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ABOUT EATB

European Association of Tissue Banks (EATB) is a scientific society gathering medical personnel involved in various aspects of tissue and cell banking from its foundation in 1991. EATB is focussed on altruistic donation of tissues and cells and promotes WHO Guiding Principles on Transplantation providing orderly, ethical rules for the use of human cells, tissues and organs for therapeutic purposes. Tissue and cell banking is a field of a medicine whose task is to prepare and store tissues procured from deceased or obtained from living donors, as well as isolation of cells procured from living donors used for treatment of patients. Banked tissues and cells are used in many areas of medicine for purposes of surgical treatment of traumas, degenerative diseases, congenital or acquired defects and reconstructions in oncology. Blood stem cells are used in treatment of hematologic malignancies.

Tissues and cells processed in tissue banks have been recognised for years as a gift of the society to the patients subjected to surgical transplant procedures. Voluntary and unpaid donation promoted from the very beginning of tissue banking activities has been recognised as one of the pillars supporting transplantation medicine, which has been reflected in the European regulations of tissue and cell banking. The EATB has many diverse functions. EATB supports European and other international endeavours to improve tissue banking.

They works with regulators to constantly strive for greater safety and efficacy. And finally, EATB holds annual congresses to provide a forum for scientific, ethical and clinical activities relating to tissue banking and to provide a forum for presentation of research and collaborative working.

For more information about the EATB, its members in all medical, scientific and other professional activities associated with tissue banking, please visit: www.eatb.org
ORAL PRESENTATIONS
ADIPOSE TISSUES

REGENERATIVE POTENTIAL OF SUBCUTANEOUS ADIPOSE TISSUE
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In the last years, the attention of plastic surgeons and researchers has been focused on the reconstructive technique based on the homologous adipose tissue implant. Fat seems the most promising tool for the regeneration and reconstruction of numerous tissues. Surgeons employed the fat graft technique for the reconstruction of breast, vocal cord, burns, wound healing, cartilage repair. Many researchers contributed to the characterization of subcutaneous adipose tissue and described the fat as the main source of regenerative niches, characterized by mature adipocytes, mesenchymal stem cells and capillaries. Adipose tissue is easily accessible and the purification protocols have been optimized and standardized. It is possible to use adipose tissue subjected to a simple purification by gravity, or the micro fractured adipose tissue. Moreover, nowadays, it is possible to make a fat graft using only adipose tissue or adipose tissue enriched by blood components, to improve the regenerative pathways. But, actually, is not present a standardized protocol for adipose tissue cryopreservation, although the strong interest of surgeons and researchers in investigate a methodology, that ensure the maintainment of adipose tissue regenerative potential. The cryopreservation could guarantee a single harvesting procedure and the storage of adipose tissue aliquots, to preserve the patient from repeated liposuctions to harvest adipose tissue, with an important decrement of pain and discomfort.

FUNCTIONAL IMPROVEMENT IN PERIORAL SCLERODERMA BY FAT GRAFTING
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Background Perioral modifications are significant stigmata of systemic sclerosis, with functional implications that negatively impact daily life. Lips stiffening results in microstoma, dryness, easy ulceration, difficult speech, food biting, oral hygiene and dental care, besides alteration in psychosocial interactions.

Objective As fat grafting has emerged as a “regenerating” treatment for scarred and fibrotic tissues, scleroderma patients may receive significant benefits from the procedure.

Methods Fat grafting was proposed to patients with perioral fibrosis in systemically stable scleroderma. 8 patients in 2 years met inclusion criteria and accepted grafting. Patients were stadiated for functional impairments with a specifically designed questionnaire. Follow up for up to 12 months included a post-operative questionnaire for subjective functional performance of the perioral region (-5/+5 scale), serial echographic assessments of fat grafts, and lips width measurement.

Results Patients reported pre-operative impairment concentrated in three daily activities (speech, eating, oral hygiene) and three psychosocial items (un easiness with others, tension in interactions, embarrassment in interactions). After fat grafting, all patients reported significant benefit in lips softness, mouth opening and appearance at 1 and 3 months. One patient reported total regression of achieved benefits by the third month. All other patients reported a partial regression of functional benefits between the 3 and 6 months, with results then stable at 1 year, with subjective perception of improvements >=3 (in -5/+5 scale) in lips sensibility, lips/mouth dryness, movement, opening, softness, as well as aesthetics and easiness in social interactions. Serial echography confirmed a significant increase in thickness in grafted areas, with progressive reabsorption over time. No variations in lips diameters were detected.

Conclusions Fat grafting seems an option to provide functional relief to systemic sclerosis patients with perioral impairment. Even if not all patients may respond, most seem to receive long term functional benefits.

SUCCESSFUL RECELLULARIZATION OF HUMAN TENDON SCAFFOLDS USING ADIPOSE-DERIVED MESENCHYMAL STEM CELLS AND COLLAGEN GEL
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The major goal of regenerative medicine is to determine experimental techniques that take maximal
advantage of reparative processes that occur naturally in the animal body. Injection of mesenchymal stem cells into the core of a damaged tendon represents such an approach. Decellularization of native tendons as potential targets and seeding protocols are currently under investigation. The aim of our study was to manufacture a recellularized biocompatible scaffold from cadaveric tissue for use in total or partial tendon injuries. Results showed that it was possible to introduce proliferating cells into the core of a decellularized tendon to treat the scaffold with a collagen gel. The method was effective in maintaining scaffold extracellular matrix and for expressing collagen type I and cartilage oligomeric matrix protein by injecting mesenchymal stem cells.

AMNIOTIC MEMBRANE

THE SWISS EXPERIENCE WITH EPIFIX
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Introduction In chronic hard to heal wounds, regenerative biologics are a well-known treatment option. Their form and applicability is continuously improved, which makes its use easier and its storage less demanding. We present a case series of the first applications of a processed human placental tissue allograft in different highly specialized wound centers in Switzerland.

Materials and Methods 61 Patients with hard to heal wounds were treated over a mean time of 4.8 weeks (range 2-12 weeks) with weekly application of the substance. The range of the origin of the wounds was very diverse (venous and mixed ulcers, arterial ulcers, Livedo and vasculitic ulcers, neuropathic and diabetic ulcers, postoperative wounds, etc), as were the patients.

Results We achieved in 61% of the patients a complete healing of the wounds (in vascular patients even 78%). Success was defined as healing of a wound previously not responding to local best practice during more than 3 months, and / or clearly exceeding expert expectation.

Discussion We present the Swiss experience in the first treatment series of chronic hard-to-heal wounds with a processed human placental tissue allograft. This case series showed with a 61% healing rate overall and 78% in vascular ulcers very promising results. It’s easy applicability and not demanding storage makes this product a valuable treatment option for this kind of wounds. Of course, larger studies with a higher number of patients are needed.

MICRO-GRAFT INJECTION OF FRESH AMNIOTIC MEMBRANE DURING “OPEN” NERVE- SEMINAL SPARING RADICAL PROSTATECTOMY: THE RIGENERA METHOD

Aim Of The Study The amniotic membrane dehydrated was used in robotic-assisted radical prostatectomy to improve functional outcomes, with interesting results related to the properties of the membrane: the presence of growth factors, anti inflammatory and anti-fibrotic factors. Regenera™ is the quickest method to obtain micro-tissue grafts of 50 microns mean size that can be used immediately in the clinical setting. The micro-grafting is normally autologous and contextual. The tissue is placed within the Rigeneracons™ and disaggregated, adding 1 ml of saline solution, via a rotating microknife at 80 rpm, using a tangential force and no incidental: in this way is not damaged the viability of autologous grafts. The system is based on evidence which in any solid tissue there is a multi potent progenitor cell side-population responsible for regeneration, smaller than 50 microns.

Aim of this study is to evaluate the safety and efficacy of the RIGNERA method, which is used to perform micro-graft transplantation of amniotic membrane in course of nerve-seminal sparing radical prostatectomy “open” for prostate carcinoma.

Material And Methods In the video the authors show the basic steps of the technique: after removing the prostate with neuro-vascular bundle and seminal vesicles sparing, we proceed, first of the vesico-urethral anastomosis, to the injection in the urethral sphincter and in the bundles of micro-grafts of fresh amniotic membrane, processed with REGENERA method.

Results We performed 8 cases with no complications related to the procedure. All patients had an early recovery of continence (<7 days). At a median follow up of 6 months, 4 patients had erectile activity
recovery with or without pharmacological aid for os. **Discussion** Fresh amniotic membrane, compared to dehydrate, presents plenty of growth and anti-fibrosis factors; in its “natural” preparation it is difficult to apply because of its particular consistency; thanks to the RIGENERA system, it is possible to obtain an injectable solution rich in growth factors and anti-inflammatory agents that can be easily injected along the course of the bundles and the urethral sphincter, favoring a faster recovery of continence and erectile function, after radical prostatectomy.

**FROZEN AMNION MEMBRANE FROM DGFG FOR A WIDE RANGE OF MEDICAL INDICATIONS**
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The amniotic membrane (AM) is a relatively simply constructed tissue consisting of epithelium and stroma. However, it possesses unique characteristics and properties, which can be efficiently applied in many medical fields. For example, it does not express the HLA antigen, therefore being non-immunogenic and not rejected by recipient. Antimicrobial, anti-inflammatory, and antiangiogenic properties of AM have been demonstrated. The antifibroblastic activity helps to prevent scarring, an important property that has made the application of the AM so valuable in ophthalmology. In addition, AM stimulates cell migration and cell proliferation, contributing greatly to the wound healing processes. Growth promotion is mainly mediated by growth factors. The transplantation of amniotic membranes to the surface of the eye has been used in ophthalmology since 1940s (de Röth). Since then, the indications for amnion membrane transplantation (AMT) have been constantly expanded in ophthalmology.

Besides ophthalmology, distinctive properties of AM determine its application in a wide range of therapeutic approaches. As early as 1910, AM was described by Davis as skin transplant, shortly followed by works of Sabella and Stern (1913) with successful application for the treatment of burns. Since then, the use of AM has been described in numerous publications for the treatment of various pathologies, including gynecological, as well as oral and maxillofacial surgery. Lately, amnion membranes can also be obtained from the DGFG in several sizes, suitable for various fields of application. For a consistent supply, the changes in biochemical and biomechanical properties of the AM are examined after multiple cryopreservation cycles.

**THE USE AND INTEGRATION OF AMNIOTIC MEMBRANE IN PEDIATRIC PLASTIC SURGERY PATIENTS: EXPERIENCE IN AOU CITTA’ DELLA SALUTE E DELLA SCIENZA**
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The use of the membrane administered by the city of health and science in Turin has emerged (was born) from an experience that seems exceptional and potentially able to provide answers to multiple difficult treatment needs. The first use in Turin and the first case all over the world in the neonatal era was the one for which used amniotic tissue in a baby with a large loss of substance of spina bifida. The short and long term results were not only encouraging but incredibly satisfying, allowing a rapid recovery of the integuments and a great soil to allow physical and physical activity to be guaranteed and a quality almost normal life to the small patient. After this first case, others are followed. One again on a defect of bifid spine had placed to a consistent result and better than the first, another in treating a ulcer compression and then four other cases of injury of the tissue for trauma or decubitus. The point of arrival was encouraging. The therapeutic pathway is liable to be applied singularly or also associated with other regenerative medicine methods such as dermal cones. The purpose of this presentation is to share my experience with you and discuss what new ideas will be able to offer clinicians to improve the treatment of complex lesions and cases by providing answers to the needs of our patients.

**THE USE OF CRYOPRESERVED AMNIOTIC MEMBRANE IN PEDIATRIC PATIENTS: CASE REPORTS**
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The treatment of difficult-to-heal wounds, chronic ulcers and burns produce significant clinical and economic burdens, and the healing process became a long and painful process with recurrent breakdown. This problem has a wider impact in pediatric patients. The widespread use of human amniotic membrane (HAM) in surgery and the results obtained have highlighted its many potential properties, including antimicrobial, antiinflammatory, antifibrotic and antiapoptotic. HAM transplantation promotes re-epithelialization, decreases inflammation and fibrosis and modulate angiogenesis, thanks to the presence of growth factors and cytokines.

In Treviso Hospital, Plastic Surgery Unit in collaboration with Pediatric Surgery have implanted HAM in four pediatric patients for different indications with promising results. HAM was collected, processed and cryopreserved by Treviso Tissue Bank Foundation. The first case reported is the application of HAM on a 3 days old patient for the treatment of myelomeningocele, the defect was covered with a patch 10 x12.5. In addition, we reported the application of an HAM patch 3x3 on a 5 years old patient for the healing of a wound with material leakage caused by a dog’s bite. Finally, the other two young patients, 22 days and 18 months old respectively, were treated with 5x5 HAM patches for burns, one caused by extravasation of antiepileptic drug and the second one by iron contact. Long term follow for all patients demonstrate a total wound healing and the absence of infections or inflammations.

We strongly support the use of HAM as an efficient therapy and we consider this option a first surgical treatment choice.

THE USE OF AMNIOTIC MEMBRANE AS SUPPORT FOR THE GROWTH OF EPITHELIAL STEM CELLS IN A GMP SETTING

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Purpose Cell therapy has been shown to be a therapeutic alternative for disorders leading to Stem Cell Deficiency. Two culture approaches are used for growing Advanced Therapy Medicinal Products (ATMPs) containing corneal or conjunctival epithelial stem cells: the explant and the single cell suspension techniques. Amniotic Membrane (AM) and Fibrin Glue (FG) are commonly used as scaffolds. The aim of our study is to compare the 2 culturing approaches using AM or FG and to evaluate whether animal-free media could sustain the growth of corneal and conjunctival epithelial stem cells. This could be advantageous for GMP (Good Manufacturing Practices) standards, as variability needs to be minimized. The absence of animal-derived products in the culturing protocols will reduce the risks of pathogen transmission and host immune reactions that can potentially lead to stem cell graft failure.

Materials & Methods The standard medium was compared to 3 different animal-free formulations and tests carried out on plastic and scaffolds. The cultures were analyzed through life span assay, morphological analysis, immunological and molecular tests.

Results All the new formulations showed to be optimal for cell cultures on plastic, but only one for culturing cells on scaffolds. AM variability might be a challenge for GMP, as reproducibility could not be guaranteed using the cell suspension technique.

Conclusion Animal-free culturing conditions could be successfully used for manufacturing ATMPs. Batch-to-batch variability of AM remains an issue for GMP requirements and strategies aimed at reducing variability (decellularization?) should be evaluated.

CARDIOVASCULAR TISSUES

ADVANCED PRESERVATION METHODOLOGIES FOR DECELLULARIZED CARDIOVASCULAR SCAFFOLDS

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Background Development of long-term preservation methods for cardiovascular grafts is of major importance to prolong their shelf-life. The objective of this study was to evaluate the suitability of three different preservation methods: cryopreservation, ice-free cryopreservation (vitrification), and freeze-drying
for preservation of decellularized bovine pericardial (DBP) scaffolds.

**Material and Methods** DBP samples were subjected to either cryopreservation, vitrification or freeze-drying. Cryopreservation was conducted at ~1°C/min using 10% DMSO. Vitrification was performed using vitrification solution VS83 and rapid cooling. Freeze-drying was done using a programmable freeze-dryer on samples which were infiltrated with sucrose for lyoprotection. The impact of the preservation methods on the structural integrity of the scaffolds was assessed using histological staining, biomechanical and biochemical tests. Microscopic, spectroscopic and thermal analysis were also performed.

