Regenerative retinal cells: The optimal environment to improve graft quality

On March 11, 2021, Ayyad Zartasht Khan defended his thesis titled "Culture and Storage of Retinal Pigment Epithelial Cells for Regenerative Medicine Purposes and the Use of Sericin to Improve Graft Quality" at the Institute of Clinical Medicine, Faculty of Medicine, University of Oslo. The research was conducted at the Department of Medical Biochemistry, Oslo University Hospital in collaboration with the Schepens Eye Research Institute of Massachusetts Eye and Ear at Harvard Medical School. His supervisors were Jon Roger Eidet (main), Tor Paaske Utheim, and Morten Carstens Moe.

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Introduction: Transplantation of retinal pigment epithelial cells (RPE) is gaining popularity as a potential treatment modality for sight-threatening diseases. The aim of this thesis was to (1) develop a system to assess the quality of cultured epithelia prior to transplantation, (2) identify a suitable storage temperature and storage medium for the short-term hypothermic storage of mature RPE cell sheets, and (3) explore whether RPE quality (viability, maturation, and morphology) could be improved using the silk protein sericin as a culture medium additive.

Methods: Experiments were conducted on cultured primary human RPE, adult retinal pigment epithelial cell line-19 (ARPE-19), induced pluripotent stem cell-derived RPE (iPSC-RPE), human conjunctival epithelial cells, and human epidermal keratinocytes.

Results: The thesis (1) presents a computerized method to non-invasively assess cell grafts prior to transplantation, (2) suggests that temperatures between 4°C and 16°C are suitable for short-term hypothermic storage of mature RPE cell grafts, (3) describes a serum-free storage medium for short-term storage of differentiated RPE, and (4) shows that supplementing the culture medium with 10 mg/mL sericin improves RPE viability, maturation, and morphology in vitro.

Conclusion: Novel, cell-based therapies are garnering significant interest. The optimization of cell culture, storage, and transportation is important for standardization and expanding access to these treatment alternatives.

Conflict of interest: Jon Roger Eidet and Tor Paaske Utheim hold a patent on the use of sericin in culture media (European Patent Number EP3317404), filed by Inven2 (the technology transfer office of the University of Oslo and Oslo University Hospital).

Key points:

- Culture, storage, and maturation of retinal cells were investigated to find optimal conditions.
- In addition to the field of retinal regenerative medicine, the work carries potential implications for our understanding of retinal physiology, epithelial cells, and the process of melanogenesis.

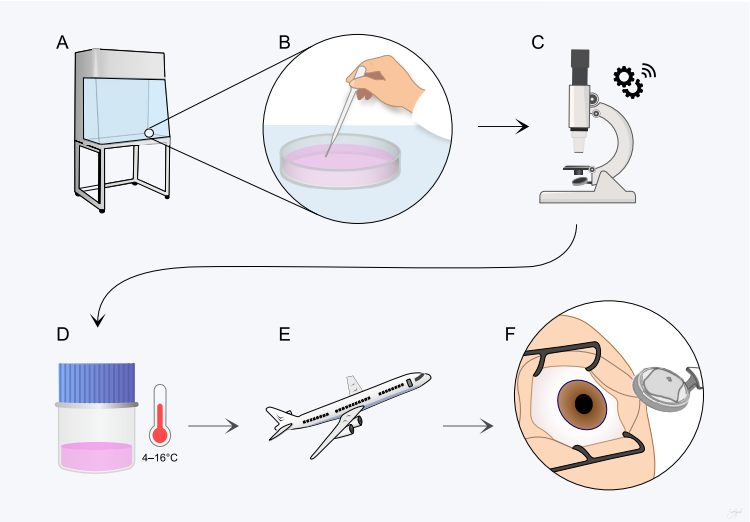

Figure 1. In this PhD thesis, the research group studied various stages in the "pipeline" of tissue engineering. Using cultured retinal pigment epithelial cells as a model, we studied in vitro culture (A) and cell culture media (B), developed a computerized method to non-invasively assess sheets of cell grafts (C), and assessed storage conditions (D) that can enable transportation (E) of tissue-engineered products from bench to bedside (F). Illustration graciously provided by Sara Nøland.

Articles in the dissertation

1. Khan AZ, et al. Nucleus Morphometry in Cultured Epithelial Cells Correlates with Phenotype. *Microsc Microanal*. 2016;22(3):612-620.
2. Khan AZ, et al. Cultured Human Retinal Pigment Epithelial (hRPE) Sheets: A Search for Suitable Storage Conditions. *Microsc Microanal*. 2018;24(2):147-155.
3. Khan AZ, et al. The Silk Protein Sericin Promotes Viability of ARPE-19 and Induced Pluripotent Stem Cell-Derived Retinal Pigment Epithelial Cells in vitro. *Curr Eye Res*. 2021;46(4):504-514.
4. Khan AZ, et al. Sericin-Induced Melanogenesis in Cultured Retinal Pigment Epithelial Cells Is Associated with Elevated Levels of Hydrogen Peroxide and Inflammatory Proteins. *Molecules*. 2020;25(19):4395.