**HUMAN PLATELET LYSATE FOR CORNEA ORGAN CULTURE**

**Methods**

32 human corneas unsuitable for transplantation from 16 human donors were cultured for 25-days in either 2%FBS or 2%HPL medium and compared by phase contrast microscopy (ECD and morphology), and next generation sequencing (NGS). Effects of 0.5%FBS, 5%FBS, 0.5%HPL, 2%HPL and 10%hPL on cultured human CSK and SF were evaluated.

**Results**

Differential cornea culture showed lower endothelial cell loss in the 2%HPL vs. 2%FBS group (ECL hPL=-0.7% vs. FBS=-3.8%; p<0.01). 2%HPL led to the upregulation of cytoprotective, anti-inflammatory and anti-fibrotic genes (e.g. HMOX1, SERPINE1, ANGPTL4, LEFTY2) and the downregulation of pro-inflammatory/apoptotic genes (e.g. CXCL14, SIK1B, PLK5, PPP2R3B). CSK/SF cell viability remained high in all groups (98-100%). Cell numbers and proliferation rates increased (p=0.024-0.001), CSK marker expression decreased with higher fractions of HPL and FBS (p<0.001). SMA1 increased with higher amounts of FBS (p=0.003) but decreased with incremental HPL substitution in both cell types (p=0.014). HPL contained more TGF-β1 (100%hPL 1.861±0.233 ng/ml vs. 100%FBS 0.015±0.010 ng/ml, p<0.001). hFGF and HGF were only detectable in 100% hPL (hFGF 0.067±0.017 ng/ml, HGF 1.074±0.050 ng/ml).

**Conclusion**

2%HPL is a suitable xeno-free substitution for 2% FBS in human cornea organ culture, inducing less ECL and potentially beneficial alterations in gene expression. CSK and SF can be cultured with xeno-free hPL. To maintain CSK characteristics substitution must remain minimal (0.5% hPL/FBS). hPL contains the anti-fibrotic HGF and bFGF, suppressing myofibroblast conversion.

**AN ARTIFICIAL-INTELLIGENCE-BASED DECISION SUPPORT TOOL FOR THE DETECTION OF CORNEA GUTTATA ON THE DONOR CORNEAS IN THE EYE BANK**

**Purpose**

Cornea guttata (CG) prevalence post keratoplasty varies from 15 to 18%, with 1 to 2% of the cases presenting with significant negative outcomes. The purpose of this research project is to create a program based on artificial intelligence (AI) that helps with the detection of CG in the donor corneas (DC) in the eye bank.

**Methods**

Preoperative corneal endothelial images (PCEI) of patients who underwent keratoplasty were collected and classified into 2 groups according to the postoperative CG grade. Group 1 included healthy corneas and those having mild postoperative CG, while group 2 included corneas with severe postoperative CG. Using previously tested semi-quantitative morphological criteria along with other characteristics such as donor age and lens status, the PCEIs were analyzed and used to create and train an AI-based tool for the detection of CG. The underlying concept of the tool compares previous cases with comparable properties to the DC in test. The postoperative CG grades of previous cases similar to the DC in test determine the prediction for its CG grade. Finally, the features and CG grade of the analyzed DC are stored in the database for future use.

**Results**

In total, 6221 PCEI belonging to 1078 patients were used to create a transparent and explainable decision support tool for the detection of CG through a hybrid approach combining 2 components. (1) Graphical analytic tools, whereby the PCEI pass multiple OpenCV-based image processing steps including the Watershed transform algorithm. In this step, cell membranes are delineated, and abnormally large cells or cell depleted areas are marked in red. Several other cell representations such as ‘honeycomb’ representation are created for an enhanced visualization of the endothelial layer (EL). (2) Machine learning (ML) classifiers including Case-Based Reasoning were created to detect CG. Initial experiments showed a performance comparable to humans (4-fold evaluation yielded precision: weighted F1 score:0.93).

**Conclusion**

We presented an AI-based program able to facilitate the detection of CG in the DC in the eye bank by comparing the PCEIs with relevant previous cases, using ML classifiers and offering an enhanced visualization of the EL. The evaluation and optimization of this program will follow as the next stage of our project.

**GUTTAE IN CORNEAL DONOR TISSUE**

**Purpose**

To report on the occurrence of guttata in corneal donor tissue.

**Material & Methods**

Retrospective database study of discard reasons for corneal donor tissue at Amnitrans EyeBank Rotterdam (AER) for the period from January 2019 to December 2021 and the outcome of an eight-question survey sent to European Eye Bank Association corresponding members addressing the occurrence of corneal guttata and the practice pattern regarding donor tissue with guttata.

**Results**

Between 2019 and 2021 6039 donor corneas were processed at AER. Average discard rate because of guttata in this period was 9 ±4% (n=552). Most corneas were discarded because of guttata at first evaluation (8%, n=481). Monthly discard rate because of guttata ranged from 3% to 19%. Yearly discard rates related to corneal guttata were 10 ±3% (8 ±3%) and 11 ±5% in 2019, 2020 and 2021, respectively. Average endothelial cell density (ECD) at the first evaluation from 2019-2021 was 2486 ±93 cells/mm², with average monthly ECD ranging from 2343 to 2642 cells/mm².
Twenty-nine eye banks completed the survey, including 4 located outside Europe. 70% reported a guttae-related discard rate of ≤4. The types of microscope used for the evaluation, the geographical location and the number of guttae permitted do not seem to influence the discard rates. 13 eye banks permitted 0 guttae while 10 banks accepted between 1-10 guttae.

