days of graft storage. Implementing an additional post-preparation step in the eye bank to evaluate cell density before graft release for transplantation may help to reduce postoperative DMEK complications.

A PORCINE CORNEA AND LAMELLAR TISSUE MODEL TO INVESTIGATE EFFECTS OF STORAGE CONDITIONS ON CORNEAL PRESERVATION

1 Umberto Rodella*, 2 Lorenzo Bosio, 3 Laura Giurgola, 4 Claudio Gatto, 5 Orietta Rossi, 6 Stefano Ferrari, 7 Jana D'amato Tothova. 8 Alchimia S.r.l., Ponte San Nicolò, Italy; 9 Fondazione Banca degli Occhi del Veneto Onlus, Venezia, Italy

Abstracts

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Background Globally, more than 12 million people are awaiting corneal transplantation and corneal donor reduction has been observed since the outbreak of the COVID-19 pandemic, negatively influencing the availability of human corneas for research purposes as well. Therefore, the exploitation of ex vivo animal models in this field is of great value.

The present study aimed at the development of a novel experimental model of porcine cornea ex vivo and lamellar tissue preparation to investigate the effects of storage conditions on corneal preservation.

Methods Twelve fresh porcine eye bulbs were disinfected by immersion in 10 mL of 5% povidone-iodine under orbital mixing for 5 minutes at room temperature. The corneoscleral rims were dissected, and stored in Tissue-C (Alchimia S.r.l., n=6) at 31°C and in Eusol-C (Alchimia S.r.l., n=6) at 4°C up to 14 days.

The evaluation of Endothelial Cell Density (ECD) and endothelial mortality was performed using vital dye Trypan Blue staining (TB-S, Alchimia S.r.l.). Digital 1X pictures of TB-stained corneal endothelium were acquired and percentage of stained area was quantified using FIJI ImageJ software. ECD and endothelial mortality were determined at 0, 3, 7 and 14 days.

Medium turbidity detected by naked eye was considered as proof of tissue contamination.

Additionally, non-vital staining of the endothelium with Alizarin Red (AR) was performed and the endothelial morphology was investigated at Day 14 in both whole corneas and dissected endothelial lamellae.

Results The contamination rate of porcine corneas corresponded to <10% and 0% in Tissue-C and Eusol-C after 14 days, respectively.

Porcine corneas stored in Tissue-C and Eusol-C showed <10% and <20% mortality in Tissue-C and Eusol-C respectively at the end of storage.

Preliminary ECD determination (range 3700-4100 cells/mm2) at Day 0 aligned with data present in the literature (Meltendorf et al., Graefe’s Arch Clin Exp Ophthalmol, 2007).

Whole cornea and dissected lamellae stained with TB and AR showed comparable endothelial morphology after incubation in Tissue-C and Eusol-C for 14 days. The lamellar tissue allowed endothelium morphology analysis at higher magnification compared to whole cornea.

Conclusion The presented ex vivo porcine model allows evaluation of the performance and safety of storage conditions. Future perspectives of this method will be the extension of the porcine corneas storage up to 28 days.

19 KILLING EFFICACY OF A NEW HYPOTHERMIC CORNEAL STORAGE MEDIUM KERASAVE AT 4°C AGAINST NINE MICRO-ORGANISMS FREQUENTLY FOUND IN DONOR CORNEAS

Laura Giurgola MSc*, Jana D’amato Tothova, Claudio Gatto MSc, Orietta Rossi. ALCHIMI.A S.R.L., Ponte San Nicolò, Italy

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Purpose The aim of the present study was to determine the killing efficacy of Kerasave (AL.CHI.MI.A Srl), a corneal cold storage medium provided with antymycotic tablet against nine contaminants associated corneal infections.

Methods The killing efficacy of Kerasave was determined after 0, 3 and 14 days of incubation at 4°C in Kerasave after inoculation of the medium with 10^5–10^6 (CFU) of Candida albicans (CA), Fusarium solani (FS), Aspergillus brasiliensis (AB), Staphylococcus aureus (SA), Enterococcus faecalis (EF), Bacillus subtilis (BS), Pseudomonas aeruginosa (PA), Enterobacter cloacae (EC) and Klebsiella pneumoniae (KP). Log10 reductions at different time intervals were determined by the serial dilution plating technique.

Results After 3 days, Kerasave induced the highest log10 decrease in the concentrations of KP, PA, CA and EC. The 2 log10 decrease was observed for SA and EF. The lowest log10 decrease was observed in BS, AB and FS concentrations. After 14 days, the microbial count of CA, FS, SA, EF, PA and EC further decreased.

Conclusions Corneal cold storage medium Kerasave effectively reduced the microbial concentration of almost all tested microorganisms after 3 days and represents a valuable tool to control the microbial contamination of human donor corneas intended for transplantation.

20 FACTORS INFLUENCING THE DISCARD OF CLINICAL TISSUE AND NHSBT METHODS TO MAINTAIN A LOW DISCARD RATE

David Usherwood*, Lauren Roberts, Toni Woodward, Sam Gallagher. NHSBT, Bristol, UK

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Introduction NHS Blood and Transplant Tissue and Eye Services (TES) is a human multi-tissue, tissue bank supplying tissue for transplant to surgeons throughout the UK. NHSBT has two Eye Banks. These are NHSBT Filton, based in Bristol and NHSBT David Lucas Eye Bank, which is based in Speke Liverpool.

