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SNBTS Tissues & Cells Directorate. UK.

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Cryobiology Unit Complejo Hospitalario Universitario A Coruña. Spain.

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BioBanco Hospital Universitario Central de Asturias-Oficina de Investigación Biosanitaria del Principado de Asturias (OIB). Spain.

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Dpt. Pharmacology, University of Valencia and IDM. Spain.

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Banc de Sang i Teixits. Spain.

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Institut für Physiologische Chemie, Universitätsklinikum Essen. Germany.

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Hospital de Jerez De La Frontera. Spain.

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Unit of Criobiology Complejo Hospitalario Universitario A Coruña. Spain.

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Hospital Universitari Mútua Terrassa, Social Work. Spain.

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Centro Vasco De Transfusión y Tejidos Humanos. Spain.

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Rodríguez, L.; Genis X.; Rodríguez L.; Tarragona E.; Ortega I.; Gomariz E.; Navarro A. - Banc de Sang i Teixits. Spain.

P-109 COMMUNICATION BETWEEN TISSUE BANK AND IMPLANTING SURGEON: SHORT-TERM FOLLOW-UP OF TISSUES FOR OCULAR SURGERIES.

Núñez, V.; Agustí E.; Martínez E.; Otero N.; Pérez M.; Casaroli-Marano R.; Vilarrodona A.
Transplant Services Foundation-Hospital Clínic. Barcelona. Spain.

P-110 A COMPARISON OF DIFFERENT CULTURE MEDIA FOR THE STORAGE OF HUMAN DONOR CORNEAS FOR GRAFTING

Zlacka D., Krabcova I., Kortusova J. and Jirsova K.
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INTERNATIONAL EXPERIENCE ON TISSUE BANKING

P-01

ACTIVITY REPORT OF THE MULTI-TISSUE BANK OF THE RED CROSS BLOOD TRANSFUSION SERVICE OF UPPER AUSTRIA

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Abstract:

In 2003 the Red Cross Blood Transfusion Service of Upper Austria started cord blood banking. It turned out as starting point for our multi-tissue bank. In 2007 all provisions were made for the first Austrian ISO 9001:2000- and GMP-certified multi-tissue bank situated at the new building of the Red Cross Blood Transfusion Service of Upper Austria. Since then our institution is specialized on several tissue banking products besides blood products. At this time we started to introduce heart valves and amniotic membrane as our first products besides cord blood. In 2008 the activity of the tissue bank was further expanded to the preparation of cornea transplants. Subsequently, in 2009 the tissue bank of the Red Cross Blood Transfusion Service was the first multi-tissue bank in Austria certified according to EC-Directives 2004/23/EC, 2006/17/EC, and 2006/86/EC and is now certified for procurement, testing, processing, storage and distribution of: * Cord blood * Heart valves and vessels * Amniotic membranes * Cornea * Ovarian tissue * Femoral heads, calvarium * Autologous platelet lysate * Serum eye drops * Semen and testes (in preparation).

P-03

ISO 9001:2008 AUDITS AND NONCONFORMITY REPORTING IN A TISSUE BANK

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Abstract:

The main ISO principles can be properly adopted in the quality management of a tissue bank. The improvement of the quality system can be maintained and measured by performance of internal audits following the ISO requirements.

Objective:

The study describes the benefits of performing ISO-complied internal audits in our tissue bank and the maintenance of an adequate system for nonconformity reporting. Such approach is necessary to achieve the quality requirements and to review the effectiveness of the arrangements for quality assurance.

Results:

All organizational processes were identified and managed in compliance to ISO standards. Quality metrics were introduced. The internal audits conducted aimed at verifying the compliance of processes performed throughout the organization with respective ISO 9001:2008 standards. An internal quality auditing program was established after an appropriate training of employees. Numerous internal audits of all organizational units and processes throughout the year have been conducted. The internal audits were carried out on the basis of a prescribed method in SOP. The methods used were as follows: interviews with employees; examination of records and documentation; complete trace of individual processes; checking of actual compliance with requirements; reporting nonconformities. Through internal audits we covered all ISO areas of requirements thus evaluating among all the state of the referring system, management of donor cases and monitoring the

reliability and accuracy of donor documentation. Focused audits have been conducted in parallel to monitor critical areas and when problems with quality have been identified. Improvements in management, training, donation and recovery processes supervisions, and statistical reporting became evident during internal audits. Although the results showed a lack of significant non-conformities, all detected deviations were reported according to the established nonconformity reporting system. A formal corrective action system has been developed and implemented. The periodical analysis of the type and severity of nonconformities was of great importance for continuous improvement of the quality system.

Conclusions:

Internal audit is a helpful tool for identification of problematic areas and opportunities for improvement in tissue banking. Positive effects on organizational quality culture has been evaluated during management review meetings. The conduction of internal audits according to ISO requirements is complementary to the audits performed to review the compliance with tissue banking standards. The implementation of a proper system for nonconformity reporting helped changing the concept of quality management from detection to prevention.

P-04

A 6 YEAR RETROSPECTIVE REVIEW OF THE KALEIDOSCOPE OF CHALLENGES ENCOUNTERED BY A SOUTH AFRICAN TISSUE BANK AND THEIR INFLUENCE ON DONOR STATISTICS AND ALLOGRAFT AVAILABILITY

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Abstract:

Purpose of study The estimated South African population stands at approximately 50 million and the Centre for Tissue Engineering - Bone Bank provides more than 16 000 bone and soft tissue allografts to the medical fraternity across South Africa annually. As a developing third world country, the study highlights the various challenges (cultural diversities, language barriers, education, public awareness and the lack of standards), that influence the concept of donation, which in turn have an effect on the availability of safe transplantable tissue to the recipient. Description of methods Donor data and results were systematically accumulated over a period 6 years (2005 – 2010), from the three main divisions in the Bone Bank – namely, Procurement, Quality Control and Production. Summary of results The challenges include the lack of standards and regulations, outdated government policies/acts relating to tissue donation and transplantation, education, public awareness, cultural and language barriers have had a crippling and curtailed consequence in the availability of tissues in South Africa. Despite these overwhelming challenges, the Bone Bank has been able to maintain an adequate monthly retrieval rate which has translated to an increase of 41.27% transplantable tissue from 2005 to 2010. A high percentage of the donors were from the caucasian population group, while the lowest donor group was identified from the African population group. Within the 6 year period, 983 QC passed tissue donors, were processed into 96 325 safe quality allografts for the much needed South African public. Conclusion In spite of these challenges, the Bone Bank has maintained excellent working relationships with the Organ Donor Foundation (ODF), Eye Cornea Bank and numerous hospital groups, such as Netcare. The continuous in-house policy, procedural and process improvements and with the implementation of both ISO 9001 and ISO 13485, the Bone Bank has optimised its production processes and significantly improved allograft delivery to its South African recipients, despite the challenges. It is the responsibility of every health care professional and healthy individual in South Africa to become conscious for the need of solid organs, corneas, bone, heart valves and skin. Therefore, it needs to become a collective obligation in a two-fold manner a) to sensitise decision makers and government to this national challenge and b) encourage and inform patients and next-of-kin to be organ and tissue donors.

P-05

COMMUNICATION & CUSTOMER SERVICE IN A TISSUE BANK

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Abstract:

Human tissues are a scarce commodity but in many cases essential for the healing of patients. Therefore, good coordination between the demand for human tissue and allograft availability is a key factor in the processes of tissue banks. Control all stages of the procedure from donation to distribution in the hospital, along with a thorough internal and external communication are the keys to success to improve the effectiveness and efficiency of the tissue bank. Fluid communication with the surgeons allows the tissue needs analysis to provide the most appropriate, depending on availability and timing needs. This is more relevant in cases where the demand is much higher than allograft availability as is the case in paediatric surgery. Aim The goal is to analyze how a good and well organize communication & customer service can improve the effectiveness and efficiency of a tissue bank, managing something with limited availability as the human tissues are. This situation is even more important when we are speaking about paediatric tissues or paediatric necessities. Study Methods We have analyze all the procedures from tissue harvesting to allograft implantation, information generated by different steps and the communication necessary to provide the surgeon with the allograft more suitable for his surgical needs, and we have correlate it with the surgeon satisfaction on the Tissue bank services. To study an specific area, we have done comparative analyses between the demand of paediatric tissues and the distributed tissue, taking into account the waiting list. It has also been studied in which cases adult tissue can be adapted for paediatrics us and when the need of tissue transplantation is crucial for life saving. Results We studied all grafts provided since January 2010 and analyzed the adequacy of what served the needs of the clinical case by: - Surgeon's Feedback (complaints, thanks, communications) - Internal communication files - Currently, in many cases, we have to adapt adult tissue for use in paediatrics Conclusions A good internal& external communication are essential to optimize the results and offer the surgeon the best opportunity available to your patient. The recovery and distribution of adult and paediatric tissue is a need in growing demand. It is a must to organize the activities and design systems to increase the effectiveness in the tissue assign in order to optimize the tissues availability.