**Results** Histological staining and microscopic analysis revealed that the extracellular matrix integrity was preserved after all preservation treatments. In addition, matrix proteins were not affected by any of the preservation treatments. Uniaxial tensile tests indicated that the cryopreserved group has a significantly increased collagen phase modulus and ultimate tensile strength compared to the control group indicating a decrease in scaffold compliance, which was not observed in vitrified and freeze-dried scaffolds.

**Conclusions** It is shown here that cryopreservation, the most widely used preservation method for cardiovascular tissues, alters biomechanical behaviour which was not observed in vitrified or freeze-dried scaffolds.

**HOMOGRAFT SURVIVAL AFTER RIGHT VENTRICULAR OUTFLOW TRACT RECONSTRUCTION RELATED TO DONOR CHARACTERISTICS AND TISSUE PRESERVATION**

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**Background** Cardiovascular homografts are used for right ventricular outflow tract (RVOT) reconstruction. The procedure has excellent survival rates but many homografts degenerate and require reintervention. Studies analyzing risk factors for reintervention often focus on patient and homograft characteristics with identification of risk factors such as young patient age and use of aortic homografts. The aim of this study was to focus on less known variables including donor characteristics and homograft management and its impact on long-term outcome of homograft implantation.

**Methods** A retrospective study was conducted, including patients undergoing RVOT reconstruction at University Hospital Lund between 1995-2008 (n = 304). Follow up was up to 22 years. Donor age, donor gender, donor type, ischemic time and retrieval to cryopreservation time were analyzed. Statistical analyses included Kaplan-Meier method with log-rank test and Cox proportional hazard regression.

**Results** Follow up was 98% complete. There were 12 deaths. 115 reinterventions were required. Donor age <30 years were identified as a risk factor with significance in univariable and multivariable analysis. Homograft from multi organ donors with no ischemic time produced the longest homograft survival, but ischemic time >24 hours in non-heart beating donors (NHBD) had a lower reintervention rate than 1-24 hours. Longer retrieval to cryopreservation times seemed to be better when compared to shorter time intervals.

**Conclusion** Homografts from donors above 30 years should be chosen when possible. There is no harm in extending the ischemic time in NHBD to 48 hours, in contrary it seems that longer ischemic times might be preferable.

**CLINICAL USES OF CRYOPRESERVED ALLOGENIC ILIAC ARTERY AND VEIN GRAFTS IN TRANSPLANTATION**

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Iliac artery and vein homografts are critical for revascularization in living-donor liver transplantation. The role of allogenic iliac arteries and veins serve as ‘jump grafts’ to lengthen portal or hepatic veins, connect multiple segmental venous outflows, and arterialise the portal vein of liver recipient. Since 2010, National Cardiovascular Homograft Bank (NCHB) and National University Hospital have collaborated in the pioneer endeavor of banking iliac vessel homografts for such surgeries in Singapore. And in 2015, NCHB collaborated with Singapore General Hospital to set up a similar iliac vessel banking programme. The processing, decontamination and cryopreservation techniques that our bank follows, help preserve iliac vessel homografts for a longer duration as compared to homografts preserved using short-term preservation techniques. Criteria for donor assessment,
techniques of iliac vessel homograft recovery, processing, decontamination, cryopreservation and storage according to the American Association of Tissue Banks standards.

From 2010 until 2016, we discovered of the 81 iliac vessel homografts processed, 77 (95.1%) were suitable for clinical use. 34 iliac artery grafts (77.3%) and 10 iliac vein graft (22.7%) were implanted. Irrespective of vessel type, homografts <50 mm in length were of little clinical use. Of the current iliac vessel homograft recipients, 32 patients had living-donor liver transplantation and one patient had reconstruction of the right internal carotid artery after resection of an aneurysm.

Our preliminary results supports existing literatures that suggest cryopreserved iliac vessel homografts can be successfully used for revascularization in liver transplantation and reconstruction of carotid artery. Encouraging short- and mid-term post-operative patient outcomes have been achieved, with no report of adverse event attributed to implanted homografts. We believe that our processing, decontamination and cryopreservation techniques have helped preserve the homografts for longer duration as compared to homografts preserved using short-term preservation techniques.

VALIDATION PROCEDURE OF HUMAN CRYOPRESERVED PERICARDIUM FOR CLINICAL USE
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**Background** In several surgical domains indications for clinical use of pericardial allograft patches exist. Especially in the field of paediatric cardiology there is an increasing demand for human pericardial allografts for clinical use.

**Objectives** Validation of cryopreserved human pericardial tissue from multiorgan/tissue donors for clinical use in humans.

**Methodology** Pericardium is harvested from 20 deceased donors (heart retrieval for transplantation or for harvesting valves or thoracic vessels). At arrival, samples for light microscopy (LM), transmission electron microscopy (TEM) and mechanical testing are taken (‘control’). Pericardium is then cryopreserved, stored at <-150°C, thawed according to protocol, and samples for LM, TEM and mechanical testing are taken (‘study’). Mechanical and morphological tissue properties are compared between study and control samples. This project is done at the European Homograft Bank (Brussels) after approval of the Ethical Committee (University Hospitals Leuven).

**Results** This abstract currently reports work in progress, to be completed within 6 weeks. Until now, no morphological alterations were seen after cryopreservation and thawing. The mechanical properties of cryopreserved and thawed pericardium were sufficient to resist physiological pressures in the human circulatory system.

**Conclusions** This work in progress indicates that cryopreservation and thawing of pericardial allografts - using the same EHB protocols and validation design as for the heart valve allografts - do not affect mechanical and morphological properties of human allograft pericardium. If completion of the test set confirms this, cryopreserved human pericardial tissue can be used in clinical practice. Further consideration is needed whether age limitations for donation of pericardium are needed.

DEVELOPMENT OF SMALL-DIAMETER VASCULAR GRAFTS THROUGH DECELLULARIZATION OF HUMAN BLOOD VESSELS
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Ready-to-use and simple-to-manage small diameter vessels substitutes are necessary for therapies of vascular diseases that need implants. Synthetic grafts frequently result in rejection and autologous substitutes are often unavailable, so tissue banks could be the best source of tissue samples to develop tissue-engineered small-diameter human blood vessels that can be used for implantation/integration during reconstructive surgery. This study aimed to develop vascular grafts through the decellularization of human small arteries/veins and to verify their integration in an experimental rabbit model. The scaffolds obtained using a detergent-enzymatic decellularization protocol were implanted at the femoral vein or artery of rabbits and maintained for two weeks in vivo. Smooth muscle cells had been removed, as confirmed by anti-α-smooth muscle actin immunohistochemistry, and collagen and elastic components were preserved, as showed by histological stainings (azan-Mallory and van Gieson) and by morphometric and transmission electron microscopy evaluations.
Quantitative evaluation of DNA demonstrated satisfactory removal; DAPI staining showed that nuclear remnants were absent. After they were surgically implanted, the vascular grafts remained patent and functional, without thrombosis or inflammatory rejection. The venous explanted grafts showed good endothelialization and cell invasion with smooth muscle cells in the media. The arterial ones, instead, showed only partial re-cellularization of the medial and adventitial layers. In conclusion, study findings showed that human small-diameter vessels can be effectively decellularized and vascular scaffolds are suitable for implantation/integration.

**MESENCHYMAL STROMAL CELL QUANTIFICATION IN RIGHT AND LEFT VENTRICLES**

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**Background** The myocardium exhibits distinct regional differences in cell composition that influence heart physiology and pathogenesis. Cardiac mesenchymal stromal cells (C-MSC) are fibroblastoid progenitors crucially involved in cardiac structure and homeostasis. The differential amounts of C-MSC in right (RV) and left (LV) ventricles have never been investigated.

**Results** We have dissected RV and LV from C57BL6 mice hearts (n=6) and obtained free wall RV and LV samples from healthy human cadaveric donors (n=5). In murine heart tissue, using immunofluorescence experiments, a difference in CD44+ or CD105+ cell number between RV vs. LV was found (CD44+ 2.16 fold, p=0.06; CD105+ 1.37 fold, p=0.04 in RV vs. LV, respectively). No viable C-MSC from murine LV tissue were obtainable, while a mean of 1.78x10^6 ± 0.89x10^6 C-MSCs/g tissue from the RV were isolated. A similar pattern was observed in human tissue: a higher ratio of CD44+ (2.05 fold, p=0.06), CD29+ (2.12 fold, p=0.08) and CD105+ (3.42 fold, p=0.05) cells has been found in the RV with respect of LV. No viable C-MSC from murine LV tissue were obtainable, while a mean of 1.78x10^6 ± 0.89x10^6 C-MSCs/g tissue from the RV were isolated.

**Conclusions** We observed in the RV of both mice and humans a significant higher amount of C-MSC with respect to the LV counterpart. Further studies may shed light on the physiological meaning of this observation, that may have implications in the pathophysiology of cardiac diseases involving fibrosis and C-MSC differentiation.

**IN VIVO REGENERATION OF MICROVASCULAR PEDICLE**

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**Introduction** The difficulty of obtaining significant long-term patency and good wall mechanical strength in vivo has been a significant obstacle in achieving small-diameter vascular prostheses. The aim of the present study was to develop a prosthetic graft that could perform as a small diameter vascular conduit for artery and vein regeneration.

**Methods** Resorbable prostheses of hyaluronan and collagen (2 mm diameter, 1 cm length) were grafted in the abdominal aorta (n = 30), and in the vena cava (n = 30) of rats as temporary absorbable guides to promote regeneration of vascular structures. Performance was assessed until 180 days after surgery by histology and immunohistochemistry.

**Results** Experiments resulted in three novel findings: 1) complete endothelialisation of the tube’s luminal surface occurred; 2) sequential regeneration of vascular components led to complete vascular wall regeneration 15 days after surgery; and 3) the biomaterial used created the ideal environment for the delicate regeneration process during the critical initial phases, yet its biodegradability allowed for complete degradation of the construct four months after implantation, at which time, a new artery and a new vein remained to connect the vascular stumps. This study assesses the feasibility to create a completely biodegradable vascular regeneration guide in vivo, able to sequentially orchestrate vascular regeneration events needed for very small artery and vein reconstruction.

**Conclusion** Resorbable prosthesis opens new innovative possibilities of surgical treatment in the field of reconstructive microsurgery and pediatric vascul-
lar surgery, and a novel experimental model to study artery and vein in vivo regeneration processes.

**HISTOLOGICAL AND APOPTOTIC EVALUATION OF PORCINE CARDIOVASCULAR TISSUE AFTER DECONTAMINATION AND FREEZING**

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**Purpose** The aim of the study is to evaluate the impact of the established decontamination, washing and cryopreservation/thawing procedure on cardiac valve integrity and cell apoptosis.

**Material and methods** Four whole porcine valves (2 aortic, 2 pulmonary: leaflets/wall) were incubated in antimicrobial solution (BASE.128) at +37°C for 14 h, washed three times (stirring at +4°C in BASE solution: 2x 5 min, 1x 6h), cryopreserved and stored in nitrogen vapour. Samples were fixed and paraffin embedded; 4-6 μm sections were stained with H/E for tissue integrity evaluation and Tunel to detect apoptotic cells. Staining was repeated for every sample (leaflet/wall): before (T0), after decontamination (T1), after freezing (T2); qualitative and quantitative analysis were performed.

**Results** H/E stained sections showed that both valves and walls substantially maintain structure, integrity and cellularity.

Tunel staining showed an increase in apoptotic cells (5-10%) between T0 and T1 in pulmonary sample (leaflet/wall), while the percentage of apoptotic cells remains stable between T1 and T2. In aortic leaflet and wall, on the contrary, a statistically significant increase in the apoptotic cells has been shown only between T1 and T2. That suggests that pulmonary valve is more sensitive to decontamination than aortic valve; the aortic valve, on the other hand, appears to be more sensitive to freezing/thawing. Nevertheless at T2, the percentage of apoptotic cells is similar in both types of valves (13-15%).

Preliminary data are promising: our procedures seems to substantially preserve tissue morphology and cell viability; confirmatory experiments will be performed on human samples.

**BIOLOGICAL MESH IN ABDOMINAL INCISIONAL HERNIA: OUTCOME FROM A RETROSPECTIVE CASE SERIES**

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General Surgery, Department of Surgery, Oncology and Gastroenterology of University of Padua, Padua, Italy

**Background** The use of biological meshes in management of potentially contaminated or incisional infected hernia and of giant wall defect is well established. The use of banked fascia lata and pericardium allograft as biological meshes is still under investigation. The aim of this study is to evaluate the outcome of homologous meshes in incisional hernia repair.

**Methods** Patients undergoing incisional hernia repair with biological mesh (fascia lata or pericardium allograft) were reviewed. A retrospective evaluation of clinical data was performed paying specific attention to hernia recurrence and wound complication.

**Result** From September 2012 to May 2017, 19 patients were treated (9 F, 10 M), mean BMI 28.6 +/-6.6, and the median follow-up was 20.5 months. All cases were complex ventral hernias: 13 patients (68.4%) were classified as grade 3 (potentially contaminated wounds) according to the VHWG risk classification, 4 patients as grade 4 (infected surgical site) and in 3 patients had a giant wall defect.

All patients were treated by using homologous mesh and in one case homologous mesh was combined with polypropylene mesh.

Recurrence was observed in 4 patients (23.5%). Two patients (10.5%) had partial skin necrosis and required dermis allograft; six patients (31.5%) required outpatient treatment.

**Conclusion** No trials have been performed to date evaluating banked fascia lata or pericardium allograft as biological materials in incisional hernia repair. According to our preliminary outcome, homologous allograft seem to be a safe and an effective method for the repair of infected or giant incisional hernia.

**THE ECONOMICS OF CARDIOVASCULAR TISSUE BANKING: A DUTCH AND EUROPEAN PERSPECTIVE**

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**Background** The use of biological meshes in management of potentially contaminated or incisional infected hernia and of giant wall defect is well established. The use of banked fascia lata and pericardium allograft as biological meshes is still under investigation. The aim of this study is to evaluate the outcome of homologous meshes in incisional hernia repair.

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**Conclusion** No trials have been performed to date evaluating banked fascia lata or pericardium allograft as biological materials in incisional hernia repair. According to our preliminary outcome, homologous allograft seem to be a safe and an effective method for the repair of infected or giant incisional hernia.
This study looks into how the fees for heart valves are set and regulated in Europe. What are the economic factors that affect the (cost) pricing of cardiac allografts in the Netherlands and other European Countries?

**Aim** Understanding the economics of cardiovascular tissue banking with a quantitative and qualitative approach. Explaining the elements composing the price of tissues: cost-process factors, organizational factors and availability factors. Identifying the economic weaknesses of the Dutch Heart Valve Bank.

**Method** The study explores and expands the limited literature with a survey to 26 European banks. The information is analyzed with modern econometric techniques and scientific data analysis tools. To assess the causal impact of cost factors and opt-out policies on the price of heart valves, an Instrumental Variable approach is adopted.

**Results** The study finds that an opt-out system decreases the price of a valve by almost 1000 Euro. It also confirms the positive impact of higher salaries in the medical sector in different countries. The empirical design can allow for causal inference of these results. No correlation is found with socio-demographic and unchangeable factors traditionally considered to influence donations.

