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## SIGNIFICANT DIFFERENCES BETWEEN SPECULAR MICROSCOPY AND CORNEAL BANK ENDOTHELIAL CELL COUNTS— A PILOT STUDY

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### Content

It was shown recently that endothelial cell count performed by cornea banks overestimates the real number of endothelial cells. The aim of this study was to investigate the internal quality of preclinical ECD in human donor corneas using two widely used methods for endothelial cell counting, transmitted light microscopy used in organ culture tissue bank and clinically used specular microscopy.

20 human donor corneas that could not be transplanted were included into this analysis. Differences in evaluating endothelial cell density (ECD) and hexagonal endothelial cell ratio (HEX) between clinical specular microscopy (CSM) and corneal bank transmitted light microscope (CBLM) were evaluated as well as differences between automated and manual cell counts.

Automated CBLM showed a higher ECD of 31,85% compared to automated CSM, while manual CBLM counting is 10,51% higher compared to manual CSM ($p<0.01$). Further, higher average ECD values results in a higher difference between CSM and CBLM measurements. The manual CBLM ECDs were significantly higher compared to automated derived ECD from CSM ($p<0.01$). However, no systematic bias can be detected when comparing the differences of the measurements with the average ECD measurements of both methods.

This preclinical pilot study confirmed a significant higher ECD using transmitted light microscopy in organ culture compared to clinical specular microscopy. This indicates that the early rapid decrease of EC universally observed after surgery might be partly artefactual.

## DECONTAMINATION OF WHOLE DONOR EYE GLOBES WITH A COMMERCIAL ANTIBIOTIC COCKTAIL MEDICAL DEVICE

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### Content

**Purpose** : The human ocular surface bears normal microbial flora and may include wide range of bacterial and/or fungal contaminants. In donor cornea transplantation a decontamination step is required in order to reduce the cornea contamination. At Eye Bank of Liege University Hospital, Belgium, the ocular surface decontamination is performed with 5% iodopovidone solution quick wash on whole globe before cornea dissection. The aim of this study is to evaluate the use of decontamination solution BASE128 (ALCHIMIA) for decontamination of the whole donor eye globe, before cornea recovery, at the Eye Bank of Liege University Hospital.

**Material&Methods:** Human donor eye globes, unsuitable for transplantation, were recovered and transported in physiological solution. All corneas were evaluated according to the procedures of the Eye Bank (Endothelial cell density (ECD), slit lamp evaluation (SL), central corneal thickness (CCT)). A microbiological test was performed on physiological transportation medium using Bact/Alert for 7 days, and a corneal swab was taken. Eye globes were immersed in BASE.128, an antibiotic cocktail containing vancomycin, gentamicin, cefotaxime, and amphotericin B deoxycholate. After decontamination phase, a microbiological and a corneal swab test were repeated. Cornea was dissected and transferred in cold storage medium (EUSOL-C, ALCHIMIA). ECD and CCT were evaluated again. Cornea was stored for 14 days at 4°C. After 14 days of storage, ECD and CCT were determined and a microbiological and a corneal swab test were performed.

**Results**: Whole globes were initially contaminated. After decontamination, all the microbiological tests resulted negative. Corneal evaluation parameters including ECD and CCT remained unvaried according to expected values.

**Conclusion**: Decontamination of whole donor eye globe with BASE.128 allowed total elimination of cornea contamination without altering corneal quality.


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## NEW DEVICE FOR PENETRATING KERATOPLASTY SURGERY TO STOP AND TREAT AN INTRAOPERATIVE EXPULSIVE HEMORRHAGE

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### Content

**Purpose**: IEH is one of the most serious complications in intraocular open-sky surgery, specially in PK when the eye is open after trephination. We present a new device for PK surgery to stop and contain IEH. The device consists of a magnetised cover and a metallic scleral ring sutured to the sclera, which allows complete sealing of the ocular cavity thanks to magnetic forces. In order to achieve the correct alignment of the magnets, the cover was designed using designing, testing and simulating software to evaluate the device adaptability to the human eye geometry. The final prototype was fabricated through additive manufacturing.

**Materials & Methods**: We performed an experimental study simulating an IEH during PK in human eyes from the Eye Bank. Thus, the scleral ring was sutured with 6 Nylon 10-0 interrupted sutures and an infusion for retinal vitreous surgery was inserted via pars plana using a 23G needle before trepanation. After starting the infusion, the device was placed over the scleral ring in order to seal the ocular cavity. Infusion pressure was increased gradually and monitored until fluid leaked.

**Results**: We observed that the device was able to handle pressures between 40 and 85 mmHg before leaking occurred. The device implantation was easy and quick, and performed successfully for high intraocular pressures.

**Conclusion**: This new magnetic device may represent a valid and safe alternative for successfully arresting and controlling IEH.

## VALIDATION AND ASSESSMENT OF AN ANTIBIOTIC DECONTAMINATION MANUFACTURING PROTOCOL FOR VACUUM-DRIED HUMAN AMNION

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### Content

**Purpose**: Elective caesarean section-delivered amniotic membrane (CAM) contains microbial bioburden that requires decontamination before transplantation. This study assessed the decontamination ability of the Tereo® manufacture process to produce the commercial dry amnion product Omnigen® (NuVision Biotherapies, UK).

**Methods**: Bioburden of 10 fresh CAM were assessed using in vitro microbial culture. Subsequently, the Tereo process was assessed for decontamination ability. Five CAM were artificially loaded (106CFU/mL) with Staphylococcusepidermidis at three different stages of processing; i) before the full manufacture processing; ii) prior to antibiotic treatment; and iii) immediately before drying, and resulting products assessed by microbial cultures. The long-term stability and antimicrobial activity of 10 Omnigen was assessed after 2.3 year storage; antibacterial activity of non-antibiotic treated CAM compared to Omnigen was evaluated using MIC/MBC, and disc diffusion assays against Meticillin-resistant Staphylococcus aureus, Meticillin-resistant S.epidermidis, Escherichia coli, Pseudomonas aeruginosa and Enterococcus. faecalis.

**Results**: Bioburden of CAM was found to be very low. The Tereo process was highly effcient at removing bioburden introduced at any stage of processing. The combined process was comparable to a sterilising process. CAM not treated with antibiotics was shown not to be antimicrobial. However, Omnigen demonstrated ffective antibacterial capacity against all bacteria tested. Antimicrobial activity of Omnigen was not reduced after 2-year storage.

**Conclusion**: Antibiotic decontamination is a reliable method for sterilization of CAM and the resultant antibiotic reservoir is effective against gram-positive and –negative bacteria. The research suggests, amnion products manufactured without the use of antibiotics possess little antimicrobial activity. However, Omnigen may be useful in the treatment strategy of microbial keratitis.


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## INNOVATIVE AND EASILY ACCESSIBLE DRY PREPARATION OF HUMAN AMNIOTIC MEMBRANE FOR ENHANCED TREATMENT OF OCULAR SURFACE AND WOUND CARE CONDITIONS

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### Content

**Purpose**: Human amniotic membrane (hAM) has considerable utility in the treatment of many ocular surface indications. Unfortunately, in many countries, availability of hAM suitable for clinical use is limited to frozen hAM products, which are difficult to store and use. Freezing also damages the tissue, compromising the biochemical and functional properties. We propose a novel dry-preserved hAM which retains the biochemical and functional properties for improved utility, enhanced wound-healing and regenerative function.