P-06

TISSUE PROCESSING RESULTS IN 2010 IN THE BANK TISSUE AREA OF BLOOD AND TISSUE BANK OF ARAGON

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Abstract:

The Blood and Tissue Bank of Aragon carries out activities relating to processing, preservation, storage, conservation and distribution of human tissues and blood components. The Area of Tissue Bank began operations in 2009. This area handles the processing, preservation, storage and distribution of tissues from living donors and cadaver donors that will be intended for clinical application in humans is regulated by the RD1301/2006. The aim of this study is to analyze the results for tissue donation, processing activity in the Tissue Bank Area and the implants performed in hospitals of the Autonomous Community of Aragon. To determine the suitability of tissue were used AEBT standards. All tissues met the requirements of the RD1301/2006. Tissue processing was carried out in Class B clean rooms and in class A laminar flow. To ensure the adequacy of tissue, we performed all the necessary microbiological controls (both the tissue processed as environmental

control and surface clean rooms used in processing). During 2010, the Tissue Bank Area processed 28 cadaveric donations. Of these, 28 donated eye tissue and 13 bone tissue. We obtained from bone tissue, cancellous bone, femoral condyle, proximal tibia, diaphysis and tendons (Achilles, patellar, tibial and semitendinous). In the case of eye tissue, maintenance of the corneas was performed at 4 °C or 31 °C, depending on the needs of the implanted equipment. The Tissue Bank also has pieces of amniotic membrane for ocular defects obtained from placenta. The number of implants in the community of Aragon, with tissue provided by our bank was 211 (74.8% bone tissue, 22.7% eye tissue and 4.2% amniotic membrane which led to 307 pieces of tissue. Only 2.6% of the implanted tissues were requested from other regional banks. The Tissue Bank area also performs serological and microbiological studies of implants. The Bank Tissue of Aragon has improved the accessibility of tissues to different hospital authorized to implant in the Community of Aragon. During 2010, we have supplied more than 97.4% of the tissue needs of the Community of Aragon. For these reasons, the control of the implants in the different hospitals (serology and microbiological serum bank of the implants) has also been greatly improved.

SPECIFIC CHALLENGES OF DONATION; PROCESSING AND CLINICAL APPLICATION IN PAEDIATRICS

P-07

EFFECTS OF NEW EU DIRECTIVE ON POSTMORTEM DONATION ACTIVITY IN SWEDEN

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Abstract:

The EU directive 2004/23/EG implies that today, a blood sample from deceased donors must be collected within 24 hours after death for analysis of possible contagious agents in order to reduce the risk that serious diseases are transmitted to recipients during a transplantation procedure. This directive has become a Swedish law (2008:286), which require a more urgent handling of possible donors. Given the many obstacles during the identification of relatives and the procedures to obtain an informed consent in addition to logistic problems regarding the transport of a possible donor, it was expected that this directive would reduce the number of donation procedures. Hence, the Swedish National Tissue Project was started, and involved the Swedish National Board of Medicine, since this is a national agency running all forensic medicine departments in the country, at which most of the postmortem tissues are collected. To determine the impact of increased resources on the success rates, one department with a good track record of many donors, and two with a dormant activity were given support for extra personnel and compensation for on-call duty to collect blood samples within 24 h. A fourth forensic medicine department given no extra resources was included as a control site. During the project period, Sept 2009-Aug 2010, tissues from 94 donors were collected at the forensic medicine departments, including 40 donations of heart valves and 70 corneas, and in addition, from mostly the same subjects, tissues for research purposes could be obtained in 46 cases. The preceding 12-month-period the corresponding figures were 23, 36 and 29, respectively. The increase was largest in Stockholm, where heart valve and cornea donors increased from 4 to 18 and from 13 to 45, respectively. At the department in Lund, not obtaining any extra resources, heart valve and cornea donors decreased slightly from 19 to 16 and from 18 to 16, respectively, partly as an effect of the EU directive's requirement for blood sample collection within 24 hours. It is concluded that increased resources are necessary to compensate for the reduction of formally acceptable donors caused by the EU directive. Studies are running to examine the rationale for the 24 hour rule by comparing blood test results at different time points after death. Preliminary observations suggest that there are no significant differences in false negative results regarding HIV and HCV detections between samples collected before and after 24 hours.

P-08

LEGAL MEDICINE VERSUS HEART VALVE DONATION

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Abstract:

As human heart valves are in high demand for certain surgical indications and the use of hearts from non beating heart cadavers is possible, institutes of legal medicine present a interesting source of potential donors. In Germany, the Institute of Legal Medicine in Munich has a tradition of heart valve explantation since 1982. In 2009, the Bavarian Tissue bank was founded as a response to the demands of the German Tissue Law. Further conditions to be met are formulated by the controlling government agency, the Paul Ehrlich Institute. With higher requirements, the difficulties to integrate a program for heart valve donation in the special setting of an institute for legal medicine are rising as well. This concerns e.g. organisational matters, quality management and especially extensive disinfection/sterility measures during explantation to ensure recipient safety against the background of a high daily workload of postmortem examinations in the setting of a forensic autopsy room. We will present our procedure to balance the requirements of legal medicine on one hand against those of heart valve donation on the other, and discuss assets and drawbacks to this approach.

P-09

DONATION FOR RESEARCH USING A PROSPECTIVE STRATEGY

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Abstract:

In 2005, a core facility for donation for research, KI Donation, was founded at Karolinska Institutet, Stockholm, and today tissues from 218 deceased donors have been collected and used for different research projects, resulting in important scientific contributions. The activities of KI Donatum are integrated with both the activities for donation for transplantation, and the routine forensic casework. Several donors have signed up both for donation for transplantation and for research (or the relatives have given consent for both purposes), and the collection procedures can often be performed in parallel. KI Donatum has a prospective strategy, implying that tissue regions of interest are carefully dissected out, and handled and processed according to protocols designed by the researcher responsible for each particular project. It also means that specific information, critical for the project, is gathered from different sources and carefully evaluated. Hence, a project regarding cardiomyocyte turnover requires detailed information about previous heart problems, and careful cardiac pathology examination whereas a project regarding neuropharmacological changes due to heroin abuse necessitates a thorough review of the drug use history, from relatives and medical charts, and comprehensive toxicological analyses of blood, urine and hair. The concept of KI Donatum also implies some ancillary services, e.g. certain tissue processing (from simple centrifugation to advanced procedures such as cell sorting), immunohistochemistry and specific biochemical analyses, in order to further characterize the individuals. Today research on postmortem tissues is typically performed on material available at tissue banks, but then the researchers have to accept that certain information important to the particular project may be missing or uncertain. Therefore a few tissue banks have started prospective sampling in parallel with their routine donation procedures. To improve the quality of research on postmortem tissues, we recommend tissue facilities to consider the strategy outlined above when procuring material for research purposes. Below are some selected publications based on the activities of KI Donatum. 1. Philipova et al. Differential forms of p53 in medulloblastoma tumor, cell lines and xenografts. *Int J Oncol.* 2011 Mar;38(3):843-9 2. Brennan et al. Antibody-based proteomics: fast-tracking molecular diagnostics in oncology. *Nat Rev Cancer.* 2010 Sep;10(9):605-17 3. Uhlen et al. Towards a knowledge-based Human Protein Atlas. *Nat Biotechnol.* 2010 Dec;28(12):1248-50 4. Bergmann et al. Evidence for cardiomyocyte renewal in humans. *Science* 2009;324(5923):98-102 5. Bhardway et al. Neocortical neurogenesis in humans is restricted to development. *PNAS* 2006;103:12564-8 6. Spalding et al. Retrospective birth dating of cells in humans. *Cell.* 2005;122:133-43.

P-10

DESIGN OF AN INTEGRATED CAMPAIGN FOR DONATION AND PROCUREMENT OF SKIN AND TISSUES THROUGH SOCIAL NETWORKING AND MICROBLOGGING SERVICES: THE MEXICAN EXPERIENCE OF THE NATIONAL RESEARCH CENTER AND BURN CARE (CENIAQ)

MARTÍNEZ-F, F.; SANDOVAL-Z, H., *Skin and Tissue Bank of the National Institute of Rehabilitation. Ministry of Health, Mexico*

Abstract:

Mexico and other emerging countries worry about a fragile culture for the donation of organs and tissues. Loopholes, idiosyncratic problems, inappropriate public policies, ethical dilemmas, private interests and insufficient campaigns in mass media, combine with public budget deficits of the health system. All this negative background represents a considerable shortfall in the procurement and subsequent transplantation of organs and tissues such as human skin, necessary for the care, treatment and rehabilitation after severe burns. In order to find alternatives, the Skin and Tissue Bank in CENIAQ has the task of designing a new campaign through social networking and microblogging services (Facebook, Twitter, Google+ LinkedIn and others), with the aim of reaching young population groups with higher levels of education and income as potential future donors. The object is to develop open, public, horizontal, participatory and democratic networks, as opposed to limited success of institutional networks that operate in closed, vertical and hierarchical bureaucratic systems. The goals for the campaign are: 1) Take advantage of lower costs and mass diffusion associated with the dissemination of messages through social networking and microblogging services; 2) Develop integral ongoing campaigns to raise awareness of potential donors; 3) Use educational techniques through micro-stories and other massive intervention methodologies, and 4) Create a database --with appropriate safeguards for privacy-- where all the members can sign up as potential donors of organs and tissues in the near future. The campaign, divided in four phases to complete in a maximum period of one year, includes the design and dissemination of intervention techniques and feedback from participants. We expect to get international financial resources and specialized advice from global NGOs and private enterprises like Google. Once implemented, the model will be available to any Latin American public health institution under the canons of a Creative Commons license. This strategy aims to replicate the methodologies and increasing the rate of altruistic donation of organs and tissues for therapeutic purposes in public health systems.