**Implications** The results suggest that there is room for concrete action in The Netherlands. An optimal policy combination should include an improvement of the organizational structure and a change in legislative defaults from opt-in to opt-out. Tissue establishments should be set free to regulate their prices. To improve scale economies, integration with hospitals and mergers into bigger, multi-tissues structures might be encouraged.

**CORNEA**

**HISTOPATHOLOGY OF DESCemet MEMBRANE ENDOTHELIAL KERATOPLASTY GRAFT REMNANTS, FOLDS, AND DETACHMENTS**

Eva Prosecka

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**Purpose** To describe the histologic features of post mortem eyes after Descemet membrane (DM) endothelial keratoplasty (DMEK) and their potential clinical implications.

**Methods** Eleven post mortem DMEK corneas of eight patients, operated for Fuchs endothelial dystrophy (FED), with an average postoperative time of 3.5 \( \pm 1.9 \) years (range 7 months to 6 years), were procured after death and processed for light microscopy evaluation.

**Results** Nine corneas showed a ‘normal’ central anatomy, i.e. the donor-to-host interface resembled that of a virgin eye. One cornea also showed an anatomic centrally ‘normal’ periphery. Ten corneas showed peripheral abnormalities. In 9, the DMEK graft overlapped the edge of the descemetorhexis. 1 eye showed scarring overlying a portion of the graft with a previous detachment followed by spontaneously adherence: 3 eyes showed graft folds with scarring; in 2 eyes, the anterior banded layer of the host DM was still in-situ across the cornea (both of these eyes had required re-bubbling); and 2 eyes showed host DM remnants within the corneal incision that may have interfered with wound healing.

**Conclusion** Incomplete host DM removal may relate to postoperative DMEK graft detachment and wound instability. Graft detachments may re-attach with interface scarring. Re-bubbling procedures may be performed within 4-6 weeks, before scarring of detached graft portions occurs. Subtle DMEK graft folds may explain subjective complaints of monoculaire diplopia.

**DESCEMET MEMBRANE KERATOPLASTY (DMEK): SECURITY AND Efficacy ANALYSIS THROUGH CLINICAL FOLLOW-UP.**

Nausica Otero(1) - Anna Vilarrodona (1) - Elba Agusti (1) - Jorge Peraza(2) - Josep Torras(2) - Eva Maria Martinez (1) - Jaime Tabera(1) - Esteve Trias(1)

Banc de Sang i Teixits, Barcelona Tissue Bank, Barcelona, Spain

**Purpose** After establishing a standardized and reproducible procedure for the stripping of Descemet membrane for DMEK, we wanted to analyze the security and the efficacy of the transplanted endothelial membranes to close the validation process.

**Methods** 11 endothelial membranes (i.e. age \( \geq 50 \) and cells \( \geq 2500 \)) for DMEK were obtained and transplanted from September to November of 2016. Early tissue engraftment efficacy was analyzed by a questionnaire form to know about tissue handling by the surgeon and the immediate postoperative attachment status. Lately, a follow-up after one month was done in order to know the evolution.

**Results** 5 different experienced surgeons from 3 transplanting centres were participating in the validation. Several factors such as graft marking, kind of injector...
used, graft unfolding time, immediate post-operative status, immediate rebubbling were analyzed. The immediate post-operative attachment was full in 9 cases and peripheral detachment in 2 cases. The final tissue quality was evaluated as excellent in 9 cases, good, in one case and average in other case. From the 11 cases, 9 resulted in satisfactory evolution and 2 in non-satisfactory evolution. Fifty four endothelial membranes have been transplanted by 13 surgeons, from December 2016 to August 2017. In 51 of the cases successful engraftments were reported and 3 of them were not successfully unfolded and attached. 

**Conclusion** Apart from knowing about the immediate postoperative attachment status, follow-up programs are carrying out to assure safety and efficacy of the tissue transplanted.

**DESCEMET MEMBRANE ENDOTHELIAL KERATOPLASTY (DMEK) PREPARATION OF CHALLENGING DONOR CORNEAS BY EYE BANK SPECIALISTS TO REDUCE THE WAITING LIST FOR KERATOPLASTY**

Kristin Mangundap(1) - Esther Groeneveld-van Beek (1) - Jessica Lie(1) - Anita Sajet(1) - Jet Kok(1) - Eva Prosecka(1) - Jacqueline van der Wees(1) - Gerrit Melles(2)

Amnitrans Eye Bank, Netherlands Institute for Innovative Ocular Surgery, Rotterdam, Netherlands

Purpose To evaluate the increase in endothelial keratoplasties (EK) by using eye bank prepared Descemet Membrane Endothelial Keratoplasty (DMEK) grafts from donor corneas unsuitable for Penetrating (PK), Descemet Stripping Endothelial keratoplasty (DSAEK) or DMEK preparation by the surgeon.

Method Donor cornea selection criteria for eye bank and surgeon cut tissue were evaluated. The percentage of DMEK grafts that were transplanted from otherwise unused corneas prepared by the eye bank was evaluated for the last 2000 donor corneas designated for EK.

Results Out of 2000 corneas designated for EK, 244 surgeries (12%) were performed using DMEK grafts prepared and distributed from corneas that were unsuitable for graft preparation by the surgeon.

Conclusion The keratoplasty waiting list shortens by employing eye bank specialists for DMEK preparation of corneas unsuitable for sending to the surgeon for preparation.

**Financial Disclosure** Dr. Melles is a consultant for DORC International/Dutch ophthalmic USA and Surgicube International. The other authors have no potential conflict of interest to disclose.

**COMPARISON OF PRESERVATION AND SHIPPING PROTOCOLS FOR PRELOADED DESCemet’s MEMBRANE ENDOTHELIAL KERATOPLASTY TISSUES**

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Aim Descemet Membrane endothelial keratoplasty (DMEK) preparation is challenging and therefore limits the uptake of this kind of surgery. Supply methods that simplify the surgical steps are key to increasing DMEK. This study compares two different shipping protocols for DMEK currently pursued in Europe and the America.

Methods 8.5 mm DMEK graft was punched, marked and loaded for transportation in two different conditions, A) endothelium tri-folded inwards in organ culture at 31°C (n=7) and B) endothelium rolled outwards in hypothermic conditions at 4°C (n=7). Tissues were shipped from Italy to the UK where they were analyzed for orientation, endothelial cell density (ECD), denuded areas, cell mortality, triple viability staining (HEC) for apoptosis, immunolocalisation of ZO-1 and Na/K-ATPase proteins, visualisation of actin filaments using phalloidin and histological analysis using haematoxylin and eosin on paraffin embedded sections.

Results All tissues clearly showed the ‘F’ mark used for graft orientation. After shipping in condition A, an increase in cell mortality of 8.1% and denuded areas of 22.4% was seen, whereas, for condition B, an increase in cell mortality of 14.2% and denuded areas of 34.3% was observed after shipping. HEC staining revealed areas of viable cells and apoptotic cells with large denuded areas mostly found in the periphery for condition B and within folds for condition A.

Conclusions Prestripped preloaded DMEK grafts retained sufficient viable cells for transplantation with condition A (endothelium-in). Endothelium tri-folded inwards gives additional advantage in terms of tissue unfolding during transplantation.
EUROPEAN REGISTRY FOR QUALITY IMPROVEMENT IN CORNEAL TRANSPLANTATION SURGERY

John Armitage(1)
University of Bristol, School of Clinical Sciences, Bristol, United Kingdom(1)

Purpose The European Cornea and Cell Transplantation Registry (ECCTR) is a project aimed at creating a multi-national database for corneal transplantation (CT) surgery. ECCTR is co-funded by the Health Program of the European Union and ESCRS, and supported by EuCornea and seven EU institutions.

Setting Multi-national, web-based, quality registry for CT surgery.

Methods We describe the set-up of a web-based system with a software interface for input and output of data related to CT surgery. Output of reports or export of own data is available on the web. Data is anonymous to all users, with the exception that reporting surgeons have access to their own data. The system does not include any patient identification. A patient-reported outcome extension will be linked to the system.

Results The system was designed to allow both manual input of data via the web and transfer of data from existing national registries and large electronic medical record systems. Interfaces have so far been created for direct transfer of data from three national quality systems: The United Kingdom Transplant Registry (UKTR), the Netherlands Organ Transplantation Registry (NOTR) and the Swedish Corneal Transplant Register.

Conclusions A European quality registry has been set-up for CT surgery. It incorporates one of the largest web-based databases in the field. ECCTR will offer surgeons a tool for quality improvement by comparison and benchmarking with state-of-the-art epidemiological data.

NEW CONTROL INTRODUCED IN THE LAMELLAR PROTOCOL TO ASSURE THE ENDOTHELIAL QUALITY AFTER THE CUTTING PROCEDURE

Nausica Otero(1) - Eva Maria Martinez(1) - Mari Carmen Sole(1) - Yolanda Plaza(1) - Elba Agusti(1) - Anna Vilarrodona(1) - Esteve Trias(1)
Banc de Sang i Teixits, Barcelona Tissue Bank, Barcelona, Spain(1)

Purpose Following the report of 18 primary graft failures with endothelial lamellae sent for transplantation during 2016 and 2017, we decided to study the state of the endotheliums after cutting the corneas with a semiautomatic microkeratome.

Methods In this study, 11 non suitable corneas for transplantation were included. We did an endothelial evaluation (cell counting with sacarose and vitality with trypan blue) prior to the cut, another one after cutting with the microkeratome apart from other assessments at 24 h, 48 h and 72 h.

Results All 11 samples showed an initial viability of more than 90% before the cut. After cutting, 10 of 11, showed a viability of more than 85%. In one case, the viability was 30% (which would have been ruled out for implant) In only 3 cases out of 11 a cell counting could be done after cutting. In 9 cases in which a count was made at 24 h (5 cases) or 48 h (3 cases) or 72 h (1 case), an endothelial counting was possible and the viability was more than 85%. A general pattern of staining with trypan blue was found in diffuse stress lines and also a blue area where the cut begins.

Conclusions Just after cutting the tissue, an endothelial counting could not be performed in the majority of cases. We introduced this parameter of acceptance of ≥80% of viability. So by implementing this new step in our protocol we assure an optimum tissue quality for the implant.

MICROBIOLOGICAL CONTAMINATION RATE OF CORNEAS EXCISED IN SITU VERSUS WHOLE GLOBE ENUCLEATION IN EYE BANK AT UNIVERSITY HOSPITAL CENTRE ZAGREB, CROATIA

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Background The safety and success of a cornea transplant depends on the application of procedures aimed to minimize the risks of transmitting systemic pathologies and eye infections from the donor to the recipient. Our goal was to evaluate whether the retrieval method influence the microbiological contamination rate of the cornea culture medium.

Methods We retrospectively analyzed the difference between the contamination rates of donor corneas retrieved by whole globe enucleation (group 1) and in situ corneoscleral disc excision (group 2) in Eye bank at University Hospital Centre (UHC) Zagreb, Croatia in the period from 2014 to 2016.

Results In the 3-year period, total of 1542 corneas were received in Eye Bank. 452 of them from group 1 and 1090 from group 2. In total 37 organ cultured corneas were microbial contaminated (2.39%), 4
of them were in the group 1 (0.88%) and 33 in the group 2 (3.03%). The contamination rate in group 1 was 1.46% in 2014, 1.11% in 2015 and 0.00% in 2016. The contamination rates in group 2 decrease from 8.90% in 2014 to 2.70% in 2015 and 2.36% in 2016.

**Conclusion** In our study the contamination rates of the two methods depend on the year analysed. The reeducation of the procurement teams in January 2015 resulted in lowering the contamination rate of corneas excised in situ. However, there is still room for improvement in disinfection methods and recovery technique, and the regular evaluation of the team work should be performed.

**CRYOPRESERVATION**

**NOVEL PRESERVATION METHODOLOGIES FOR DECELLULARIZED CARDIOVASCULAR SCAFFOLDS**

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**Background** Decellularized xenogeneic scaffolds have the potential to be used as starter implants for guided tissue regeneration in heart valve surgery. Development of long-term preservation methods for these grafts is of major importance to prolonging their shelf-life. The objective of this study was to evaluate the suitability of three different preservation methods, including slow-freezing-rate cryopreservation, vitrification, and freeze-drying, for preservation of decellularized bovine pericardial (DBP) scaffolds.

**Materials & methods** Bovine pericardium was decellularized using Triton X-100, cholic acid and endonucleases. Following decellularization, DBP samples were subjected to either slow-freezing-rate cryopreservation, vitrification or freeze-drying (n=6 in all cases). Slow-freezing-rate cryopreservation was conducted at -1°C/min using 10% DMSO as cryoprotectant. Vitrification was performed using VS83 (4.65 mol/L formamide, 4.65 mol/L DMSO and 3.31 mol/L Propylene glycol in EuroCollins solution) and cooling above the vapour phase of liquid nitrogen. Freeze-drying was done using a programmable freez-eze-drier with temperature-controlled shelves, whereas the samples were infiltrated with sucrose for lyo-protection. The impact of the preservation methods on the structural integrity of the scaffolds was assessed using histological staining, scanning electron microscopy (SEM), Multiphoton microscopy (TPM) and uniaxial tensile testing. Fourier transform infrared spectroscopy (FTIR) was used to study the overall protein secondary structure and differential scanning calorimetry (DSC) was used to determine thermal protein denaturation profiles.

**Results** The histological staining, SEM and TPM revealed that the extracellular matrix (ECM) integrity was maintained after the preservation treatments compared to the non-preserved control. Inspection of the protein amide-I band (1600-1700 cm⁻¹) in the FTIR spectra showed no statistically significant differences in the overall protein secondary structure after preservation and reconstitution. The DSC results indicated that the protein denaturation temperature was not significantly affected by any of the preservation protocols. The cryopreserved group demonstrated a significantly increased collagen phase modulus compared to the non-preserved control group (p<0.001) indicating a decrease in scaffold compliance. The collagen phase modulus of the cryopreserved group was also significantly increased compared to the vitrified group (p<0.001). All preservation methods resulted in increased scaffold thickness compared to the non-preserved control (p≤0.05).

**Conclusion** The most commonly used preservation method for cardiovascular tissue banking is cryopreservation by slow rate freezing. It is shown, however, that cryopreservation of pericardial tissues using 10% DMSO and slow rate freezing results in more rigid tissues compared to vitrified or freeze-dried tissues. This change in mechanical properties might be caused by damage due to ice crystal formation disturbing the ECM histoarchitecture. Uniaxial tensile testing studies indicated that vitrification and freeze-drying did not modify the biomechanical behaviour of the scaffolds. Histology indicated no differences in the gross histo-architecture after any of the preservation procedures, and also proteins were found to be stable. Whereas cryopreserved or vitrified scaffold are usually stored in liquid nitrogen or a mechanical freezer and include the use of toxic cryoprotective agents, freeze-drying is done with non-toxic protective agents and samples can be stored at room temperature which has clear advantages for biobanking and transport. It is suggested that freeze-drying could replace currently used cryopreservation and vitrification approaches.
for preservation of decellularized scaffolds.