**Methods**: Cryopreserved human amnion (CPAM) and AM delicately dried using low temperature vacuum evaporation (Omnigen®) were prepared and assessed in vitro for changes in composition; in vitro biocompatibility; and in vivopre-clinical biocompatibility and wound healing, supported by ex vivoanalyses of wound healing using electron microscopy for tissue structural changes, and RT-qPCR to compare changes in wound healing gene expression.

**Results**: Structural: Drying AM devitalised cells, but unlike CPAM, cellular and structural integrity of Omnigen is preserved. Biochemical: Omnigen demonstrated highest biochemical retention efficiencies and bioavailability compared to frozen-AM. *In vitro* culture: Omnigen demonstrated enhanced cell viability andhealth, and wound healing.

*In vivo and ex vivoanalysis*: Xeno-transplanted Omnigen was easily orientated and applied directly dry. Omnigen promoted improved epithelial defect recovery rates comparable to frozen AM, with increased expression of wound healing factors (EGF, TGF-β 1, Collagen-IV and MMP9).

Conclusions: Omnigen is an enhanced hAM product that overcomes existing limitations of frozen AM. It is easy to store and ship, simple to use, and possess enhanced wound healing properties. Omnigen has the potential to be available at the point of care providing ophthalmologists with an improved sight saving therapy.

## EXTRACTION OF CRYSTALLINE PROTEINS FROM PORCINE EYE LENS AND EVALUATION OF THEIR RELATION WITH OXIDATIVE STRESS

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### Content

**Purpose**: To extract crystalline proteins from porcine eye lenses and to evaluate their relation with oxidative stress.

**Methods**: 10 lenses were extracted from porcine eyes. The lenses were washed with phosphate-buffered saline (PBS)and homogenized in extraction buffer (20 mM Tris HCl, 5 mM EDTA, 2.6 ml/g of lenses) using Polytron homogenizer. The water insoluble residues were eliminated from the homogenate by subsequent centrifugations at 12000 rpm for 10 minutes. Crystalline proteins concentration of the extract was assessed with Bradford assay using Bradford reagent (Sigma Aldrich) and HPLC using Jupiter 5 µm C18 300 Å column.

The reactive oxygen species (ROS) probe Dihydrorhodamine-123 (DHR123, Sigma Aldrich) was added to the lens homogenate solution containing 3 mg/ml of crystalline proteins and irradiated by UV light (254 nm) for 0, 5, 10, 15 minutes. Irradiated samples were analyzed both by HPLC for protein characterization and fluorimetry recording emission fluorescence spectra from 510 to 700 nm, using a Perkin Elmer luminescence spectrophotometer with excitation at 505 nm to determine ROS production.

**Results**: HPLC analysis showed the presence of a specific peak pattern of crystalline proteins in the porcine eye lens extract corresponding to 21% of α, 66% of ß, and 13% of γ crystalline proteins.

The concentration of each crystalline protein decreased after 15 minutes of UV irradiation.

The emission fluorescence spectra showed a peak at 527 nm corresponding to the presence of Rhodamine123, as a result of the oxidation of DHR123 probe induced by the presence of ROS.

**Conclusions**: α, β and γ crystalline proteins were extracted from porcine eye lens and quantified. UV irradiation of crystalline proteins solution induced the protein degradation that could be related to ROS production. Additional studies are necessary to evaluate the oxidative stress mechanism that induce crystalline proteins degradation.


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## THE SAFETY OF THE DONOR CORNEA FOR THE PATIENT

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### Content

**Introduction and Purpose**: To guarantee the safety of donor tissue there are a lot of safety procedures in an eye bank. Besides these local facilities for providing safe corneas in the Netherlands the Dutch Transplant Foundation ( NTS) is responsible for screening of the general health of the donor. The national institute responsible for Biovigilance (TRIP) receives and analyses all donor related side effects. A bank is obliged to report all the possible risks associated with the tissue. In the Netherlands there is a National Follow up Registry (NOTR in which all clinical data of the patients with cornea transplants are stored.

The purpose of this study is to investigate the safety of the corneas delivered at the bank for the patients, who received these corneas.

**Methods**: In this retrospective study all cornea donor related reports to TRIP, concerning tissue from the cornea bank ETB-BISLIFE are analyzed. The reports are categorized in Adverse Events and Adverse reactions according the national system. All the adverse events and reactions were analyzed if they had an effect on the patients or not. The value of the NOTR for the predictability of the safety of the donor was analyzed as well.

**Results**: More than 10.000 donor corneas were delivered in the Netherlands form 2009-2018.90 adverse events and reactions were reported during this period, of which 52 were severe. Of these 90 cases 35 x there was not an effect on the patient and NOTR is not of value. In 40 cases there was a possible effect and in 15 cases a new surgery, so in 61 % the NOTR could be of value to say something about the safety of the donor cornea for the patient.

**Conclusion**: By analyzing SARS and SAES it is concluded that donor corneas from the cornea bank are safe for the patient. With a national follow up registry as developed in our country it could be possible in the future to be more secure about the risk assessment for the patient.

## REVIEW OF REPORTED DMEK GRAFT PREPARATION FAILURES AND ANALYSIS OF POSSIBLE CONTRIBUTING FACTORS

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### Content

**Introduction and purpose**: The two eye banks in our organisation together distribute over 4000 corneal tissues for transplantation per annum, with a steadily rising number for DMEK. Our tissue allocation for DMEK requires min. donor age (55 yrs) and no cataract surgery; diabetes mellitus (DM) is acceptable. DMEK grafts are prepared by surgeons in theatre.

DM in eye donors might increase the risk of unsuccessful DMEK graft preparation. The rate of DM in our country is estimated at 6%.

Adverse reactions & events in relation to ocular tissue grafts are reported to the issuing eye bank for investigation.

We identified and reviewed relevant reports relating to DMEK graft preparation failures, to better understand poss. contributing factors, esp. the role of donor DM.

**Material & Methods**: All adverse reports between July 2015 and September 2019 were reviewed, and those relating to DMEK preparation failures analysed further with regards to donor factors (DM status, age, cataract surgery), review of eye bank records and surgeon's description of the problem.

**Results**: During this time, over 16000 corneal grafts incl. over 2200 DMEK grafts were issued, and 158 adverse reports received in relation to tissue quality & safety.

30/158 (19%) related to problems specific to DMEK graft preparation, with 28/30 suitable for further analysis.

11/28 implicated donors had a history of DM. In 5/11, this was the only identifiable factor, and surgeons reported "very friable tissue" or "very adherent Descemet membrane". In the remaining 6 cases, additional factors likely contributed to graft preparation failure.

In 17/28 cases no donor DM was found, but one or more other factors (13/28), e.g. tissue misallocation, or none (4/28).

**Conclusions**: Given the large number of grafts issued for DMEK and prevalence of DM, the very low number of reported graft preparation failures associated with DM is reassuring. Future preparation of DMEK grafts in the eye bank will provide valuable further insight.


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## COMPARISON OF THE PERFORMANCE OF TRADITIONAL COLD CORNEAL STORAGE MEDIA TO A NEW COLD CORNEAL STORAGE MEDIA WITH ANTIMYCOTIC TABLET

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### Content

**Purpose**: To compare the performance of Kerasave and Optisol GS corneal storage media across metrics of donor evaluation and processing.

**Material & Methods**: Forty paired corneas were swabbed for microbiological testing prior to recovery. Donor decontamination occurred per eye bank SOP, tissue was recovered and placed into a CVC containing Kerasave (Alchimia) or Optisol GS (B&L) at 4°C. Tissue simulated the eye bank process for PK, DSAEK, and DMEK. Endothelial density (ECD), central corneal (or DSAEK graft) thickness (CCT), slit lamp exam (SL), and endothelial cell mortality (ECM) were assessed following incubation. These points in time varied for PK and DMEK/DSAEK groupings. Media was collected at the time of tissue processing for sterility testing, and at time of final evaluation.

