P03-A138 COMPARISON OF SPECULAR AND LIGHT TRANSMISSION MICROSCOPY FOR ENDOTHELIAL CONTROL OF CORNEAS STORED IN THE ACTIVE STORAGE MACHINE

[1]Emmanuel Crouzet, [2]Gabrielle Dumont, [1]Benjamin Peyret, [2]Pascal Herbepin, [1,3]Sylvain Poinard, [1,3]Oliver Dorado, [4]Sandrine Ninotta, [4]Sophie Acquart, [1,3]Philippe Gain, [1,3]Gilles Thuret. [1]*Biology, Engineering and Imaging for Ophthalmology, BiiO, Faculty of Medicine, Health Innovation Campus, Jean Monnet University, Saint-Etienne, France*; [2]*Sincler, Clermont-Ferrand, France*; [3]*Department of Ophthalmology, University Hospital, Saint-Etienne, France*; [4]*Eye Bank. French Blood Center Auvergne Rhône Alpes, Saint-Etienne, France*

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Purpose The Active Storage Machine (ASM), designed by Sincler (a company of group Laboratoires Théa) for eyebanks, will be used for long term donor corneas preservation at 31°C before transplantation. In this device, the endothelial cell density (ECD) counting is expected to be performed non-invasively throughout the storage, thus without changing the storage medium nor handling the cornea. To meet these constraints, specular microscopy (SM), also used for cold storage was selected, instead of the standard light transmission microscopy (LTM-NaCl) used in eye banks storing corneas in organ culture at 31-34°C. The purpose of this study is to compare both imaging methods for ECD measurement of corneas preserved in ASM.

Methods Five human corneas from body donation to Science were preserved in a prototype ASM with 35mmHg in the endothelial chamber, 2.5µl/min of Corneamax® (Eurobio, France) at 31°C for 5 days. The endothelium of the cornea was imaged through the ASM window using the CellChek® D+ SM (Konan Medical, California, United-States) equipped with an add on device at customized stage. The cornea was then immediately removed from the ASM and prepared for standard endothelial assessment (dilation of the intercellular spaces using 0.9% NaCl and light transmission imaging). Finally, the endothelium was stained with alizarine red and trypan blue and observed again with the same microscope, to determine ECD using the referenced method up to now. For each cornea and each observation method, 5 images were acquired: 1 central and 4 paracentral. The SM images were counted with the Konan software. The LTM-NaCl and 'Alizarin Red' counts were performed with a dedicated plugin of ImageJ after microscope calibration.

Results The means ± SD of the ECD calculated for SM, LTM-NaCl and 'Alizarine' images were respectively of 2314 ± 537, 2243 ± 506 and 2354 ± 543 cells/mm^2. There was no significant difference between the 3 methods (ANOVA one-way, p-value = 0.1066). The percentage error was -1.7% +/- 3.3% for SM and -4.7 +/- 4.0% for light transmission microscopy.

Conclusion Quality control of the endothelium of corneas stored in ASM can be performed non-invasively with a standard eye bank SM. The ECD measured by SM does not differ from that measured by the conventional microscopy technique used until now in organoculture.

P04-A115 SERUM CONTAINING ORGAN CULTURE OF THE HUMAN CORNEA – STILL STATE OF THE ART?

[1]Nicola Hofmann, [1]Anna-Katharina Salz, [1]Martin Börgel, [2]Katrin Engelmann. [1]*Deutsche Gesellschaft für Gewebetransplantation, DGFG, Germany*; [2]*Augenklinik, Klinikum Chemnitz gGmbH, Germany*

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Purpose For decades, human corneas are prepared and stored in specialized tissue banks prior to transplantation. Especially in Europe, storage takes place in 'organ culture', the storage in cell culture medium at approximately physiological temperature. Traditionally, a serum-containing medium is used for this purpose. However, the use of fetal calf serum has considerable disadvantages: there is a risk of disease transmission, availability may not always be guaranteed in the necessary quality, there are considerable differences from batch to batch, which is associated with batch testing required in each case, and last but not least, the extraction of serum from unborn calves is an ethical issue.

Methods In recent years, several studies have focused on the improvement of organ culture conditions for donor corneas, including different serum-free media and alternative deswelling substances. Meanwhile, media are on the market which seem to be equivalent to serum-supplemented MEM. Nevertheless, serum-free medium has not yet found its way into routine organ culture of corneas.

Results Our own preliminary studies have shown that despite the promising approaches, no satisfactory overall result could be achieved. Since only maintenance metabolism is required for storage of corneas until transplantation, in principle cultivation in the conventionally used medium seems possible without addition of serum at all. Corneas stored in this way had comparably endothelial cell density (ECD) to their counterpart stored in serum-supplemented medium. However, during the final evaluation after deswelling, the ECD dropped drastically.

Engelmann et al. started research on the use of serum-free culture medium (SFM) for a long time and comparable or even superior ECD and viability could be demonstrated. So far, however, it has not been possible to define a deswelling medium adapted to these conditions.

Also, a serum-free storage medium developed by Eurobio (CorneaSyn) could not completely convince, because although ECD of the examined corneas remained constant, the morphology of the cells changed.

Conclusion Since it is essential to intensify efforts towards a serum-free system it is planned to test serum substitutes and, if possible, also to replace the de-swelling additive dextran with a less harmful alternative to guarantee the quality of cornea grafts in the future.