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EUROPEAN PROJECTS

EUDONORGAN, TRAINING AND SOCIAL AWARENESS IN ORGAN AND TISSUE DONATION
Melania Istrate(1) - Gloria Paez(2) - Marti Manyalich(1) Donation and Transplantation Institute, University of Barcelona, Barcelona, Spain(1) - Donation and Transplantation Institute, Transplant Procurement Management, Barcelona, Spain(2)

EUDONORGAN, acronym for ‘Training and social awareness for increasing organ donation in the European Union and neighbouring countries’ (SANTE/2015/ D4/037) is a service contract awarded by the European Commission from the European Union budget, on the initiative of the European Parliament.

The main aim of the project is to provide training and increase social awareness in the European Union (EU) and neighbouring countries to enhance the positive attitude towards organ and tissue donation, and ultimately help improving donation rates.

The EUDONORGAN project is developed by an international consortium led by University of Barcelona, Spain, and it includes universities and organizations located in Spain, Slovenia, Croatia and Italy.

The project is based on two work packages (WPs) – Train the Trainers and Social Awareness. The Train the Trainers program provides participants with medical knowledge in organ and tissue donation, quality management insights and communication techniques under the overarching umbrella of educational strategies. The training program employs a blended learning methodology based on active learning methods and main adult learning principles, and is carried out by means of e-learning (via WebApp) and face to face training sessions. Upon completion, training beneficiaries will become advocates in organ and tissue donation, and one of their tasks will be to organize training programs and social awareness events on organ and tissue donation in their centres. The EUDONORGAN training program is being attended by 104 participants, from 28 different countries (Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Malta, Montenegro, Netherlands, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Turkey).

By 2019, a total of 350 to 400 participants from the EU and neighbouring countries, including healthcare professionals and other relevant players will benefit from this project.

Acknowledgement to EUDONORGAN consortium partners: DTI Foundation, Spain, the Institute for Organ and Tissue Transplantation of the Republic of Slovenia, Slovenija-transplant, the Institute for Transplantation and Biomedicine - Ministry of Health of the Republic of Croatia, Italian National Transplant Centre - Italian National Institute of Health, Italy, and Dinamia S. Coop, Spain.

MICROBIOLOGICAL REQUIREMENTS

EVALUATION OF ALLOGRAFT DECONTAMINATION WITH TWO DIFFERENT ANTIBIOTIC COCKTAILS AT TREVISO TISSUE BANK FOUNDATION
Diletta Trojan(1) - Lisa Spagnol(1) - Elisa Cogliati(1) - Adolfo Paolin(1) Treviso Tissue Bank Foundation, Tissue Bank, Treviso, Italy(1)

This study reports the results of the analysis of the decontaminating efficacy of a new antibiotic cocktail compared to the one previously used at FBTV. Cocktail A, consisting of RPMI medium, Ceftazidime, Lincomycin, Polymyxin B and Vancomycin, was compared to cocktail B, consisting of BASE medium, Gentamicin, Meropenem and Vancomycin. Decontamination was carried out twice, i.e., immediately after retrieval and after processing. Microbiological analyses were carried out before processing (Time 1) and before freezing/cryopreservation (Time 2). Cocktail A was used for the decontamination of 3,548 tissues of which 266 were cardiovascular (CVT) and 3,282 musculoskeletal (MST). The total percentage of tissue contamination was 18.63% at Time 1 and 0.81% at Time 2 with contamination of 15.69% of MST at Time 1 and 0.36% at Time 2 and contamination of 54.89% of CVT at Time 1 and 6.39% at Time 2. Cocktail B was used for the decontamination of 3,634...
tissues of which 318 were CVT and 3,316 MST. The total percentage of tissue contamination was 8.70% at Time 1 and 0.19% at Time 2 with contamination of 7.57% MST at Time 1 and 0.03% at Time 2, and contamination of 20.44% of CVT at Time 1 and 1.89% at Time 2. Our results have shown that, compared to cocktail A, cocktail B is more effective in killing bacteria during the two step decontamination procedure in both CVT and MST.

VACUUM-DRYING AND ANTIBIOTIC DECONTAMINATION PROCEDURES ARE EFFECTIVE FOR BIOBURDEN ELIMINATION FROM CLINICAL AMNIOTIC MEMBRANE MANUFACTURED FOR THE TREATMENT OF OCULAR SURFACE DISEASE

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Amniotic membrane (AM) must be presented sterile for surgical applications, regardless of the preservation technique it has gone through, to prevent disease transmission to recipients. Terminal sterilisation techniques such as γ-irradiation are reported to damage tissue constituents. We developed a vacuum-dried AM (VDAM) product, which delicately preserves the beneficial wound healing properties of AM. This presented work validates the ability of the novel processing method to decontaminate potential bioburden. AM was collected via consented caesarean sections and the natural bioburden assessed using a colony forming unit (CFU) growth assay. Due to low natural bioburden (40% of freshly collected AM had no contamination), the novel processing steps were then assessed for decontamination using AM preloaded with 106 CFU/mL of Staphylococcus epidermidis. Steps investigated were: A) washing in 0.9% NaCl; B) raffinose wash; C) antibiotic treatment; D) low-temperature vacuum-drying; E) all combined steps. Each of the individual processing protocol steps contributed in the overall elimination of 99.98% of loaded baseline bioburden (CFU/mL =1.5x105). Step A reduced 95.65% of bacterial load. Step B further removed bioburden by 98.17% reduction of the 106 CFU. Drying (Step D) was able to remove 94.26% CFU. Step C (Antibiotics) completely removed all CFU. The combined process completely eliminates 106 CFU, with drying and antibiotic treatment being considered a sterilisation step. The risk of major bioburden contamination of caesarean section-AM is low. Nevertheless our VDAM processing method is a robust approach to removing any bioburden, sub-clinical infection, and creates a reliably sterile product suitable for clinical application.

LOW ENERGY ELECTRON IRRADIATION – A SAFE STERILIZATION PROCEDURE FOR TISSUE TRANSPLANTS RENDERING THE TISSUES FUNCTIONAL NON-COMPROMISED?

Simona Walker(1) - Jessy Schönfelder(3) - Christiane Wetzel(1) - Richard H Funk(2) - Michael C Hacker(3) - Michaela Schulz-Siegmund(3)

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Current decontamination regimes in German tissue banks mainly focus on antibiotic treatment. Although the tissue functionality remains non-compromised, decontamination is often not sufficient. As a result, approximately one third of the donor tissue has to be discarded. Additionally, sterilization with gamma irradiation is used in several tissue banks. Since impairments in mechanical strength occur, this procedure is only applied to tissue grafts that do not depend on their mechanical strength. Low-energy electron irradiation (LEEI) is a rapid sterilization procedure that is believed to be less detrimental to the mechanical strength of tissues because of high dose rates. Thus, LEEI is a promising technology for specific tissue grafts that have particular quality criteria. Porcine pericardial tissue was irradiated using LEEI. After application of a dose of 30.9 ± 3.1 kGy, no viable pathogens could be detected. Thus, the natural bioburden of 5.1*105 ± 4.6*105 viable bacteria was successfully inactivated. Ultimate tensile strength and elastic modulus remained unchanged after irradiation compared to non-irradiated pericardia. The overall metabolic activity of human umbilical vein endothelial cells seeded on LEEI treated pericardia as well as the cell number remained unchanged compared to non-irradiated pericardia. Using LEEI we could reproducibly sterilize porcine pericardia. Concurrently, the tissue mechanical stren
gth remained unaffected by the irradiation. Thus, LEEI represents a promising technology for routine application in tissue banks for grafts with particular quality criteria including pericardia, ligaments and tendons. Further analysis of this procedure shall be carried out in cooperation with the German Society for Tissue Transplantation (DGFG).

STERILITY TESTING OF TISSUE SAMPLES ACCORDING TO EUROPEAN PHARMACOPOEIA
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Purpose The aim of this study was to validate the sterility testing of tissue samples according to the “Method suitability test” defined by the European Pharmacopoeia (EP, chapter 2.6.), using the MEB buffer (ALCHI.MIA. S.r.l.) for extraction of microorganisms from tissue samples and RESEP (ALCHI.MIA. S.r.l.) for elimination of antimicrobials before direct inoculation of growth media.

Materials and methods Samples consisting of one gram of sterile porcine aortic valve were immersed in BASE.128 (ALCHI.MIA. S.r.l.) at 4°C for 24 h to simulate the tissue decontamination process with an antibiotic cocktail. The samples were then contaminated with 10-100 cfu of EP reference strains (Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Bacillus subtilis, Aspergillus niger, Clostridium sporogenes), and introduced in a vial containing MEB extraction buffer and stirred at room temperature for 20 min in order to extract microorganisms from the tissues. The buffer was then treated with RESEP syringe for removal of antimicrobial residues and inoculated in Tryptic Soy Broth (TSB) or Thioglycolate (TG). The turbidity of the growth media was determined visually after 5 days of incubation at 22°C (TSB) or 33°C (TG).

Results MEB buffer extracted the whole 10-100 cfu of all tested microorganisms from aortic valve samples. All microorganisms showed growth in TG or TSB media after RESEP treatment indicating vitality and absence of BASE.128 antimicrobial residues. Turbidity of growth media was detected within 5 days after inoculation in all tested conditions.

Conclusions The sterility test of the tissue samples, including the extraction of microbial contaminants from tissues using MEB buffer and removal of antimicrobials using RESEP, before direct inoculation, was successfully validated according to the “Method Suitability Test” of the European Pharmacopoeia (chapter 2.6.1.).

RETROSPECTIVE EVALUATION OF MICRO-BIOLOGICAL RESULTS: LAST FIVE AND HALF YEARS CARDIOVASCULAR TISSUE BANKING IN MILAN
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Introduction Cardiovascular Tissue Bank of Milan activity is 25 years old; we analyzed increasing microbiological positive tissues, after decontamination, observed in last period.

Methods From January 2012 to June 2017 842 cardiovascular tissues are collected/processed only and always by our staff. 371 valves (V), 374 arterial (A) 97 venous vessels (VV) from HB (heart beating) and NHB (no heart beating) donors.

Results After procurement and before preparation/decontamination:  
positive results (transport solution or/both tissue) at least one bacteria: 
donors HB 58% (49-67); Gram (+) 89 %, Gram (-) 8%, both 3% vs NHB 82 % (60 -100) Gram (+) 80 %, Gram (-) 15 %, both 5%.

After decontamination (antimicrobial solution 24 - 96 h +4°C) and before cryopreservation:  
donors positive results (tissue or/both washing, antimicrobial, freezing solution) at least one bacteria: HB: 14% (4-30) Gram(+) 86 %, Gram (-) 14%, vs NHB 26 % (0 -42), Gram (+) 82 %, Gram (-) 18%

Discussion As expected NHB donor’s tissue are much more contaminated (79% vs 53 %) and difficult to decontaminate (positive 19% vs 8%).

HB contamination is essentially skin bacteria (probably procurement contamination), antibiotic sensitive and positive percentage unvaried in this years (4-5%).

NHB contamination is mostly enteric bacteria (donor’s necrosis), more resistant to our usual decontamination, slowly but continually growing in positive amount (to % to 11%).

We explain this with diffusion of multi resistant strains (no environmental bacteria) and requirement modifying NHB donors decontamination time and temperature or finally change composition of antimicrobial solution.
HEPATITIS B MARKERS IN TISSUE DONORS. NATIONAL MULTICENTER STUDY. PRELIMINARY RESULTS
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Introduction In tissue donors is mandatory to perform the screening of hepatitis B virus. Traditionally, when core antibody (HBcAb) positive with hepatitis B surface antibody (HBsAb) positive, the donor was considered suitable. However, in the literature were published isolated cases with DNA positive.

Methodology We collected prospectively data for all tissue donors in Spain (except living donors) during 2017: age, type of donor (organ and tissue donor or tissue donor alone), cause of death, tissues procured and the hepatitis B markers. The incident of HBcAb, HBsAb and DNA was analyzed. We present the preliminary results of the first 6 months.

Results and discussion In this period 734 tissue donors were collected. 461 were male (62.8%). Mean age 63.4±15.1 years old, 420 (57.2%) were only tissue donors. HBsAg was positive in one case. HBcAb was positive in 68 cases (9.3%), in those cases: HBsAb was negative in 9 cases (13.2%), in 2 of them, DNA was positive (22.2%); HBsAb was positive in 59 cases (86.8%) and DNA was positive in 3 cases (5.1%). In 20 cases the titer of HBsAb was less than 100. We found one case HBcAb negative, HBsAg negative and DNA positive. HBcAb positive had higher mean age (67.7 vs 62.9) (p=0.010).

In seems clear that it is mandatory to do DNA with HBcAb positive. Another concern would be which the level of HBsAb to be considered as positive, and how to proceed with those donors. Each Tissue establishment should have a clear protocol regarding hepatitis B.

HUMAN SCLERAL PATCHES: MICROBIOLOGICAL SAFETY EVALUATION
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Human scleral tissues are frequently used in ophthalmic surgery and prevention of infections by means of the use of preserved tissues is crucial. The purpose of this study was to determine the ability of bacteria to survive in sclera preserved in 70% ethanol/water solution (70ES), the most common preservation solution. To evaluate the effectiveness of 70ES as germicidal, we tested some of the most common microorganisms contaminants of human ocular tissues: Staphylococcus aureus, Candida albicans, Enterococcus faecalis, Pseudomonas aeruginosa (the most common etiologic agent of infectious scleritis, difficult to be treated) and Bacillus cereus (able to form spores and more resistant to the action of germicides). After contamination, scleral patches were transferred to a sterile vial containing 50 mL of 70ES. The vials were maintained at room temperature to simulate normal storage conditions.

From every vial, scleral patches were removed after 2, 24, 48, 72, 96 hours and 7, 10 and 14 days of immersion and incubated at 37°C in thioglycollate enriched medium. At the same time 3 mL of the preservation medium were inoculated in Bactalert FA, and a total amount of 1.5 mL in HB&L (aerobic, anaerobic and Sabouraud).

As contamination control, all the five tested species of bacteria were isolated from the contaminated scleral disks, incubated for 48 hours in thioglycollate medium. Staphylococcus aureus, Candida albicans, Enterococcus faecalis, Pseudomonas aeruginosa presented no growth since 2 hours of immersion. Instead, Bacillus cereus was recovered from scleral disks immersed in 70ES until day 14, and also after renewal of 70ES, until day 43. Bactalert and HB&L tests were negative, at any time.

70ES is an excellent medium for preserving human scleral tissue, due to his germicidal activity and easy handling in preparation and storage of human scleral grafts. Nevertheless, resistant microorganisms can survive in scleral tissues preserved with 70ES. Our study suggests that a routine biopsy of the preserved scleral tissue before its releasing for clinical use is needed and advantageous, as a direct and reliable investigation for tissue contamination.
Clinical and MRI Evaluation of MAT with Soft Tissue Technique: A 7 Years Follow-Up
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Methods
Since 2005 more than 80 patients have been treated with MAT in our Unit. At present time 36 of them performed at our hospital an MRI and a clinical evaluation. The mean age is 38 yrs, 29 are males and 7 females, 20 left knees and 16 right knees, 18 lateral and medial meniscus respectively. All patients except one, have a history of at least one previous surgery, in 28 meniscectomy or meniscal suture. In 23 patients MAT has been associated to one or more surgical procedures: ACL reconstruction (10), chondral or osteochondral treatment (9), tibial osteotomy (5)

MAT was performed with soft tissue technique.