**Results**: Initial swab tests showed 90% and 86% contamination of corneas being stored in Kerasave and Optisol GS respectively, 24% and 19% of which were due to fungal contamination. Kerasave was free of fungi at all points in time, and fungi was detected in one Optisol GS DSAEK media at the end of storage. Initial mean CCT measurements in the Kerasave group were on average 45 ± 3 µm greater than Optisol (p =0.006), but over time this number decreased in the PK group. No statistically significant changes were noted when the Kerasave group was compared to Optisol with respect to ECD or ECM, or when either group was compared to itself over time.

**Conclusion**: Key metrics of corneas stored in Kerasave and Optisol GS and processed for DSAEK and DMEK were comparable. Over time, and with the exception of DSAEK grafts, the degree of swelling was greater for Optisol GS than Kerasave, while absolute thicknesses were greater in Kerasave.

## CHANGES IN THICKNESS AND CURVATURE OF HUMAN CORNEAL GRAFTS IN DEXTRAN-CONTAINING CULTURE MEDIUM BEFORE KERATOPLASTY

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### Content

**Purpose**: With organ culture, the graft is typically transferred from an isotonic dextran-free preservative culture medium (medium I) to a hypertonic medium (medium II, containing dextran T-500 6%) for deswelling before corneal transplantation. The purpose of this study was to determine the kinetics of this deswelling process in medium II.

**Material & Methods**: We prospectively examined the tomography of 24 corneas before and every hour after transfer from medium I into medium II. We used an anterior segment OCT, without extracting the tissue from the container. The central corneal thickness (CCT) was measured with the manual measurement tool of the AS-OCT (CASIA 2, Tomey, Japan). The radii of curvature (RC) (anterior flat (Af) and steep (As) and posterior flat (Pf) and steep (Ps)) were measured with a MATLAB self-programmed Software according to Mäurer & Langenbucher. We analyzed the changes of corneal thickness and radii hour by hour.

**Results**: The CCT (±2SD) at baseline (T0) was 727±156µm. It reached 581±103 (55.5%±20.4% of the measured deswelling after 24h), 506±84 (82.9%±13.9%), 472±79 (94.2%±6.4%) and 456±7µm after 6, 12, 18 and 24 hours, respectively. The RC (±2SD) at baseline (T0) were (Pf, Ps, Af and As): 6.6±0.5, 6.2±0.5, 7.7±0.4 and 7.4±0.4, respectively. After 24h, it reached: 6.8±0.1, 6.6±0.3, 7.6±0.1 and 7.4±0.2mm.

**Conclusion**: The kinetics of the deswelling process in medium II follow a non-linear hyperbolic curve on average. Considering a deswelling of 82.9% (CCT 506±84µm) at 12h, we assume that a time interval of 12 hours in medium II might be enough for clinical purposes. This result might help to keep storage in medium II as short as possible in order to escape potential toxicity effects of dextran in culture medium II. The radius of curvature does not seem to significantly change within 24 hours for all measured surfaces and meridians.


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## CORNEA DONATION IN GERMANY: OBTAINING CONSENT

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### Content

According the World Health Organization (WHO), about 4.9 million bilaterally blind people world-wide are in need of corneal transplants. Unfortunately, availability fails to meet demand year after year, so raising the number of donations is one of the most important challenges.

In Germany with it's opt-in donor policy, it is important to express explicit consent to donation while still alive. While relatives may also decide according to presumed will or own values it has been shown in several studies that the known attitude of the deceased towards donation facilitates the family's decision. Interestingly, consent expressed in surveys does not necessarily translate into actual donation.

Therefore, we analyzed 25654 family interviews from September 2013 to February 2018 for several factors that might influence the family's decision in an actual situation after bereavement as well as the development of consent to cornea donation over this period.

Only 20.8 % of all potential cornea donors had declared their own wishes and the numbers are declining. Spouses and parents were more often informed about their beloved's donation wishes than any other relative— including adult children who were the highest ranking decision-maker in most cases (47.6 %). Refusal by potential donors was communicated more often than consent. Consequently, consent to cornea donation was declining throughout the analyzed years.

Personal interviews and interviews via telephone handled by staff known to the family resulted in better consent rates (up to 75.6 %) with male interviewers receiving higher consent rates in general and male relatives consenting more frequently than females. The sex of the approached relative(s) in relation to a male/female interviewer was of low importance. The results also show that it is important to allow discussion about that topic between family members- the more relatives were involved the higher was the probability of consent.

## TOWARDS A LIMBAL STEM CELL XENOFREE GMP PRODUCTION IN EYE BANK.

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### Content

**Purpose**: Our purpose was to investigate the efficacy of a xenofree culture system composed of supplemented hormonal epithelial medium complemented with human serum (XSHEM) and a feeder layer of human adipose tissue processed lipoaspirated cells (PLA) as a xenofree alternative for autologous cultured limbal epithelial stem cell transplantation (CLET).

**Material & Methods**: In order to achieve our goal, we compared limbal stem cells (LSC) cultured on two different irradiated feeder layers with a modified XSHEM. Human limbal stem cells (LSC) were cultured on gold standard murine 3T3 Swiss Albino fibroblasts obtained from Kerafast (3T3-J2, EF3003) and on human PLA obtained by stromal vascular fraction isolation in planned lipoaspiration surgeries. These AT-MSC accomplished the criteria for mesenchymal stem cells characterization. We performed studies for viability, clonogenicity, proliferation and cytotoxicity. Furthermore, we quantified the expressions of ΔNp63α, ABCG2, Bmi-1, cytokeratin 3 and 12 by qPCR and immunofluorescence. Finally, we also analyzed the expression of interleukin 6 (IL-6) and stromal derived factor 1 (SDF-1).

**Results**: The results showed that irradiated human PLA feeders improved the undifferentiated state of LSC, enhanced its growth and clonogenicity stimulating IL-6 secretion and SDF-1 expression, as well as decreased the cytotoxic activity when compared with irradiated 3T3 fibroblast feeder cells.

**Conclusions**: Our results concluded that the combination of modified XSHEM medium and PLA feeder layers improved the progenitor potential and quality of LSC cultures, demonstrating that this is an efficient approach for xenofree LSC production in Eye Banks for cell therapy. Moreover, PLA feeder layers could enhance the clinical outcomes of CLET due the increased progenitor potential of LSC.


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## IS CATARACT SURGERY WITH THE NANO-LASER AN ENDOTHELIAL PROTECTIVE PROCEDURE? CORNEAL ENDOTHELIAL CELL COUNTS AND CENTRAL CORNEAL THICKNESS FOLLOWING NANO-LASER CATARACT SURGERY.

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### Content

**Purpose**: Evaluation of modifications of corneal endothelial cell counts (ECC) and central corneal thickness (CCT) following Nano-Laser cataract surgery (NLCS) using the fellow unoperated eye as a control.

**Method**: Single-center retrospective study involving the medical records of 131 eyes of 131 patients who underwent NLCS ( Cetus Nanolaser System, A.R.C. Laser, Germany) , year 2015-2018. All patients underwent a 2.4 mm cc- incision, ccc- capsulorhexis, nanolaser photofragmentation and implantation of an acrylic foldable IOL.

The main outcome measures were modifications of ECC and CCT measured before and at a subsequent follow up visit.

