
CULTURAL FACTORS INFLUENCING RATES OF CORNEAL TISSUE DONATION

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Purpose: Several studies have shown that minorities are less likely to authorize organ donation in the United States, and a preliminary review of data at Miracles in Sight (MIS) confirmed that this was also true for corneal tissue donation locally. The purpose of this qualitative study was to explore the perceptions and experiences of African Americans and Hispanic Americans regarding corneal tissue donation.

Methods: Two focus group discussions were conducted with a total of 19 participants, all age 40 and over. The discussion groups were separated by race, with 8 participants in the African American group and 11 participants in the Hispanic group. The discussions lasted approximately 90 minutes, and participants received a $25 gift card for completing the group. All sessions were digitally recorded and files were transcribed verbatim by a research staff member. Each transcript was coded, based on a codebook that was developed collaboratively with the research team, and entered into ATLAS. ti 7.5.18, a qualitative data analysis software program, to facilitate analysis.

Results: We conducted a thematic qualitative analysis of the focus group discussions to summarize participants’ perceptions on four key areas: 1. Knowledge about corneal tissue donation. 2. Decision to become a corneal tissue donor. 3. Familial involvement in decisions about corneal tissue donation. 4. Cultural perspectives on corneal tissue donation. The knowledge base regarding specifics about corneal tissue donation was similarly low in both groups.

The African American participants expressed concern with the history of inequality or mistreatment in research, citing Tuskegee. The Hispanic American participants discussed classism and feared favoritism being shown to those with more money, and who are American citizens. Both groups felt that family opinions and religion would have significant effects on the decision to authorize corneal tissue donation. Both groups agreed that a lack of education contributed to lower donation rates within their communities, and were eager to think of ways to improve education.

Conclusions: There were cultural differences among perceptions and thoughts related to corneal tissue donation rates in the African American versus Hispanic American groups. African American participants expressed an underlying mistrust of almost all healthcare providers, including medical researchers, which they felt would influence many of them to avoid authorizing donation of corneal tissue. Hispanic American participants felt more suspicious of possible unjust profitability in corneal tissue donation and feared that their corneal tissue might not go to the patient with the highest need, but instead could end up benefitting the patient with the most money or highest position in society. These differences are understandable given each group’s respective history in the United States, as well as in their countries of origin. Alternatively, both African American and Hispanic American groups agreed that the best way to increase authorization of corneal tissue donation in their communities would be increasing educational efforts by members of their own community, and specifically, more personal stories about the effects of donation (on the donor and recipient). These cultural differences will be helpful to consider as eye banks and Donate Life focus efforts on educating African American and Hispanic American groups regarding corneal tissue donation.

**Corneal Fellow

COST-BENEFIT ANALYSIS OF AMPHOTERICIN B SUPPLEMENTATION OF CORNEAL STORAGE MEDIA WITH EK-PREPARED TISSUE

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Purpose: Post-EK fungal keratitis and endophthalmitis are relatively uncommon but severe adverse events. There have been increased concerns regarding this condition and supplementation of amphotericin B in corneal storage solutions has been proposed as a solution. Here, we systematically examined the course of Post-EK fungal infections and studied the economic impact of amphotericin supplementation by conducting cost-utility and cost-benefit analysis.

Methods: We found 31 separate cases of post-EK fungal infections, including 22 cases from the US and 9 cases from abroad derived from the published and unpublished literature. We also consulted with eye bank professionals regarding the possible costs associated with amphotericin supplementation. Cost-benefit studies were completed with a decision-tree model with 100,000 USD per QAL Y as thresholds. Bivariate sensitivity analyses were used to analyze if cost-effectiveness can be achieved under varying degree of amphotericin effectiveness and post-EK fungal infection incidences.

Results: Statistically significant differences existed between post-EK fungal keratitis and endophthalmitis regarding inpatient stays and costs. Threshold analysis revealed that amphotericin supplementation is cost-effective at 100,000 USD/QAL Y level under conservative assumptions with amphotericin supplementation being moderately effective, i.e. preventing >32% of post-EK fungal infections and incidence of post-EK fungal infection remained at the average level between 2012 and 2017.

Conclusions: Supplementation of amphotericin B in corneal storage solution is cost-effective in preventing post-EK fungal infection assuming the measure is effective in reducing fungal infection incidences.
POST-KERATOPLASTY INFECTIONS ARE NOT ASSOCIATED WITH EYE BANK PREPARED CORNEAS: A SINGLE EYE BANK ANALYSIS OF A LARGE DATASET

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Purpose: To perform a retrospective study examining our eye bank’s post-operative results of infection rates associated with eye bank prepared or non-prepared corneas from January 2006-December 2017.

