

Scientific Abstracts

Page	Page	Page
<p>2 CHANGES IN PANEL-REACTIVE ANTIBODY FOLLOWING PENETRATING KERATOPLASTY K. Boden, S. Wahl, A. Rickmann, K. Boden, P. Szurman, K. Januschowski</p> <p>EFFECT OF A SECOND BETADINE EXPOSURE ON CORNEAL TOXICITY TO DECREASE DONOR TISSUE FUNGAL BIOBURDEN Onkar B. Sawant, PhD, Eversight Co-Authors: Kayla Jones, CEBT; Michael S. Titus, CEBT; Shen Xiang, PhD; Ali R. Djalilian, MD</p> <p>3 EFFICACY OF AMPHOTERICIN B IN CORNEAL PRESERVATION MEDIA AFTER EXTENDED FROZEN STORAGE Doowon Huh, PhD,* Weill Cornell Medical College Co-Authors: Khoa D. Tran, PhD; Megan M.W. Straiko, PhD; Christopher S. Sales, MD, MPH</p> <p>PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF CORNEAL ENDOTHELIAL CELLS DURING IN VITRO EXPANSION Anthony Aldave, MD, Stein Eye Institute Co-Authors: Ricardo F. Frausto; Gary S.L. Peh; Jodhbir S. Mehta</p> <p>4 DIRECT IN SITU CELL VIABILITY ASSESSMENT OF DMEK GRAFT IN A MODIFIED JONE'S TUBE Sung Lee, BS, Lions Gift of Sight Co-Authors: Peter Bedard, MS; Aaron Bae; Ching Yuan, PhD, CEBT; Joshua Hou, MD</p> <p>EVALUATION OF A NOVEL, HIGHLY EFFICIENT DMEK PREPARATION DEVICE IN NON-DIABETIC AND DIABETIC CORNEAS Katie Solley, MSE Johns Hopkins University Co-Authors: Kendall Frank, CEBT; Darrell Lee, CEBT; Juan Guerrero, CEBT; Shannon Schweitzer, CEBT; Sudeep Pramanik, MD; Kunal S. Parikh, PhD</p>	<p>5 DESCOMET'S MEMBRANE VS. AMNIOTIC MEMBRANE FOR CORNEAL EPITHELIUM REGENERATION Peter Bedard, MS, Lions Gift of Sight Co-Authors: Ching Yuan, PhD; Sung Lee, BS; Heidi Röhrich, Dipl.-Biol.; Joshua Hou, MD</p> <p>CLINICAL OUTCOMES OF DESCOMET MEMBRANE ENDOTHELIAL KERATOPLASTY PERFORMED IN EYES WITH COMORBID KERATOCONUS AND CORNEAL ENDOTHELIAL DYSFUNCTION Philip W. Dockery, MPH,* Parker Cornea Co-Authors: Jack S. Parker, MD, PhD; Renuka S. Birbal, MD; C. Maya Tong, MD; John S. Parker, MD; Katelyn P. Joubert, BS; Gerrit R. J. Melles, MD, PhD</p> <p>6 SAFETY AND EFFICACY OF USING PAIRED RADIAL INCISIONS TO FACILITATE UNSCROLLING IN DMEK Joshua Hou, MD, University of Minnesota/Lions Gift of Sight Co-Authors: Jeff J. Justin, BS, and Mark S. Hansen, MD</p> <p>LOWER CORNEAL HAZE AND ABERRATIONS IN DMEK VERSUS DSAEK IN FELLOW EYES FOR FUCHS' ENDOTHELIAL CORNEAL DYSTROPHY William H. Waldrop, MD, UT—Southwestern Medical Center Co-Authors: Matthew Gillings; Danielle Robertson, PhD, OD; Matthew Petroll, PhD; Vinod Mootha, MD</p>	<p>7 UNBIASED, AUTOMATED ECD ANALYSIS USING GABOR-DOMAIN OPTICAL COHERENCE MICROSCOPY AND MACHINE LEARNING Cristina Canavesi, PhD, MBA, LightTopTech Corp. Co-Authors: Andrea Cogliati, PhD, Holly B. Hindman, MD, MPH</p> <p>MID TO LONG TERM INTERNATIONAL OUTCOMES OF THE BOSTON TYPE I KERATOPROSTHESIS IN STEVENS-JOHNSON SYNDROME Angela Chen,* Stein Eye Institute, David Geffen School of Medicine Co-Authors: Clemence Bonnet, MD; Reza Ghaffari, MD; Anthony Aldave, MD</p> <p>8 ANALYSIS OF REGULATIONS PROHIBITING CORNEAL DONATION BY MEN WHO HAVE SEX WITH MEN Michael A. Puente, MD, University of Colorado School of Medicine Co-Authors: Jennifer L. Patnaik, PhD; Anne M. Lynch, MD, MSPH, Blake M. Snyder, MD, Chad Caplan, MD, Binhan Pham, BS, Helio Neves da Silva, BS, Conan Chen, BS, Michael J. Taravella, MD, Alan G. Palestine, MD</p> <p>VISION-RELATED QUALITY OF LIFE AMONG CORNEAL TRANSPLANT RECIPIENTS Grace E. Dunbar, MD,** University of Michigan Kellogg Eye Center Co-Authors: Autumn N. Valicevic, MS; Tomás E. Meijome, MD, MS; Michael Titus; Joshua D. Stein, MD, MS; Maria A. Woodward MD, MSc; Shahzad Mian, MD</p>