Parameters were measured by means of a nc.– specular microscope (EM 3000 Tomey) and an anterior segment-OCT ( RTVue 100). The unoperated contralateral eyes were used as a control group. Statistical analysis was performed using SPSS Stat. 25.0.

**Results**:15 of the 131 patients met the inclusion criteria. In a variable period of follow-up ranging from 3 months to 2.5 years, a comparison in the enrolled patients of the modification of the primary outcome measurements (ECC) between the two groups of eyes using a paired t-test indicated there were no statistically significant differences between the eyes that underwent NLCS and the untreated eye, except for CCT ($p<0.04$). Although the difference was statistically significant, the difference of means between the two groups of eyes was only 1.18%, with a lower decrease in NLCS treated eyes.

**Conclusion**: Cataract surgery with the Nano-Laser is an endothelial protective procedure. The modification induced by NLCS over a variable post-operative time ranging up to 2.5 years did not present statistically significant differences in the ECC.

The modification in CCT was statistically significant. Although these results will have to be confirmed in a multicenter prospective study, they are in accordance with previously published studies that report a sparing of endothelial cells when performing NLCS.

## ESTABLISHMENT OF AN ELECTRONIC DOCUMENTATION SYSTEM TO STEER HIGHLY REGULATED PROCESS STEPS OF HUMAN TISSUE DONATION, TISSUE MANUFACTURING AND TISSUE ALLOCATION

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### Content

Due to increasing regulatory requirements, the establishment of an electronic database is an almost essential prerequisite for the manufacturing of human tissue-derived transplants. Since 2016, we have been developing a database that enables process-controlled documentation by authorized users with full audit trail on all changes. Among other functions the database consists of three main modules: (i) human tissue donation (under the permission of section 20b German drug law), (ii) tissue processing of various tissue specificities and (iii) allocation (distribution and billing of transplants). Additional features like contact-, warehouse- and basic human resource management (coordination of services, holiday and working time) complete our versatile system.

The processing module e.g. enables full traceability of the materials used as well as the automated generation of the SEC. All in-process controls, the testing and release of transplants are electronically monitored, and therefore ensure a valid manufacturing process. The allocation module aids the user with the documentation of transplant inquiries, the generation of request - and waiting lists, and the allocation of transplants to a patient.

If requested, all modules provide forms of every individual work step in pdf format, for example to create donor files or to exchange information with co-operation partners. Furthermore, data can be imported and exported in csv format, facilitating interchangeability with computing interfaces of cooperating medical facilities. The database allows a real-time documentation of every step involved in the donation and transplantation process, and ensures the storage of transparent documentation for transplantation institutions.


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## THE ACTIVE STORAGE MACHINE (ASM) ALLOWS DETECTING TRACES OF CORNEAL REFRACTIVE SURGERY: CASE REPORT

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### Content

**Introduction**: The detection and selection of corneas operated on for refractive surgery (LASIK or photorefractive keratectomy (PKR) by excimer) will become a major concern for eye banks in the coming years because this surgery is often forgotten during the interview with the deceased's relatives. Passive storage in organoculture (OC) prevents the detection of the traces of this surgery because of stromal edema making the cornea less transparent and wrinkled. On the other hand, the active storage machine (ASM) restores intraocular pressure, restores the cornea to its original shape, respects transparency and incorporates non-invasive controls. It is therefore likely to facilitate the detection of refractive surgeries. We present here the case of 2 corneas operated on with PKR and stored successively in OC and ASM.

**Methods**: The 2 corneas of a 49-year-old donor operated 20 years earlier by PKR for -2 and -3 diopters myopia were stored in OC for 14 days and then placed in ASM for 48 hours. Transparency (transparometer, BiiGC), thickness map and OCT topography (Casia 1, Tomey) were performed under the 2 storage conditions. At the end of the observation, histology and electron microscopy were performed.

**Result**: Traces of PKR remained unnoticed in OC while they were evident in ASM: central epithelial anomaly, central thinning and flattening of central keratometry were found respectively in transparometry, pachymetry map and OCT topography. Histology and ultrastructure confirmed the absence of Bowman's membrane, pathognomonic of PRK.

**Conclusion**: By placing the cornea under physiological conditions, and in particular by triggering its deswelling thanks to the intraocular pressure and by restoring its natural curvature, the ASM should allow effective detection of refractive surgery traces.

## NEW HECPLUS PROTOCOL TO FURTHER IMPROVE THE ACCURACY OF PAN-ENDOTHELIAL VIABILITY MEASUREMENT

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### Content

**Purposes**: The HEC (Hoechst-Ethidium-Calcein) labeling, which we described in 2011, is a simple destructive test unanimously adopted to determine the pan-endothelial viable endothelial cell density (vECD) using image analysis (e.g. using CorneaJ, ImageJ dedicated plugin). Sometimes, the endothelial surface, folded by stromal edema remains difficult to analyze. We present here the HECplus technique, which, with an optimization of image acquisition and analysis, further increases the accuracy of vECD determination, particularly in endothelial folds.

**Methods**: 10 pairs of corneas stored in organoculture at 31°C were analyzed. The endothelial side was incubated with 4µM Calcein-AM, 1µM Ethidium and 11µM Hoescht 33342 for 45 min at RT. Fluorescence was acquired in 3 channels first with a macroscope, in 3D with an image stacking of the entire graft then with a microscope after flat mounting. A 2mm plastic pellet emitting constant fluorescence at 510nm was imaged at the same time to normalize the images. Only one sharp image was reconstructed in ImageJ from the stacks of images. The final standardized images were then analyzed by CorneaJ (HEC group) and then manually by delimiting all viable surfaces on a graphic tablet (HECplus group)

**Results**: The vECD was significantly higher by 8.2 +/- 1.9% in the HECplus group versus HEC (2059 +/-127 vs 1989+/-156 cells/mm²). Endothelial cells died preferentially in the top of the folds and were better preserved in the bottom of the folds. While HEC wrongly considered some folds as dead, HECplus allowed the error to be corrected. The folding pattern was similar between the 2 corneas of the same pair but differed from one donor to another. The normalization of images for calcein showed overall differences in the level of fluorescence between corneas, suggesting differences in metabolic activity.

**Conclusions**: We recommend applying the HECplus protocol when a very high degree of accuracy is required, for example when comparing storage media.


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## EXPERIENCE IN WILLINGNESS OF CORNEAL DONATION IN AN INSTITUTE FOR LEGAL MEDICINE WITH FORENSIC CASES OF DEATH AND DECEASED IN CLINICAL MORTUARY

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### Content

The Institute of Legal Medicine as part of the University Medical Center Hamburg-Eppendorf (UMC) not only acts as clinical mortuary for the UMC receiving all deaths from the hospital (group a) but also works on order by authorities and the departments of public prosecution to perform postmortem examination of all sudden, possible non-natural and other unexplained deaths in Hamburg and its surrounding regions (group *b*).

Thus, it is possible to include a significant larger group of deaths into corneal donation than in other facilities. For all incoming bodies of both groups (*a* and *b*), physicians conduct a standardized assessment regarding the possibility of corneal donation including the examination of criteria of exclusion. This process bases upon long-lasting practice in the field of tissue donation in Hamburg.

As the shortage of corneal allografts affects the UMC in Hamburg-Eppendorf too, an initiative has been started in order to obtain more corneal donations. Aside from an intensified cooperation with external hospitals that report cases of death for possible donation (potential group *c*), there is an analysis of those two groups above (a and b) as they vary in several points regarding preconditions such as different medical histories and unequal circumstances of death. Especially the different circumstances of death may cause an increased rate of denial by relatives. This observation could lead to an approach for decreasing the shortage of corneal transplants.

