

adherence (n=16 (12%) in the no-touch group, n=10 (10%) in the liquid bubble group; P=0.37).

Conclusion Clinical outcomes after DMEK are comparable for grafts prepared by either the manual no-touch peeling technique or the modified liquid bubble technique. While both techniques are safe and useful techniques to prepare DMEK grafts, the modified liquid bubble technique offers advantages for corneas with scars.

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SIMULATION OF EYE SURGERY IN PORCINE EYE GLOBES AND EVALUATION OF RETINAL CYTOTOXICITY

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Purpose To simulate pars plana vitrectomy in porcine eyes ex vivo using intraoperative devices and to evaluate viability of retinal cells.

Methods 25 enucleated porcine eyes were divided in following groups Group A) No surgery control; Group B) Sham surgery; Group C) Cytotoxic control; Group D) Surgery with residues; Group E) Surgery with minimal residues. The retina was extracted from each eye bulb and the cell viability was determined by MTT assay. The in vitro cytotoxicity of each used compounds was tested on ARPE-19 cells.

Results No cytotoxicity was detected in retinal samples in groups A, B and E. Samples from eye bulbs that had undergone surgery with minimal removal of residues (group D) and cytotoxic controls (group C) showed high retinal cytotoxicity. The simulation of vitrectomy indicated that the combined use of compounds does not affect retinal cells viability if all the compounds are properly removed, whereas the cytotoxicity detected in group D may suggest that the presence and accumulation of the residues of the compounds used intraoperatively could negatively impact retinal viability.

Conclusions The present study demonstrate the crucial role of an optimal removal of the intraoperative devices used in eye surgery to ensure safety to the patient.

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DEVELOPMENT OF HUMAN AMNIOTIC MEMBRANE PRODUCTS FOR REGENERATIVE MEDICINE APPLICATIONS

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Introduction Human amniotic membrane (HAM) has important biological properties that make this tissue an ideal substrate for regenerative medicine applications, including treatment of ocular diseases and wound healing. NHSBT can successfully decellularise HAM for promoting enhancement of limbal stem cell expansion in vitro more efficiently than the cellular HAM.¹ In this study we present new formulations of decellularised HAM as freeze-dried powder and derived natural hydrogel. The aim was to develop a variety of GMP-compliant allografts to treat ocular diseases.

Materials and Methods Six HAM, obtained from elective caesarian deliveries, were dissected, decontaminated and subjected to an in-house developed decellularisation protocol including a

mild SDS concentration as detergent and nuclease steps. Following decellularisation, the tissue was placed in a sterile tissue culture flask and freeze dried. The freeze-dried tissue was cut into pieces of ~1g each, dipped into liquid nitrogen, then ground with a pulverisette. Ground tissue was solubilised using porcine pepsin and 0.1M HCl (stirred for 48 hours, 25°C). At the end of solubilisation, the pre-gel solution was kept on ice to adjust the pH back to 7.4. Gelation was induced when the temperature of the solution was increased to 25°C and aliquots were used for both in vitro cytotoxicity (up to 48 hours) and biocompatibility (up to 7 days) testing (MG63 and HAM cells). Cells were added into the solution before gelling and on top after gelling.

Results The pre-gel solution obtained from decellularised HAM appear homogenous without undigested powder, and it was able to gel within 20 minutes at RT. Gels with a concentration of 4-8mg/mL tissue powder retained shape (including in an aqueous environment). When added on top of gels, cells were observed to attach and proliferate over time. When added into gels, the cells were observed throughout the gels and appeared to be migrating through the gel.

Conclusion Acellular HAM can be successfully freeze dried and converted into new formulations for topical application (powder and hydrogel). The new formulations could improve HAM delivery and provide a better scaffold for tissue regeneration. To our knowledge, this is the first time an amnion hydrogel formulation has been developed in GMP compliant setting for tissue banking purpose. Further studies will also investigate the ability of amnion hydrogel to promote stem cells differentiation into the three lineages (adipogenic, chondrogenic, osteogenic) in and/or on the gels.

REFERENCES

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ADVANCING THE BASIC UNDERSTANDING AND MANAGEMENT OF POSTERIOR CAPSULE OPACIFICATION FOLLOWING CATARACT SURGERY USING HUMAN DONOR EYES

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The human lens is an extraordinary tissue. It has no innervation or blood supply and receives the key building blocks of life from the aqueous and vitreous humours that bathe it. The primary objectives of the lens are to remain transparent and refract light in order to focus light on the retina. These are achieved through exquisite cell organisation and order. However, in time this order can be disrupted and visual quality can deteriorate through the formation of cataract (a clouding of the lens). At present there is no cure for cataract with surgery the only means of resolution. This procedure is performed in ~30 million patients per annum across the globe. Cataract surgery involves making a circular opening (capsulorhexis) in the anterior lens capsule and removal of central lens fibre cells. The product of cataract surgery is known as a capsular bag, which comprises a ring of the anterior capsule and entire posterior capsule. The capsular bag remains in situ, partitions the aqueous and vitreous humours, and in the majority of cases, houses an intraocular lens (IOL). Initial results are superb, but a significant number of patients