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CHANGES IN PANEL-REACTIVE ANTIBODY FOLLOWING PENETRATING KERATOPLASTY

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Purpose: Panel-reactive antibody (PRA), a measure of sensitization, is used in solid organ transplantation to stratify treatment and outcomes for transplant candidates, yet its use in ophthalmology is uncommon. The purpose of this study was to determine if multiple corneal transplants were associated with an increased PRA level.

Methods: A retrospective chart review was performed at CVP to identify patients that had undergone a penetrating keratoplasty (PK or other full thickness keratoplasty i.e. keratoprosthesis). Exclusion criteria included a history of solid organ transplantation and multiple prior blood transfusions. Patients were divided into those that have had a single PK without history of rejection and those that have had multiple PK grafts. Consent was obtained to have the patient submit a blood sample for PRA testing. The retrospective chart review was also used to identify additional patients that had previously had PRA testing and undergone a penetrating keratoplasty.

Results: A total of 31 eyes from 31 patients met the inclusion criteria. There were 21 eyes with multiple PKs and 10 eyes that received a single PK. From the multiple PK group, 48% (10/21) demonstrated an elevated PRA level compared to 20% (2/10) from the single PK group ($p = 0.021$). The relative risk for elevated PRA was 2.4 ($p = 0.19$).

Conclusions: An increased PRA level may be associated with a history of multiple PKs. Certain patients may have developed an increased PRA from PK failure and rejection; however, there are likely other factors that determine whether or not a patient's PRA changes in response to PK.

EFFECT OF A SECOND BETADINE EXPOSURE ON CORNEAL TOXICITY TO DECREASE DONOR TISSUE FUNGAL BIOBURDEN

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Purpose: The purpose of this study is to assess subsequent corneal toxicity of a second Betadine/povidone-iodine (PVP-I) application at the time of donor tissue recovery. Recent reports suggest that a second 5-minute application of PVP-I at the time of tissue recovery significantly reduces fungal bioburden. Toxicity of the epithelium is of particular concern as damaged epithelium leads to increased corneal edema. We were also interested in analyzing incidences of positive rim culture and post-operative infections before and after implementing double soak procedure

Methods: The left eye of each donor was prepared using one 5-minute application of PVP-I followed by a rinse with sterile solution, while the right eye received one 5-minute application of PVP-I, a rinse with sterile solution, conjunctival removal, and a second 5-minute application of PVP-I followed by a final rinse. Death to preservation time for all donors was under 15 hours and tissues were processed for staining the morning following recovery. The corneas were stained with Calcein AM and Propidium Iodide (PI) for detection of live and dead cells, respectively. After staining, corneas were cut into halves. One half was placed on the slide for flat mount imaging, the other half was embedded in OCT compound for cryosectioning. Corneal epithelial cell imaging was performed using either Zeiss HAL100 or Leica DMI8 wide-field fluorescence microscope. Imaging was performed at the central, limbal and scleral regions. Cell counting for live and dead cells was performed using corneal flat mounts at 10x.