## GAINING CONSENT FOR CORNEA DONATION FROM DECEASED PATIENTS' RELATIVES — EVALUATION OF A COMMUNICATION TRAINING OF EMPLOYEES IN CORNEA EYE BANKS USING THE AACHEN MODEL AS AN EXAMPLE

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### Content

**Purpose**: Due to a lack of donor corneas in Germany the quality of acquisition telephone calls is becoming more important. It has been shown scientifically that physicians in cornea eye banks feel under-prepared in the skills necessary for donor acquisition. Therefore specific communication training was developed, which needs to be evaluated and any improvement potential described.

**Materials & Methods**: A total of 108 employees from 14 different locations in Germany underwent training, between one and a maximum of 11 per training session. A structured questionnaire-based participant evaluation of 25 communication training sessions since 2008 has been evaluated with descriptive and frequency analyses. A structured evaluation of n=56 participants is available.

**Results**: The 'benefit of the seminar for my daily work' was assessed by the majority of participants with 100% (76.8% of participants) or 75% (17.9% of participants). For 87.5% of the participants it was entirely true to have 'acquired new insights and made new experiences' from this training.

**Conclusions**: The communication training was well received in Germany and positively evaluated by participants. A short form of the training is currently being developed to meet the request for an 'update' on the communication training. International formats and comparable offers for non-physician staff could be offered in the future.


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## TISSUES WITH ISSUES: HOW TO PREVENT WASTE OF SUB-OPTIMAL DMEK GRAFTS?

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### Content

**Introduction**: Descemet membrane endothelial keratoplasty (DMEK) and other EK techniques are nowadays the preferred surgical choice worldwide for the treatment of endothelial dysfunction. More and more eye banks now provide pre-cut DMEK transplants to corneal surgeons, a service that was first offered by Amnitrans Eyebank Rotterdam.

Initially, surgeons accepted the pre-cut tissue quite promptly, understanding the scarcity of altruistically donated human tissue and especially pre-cut DMEK tissue. However, since a few years surgeons have become more and more critical about the properties of the proposed DMEK graft, and grafts are sometimes refused because of small tears, small chips out of grafts or tight rolls while the quality of the endothelium is good.

**Methods**: The number of DMEK tissues not accepted by surgeons and the underlying reason was analyzed from 2017 to 2019.

**Results**: Overall, about 13% of the DMEK grafts prepared by Amnitrans Eyebank had small issues (tear/small chip). The reasons to refuse DMEK tissue by surgeons changed over the years. The only reason to refuse DMEK tissue in 2017 was the tightness of the scroll. In 2018, tightness was still the main reason for refusal, while in 2019 the main reason was the presence of a small chip or small tear in the graft. The number of unaccepted DMEK tissues did not increase over the years because the offered tissue was better matched beforehand with the demands of the surgeon regarding tightness of the scroll or other features.

**Discussion**: It is vital to raise awareness on this issue which may otherwise lead to unnecessary tissue waste. While eye banks should closely work with surgeons and know their preferences to be able to optimally place transplants, surgeons should bear in mind that donated tissue is not a standard product but has intrinsic features that cannot be changed and ask themselves if they would not use certain grafts when they had prepared them themselves?

## SIMPLE CONJUNCTIVAL EPITHELIAL TRANSPLANTATION (SCET) IN PRIMARY PTERYGIUM SURGERY

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### Content

**Introduction**: Primary pterygium is one of the most common eye diseases, affecting a large percentage of the population. The estimate prevalence ranges from 0.7% to 31%, and the incidence is higher in geographic areas with high ultraviolet radiation. Hereditary factors play a role. Surgery is the only effective treatment. There is no consensus about the best methods for pterygium excision and variable recurrence rates have been reported.

**Purpose**: To assess the effectiveness of a new surgical option for patients with primary pterygium based on the Simple Conjunctival Epithelial Transplantation (SCET) technique.

**Methods**: SCET is a single stage technique consisting in the seeding of fragments of conjunctival epithelium onto human amniotic membrane (HAM) large enough to cover the bare sclera resulting from the pterygium excision. The HAM with fragments seeded are glued in place with fibrin sealant. We perform excision of the pterygium by dissection of the head off the cornea, exposure of the underlying fibrovascular tissue accurately removed by dissection, preserving surrounding conjunctival tissue. Abnormal scar tissue is removed. The eye is maintained closed for 3 days and then corticosteroid/antibiotic combination eye drops are used. Evaluations are scheduled weekly in the first month and monthly in the following months, up to 6.

**Results**: Three patients are in the study. Evaluation during the first weeks showed good adhesion of the HAM over the sclera and its quite fast disappearing. Initial results on the recovery of normal conjunctival tissue on the area of the pterygium up to 3 months are encouraging toward an effective intervention. No adverse reaction, inflammation, or infection occurred.

**Conclusion**: Our data pave the way for the SCET as a new therapy for the restoration of the integrity of the ocular surface after pterygium surgery. In case at 12-month evaluation no recurrence will occur, we could be confident on the efficacy of SCET also to prevent relapse.


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## DESCEMET'S MEMBRANE BIOMIMETIC MICRO-TOPOGRAPHY DIFFERENTIATES HUMAN MESENCHYMAL STEM CELLS INTO CORNEAL ENDOTHELIAL-LIKE CELLS

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### Content

**Purpose**: The loss of corneal endothelial cells (CEC) bears disastrous consequences for the patient, including corneal clouding and blindness. Corneal transplantation is currently the only therapy for severe corneal disorder. However, the worldwide shortages of corneal donor material generate a strong demand for personalized stem cell based alternative therapies. As human mesenchymal stem cells (hMSC) are known to be sensitive to their mechanical environments, we investigated the mechanotransductive potential of a Descemet's membrane-like micro-topography (DLT) to differentiate hMSC into CEC-like cells.

**Methods**: Master molds with inverted DLT were produced by 2-photonic lithography (2-PL). To measure the mechanotransductive potential of the DLT, MSC were cultivated on silicone or collagen imprints with DLT. Changes in morphology were imaged and changes in gene expression of CEC typical genes such as zonula occludens (ZO-1), sodium/potassium (Na/K)-ATPase, paired like homeodomain 2 (PITX2) and collagen 8 (COL-8) were measured with Real Time (RT) PCR. At least immunofluorescence analysis have been conducted to confirm gene data on protein level.

**Results**: The adhesion of MSCs to DLT molded in silicon and particularly in collagen initiate polygonal morphology, monolayer formation and enhance not only the transcription of CEC typical genes such as ZO-1, Na/K-ATPase, PITX2 and COL-8, but also the expression of the corresponding proteins.

**Conclusion**: The artificial reproduction of a Descemet's membrane with respect to the topography and similar stiffness, offers a potential innovative way to bioengineer a functional CEC monolayer from autologous stem cells.

**Keywords**: Corneal endothelial cell loss, mechanotransduction, mesenchymal stem cells, descemet topography

## SHOULD DONOR AGE BE INCLUDED AS EXCLUSION CRITERIA FOR THE CORNEA DONATION?

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### Content

Life expectancy increased dramatically in the last decades, particularly in western countries. Since medical contraindications for cornea donation don't exclude the main causes of death in the elderly, an important percentage of corneal graft comes from aged individuals. Low endothelial cell density (ECD) may have a negative influence on graft survival, in particular for penetrating and endothelial keratoplasties (PK, EK), justifying that corneas with ECD lower than 2000/mm2 should be excluded. Because ECD decreases with age, we proposed to reevaluate the impact of donor age on the ECD-based exclusion rate of collected corneas.