P-11

THE CHALLENGE TO COORDINATE DEATH IN THE NAME OF LIFE

POPOVA, P.; KALAYDJIEVA, V., *Tissue bank "Osteocentre Bulgaria". Sofia. Bulgaria.*

Abstract:

The tissue donation starts from "the end" of one's life in order to donate new "beginning" of other life or to get better it's quality. The successful donor coordination requires strict observance of the fundamental principle in the medical philosophy "Primum non nocere". This principle faces each doctor to the challenge to remain faithful to the Hippocratic oath. The donor coordination is complicate and difficult process. Each hospital coordinator is obliged to be acquainted with and observe the following regulations and requests: 1. Strict observation of the donor medical suitability criteria. 2. Observation of the current legislation and the moral and ethical norms. 3. Compassion and psychological support to the donor's family. 4. Confidentiality about the mystery of donation. 5. The donated tissue is priceless gift of life, granted only to the patients in need as a gift. 6. Equal standing for donation- irreversible right to everyone. The comprehensive approach of donor evaluation determines the best way for the donor medical assessment. The selection of the potential donors should follow

the maxim "maximal benefit and minimal risk" for the potential recipients, needed transplantation. The coordination of death in the name of life requires: many knowledge, developed sense of partnership, strong believe in donor mission, determination to win in the fight for standing up for the right to live. The donor coordination requires one dedicated team of partners. The fundamental columns in each donor process are: hospital coordinator, clinical psychologist, hospital surgical recovery specialist and in house donor coordinator on call in the Tissue Bank. The basic stages in donor coordination are: management of the donor referral system, report for each potential donor case – discussion, timely contact with the relatives and inform about the opportunity of donation, serological screening, signing of a consent form, recovery procedure with observation of all medical criteria for septic and antiseptic, diligent reconstruction of the body, properly final completion of the documents, storage of the recovered tissue. The coordination of the combined organ and tissue donors is even greater challenge for the hospital coordinators. The simultaneously representation of the opportunity for organ and tissue donation is the most correct and gentle approach toward the relatives and is the key to success in such donor cases. The strict observation of the moral and ethical norms during the implementation of medical occupation ensures the positive relation of the patients, their relatives and the society as a whole to the Healthcare and donation. Donation gives recipients last chance for treatment as a present. For that reason it is very important to have "opened for donation" National Health policy. The donor coordination is a great challenge to remain a doctor, even after the death of his patient.

P-12

IMPACT ASSESSMENT OF SKIN DONATION PROGRAM IN COSTUMERS OF A EMERGENCY ROOM AND UNIT OF INTENSIVE CARE

MARTÍNEZ-F, F.; GUZMAN-M, K.; LOMELI-R, C.; MADINAVEITIA-V, J.; ELIZONDO, B.; CARRILLO, E., *Skin and Tissue Bank of CENIAQ. National Institute of Rehabilitation. Mexico.*

Abstract / Background:

In a study by fattum BTL to 40 public transport users in Mexico City during 2010 to assess the knowledge of the donation of skin and its therapeutic use, important results were obtained indicating 100% of respondents agree with the donation of organs and tissues and therefore be altruistic donors; 32% (13/40) respondents are unaware the skin is an organ that can be donated, however, 100% are willing to be a donor of skin after death. Therefore we assume that according to data obtained in 2010 more than 50% of respondents would know that the skin is an organ and can be donated but the vulnerability of the user in a hospital that has an impact on the acceptance of the donation.

Material and Methods:

We conducted a survey of 30 users of the emergency department and intensive care unit at the National Institute of Neurology and Neurosurgery (INNN) prior to an induction course on skin donation, which included 11 questions that were designed for data collection about the knowledge of donation of organs and tissues besides the willingness to accept the donation of skin in case of loss of a relative.

Results:

87% (26/30) agree with the donation of organs and tissues, 57% (17/30) know that the skin is an organ and therefore may be donated, 70% (21/30) are unaware of the use of donated skin, 73% (22/30) don't know the process of donating skin; instead 30 % (9 / 30) is willing to accept the donation of skin when they suffer the loss of their relative.

Conclusions:

The results obtained by fattum BTL show that 100% of the population is willing to be a donor, but it is important to note that their emotional state is not subject to a time of stress and fear. However the population studied in the INNN indicates that 30% of respondents are willing to donate if they suffer loss of a relative, taking into account that these are in a situation where their relative's health is compromised. It's important to design a massive campaign where information is more extensive, for

the results show that 70% do not know the process and the use of skin that is donated. Additionally the education to fomenting culture of donation has been traditionally focused to Organs making relevant the necessity of a massive campaign and restructured program for tissues.

P-13

REVIEW OF THE TISSUE DONATION PROCESS AT THE TATA MEMORIAL HOSPITAL

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Abstract / Introduction:

The Tata Memorial Hospital (TMH) Tissue Bank provides frozen and freeze-dried, irradiated bone grafts, and freeze-dried, irradiated amnion dressings. These tissues are obtained mainly from living donors. This study is a review of the tissue donation process from 2006-2010.

Material and Methods:

During the study period there were 3927 bone donors and 6444 amniotic membrane donors. 1088 (27.70%) of the bone donors and 992 (15.39%) of the amniotic membrane donors were rejected. 3112 bones and 5452 amnion were processed generating 5929 bone grafts and 9518 amnion dressings. Of the total grafts generated, 0.82% of the bone grafts were rejected due to machine failure and 0.50% of the amnion dressings were rejected during processing. From 2009-2010 a number of measures were used to reduce tissue donor rejections. These included intensified efforts to motivate potential donors, continuous follow up with donating surgeons to ensure blood samples or blood reports, collection of tissues at regular intervals to avoid tissue deterioration, conducting programmes to educate staff on the importance of donor screening, labeling and storage of donated tissues.

Results:

Rejection of the bone and amniotic membrane donors was due to inadequate screening (14.14%) because of unavailability of blood samples, non compliance with screening criteria (4.57%) including a recent blood transfusion, and improper storage of the tissue (1.25%). The number of rejections of amniotic membrane donors fell by more than 50% in 2009 and 2010. Similar results were obtained with bone donors.

Conclusion:

Frequent and consistent communication between the tissue bank and the donating agencies, along with awareness programmes to educate staff, results in greater co-operation and compliance with the Tissue Bank's requirements, with a consequent reduction in the number of rejected bone and amniotic membrane donors.

P-14

CT SCAN IMAGING AS A TOOL IN TISSUE DONOR SCREENING PROCESS

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Abstract:

The Victorian Institute of Forensic Medicine is a statutory body established to provide the Victorian Coroner with further information regarding cause of death of individuals deceased within the state of Victoria. Along with the State Coroner's Office, the Institute (incorporating the Donor Tissue Bank) is one of the occupants of the Coronial Services Centre, located in Southbank, Melbourne, Australia. In excess of 4000 cases are admitted to the VIFM Mortuary facility every year. As part of the admission protocol, bodies will be photographed and submitted to a full body CT-scan. The circumstances of death provided by Police, the findings of the physical external examination and the admission images, in particular CT scan, will be used by VIFM forensic pathologists to inform the Coroner who will direct the further steps of the investigation of the cause of death. The specific information provided by the CT scan imaging quite often will influence in the need or not to proceed with a full autopsy. The privileged access to human tissue for transplantation led the VIFM to establish the Donor Tissue Bank of Victoria (DTBV) in 1989. The DTBV rely its multi banking operation (skin, muscle skeletal and cardiac tissue) on an "all in house" capacity including a team of Tissue Donor Coordinators. The DTBV benefits from the unique relationship with the Coroner's Court of Victoria which allows for the early access to all referred cases to the Victorian Coroner as much as to the information collected by VIFM. The DTBV Tissue Coordinators will evaluate all cases within hours of admission for their potential as to tissue donation. Access to the CT scanned images has provided an increased capacity for the Tissue Donor Coordinators to establish in potential donors:

- Extent of chest trauma and cardiac tissue compromise, in particular after direct trauma (MVA) and post cardiac resuscitation.
- Extent of and precise anatomy of clinically identified bone trauma.
- Correlation between identified scars on physical examination and underlying soft and hard tissue involvement.
- Measurement of tissues to match customized graft requests (e.g. meniscus and tendons).
- Measurement of cardiac valves.