Results: The mean percentage cell death rate for the left ($34 \pm 5\%$) and right ($37 \pm 7\%$) eyes did not differ significantly. Total number of live (L: 833 ± 146 cells/mm², R: 774 ± 120 cells/mm²) and dead (L: 494 ± 148 cells/mm², R: 551 ± 204 cells/mm²) cells were unaltered between left and right eyes. Preliminary analysis did not reveal any significant alterations in the corneal thickness indicating healthy corneal endothelial function after additional PVP-I exposure. Increasing povidone-iodine exposure appears to decrease incidences of corneal rim contamination. Since implementing, double PVP-I soak procedure in November 2019, we have not observed any incidences of post-operative infections.

Conclusion: These results indicate that new donor preparation methods with an additional 5-minutes Betadine exposure does not affect tissue quality and could lower the incidences of post-operative infection.

EFFICACY OF AMPHOTERICIN B IN CORNEAL PRESERVATION MEDIA AFTER EXTENDED FROZEN STORAGE

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Purpose: To investigate the antimycotic activity of amphotericin B that has been previously frozen for 28 days prior to supplementation of Optisol-GS.

Methods: Triplicate Optisol-GS samples were inoculated with 106 colony-forming units (CFU) of *C. albicans*. Each set of triplicate cultures was supplemented with 2.5 µg/mL of amphotericin B that was either freshly resuspended and never frozen, frozen overnight at -20 °C and thawed, or frozen at -20 °C for four weeks and thawed. The cultures were stored at 4 °C with aliquots taken at 0, 6, 24, and 72 hours for quantification. The efficacy of each preparation of amphotericin B in reducing *C. albicans* growth was assessed at these time points.

Results: Six hours after antifungal supplementation, there was a 1.33 log₁₀ CFU reduction with freshly resuspended amphotericin B, compared to a 1.31 log₁₀ reduction with amphotericin B that was frozen overnight (p=0.20), and a 1.18 log₁₀ reduction with amphotericin B that was frozen for 4 weeks (p=0.05). After 72 hours, there was a 2.72 log₁₀ CFU reduction with freshly resuspended amphotericin B, a 2.64 log₁₀ CFU reduction with amphotericin B that was frozen overnight (p=0.45), and a 2.18 log₁₀ CFU reduction with amphotericin B that was frozen for 4 weeks (p=0.05).

Conclusions: Previously frozen amphotericin B remains highly effective against *C. albicans*. Optisol-GS supplemented with 2.5 µg/mL amphotericin B that was frozen for four weeks at -20 °C results in >90% CFU reduction by 6 hours and >99% reduction by 72 hours.

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PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF CORNEAL ENDOTHELIAL CELLS DURING IN VITRO EXPANSION

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Purpose: To determine the impact of in vitro expansion of corneal endothelial cells (CEnC) on gene expression through transcriptomics analysis and on CEnC morphology and function.

Methods: Seven independent primary CEnC cultures were established using two previously described protocols. RNA was isolated at each passage, and RNA-sequencing was performed, followed by differential expression, gene ontology and pathway analyses. Morphometric analysis was performed at each CEnC culture passage, while cell barrier and transporter assays were performed at passage 0.

Results: Analysis of the expression of genes reported to be markers for CEnC identity and/or to determine the quality of CEnC in culture revealed that many were neither specific nor highly expressed in CEnC, or did not correlate well with other quality metrics. Based on transcript abundance, SLC4A11 and CD44 may represent the optimal markers for selection of cultured CEnC. Passage 3 and 4 showed the greatest number of differentially expressed genes, with enrichment of cell senescence-associated gene ontology and pathway terms. Robust cell barrier (determined by measuring resistance of CEnC to an electrical current) and pump functions (determined by measuring the function of several membrane bound transporters (SLC4A4, SLC4A11 and SLC16A1)) were observed.

Conclusions: Early passage in vitro expanded CEnC have similar morphology, gene expression profile, and activity as in vivo corneal endothelial cells. The progression to senescence in vitro remains a significant barrier to the expansion of cultured CEnC and maintaining the CEnC morphologic and functional phenotype at higher passage numbers will require targeting of pathways associated with CEnC senescence. Doing so may permit sufficient expansion of CEnC to serve as a viable approach to the management of CEnC decompensation and challenge the “one donor-one recipient” paradigm.