1357 corneas collected in EUROBIO° Media in 2017 and 2018 were analysed for ECD, without considering the other potential causes for destruction. Average ECD slightly decreased with age (about 90/mm2 every 10 years) from 2700 at age 20 years to 2090 at 90 years. Accordingly, the percentage of corneas with low ECD (mean:26%) increased with age, as well as the percentage with unquantifiable ECD (mean:18%). In older donors (>85 years), the percentages were 38% and 24% respectively: only 38% of the corneas were eligible for PK or EK after ECD evaluation, without considering the additional causes of destruction. Older donors represented 18% of the total collected corneas, and 12% of the corneas with ECD>2000/mm2.

Exclusion of aged donors could be an opportunity to optimize the cost/effectiveness ratio of the eye banking, and to avoid the ethical issue of discarding the major part of the corneas collected in the elderly. However, most eye banks can not afford to lose that 12% of usable grafts, unless they have enough procurement teams. In addition, it should be considered that corneas with low ECD can be used for deep anterior lamellar keratoplasty because the endothelium of the recipient is left intact. In conclusion, each eye bank must define a limit age in its exclusion criterias for donors, depending on its activity and its procurement capacity.


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## TO RAISE PEOPLE'S AWARENESS THROUGH YOUR WEBSITE – SUCCESS FACTORS FOR ONLINE COMMUNICATION FOR NON-PROFIT ORGANISATIONS

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### Content

Tissue donation and its processes in Germany are still completely unknown to most people. The DGFG wants to improve awareness and sensitize people of tissue donation. Therefore, the DGFG has restructured and renewed its website and content as a supplement to the information provided by the Federal Centre for Health Education (BZgA). The relaunch was in April 2019.

On the main pages Tissue Donation, Tissue Transplantation, Network, DGFG and News, the DGFG addresses its main target groups: patients, hospitals, physicians, cooperation partners and general interested people. While previously networking activities were in the foreground, now general information on tissue donation combined with patient stories play a prominent role.

The reorientation of the website's content has been very well perceived by the visitors (source: Google Analytics): In addition to the landing page, the page Tissue Donation increased from rank 7 up to the second most visited page.

Overall, the number of page users (PU) and page views (PV) increased: While in January 2016 the number of monthly PU was 646 (3,076 PV), a total of 1,997 PU (5,113 PV) visited the website in September 2019. Even five months before and after the relaunch, a considerable improvement can be seen (9,448 PU/23,288 PV vs. 9,920 PU/27,543 PV).

Cross-media communication of new content shows also positive effects on the website's statistics: Around the release dates of the newsletter, the usage statistics are approx. 10 percent higher than the average PU. Also, more users are entering gewebenetzwerk.de through social media postings: 20 percent of the PU in May 2019 (n= 1,922) came from Facebook.

Graphics and strong images led to a higher engagement on social media and therefore higher traffic on the website. Users are more informing themselves about tissue donation and consuming patient stories. Cross-media communication based on continuous content production can also be marked as success factors in online communication.

## ESTABLISHMENT OF A PROCESS-ORIENTED DATABASE SYSTEM FOR TISSUE INSTITUTIONS FOR TISSUE DONATION, PROCESSING AND ALLOCATION

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### Content

In October 2019, the DGFG launched an online database system for ophthalmological tissues. Until then, there had been no comprehensive validated database system for tissue institutions in Germany that mapped the process chain of tissue donation, processing and allocation and allowed process-controlled work.

In 2005, the DGFG had already introduced a system which integrated processing and allocation data. Now, the successor system enables all three process steps in one database and integrates evaluation and economic functions as well as medical and commercial controlling.

In close cooperation with United Information Systems Animal Husbandry (VIT) a system validated according to GMP Annex 11 was programmed. Strictly process-oriented, the database system replaces the previous IT system, in which the donation and parts of the processing and allocation still had to be processed by hand. Using Qlik anlaytic (data warehouse and evaluation) tool, the data is available at any time for research and analysis to the parties involved according to their function (donation, processing, allocation, transplantion). Transplant centres can now gain insight into the progress of the registered patients, the quality of the tissues offered and the final care of the patients.

The responsive system can be accessed location-independently via computer or mobile device through strict user authorization. All processes are completed by printing the donor, manufacturing and tissue allocation file for the purpose of physical archiving for thirty years. More than 14.000 donor files (2013-19) have been recorded in the new database to date.

Overall, the new DGFG database ensures a transparent, effective and time-saving process chain and combines the three central areas of donation, processing and procurement of tissues. The complete recording of all process steps of the dispenser and fabric batch production allows a detailed statistical evaluation, which meets the requirements of the authorities.


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## AA NOVEL PRESERVATION TECHNIQUE FOR LONGTERM STORAGE AND AMBIENT DISTRIBUTION OF TRANSPLANTABLE HUMAN CORNEAS

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### Content

**Purpose**: Worldwide, only 100,000 corneal transplants are performed annually, despite the fact that there are 1.5 million cases of corneal blindness diagnosed each year. One limitation to corneal transplantation is access to quality donor tissue, due to inadequate eye donation services and infrastructure in developing countries. This is compounded by the fact that there is no long-term storage solutions for effectively preserving spare donor corneas collected in countries with a surplus. Eye banking infrastructure requires large local investment and labour to put in place, however, increased access to tissue can be achieved by development of preservation techniques to increase corneal storage times and allow for global shipping at ambient temperature.

**Methods**: We used a novel drying technique to preserve human corneas collected in the US and UK. We assessed weight, thickness, transparency, cell viability, cell membrane permeabilisation, ECM content and structure, comparing to non-dried donor corneas. A subcutaneous implantation model was performed in rats to assess biocompatibility and cell integration of the dry corneas. Clinical suitability was assessed through market access research targeting corneal consultants in the UK.

**Results**: Dried corneas were comparable to non-dried donor corneas in all investigated aspects other than cellular viability. When implanted subcutaneously in rats, the dried cornea was well tolerated, with cellular migration into the matrix and no visible immune rejection. We spoke to 12 corneal consultants, at 7 different hospitals, all gave positive feedback regarding future use and potential clinical indications.

**Conclusion**: Our preservation technique provides an easy-to-manufacture, non-viable, dehydrated cornea sutibale for a range of clincial indications and tectonic support. It can be stored on the shelf in hospitals for over 2 years and can be shipped at ambient temperatures worldwide, relieving the global shortage of corneal tissue.

## PRE-CUT LAMEK ENABLES CELL RECOVERY THROUGH REST PERIOD BEFORE SURGERY

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### Content

Descemet membrane endothelial keratoplasty (DMEK) is an important innovation in ophthalmology. Since 2015, a preparation procedure was established in the DGFG to supply surgeons with pre-cut cornea grafts from tissue banks for a safe performance of DMEK. This pre-preparation might also have an influence on the viability of endothelial cells. Stress that affects the cells during the preparation and surgery in the operation theatre (OT) might be reduced by the resting phase between tissue bank and OT.

Pre-cut LaMEK were prepared as usual. After deswelling endothelial cell density (ECD) was estimated, the lamella was stripped and divided into two halves. With the first half a life/dead staining was done. The second half was placed back to the medium filled culture container. After 72 hours the ECD was counted again and a DMEK surgery was simulated. Subsequently, a final life/dead staining was carried out. With the corresponding cornea all steps were carried out analogously, but the simulated operation took place directly after the preparation.