Access to such information has critically contributed to the outcomes of tissue donation coordination. Firstly, it has avoided approaching donor families where preliminary findings in the CT scan preclude donation. Secondly, being able to evaluate the status of soft tissues in closed trauma has provided increased opportunities to request tissues in cases overlooked otherwise. Finally, there is the possibility to cross-match specific tissue requests and progress with donation request. Provided cases, and images, illustrate the contribution of CT scan imaging in decision making within diverse scenarios and the further potential in tissue donation.

CARDIOVASCULAR

P-15

IN VITRO SUSCEPTIBILITY OF HIGH VIRULENCE MICROORGANISMS ISOLATED IN HEART VALVE BANKING

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Abstract:

Heart valves are not retrieved under conditions where sterility can be absolutely guaranteed. In this sense, measures such as antibiotic incubation are normally taken to eliminate microbial contamination. The aim of this study has been an evaluation of in vitro susceptibility of high virulence microorganisms, isolated in our Heart Valve Banking on basis to monitor its

susceptibility pattern as well as a basis to improve further antibiotics mixtures. The study included hearts retrieved from human donors who had been received in the Tissue Establishment of Córdoba (Spain) since 1996 to 2009. A total of four samples of each valve were obtained for microbiological analysis Sample 1. After dissection and previously to antibiotic immersion, a small tissue fragment (100 – 200 mg) of the subvalvular myocardium was obtained for microbiological analysis. Sample 2. After antibiotic disinfection 30 ml sample of cocktail antibiotic was drawn with a syringe and distributed for analysis . Sample 3. Of each heart valve that was approved as suitable for further processing, another small tissue fragment (100 – 200 mg) was obtained after incubation period. Sample 4. After putting the graft and the cryoprotective medium in a freezing bag, a remaining volume of 20 ml cryoprotective medium was used for microbiologic examination. Microbiological test were carried out under aseptic conditions. Samples were incubated in fluid thioglycollate medium, trypticase soy broth and sabouraud dextrose chloramphenicol agar. Incubation was establish for not less 14 days. Cultures were observed several times during incubation period. In vitro susceptibility to antibiotics were performed using WIDER automatic system (Soria Melguizo, Spain) under susceptibility and resistance criteria recommended by the MENSURA Spanish group. Between 1996 and 2009 a total of 849 valves were processed in the Tissue Establishment of Cordoba (Spain). A total of 92 positive cultures were obtained after microbiological analysis, 56.2% due to presence of low virulence microorganisms and the rest of 43.8% due to the presence of high virulence microorganisms. Aminoglicosides, such as Amikacin, Gentamicin and Tobramycin, in addition to Quinolone (Ciprofloxacin) and Imipene were more efficient drugs against gram negatives isolates. On the other hand Vancomycin remains as a effective antibiotic against Gram positives. Amoxicillin / Clavulanic and Teicoplanin could be alternatives to be used. On basis to these data, high virulence microorganisms isolates in Heart Valve Banking shown a pattern of susceptibility similar to those shown in other clinical circumstances and the most commonly used antibiotics regimes are useful to date. One antibiotic mixture with an aminoglicoside in addition to ciprofloxacin and vancomycin is efficient to be used. Further studies will be necessary to monitoring patterns changes in vitro susceptibility of microbiological isolates in tissue banking.

P-16

SECURING DONOR SAFETY AND OPTIMIZING RECIPIENT OUTCOMES IN LIVING DONOR LIVER TRANSPLANTATION: SYSTEMATIC SELECTION OF GRAFT TYPES AND USE OF CRYO-PRESERVED VEIN GRAFT

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Abstract / Introduction:

Donor safety is the most important factor in living donor organ transplantation. In living donor liver transplantation, preservation of functional hepatic mass in the donor is crucial for securing donor safety. Handling of the middle hepatic vein [MHV], a large outflow route for both the right and left liver, has been the key issue in donor hepatectomy. In case in which left liver graft may not provide sufficient graft volume, a right liver graft becomes an option. However, resection of the right liver with the MHV may result in significant congestion of the remnant left lobe in the donor. In our adult to adult living donor liver program, we have continued to apply systematic selection of graft types and use of cryopreserved vein graft for outflow reconstruction of the middle hepatic vein [MHV]. In the current study, we evaluate the efficiency of our approach.

Method:

Outcomes of one hundred consecutive living donor liver transplantation cases were studied. Donor complication was assessed with Clavien system.

Results:

Among the 100 donors, right liver graft was procured in 67 cases (67%), and left liver graft in 33 cases (33%). Left liver graft with the caudate lobe was selected in 24 cases (73%). In the right liver graft group, 9(14%) were with the MHV, and

54(86%) were without the MHV. No grade IV or mortality was observed. Overall, complications were observed in 89%, 52%, and 57% of the donors who were retrieved right liver graft with the MHV, right liver graft without the MHV and Left liver graft with the caudate lobe, respectively. The severity of complications were, Grade I-II;78%, 39%, 44%, Grade IIIa;11%, 13%, 9%, and Grade IIIb;0%, 0%, 4%, respectively. No complications were reported in 11%, 48%, and 48% of each group. Five year survival of the recipients with right liver graft with the MHV, right liver graft without the MHV and Left liver graft with the caudate lobe were, 78%, 88% and 92%, respectively.

Conclusion:

Use of cryopreserved vein graft for venous outflow reconstruction allows avoidance of right liver graft with the MHV under algorithm based systematic selection of graft types. The approach may be effective in securing donor safety and optimizing recipient outcomes in living donor liver transplantation.

P-17

THE EFFECT OF FREEZING RATES ON MOISTURE CONTENT AND SURFACE TEXTURE OF FREEZE-DRIED BOVINE PERICARDIUM

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Abstract:

Bovine pericardium has been used as tissue graft application especially for soft tissue repair. The continuance of the characteristic of this tissue graft is important for a good tissue repair performance. Freeze-drying is a process used to remove water and make the tissue product relatively inactive and able to be stored at room temperature. The freeze-drying formulation is very important in controlling moisture content on tissue graft which will affect the storage duration and tissue structure after sterilization process. Freeze-drying comprises of three stages: freezing, primary drying and secondary drying. The freezing phase is considered as the most critical step in the freeze-drying process. The aim of this study was to investigate the effect of freezing rates on moisture content and surface texture of freeze-dried bovine pericardium. The bovine pericardium was trimmed into sheets after the removal of adherent fat. The pericardium was cleaned, disinfected in 0.05% sodium hypochlorite and stretched on perspex frame. The samples were subjected to either slow freezing (0.8°C/min) or fast freezing (15°C/min) until -50°C. Primary drying was conducted at -5°C and 100 mTorr and for secondary drying the temperature was increased to 25°C at the same pressure for both groups. The residual moisture of the freeze-dried samples (6 cm x 4 cm) were analyzed using moisture analyzer at a constant temperature of 100°C. The surface texture was observed by optical 3D measurement device. The average moisture content of the slow freezing samples was 3.27% compared to fast freezing samples which was 10.45%. The lower moisture content was caused by easy sublimation of larger ice crystal formed during slow freezing. Optical 3D observation revealed higher surface texture roughness on the slow freezing sample compared to fast freezing sample. These finding may be due to the lower moisture content of the samples. In addition rougher surface textures induce cells attachment. In conclusion this study showed that slow freezing rate led to low moisture content hence optimizing the storage conditions of freeze-dried bovine pericardium and suitable for tissue scaffold.

P-18

ANATOMICAL FREQUENT ANOMALIES IN CARDIAC VALVES. 15 YEARS OF EXPERIENCE ALVAREZ MV, ALVAREZ PV, BERTOLOTTI AM, FAVALORO RR. CARDIOVASCULAR TISSUE BANK, FAVALORO FOUNDATION, BUENOS AIRES ARGENTINA

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Abstract / Introduction:

During the heart valve cryopreservation process there are anatomical findings that determine which harvested tissue will be finally discarded. International literature provides little data on the prevalence of these anomalies.

Objective:

To analyze the number of heart valves discarded due to anatomical anomalies (AA) during the dissection stage at the Cardiovascular Tissue Bank of the Favaloro Foundation.

Material and Methods:

A retrospective analysis was made of all the hearts processed from September 1996 to April 2011. A classification was made of the causes that led to discard the valves according to the anomalies found. The anatomical findings were compared according to the type of valve: aortic (AoV) or pulmonary (PV). The AA were: bicuspid valve leaflets (Bi), >20% calcification (Cf), intimal peel (IP), hemorrhage with fibrosis (H) and fenestrations (Fe) with >1 cm diameters. The chi square method was used to compare qualitative variables.

