SARS-CoV-2 REAL TIME POLYMERASE CHAIN
CYTOPROTECTIVE EFFECTS OF HUMAN PLATELET REACTION TESTING OF CORNEAS FROM HUMAN DONOR CORNEAS:
POST-MORTEM SARS-COV-2 POSITIVE DONORS

P31-A150

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Purpose Possible transmission of SARS-CoV-2 from donors to recipients via cornea grafts is still a concern of the transplantation community. Current recommendations are to avoid corneal transplants from donors with ongoing SARS-CoV-2 infection or those recently exposed to it. During pandemic period in Croatia 21/1113; (1.9%) corneas were procured from donors positive for SARS-CoV-2 by postmortem nasopharyngeal swab tests. That tissue was discarded. Due to the lack of knowledge about the infectivity of such corneas, we started prospective study of SARS-CoV-2 presence in cornea tissue. Here we show our first results.

Methods In the study period we had four corneas procured from two post-mortem SARS-CoV-2 positive donors. For the presence of SARS-CoV-2, analysis is performed on donor serum, hypothermic storage medium and cornea tissue lysate. Corneas were stored in hypothermic condition for 8 to 10 days, after which tissue was macerated and washed with PBS. The intracellular content was released by incubation with lysis buffer, followed by centrifugation. Next, tissue lysate, serum and hypothermic storage medium were in parallel subjected to fully automated nucleic acid isolation and RNA expression was analyzed by qRT-PCR. During isolation, RNA pur was used as internal control for successful nucleic acids isolation.

Results No SARS-CoV-2 RNA was detected in the donors serum, storage medium and cornea tissue from donors who were SARS-CoV-2-positive upon tissue procurement. In nasopharyngeal swabs of post mortem positive donors cycle threshold values of viral copies were high (CT>34), indicating that there was small number of viral particles in infected donors that could have impact on negative results in tested tissue. Conclusion Our data suggested that corneas may not be SARS-CoV-2 permissive if the donor was postmortem positive. Further research is required to gain more coherent insight into SARS-CoV-2 transmission via corneal transplantation.

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CYTOPROTECTIVE EFFECTS OF HUMAN PLATELET
LYSATE DURING THE XENO-FREE CULTURE OF HUMAN DONOR CORNEAS

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Purpose We evaluated the suitability of 2% human platelet lysate (2%HPL) to replace 2% fetal bovine serum containing medium (2%FBS) for the xeno-free culture of human donor corneas.

Methods 32 human corneas unsuitable for transplantation from 16 human donors (age 69.3±15.7years) were collected. 38.5±17.1 hours after death. They were first cultured in 2% FBS containing medium for 3 days (time point TP1), then evaluated by phase contrast microscopy (endothelial cell density (ECD) and cell morphology). Following an additional 25-days culture period (time point TP2) in either 2%FBS or 2%HPL medium the pairs were again compared by phase contrast microscopy (ECD and morphology), stroma and Descemet membrane/endothelium (DmE) were processed for next generation sequencing (NGS). Results ECD did not differ between the 2%HPL and 2%FBS group at TP1 (p=0.87). At TP2 the ECD was higher in the 2%HPL group (2179±288cells/mm2) compared to 2%FBS (2113±313cells/mm2; p=0.03), and endothelial cell loss was lower (ECL hPL=−0.7% vs. FBS=−3.8%; p=0.01). There were no significant differences in cell morphology, neither between TP1 and 2 nor between 2%HPL and 2%FBS. NGS showed the differential expression of 1644 genes in endothelial and 217 genes in stromal cells. 2%HPL led to the upregulation of cytoprotective, anti-inflammatory and anti-fibrotic genes (e.g. HMOX1, SERPINE1, ANGPTL4, LEFTY2, GADD45B, PLIN2, PTK3, GFRA1/2) and the downregulation of pro-inflammatory/apoptotic genes (e.g. CXCL14, SIK1B, PLK5, PPP2R3B, SLURP1, FABP5, MAL, GATA3).

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.

P33-A110

EX Vivo MODELS OF CORNEAL EPITHELIAL
REGENERATION IN BIOREACTOR: RESPECTIVE ROLES OF LIMBAL, CONJUNCTIVAL AND CORNEAL EPITHELIUM

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Purpose To evaluate the potential of ex vivo limbal, conjunctival and corneal epithelial models in a bioreactor to investigate the respective roles of limbal, conjunctival and corneal cells in corneal epithelial regeneration.

Methods Human limbal, conjunctival and corneal epithelial cells were isolated from healthy donors and cultured in a bioreactor. The long-term maintenance in a bioreactor was assessed by analyzing the expression of specific markers (e.g. cytokeratin 19 for limbal cells, occludin for conjunctival cells, and corneal epithelial markers). The differentiation of limbal cells into corneal epithelial cells was also evaluated by examining the expression of corneal epithelial markers. The potential of these models to regenerate corneal epithelium was assessed by examining the ability to form stratified epithelial sheets and the expression of corneal epithelial markers.

Results Limbal cells maintained their limbal phenotype in the bioreactor and differentiated into corneal epithelial cells, as evidenced by the expression of corneal epithelial markers. Conjunctival cells also maintained their conjunctival phenotype in the bioreactor and did not differentiate into corneal epithelial cells. Corneal epithelial cells maintained their corneal phenotype in the bioreactor and did not differentiate into limbal or conjunctival cells.

Conclusion The ex vivo models of corneal epithelial regeneration in a bioreactor showed that limbal cells are more capable of differentiating into corneal epithelial cells compared to conjunctival cells. This indicates that limbal cells play a more significant role in corneal epithelial regeneration compared to conjunctival cells. Further research is required to investigate the potential of these models to regenerate corneal epithelium in a clinical setting.

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