The 16 eye banks that responded ‘no’ to the question whether the contralateral cornea of a guttata-cornea was automatically discarded did report a lower guttae-related discard rate than the other eye banks.

Conclusion The high variability of the discard rate due to guttae in donor corneas (ranging from <1% and >12%) is an indication that it is not always easy to detect guttae in donor corneas. Although transplanting corneal grafts with guttae does not necessarily mean that a re-transplantation will be needed on the short term, a vital method to unequivocally determine that it is not always easy to detect guttae in donor corneas. Although transplanting corneal grafts with guttae does not necessarily mean that a re-transplantation will be needed on the short term, a vital method to unequivocally determine the presence of guttae in the eye bank seems essential to prevent unnecessary waste of suspect tissue and unnecessary re-surgeries.

**P08-A131 COMPARISON OF STERILE DONOR TOMOGRAPHY IN THE EYE BANK AND PREVIOUS KERATOMETRIC MEASUREMENTS DURING THE DONOR’S LIFETIME**

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Purpose Sterile donor tomography in the eye bank can be used to minimise refractive surprises after corneal transplantation.

The aim of this study was to compare sterile tomography of donor corneas in the eye bank with keratometric measurements of the same donors performed prior their death.

Methods Since 2018, 1246 donor sclerocorneal discs have been routinely measured using donor tomography, taken steriley through their cell culture flask in medium II using the anterior segment optical coherence tomograph Casia 2 (Tomey Corp., Nagoya, Japan) and a custom-made Matlab software (The MathWork Inc., Natick, Massachusetts, USA). Of all these donor corneas, 19 (1.5%) appeared to have been measured with Pentacam (Oculus Optikgeräte GmbH, Wetzlar, Germany) in the donors before death. Both measurements, taken at a mean interval of 35 ± 26 months, were compared using a Wilcoxon signed-rank test.

Results The mean steepest/flattest front surface radius and anterior astigmatism of the corneas measured with Pentacam amounted 7.66±0.35/7.93±0.37 mm, and 0.27±0.43 mm. Corresponding values of sterile donor tomography were respectively 7.48±0.31 [p<0.01]/7.77±0.25 [p=0.01] mm, and 0.29±0.35 [p=0.78] mm.

At the posterior corneal surface, the Pentacam measured a mean steepest/flattest surface radius and astigmatism of 6.27 ±0.33/6.72±0.48 mm and 0.45±0.47 mm, whereas values of sterile donor tomography amounted 6.55±0.30 [p<0.01]/6.94 ±0.33 [p=0.04] mm and 0.39±0.26 [p=0.63] mm, respectively.

The central corneal thickness amounted 575±52 μm with Pentacam, and 597±80 μm [p=0.20] with sterile donor tomography.

Conclusion The front and back surface astigmatism as well as the central corneal thickness remained statistically unchanged after corneal excision and preservation in organ culture in comparison to measurement of the donor prior death. The statistically non-similar anterior and posterior radius of curvature between both methods must be seen in light of the known differing corneal topography between swept-source anterior segment optical coherence tomography and Scheimpflug imaging. These results suggest a merely minimal deformation caused by the storage and attachment of donor corneas to their holder in the cell culture flask for sterile donor tomography, causing a steeper anterior surface curvature but leaving the astigmatism still congruent with previous in situ conditions.

**P09-A130 MANAGEMENT OF AN EYE BANK WITH ORGAN-CULTURED AND HYPOTHERMIC CORNEAS: MICROBIOLOGY IN ENDOTHELIAL GRAFTS**

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Purpose To analyse the microbiologic control results taken during the processing of hypothermic and cultured corneas for endothelial transplants comparing the two groups from January to September 2022.

Methods The microbiologic controls of hypothermic corneas prepared for DSAEK or DMEK are: Transport Eusol control (pre-manipulation) and new Eusol control (post-manipulation). In cultured corneas the number of controls is increased to 4: first culture medium, evaluation culture medium, transport medium 24 hours post-evaluation and transport medium post-manipulation.

Results A total of 1438 corneas were processed for transplant during the 9 months studied (321 fresh corneas and 1113 cultured corneas). A total of 557 corneas were prepared for DSAEK or DMEK, from which 89 (15,98%) were hypothermic corneas and 468 (84,02%) were cultured. From hypothermic corneas, 65 were cut for DSAEK and with 24 corneas, pre-stripping for DMEK was done. In the case of cultured corneas, 187 were cut for DSAEK and with 281 pre-stripping for DMEK was done. The number of corneas with positive results in the microbiologic controls were 15 (16,85%) in the case of fresh corneas (in 7 corneas that were prepared for DSAEK and in 8 for DMEK) and 4 cases (0,85%) in cultured corneas (in 3 corneas for DSAEK and in 1 cornea for DMEK) resulting in a clear difference between both preservation methods. Bio-surveillance notifications notified during the studied period have been a total of 5, from which 2 were SAE in hypothermic corneas and other 2 were SAE and 1 SAR, in cultured corneas, all for endothelial transplantations.