Scientific Abstracts

** Medical Student ** Resident*

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COVID-19 PANDEMIC AND EYE BANKING: IMPACT OF THE SECOND WAVE

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Purpose: To examine the impact of the second wave of the COVID-19 pandemic on eye banking.

Methods: Retrospective review of Eversight eye bank eligible donors from July through December 2020. Referrals and donors ruled out due to Eye Bank Association of America (EBAA) COVID-19 eligibility criteria were counted. Results of routine post-mortem COVID testing were examined.

Results: EBAA COVID guidelines resulted in an average of 3.01% of eye bank referrals to be ruled out for potential donation between July and December 2020. There was a shortage of 9.38% donor corneal grafts when comparing available tissue to number of scheduled surgeries. The eye bank performed 1,602 post-mortem COVID tests over this six-month period of which 39 tests (2.7%) were positive or indeterminate. There was a strong correlation between COVID-19 positive cases in states compared to Eversight post-mortem positivity (r = 0.88).

Conclusion: During the second wave of the pandemic, when corneal transplant procedures were back to near-normal levels, there was a shortage of corneal tissue. Routine post-mortem testing captures asymptomatic donors where corneal grafts would have been used for transplantation

**Medical Student — Graduated May 20*

A NOVEL VIRTUAL WET LAB — USING A SMARTPHONE CAMERA ADAPTER AND A VIDEO CONFERENCE PLATFORM TO PROVIDE REAL-TIME SURGICAL INSTRUCTION

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Purpose: Proctored surgical instruction has traditionally been taught through in-person interactions in either the operating room or an improvised wet lab. Eye banks have utilized wet labs to train current surgeons and the next generation of ophthalmologists. Due to the COVID-19 pandemic, live in-person instruction was not feasible due to social distancing protocols, so a virtual wet lab (VWL) was proposed and implemented. Our purpose here is to describe our experience with a VWL as a Descemet membrane endothelial keratoplasty (DMEK) skills transfer course. This is the first time that a VWL environment has been described for the instruction of ophthalmic surgery.

Methods: Fourteen participant surgeons took part in VWLs designed for DMEK skills-transfer in September and October 2020. A smartphone camera adapter and a video conference software platform were the unique mediums for the VWL. Following a 60-minute didactic session, participants were divided into break-out rooms for the next 45-minutes where their surgical scope view was broadcasted live, allowing instructors to virtually proctor their participants in real-time. Participants were surveyed to assess their satisfaction with the course.

Results: 100% of participants successfully injected and unfolded their DMEK grafts. Nine of 14 participants completed the survey. Respondents rated the experience highly favorably.

Conclusion: With the use of readily available technology, VWLs can be successfully implemented in lieu of in-person skills-transfer courses and eye banks can continue with their long-standing tradition of training and education. Further development catering to the needs of the participant might allow VWLs to serve as a viable option of surgical education currently limited by geographical and social-distancing boundaries.

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BOWMAN LAYER ONLAY GRAFTING: PROOF-OF-CONCEPT OF A NEW TECHNIQUE TO FLATTEN CORNEAL CURVATURE AND REDUCE PROGRESSION IN KERATOCONUS

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Purpose: To describe a new surgical technique for flattening the corneal curvature and to reduce progression in eyes with advanced progressive keratoconus by using Bowman layer (BL) onlay grafting, and to report on the preliminary outcomes of this procedure.

Methods: Five patients with advanced progressive keratoconus, underwent BL onlay grafting. After removal of the epithelium, a BL graft was placed and "stretched" onto the stroma, and a bandage lens was placed to cover the BL graft. Best spectacle- and/or best contact lens-corrected visual acuity (BSCVA/BCLVA), refraction, biomicroscopy, corneal tomography, anterior segment optical coherence tomography and complications were recorded at 1 week, and 1, 3, 6, 9, and 12-15 months postoperatively.

Results: All five surgeries could be performed successfully. Average Kmax went from 75D preoperatively to 70D at one year postoperatively. All eyes showed a completely re-epithelialized and a well-integrated graft. BSCVA improved at least 2 Snellen lines in 3/5 cases and BCLVA remained stable, improving by 3 Snellen lines in case 1 at 15 months postoperatively. Satisfaction was high and all eyes again had full contact lens tolerance.