DIRECT IN SITU CELL VIABILITY ASSESSMENT OF DMEK GRAFT IN A MODIFIED JONE'S TUBE

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Co-Authors: Peter Bedard, MS; Aaron Bae; Ching Yuan, PhD, CEBT; Joshua Hou, MD

Purpose: To identify a reliable method for assessing endothelial cell viability of DMEK tissue stored in a modified Jones tube without physical manipulation of the graft.

Methods: In-situ viability of endothelial cells was determined using a cell-permeable fluorescence dye, alamarBlue (Resazurin), prepared in cell culture medium (DMEM). The DMEK graft was loaded into the Jones tube and stored in Life4°C. A two-peristaltic pump system was used to perfuse the graft and replace Life 4°C with alamarBlue/DMEM followed by an hour incubation at 37°C. alamarBlue/DMEM was collected under a dissection microscope to visually confirm thorough perfusion. Endothelial cell viability was measured on the day of the peel/loading and after two days of storage in the tube. Fluorescence intensity was determined using a Synergy HTX Plate Reader.

Results: Variation of the fluorescent readout was within 5% between samplings of the same graft. Storage of DMEK grafts in Jones tube for two days led to a ~25% decrease of fluorescence signals. DMEK grafts stored in a capped tube displayed substantial difference in fluorescence signals (up to 43%) compared to uncapped tube.

Conclusions: alamarBlue can be used as an effective cell viability assay for preloaded DMEK grafts. Due to the sensitivity of fluorescence measurement and no evident toxicity to endothelial cells, alamarBlue could complement other validation methods as a tissue evaluation standard.

EVALUATION OF A NOVEL, HIGHLY EFFICIENT DMEK PREPARATION DEVICE IN NON-DIABETIC AND DIABETIC CORNEAS

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Purpose: To evaluate the efficiency, efficacy, and viability of grafts prepared by DescePrep, a new method of Descemet's Membrane Endothelial Keratoplasty (DMEK) tissue preparation, in non-diabetic and diabetic donor corneas.

Methods: 15 non-diabetic donor corneas and 5 diabetic corneas were processed using DescePrep. Corneas were stained with Trypan Blue, processed with DescePrep, stained again, and imaged via light microscopy. 10 non-diabetic corneas and 5 diabetic corneas were processed to evaluate yield and efficiency. 5 non-diabetic corneas were evaluated for graft viability via cell counts, optical coherence tomography (OCT) imaging, and slit-lamp analysis. Cell counts were recorded by averaging cell density data from 3 images using the CellChek D+.

Results: 95% (19 of 20) corneas were prepared successfully, with a single failure occurring in a non-diabetic cornea due to neovascularization. The average non-diabetic cornea preparation time was 2 min and 4 sec. Non-diabetic corneas showed complete separation of Descemet's membrane, as confirmed by OCT imaging. There was no significant change in endothelial cell density following preparation ($p = 0.438$). 100% (5 of 5) of diabetic donor corneas were prepared successfully and processed in less than 10 min, each, including a cornea with a diabetic mellitus scale rating of 5.

Conclusions: DescePrep is a new DMEK preparation technique that can process donor corneas at high yields in minutes without significant endothelial cell loss.

DESCEMET'S MEMBRANE VS. AMNIOTIC MEMBRANE FOR CORNEAL EPITHELIUM REGENERATION

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Purpose: To compare cell characterization and proliferation for limbal stem cells cultured on Descemet's membrane (DM) vs. amniotic membrane (AM). To test the suitability of DM as an epithelial substrate in organ culture.

Methods: Cadaveric corneal limbal explant cultures were split and reseeded onto decellularized DM and AM. Cell morphology was compared. The expression of limbal stem cell (LSC) markers was determined by In-cell Western technique. Cell replication rates were quantified by BrdU incorporation. Cadaveric corneas with the epithelium debrided had DM attached by fibrin and were maintained as organ cultures for re-epithelialization. Fluorescein, H&E and immunostaining were performed to characterize cell growth in comparison to mated control without DM.

Results: Cell morphology on DM and AM was consistent with LSCs. The expression of ABCG5, ABCG2 and p63a (LSC markers) was comparable for DM and AM. Cells seeded on DM had higher BrdU incorporation compared to AM. Re-epithelialized layers were observed on DM after organ culture. Organ culture restored barrier function and new cells expressed corneal epithelial, not conjunctival, cell markers.