Results:

During the period established for the study, 950 hearts were received and processed. From 1620 potential valves, 324 (20%) were discarded for different causes: 89 (27.6%) for AA and 235 (72.4%) for other causes (+ serology, + control culture, technical errors). Of the valves discarded for AA, 61 (68.6%) were AoV and 28 (31.4%) were PV. The AA according to AoV or PV were: Cf 22 (37%) vs 3 (12.5%) P=.06; Fe 10 (17%) vs 16 (56.3%) P=.007; H 3 (3%) vs 2 (6%) P=.5; IP 5 (8.6%) vs 3 (12.5%) P=.5; Bi 21 (34.3%) vs 4 (12.5%) P=.09.

Conclusions:

An accurate and thorough examination of the above mentioned AA during dissection proves that there is a higher percentage of AoV discarded for Bi or Cf, while in PV there is a prevalence of Fe. The examination of these anomalies they generate a better quality of its implants.

P-19

MECHANICAL PROPERTIES OF MITRAL ALLOGRAFTS ARE NOT REASONABLY INFLUENCED BY CRYOPRESERVATION

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Abstract / Introduction:

During last half century the heart valve surgery has been developed – recently, heart valves are repaired, if possible, or replaced. Both lines - mechanical valvular substitutes as well as biological ones underwent huge technological development.

Mechanical prostheses remain popular for their durability, while the biological (xenograft tissue or allograft tissue) valves are preferred for low thrombogenicity. Although first experiments with mitral allograft (MA) have been reported even earlier than those with aortic allografts, MA was never widely used in clinical practice. The durability of MA was very disappointing even when operated by very experienced surgeons. Many disadvantages of MA in mitral position disappear when it is implanted into tricuspid position, e.g. to low pressure system. Patients with tricuspid valve bacterial endocarditis, in particular, can theoretically benefit from MA transplantation, and that awakened our interest. We decided to evaluate methods of tricuspid valve replacement by MA in a sheep model. As a basic step we decided to use the same protocol, which our tissue bank uses for human aortic and pulmonary allografts. At experimental settings the simple tearing test performed by the surgeon proved quite reliable for determining mechanical tissue properties. Short term as well as long-term sheep experimental results proved to be promising. In aortic allografts no detectable differences were found between the mechanical behavior of the cryopreserved allograft aortic leaflets and fresh tissue. There is not much data concerning mechanical properties of MA.

Material & Methods:

A control group of 39 fresh sheep MA and a test group of 13 cryopreserved sheep MA were studied. Sterile surgical exposure for the MA harvesting was achieved via a right anterolateral thoracotomy under general, intravenous anesthesia. Afterwards the animals were put to death by intravenous administration of Thiopental (10mg/kg) and potassium chloride (20ml/kg). Their hearts were explanted, mitral valves were harvested with rims of left atrial and left ventricular muscle and with the entire subvalvular apparatus, including both papillary muscles. After harvesting, 39 fresh MA were stored in saline, at +5 to +7 oC, and were mechanically tested within 24 hours. Another 13 MAVs were processed according to the standard protocol of the Tissue Bank. They were deposited directly into the cultivation medium E 199 with the antibiotic cocktail - cefuroxime 0.2 mg/ml, (Zinacef, GlaxoWellcome) + piperacillin 0.2 mg/ml (Pipril, Lederle) + amikacin 0.1 mg/ml (Amikin, Bristol-Myers Squibb) + fluconazol 0.1 (Diflucan, Pfizer). After 24 hours storage at the temperature of 37.0 oC the valves were kept at + 5 to + 7 oC over the period of 3-5 days. Then they were transferred under sterile conditions into the cryoprotective solution (E 199 with 10% dimethylsulfoxide) in a laminar flow box, and sealed into plastic bags (Gambro Hemofreeze/Haemo bags NPBI BV DF 1200, the Netherlands) using two-layer technique. Finally, MA were programmed cooled (between temperatures of + 10 to - 60 oC at the rate of - 1 oC/min.) and than stored in liquid phase of liquid nitrogen (- 196 oC) in a separate container. Average storage time in tissue bank was 5.3month (from 3.5 to 12 month). Thirty minutes before the operation MA were removed from the container and thawed in a standard way (15 minutes in room temperature followed by 15 minutes in 37 oC water bath). In the experimental laboratory we used a similar shape of sample in all cases so we were measuring the mechanical properties of all parts of mitral valve at one time. Samples always contained the mitral annulus, part of the anterior leaflet, corresponding chordae tendineae and the postero-medial papillary muscle. For mechanical testing, the MA tissue was fixed to the traction machine jaws with mitral annulus and papillary muscle. Stepwise stress-relaxation measurements were made on all tissue samples. Zwick Roell Z050 (Zwick GmbH & Co, Germany, <http://www.zwick.de>) traction machine equipped with pneumatic grips and 200 N loading cell was used for the MA tissue testing. The specimens were fixed between the grips of the apparatus with a free length corresponding to the specimens' dimensions. The samples were stretched in steps of 1 millimeter every 5 minutes and the loading protocol consisted in six loading cycles. The time elapsed from the beginning of the step was chosen to reach approximately the steady state. The force-elongation curves were recorded. A five element generalized Maxwell model was used for the description of the relaxation behavior of the tissue. A simple Maxwell body includes a viscous element, η , and an elastic element, E , connected in series. Generalized Maxwell models consist of some simple Maxwell bodies coupled in parallel. In addition, an elastic element, EP , may be connected in parallel to them. In stress relaxation experiments, the applied stretch $e(t) = \hat{e}$ is instantaneous and constant throughout the loading cycle. Constitutive equation of the generalized Maxwell model consisting in n Maxwell bodies with parallel elastic element EP may be written as: $F(t) = \hat{e} EP + \sum_{i=1}^n \hat{e} E_i \exp(-t/ \tau_i)$, where the time constant $\tau_i = \eta_i/E_i$ represents the corresponding relaxation time for one step of stretching and η_i and E_i denote the viscous and elastic modulus of i -th Maxwell body, respectively. For five element Maxwell model with $n = 2$, the above equation is the final force-time relation with five material parameters EP, E_1, E_2, η_1 , and η_2 to be identified.

Results:

The above equations describe a single step of stretching at constant values of the parameters EP, E_1, E_2, η_1 , and η_2 . Their values are different for each relaxation step. To identify these parameters, a direct exponential fitting to the experimental data was performed using statistical software R. The fitting process was the Gauss-Newton algorithm based on the nonlinear

least-squares method. A set of material parameters was determined for each stretching step and for each specimen of the control group of fresh tissue and the test group of thawed cryopreserved MA, respectively. Coefficient of correlation R of the fitting procedure was better than 0.99 for all specimens and every loading cycle. From the total number of 52 heart valves, the control group of fresh tissue contained 39 specimens, while the test group of cryopreserved MA was represented by 13 specimens. The fitted values of material parameters were examined cycle by cycle. For each loading cycle, the outliers were carefully detected using standard techniques of exploratory data analysis and they were removed from the statistical files. For each viscoelastic parameter and each loading cycle, null hypotheses were formulated stating that the means and the variances of the two groups under consideration do not differ significantly. To test the hypotheses, t-test and F-test were carried out in R statistical package. The assumed normality of the populations was tested and confirmed before the execution of the above mentioned tests. Both statistical tests were carried out at 95% confidence level. The mean values of identified elastic moduli EP, E1, and E2 do not manifest any pronounced dominance of one group of specimens over the other. Order of magnitude of all three elastic moduli ranges from 102 to 103 N/m. The identified values of the parallel elastic modulus EP range from 0.44 ± 0.24 kN/m in the first loading cycle to 4.65 ± 1.70 kN/m in the last loading cycle for the control group and from 0.58 ± 0.19 kN/m to 3.91 ± 1.79 kN/m for the test group. In the first loading cycle, the modulus of the test group is slightly higher than that of the control group while its standard deviation remains lower. Control group modulus is then slightly higher in the next loading cycles. Serial elastic modulus E1 is lower than parallel elastic modulus EP but of the same order. For the control group, the values range from 0.29 ± 0.13 kN/m to 1.40 ± 0.43 kN/m, and for the test group from 0.36 ± 0.12 kN/m to 1.30 ± 0.56 kN/m. The values of the test group are slightly higher than the control group values up to the fourth loading cycle, almost equal in the fifth loading cycle, and finally little bit lower in the last loading cycle. The identified values of serial elastic modulus E2 are very similar to those of serial elastic coefficient E1. The identified data encompass the interval from 0.25 ± 0.12 kN/m in the first loading cycle to 1.28 ± 0.48 kN/m in the last loading cycle in the case of the control group and from 0.23 ± 0.12 kN/m to 1.18 ± 0.64 kN/m in the case of the test group. The mean values of the test group are higher than means of the control group in the second and third loading cycle. This difference, however, is less than 0.05 kN/m up to the fourth loading cycle. No significant difference ($p > 0.05$) was found for elastic parameters between the two groups of specimens, neither between the mean values, nor between the variances. Test group serial viscous modulus η_1 has higher values than that of the control group. Order of magnitude of viscous modulus η_1 lies between 104 and 105 Ns/m. It is the only viscoelastic parameter determined with such strongly pronounced dominance of one specimen group. The values range from 33.75 ± 13.36 kNs/m to 159.99 ± 48.48 kNs/m for the control group and from 44.08 ± 15.19 kNs/m to 163.30 ± 90.85 kNs/m for the test group. No significant difference ($p > 0.05$) was found between the mean values of the two groups of specimens. Significant difference in variances was observed in the third, fifth, and sixth loading cycle with p equal to 0.027, 0.042, and 0.004, respectively. Test group standard deviation is more than 1.65 higher than control group standard deviation in these three critical loading cycles. As the equality of variances is one of the assumptions of the Student's t-test, the relevance of its results in the third, fifth, and sixth loading cycle may be compromised. Nevertheless, the difference of the mean values expressed as percentage of the higher mean gives 23%, 25%, 27%, 14%, 19%, and 2%. It is obvious that the differences 27% in the third cycle and 19% in the fifth cycle fall into reasonable limits. Note only 2% difference in means in the sixth loading cycle. In this respect, the difference in means between the two groups remains negligible. Viscous modulus η_2 of the test group has lower values than that of the control group and it has values of order of magnitude 103 Ns/m. Identified values of the viscous modulus η_2 encompass the interval from 1.63 ± 0.79 kNs/m to 7.57 ± 2.49 kNs/m in the control group and from 1.74 ± 1.13 kNs/m to 5.84 ± 3.56 kNs/m in the test group. As well as in the previous cases, no significant difference ($p > 0.05$) was found between the mean values of the two groups of specimens. Significant difference ($p = 0.031$) in variances was observed in the fourth loading cycle. However, the difference between mean values in this loading cycle is practically negligible.

