processed within 2 h after death. Porcine eye bulbs were decontaminated by immersion in 10 mL of 5% povidone-iodine and corneas were dissected under aseptic conditions, leaving approximately 2 mm of the scleral rim. DMEK grafts were prepared by means of mechanical stripping technique using specific surgical instruments for DMEK (Moria, France) on fresh corneas (n=2) and on corneas stored in Eusol-C (AL.CHI.MI.A. Srl, Italy) at 4°C for 7 days (n=4) and for 14 days (n=4). Endothelial cell (EC) density was compared before DMEK-preparation (specular and light microscopy on trypan blue stained tissues) and after DMEK-preparation (fluorescence microscopy on Calcein-AM stained tissues). DMEK graft injection was simulated in anterior chamber of fresh porcine eye bulbs.

Results The porcine DMEK grafts preparation resulted to be more challenging compared to human DMEK grafts. Despite similarity between human and porcine corneas, porcine Descemet membrane (DM) firmly adheres to the underlying stroma. DMEK grafts preparation was not successful at day 0; DMEK preparation was possible by mechanical stripping technique on corneas stored in Eusol-C for 7 and 14 days obtaining naturally rolled endo-out porcine DMEK grafts. An EC mortality increase up to 20% was observed on DMEK graft compared to initial whole corneal tissue. DMEK roll injection was successfully simulated in anterior chamber of the porcine eye bulb.

Conclusion Naturally rolled DMEK endo-out grafts were successfully prepared by mechanical stripping technique on porcine corneal tissues stored in Eusol-C at 4°C (up to 14 days). DMEK Surgery including the tissue injection in anterior chamber could be simulated. Further studies will be performed to improve ex-vivo-porcine DMEK surgery model.

P16-A111 FACTORS AFFECTING THE DENSITY OF CORNEAL ENDOTHELIAL CELL CULTURES OBTAINED FROM DONOR CORNEAS

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Purpose The shortage of donor corneas represents a worldwide problem, and corneal endothelial cell (CEC) therapy might be a promising alternative approach. CEC can be implanted alone, which has shown limited efficacy, or with a scaffold that holds the cells together as a monolayer tissue, thus imitating Descemet membrane endothelial keratoplasty. We believe that endothelial cell density (ECD) >2000 cells/mm2, a cut-off value that eye banks use to provide quality tissues for transplantation to surgeons, should also be adopted as a parameter to define the quality of CECs as a new Advanced Therapy Medicinal Product for clinical applications in patients with endothelial dystrophies.

Methods We isolated and cultured CECs from one or more corneas of elderly age donors with ECDs higher than or below 2000 cells/mm2. CEC cultures were carried out on coated plates and on hydrogels with a preformed basement membrane (from TissueGUARD, Germany). Immunofluorescence with antibodies against ZO-1 was performed to evaluate the ECDs of the CEC graft obtained.

Results Our results suggest that primary cultures with ECDs>2000 cells/mm2 can be obtained on coated plated only when (1) CECs are isolated from one or more corneas of young donors; (2) CECs are isolated and pooled together from at least 2 elderly age donor corneas (if ECD>2000 cells/mm2) or 3 elderly age donor corneas (if ECD<2000 cells/mm2). Secondary cultures are all characterized by low ECDs. Hydrogels have been shown to be able to lead to increased ECDs after their release.

Conclusion Our protocol highlights the difficulties in obtaining cultures with ECDs>2000 cells/mm2. Despite being achievable with corneas from young donors, this becomes challenging when corneas from elderly donors are used, i.e., the overall majority of those collected by eye banks, particularly when corneas from elderly age donors with ECD<2000 cells/mm2 are considered as a source. One alternative would be to isolate CECs from more corneas, but this might raise the issue of antigenic stimulation, which could eventually lead to transplantation failure. Our strategy to overcome these challenges is the use of a preformed basement membrane as a scaffold for CECs. However, this challenging approach should be investigated more before proceeding to clinical application.

P17-A140 CLINICAL OUTCOMES OF THE FIRST ORGAN-CULTURED PRELOADED DMEK-A SINGLE CENTRE STUDIE

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The use of ready-to-use grafts from specialized eye banks might provide a number of benefits, including graft quality control, assured availability at a certain operation time, a decreased likelihood of case cancellation, and a shorter and less sophisticated DMEK surgery, with a resulting lower risk of graft damage. However, it is critical to thoroughly establish the clinical safety of employing these preloaded tissues. Especially since most of the studies were prepared and stored under hypothermic conditions. There are only a few studies on preprepared tissues in organ culture, which are partly controversial.

In this prospective study we included patients who received DMEK surgery at the Knappschaft Eye Clinic Sulzbach. Patients received either a preloaded DMEK (pDMEK), prepared five days before surgery in the eye bank, or a conventional, directly before surgery, surgeon-prepared DMEK (sDMEK).

The preliminary data show a trend towards more frequent need for rebubbing in the pDMEK group and a statistically non-significant lower postoperative endothelial cell count compared to the sDMEK group. However, the development of visual acuity and decrease in corneal thickness is comparable in both groups.

Therefore, we investigated the clinical outcomes of the first organ-cultured preloaded DMEK cases and compared these outcomes with those from our very last cases with surgically loaded tissues from a single centre.

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