Clinical evaluation was conducted by Lysholm, IKDC, VAS, Koos and Tegner scores. MRI evaluation was performed during the final follow-up. MRI was evaluated for the morphology of the new-meniscus and the interface with the remnant, retear, root tear, meniscus extrusion, advanced osteoarthritis and visualization of the tunnels, bone marrow edema and other findings that could explain loss of function or pain.

Results
The score’s improve was: Lysholm 27.5 (p<0.0001); IKDC 19.95 (p=0.0001); VAS 30.08 (p=0.0001); Koos 17.23 (p=0.0004); Tegner 0.97 (p=0.0137).

Adverse events: 2 de novo lesions of the transplanted meniscus; 1 detachment of the anterior horn; 1 joint stiffness.

MRI evaluation: normal postoperative meniscus-graft interphase is sharp. The graft demonstrates higher signal than the native rim. The tunnels are well seen as ‘ghost holes’. Findings that could explain pain and limited range of motion included advanced OA with bone marrow edema, anterior and posterior root tears with extrusion, Cyclops lesion in the notch, new and re-tears of the graft. Metal artifacts may obscure the area of interest and a metal protocol should be used.

Conclusions
MAT may restore the articular homeostasis and represents a useful therapeutic option for the treatment of a symptomatic knee previously submitted to meniscectomy.

Extending the Width and Height of the Alveolar Process and the Alveolar Part of the Mandible with Allogeneic Bone Blocks
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One of the common issues in implant-prosthetic treatment is the insufficient amount of the patient’s bone tissue, which hinders correct submergence of an implant used to restore a dental defect. In such cases augmentation is necessary. Otherwise, the implant will not be adequately supported by the bone structure or the final functional or aesthetic outcome will be unsatisfactory.

Bone regenration was performed with frozen allogeneic bone blocks, which were sterilised with a radiation dose of 35 kGy and prepared by the National Banking Centre for Cells and Tissues.

The authors present their 7-year experience of using allogeneic bone blocks with 204 implant locations prepared. The procedures were conducted in patients with severe bone atrophy within the transverse or vertical dimension or within the transverse and vertical dimension. The bone blocks were formed to place the lamellar bone outside and towards the alveolar ridge, whereas their medial side reflected the recipient site accurately. Then the blocks were stabilised with titanium screws or miniplates and finally covered with PRF membranes.

Allogeneic bone blocks were used to extend the width and height of the alveolar process both, in the lateral and frontal sections. There were also cases where an implant was submerged in grafted bone only.

After several months of bone remodelling, the optimal width of the process was found allowing implantation and aesthetic implant-prosthetic restoration. CT scans did not show biomaterial resorption. Histopathology testing of the grafts confirmed de novo bone formation.

Several years of clinical observation confirmed that allogeneic bone in the form of a block constitutes adequate material to extend the width and height of the alveolar process, particularly in complex and complicated cases both, from the outside of the process and from the side of the maxillary sinus. Appropriate preparation of a bone block eliminates the risk of graft resorption.
In view to improve the bioactivity of bone allograft by combination with adipose stem cells (ASCs), we postulate to demineralize human cancellous bone allografts for a better stem cells colonization and function. Bone allografts (n=16) were treated for decellularization and demineralization (during 0,4,8,12hrs). Each implant was studied by ionometry/pQCT (for residual calcium/mineral density), microtomography (for macroporosity/open porosity), Helium pycnometry/Hg porosimetry (for the absolute density/microporosity) and X-Ray photoelectron spectroscopy (for bone surface analysis). The graft recolonization by ASCs was assessed by SEM, histology, growth factors content (VEGF,SDF-1α,IGF-1,Osteoprogeterin,BMP-7) and DNA extraction at 24 hours/day 15 post-cellular seeding. Finally, non-/demineralized bone allografts (alone/-recolonized with ASCs) were implanted in 10 nude rats to study the osteoinductivity/angiogenicity function of ASCs in view to return the bioactivity for bone healing.

**BONE GRAFT IN TREATMENT OF AVASCULAR NECROSIS OF THE FEMORAL HEAD**

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Avascular necrosis of the femoral head (ANFH) is a debilitating disease that usually leads to destruction of the hip joint in patients who are in the third, fourth, or fifth decade of their life. It has been estimated to afflict 10,000–20,000 new patients a year only in United States.

This progressive disease is characterized by reduced local blood flow, death of the osteocytes and the bone marrow, leading to a progressive destruction of bone architecture, subchondral fracture, extensive hip pain and loss of joint function.

Ultimately, after collapse of femoral head, a standard hip arthroplasty is indicated.

Several treatment methods and procedures have been proposed to avoid or postpone THA: there’s an increasing interest in surgical techniques using homologous bone graft in association with biological adjuvants. In Rizzoli Orthopaedic Institute (SSD Chirurgia Conservativa e Tecniche Innovative), 112 patients with ANFH (stage 2 A to 3 A of ARCO Classification) were treated with Core Decompression, removal of necrotic tissue, filling in a retrograde manner with lyophilized homologous bone tissue augmented with autologous BMSC and autologous PFR.

The patients underwent a 6 weeks, 3 months, 6 months, 12 months and 24 months follow up. Follow up examination revealed improvement of Harris Hip Score (from 60,2 to 72), Vas scale (from 60 to 23), WOMAC Score (from 53,4 to 35,8). Comparison of preoperative and 24 months follow up x-rays revealed no significant differences in size of necrotic area in 67% of patients, increasing of necrotic area in 13% of patients, collapsing of femoral head in 20% of patients.

The 18% of patients underwent THA during follow up. Despite the controversial indications, limits and results of these innovative techniques in scientific literature, performing CD for ANFH seemed to be effective for preventing femoral collapse within a short-term follow-up. Basing on our experience, patients have good clinical outcomes.

**IMPROVEMENT OF BONE ALLOGRAFT RECOLONIZATION BY ADIPOSE STEM CELLS: IMPACT OF BONE GRAFT DEMINERALIZATION.**

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In conclusion, the demineralization of cancellous bones significantly improves the colonization and function of ASCs in view to return the bioactivity for bone healing.
ALTERNATIVE APPROACHES FOR EXTRACTING BONE MORPHOGENETIC PROTEINS (BMPS) FROM HUMAN BONE

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Background Marshall Urist, an American scientist hypothesized the existence of BMPs in 1964 (Urist, 1965: 893). Bone morphogenetic proteins (BMPs) are termed osteoinductive, meaning they have the ability to stimulate bone growth naturally in humans and animals (Skyras & Opperman, 2003). The processes of extraction has however proven to be expensive and labour intensive. This is especially the case with the current methods employed at the CTE, which involve utilising urea for extraction.

Objective To explore alternative extraction methods that will yield large quantities of the extract, with fewer purification steps.

Methods Extraction Method 1: Guanidine Hydrochloride Extraction (Jiang et al., 2007 – Method 2). The DBM fraction was subjected to extraction in 6.5 volumes of 6 M guanidine hydrochloride (GuHCL) in Tris, pH 7.4 and protease inhibitors for 72 hours.

Extraction Method 2: Ammonium Acetate Extraction (with reference to Ammonium Bicarbonate Extraction, Buckley et al., 2009). The DBM fraction was subjected to extraction in 10 volumes of 50 mM ammonium acetate with protease inhibitors for 5 hours at 65°C.

Extraction Method 3: Collagenase Extraction (Zhang et al., 2012). The DBM fraction was subjected to extraction in 10 volumes of 0.2 M Tris (pH 7.4) with protease inhibitors for 2 hours. After 2 hours collagenase was added to a final concentration of 20 collagenase degrading units (CDU)/ml to aid extraction for a further 16 hours. The extracts were collected and particulate matter removed by filtration (using coffee filters).

Results Successful extraction of proteins particularly BMP-2 was achieved with all three extraction methods. Based on the data obtained, Method 1 had the highest protein concentration, but GuHCl also requires purification steps. Therefore method 2 was the preferred option in terms of cost and labour.

Conclusion The methods that were explored in this study yielded sufficient amounts of BMP-2 to substantiate additional studies. It was concluded that more research work is required for further analysis of method 1 and method 2.

CUSTOM GRAFTS, MANUAL AND COMPUTERIZED PROCESSING - THE EXPERIENCE OF MUSCULOSKELETAL TISSUE BANK OF BOLOGNA

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Health care over the last decade has been pushing more and more to personalized treatments, also affecting regenerative medicine. The production of preformed and precision tissues is an increasingly stringent requirement for tissue banks, as the evolution of surgical techniques requires innovation in distributed tissues, both in orthopedic, orthodontic, maxillofacial and otolaryngology surgery. Musculoskeletal Tissue Bank of Bologna can provide different customization solution: manual processing and computerized machining.

The long-standing experience of laboratory technicians led to the development of skills in manual processing, today custom-made tissues, of different shapes or sizes, are distributed on a specific request. Computerized processing derives from the need to produce both high precision personalized grafts, both standardized grafts with a high degree of repeatability. For many years, tissue banks in the US use CNC machines for processing bone portions, as well as xenogenic bone processing centers in Europe; However, European tissue banks have not yet implemented this technology. At present BTM has a six years experience in using a 4-axis CNC milling machine suitable for Clean Room Class A (annex 1 GMP).

Thanks to this technology it is possible to machine tissues with a precision of less than one tenth of a millimeter, ideal for producing personalized blocks for dentistry as well as thin struts and spinal cages. However, mechanical limitations of milling machines prevent the creation of complex geometries. Thanks to a research project, an innovative robotic arm milling machine will be installed in our clean rooms, with which complex machining will be possible.

PREDISPOSING FACTORS FOR CONTAMINATION OF MUSCULOSKELETAL ALLOGRAFTS

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**Background** Musculoskeletal tissue grafts are widely used in orthopedic and reconstructive surgery. Epidemiological data shows incidences of graft contamination that vary from 5 to 50%. The identification of factors that favor this serious complication allows us to make an adequate evaluation of the donor in order to obtain safe grafts. The aim of this study was to determine the factors that contribute to the contamination of the osteotendinous tissue.

**Methods** A prospective study was conducted with 8024 musculoskeletal tissue grafts from 464 donors during the period from January 1, 2016 to June 30, 2017. Several factors such as age, gender, ICU stay, organ donation, and positive culture (blood, urine and bronchial aspirate) were related to graft contamination.

**Results** The contamination rate of musculoskeletal allografts was 10%. The majority of microbial species recovered from tissue cultures belonged to species normal to the skin microflora: coagulase-negative Staphylococcus and Bacillus species. Age, sex, ICU length of stay and urine culture were not associated with an increase in the contamination rate of osteotendinous grafts, while a positive blood or bronchial aspirate culture, as well as the donation of some organ previously, affected the risk of graft contamination.

**Conclusions** In conclusion, according to our study, organ donation and a positive blood or bronchial aspirate culture were associated with an increase in the grafts contamination rate, so we believe these factors must be taken into account, at the time of donor evaluation, in order to assess the quality and safety of musculoskeletal tissue.

**NEW TECHNOLOGIES**

**3D BIO-PRINTING AND MUSCLE DERIVED PERICYTES FOR ARTIFICIAL SKELETAL MUSCLE HUMAN-LIKE SIZE**

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The skeletal muscle tissue exhibits good regenerative capabilities, which are however limited by injury size. As a matter of fact, large muscle lesions are characterized by poor recovery accompanied by scar formation and functional detriment, condition common to people suffering from volumetric muscle loss and needing reconstructive therapeutic approaches. Even if surgical autologous transplantation is a standardized procedure, the outcomes are often unsatisfactory. Hence, the pressing need to develop engineered artificial tissues to replace wasted muscle. Tissue engineering (TE), exploiting stem cells embedded in biomimetic scaffolds, aims to mimic organogenesis by building artificial tissues to replace the damaged ones. Skeletal muscle TE is an up-and-coming biotechnology with great potential for muscle repair, but no conclusive strategy has been demonstrated yet. Reconstructing the skeletal muscle architecture and function is still a challenge requiring the parallel alignment of myofibrils arranged into organized sarcomeres. Recently we demonstrated the great potential of a hybrid biomimetic matrix, namely PEG-Fibrinogen, for enhancing the engraftment of myogenic cell progenitors by providing a suitable 3D environment for mouse muscle reconstruction. Starting from these observations, we developed a novel approach for the regeneration and/or reconstruction of skeletal muscle tissue segments of human-like size by exploiting a population of adult myogenic stem cells, namely pericytes, in combination with 3D bio-printing technology to guarantee a functional architecture. In vitro characterization of cell-laden constructs showed enhanced myogenesis and positive myostructure alignment. Thanks to the enhanced control over cell deposition and alignment, the presented technology has the potential to support skeletal muscle repair and regeneration.

**TISSUE BANK APPROACHES TOWARD THE GENERATION OF DECELLULARIZED TISSUES FOR IN VIVO AND/OR IN VITRO REPOPULATION**

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There is a clinical need for improved tissues that act as effective and preferably superior to traditional processed tissues. Decellularized tissues are advantageous compared to soft tissues preserved with cellular components that finally are implanted into the patient. Mainly due to their lack of immunogenicity with the maintenance of its native matrix ultrastructure and biological function. Currently the...
ABSTRACT BOOK

majority of commercialized pericardium for cardiovascular applications is from bovine origin. We present a method to decellularize human pericardium for clinical purposes.

**Methods** Once pericardium is thawed and defatted, 1M NaCl treatment followed by 1% SDS treatment was carried out. After several washes, tissue is lyophilized and stored at room temperature. DNA presence was measured with commercial kits. Histological sections stained with hematoxylin & eosin were performed to ensure cell removal, scanning electronic microscopy (SEM) was used to evaluate tissue porosity. After this treatment, tissue retraction and biomechanic parameters were analyzed.

**Results** Protocol probes to be effective to remove DNA (concentration < 50ng/mg of tissue) and cellular debris while preserving collagen and glycosaminoglycans (GAGs) tissue concentration. Moreover, the method generates porosity (porus size ≥20 μm) in the matrix, which would enable cells to adhere and repopulate.

**Conclusions** Our approach demonstrated effectiveness in removing cells from pericardium and it has been displayed as GMP complying process. Preliminary results show an effective tissue decellularization maintaining collagen and GAGs matrix composition that preserves the elasticity of the tissue enabling downstream transplantation applications. Furthermore, porosity which is essential to cell adhesion and repopulation, is also maintained.

**THE ENZYMATIC DE-EPITHELIALISATION TECHNIQUE DETERMINES THE VIABILITY OF HUMAN AMNIOTIC EPITHELIAL CELLS**

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**Aims** Human amniotic membrane (HAM) is increasingly used as an allograft in regenerative medicine or as a source of pluripotent cells for stem cell research. The aim of this study was to find effective and safe enzymatic HAM de-epithelialisation method which leads to both obtaining of well-preserved denuded HAM and harvesting viable hAECS for subsequent culture.

**Methods** Ten human placenta were used for experiments. The efficiency of HAM de-epithelialisation using Tryple Express, trypsin/EDTA, and thermolysin was controlled by hematoxylin and eosin staining and by DNA concentration measurement. The cell viability was checked by trypan blue staining and by cell counting using a hemocytometer. Immunostaining for collagen type IV and laminin 5 was used to check the integrity of HAM basement membrane. Harvested hAECS were cultured and their stemness was assessed using RT-PCR (NANOG, SOX2), and proliferation potential was determined by WST-1 assay after each passage.

