procured and 25254 Corneas supplied for transplant. This is a discard rate of 41%.

Based on the EEBA 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

1. EEBA Statistical report of Eye Banking Activity in Europe for 2019 and 2020.

Theme 4 – New paradigms in eye banking

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

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Introduction Recent clinical studies suggest that RPE-cell replacement therapy may preserve vision and restore retinal structure in retinal degenerative diseases. New developments enabled the differentiation of RPE cells from pluripotent stem cells. Scaffold-based methods are being tested in ongoing clinical trials for delivering these cells to the back of the eye. Borrowed materials from donor tissues can be used as cell supports in subretinal transplantation. These biological matrices resemble the extracellular matrix microenvironment of the native tissue. The Descemet's membrane (DM) is an example of high collagen-rich basement membrane (BM). The potential of this tissue in retinal repair remains to be uncovered.

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 trans-epithelial 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.

22 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

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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 preloaded 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.

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Results 65-80% of fragments generated outgrowth 48-72h after gluing, without differences between type of AM preparation and fragment size. Within 6-13 days, a full epithelium covered the surface of the amniotic membrane. Specific marker expression (Muc1, K19, K13, p63, ZO-1) was detected. The shipping test showed after 24h the 31% of the fragments glued over the AM epithelial side, compared to more than 90% of fragments stayed attached in the remaining conditions (stromal side, stromal without spongy layer, epithelial side without epithelium).

Surgical excision and SCET for nasal primary pterygium were performed in 6 eyes/patients. No graft detachment and recurrence occurred within 12 months. In vivo confocal microscopy showed progressive expansion of the conjunctival cell population and formation of a clear cornea-conjunctiva transition.

Conclusions We established the most suitable conditions for a novel strategy based on in vivo expansion of conjunctival cells from conjunctival fragments glued over the AM. The application of SCET seems to be effective and replicable for the renewal of conjunctiva in patients requiring ocular surface reconstruction.

23 CRYOPRESERVATION OF HUMAN AMNIOTIC MEMBRANE (HAM) FOR OCULAR SURFACE RECONSTRUCTION: A COMPARISON BEETWEEN PROTOCOLS

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Purpose In the past decades, the human amniotic membrane has been largely applied for several surgical and non-surgical procedures. It has been farther demonstrated that both hAM and cornea share similar patterns of expression of structural components of the basement membrane (like laminin 5 and collagen IV) making hAM an useful tissue for ocular surface reconstruction. Since 1996 in fact, amniotic membrane transplantation has been applied to a large number of ocular surface diseases including Stevens-Johnson syndrome, pterygium, corneal ulceration, ocular surface reconstruction after chemical/thermal burns and in the reconstruction after excision of ocular surface neoplasia. During the previous decades, hAM has achieved a pivotal role in regenerative medicine too.

The possibility to preserve human amniotic membrane, without affecting membrane's features, has become pivotal, allowing virological and microbiological analyses to be carried out before grafting. The purpose of the present study is to investigate an easier and cheaper protocol for human amniotic membrane preservation without affecting its properties and structure, ensuring the safety profile of the tissue. We compared the effects on adhesive and structural properties of newer preservation conditions to those obtained with an established, standardized protocol (dimethyl sulfoxide at -160°C). In attempt to simplify and enhance the safety of the procedure, we tested dextran-based freezing medium and a dry condition (no medium) at temperatures of -80°C.

Methods Five patches of human amniotic membrane were obtained from three different donors. For each donor, five preservation condition were tested: dimethyl sulfoxide at -160°C, dimethyl sulfoxide at -80°C, dextran-based medium at -160°C, dextran-based medium at -80°C and dry freezing at -80°C (no medium). At the end of four months storage period, adhesive properties and structure were analyzed.

Results None of the newer preservation protocols showed differences in adhesive and structural properties of the tissues. The stromal layer always kept its adhesiveness, while both structure and basement membrane were not altered by any the preservation protocol.

Conclusions Switching from liquid nitrogen cryopreservation to -80°C would reduce manipulation, simplify the procedure, making it also cheaper. The use of dextran-based freezing medium or no medium at all (dry condition) would avoid the potential toxicity of the dimethyl sulfoxide-based freezing media.

24 EFFECT OF GRAFT PREPARATION TECHNIQUES ON CLINICAL OUTCOMES AFTER DESCEMET MEMBRANE ENDOTHELIAL KERATOPLASTY (DMEK)

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Purpose To compare the clinical outcomes after Descemet membrane endothelial keratoplasty (DMEK) for grafts prepared by the manual no-touch peeling technique and grafts prepared by a modified liquid bubble technique.

Material and Methods For this study, 236 DMEK grafts were included that were prepared at Amnitrans EyeBank Rotterdam by experienced eye bank personnel. 132 grafts were prepared by using the 'no-touch' DMEK preparation technique and 104 grafts by using a modified liquid bubble technique. The liquid bubble technique was modified to render it a no-touch technique while maintaining the ability to save the anterior donor button as a potential Deep Anterior lamellar keratoplasty (DALK) or Bowman layer (BL) graft. DMEK surgeries were performed at Melles Cornea Clinic Rotterdam by experienced DMEK surgeons. All patients underwent DMEK for Fuchs endothelial dystrophy. Average patient age was 68 (±10) years and average donor age was 69 (±9) years with no difference between the two groups. Endothelial cell density (ECD) was evaluated after graft preparation by light microscopy in the eye bank and at 6-month postoperatively by specular microscopy.

Results Endothelial cell density (ECD) decreased from 2705 (±146) cells/mm2 (n=132) before to 1570 (±490) cells/mm2 (n=130) at 6 months postoperatively for grafts prepared by the no-touch technique. For grafts prepared by the modifiedliquid bubble technique, ECD decreased from 2627 (±181) cells/mm2 (n=104) before to 1553 (±513) cells/mm2 (n=103) after surgery. Postoperative ECD did not differ for grafts prepared by the two techniques (P=0.79). Central corneal thickness (CCT) decreased from 660 (±124) µm to 513 (±36) µm postoperatively in the no-touch group and from 684 (±116) µm to 515 (±35) µm postoperatively in the modified liquid bubble group, with no postoperative CCT difference between groups (P=0.59). In total 3 eyes underwent re-surgery within the study period (n=2 (1.5%) in the no-touch group, n=1 (1.0%) in the liquid bubble group; P=0.71) and 26 eyes required a re-bubbling procedure for incomplete graft

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