

Purpose Human corneas preserved in bioreactor (BR) are characterized by not only a better endothelial viability, but also a more differentiated and stratified epithelium compared to corneas preserved in organoculture. By using corneal preservation in BR, we aimed to analyze the respective contribution of corneal (C), limbal (L), and conjunctival (Conj) epithelia in corneal epithelial regeneration.

Methods Five pairs of corneas from body donation to Science were used with a death-to-collection time <20 hours. A 3- to 5-mm-wide conjunctival flange was kept intact. Five patterns were set up by complete mechanical removal of 1, 2, or 3 epithelia (-): C-L+Conj+, C-L-Conj+, C-L+Conj-, C+L-Conj-, C-L-Conj- (control) (n=2 for each pattern). The L epithelia was destroyed by scraping and thermocoagulation. Corneas were then kept in BR (21mmHg, 2.5µl/min of Corneamax Eurobio, 31°C) for 3 weeks to allow epithelial regeneration. The epithelium was then analyzed using immunofluorescence (IF) on flat mounted cornea by targeting CK12 (corneal epithelium) and CK15 (limbal epithelium). Cell nuclei were counterstained with DAPI. Corneal transparency was quantified using a transpometer.

Results No epithelium was reconstituted in the C-L-Conj- control group. In the other 4 models including the C-L-Conj+ group, the cornea was transparent and covered by a pluristratified corneal epithelium, characterized by CK12 expression.

Conclusion In this BR model, conjunctival epithelial cells alone allowed the regeneration of a typical corneal epithelium whereas corneal epithelium was able to migrate to the limbus and conjunctiva. We hypothesize that all 3 ocular surface epithelia contain stem cells or progenitors able to migrate throughout the cornea and restore the corneal epithelium independently of each other. The main difference between our ex vivo model and in vivo situation is the absence of neovascularization. This suggests that the main cause of limbic insufficiency is due to the loss of the anti-angiogenic barrier rather than the loss of limbic stem cells.

P34-A132 AIR-LIFT CULTIVATION OF HUMAN CORNEAL DONOR TISSUES

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Purpose Corneal donor tissue can be used for a number of different reconstructive surgical operations involving the rehabilitation of injured or degraded anterior, posterior and intermediate corneal lamellas.

Potential corneal donor tissues undergo a rigorous screening process including medical evaluation of the endothelial and stromal layers. Depending on this assessment, the tissue's scope of use is often narrowed down to few types of emergency procedures mainly due to an insufficient number of viable endothelial cells or divergent cell morphology.

In addition to all these limitations, one must not ignore the sometimes critical post-preparation degeneration caused by the submerged culturing process itself, leading to epithelial debridement and stromal edema. All these factors reduce the already short supply of donor corneas. In this study, we aim to optimize this culturing process to avoid tissue degradation.

Methods We used an organ culturing system based on our long established Ex Vivo Eye Irritation Test System (EVEIT) (Spöler et al., 2015). This bioreactor has been modified in size and shape to accommodate human-sized corneal explants. The established mechanisms for supplying the cornea with nutrients and physiologically relevant pressure conditions were adapted to support sterility. Human donor corneas that failed the initial quality protocol and which are released for research were obtained from our cornea bank and inserted in the EVEIT culture system. Corneal integrity was observed during the cultivation period of 19 days.

Results The human cornea observed maintained transparency in contrast to what generally can be observed in the established European culturing system with submersion of the cornea. Final endothelial layer examinations confirmed the presence of viable endothelial cells, as documented during initial corneal bank quality control.

Conclusion With this proof of principle, we confirmed that we can maintain the integrity of the human donor cornea in our modified EVEIT organ culture system. Further investigation, optimization and confirmation will be pursued to meet medical regulations.

P35-A133 NOVEL ECCENTRIC CORNEOSCLERAL DONOR PREPARATION TECHNIQUE PROVIDING CORNEOSCLERAL TECTONIC AND CENTRAL SPLIT CORNEAL GRAFTS FOR MULTIPLE RECIPIENTS

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Today, split cornea technique is an established procedure and is mostly used for two recipients by combining deep anterior lamellar keratoplasty (DALK) and Descemet membrane endothelial keratoplasty (DMEK) surgeries. However, for some surgical interventions including block excision with tectonic corneoscleral grafting, split cornea procedure is not planned regularly up to now. In the run-up for this procedure, normally a donor cornea with a bigger scleral ring is gained. Nonetheless, the preparation of the tectonic graft for covering the corneoscleral defect after block excision results in a rest donor cornea transplant which is normally too small for further regular size penetrating keratoplasties (PKs) or combined DALK/DMEK surgeries. However, using a modified donor transplant trephination technique, a corneoscleral transplant for regular size keratoplasties can be gained, also after preparation of a tectonic graft for block excision. Herein, we describe shortly this novel donor preparation technique, the differences compared to the standard procedure, possible applications, and the advantages and disadvantages for the first time.