Conclusions: DM is able to support LSC proliferation, morphology, and stem cell marker expression as well as AM in culture. In addition, DM supports re-epithelialization on organ-cultured corneas. Therefore, it is a viable alternative to AM for epithelial regeneration therapy studies.

CLINICAL OUTCOMES OF DESCEMET MEMBRANE ENDOTHELIAL KERATOPLASTY PERFORMED IN EYES WITH COMORBID KERATOCONUS AND CORNEAL ENDOTHELIAL DYSFUNCTION

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Purpose: To evaluate the clinical outcome of Descemet membrane endothelial keratoplasty (DMEK) performed in eyes with comorbid keratoconus (KC) and corneal endothelial dysfunction.

Methods: Twenty-three consecutive eyes of 14 patients with comorbid KC underwent DMEK for corneal endothelial dysfunction; best spectacle corrected visual acuity (BSCVA), maximum corneal curvature (Kmax), central corneal thickness (CCT), and intra- and postoperative complications were assessed.

Results: Excluding eyes requiring re-transplantation for primary graft failure (n=3), all eyes showed improvement in BSCVA, reaching > 20/40 (0.5) in 85%, > 20/25 (0.8) in 50%, and > 20/20 (1.0) in 25% by one month postoperatively; 88%, 69%, and 38% by 6 months postoperatively; and 83%, 67%, and 42% by 12 months postoperatively. CCT decreased from 594µm preoperatively to 484µm at 1 month (p<0.001) and 489µm at 12 months (p<0.001). Kmax decreased by a median of 1.9 diopters (D) at 1 month (p=0.003) and 2.8 D at 12 months (p=0.012), and every eye with a preoperative Kmax > 46 D demonstrated flattening.

Conclusions: DMEK is technically feasible in eyes with comorbid KC and may give excellent visual and refractive outcomes, including significant corneal flattening, which may potentially create a visually significant hyperopic shift in patients with severely ectatic corneas.

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SAFETY AND EFFICACY OF USING PAIRED RADIAL INCISIONS TO FACILITATE UNSCROLLING IN DMEK

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Co-Authors: Jeff J. Justin, BS, and Mark S. Hansen, MD

Purpose: Adding paired radial incisions to DMEK grafts has been reported to induce a triple scroll conformation that facilitates unscrolling. The purpose of this study was to test the safety and efficacy of adding incisions by assessing associated endothelial cell loss (ECL), risk of intraoperative tearing, impact on scrolling patterns, and impact on surgical unscrolling times.

Methods: A prospective laboratory study was performed using 7.75mm DMEK grafts. For safety testing, ECL was assessed using calceinAM before and after incisions. Risk of intraoperative tearing was evaluated by comparing incision lengths before and after DMEK surgery in the wet lab. For efficacy testing, the impact of incisions on scrolling pattern was evaluated by observing grafts with incisions under submersion. The impact of incisions on unscrolling times was evaluated by comparing unscrolling times in mock DMEK surgery before and after incisions.

Results: Mean ECL was 0.8% for incisions vs. 4.0% for S-stamps ($p=0.02$). There was no difference between mean incision length pre ($503\mu\text{m}$) and post ($528\mu\text{m}$) mock DMEK surgery ($p=0.30$). For grafts age ≤ 65 years, 60% (6/10) achieved a stable triple scroll with incisions. For grafts age >65 years, 0% (0/4) achieved a stable triple scroll. Mean unscrolling time in mock DMEK surgery was shorter with incisions (2.61min) compared to without (5.44min) ($p=0.015$).

Conclusions: Paired radial incisions can be safely added to DMEK grafts with minimal endothelial cell loss and risk of intraoperative tearing. Adding incisions is effective for inducing a favorable triple scroll conformation in some DMEK grafts age ≤ 65 . Adding incisions may also reduce unscrolling times for some surgeons.

LOWER CORNEAL HAZE AND ABERRATIONS IN DMEK VERSUS DSAEK IN FELLOW EYES FOR FUCHS' ENDOTHELIAL CORNEAL DYSTROPHY

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Purpose: To investigate the long term corneal changes in patients with Fuchs' endothelial corneal dystrophy (FECD) contributing to superior postoperative visual outcomes after Descemet membrane endothelial keratoplasty (DMEK) compared to Descemet stripping automated endothelial keratoplasty (DSAEK).