**Results** The HAM was successfully de-epithelialised using all three types of reagents. In some cases (40%), stromal fibres were damaged after the thermolysin application. About 60 and 6% of hAECS remained viable using trypsin/EDTA and Tryple Express respectively. All cells were lethally damaged after the thermolysin application. The hAECS isolated using trypsin/EDTA were successfully cultured up to the 5th passage but in later passages, the cells changed their morphology to more fibroblast-like form and their proliferation potential increased.

**Conclusion** Trypsin/EDTA technique showed to be the most efficient for both obtaining undamaged denuded HAM and highest amount of viable hAECS for consequent use.

COMPARISON OF IN VITRO EFFECT OF OSTEOCARTILAGINEAL DEMINERALISED AND DEMINERALISED-DECELLULARIZED GRAFTS ON CELLS USED IN CARTILAGE REGENERATION

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The aim of this study is to determine in vitro effect of osteochondral demineralised graft (ODC) and demineralised-decellularized graft (ODDG), enriched with alogenous mesenchymal stem cells (MSC) and chondrocytes.

**Materials and methods** The MSC and chondrocytes were isolated from domestic rabbits four months old, with approval of ethical committee. MSC were cultivated during 4 passages and chondrocytes in 3, each in their own nutritional media, at 37°C with 5%CO2. From three freshly sacrificed rabbits were harvested the distal femurs, which were demineralised in 0.6M HCl. With 3.7mm biopsy punch 70 pieces of osteochondral tissue were cutted. Half of them were
decellularized in 0.5% SDS. All grafts were stored in DMEM at 4°C.
The cytotoxicity (MTT) and cell population tests were performed according to ISO 10993-5 standard. The MTT test was evaluated at 24, 48 and 72 hours with 570 nm wavelength in a spectrophotometer (TECAN).
The population of grafts with cells were evaluated by SEM and histological examination at the eighth day.

**Results** At MTT assay with CSM, the ODG presented a lower cell viability than with ODDG: 87.58% at 24h that decreased to 79.90% at 72h, respectively 89.66% at 24h and 89.72% at 72h for ODDG. At MTT assay with chondrocytes in both cases the viability were more than 80% with insignificant increase for ODG. AT SEM and histological examination the cells graft population were practically the same.

**Conclusion** Utilization of MSC is more indicated with ODDG, but chondrocytes can be used with both graft types.

**PROCESSING STANDARDS**

**IMPLEMENTATION OF THE SINGLE EUROPEAN CODE – FIRST EXPERIENCES AT OUR MULTI-TISSUE BANK**

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**Introduction** A consistent system for coding of tissues and cells among all EU member states became mandatory with the introduction of the Single European Code in April 2017.

**Material and Methods** The regulations for the Single European Code in Germany will be presented. Our own experiences with the implementation of the SEC in our multi-tissue bank are reported. Different options for the assignment of the unique donation number will be demonstrated.

**Results** The implementation of the SEC in our multi-tissue bank could be successfully realized. However, it revealed a number of difficulties, especially the sterile labelling of certain tissue transplants and the design of the unique donation number.

**Conclusion** The introduction of the SEC has made a contribution to the safety of recipients of tissue and cells transplants through a system of comprehensive and transparent traceability on a European level.

**EUROCET: BECOMING THE EUROPEAN HUB FOR TISSUES AND CELLS DATA ACTIVITY COLLECTION**

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Starting from 2008, the European Registry for Organs, Tissues and Cells (EUROCET) collects and publishes the annual report of the data on tissues and cells (T&C) donation and transplantation activities in Europe. All figures included in the EUROCET website (http://www.eurocet.org) are official and certified, since they are provided by the accredited European T&C Competent Authorities (CAs) which receive the information from all their authorized Tissue Establishments.

The ever-growing collaboration with data providers raised also doubts, suggestions and concerns about many different aspects of the data collection. All these contributions gave the Eurocet team the input to start a process for streamlining the data collection forms, dataset and to further standardize the data set definitions. The new updated data forms will be shared with all EU T&C CAs before being consolidated and circulated for the collection of next year.

This restyling aims at making the Eurocet collection easier, quicker and friendlier to the professionals who collect the data and will find in Eurocet a useful tool to comply with the Directive 2004/23/EC (art. 10.1).

Also the trust of the general public in this field of healthcare sector will benefit from transparent, standardized, and certified information on T&C donation and transplantation activities in Europe.

The increasing participation of EU T&C CAs in the data provision during these nine years will be showed here, together with the tissue activity data collected from 2016.

**ADOPTING A RISK BASED THINKING QUALITY MANAGEMENT SYSTEM APPROACH IN A TISSUE ESTABLISHMENT**

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**Introduction** During a recent competent authority inspection, it was suggested that Oxford Cell & tissue Biobank could review their quality management system using a risk based thinking, process approa
ch based upon the principles of updated ISO standards. Using RBT, it was proposed that risks could be identified, considered and controlled throughout design and use of the QMS.

**Method** OCTB Quality Manager reviewed updated ISO standards and together with DI, identified elements which could be implemented to improve OCTB QMS. OCTB staff and other relevant staff were given appropriate training regarding changes to standards and asked for their input. Critical stages of processes and resources for each tissue/cell were itemised. Potential risks which may occur and opportunities to develop improved practices were identified documented using flow diagrams. Additional use of resources and interaction between processes which could deliver an improved product/service were then added. Additional measures to mitigate risk and reduce occurrence of risks were then planned. Requirement for additional knowledge and skills was assessed. Benchmarking with tissue establishments, surgical centres and other stakeholders to identify areas of best practice was undertaken. Political/economic Organizational factors were considered (e.g. NHS funding pressures). Proposed changes were critically assessed, considering impact, implementation and assessment of effect on quality and safety. Improvements in effectiveness and efficiency of OCTB products/service were monitored and measured (results achieved versus resources used).

**Results** Staff working at all levels of the organisation were engaged and became capable of assessing risk and empowered to take preventative and corrective action. More proactive knowledge and skills shared through training enabled a much wider skills base. Additional risks were identified, prioritised and actions planned and implemented quickly. Each tissue/cell processing pathway was developed into more collaborative processes highlighting interactions contributing to an improved process. Process mapping recorded where multiple resources and interaction between processes could be beneficial. Risk register was reviewed and improved and contingency plans enhanced. Proposed changes were controlled using methods including additional training sessions, improved SOP’s, development of additional MDT meetings, enhanced interaction and communication with customers. Efficiency improvements were monitored and measured (results achieved versus resources used).

**REPRODUCTIVE TISSUES**

**ART EVENT AND ADVERS REACTION NOTIFICATIONS IN 2014: CATALONIA VERSUS EUROPE**

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**Introduction** As a result of the amendment of Law 14/2006, of 21st May on assisted reproductive techniques (ART), it was mandatory for Spanish centres to report their activity and their results. The first year for which results are available is 2014. Also, since 2013, the Catalan Organization of Transplantation (OCATT) manages the notifications of Vigilance in the field of Assisted Human Reproduction and prepares the corresponding annual report.

**Objective** The objective of this study is to compare the notifications to the OCATT Vigilance Register of the year 2014 in the field of ART with the report published by the European Commission in the same period and to analyze the effects and adverse reactions reported.

**Results** Catalan ART centres reported 13,133 embryo transfers and 7,778 cycles of artificial insemination. 48 vigilance cases were reported to OCATT, of which only 9 were considered adverse reactions and 7 adverse effects, representing 0.002 of the activities performed.

In Europe, more than 328,534 embryo transfers were performed and more than 78,863 cycles of artificial insemination. The different countries reported to the European Commission 81 adverse reactions and 551 adverse effects, with a 0.001 on the activities performed.

**Conclusions** Results show that in Catalonia centres are notified to the competent authority more events and adverse reactions that in the global of Europe, besides the short time that OCATT is managing vigilance in ART.

However, it is considered necessary to take concrete actions to involve all authorized centers and all the actors involved.
SKIN/DERMIS

MULTIPLE INTERVENTIONS FOR DIABETIC FOOT ULCER TREATMENT (MIDFUT) TRIAL PROTOCOL


Background

In the UK diabetes affects 4.5 million people of whom approximately 2.5% (112,500) have a diabetic foot ulcer (DFU). In 2014-15 NHS England spent an estimated £972 million - £1.13 billion, 0.7-0.8% of its budget on DFU treatment, not taking into account additional social and public health sector costs. Despite implementation of multidisciplinary care, healing rates for DFUs at 12 weeks with standard therapies are only 7.7-46%. Delays in healing increase the risks of infection, hospitalisation and amputation. Of those who do not achieve 50% reduction in ulcer size at 4 weeks, an estimated 30-91% will not heal by 12 weeks, which is a marker of the need for early adjuvant therapies. Several treatment options are available for hard-to-heal ulcers with few randomised comparisons evaluating them. Therefore, there is substantial uncertainty surrounding current best management.

Aims

The MIDFUT trial aims to assess the clinical-and cost-effectiveness of hydrosurgical debridement alone, or in combination with (i) negative pressure wound therapy or (ii) decellularised human dermal allograft or (iii) both, in the treatment of hard-to-heal diabetic foot ulcers.

Methods

MIDFUT is a NIHR HTA-funded, multi-centre, seamless Phase II/III, open-label, parallel group, multi-arm-multi-stage RCT trial of patients with DFU. The main eligibility criteria are adult patients with diabetes and a chronic DFU or surgical debridement wound or open minor amputation, of at least 1 cm² in area, that shows limited response to standard care, defined as having <40% reduction in index ulcer area over a period of at least 4 weeks. Target recruitment is 660 patients from at least 24 centres over three years. Phase II will randomise 324 patients into one of four intervention arms or TAU in a 1:1:1:1:2 allocation. At 4 weeks post-randomisation the short term outcome of ulcer area reduction by at least 50%, assessed by clinicians masked to treatment group, will be used to select at most two of the four intervention arms to be taken through to Phase III. These intervention arms will have achieved at least 10% absolute improvement in outcome versus TAU. If more than two interventions show a sufficient response then information on the safety profile and costs of treatments will also be considered. There will be a seamless transition to Phase III of the trial. Phase III will randomise a maximum of 336 patients to (at most) two intervention arms or TAU (1:1:1 allocation). The Phase III primary outcome will be time to healing of the index ulcer, assessed by clinicians who are masked to treatment group. Secondary outcomes are: proportion of DFU healed by 12/20/52 weeks; re-ulceration; infection; revascularisation; SAEs (e.g. amputation, admission to hospital); QoL (DFU-SF and EQ-5D-5L) at 4/12/20/52 weeks; health resource utilisation.

HRA approval has been granted and NIHR portfolio adopted. The trial is currently seeking multi-disciplinary diabetic foot clinics interested in participation.

Acknowledgements


References


HOMOLOGOUS SKIN ALLOGRAFT: SKIN BANKING IN TISSUE BANK OF VERONA AND CLINICAL USE IN THE DIVISION OF PLASTIC AND RECONSTRUCTIVE SURGERY OF VERONA (ITALY)

**Introduction** In severe burned patient, the use of homologous skin allograft is a valid treatment. We describe the experience of Verona Tissue Bank during 13 years of activity and clinical use in burned patient. Cadaveric donors are evaluated according to national and international standards in order to avoid the risk to transmit communities disease, malignancies and other infectious diseases. Skin collected were processed, cryopreserved and banked.

From April 2003 to December 2016 Tissue Bank of Verona distribute 2,318,746 cm² for 1,714 patients admitted to Verona Burn Center (720 patients) or Padova Burn Center (994 patients). Of these we describe a cohort of 236 patients (168 adult - average 53.1 y - and 68 paediatric - average 3 y) that received homologous skin grafts in the Verona Burn Center. The burned body surface area (TBSA) was > 25% in 56/236 patients, and < 25% in 180/236. Only 8/236 (3.4%) died for complication due to concomitant pathologies (diabetes mellitus, heart disease, liver disease, kidney disease). In all these cases TBSA was > 40% with the median age of 73 years. In the 228 survival group, 140/228 patients (61.4%) underwent to one or more surgical treatment (average 1.4), while 88/228 (38.6%) healed without surgery. The hospitalisation was respectively 42 days (TBSA >25%) and 14 days (TBSA <25%) in adult, and respectively 25.6 days (TBSA >25%) and 9.6 days (TBSA <25%) in paediatric patients.

In burned patients, both adult and paediatric, use of homologous skin graft is a well known procedure to promote and facilitate reepithelization in superficial burns, improving clinical course, reducing pain, risk of infectious and fluid loss. In more deep lesions homologous skin grafts can be utilized after necrectomy for a temporary cover in order to obtain the best wound bed before autologous grafting. No side effects or adverse reactions in long term follow up were observed in recipient site.

**PRODUCTION AND CLINICAL DISTRIBUTION OF A DECELLULARIZED HUMAN DERMIS: THE EXPERIENCE OF EMILIA ROMAGNA REGIONAL SKIN BANK**

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The continuous scientific innovation in the field of Medicine has led in the last decades to the development of Tissue Banks dedicated to activities of harvesting, processing, storage and distribution of tissue for different clinical applications. In particular, the Emilia Romagna Regional (ERR) Skin Bank is one of the 5 Italian Skin Tissue Banks accredited by the National Transplant Center (CNT) and the Superior Health Institute (ISS) for the procurement, processing, storage and distribution of cutaneous tissues, according to current regulation (CNT 09/2016). In addition, ERR Skin Bank carried out also activities in the field of Regenerative Medicine that was able to produce a patented decellularization method (PTC/IB2008/002753), able to remove cellular component from dermis maintaining unaltered its structural integrity. The Decellularized dermis thus obtained is a biological product of tissue engineering widely distributed as a permanent dressing by ERR Skin Bank for different clinical applications since it avoids problems deriving from rejection on the receiving patients. Here we described the clinical distribution of the Decellularized dermis in the year 2016, taking into account the different medical fields in which it was required.

**THE NEW TISSUE AND CELL FACTORY TURIN SKIN BANK**

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The Tissue and Cell Factory Turin Skin Bank, has been the Regional Reference Centre for the skin preservation for the last 18 years. It has been authorised by the Italian National Transplant Centre to harvest, manipulate and distribute human alloplastic skin and acellular dermal matrix (HADM) from multi-tissue and multi-organ donors and to store autologous skin and adipose tissue. Before distribution tissues undergo strict quality controls, which include a microbiological and viability screening to certify their suitability for clinical use. The Bank operates on the basis of GMP regulation in new laboratories (composed by 4 sterile rooms + 2 research labs) opened in 2016. An articulated quality system documentation has been realized in order to codify and regulate all the operative procedures concerned in the handling and preparation of the tissue products, in the laboratories and in the training and updating of the specialised personnel.
Two new products are now distributed by our facility: Human Acellular Dermal Matrix (HADM) employed in various reconstructive procedures (burns, breast, pelvic and abdominal wall reconstruction) as a scaffold for autologous tissue regeneration. The second product is autologous adipose tissue for reconstructive plastic and orthopedic purpose.

The Turin Skin Bank is active in the field of research and scientific innovation carrying out studies on new dermal products, bio-substitutes and adult mesenchimal stem cells from adipose tissue.

