preliminary investigations and can be used for testing new media, substances, drugs, or preservation conditions and their impact on corneal tissue quality and safety. Furthermore, the quantitative method to assess whole endothelium mortality can be implemented at eye banks for the evaluation of corneas intended for transplantation.

THE EVEIT BIOREACTOR SYSTEM AS PLATFORM FOR ARTIFICIAL CORNEA PROTOTYPES

Purpose: The corneal donor tissue is in short supply. Only a fraction of the demand is met. The tissues can vary in quality and sometimes have limited use. To address the issue, generation of artificial corneal grafts is intensively researched.

Various aspects of these prototypes need to be tested, ranging from structural integrity to cellular morphology. Our Ex Vivo Eye Irritation Test (EVEIT) is based on an air-lift organ culture system, where we currently are using rabbit corneas from food industry. We constantly expanded our capabilities in quantifying various parameters concerning metabolism, structural integrity and optical properties. This also opens up the possibility of using the system as a testing platform for prototypical artificial corneal constructs.

Methods: Various ophthalmological aspects can be investigated using the EVEIT system:

- Self-healing of superficial injuries and morphological characteristics can be observed over several days by live–tissue staining macroscopy.
- Metabolic parameters are recordable via the endothelial nutrient supply mechanism.
- Acute changes in internal pressure can be measured in the artificial anterior chamber with high resolution.
- Corneal barrier functions and pharmacokinetic properties can be quantified using photometric analysis methods.
- Dry–Eye model and established corneal edema models can be employed to test the efficacy of potential therapeutics.
- Advanced 3D design and printing methods allow us to quickly adapt the bioreactor, for example, to incorporate human corneas or to improve the mobility of the system.
- In order to comply with the 3Rs principle, testing of several different chemicals on one cornea is now also possible with the aid of automated multi–application.
- Recent developments of the EVEIT system include the engineering of an artificial eyelid model.

Results: Our long experience in using and optimizing the EVEIT system led to a unique adaptability to accommodate different testing conditions and requirements. Established disease models such as corneal edema and in dry eye syndrome (in process) are involved in testing new drugs.

Conclusion: Our established EVEIT system, in addition to its experimental capabilities, could contribute to the development of artificial corneal grafts in the future, as we have shown in previous work. The flexibility of the system allows us to adjust and improve an enormous range of test conditions and parameters.

ASSESSMENT OF PERFORMANCE AND SAFETY OF CORNEAL CHAMBER HYPERTHERMIC STORAGE AND PSS-L CORNEAL RINSING IN HUMAN AND PORCINE CORNEAS

Purpose: To prove the safety and performance of the hypothermic corneal storage medium Corneal Chamber, containing Eusol-C solution (AL.CHI.MI.A. S.r.l.) and of the rinsing solution PSS-L (AL.CHI.MI.A. S.r.l.) in support to the new CE certification process in accordance to the EU 2017/745 Medical Device Regulation.

Methods: Fifteen (n=15) human donor corneas unsuitable for transplantation and n=11 porcine corneas were evaluated for the following quality parameters: ECD, HEX%, CV%, endothelial morphology, endothelial mortality and transparency at day 0 and after 14±1 days (day 14) of storage in Corneal Chamber at 2-8°C. Then, corneas were rinsed in PSS-L for 1' at room temperature (RT) and the same parameters were assessed Post Rinsing (Day 14PR). In order to evaluate the antimicrobial carryover after the corneal storage in Corneal Chamber (14 days at 4°C), gentamicin sulphate was quantified in human and porcine corneas homogenates by UHPLC.

Results: Human and porcine corneas stored in Corneal Chamber at 2-8°C for 14 days showed a good overall quality of the tissue according to quality parameters evaluated. In particular, mean ECD, HEX% and CV% did not show statistically significant changes at the end of storage and endothelial mortality increased of 3.1±3.3% in human corneas and 7.8±3.5% in porcine corneas. Slight variations in endothelial morphology score and corneal transparency were observed. Rinsing with PSS-L did not negatively affect the quality parameters evaluated before and after rinsing and gentamicin sulfate residues were completely removed.

Conclusion: The storage of corneal tissues in Corneal Chamber at 2-8°C for 14 days and the corneal rinse with 30 ml of PSS-L at RT for 1 min are safe and effective procedures allowing the preservation of the corneal quality parameters including ECD, endothelial mortality, endothelial morphology, HEX%, CV%, and corneal transparency and the elimination of gentamicin sulfate from the tissues before transplantation.
FACTORS AFFECTING THE DENSITY OF CORNEAL ENDOTHELIAL CELL CULTURES OBTAINED FROM DONOR CORNEAS

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Factors affecting the density of corneal endothelial cell cultures obtained from donor corneas

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.

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 rebubbling 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.