Method: We examined reports of fungal and bacterial infections associated with corneas distributed by our eye bank that had postoperative information (N=17035 corneas). We quantified the number of infections for corneas that underwent preparation by our eye bank and those that were distributed without preparation. We investigated the association between increased use of eye bank prepared corneas to determine if there is a relationship to the infection reports.

Results: Out of 17035 corneas, 9554 (56%) were prepared at our eye bank while 7481 (44%) corneas were not prepared. A total of 11 infections were reported for eye bank prepared corneas and 42 for non-prepared corneas (0.12% vs 0.56%, P=0.0001). Of these 53, only 4 fungal infections (1 prepared and 3 non-prepared) were attributed to the tissue after investigation by eye bank Medical Directors. There was no ascending trend of reported infections associated with eye bank processed corneas going from the first 3 years (2 of 1054 corneas, 0.19%) to the last 3 years of the study period (6 of 3500 corneas, 0.17%, P=0.901).

Conclusions: Reported infection rates were up to 5X higher for corneas distributed directly to surgeons for transplant compared to eye bank prepared corneas. Reports of infections remained low despite the use of eye bank prepared tissue for corneal transplants increasing from 30% to 75% over the course of our study period.

A COMPARISON STUDY: CLARITY OF GENTIAN VIOLET S STAMPS OVER TIME USING TWO PREPARATION METHODS

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Purpose: The purpose of this study was to evaluate size and visibility of the S stamp when prepared with Optisol-GS versus Balance Salt Solution (BSS).

Methods: A total of 18 donor corneas were assigned a random number and divided into two equal groups for DMEK preparation. All DMEK grafts were prepared according to standard procedures by a single technician. The BSS group served as the control, using BSS to float the graft back into position after partial stripping of Descemet’s membrane, prior to the application of the S stamp. The second group used Optisol-GS to reposition the graft. The S stamps were imaged at 15 minutes, 18, 24, 48 and 72 hours post preparation. The images were analyzed in ImageJ by a masked reader for endpoint measures of S stamp length, top and bottom width, and visibility. Visibility was graded using an Ink Grading Scale, a composition of S stamp images arranged numerically from 0 to 5; 0 representing a poor, non-visible S and 5 representing the optimal, most prominent S. Statistical analysis was completed using repeated measure ANOVA methods.

Results: Statistically significant differences were identified for all end measures but not at each time interval. The Ink Grading Scale produced the most consistent results with the Optisol-GS group having significant/near significantly higher least-square means at each time interval: p=0.217, p=0.012, p=0.058, p=0.05, p=0.03 (arranged 15 minutes to 72 hours). The average S stamp grade at 15 minutes was 4.44 vs. 4.67 for BSS vs Optisol-GS. Over the next 72 hours, S visibility reduced by an average of 4.2 vs. 2.78 points for BSS vs Optisol-GS, resulting in a lesser degree of fading for the Optisol-GS group.

Conclusions: Optisol-GS yielded more consistent results with a lesser degree of fading and maintained S stamp size better than BSS.
A NOVEL APPROACH TO DETERMINE AND QUANTIFY TOTAL CORNEAL LIMBAL STEM CELL HEALTH USING OPTICAL COHERENCE TOMOGRAPHY

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Purpose: One of the major limitations in the field of limbal stem cell evaluation and treatment with transplant is our inability to quantitate in real time the total amount of limbal stem cell deficiency (LSCD) in a given patient. To address this limitation, we developed a structural map of the entire human corneal limbus using spectral domain-optical coherence tomography (SD-OCT).

Methods: Seven donor corneas of various ages from Miracles In Sight were included in this study. We applied a commercially available, micrometer-resolution SD-OCT system to characterize the limbal palisade region. OCT scanning was performed on a rotatable platform at 30 degree intervals for 360 degrees. Scans were 3 mm in diameter and 1.5 mm wide to include the corneoscleral limbus. The images were mapped, processed and analyzed using Matlab software.

Results: En-face SD-OCT successfully visualized the Palisades of Vogt (POV) in four of the corneas as a radially oriented network, located in the superficial limbus. The mean number of palisade ridges was 58 ± 34.17 per cornea. The POV were found predominantly in the superior and inferior limbus (p<0.05). No discernible POV structures were seen in 3 subjects.

Conclusions: OCT is a useful technique to assess the limbal stem cell population in donor corneas. Future work will include histology to correlate the presence of stem cells in the visualized structures. This imaging could be used to assess stem cells for transplantation and improve our outcomes in the medical and surgical management of patients with LSCD.