Work supported by Fondazione Piemontese per gli Studi e le Ricerche sulle Ustioni “Simone Teich Alasia” and by Compagnia di San Paolo.

USE OF DECELLULARIZED HOMOLOGOUS DERMIS IN RECONSTRUCTIVE SURGERY: EXPERIENCE IN AUSL ROMAGNA
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Background Recently, thanks to tissue engineering methods, several synthetic and biosynthetic cutaneous substitutes have become available for reconstructive surgery, in particular for the treatment of severe wounds.

In collaboration with the Istituti Ortopedici Rizzoli of Bologna, Centro Grandi Ustionati, Skin Bank of Emilia Romagna and Cell Factory, a new bioproduct to be used as a cell-free scaffold has been developed to heal severe wounds involving osteo-tendinous structures.

Materials and Methods The cell-free scaffold is obtained through minimal manipulation of homologous derma derived from multi-tissue or multi-organ donors. Skin tissue is first decellularized using a patented method (PCT / IB2008 / 002753) and then cryopreserved in nitrogen at -196°C to maintain its biological properties such as sterility, absence of viable cells and DNA, presence of collagen and traction resistant elastic fibers. According to our experience at AUSL Romagna, there are several applications of decellularized human dermis in the field of reconstructive surgery, such as oncology.

Results The authors present the most critical cases with hard-to-heal wounds treated with homologous dermis.

Conclusions Decellularized human dermis has good biocompatibility and essential biological properties for clinical use, with good clinical outcome in the absence of complications.
POSTERS
P1. TWO DECONTAMINATION SOLUTIONS FOR HUMAN AMNION - COMPARISON OF MICROBIOLOGICAL EFFICIENCY AND THE CELL VIABILITY
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Aims The antimicrobial efficiency and toxicity of two decontamination solutions, commercially produced BASE•128 and laboratory decontamination solution (LDS) with an analogous content and concentration of antibiotic-antimycotic compounds, were compared using human amniotic membrane (HAM).

Methods The antimicrobial efficiency against five human pathogens (Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli, and Enterococcus faecalis) has been assessed using agar well diffusion method in fresh and frozen solutions stored for 1, 3, and 6 months. HAM was prepared by blunt dissection, placed on nitrocellulose scaffold, and decontaminated, following three protocols: 1) 6 h, 37 °C; 2) 24 h, room temperature; 3) 24 h, 4 °C. The viability of epithelial (EC) and mesenchymal stromal cells (MSC) was assessed via trypan blue staining. The percentage of apoptotic cells was detected via TUNEL method.

Results No statistical differences between the growth inhibition of all decontamination solutions have been found. The mean % (± SD) of dead EC (%DEC) from six fresh placentas was 12.9 ± 18.1. Decontamination increased %DEC compared to culture medium. Decontamination with BASE•128 for 6 h, 37 °C led to the highest EC viability (81.7 %). Treatment with LDS at 24 h, 4 °C resulted in the lowest EC viability (55.9 %) in the set. MSC were more affected by apoptosis than EC.

Conclusion Both used solutions exhibit the same antimicrobial efficiency. Although the BASE•128 expresses lower toxicity compared to LDS, we present LDS as an alternative and cheaper decontamination solution with a satisfactory preservation of cell viability.

P2. EVALUATION OF RESEP DEVICE TREATMENT FOR ANTIBIOTIC REMOVAL ON HOMOGENIZED TISSUES
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Purpose To evaluate the use of RESEP device (ALCHIMIA S.r.l.), intended for antibiotic removal from liquid samples, on tissue homogenates.

Material and method RESEP treatment of tissues:
- Homogenization: 1 gr in 10 ml of BASE solution with IKA ULTRA TURRAX homogenizer
- 2 passages in RESEP, 20 min/each at RT RESEP was evaluated for the following parameters:
  1. Bacterial retention: sterile human fresh aorta fragments were homogenized, contaminated with P.aeruginosa (3 doses) and treated with RESEP.
  2. Interference with bacterial growth: sterile human fresh aorta and pericardium fragments were homogenized, untreated/treated with RESEP and contaminated with P.aeruginosa and S.aureus (3 doses).
  3. Antibiotic removal ability: decontamination (BASE.128_12.5ml/g; 14 h, 37°C), washing (BASE 6.5 ml/g, 2x 5 min, 1x 6h, 4°C) and homogenization procedures were applied on sterile porcine cardiovascular tissues. Microbial strains were spiked on RESEP treated/untreated homogenates. All the homogenates were tested on agar plates for microbial quantification and in BactAlert.

Results Two sequential passages of the homogenates on RESEP only slightly reduced Paeruginosa load tested on agar plates. Samples were positive in BacAlert. P.aeruginosa and S.aureus in RESEP treated and untreated homogenates showed similar growth capability on agar plates and were positive in BacAlert. RESEP-treated homogenates showed a slightly better bacterial recovery than RESEP-untreated samples both in agar plates and in BacAlert.

Conclusions RESEP device is compatible with homogenized tissues ensuring a good bacterial recovery and absence of interference with bacterial growth. Preliminary data suggest also the capability of RESEP to remove antibiotic in tissue homogenates.
P3. METHOD VALIDATION FOR STERILITY TESTING OF CORNEAL PRESERVATION MEDIA ACCORDING TO “METHOD SUITABILITY TEST” OF EUROPEAN PHARMACOPOEIA (CHAPTER 2.6.1.)

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**Purpose** The aim of this study was to validate the method for sterility testing of corneal storage media Tissue-C and Carry-C according to the “Method suitability test” (EP) using BACTEC (Becton Dickinson) automated system in a multicentric study.

**Material & Methods** The validation study was performed at the Eye Bank of Rome and Eye Bank of Monza, Italy. Samples of organ culture medium (Tissue-C, ALCHI.MI.A. S.r.l.), deswelling/transport medium (Carry-C, ALCHI.MI.A. S.r.l.), and optimal growth media (growth control) were inoculated with 6 EP reference strains to obtain final microbial concentration of 10 cfu/ml, and tested at least in triplicate with BACTEC automatized system.

Method sensitivity, specificity and robustness were determined for each medium, with and without antibiotic removal from samples with RESEP (ALCHI.MI.A. S.r.l.).

**Results** Both eye banks obtained the same method sensitivity and specificity results. The method for sterility testing of Tissue-C and Carry-C samples after RESEP-treatment using BACTEC system showed 100% sensitivity and specificity. Samples treated with RESEP showed similar times to detection as compared to growth controls.

**Conclusions** BACTEC system can be considered validated with 100% sensitivity and specificity, and robustness for samples of corneal storage media contaminated with 1-10 cfu/ml, and treated with RESEP.

P4. VALIDATION OF MICROBIOLOGICAL TESTING ON CARDIOVASCULAR TISSUE USING BACT ALERT BLOOD CULTURE BOTTLES IN EMILIA ROMAGNA CARDIOVASCULAR TISSUE BANK

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Our bank (BTCV) has been cryopreserved cardiovascular tissue since 2011 over the years we have noticed a minor sensitivity of the broths in presence of bacteria in cardiovascular tissues against positive results on freezing fluid using blood culture bottles. We decided to investigate the efficacy of the bact alert bottles for hemoculture by performing microbiological tests on cardiovascular tissues. We have broken up the cardiovascular tissues so we could inoculate the bact alert bottles using a large-caliber needle. For aerobic fertility tests we inoculated blood bottles using S. Aureus, B. Subtilis, P. Aeruginosa, C. Albicans, A. Brasiliensis: 15 bottles containing sterile cardiac tissue and 15 bottles containing sterile venous tissue. For anaerobic fertility tests we inoculated blood bottles using C. Sporogenes, 3 bottles containing sterile cardiac tissue were inoculated, 3 bottles containing sterile venous tissue. For the sterility test, 1 aerobic blood bottle and 1 anaerobic blood bottle were used. The growth of most microorganisms is detectable in the first 48 hours of incubation and for all species tested is detectable within 7 days. It has also been shown that the presence of cardiovascular tissue does not interfere with microbial growth for both tested microbial concentrations. Furthermore, the absence of microbial growth in non-inoculated bottles and the consistency between the inoculated strain and the observed growth were demonstrated. The minimum microbial concentration tested is 10 cfu. The use of bact alert bottles appears to be more sensitive than using broths.
P5. THE ROLE OF BONE GRAFT IN NON-UNION MANAGEMENT
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There are several orthopedic tissues known to be resistant to the healing process, such as cartilage tissue, meniscus and ligaments. Bone tissue instead, is known to repair itself, if the conditions are favorable. There are some cases, however, where the bone fragments fail to fuse, which increases the chance of patients’ morbidity and affects several economic and social aspects of their lives.

The factors which may lead to this complication are manifold. In particular, lack of stability of fracture, devitalization or a gap between bony fragments can contribute to this pathology.

Treatment is based on the creation of a biomechanical environment that allows the reactivation of the bone healing processes, as explained in the Diamond concept, which emphasizes the stability of the point of fracture, the presence of osteogenic cells and growth factors, and finally the use of grafts.

Recently, the use of matrices is generating great interest, spanning from the use of the autologous graft as the gold standard, to the development of biocompatible materials.

Between these two extremes lies the use of homologous bone tissue from a donor, which combines the absence of negative aspects such as the comorbidity of the donor site and the low availability of the autograft, with the clear superiority of using biomaterials.

This report seeks to help explain the importance of bone tissue donation and its use to treat non-union or to expedite the process of fracture consolidation, leading to great biological, psychological, and social advantages for the patient.

P6. BONE GRAFTING IN ONCOLOGICAL ORTHOPAEDICS: THE EXPERIENCE OF THE REGINA ELENA NATIONAL CANCER INSTITUTE
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Bone tissue can be site for benign and malignant tumor. In benign lesions the main approach is observation; when necessary intralesionale surgery and bone grafting is the main treatment; the residual cavity is usually filled with homoplastic bone chips and struts to obtain a higher biomechanical stability.

In malignant lesions wide surgery is the mainstay treatment; in those case reconstruction can be done with prosthesis, massive homograft, or composite.

The aim of presenting paper is to value the main indication to bone grafting in oncological orthopedics. All case operated for bone tumor undergone reconstruction with bone grafting at Regina Elena National Cancer Institute in Rome were considered.

Indication for bone filling, chips and struts and for massive homograft were valued considering possible advantages and complications.

P7. HOW WE DO IT: “SPACE TECHNOLOGY” SECURES ADEQUATE AIR CLEANLINESS FOR PRIMARY ALLOGRAFT HEART VALVES PROCESSING
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Introduction and Aim of the Presentation: In our department the donor heart dissection and primary allograft heart valves (AHV) processing perform dedicated cardiac surgeons. Advantages:

1) Donor heart inspection and dissection has been legally qualified as “Partial donor heart postmortem examination”

2) Low discard rate due to the technical errors.

From logistic reasons the laboratory has to be in the extremely busy area of central operating theaters (COT). Building of clean room was not possible. We decided to secure the required environment cleanliness level by means of HEPA filtration and microbial destruction technology using cold plasma (Plasmair Sentinel, Aerinspace, France).

Material and Methods: In COT the laboratory for primary AHV processing was adopted from storeroom. Working space with laminar box and decontamination unit was included. Facility observes the standard
cleaning and maintenance protocol. Environment cleanliness level control comprised airborne particle count and microbiological testing, always at rest, and during the simulated operation. It was scheduled twice a year at least. The level C is required in the laboratory, while level A has to be reached in the laminar hood.

**Results:** All but one measurement in laboratory room (airborne particle count) were within the limit, and introducing proper drape was enough for rectification. All but one measurement (microbiological testing) in the laminar box fulfilled the criteria, and tighten up regime solved it.

**Conclusions:** Our data shows that HEPA filtration and microbial destruction technology using cold plasma is capable to secure required environment cleanliness level in primary AHV processing laboratory.

**P8. THE FIRST CARDIOVASCULAR TISSUE BANK IN SWITZERLAND: ESTABLISHMENT OF AN EFFICIENT METHOD TO DECONTAMINATE, WASH AND FREEZE CARDIOVASCULAR TISSUES**

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(2)

**Purpose** Establish a method to decontaminate, wash and freeze cardiovascular tissues.

**Methods:**
- Samples: sterile porcine cardiovascular tissues.
- Decontamination: antibiotic solution BASE.128_12.5 ml/g, 14 h, 37°C.
- Washing: BASE 6.5 ml/g, 2x 5 min, 1x 6h, 4°C.
- RESEP treatment of homogenates: 2x 20 min, RT.

**Evaluation of:**
1. Decontamination efficiency: samples were immersed in contaminated solutions (103-108 cfu/ml: 10 bacteria and 2 fungi strains), decontaminated, washed and homogenised. Homogenates (RESEP treated/untreated) were tested for contamination on agar plates and BactAlert.
2. Capability of washing procedure to remove antibiotic: decontamination and washing procedure was applied. 12 microbial strains were spiked on RESEP treated/untreated homogenates, tested on agar plates and BactAlert.
3. Freezing method: several profiles were tested on aortic, pulmonary and pericardium specimens.

**Results**
1. Decontamination resulted in 6 Log10 reduction for 5 strains (P.aeruginosa, B.atrophaeus, S.marcescens, P.acnes, E.coli), 5 Log10 for 4 strains (S.epidermidis, K.pneumoniae, C.sporogenes, B.fragilis), 4 Log10 for S.pyogenes, 1.0 Log10 for C.albicans and A.brasiliensis.
2. Microbial growth was demonstrated on:
   - RESEP-treated tissues: 12/12 strains (agar plates and BactAlert)
   - RESEP-untreated tissues: 11/12 strains on agar plates (at less extent for 4 strains), 10/12 strains on BactAlert.
3. A Freezing profile was defined leading to optimal freezing curve (-1°C/min to -40°C, -3°C/min to -120°C).

**Conclusions**
Decontamination was effective (4-6 Log10) for 10/12 strains. The extensive washing procedure, able to remove antibiotic from the solutions and tissues, in combination with the RESEP ensures a reliable sterility assay. An optimal freezing profile was set-up.

**P9. RETROSPECTIVE ANALYSIS OF 32 YEARS OF ALLOGRAFT HEART VALVE BANKING IN CENTRAL SOUTH AFRICA**

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**Introduction** The history of homologous cardiac valves dates back more than 40 years. Much emphasis was placed on the optimization of graft retrieval, preservation techniques and clinical application. A cardiac homograft valve bank was established at the Department of Cardiothoracic Surgery,University of the Free State.Bloemfontein in 1982.

**Methodology** A 32-year retrospective analysis was performed since the first valve was successfully procured and transplanted in 1984.

**Results** 3182 valves (2179aortic and 1003pulmonary) were harvested from 2185 donors, of which 1181(54%) aortic and 738(73.6%) pulmonary homo
grafts respectively were released for clinical use. Main reasons for discarding valves were HIV (30.7%), Hepatitis B (9.4%), venereal diseases (7.4%), atherosclerosis (8.7%) and positive cultures (9.9%). Some years yielded no positive cultures post-sterilization, while the highest percentage of valves lost in one year due to positive cultures was 20%. Mean donor age was 26.43 years with male predominance of 1651 males versus 534 females. Average ischemic time was 29.75h mainly due to medico-legal autopsies exceeding the desired 24h time limit. Valves were disinfected in an antibiotic cocktail of Vancomycin, Piperacillin, Amikacin and AmphotericinB prior to cryopreservation. Surgical procedures utilizing the majority of homografts were aortic valve replacements (44.2%), aortic root replacements (6.7%) and right ventricular pulmonary artery conduits (49.1%). The bank also supplied 32 other centres with homografts (563 aortic and 395 pulmonary).