Discussion:

The lifelong postoperative stress on a heart valves tissue prosthesis is known to be enormous. That is why the mechanical testing of the commercial heart valve prostheses of all types became routine during the last 50 years. Special simulators were developed for mechanical as well as for biological prostheses, and precise, scientific methods of tissue mechanics and tensile strength measurements were introduced. As our experimental MA transplants into the tricuspid position on the sheep model were intended as a first step of clinical project we decided to examine allograft tissue quality objectively. In other words we wanted to know if our aortic & pulmonary allograft processing and cryopreservation method was feasible for MA as well, or if we have to develop a special MA processing protocol. For aortic and pulmonary allografts some data on mechanical properties are available. The ultrastructure and mechanics of fresh, cryopreserved, and cellular extracted porcine aortic valve

leaflets were tested. Reduction in the fracture tension and increased tissue extensibility were observed. Cellular extraction preserves matrix structure and mechanics over the physiological loading range. On the contrary, the combination of extraction and fixation may lead, according to these authors, to early degenerative failure. When the mechanics of fresh, refrigerated, and frozen porcine arterial tissue was measured and statistically compared the effects and impact of common storage protocols on tissue mechanics were revealed. Subfailure stress, ultimate stress, and Young's modulus decreased significantly in refrigerated specimens while physiological, subfailure, and ultimate failure mechanics between fresh and frozen specimens were not significantly different (Stemper et al. 2007). It was reported that cryopreservation of decellularized arteries does not affect the structure and mechanical properties of the rabbit carotid artery (Fonck et al. 2008). Exhaustive overview of the biomechanics of heart valves and their function including solid and fluid mechanics and fluid-structure interaction studies have been published (Sacks et al. 2009). This paper shows that most of the research concerned with heart valve mechanics has been conducted on aortic valves. Indeed, only a few studies deal with mitral valves and they are usually limited to leaflets with no interest to related heart structures. Surface strains were determined for anterior mitral valve leaflet (Sacks et al. 2002). Biaxial mechanical testing of porcine mitral valve leaflets revealed significant difference in material properties between the anterior and posterior leaflets (May-Newman and Yin 1995). In order to quantify the influence of the cryogenic treatment on tissue mechanics, a testing protocol was defined and viscoelastic parameters were determined for a control group of fresh tissue specimen and a test group of cryogenically processed samples. A five-element Maxwell viscoelastic model was applied to characterize the tissue's mechanical behavior. The tissue mechanics were studied in terms of forces and elongations rather than in terms of stresses and strains since the specimens' geometrical form was very complicated and irregular that would cause the difficulties in assessing of geometrical characteristics like cross-section area. The actual configuration of the experimental device did not allow us to immerse the specimen during the test into any liquid media, e.g. saline. Thus, the testing conditions are somewhat removed from the situation in the animal's body. In order to approach real conditions and to prevent dehydration of the specimen, the specimens were moistened during the test, the total testing time was reduced to 30 minutes and there was no preconditioning phase in the testing protocol. Preconditioning is recommended for stabilizing the internal structure of the tissue that should decrease variability and lead to interpretable results (Fung 1993). Freezing is considered as one type of preconditioning since it also may change the mechanical properties of soft tissue (Gao et al. 2010). According to the authors, results from the thawed liver tissue were generally consistent, interpretable and no other preconditioning was applied. The preconditioned state of the porcine aortic valve material is a function of the deformation history that has occurred before the preconditioning cycles and preconditioning without an adequate rest period between tests increases predictive errors (Carew et al. 2000). However, may introduce a loss of stiffness (Liao et al. 2009) or plastic deformation of tissue specimen. Anomalous decrease of stiffness of skin and myocardium tissue was attributed to the small number of cyclic loads and it was concluded that this behavior is a true phenomenon unique to load controlled deformations that results from the interplay of nonlinear effects and creep behavior (Giles et al. 2007). Test specimens are usually preconditioned by applying a cyclic load to reduce the viscoelastic effects (Ghaemi et al. 2009). However, the viscoelastic effects are of the utmost interest in our study as they introduce time-dependent phenomena like creep and/or relaxation. The aim of this study was to evaluate the overall viscoelastic behavior and to compare the influence of a specific treatment on the viscoelasticity of the tissue that is represented by a set of viscoelastic coefficients. We decided to omit the preconditioning phase as this allowed us to examine the inherent material properties of the tissue under consideration that are not modified by previously introduced deformations and/or stresses. There are only a few studies focused on the influence of freezing on tissue mechanical behavior. The effect of freezing on the mechanical properties of spleen tissue has been reported to be negligible (Davies et al. 2000). No significant gross histological damage was observed in frozen liver tissue samples that were loaded within the elastic regime. Histological changes due to mechanical stresses were associated with permanent plastic deformations related to structural irregularities such as the blood vessels and bile ducts (Rabin et al. 1997). Similarly, the results of the present study do not reveal any significant difference in viscoelastic properties between fresh and frozen heart tissue. In the present study, no significant difference between the control and testing group was found in mean values of determined parameters suggesting that the tissue processing and cryopreservation do not alter the tissue's structural components that play crucial role in its viscoelasticity. Tissue elasticity compensating mostly for the instantaneously applied load may be attributed to collagenous tissue, more precisely to the anterior leaflet and especially to the corresponding chordae tendineae. The viscous response corresponding to the relaxation of the specimen would be dominant mostly in muscular tissue. Significant differences observed in the variances of viscous parameters in certain loading cycles reflect a wider range of values of the test group compared to the control group. This difference cannot be attributed to the lack of a precondition phase since it would affect the control group as well. The difference may be due to variability in the geometry of the papillary muscle that contributes to the viscous response of the

specimen and, so, to variability in its cross-section bearing the mechanical load. A similar size and geometrical shape of papillary muscle of harvested MA samples cannot be guaranteed since it depends on many factors including surgeon's judgment and experience. Moreover, attaching the muscle between the grips of the testing device may considerably change the free size of the muscle tissue that is not squeezed between the jaws and that participates on the viscous response to the applied load. Together with relatively low number of specimens in testing group and different number of specimens in each group, this factor may play a role in statistical analysis. Nonetheless, the results of the present study are very encouraging as they show that the tissue processing and cryopreservation do not alter significantly the overall viscoelastic behavior and mechanical performance of the tissue.

Conclusions:

Our study shows that current allograft heart valve tissue processing and cryopreservation protocol could be applied on MA tissue as well. The mechanical properties of cryopreserved MA tissue do not differ significantly from the quality of the native mitral valve tissue in sheep model. On the basis of this experimental mechanical testing the standard allograft heart valve bank protocol will be used even for MA processing for clinical purposes. Acknowledgments: We would like to thank the laboratories of the University of West Bohemia, PILSEN, Czech Republic, namely Department of Mechanics and New Technologies Research Centre for enormous help during our experiments.