Materials and Methods NHSBT monitors our monthly discard rates with the aim to review for any patterns. Due to the NHSBT Eye Banks using a computer system called PULSE we can categorise all our discard for further analysis. Focusing on key areas such as Contamination, Corneal Assessment failure such as Low Endothelial Cell count, Medical deferrals and blood sample quality.

Results 2019- NHSBT Procured 5705 Eyes and Issued 4725. This is a discard rate of 19%

2020- NHSBT Procured 3725 Eyes and Issued 2676. This is a discard rate of 28%

2021- NHSBT Procured 4394 Eyes and Issued 3555. This is a discard rate of 19%

Based on the EEEA Statistical report of Eye Banking Activity in Europe for 2019- 42663 Eyes/Corneas in situ were
processed and 25254 Corneas supplied for transplant. This is a discard rate of 41%.

Based on the EEEBA Statistical report of Eye Banking Activity in for 2020-33460 Eyes/Corneas in situ were procured and 21212 Corneas supplied for transplant. This is a discard rate of 37%.

Discussion Based on this data, NHSBT discard rate is below the European Average. Key factors which contribute to this low discard rate. Independent Clean rooms for excision and assessment operating to a Grade A Level. A centralised National Referral Centre and 4 dedicated Retrieval Teams ensure retrievals are within 24 hours of Death, and excision occur within 24 hours of enucleation. A dedicated Admin and Clinical Nursing Team performing the medical release ensures the Tissue is released promptly after Microbiological Testing (Day 10) for Assessment. During 2020 due to COVID all routine operations were cancelled suddenly. This resulted in an Increase of discard due to time expiry.

REFERENCES

Theme 4 – New paradigms in eye banking

DENUDED DESCEMET’S MEMBRANE AS POTENTIAL TOOL TO SUPPORT HUMAN EMBRYONIC STEM CELL DERIVED RETINAL PIGMENT EPITHELIAL CELLS CULTURE

Aims To investigate human embryonic stem cell-retinal pigment epithelium (hESC-RPE) cells survival and behaviour on a decellularized DM, which may be of clinical relevance in retinal transplantation.

Materials DMs were isolated from human donor corneas and treated with thermolysin. The DM surface topology and the efficiency of the denudation method were evaluated by atomic force microscope and histology. hESC-RPE cells were seeded onto the endothelial-side surface of acellular DM in order to determine the potential of the membrane to support hESC-RPE cell culture, alongside maintaining their viability. Integrity of the hESC-RPE monolayer was assessed by measuring transepithelial resistance. RPE-specific gene, protein expression and growth factors secretion were assessed to confirm maturation and functionality of the cells over the new substrate.

Results Thermolysin treatment did not affect the integrity of the tissue, thus ensuring a reliable method to standardize the preparation of decellularized DM. hESC-RPE cell attachment 6 days post-seeding and proliferation rates over the acellular DM were similar to hESC-RPE cells cultured on tissue culture inserts.

On the new matrix, hESC-RPE cells succeeded in forming an intact monolayer with mature tight junctions. The resulting cell graft showed the characteristic RPE morphology. The expression of typical RPE genes, proper protein localization and key growth factor secretion further confirmed the correct RPE phenotype. The viability of the cells was maintained for up to 4 weeks in culture.

Conclusion Acellular DM was shown to be capable of sustaining hESC-RPE cells growth, thus confirming to be potentially a valid alternative to the Bruch’s membrane.

Further in vivo studies will need to verify if this product can represent a feasible tool to deliver RPE cells in the back of the eye.

Our study highlights the possibility of recycling unsuitable corneal tissues, which would otherwise be discarded by the eye banks for clinical application.

RENEWAL OF CONJUNCTIVAL EPITHELIUM OVER AMNIOTIC MEMBRANE TO PERFORM AUTOLOGOUS SIMPLE CONJUNCTIVAL EPITHELIAL TRANSPLANTATION (SCET): IN VITRO VALIDATION AND RESULTS OF CLINICAL APPLICATION FOR PRIMARY PTERYGIUM

Background Transplantation of ex vivo cultured conjunctival cell layers, generated on amniotic membrane or other scaffolds, provides a viable option in treating heterogeneous ocular surface conditions. By comparison, cell therapy is costly, labour-intensive and subject to good manufacturing practice requirements and regulatory approval; no conjunctival cell-based therapy is currently available. Several techniques are available after primary pterygium excision to recover the ocular surface anatomy by restoring healthy conjunctival epithelium and preventing recurrence and complications. However, application of conjunctival free autograft or transpositional flap to cover the bared scleral area is limited when the conjunctiva are to be spared for future glaucoma filtering surgery, in patients with large or double-headed pterygia, in recurrent pterygia, or when the harvesting of donor conjunctival is precluded by scarring.

Aim To develop a simple technique to obtain expansion of the conjunctival epithelium when applied in vivo in diseased eyes.

Methods We evaluated in vitro the best way of gluing conjunctival fragments over the AM, the efficiency of the fragments to generate conjunctival cell outgrowths, the molecular marker expression, and the feasibility of shipping preloaded AM.

We performed simple conjunctival epithelial transplantation (SCET) in which we glued an amniotic membrane patch pre-loaded with autologous conjunctival tissue fragments over the scleral defect after pterygium excision and evaluated the recovery of the normal conjunctival epithelium and the disease recurrence up to 12 months after surgery.