**Corneal Fellow

ADOPTION OF GEBAUER SLE MICROKERATOME FOR DSAEK AND UT-DSAEK PREPARATION

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Purpose: Determine the feasibility of the Gebauer SLe microkeratome for DSAEK and UT-DSAEK preparation using a metrics based approach (nomogram).

Methods: Modify existing SOP and apply EK process principles of corneal thickness measurement and head size for desired outcome. Using the same technician (experience N=402 cuts in 2017) for each cut. Develop nomograms with modifications after the first 8 cuts. Review factors that introduce outcome variation. Identify possible errors.

Results: With a set goal of 80-120 µm the technician performed 8 cuts which resulted in a range of cuts from 17-92 µm or an average of 64 µm. The nomogram was modified and an additional 10 cuts were performed which resulted in a range of cuts from 38-126 µm or an average of 81 µm.

Conclusions: Initial data with Gebauer SLe microkeratome. System is capable of DSAEK or UT-DSAEK graft preparation. After as few as 18 cuts the development of a nomogram resulted in an acceptable range for graft preparation. Further efforts are needed to refine the technique required to limit variations from expected results.

QUANTITATIVE ASSESSMENT OF HUMAN CORNEAL ENDOTHELIUM WITH GABOR-DOMAIN OPTICAL COHERENCE MICROSCOPY

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Purpose: Corneal endothelial cell (EC) failure is nowadays the leading cause of corneal transplantation in the United States. Eye banks around the world have the difficult task to select appropriate tissues for corneal transplantation. This task requires an advanced qualification of the tissues in order to reduce the risk of transplantation failure. Current standard of care for EC evaluation is specular microscopy. Yet this technique needs the mechanical translation to assess all corneal layers from the epithelial layer to the endothelial layer, and it has been in large part limited by a small field of view that introduces variability in the estimation of endothelial cell density (ECD). The purpose of this pilot study is to investigate the use of a high definition imaging system throughout the entire corneal layers—Gabor domain optical coherence microscopy (GDOCM)—for corneal EC evaluation to the clinical level.

Methods: Donor corneas intended for research were obtained from Lions Eye Bank at Rochester. Each cornea was mounted onto an artificial anterior chamber placed on the deck of a tip, tilt & rotation stage. GD-OCM images were taken at five different locations, one central and four peripheral (nasal, temporal, superior, and inferior). The cornea was at a five-degree tilt for imaging peripheral locations. A total of six images were taken at every location (thirty images per cornea in total). The images, which are affected by corneal curvature, were flattened using custom MATLAB® code. The en face images of the flattened corneal endothelium were analyzed by three blinded examiners, using the custom corner method in MATLAB® and the cell counting plugin in ImageJ. The total counted cell number (ECN), the cell area (ECA), and the endothelial cell density (ECD) were quantified in a circle of 250 micron radius.

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**RESULTS:** The imaging field of view was 1 mm x 1 mm, and the maximum area of analysis was 0.385 mm² (a circle of 350 micron radius). For the following analysis, the endothelial cells were evaluated in the area of 0.196 mm² (a circle of 250 micron radius). (C: center, N: nasal, T: temporal, S: superior, and I: inferior)

For cornea 1 (age: 66), ECD [cells] was 257 ± 33 (C), 236 ± 28 (N), 209 ± 32 (T), 272 ± 50 (S), and 216 ± 36 (I). The mean ± SD of ECD at five zones were 238 ± 42. ECA [x 10-4 mm²] was 3.723 ± 0.065 (C), 3.632 ± 0.035 (N), 4.071 ± 0.063 (T), 3.919 ± 0.063 (S), and 3.543 ± 0.054 (I). The mean ± SD of ECA at five zones were 3.778 ± 0.203.

ECD [cells/mm²] was 2687 ± 46 (C), 2753 ± 26 (N), 2457 ± 38 (T), 2253 ± 41 (S), and 2823 ± 43 (I). The mean ± SD of ECD at five zones were 2654 ± 140.

For cornea 2 (age: 60), ECD [cells] was 338 ± 62 (C), 310 ± 32 (N), 392 ± 31 (T), 366 ± 42 (S), and 415 ± 36 (I). The mean ± SD of ECD at five zones were 362 ± 55. ECA [x 10-4 mm²] was 3.273 ± 043 (C), 3.169 ± 0.033 (N), 3.212 ± 0.039 (T), 3.259 ± 0.043 (S), and 3.146 ± 0.037 (I). The mean ± SD of ECA at five zones were 3.212 ± 0.062.