P-20

FACTORS AFFECTING THE EFFECTIVENESS OF A CARDIOVASCULAR RECOVERY TEAM

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Abstract / Introduction:

Advances in cardiac surgery, such as the use of cardiovascular homograft in paediatric surgery to repair congenital diseases, and the increase of life expectancy have caused an increase in tissue demand. At the same time, the decrease in the number of donors due to a lesser degree of traffic and industrial accidents, and lower hospital economical resources is forcing us to optimize the cardiovascular tissue recovery. Aim: The aim of this study is to assess the recovery team effectiveness analyzing tissue characteristics and its viability, and if there is any relation with medical history, cause of death and the age of the donor. Methods: The study has been carried out with 112 heart donors, recovered and processed during 2010. 73 % of the above mentioned hearts were recovered by our own retrieval team (Team A), who has been specially trained in multi tissue recovery, and the 27% left have been retrieved by organ transplantation teams (Team B). The following factors have been analyzed; tissue viability, presence or absence of aortic arch and pulmonary bifurcation and its relation with the recovery team. It has been also studied (i) types of homografts obtained (ii) the total homografts retrieved per donor, (iii) and the total homografts obtained by the different recovery teams. Other factors considered were; age, medical history (cardiovascular and neurological diseases, obesity, diabetes, and surgical history), cause of death of the donor and type of donor (non heart beating donors, exitus, or brain death donors).

Results:

It has been found out that there are no differences between recovery teams in relation to the total amount of recovered tissue per donor (mean: 1.6 allograft per each recovered heart) However, there is a difference between recovery teams in relation to the type of recovered allograft; the total amount of aortic archs and pulmonary bifurcations retrieved by Team A is higher than the one recovered by Team B; by a 19.8% of aortic archs and by a 27.2% in. pulmonary bifurcations.

Conclusions:

The study shows the importance of having a trained recovery team in multi tissue recovery. This fact highlights this is the way to get a better quality tissue that can be used for different clinical applications. A longer pulmonary artery that includes a pulmonary bifurcation, allows us during processing, to split it in a pulmonary valve and a pulmonary artery. Nowadays this is one of the best options to increase the number of tissues availability for paediatric congenital diseases surgery.

P-21

MINIMISING INFECTION & MAXIMISING POTENTIAL DURING CARDIECTOMY OF OXFORD DONORS

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Abstract:

Oxford Heart Valve bank has routinely cultured the fluid surrounding the retrieved Donor Hearts and compared this to samples of untreated tissue segments since 2004. The heart samples are consistently <5% contaminated which appears to be satisfactory, but this may be improved.

There are many factors that may minimise the risk of infection. Over the last three years, Oxford has retrieved more hearts using its own dedicated team with strict pathology-based procedures. The majority of the other hearts are removed by a variety of UK organ teams using a range of different surgical procedures. Despite this variation, there is an increased number of infected results in the samples from Tissue only donors - there are infected results from donors with increased time from death to cardiectomy (warm ischaemic time). Heart Beating donors who have had prolonged ventilation have been found to have increased incidence of positive culture results.

Environmental monitoring of the mortuary facility has been routinely performed and compared to that of the operating theatre and to that of the contaminants found in the incoming heart tissue/fluid. It might be assumed that operating theatres are cleaner than mortuaries but this was not proven by Oxford. Very similar results were recorded. Cleaning procedures in operating theatres were not consistent. A detailed air monitoring study found 1% contaminants were derived from the environment (i.e. moulds), 80% from the donor's bodies (i.e. alpha haem strep, e.coli, enterococci) and 19% derived from either environment of donor (i.e. staph aureus, pseudomonas).

Thus the environment had little effect upon the infection risk of the tissue.

The organ teams open the abdomen from xiphisternum to symphysis pubis using a scalpel and diathermy. Anterior chest wall is then opened using a Gigli saw and chest retractors. Organs are identified, labelled and warm-phase dissection completed. Vessels are then cannulated and cold perfusion started just above or below the diaphragm. At this stage, some of the organ teams move quickly to exsanguinate and often cut into the ventricles to drain blood quickly. After all other organs are retrieved, the organ teams use a variety of methods to dissect out the heart for valves. Extra vessels for transplantation of liver and pancreas may have already been taken (iliacs, aortic arch). The Oxford heart valve bank team uses a midline or y shaped incision to gain access to the pericardium and then removes the heart by blunt, blind dissection, the process taking 30 minutes from start to finish (including suturing incision).

The bifurcation of the Pulmonary artery is the most commonly requested tissue in the UK currently. The Oxford heart valve bank team successfully retrieved bifurcated pulmonary valves in every cardiectomy they performed in 2011, however the Organ teams cut 35% of pulmonary donor valves too short. A significant difference was also found between the performance of heart beating and non heart beating organ donor teams – less bifurcated pulmonary valves were retrieved by heart beating donor teams. This may be related to length of overall procedure and whether lungs removed for transplant or not. The apex of the heart is removed when the heart valve bank team performs the cardiectomy. This is to facilitate washing of the interior of the heart before and during transport to the tissue banks. It also enables a pathologist to keep it for testing if required or it can be returned to the donors' body. The Organ teams don't remove the apex of the hearts but they do appear to wash the hearts in some way because they usually arrive at the tissue bank in good condition. There doesn't seem to be a noticeable connection between positive culture and apex removal.

The Oxford incoming heart tissue and fluid studies have shown that most infections are derived from the bowel of the donor's body and not external contaminants. Any movement would thus accelerate the proliferation of bacteria. The Heart Valve Bank team will always ensure that the movement of the body is minimised until after cardiectomy (e.g. heart valves will always be first tissue to be retrieved). Organ teams may have to move the body and the heart whilst dissecting and removing the other organs. No significant difference has yet been observed. Donor's who have suffered a significant road traffic collision have, however, been shown to have a significant incoming positive results.

The evidence regarding movement of a donor's body increasing risk of infection would therefore not appear to support the movement of bodies to a central clean facility.

The Heart Valve Bank team have made a record of the incoming requests and compared this to outgoing distributed tissue, the tissue in stock and the waiting list. This data analysis has been used to update cardiectomy practice. Oxford have thus recently decided to increase the minimum age limit for baby donors to reflect the current UK demand. There is a surplus of mid size aortic valves in supply and so Oxford is choosing not to keep all aortic valves if, at cardiectomy, they appear to be substandard and mid size. Instead of processing them in the tissue bank where they are unlikely to be used, the decision has been made to leave them in the donor's body.

Organ teams do not usually retrieve organs from baby donors less than two years of age. Oxford Heart Valve Bank however, is referred baby donors from various areas around the UK. Many babies are transferred prior to death to a hospice. Cardiectomy is usually performed in a cold room in the hospice. One of the main risks with baby donors is getting a good quality and sufficient blood sample for testing. A maternal sample has always to be taken and a repeat at one hundred and eighty days. As a result, these baby donor valves (which are very much in demand) are not discarded due to lack of a blood sample.

In conclusion, there are many ways to try to minimise infection which have been investigated in Oxford, some of which are already having an effect. The Heart Valve Bank team and the Oxford Organ teams are working very closely together to set up a standard procedure for cardiectomy for UK Organ teams which will increase the number of bifurcated pulmonary valves in future.

P-22

RECIPIENT OUTCOME STUDIES IN OXFORD

DAVIES, J.; CHARLESWORTH, K.; FOSTER, R.; THOMAS, Y.; AUCKBURALLY, F.; RATNTUNGA, C., *Oxford Heart Valve Bank. UK.*

Abstract:

Routine assessment of clinical efficacy by evaluation of surrogate markers is common but the risk is that the markers chosen may be misleading. Variations exist in Cardiovascular Tissue processing methods which may be due to designs to maximise viability of the tissue. Quality assessment however, is most pertinent if it evaluates a property of the tissue which is a proven key performance indicator. Viable cells may not be required for long term tissue function. Recipient outcome studies are direct evaluation methods and measure patient mortality or morbidity which may be more relevant markers. However, these studies are time consuming and costly. There are no Tissue Banking Association guidance for recipient follow up studies however many lessons have been learnt with respect to follow up studies of recipients of bioprosthetic & prosthetic valves. Currently in the UK, there is a clinical database – Central Cardiac Audit Database (CCAD) to which surgeons submit data following each operation. Reports are issued every two – three years and remain anonymised. There is also an administrative dataset or coding system – Hospital Episode Statistics (HES). However, volunteered data sometimes over estimates survival by 20% thus it is has become important in UK to validate data by link to National statistics (e.g. to NHS number). There are lessons to be learnt from UK studies when analysing cardiovascular tissue data. To minimise the effect of varying case mix when comparing recipient outcomes in different centres, benchmark procedures should be used. One year survival is a better performance indicator than peri-operative mortality. Freedom from re-intervention is a less crude indicator than patient survival. This paper summarises different follow up studies performed in Oxford. 1704 individual tissues despatched by Oxford were followed up with written surgeon surveys. 1180 surveys were returned. Surgeons were verbally interviewed

about remaining tissues. 0% post operative infections were reported. A more detailed retrospective study of mortality and morbidity of 77 patients in one surgical centre have been followed up for 11 years. Oxford distributed 214 tissues in 2010. Detailed prospective outcome study forms have been sent out to each surgeon to complete relevant data such as NHYA grades and echocardiography results. Oxford has started a valve registry for comparison of all types of valves at yearly intervals and also a valve explant study both of which include cardiovascular tissue valves/patches. These follow up outcome studies may collectively be a more accurate marker of the Oxford tissue quality.