Methods: Using retrospective analysis, we evaluated nine patients with FECD who underwent DSAEK in one eye and DMEK in the fellow eye. Patients were genotyped for the triplet repeat expansion in the TCF4 gene and imaged using optical coherence tomography, Scheimpflug imaging, and in vivo confocal microscopy through focusing (CMTF).

Results: Eight of nine subjects were genotyped and all were found to harbor the triplet repeat expansion. Average time between endothelial keratoplasty and imaging was 76 ± 22 and 37 ± 9 months after DSAEK and DMEK respectively. Mean best spectacle corrected visual acuity (logMAR) was 0.04 ± 0.05 and 0.11 ± 0.03 in the DMEK eyes versus DSAEK eyes ($P=0.02$). Posterior corneal higher order aberrations (HOAs) were less in DMEK eyes compared to fellow DSAEK eyes (0.25 ± 0.06 and 0.66 ± 0.25 respectively, $P < 0.01$). Using CMTF, we found the persistent anterior stromal haze is correlated between right and left eyes ($R = 0.73$, $P < 0.05$) but total stromal backscattering was higher for DSAEK eyes ($P < 0.05$).

Conclusions: DSAEK inherently results in higher total stromal backscattering (haze) compared to DMEK due to the addition of stromal tissue. Lower HOAs of posterior cornea and lower total stromal backscattering (haze) may both contribute to superior visual outcomes after DMEK compared to DSAEK.

UNBIASED, AUTOMATED ECD ANALYSIS USING GABOR-DOMAIN OPTICAL COHERENCE MICROSCOPY AND MACHINE LEARNING

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Purpose: To improve accuracy and reduce bias in endothelial cell density (ECD) quantification by combining Gabor-domain optical coherence microscopy (GDOCM) for three-dimensional (3D) wide field of view (1 mm²) corneal imaging and machine learning for automatic delineation of endothelial cell boundaries.

Methods: Human corneas stored in viewing chambers were imaged over a wide field-of-view with GDOCM without contacting the specimens. Numerical methods were applied to compensate for the natural curvature of the cornea and produce an image of the flattened endothelium. A convolutional neural network (CNN) was trained to automatically delineate the cell boundaries using 180 manually annotated images from six corneas. Ten additional corneas were imaged with GDOCM and compared with specular microscopy (SM) to determine performance of the combined GDOCM and CNN to achieve automated endothelial counts relative to current procedural standards.

Results: Cells could be imaged over a larger area with GDOCM than SM, and more cells could be delineated via automatic cell segmentation than via manual methods. ECD obtained from automatic cell segmentation of GDOCM images yielded a correlation of 0.94 ($p < 0.001$) with the manual segmentation on the same images, and correlation of 0.91 ($p < 0.001$) with the corresponding manually counted SM results.

Conclusions: Automated endothelial cell counting on GDOCM images with large field of view eliminates selection bias and reduces sampling error, which affect the gold standard of manual counting on SM images.

MID TO LONG TERM INTERNATIONAL OUTCOMES OF THE BOSTON TYPE I KERATOPROSTHESIS IN STEVENS-JOHNSON SYNDROME

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Purpose: To determine mid to long term outcomes of Boston type I Keratoprosthesis (KPro) implantation in individuals with Stevens-Johnson syndrome (SJS) in a multi-center, international study.

Methods: This is a multi-center, retrospective, comparative case series of KPro procedures performed at 11 international sites (5/1/2006 – 12/31/2013) and at Stein Eye Institute, UCLA (5/1/2004 – 12/31/2018). Statistical analysis was conducted to identify significant differences in visual outcomes, complications, and retention rates between SJS and non-SJS study groups.

