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 neurotrophic factor (BDNF) into the genome of primary human retinal pigment epithelial cell line. The results of transfection were evaluated by different methods including microscopy, immunoblotting, ELISA, and quantitative PCR (qPCR).

Results Seedling 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 ± 9.94 a, min: 49 a, max: 83 a, n = 12), post-mortem time of isolation (37.3 ± 17.0 h, min: 16 h, max: 68 h), and cultivation time (27.6 ± 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.

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°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.
and saved 40% (p=0.0002, n=10) of count time. To perform IF after HEC, prolonged washing in PBS is an effective method to remove residual Calcein fluorescence and allows release of the FITC/Alexa 488 filter.

**Conclusion** This study provides effective technical tips for optimizing the endothelial viability assay using Calcein AM and for performing IF after the viability assay.

**P39-A145 ANALYSIS OF CORNEA DONATION PROGRAM IN CROATIA**

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**Purpose** Croatian Tissue and Cell Bank (CTCB) regularly monitors the effectiveness of cornea donation program on the national level. All hospitals are required to have designated tissue donation coordinators in charge of detection, family interview and tissue procurement. If hospital has cornea donation program only from donors after brain death (DBD), tissue donation coordinator can be the same as for organs. Five collection centres have cornea donation program for donors after circulatory death (DCD) with designated cornea donation coordinators.

**Methods** We retrospectively analyzed all monthly reports from tissue donation coordinators in the period from May 2019 to September 2022. Additional data was collected from national organ and tissue database Croatian National Transplantation Network (NTM).

**Results** During the analyzed period, 25,753 deaths were recorded, from which 38.6% to 54.7% of DCD and 0.6% to 1.1% of DBD donors were considered for cornea donation, depending on the hospital. Out of all deceased, 2.4% to 5.2% of patients were realized as cornea donors, 0.4 to 0.5% of which were DCD and 2 to 4.7% were DCD. Cornea donations were realized in 18.2% to 38.9% cases of all DBD donors. As SARS-CoV-2 pandemic has stratched in March 2020, the cumulative number of donations declined for 26.1% in 2020 and 12.1% in 2021, compared to the pre-pandemic 2019. Moreover, CTCB received 30.5% less DCD in 2020 and 21.9% less in 2021. Despite that, we recorded increase in DBD during 2020 and 2021, for 13.3% and 44.7%, respectively. The same trend continued throughout 2022, where only until September 16,1% more DBD were received than in the whole 2019.

**Conclusion** Hospitals involved in cornea donation program record high number of deaths, however only a small proportion of which are realized for cornea donation. This is particularly pronounced in DBD donors. SARS-CoV-2 pandemic left significant impact on donation program. However, CTCB recorded higher number of DBD donors during that period. The current situation leaves plenty of room for improvement of CTCB and corresponding donation hospitals, to increase disproportionately low rate of cornea procurement in respect to the total rate of deaths and considered donors.

**P40-A122 HOW TO ESTABLISH SUCCESSFUL NETWORKING: EDUCATIONAL TRAINING & EXCHANGE OF EXPERIENCES**

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**Introduction** Educational training within eye bank staff is needed to fulfill legal requirements and to keep staff up-to-date in times of rapid change and innovation.

Especially when starting with eye banking a good and close contact to experienced colleagues could be of great benefit for both parties - the newcomers and old stagers.

**Purpose** The exchange of experiences and the mutual support in key processes of eye retrieval and banking contributes to the establishment of a structured and functional cooperation with the further goal of establishing a successful network far beyond the national borders.

**Materials and Methods** In July 2018 a first visit of Hornhautbank Munich team in Malta was organized followed by a visit of Malta staff members in November 2018 and July 2019 and a further visit in August 2022 after a longer pause related to Covid-pandemic.

The SOPs of both facilities were compared with regard to local regulations and analyzed to assess how they can best be implemented taking into account local regulations and conditions.

Hands-on training in in-situ-excision and the evaluation of the retrieved donor corneas using slit lamp- and endothelial-microscopy deepened the theory for practical implementation.

**Results** Training materials have been loaned to the team in Malta for further training, and joint online meetings are planned for further training and sharing of difficult case reports to provide the team with appropriate assurance in all eye bank areas.

Such cooperation has increased the confidence of the teams and supported the licensing inspections by competent authorities.