

EEBA 2023 Session I – Tissue banking: Lamellar Grafts; Corneal Storage Methods; Quality Assurance

P01-A120 PRE-DISSECTED DMEK STORED IN AN ACTIVE STORAGE MACHINE: FEASIBILITY STUDY

^{1,2}Paul Goin, ^{1,3}Sylvain Poinard, ^{1,3}Oliver Dorado, ¹Chantal Perrache, ¹Zhiguo He, ^{1,4}Sandrine Ninotta, ^{1,5}Frédéric Mascarelli, ^{1,3}Philippe Gain, ^{1,3}Gilles Thuret, ²Anne-Sophie Gauthier. ¹Biology, Engineering and Imaging in Ophthalmology (BiiO), EA2521, Faculty of Medicine, Jean Monnet University, Saint-Etienne, France; ²Department of Ophthalmology, University Hospital of Besançon, France; ³Department of Ophthalmology, University Hospital of Saint Etienne, France; ⁴Cornea Bank, EFS Rhône-Alpes-Auvergne, Saint Etienne, France; ⁵Cordeliers Research Center, UMR S1138, Paris Descartes University, Paris, France

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Purpose The number of endothelial grafts precut by eye banks increases. Their shelf life is limited to a few days. We previously demonstrated the superiority of an active storage machine (ASM) over organ culture (passive) for whole corneas. Aims: To measure the endothelial viability of pre-dissected DMEK after 3 and 10 days of storage in our ASM in a preclinical study.

Methods Pairs of human corneas were included. The endothelial cell density (ECD in cells/mm²), thickness and transparency of corneas were measured before graft preparation. Descemet's membrane (DM) was peeled using the no-touch technique leaving the graft attached to the center of the cornea (on approx. 1mm²). After randomization, one cornea was kept in organ culture (OC) and the other in the ASM (21 mmHg, 2.6 µL/min) in the same medium (CorneaMax, Eurobio). The final viable ECD was determined using the triple staining with Hoechst-Ethidium-Calcein-AM. In addition, the expression of CD166 and NCAM (lateral membranes), ZO-1 (apical junctions), Na⁺/K⁺ ATPase (endothelial pump function) and COX-IV (mitochondrial content) was studied by immunostaining to characterize endothelial cells after the storage.

Results Initial ECDs were comparable: 2185±232 cells/mm² in the ASM versus 2276±328 in OC for the 3-day period and 2680±416 cells/mm² in the ASM versus 2644±420 in OC for the 10-day period. The DMs did not fold back in either BR or OC. The viable ECD did not differ significantly between the ASM and OC for either storage period: 2378±501 (ASM) versus 2342±503 (OC) for the 3-day period (n=8 pairs and p=0.624) and 2482±288 (ASM) versus 2579±315 (OC) for the 10-day period (n=5 pairs and p=0.176). Corneas were more transparent and thinner in the ASM than in OC after 3 days (916±86 versus 1193±136µm, p=0.0001) and 10 days (957±128 versus 1220±105µm, p=0.0625). The functional and structural markers studied were expressed in both groups after 3 and 10 days, some better preserved in the ASM.

Conclusion The storage of precut DMEKs is possible in ASM and OC for at least 10 days. Interestingly, a pre-dissected endothelium continues to partially exert its pump function into the ASM. In practice, this could allow the stroma to be used for DALK without further deswelling. In addition to

improving the storage of whole grafts, the ASM allows the storage of precut DMEKs for up to 10 days with excellent endothelial survival.

P02-A121 PRECUT DSAEK STORED IN AN ACTIVE STORAGE MACHINE: FEASIBILITY STUDY

^{1,2}Sylvain Poinard, ^{1,2}Oliver Dorado, ^{1,3}Paul Goin, ¹Chantal Perrache, ¹Zhiguo He, ¹Sandrine Ninotta, ^{1,4}Frédéric Mascarelli, ^{1,2}Philippe Gain, ^{1,2}Gilles Thuret, ^{1,3}Anne-Sophie Gauthier. ¹Laboratoire de Biologie, Ingénierie et Imagerie pour l'Ophthalmologie, BiiO, Faculté de Médecine, Université de Jean Monnet, 10 rue de la Marandière, 42270 Saint-Etienne, France; ²Département Ophthalmologie, Centre Hospitalier Universitaire, Avenue Albert Raimond, 42055 Saint-Etienne Cedex 02, France; ³Département Ophthalmologie, Centre Hospitalier Universitaire, 25000 Besançon, France; ⁴Centre de Recherche des Cordeliers, UMR S1138, Université Paris Descartes, Paris, France

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Purpose The number of endothelial grafts precut by eye banks increases. Their shelf life is limited to a few days. We previously demonstrated the superiority of an active storage machine (ASM) over organ culture (passive) for whole corneas. Aims: to measure endothelial viability of precut DSAEK after 3 or 10 days of storage in our ASM in a preclinical study.

Methods Human pairs of corneas were included. The endothelial cell density (ECD in cells/mm²), and central corneal thickness (CCT in µm) were measured to ensure their initial intra pair comparability. After deswelling (CorneaJet, Eurobio) grafts preparation was performed by cutting the anterior stroma with a Moria linear microkeratome and keeping the anterior lamellae attached during storage. After randomization, one cornea was kept in the corneajet bottle (CJ) and the other was inserted into the ASM allowing a renewal or storage medium (CorneaMax, Eurobio) at 2.6 µL/min with 21 mmHg of pressure in the endothelial chamber. Both group of corneas were stored for 3 or 10 days at 31°C. The final viable ECD (vECD) was determined using the triple staining with Hoechst-Ethidium-Calcein-AM by an independent experimenter in a masked fashion.

Results Initial ECDs were comparable: 2595±878 in ASM versus 2654±954 cells/mm² in CJ for the 3-days period (n=5 pairs) and 2416±712 in ASM versus 2492±764 cells/mm² in CJ for the 10-period (n=5 pairs). CCTs were also comparable. The anterior lamellae stayed attached in either the ASM or CJ. vECD was significantly higher in ASM than in CJ with respectively 2062±695 cells/mm² versus 1632±633 cells/mm² after 3 days either a cell loss of 20.5% and 38.5% respectively (p=0.0062) and 1082±649 versus 935±691 cells/mm² for the 10-day period either a cell loss of 132% and 164% respectively (p=0.005). Grafts thickness did not differ after 3 days 219±25 µm in ASM versus 182±39 µm (p=0.063) or 10 days respectively 221±58 µm versus 189±48 µm (p=0.06).

Conclusion The storage of precut DSAEKs into the ASM allows a better preservation of grafts without use on deswelling storage medium. Nevertheless, the cell loss remains high after 10 days, suggesting a significant cell stress.