ECD [cells/mm²] was 3056 ± 40 (C), 3156 ± 33 (N), 3114 ± 38 (T), 3068 ± 41 (S), and 3179 ± 37 (I). The mean ± SD of ECD at five zones were 3115 ± 60.

**CONCLUSIONS:** In this pilot study, we demonstrate a pathway for GDOCM to assess corneal endothelial layers. Results of two donor corneas show that (1) the SD of ECD is approximately 1.5% of the mean of ECD, which points to repeatability of the technique and (2) the ECD value varied with the location of the assessment, which points to the need to assess ECD at multiple locations.

**EFFECT OF TRANSFER OF DONOR CORNEAL TISSUE FROM ONE STORAGE MEDIA TO ANOTHER ON CORNEAL ENDOTHELium**

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**Purpose:** The purpose of this study is to evaluate the effect of transfer of donor corneal tissue from McCarey–Kaufmann (MK) medium to Optisol-GS on corneal endothelium.

**Methods:** This was a prospective, randomized comparative study. Twenty paired human donor corneal tissues of optical quality were retrieved. One tissue of the pair was preserved in Optisol-GS preservative medium (Group A) and the other tissue of the pair in MK medium (Group B) at the time of corneoscleral disc excision. Within 12 hours of retrieval, each cornea was evaluated using slit-lamp biomicroscopic examination and specular microscopic analysis. Group B corneas were transferred to Optisol-GS medium within 48–53 hours of retrieval. Specular analysis of the paired corneas was repeated 3 hours after transferring to Optisol-GS. On day 7 of storage, specular analysis of both the tissues was repeated.

**Results:** The average age of the donor at the time of death was 29 years (16–68 years). The reduction in endothelial cell count, from baseline, in Groups A and B was 5.5% and 5.8% (P = 0.938) on the 3rd day and 8.2% and 12.6% (P=0.025) on the 7th day, respectively, postretrieval. The coefficient of variation (CV) increased by 36% (P=0.021) and hexagonality reduced by 19% (P=0.007) on day 7. All tissues retained an endothelial cell density higher than the accepted critical level for penetrating keratoplasty.

**Conclusions:** Significant endothelial cell loss was noted while transferring tissues from one medium to another, necessitating the need for reevaluation of transferred tissues before utilization.

**IMPACT OF INFLATION PRESSURE ON ENDOTHELIAL IN PRE-DESCEMET’S ENDOTHELIAL KERATOPLASTY (PDEK) PREPARATION**

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**Purpose:** To evaluate the impact of inflation pressure on endothelial quality after PDEK prep.

**Methods:** Donor corneas were randomized to inflation for PDEK using air vs. Optisol GS. PDEK prep was performed under continuous pressure monitoring using an in-line pressure gauge (DPI 705 series). Bubble type (1 vs. 2), peak inflation pressure (PIP), and mean expansion pressure (MEP) during bubble formation, were documented. Trypan blue and Fiji-imageJ software were used to grade the PDEK tissue as acceptable (<25% global cell loss) or unacceptable. A real-time live-dead assay was performed during inflation on additional corneas to evaluate mechanism of cell death.

**Results:** In total, 25 corneas were tested. 56.0% of cases resulted in a type 1 bubble and 32.0% of cases resulted in a type 2 bubble. 29% of type 1 bubbles had acceptable endothelial cell quality compared to 88% of type 2 bubbles (p=0.012). Mean PIP, but not MEP, was statistically higher for type 1 (1030.5 mm Hg) vs. type 2 (593.1 mm Hg) (p=0.012) bubbles. There was no difference in mean PIP or MEP between use of air (706.0 mm Hg) vs. type 2 (510.7 mm Hg) and use of Optisol (852.9 mm Hg, 653.0 mm Hg) (p=0.012) bubbles. There was no difference in mean PIP or MEP between use of air (706.0 mm Hg) vs. type 2 (510.7 mm Hg) and use of Optisol (852.9 mm Hg, 653.0 mm Hg). Increasing PIP and MEP was associated with increased post-processing endothelial cell loss. On real-time live-dead assay, increased inflation pressure was associated with stromal swelling and cell loss prior to bubble formation.

**Conclusions:** Significant endothelial cell loss can occur during PDEK bubbling. Type 1 bubbles are associated with higher inflation pressures and more endothelial cell loss compared to type 2 bubbles. Increasing PIP during bubble formation is associated with higher inflation pressures and more endothelial cell loss compared to type 2 bubbles.