Results: 577 KPro procedures were performed in 525 eyes of 509 individuals, with 43 procedures performed in 30 eyes of 29 individuals with SJS. Repeat KPro was a significantly more common surgical indication in the SJS group than in the non-SJS group (30.2% vs. 7.3%, $P < 0.0001$). A significantly greater percentage of individuals with SJS had CDVA $\geq 20/200$ each year up to 5 years after surgery ($P = 0.0326$). Postoperative complications that were more common in the SJS group included persistent corneal epithelial defect ([PED] 53.2% vs. 11.7%, $P < 0.01$) and corneal stromal necrosis (64.9% vs. 23.2%, $P < 0.01$), which led to a higher retention failure rate (0.177/eye-year vs. 0.054/eye-year, $P < 0.01$), all of which were most common in the first year after surgery.

Conclusions: At each of the first five years after surgery, a larger percentage of individuals with SJS had CDVA $\geq 20/200$ than those with other indications, likely due to a significantly lower incidence of glaucoma. Sterile corneal stromal necrosis and persistent corneal epithelial defect formation were significantly more common in the SJS group, while sight-threatening complications such as endophthalmitis and retinal detachment were not. However, the significantly higher retention failure rate in the SJS group leads us to recommend a conservative approach to the use of the KPro in SJS.

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ANALYSIS OF REGULATIONS PROHIBITING CORNEAL DONATION BY MEN WHO HAVE SEX WITH MEN

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Purpose: A federal policy in place in the United States since the early years of the AIDS pandemic prohibits corneal donation by men who have had sex with another man (MSM) in the preceding five years, regardless of the results of infectious disease testing. Canada similarly enforces a 12-month ban on MSM corneal donation, even though modern virologic testing is reliable within days to weeks of viral exposure. We sought to determine how many corneal donations were turned away in 2018 due to these policies.

Methods: We contacted all EBAA-certified eye banks in the US and Canada by phone (as well as the independent eye bank of Quebec) and asked how many referrals they rejected in 2018 due to MSM status.

Results: Twenty-four of the 65 eye banks were able to provide a specific number of referrals disqualified in 2018 specifically due to MSM status. Those 24 eye banks turned away 360 referrals in 2018 specifically because of MSM sexual activity, equating to 720 eyes. Since those 24 eye banks accounted for 46.2% of the corneas recovered in the US and Canada in 2018, this suggests at least approximately 1600 eye donations were disqualified solely because of MSM status.

Conclusions: With global shortages of corneal donations, these policies are depriving patients of vision-restoring surgery. Since modern virologic testing is reliable within days to weeks of HIV or hepatitis exposure, the 5-year and 12-month MSM deferral periods are no longer scientifically justified. The FDA and Health Canada should revise their MSM deferral policies in light of current scientific evidence.

VISION-RELATED QUALITY OF LIFE AMONG CORNEAL TRANSPLANT RECIPIENTS

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Purpose: To assess patient-reported outcomes following penetrating keratoplasty (PKP), deep anterior lamellar keratoplasty (DALK), Descemet stripping automated endothelial keratoplasty (DSAEK), and Descemet membrane endothelial keratoplasty (DMEK).

Methods: Records of 103 PKP, 24 DALK, 42 DSAEK and 50 DMEK patients were analyzed from the Sight Outcomes Research Collaborative ophthalmology electronic health repository. Participants completed the Abbreviated National Eye Institute Visual Function Questionnaire (NEI-VFQ 9). Nonparametric tests and linear regression models were performed to analyze associations between clinical characteristics and NEI-VFQ 9 scores.

Results: Median composite NEI-VFQ 9 score for all eyes following keratoplasty was 72.7 ± 19.1 (range 9 to 100), with an average time of questionnaire administration 1.5 years after surgery. DALK, DMEK, DSAEK, and PKP surgery types had median NEI VFQ-9 total scores of 77.8, 84.2, 76.1, and 70.6. Higher postoperative acuities in the operative and fellow eyes were associated with higher NEI-VFQ 9 scores ($p < 0.001$ and $p < 0.001$). When controlling for acuity, the adjusted difference in postoperative NEI-VFQ 9 scores in PKP patients compared to DALK patients was 1.42 points lower ($p = 0.71$). The adjusted difference in postoperative composite NEI-VFQ 9 scores was 1.57 points lower among DSAEK patients compared to DMEK patients ($p = 0.66$).

Conclusions: Postoperative acuities were strongly associated with vision-related quality of life among corneal transplant recipients. When controlling for postoperative acuity, vision-related quality of life was similar between PKP and DALK patients and between DSAEK and DMEK patients.

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