Conclusion: BL onlay grafting may be a feasible surgical technique, providing up to -5D of corneal flattening in eyes with advanced keratoconus

EYE BANK PREPARED NANOTHIN DSAEK TISSUE: 3-YEAR PROCESSING EXPERIENCE

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Purpose: (1) To evaluate surgeon preference for nanothin (≤50 µm), near nanothin (50-70 µm), ultrathin (≤100 µm), and standard DSAEK (≤250 µm). (2) To evaluate rates of tissue loss, on-target cuts, and successful tissue placement for eye bank prepared DSAEK of various thicknesses.

Methods: Retrospective review of surgeon preferences, surgeon tissue requests, and tissue processing records from 2017 to 2019.

Results: In total, there were 1,303 requests for DSAEK tissue over the study period. Among surgeons reviewed, 2.1% preferred exclusively nanothin DSAEK, 14.9% preferred near nanothin DSAEK, 25.5% preferred ultrathin DSAEK, and 57.4% preferred standard DSAEK. Over the study period, 9.2% of tissue requests were for nanothin DSAEK, 7.0% were for near nanothin, 27.7% were for ultrathin, and 56.1% were for standard DSAEK. Overall, there was a 4.4%, 3.4%, 0.3%, and 0.8% tissue loss rate per attempted cut for nanothin, near nanothin, ultrathin, and standard DSAEK, respectively. Excluding tissues lost, there was a 68%, 52%, 71%, and 92% success rate for on-target cuts in the preferred thickness range for nanothin, near nanothin, ultrathin, and standard thickness DSAEK, respectively. For off-target cuts outside of the preferred thickness range, 79% of tissues were still accepted by the requesting surgeon, 5% of tissues were redistributed to a different surgeon, and 16% of tissues could not be placed elsewhere.

Conclusion: DSAEK can be reliably cut down to nanothin range (≤50 µm) with minimal tissue loss. Rates of achieving on-target cuts in the preferred thickness range decreased as the range narrowed. However, most off-target cuts were still accepted by the primary requesting surgeon.

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SAFETY OF EYE BANK PREPARED PRE-STRIPPED, PRE-STAINED, PRE-LOADED DESCEMET'S MEMBRANE ENDOTHELIAL KERATOPLASTY (P3DMEK) TISSUE

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Purpose: Despite better visual outcomes, faster healing, and reduced risk of rejection, some surgeons have been hesitant to adopt Descemet membrane endothelial keratoplasty (DMEK) due to a steeper learning curve and difficult tissue preparation. Use of eye bank pre-stripped, pre-stained, and pre-loaded (p^3) DMEK tissue can reduce the learning curve and risk of complications.

Methods: We conducted a prospective study (3/7/2017-6/11/2019) including 105 eyes undergoing DMEK with p^3 tissue. Primary outcomes were primary graft failure, graft detachment, and re-bubbling. Secondary outcomes included baseline and post-operative visual acuity at week 1 and months 1, 3, 6, and 12. Baseline and post-operative pachymetry and specular microscopy endothelial counts were also collected.

Results: 88 and 67 eyes achieved the 6-month and 1 year follow-up, respectively. No primary graft failures occurred. A total of 33 eyes had at least a partial graft detachment. 21% of eyes required re-bubbling. Mean difference between pre-operative and 12-month post-operative graft endothelial cell counts and pachymetry was -655 cells/mm2 (95% CI -505 –-805), a 24% decrease, and -122 µm (95% CI -103–-143), respectively. Mean case time for p^3DMEK with phaco or p^3DMEK alone was 34 (95% CI 33 –36) and 24 minutes (95% CI 23 –27), respectively. 46% of eyes were 20/20 or better at 3 months. In comparison, mean case time for eyes undergoing DMEK (N=21) with phaco or DMEK alone was 46 (95% CI 43 –49) and 36 minutes (95% CI 29 –43), respectively ($p<0.05$).

