

## EEBA 2023 Session VI – Poster II

**P36-A151 PRIMARY HUMAN RPE CELLS AS INDIVIDUAL STARTING MATERIAL FOR NON-VIRAL GENE TRANSFER OF THERAPEUTICALLY ACTIVE FACTORS**

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**Purpose** Our aging society leads to an increasing incidence of neurodegenerative diseases. To date, the development of defined therapies has been hampered because the pathological mechanisms are poorly understood. Cell-based additive gene therapies to enhance the expression of protective factors are considered a promising modality for the treatment of neurodegenerative diseases, such as age-related macular degeneration (AMD). We have developed a method to stably overexpress the genes encoding pigment epithelium-derived factor (PEDF) and brain-derived neurotrophic factor (BDNF) into the genome of primary human retinal pigment epithelial (RPE) cells by electroporation using the Sleeping Beauty (SB) transposon system. BDNF is the most abundant neurotrophin in the central nervous system. PEDF is a multifunctional protein with anti-angiogenic and neurotrophic properties.

**Methods** Primary RPE cells were isolated from various human donor eyes and maintained individually in culture. After reaching confluence, RPE cells were trypsinized and co-transfected in suspension with two plasmids encoding SB100X transposase and the transposon carrying a PEDF and BDNF transcription cassette, respectively. The results of transfection were evaluated by different methods including microscopy, immunoblotting, ELISA, and quantitative PCR (qPCR).

**Results** Seeding of sufficient numbers of primary human RPE cells allows cultivation and growth into an integrated monolayer of pigmented, hexagonally shaped cells, independent of the donor age ( $65.3 \pm 9.94$  a, min: 49 a, max: 83 a, n = 12), post-mortem time of isolation ( $37.3 \pm 17.0$  h, min: 16 h, max: 68 h), and cultivation time ( $27.6 \pm 14.1$  d, min: 13 d, max: 61 d). Successful transfection was demonstrated in experiments performed independently. Applied electrical pulses had no negative effects on cell morphology. Gene expression of PEDF and BDNF was significantly increased compared with non-transfected control cells. Secretion of recombinant PEDF and BDNF proteins was also significantly elevated and remained stable over time.

**Conclusion** The studies using primary human RPE cells are an important step in the development of a cell-based PEDF or BDNF gene therapy that could be applied as an advanced therapy medicinal product to treat AMD or other degenerative retinal diseases.

**P37-A124 SMILE BANK – CORNEAL STROMAL LENTICULES FROM RELEX SMILE – THE NEW EYE BANK PRODUCT**

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**Purpose** The aim of our presentation is to introduce future eye bank product - corneal stromal lenticule from living donors, which can be used for allotransplantation.

**Methods** ReLEx (refractive lenticule extraction) SMILE (small incision lenticule extraction) is a common approach in laser eye surgery. It is minimally invasive and flap-free procedure. During this procedure part of corneal stroma (lenticule) is created by femtosecond laser and consequently removed through small incision. The lenticule is basically waste material of the ReLEx SMILE procedure. In the International Eye Bank of Prague, we decided to establish new protocol for lenticule withdrawal, storage and release for transplantation.

**Results** All donors signed an informed consent, and their serum was tested for the presence of infectious diseases. After ReLEx SMILE procedure the lenticule was stored in container with cryopreservation solution and frozen in the eye bank using the same protocol for frozen amniotic membrane. After 6 months in  $-80^{\circ}\text{C}$  tissues were defrosted and examined histologically, using conventional light histology staining and electron microscopy.

**Conclusion** We believe, that lenticule from living donor is a safe and effective tissue, that can be used for many indications and in particular situations represents good alternative to whole donor cornea and amniotic membrane.

**P38-A108 OPTIMIZATION OF THE TRIPLE HEC STAINING FOR LABORATORY ASSESSMENT OF DMEK GRAFT QUALITY**

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**Introduction** The quality of the endothelial graft is critical to the success of DMEK and to the survival time of the graft. The peeling technique, preservation method, and skill level of graft preparers need to be evaluated and validated. The most reliable method of evaluation is the viability test based on a triple staining of Hoechst- Ethidium-Calcein AM (H-E-C) which allows the determination of the total number of viable cells on the graft. However, this test has some shortcomings for DMEK grafts: 1) The undesirable fluorescence of the Calcein AM stain prevents accurate viability analysis, especially in cases where the graft is attached to the cornea for preservation; 2) Incompatibility with immunofluorescence (IF) that could provide additional information. The objective of this study is to develop technical tricks to overcome these drawbacks.

**Methods** Two strategies were employed to improve Calcein AM staining: 1. Increase the specific fluorescence intensity by changing the diluent and the concentration of Calcein AM; 2. Decrease undesired fluorescence from keratocytes by adding Trypan Blue (BT). In order to combine the IF after the HEC test, an extension wash in PBS was performed.

**Results** Calcein AM at  $4\mu\text{M}$  diluted in OptiMEM increased fluorescence intensity 3-fold ( $p=0.0017$ ,  $n=5$ ) compared with conventional staining at  $2\mu\text{M}$  in PBS. BT decreased the undesired fluorescence of Calcein and thus optimized count variability between different operators by 42% ( $p=0.0027$ ,  $n=10$ )