The first results show that the ECD in the OT-lamellas remains constant until the simulated operation, but subsequently decreases noticeably. The ECD of the pre-cut lamella also remains the same or decreases slightly. After the resting phase in culture medium however, ECD increases considerably and is also higher after the simulated operation. Corresponding the final life/dead staining shows visibly more red stained dead cells in the OT-lamellas compared to the LaMEK.

With the introduction of pre-cut corneal transplants for DMEK, the technique for the surgeon has significantly simplified, since a preparation risk is excluded and time and costs in the OT are reduced. In addition, the pre-preparation of LaMEK seems to offer a recovery phase for the cells, since the entire process of preparation with subsequent operation may induce an apoptosis reaction in some cells, which is reversible in the storage phase of the pre-cut tissue.


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## EXPERT AND USER FEEDBACK ON THE APPLICATION OF AMNIOCLIP PLUS

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### Content

Surface disorders of the eye can lead to defects of the cornea, which are often difficult to treat. In addition to the pharmacological therapy the amniotic membrane transplantation (AMT) is an excellent treatment option. A significant disadvantage of the conventional AMT is the membrane suture fixation on the ocular surface which causes an extra trauma. To avoid this additional surgical manipulation a ring system in which the amniotic membrane (AM) is clipped (AmnioClip-plus, AC+) was developed. The AC+ can easily be used similar to a contact lens. Since the beginning of 2019, the DGFG has the authorisation to distribute the AC+. However, one requirement of the competent authority PEI (Paul Ehrlich Institute) is the evaluation of further applications of the AC+.

Two questionnaires on the evaluation of the AC+ were developed, the first is filled in by the surgeon from a professional point of view and the second by the treated patient. The information is evaluated anonymously according to handling, safety and effectiveness of the AC+.

The first questionnaires returned show a positive feedback by both physicians and patients: The application is perceived as very comfortable. The AC+ is quick and easy to use and patients assure that they quickly become accustomed to the light foreign body sensation. The effect of AM in AC+ is described by surgeons as analogous to conventional AMT, although the side effects of the suture are of course eliminated. In some cases, the patients themselves wish a further application. In an exceptional case, partial degradation of the membrane in the ring occurred before the end of the possible 14-day wearing period.

The feedback of the users confirms the good and safe application of the AC+. The easy repeatability also makes it possible to treat other diseases such as dry eye syndrome. At the same time, however, the answers also show that a financial billing option can be decisive for the therapy decision.

## DECELLULARISATION OF CONJUNCTIVA FOR DEVELOPMENT OF A NEW COMPOSITE ALLOGRAFT TO TREAT SEVERE OCULAR DISEASES

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### Content

**Purpose**: Cicatrising conjunctivitis is a chronic condition affecting the ocular surface, caused by trauma, infections, neoplasia, autoimmune etc. The inflammation and scarring lead to loss of the conjunctiva and distortion of the eyelids. Tissue regeneration is needed to re-establish homeostasis of the ocular surface and to preserve vision. Present grafting strategies have limitations including low strength and the limited availability of autologous or allogeneic conjunctiva. The aim was to develop a new composite conjunctival allograft, by incorporating decellularised tissue into a biomaterial (electrospun fibres) to support conjunctival cell expansion.

**Material & Methods**: Porcine conjunctivas (N=14) were decellularised over 3 days in a closed system by adapting a published protocol. DNA was quantified by PicoGreen assay. Powdered decellularised conjunctiva was compared to small intestine submucosa as a source of natural extracellular matrix (ECM) for incorporation into electrospun fibres. Solutions of polycaprolactone dissolved in 1,1,1,3,3,3hexafluoroisopropanol were prepared at 12%w/v plus natural ECM (1-10%) for electrospinning. Human conjunctival epithelial (HCjE) cells were seeded on the whole decellularised tissue or electrospun scaffold, and cultured for 7 days then analysed by SEM.

**Results**: The tissue was decellularised (residual DNA below 50ng/mg dry tissue) with no effect on the ECM or biomechanics. Altered fibre morphologies were observed for electrospun scaffold with decellularised tissues. The electrospun scaffold with ECM promoted growth of HCjE cells, in comparison with the whole decellularised tissue used as control.

**Conclusion**: These data support the development of a novel conjunctival substrate for ocular surface regeneration. The composite graft can be part of an advanced strategy to regenerate tissue tailored to specific areas of the conjunctiva that require increased strength (fornices).


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## ASSESSMENT OF EFFECTIVENESS OF SERUM EYE DROPS USING A CORNEAL WOUND HEALING MODEL *IN VITRO*

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### Content

**Purpose**: Serum eye drops (SE) are widely used by ophthalmic patients to treat dry eye conditions. Clinical guidelines from the Royal College of Ophthalmologists report a beneficial use of SE as adjunctive treatment for patients with severe ocular surface diseases. However, functional data to prove efficacy of the treatment to promote ocular tissue regeneration are missing in the literature. We used an established *in vitro* model to assess the pro-healing effect of SE on wounded corneas.

**Material & Methods**: Human (N=7) and porcine (N=23) dissected corneas were used to set up the corneal wound healing model *in vitro*. An alkali burn epithelial wound was induced by using a sterile 5-mmdiameter round filter paper soaked in 0.5 M NaOH, applied for 20 seconds to the central cornea. After extensive washing, corneas were kept in culture for up to 72 hours in high-glucose DMEM medium, 5% FCS and 2% pen/strep, with or without application of SE on the corneal surface (3 drops, 5 times a day). Corneas were washed, processed for histology and stained by Van Gieson or DAPI. Wound closure was quantified microscopically by measuring the length of corneal epithelium outgrowth from the wound edge and epithelium thickness up to 72 hours post-wounding.

**Results**: The corneal epithelium was fully regenerated in culture within 72 hours post-alkali burn in both human and porcine tissues. A larger amount of regenerated epithelium (+20.1%; CI: 4.7) was observed at 48 hours when SE were added to wounded corneas. Regenerated corneas were also thicker in presence of SE: +45.5% (CI: 5.7) at 48hrs, +5.5% (CI: 0.5) at 72hrs.

**Conclusion**: We have shown that the addition of SE stimulates enhanced corneal wound healing *in vitro*, thus confirming the effectiveness of SE treatment for ocular surface regeneration. The present model can be used in future to prove effectiveness of newly developed ophthalmic preparations.

## OPTIMISATION OF HUMAN AMNIOTIC MEMBRANE DECELLULARISATION

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### Content

**Purpose**: Human amniotic membrane (HAM) has numerous applications in a variety of different fields within medicine, including ophthalmic applications to promote healing and ocular surface regeneration. We have previously shown that decellularised HAM can enhance limbal stem cell expansion in vitro. Although residual DNA amount was very low in decellularised HAM, occasionally parts of the epithelium remained. Here we investigated the incorporation of a specific de-epithelialisation step for more effective removal of HAM epithelium. The aim was to develop a GMP-compliant decellularised HAM allograft, which could lead to a new clinical product in the UK.

**Material & Methods**: Four HAM, obtained from elective caesarian deliveries, were dissected, incubated with antibiotics and subjected to 2 different decellularisation protocols (total length 5 days): 1) standard NHSBT protocol (10mM Tris hypotonic buffer, 0.07% SDS detergent buffer, nuclease); 2) deepithelialisation (1M NaCl solution). Biopsies were used for histological analysis (Van Gieson, Haematoxylin & Eosin and DAPI), and DNA was quantified by Pico Green assay.