**Conclusion** The Bloemfontein bank has established itself over the years as a viable and functional cardiac homograft bank, and is currently the only heart valve bank in SA. Despite major advances in cardiology regarding valve replacement procedures, the demand for and application of homografts remain high, but availability remains a major concern.

**SKIN/DERMIS**

**P10. SKIN BANKING AND DONOR ASSESSMENT IN TISSUE BANK OF VERONA 13 YEARS OF EXPERIENCE**

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In severe burned patient, the use of homologous skin allograft is a valid treatment. We describe the experience of Verona Tissue Bank, in collaboration with the regional procurement and recovery equipe, in evaluating cadaveric skin donors, recovery homologous skin, processing and banking validated cryopreserved skin, during 13 years of activity. Cadaveric donors are evaluated according to national and international standards in order to avoid the risk to transmit communities disease, malignancies and other infectious diseases. Skin collected were processed, cryopreserved and banked from April 2003 to December 2016. 713 cadaveric donors were evaluated for skin donation, 24% HB donors and 76% NHB donors. From 2003 to 2008, during the “First phase” of activity of the Tissue Bank, 178 donors were evaluated and skin was collected (average 29 donors / year) by a local procurement/recovery equipe (that recovered only skin tissue). From 2009 a “second phase” of activity started, with a different organization: a regional procurement/recovery equipe performs assessment of cadaveric donors and recovery of all tissues; from 2008 to 2016 582 donors were evaluated and skin was collected (average 65 donors /year). The procurement/recovery equipe collected 2,682,069 cm² (average 266,696 cm²/year and 4,182 cm²/donor) that was validated, processed (antibiotic treatment) and cryopreserved in mechanical freezer (-80°C) using DMSO 10% in tissue bank laboratories, for 2 years. The cryopreserved skin was in all cases negative for microbiological test. According to European Directives, the processing is performed in B grade Laboratory.

**CORNEA**

**P11. CORNEA TRANSPLANTATION IN THE REPUBLIC OF MOLDOVA**

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State medical and Pharmaceutical University“Nicola Testemitanu”, Laboratory of Tissue engineering and Cells cultures, Chisinau, Moldavia(1)

**Introduction** The successful history of cornea transplantation in the Republic of Moldova has its origin in the early 1960s. Numerous problems in the field were reported within the Joint Programme CoE-EC for 2004 – 2006 by the Council of Europe’s experts with further support in the implementation of priority strategies related to human substances procurement and transplantation. The need to develop tissue transplantation, especially cornea transplantation, is the result of the analysis of the continued growth of the waiting list for cornea transplantation, which nowadays has reached the number of 140 patients.

**Materials and Methods** Since 2011, the number of authorized medical institutions has increased. Thus in 2011 in the country there were 3 institutions authorized for cornea transplantation, and today their number has doubled and represents 6. The multi-tissue human bank started the procurement and storage activities in 2013. The National Clinical Protocol “Cornea Transplantation” was developed and approved
Results Cornea procurement and transplantation were relaunched in March, 2013. Pursuing the scope to improve donation and transplantation of tissues and cells the “Standard on organization and performance of procurement and transplantation of human organs, tissues and cells” has been approved in June, 2017. The total number of cornea transplanted has reached 155.

Conclusions There is an effective collaboration between the multi-tissue human bank, Transplant Agency and the hospital sector with the main scope to cover the country’s needs in cornea for the patient’s treatment.

DONATION’S NETWORK

P12. CORNEAL INVOLVEMENT IN DONORS WITH ANKYLOSING SPONDYLITIS
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Summary The Ankylosing Spondylitis (AS) is associated with HLA-B27 antibody in up to 96% of cases, thus causing a greater inflammatory response and manifests recurrent outbreaks of anterior uveitis, because Fas gene is activated inducing the apoptosis of endothelial cells. The aim of this study is to evaluate corneal involvement in AS, in order to optimize donor evaluation and corneal tissue processing.

Method Case-control study. Corneal donors (N=6) between 2011 and October 2016 with AS were duly reviewed, and a control group (N=12) was established at 1:2 per case. The inclusion criteria were: year of death, age, gender, relevant pathology in common other than AS. Corneal viability: endothelial count ≥2000cells/mm2 and impossible count: ≤499cells/mm2.

Results Surveyed group: 4 viable corneas (2560cells/mm2) and 8 not viable (1412cells/mm2). Control group: 23 viable (2481cells/mm2) and 1 not-viable (499cells/mm2)

Comparing the corneal viability, we observed a statistically significant association of risk OR=46 and Fisher’s test = 0.000129 (p<0.05) and the guttata in the non-viable corneas. Fisher’s test of 0.018 (p<0.05).

Conclusions This study evaluates a significant association (especially from the age of 60), of risk between AS and non-viability of corneal tissue in these donors, limiting damage to the endothelium.

It is necessary that both the Transplant Procurement Management and the staff of the tissue bank investigate this pathology and register it in the donor’s protocol.

P13. ANALYSIS OF THE IMPACT OF A MULTIFACtorial INTERVENTION PLAN TO INCREASE TISSUE DONATION RATES IN A THIRD LEVEL HOSPITAL
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Third level hospitals are the main producer of organ and tissue donor accounting 61% of tissues generated. Tissue donors in our center did not reach the usual rates of the other hospitals with the same characteristics. The need to analyze the process of detection and obtaining of tissue donor was detected.

Determine the impact of a multifactorial intervention plan to increase tissue donation rates in a third level hospital from June 2016 to June 2017.

A multifactorial analysis was performed following the cycle of continuous quality improvement (E.Deming) from June 2016 to 2017. The following categories were detected: late or ineffective detection, lack of knowledge of professionals and families, and lack of required spaces.

The interventions carried out were: reorganization of the detection circuit, training sessions to the professionals and information points about tissue donation.

During the period from June 2015 to 2016 the total of valid donors was 29 (N=32). After the reorganization it increased to 73 (N=82) of which 21 were organ and tissue donor, 7 were only tissue donor and 54 were only corneas donor. The training received from the professional had no impact on donation rates.
The only intervention that had impact on the increase of tissue donation rates was the reorganization of the circuit increasing tissue donation by 256%.

**P14. IDENTIFYING CHALLENGES AND OPPORTUNITIES IN TISSUE DONATION IN THE NETHERLANDS**
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**Background** The Dutch Transplant Foundation (DTF) has a central, independent and coordinating role with regard to tissue donation in the Netherlands. For this purpose, the DTF works closely with tissue banks and donation professionals in hospitals. All doctors in Dutch hospitals are obligated to fulfil tasks regarding tissue donation. Donation coordinators support these doctors through education and administrative support. Despite this system, the number of tissue donors declined by 7.5% in 2016 compared to 2012.

**Methods** The current donation process was analysed for possible improvements to achieve an increase in tissue donors. The donation process was divided into four phases: donor recognition, consulting the Donor Registry, requesting permission for donation from the family and reporting the donors to the DTF.

**Results** A large number of potential tissue donors is missed in the phases of donor recognition and permission for donation.

- Consultation of the Donor Registry takes up to 10 minutes because of incompatible IT systems.
- Reporting donors to the DTF takes a lot of time because of the extensive list of mandatory questions.

**Conclusion**
- Because of the large number of doctors responsible for tasks regarding tissue donation, it is a challenge to keep their knowledge of tissue donation up to date.
- A link between IT systems will be developed to reduce time for consulting the Donor Registry.
- In 2018 the DTF starts several projects in cooperation with donation professionals. These projects will focus on better donor recognition and increasing the rates of permission for donation.

**P15. DGFG - A SUCCESSFUL TISSUE NETWORK FOR GERMANY**
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The German Society of Tissue Transplantation (DGFG), a non-profit organization, is dedicated to providing patients with safe and durable, high-quality tissue transplants, like corneas, amniotic membranes, heart valves and blood vessels. With the enactment of the Tissue Act in Germany 10 years ago - in 2007 - and the changes that were made to German Drug Act and the Transplantation Act, tissue storage and transplantation processing must adhere to strict legal regulations. The DGFG has developed in the last decade the largest tissue network in Germany - a network of German hospitals and tissue banks, operating exclusively in the field of non-commercial tissue donation. In Germany the DGFG provides 120 transplant programs for corneas, 35 clinics for heart valves and blood vessels, and about 40 facilities for amniotic membranes. Alone in 2016 over 4,100 patients have had tissue transplants from the DGFG. The author describes the whole process, exchange of knowledge, training of staff, common database and the system of tissue allocation as some of the instruments used in the DGFG. Future developments in tissue banking, new regenerative products and tissue for medicinal research are also in the focus of the network. The case example describes why the organization of tissue donation, the support of the tissue banks in the processing and distribution works better in a network like the DGFG than any standing alone activity.

**P16. DONOR SELECTION GUIDELINES – AN EVIDENCE BASED APPROACH**
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**Background** Commission Directive 2006/17/EC (EUTCD) sets out minimum selection criteria for donors of tissues and cells. Annex I of EUTCD states that donors must be excluded unless justified by risk assessment if they have a “History, clinical or laboratory evidence of HIV, acute or chronic Hepatitis B (except in case of persons with a proven immune status), Hepatitis C, HTLV I/II, transmission risk or eviden-
of risk factors for these infections."

The UK Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO) set up a working group (WG) in 2016 to review the evidence base for donor selection, deferral and exclusion regarding social behaviours that may increase the risk of acquiring blood-borne infections (BBI) (HIV, HBV, HCV, syphilis). The WG was also asked to review the risk BBI following procedures that involve skin piercing of the skin. The remit of the review included blood, tissues and cells.

**Review** The WG included SaBTO members, invited professional experts and representatives of stakeholder organisations. The WG considered many relevant issues including epidemiological data on transmissible BBI, the performance of tests for diagnosing BBI, statistical modelling of the risk of Transmissible infections, international practice, ethics and motivation.

**Outcome** The WG recommendations were approved by SaBTO in June 2017 and accepted by the UK health departments if there were no legal restrictions. The process and the recommendation will be presented on behalf of SaBTO

**Acknowledgment** This abstract is presented on behalf of the SaBTO and the WG.

**P17. DEVELOPMENT OF AN OUT-OF-HOSPITAL DONATION PROJECT**

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**Background** Tissue donation in Catalonia is still linked to multi-organ donation. The difficulty to establish mechanisms of screening among the hospitals of the health system, as well as the aging of the population, has motivated the need to look for other sources of donation.

For this reason in 2016, thanks to collaboration between the Blood and Tissue Bank, the Catalan Department of Justice, the Emergency Medical System (EMS) and the Hospital Clinic has begun the procurement of corneas in the Institute of Legal Medicine and Forensic Sciences of Catalonia (IMLCFC), where the judicial autopsies of the province of Barcelona are completed. In a second phase an autopsy room at IMLCFC has been adapted as an operating room for tissue retrieval in order to accept multi-tissue donors.

**Methods** A nurse from the Donor Center (Management Unit of the donation) has been assigned to the IMLCFC to make the assessment of all the deaths that come in this Institute. Previously, the EMS has provided the clinical records as well as the contact information of the deceased for seeking consent from their next of kin. Meanwhile, the judge’s authorization is requested. If is granted, the serologies are taken and a retrieval team goes to the IMLCFC to obtain the tissues in agreement with the forensic responsible for the donor.

**Results** 5 multi-tissue donations were obtained in one month, with an average age of 50 years.

**Conclusion** Out-of-hospital donation has been achieved through the collaboration between the different work teams.

**P18. THE BODY DONATION PROGRAM: A NOVEL CONCEPT OF TISSUE BANK**

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The Institute of Human Anatomy has developed a Body Donation Program which was approved in 2003 by the Council of the Faculty of Medicine and Surgery of the University of Padova. The main aim was to promote dissection as a necessary formative instrument for students, residents, and surgical specialists. Furthermore, the Body Donation Program collects donated bodies and body parts and manages this anatomical material, through a series of procedures developed in the context of a specific policy for quality assurance. The quality management system achieved ISO 9001:2008 certification in 2011, which aimed to guarantee competence and privacy all over the processes, to develop a monitoring system and to continuously update the personnel of the Program. Bodies can be stored frozen or fixed in embalming solutions for future anatomical training, with quality of tissue preservation very similar to that of the body in vivo. Moreover, several types of tissues have been used for research in tissue engineering and regenerative medicine. Thus, in our experience, the role of Body Donation Program may be implemented as a sort of bio bank since all tissues and organs could be sampled and catalogued to be available for research purposes.
P19. ASSESSING THE PROBABILITY OF UNDIAGNOSED CONNECTIVE TISSUE DISEASE IN YOUNG TISSUE DONORS WHO DIED OF AN ARTERIAL DISSECTION
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Background An arterial dissection might occur at a relatively young age (<50) as a manifestation of a connective tissue disease. In the Netherlands, connective tissue diseases such as Marfan syndrome are a contraindication for tissue donation due to the poor quality of the tissues. The medical staff at the Dutch Transplant Foundation (NTS) is regularly confronted with relatively young donors who died after arterial dissection without a prior history of connective tissue disease. This raises the question if these donors might have had an undiagnosed connective tissue disease.

Methods We searched the literature for detailed information about the different connective tissue diseases including, but not limited to, pathogenesis, risk-factors, clinical criteria, genetic determinants and associations with aneurysmal formation and dissection.

Results & discussion Based on the information we found, we developed a donor screening guideline to assess the probability of an undiagnosed connective tissue disease in donors who died of an arterial dissection. We would like to present this guideline because it provides a tool to quickly come to a decision whether the tissues are suitable for transplantation. We would also like to learn how other organizations deal with these issues. A comparison or collaboration with other organizations is desired.

P20. IMPORTANCE OF ECONOMICAL ANALYSIS IN ALL STEPS OF TISSUE BANKING ACTIVITIES: SURGICAL KITS FOR TISSUE RETRIEVAL
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Background Tissue retrieval requires specific instruments to be performed. In 2014 the retrieval team from the Donor Center-Barcelona Tissue Bank started to work with surgical kits containing all material needed for retrieval, from the surgical tooling to sterile gowns and gloves for the team or specimen bags, but except in multi-tissue donations a large part of the contents of the kit were discarded after each retrieval because weren’t needed.

Methods Since 2016 4 new models of specific retrieval kits have been developed that allows to perform the different kind of retrievals (eye, skin, bones, bones and tendons) adjusting the surgical supplies and having a better a better follow-up on expenditure.

Results In 2015 were performed 1204 retrievals using the full kits with an average monthly consumption (including both expendable material and full retrieval kits) of 28539 Euro while in 2017, 2242 retrievals were performed using the specific kits with an average monthly consumption (including both expendable material and specific kits) of 22114 Euro. This results in a saving of 6425 Euro per month. Kits have been positively accepted by professionals and are currently being reviewed for improvement taking into account their opinions.

Conclusion The use of specific kits reduces storage needs, facilitates the transport of material for the retrievals without providing expendables that are not used and allows the economical monitoring and the control of expenditure ensuring efficiency and economic sustainability.
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