Conclusion: P^3DMEK tissue is safe and can provide excellent clinical outcomes that are comparable to surgeon prepared and loaded DMEK tissue. Eyes undergoing p^3DMEK benefited from decreased intraoperative times, risk of endothelial cell loss and need for re-bubbling.

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COMPARISON OF DONOR CORNEA ENDOTHELIAL CELL DENSITY DETERMINED BY EYE BANKS AND BY A CENTRAL IMAGE ANALYSIS READING CENTER

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Purpose: To evaluate agreement between eye banks (EBs) and an image analysis reading center on endothelial cell density (ECD) determinations using same image analysis method.

Methods: The Cornea Image Analysis Reading Center (CIARC) determined ECD with a single experienced analyst on EB-obtained-preoperative central endothelial images from two eye banks, Eversight and Lions VisionGift, employing the Konan center analysis method. The EBs performed ECD determination on their respective sets of images employing the same analysis method with experienced eye bank technicians.

Results: The mean age of the 196 donors was 54 years (range 30 to 75 years). Seventy (36%) of the 196 patients were women, and 54 (28%) were diabetic. The mean preoperative ECD was 10 cells/mm² greater by the EBs than by CIARC (N = 196, p=0.90) with 95% limits of agreement of [-305, 324 cells/mm²]. Agreement was not substantially changed when the difference between the EB and CIARC ECD was adjusted for gender, donor age, donor diabetes, and number of cells analyzed (mean 23 cells/mm², p=0.73). The EBs-determined preoperative ECD was within 5% of the CIARC-determined ECD for 140 (71%) image sets, with 31 (16%) higher by >5% and 25 (13%) lower by >5%.

Conclusion: Well trained eye bank technicians achieve comparable results for ECD determination with an experienced image analyst from a reading center when the same image analysis method is employed.

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SMOKING ASSOCIATED WITH REPEAT KERATOPLASTY AFTER ENDOTHELIAL KERATOPLASTY

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Purpose: To determine risk factors associated with repeat keratoplasty after endothelial keratoplasty (EK).

Methods: Retrospective analysis of IRIS® Registry data on EK procedures performed between 2013-2018. Kaplan Meier survival analysis was used to determine probability of subsequent keratoplasty. A multivariable general estimating equation model adjusting for demographics, indication, ocular comorbidities and complications was used to assess risk factors for repeat keratoplasty.

Results: A total of 29,412 eyes were included in the analysis; 3,646 eyes underwent repeat keratoplasty. The probability of repeat keratoplasty was 91.3% (95% CI: 91.0 – 91.6%), 88.9% (88.5 - 89.3%), and 82.6% (82.0 – 83.3%) at 1, 2, and 5 years, respectively. Factors associated with repeat keratoplasty include Black versus white race (OR 1.24, 95% CI: [1.10-1.39]), active smoking versus non-smokers (OR 1.14, [1.04-1.26]), Medicaid insurance versus private (OR 1.46, [1.12-1.90]), indication of prior graft failure (OR 1.61, [1.45-1.78]) or bullous keratopathy (OR 1.60, [1.46-1.74]) versus Fuchs, history of glaucoma (OR 1.22, 95% CI:1.12-1.33) or glaucoma surgery (OR 1.21, [1.10-1.34]) and subsequent procedures including re-bubble (OR 2.24, [2.05-2.45]), cataract (OR 1.60, [1.44-1.78]), other anterior segment (OR 2.44, [2.24-2.66]), or glaucoma (OR 1.52, [1.38-1.68]) surgery.

Conclusion: This large national study identified smoking as an independent factor associated with decreased graft survival in addition to Black race, lower socioeconomic status, surgical indication other than Fuchs, and post-operative procedures. Surgeons should advise graft recipients about this association to encourage smoking cessation.

THE IMPACT OF AMPHOTERICIN B FORTIFIED PRESERVATION MEDIA ON DONOR RIM CULTURES AND POST-TRANSPLANT INFECTION

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Purpose: Despite eye bank infection prevention measures, donor-to-host transmission of organisms is a known cause of post-transplant infection. The impact of antifungal-containing corneal preservation media on rates of positive donor rim fungal cultures and incidence of post-transplant fungal infection is unknown. The study aims were to determine whether the addition of Amphotericin B to corneal preservation media reduces the rate of positive donor rim cultures or the incidence of post-transplant infection.