**Results**: The amount of residual DNA in decellularised HAM was below 50ng/mg dry weight in all donors, for both protocols used. Mean DNA values (expressed in ng/mg dry tissue; N=3) were: Protocol 1 – native HAM, 665 (CI: 295.6); decellularised HAM 17.2 (CI: 16.2); Protocol 2 – decellularised HAM 21.8 (CI: 14.9). Although not statistically different, use of a de-epithelialisation step produced a HAM without epithelial remnants.

**Conclusion**: Pre-de-epithelialisation of HAM demonstrates a greater efficacy in the removal of residual cellular components, during the decellularisation processing. Further studies are needed to confirm de-epithelialisation is critical for glycocalyx removal, and to assess the absence of cytotoxicity. Ultimately the new decellularised HAM scaffold may promote better differentiation of co-cultured cells.


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## AMNIOTIC MEMBRANE — THE PROCESSING, STORAGE, SUPPLY, AND ITS USE IN OPHTHALMOLOGY.

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### Content

**Introduction**: Even though the main aim of the Eye Bank is to provide corneas for transplantation, there has been a growing demand for amniotic membrane grafts extensively used in ophthalmic surgery.

**Purpose**: To present the technique of amniotic membrane (AM) processing, method of storage, and also to analyze and review amniotic membrane harvest over the 20 years period in the Lublin Eye Bank, Poland.

**Material and Methods**: Amniotic membrane is prepared from placentas retrieved from donors undergoing elective caesarean section. Maternal blood sample is collected two times: at the time of delivery and 6 months later in order to perform serological and molecular tests for HBV, HCV, HIV, syphilis, and to cover the "serological window" , respectively. Amniotic membrane preparation procedure is carried out under aseptic conditions in a class 2 biological cabinet by blunt dissection from chorion. Amniotic membrane is then flattened onto nitrocellulose paper with the epithelium side up and is cut into 3x3 cm pieces/grafts. For preservation, amniotic membrane is embedded in the Dulbecco's Modified Eagle Medium (DMEM) with glycerol in ratio 1:1 in temperature -80C$^{o}$ and can be stored up to two years. During the 20 years (1999-2019) in the Lublin Eye Bank 5707 amniotic membrane grafts were prepared.

**Conclusions**: Since amniotic membrane is widely used in the treatment of various ophthalmic disorders, and there is considerable demand for it, therefore procuring, processing, and supply of amniotic membrane maintains a significant task in everyday Lublin Eye Bank's work and activity.

## ETHICAL AND LEGAL CONSIDERATIONS IN THE MANAGEMENT OF OCULAR TISSUE DONATIONS FROM NON-COMPETENT DONORS: THE EXPERIENCE OF THE VENETO EYE BANK FOUNDATION

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### Content

**Introduction and Purpose**: The transplant legislation adopted by Italy in April 1999 was for an opt-out system. Nevertheless, the full application of the law on presumed consent hasn't been fully implemented yet and the system is still based both on the Transplant Information System database (Sistema Informativo Trapianti) and on obtaining written consent from the donor's next-of-kin.

What happens to those potential donors who are not capable of self-determination and what is the role of their families?

This study illustrates the Italian experience (limited to the Veneto Region) in the management of those patients who are not competent at the time of death and the handling of a potential tissue donation, in the event that the person has no written testament.

**Materials and Methods**: The primary source is represented by law n. 91 of 1 April 1999, entitled "Provisions regarding the removal and transplantation of organs and tissues", followed by the national guidelines for the procurement, processing and distribution of tissues for therapeutic use, developed by the National Transplant Centre. In addition, there are the indications provided by the Veneto Regional Transplant Centre, as the local authority for medical-legal issues.

**Results and Conclusion**: At present, in Veneto, we consider as informed donors only those adults (aged > 18 years) who have been able, at least once in their lives, to self-determine and develop self-awareness.

Only in these cases of non-competent potential donors are family members required to be interpreters of the will expressed in life by the deceased person, either explicitly or implicitly. This process is administered by the Regional Transplant Centre for borderline situations or cases that require specific in-depth analysis.


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## SETTING UP A SERUM EYE DROPS PROGRAM IN AN AUSTRIAN BLOOD AND TISSUE BANK

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### Content

Serum eye drops (SED) are used in patients suffering from severe ocular diseases, like dry eye or persistent epithelial defects, where the patient has not responded to conventional treatment, as a last resort. Both, autologous and allogeneic SED are in use, either undiluted or diluted, containing growth factors and other components coming close to natural tears.

SED may fall within different national legal frameworks in the EU with different quality and safety requirements. In Austria SED are classified as medicinal product for named patient use.

In 2009 our Blood and Tissue Bank started processing SED for selected patients.

Whole blood of approximately 450 ml is withdrawn from healthy, unpaid, voluntary donors, using a sterile closed blood collection system (TF304 system, Meise GmbH, Germany). After clotting, centrifugation and filling of the SED applicators (TF36 applicators, Meise GmbH, Germany), a maximum of 72 applicators with each approx. 2 ml of serum inside, can be obtained and frozen at -30°C.

Asides donor testing for infectious diseases (HIV, HBV, HCV, TPHA, HSV, CMV) each product is tested for residual blood cell count and sterility.

Up to now sterility testing was done with direct inoculation according to Ph. Eur. 2.6.1. The presentation

will give an outlook on the change of the testing method to blood culture testing (according to Ph. Eur. 2.6.27).

After product release SED are delivered to the patient via pharmacy and further stored in a domestic freezer with a freezing temperature of about -18°C. Therefore a stability program was set up for SED, in order to clarify the influence of different storage temperatures, comparing various protein levels (EGF, PDGF, Fibronectin) at different time points (fresh, six and 12 months).

It could be shown that stability is not influenced by the different storage temperatures and can be retained at least for six months.

## SCREENING FOR CORNEA GUTATTA DURING ORGAN CULTURE

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### Content

Purpose: Eye bank quality controls aim at eliminating corneas with a potential risk for the recipient. During endothelial control, abnormalities in the center of the endothelium lead to the suspicion of Cornea Guttata (early stage of Fuchs Corneal Endothelial Dystrophy (FECD)). Aim: to compile these anomalies and verify their exact nature by histopathology.

**Material & Methods**: Each cornea had early endothelial control after receipt (between D2 and D5): staining with trypan blue 0.04% for 1 minute and rinse with NaCl 0.9% for 4 minutes to dilate the intercellular spaces and allow the endothelial cells to be visualized under an optical transmitted light microscope. A cornea guttata was suspected in the presence of rounded excrescences of the Descemet membrane with or without brown pigments in the central area. These corneas were systematically sent to the BiiGC laboratory for immunofluorescence on flat mounting of the main FECD markers.

**Results**: Between January 2016 and June 2019, 2368 corneas were received at our Eye Bank. Donors, 39.6% female, were 74 years old on average (18-106). Of these corneas, 624 (26%) showed endothelial nonconformity, of which 32 (5% of the 624 and 1.3% of all corneas) were suspected of Cornea Guttata. Histology confirmed all cases. These donors, 53% female, were 77 years old on average (53-93). Central endothelial counting was still possible for 26 corneas (between the guttae or in the mid periphery), 12 had an ECD<2000 cell/mm2 (average 1915 +/-562 from 854 to 2992) but 14 had an ECD>2000 (average 2331+/-346, from 2010 to 2992).

**Conclusion**: The detection of cornea guttata is an important step in endothelial quality control since the ECD figure is not sufficient to eliminate 100% of cases. The examination under an optical microscope is sensitive enough for trained technicians. Any central anomaly must raise the suspicion of a guttata. Histological verification provides confirmation that is essential for the training of technicians.


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