Method: Retrospective chart review at a single institution.

Results: A total of 1273 corneal transplantations performed between 2016 and 2021 were analyzed. 853 transplants were stored in preservation media without Amphotericin B, and 393 in media with Amphotericin B. When compared to transplants stored in media without Amphotericin B, the transplants stored in media with Amphotericin B had a lower rate of both positive donor rim fungal cultures (1.7% vs 2.9%, p=0.23) and bacterial cultures (1.2% vs 2.8%, p=0.09); however, these differences did not achieve statistical significance. There was 1 infectious complication in the group stored in media with Amphotericin B, and 3 infectious complications in the group without Amphotericin B.

Conclusion: The addition of Amphotericin B to graft storage media was associated with a decrease in incidence of positive donor rim fungal and bacterial cultures, which was not statistically significant. Given the rarity of post-keratoplasty fungal infections, the impact of preservation media containing Amphotericin B on rates of post transplant fungal infections remains unknown.

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INVESTIGATION OF OXIDATIVE STRESS AND INFLAMMATORY RESPONSE IN EX VIVO CORNEAL ENDOTHELIAL CELLS DURING CELL CULTURE AND EXPANSION

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Purpose: Ex vivo expansion of corneal endothelial cells (CEnC) has the potential to alleviate the global shortage of donor corneal tissue that limits access to corneal transplantation. However, senescence and cell-state transition are current obstacles to the successful expansion of ex vivo CEnC (evCEnC). To determine whether oxidative stress and inflammation plays a role in the expansion capacity of evCEnC, we performed RNA-sequencing analyses of expanded evCEnC at each passage. We also cultured evCEnC with and without SkQ1, an antioxidant and anti-inflammatory compound, and measured intracellular free radical (IFR) levels.

Methods: RNA was isolated from evCEnC at each cell passage and RNA-sequencing analyses were performed to identify differentially expressed genes and pathways in evCEnC during cell expansion by comparing their transcriptomic profile at passage 0 to their expression profiles at subsequent passages. HCEnC-21T, a CEnC line, was cultured in media supplemented with varying concentrations of SkQ1 and cell viability was assessed by MTT assays to determine the optimal SkQ1 dose range in CEnC. To assess the effect of SkQ1 treatment on protecting CEnC from acute oxidative stress, cells were treated with SkQ1 over 3-5 days and then subjected to varying doses of tBH, an agent inducing acute oxidative stress; cell viability was assessed by MTT assay 2-3 hours after tBH treatment. To determine the impact of SkQ1 treatment on basal IFR levels, evCEnC were isolated from donor corneas and split into two cultures (SKQ1-treated and untreated), which were each grown to confluence. DCFH-DA assay was performed to measure differences in IFR levels at each passage.

Results: RNA-sequencing analyses identified the NRF2-mediated oxidative stress response pathway to demonstrate the highest activation score (z-score 4.596) among the identified canonical pathways differentially expressed in evCEnC at passage 4, compared to passage 0. Canonical pathways associated with oxidative stress and inflammatory response were also identified to be among the top activated differentially expressed pathways at passages 3 and 4, compared to passage 0. When compared to untreated cells, HCEnC-21T treated with 250nM or less, exhibited no significant loss of cell viability. HCEnC-21T induced with acute oxidative stress (100 µM tBH) demonstrated increased cell viability protection in a SkQ1 dose-dependent manner with 0nM, 50nM, 250nM, and 500nM SkQ1 treatment leading to 14.8%, 18.2%, 36.3%, and 41.2% cell viability, respectively. evCEnC treated with 50nM and 250nM SkQ1 demonstrated 26%-32% decreased levels of IFR concentrations, compared to tBH-untreated evCEnC by passage 2.

Conclusion: The potential clinical utility of ex vivo expanded CEnC is limited by cell senescence and cell state transition, which are due in part to oxidative stress and inflammation. SkQ1, a commercially-available antioxidant and anti-inflammatory compound, decreases intracellular IFR levels and protects against oxidative stress at concentrations that do not negatively impact corneal endothelial cell viability.

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