REPRODUCTIVE

P-23

OVARIAN TISSUE BANKING - THE UPPER AUSTRIAN NETWORK FERTISAVE

HENNERBICHLER, S., *Red Cross Blood Transfusion Service of Upper Austria, Linz/Austrian Cluster for Tissue Regeneration. Austria*

Abstract:

Kinderwunschlinik Wels, Austria (Loimer L., Swoboda M.) • General Hospital Linz, Austria (Oppelt P., Krause S.) • Krankenhaus Barmherzige Schwestern Linz, Austria (Stummvoll W., Costamoling W.) • Klinikum Wels-Grieskirchen, Austria (Reisenberger K., Teiche P.) • Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria (Gabriel C., Peterbauer-Scherb A., Hennerbichler S.) Radiation or chemotherapy of oncological diseases often causes a follicular decrease as adverse reaction, which may lead to infertility and premature menopause. Therefore cryopreservation of ovarian tissue would represent a possibility for fertility protection therapy. Fertisave Upper Austria (www.fertisave.at) is a medical network with an interdisciplinary team of several Upper Austrian institutions. The aim of this network is to maintain fertility in malignant diseases. It is certified and registered within superior networks (e.g. FertiProtect) and offers ovarian tissue banking as a possible fertility protection therapy in Upper Austria. In the future also cryopreservation of testicular tissue is intended.

P-24

FREEZING OF SEMEN PEARL SAMPLES AND SPERMATOZOIDS FROM TESTICLE BIOPSIES

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Abstract / Introduction:

When the production of spermatozooids is limited or they can only be recovered from the testicles by biopsy, in these cases it is necessary to optimize the cryopreservation technique of the sample. The semen sample is frozen in small pearls to allow for several attempts of in vitro fertilization from one unique sample.

Objective:

Semen pearl samples are compared using the techniques for freezing of dry ice and liquid nitrogen.

Material and Methods:

Samples were used that were obtained from autologous semen donors (N=15) after informed consent. A recount and viability of the sperm is carried out using the Sperm Class Analyzer (SCA). After dilution of the sample with the cryoprotectant (Sperm-Cryo tm, Cryos) the sample was divided into two groups for study: Freezing was carried out on pearls directly in dry ice (group 1) or on pearls in liquid nitrogen (group 2). In the case of the pearls frozen in dry ice, small holes are hollowed out in the dry ice and by using a pipette a drop is allowed to fall from the semen suspension (15 microlites) on these same holes. After a few minutes two or three pearls are stored per cryotube. In the case of the pearls frozen in liquid nitrogen a drop is allowed to fall directly from the suspension into the liquid nitrogen. After a few minutes two or three pearls are stored per cryotube. After a month of storage in a nitrogen tank at a temperature below -150°C, the samples were thawed by immersion of the same at 37°C. After the elimination of cryoprotectant by dilution and centrifugation, count and viability of the sperm samples were carried out in SCA.

Results:

After thawing, a similar recount of spermatozoids is observed in both samples. In the samples frozen in pearls carried out with dry ice a greater number of moving spermatozoids are observed than in the pearl samples carried out using liquid nitrogen.

Conclusions:

The most effective protocol must be chosen for the cryopreservation of small semen samples that allow for the use of multiple cycles of intracytoplasmic microinjection (ICSI).

P-25

OPTIMUM CRYOPRESERVATION OF SEMEN AND SPERMATOZOIDES FROM TESTICLE BIOPSIES

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Abstract / Introduction:

Sperm cryopreservation is an ancient method of preserving male fertility in a way that will maintain sperm viability for a long period of time. Sperm quality and quantity are the two main factors that can have an effect on the success of sperm cryopreservation.

Objective:

Comparison between the classic technique of a slow-freezing method and preservation in liquid nitrogen vapour for the cryopreservation of normal sperm samples.

Material and Methods:

Samples from autologous donors of sperm (N=5) after informed consent were used. Count and viability of the sample were analyzed in the Sperm Class Analyzer. After dilution of the sample with the cryoprotectant (Sperm-Cryo tm, Cryos) the sample was divided into three groups for study. The samples were stored in cryotubes (samples of 0.25-0.50ml): Group 1: Programmed freezing was carried out using a freezing ramp (CM2000) with index of freezing of 3.1°C/min until -34°C and 15°C/min until -120°C or with index of freezing of 0.79°C/min until -30°C and 7.3°C until -120°C (group 2). Group

3: Freezing in vapour phase of liquid nitrogen placing the cryotubes directly on a floating platform in a polystyrene box with liquid nitrogen, avoiding the immersion. It is moved gently and after 30 minutes the cryotubes are submerged in liquid nitrogen. After a month of storage in a nitrogen tank at a temperature below -150°C , the samples were thawed by immersion of the same at 37°C . After the elimination of cryoprotectant by dilution and centrifugation, count and viability of the sperm samples were carried out in SCA.

Results:

After thawing, a similar recount of spermatozoids was observed in both samples although in the samples frozen in a controlled program a greater number of moving spermatozoids were observed than in samples frozen in vapor phase. Differences were observed as to mobility between the two curves used to carry out the programmed freezing. There being a greater mobility observed in the samples of programmed freezing of group 1.

Conclusions:

It is necessary to optimize the protocol of cryopreservation to diminish the damage caused to sperm on freezing.

P-26

SYSTEM FOR THE STORAGE AT ULTRALOW TEMPERATURES OF BIOLOGIC MATERIAL FOR IVF USE

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Abstract:

The need to have at our disposal safe storage systems that allow for the optimization of space in mechanical freezers and liquid nitrogen tanks has made it necessary for the tissue bank of the CHUAC to develop its own classification and storage system which is characterized by its low economic cost, safety and versatility. The storage system is based on polycarbonate cassettes composed of cells which allow for the introduction of different sized containers used frequently in IVF, labs and tissue bank. Polycarbonate is a material which tolerates very well ultra-low temperatures and temperature changes and whose transparency also allows for a simple visualization of the content of each cell. At the beginning designed to be used with the usual straws containing embryos or sperm, which are normally used in IVF, they can also be used for other purposes where it might be necessary to store small samples of biological material. In the case of the straws the system can be easily adapted to different lengths of straw by simply displacing the base of the cell. The system allows for the perfect handling and identification of diverse materials which are susceptible of being classified by colors and the system is protected by copyright.

P-27

CASE REPORT: THE USE OF PLATELET-RICH PLASMA IN ORTHOTOPIC CRYOPRESERVED OVARIAN TISSUE TRANSPLANTATION

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Abstract / Introduction:

Transplantation of viable cryopreserved ovarian tissue is a promising clinical option, mainly for young women, to restore fertility after gonadal dysfunction resulting from cancer therapy. We report here the case of a female patient with bilateral oophorectomy undergoing autotransplantation of viable cryopreserved ovarian tissue pre-treated with platelet-rich plasma (PRP) suspension to improve the ovarian function restoration by diminishing ischemic length period after implantation.

Material and Methods:

Female patient with previous unilateral oophorectomy underwent contralateral oophorectomy in 2001 due to dermoid cyst, when she was 20 year old. At the same surgical time, as iatrogenic menopause was going to be induced, little remanent ovarian tissue cryopreservation was performed in order to preserve her fertility. To cryopreserve the ovarian tissue, ovarian cortex was isolated from the medular and cut it up into small pieces of 2 mm thickness. Ovarian tissue was then transported in Flushing medium at 4°C (specific medium for the conservation of oocytes that contains heparin, human albumin solution, recombinant human insulin and gentamicin sulphate) and cryopreserved with a controlled slow cooling method in the Tissues Bank. The woman has been treated with hormonal substitute treatment until the age of 30, when the patient undergoes laparoscopic orthotopic transplantation of the cryopreserved ovarian tissue into a bilateral peritoneal pocket in the pelvic peritoneum of the ovarian pit. Transplantation of ovarian grafts without vascular pedicle requires the establishment of a new blood supply that takes, at least, 5 days, and leads to a substantial loss of follicles. We use platelet-rich plasma (PRP) to improve the mechanisms leading to graft re-perfusion with the aim of reducing the avascular period. PRP is becoming a new application in tissue engineering and a developing area for clinicians and researchers because it is a natural source of growth factors, many of which can accelerate and promote angiogenesis.

Results:

For now this patient is being monitored with monthly estradiol and FSH determinations to evaluate how her ovarian function is being restored.

Discussion:

As a number of successful human pregnancies have been possible with viable cryopreserved ovarian tissue, the protocol described here gives a real hope to patients who need an urgent treatment when ovarian function failure occurs. Although heterotopic transplantation is less complicated to perform and allows an easier monitoring and access to the tissue when assisted reproduction techniques are underwent, orthotopic technique gives the possibility of spontaneous pregnancies and when it has been performed by experienced surgeons, intervention time is low and patient recovery is extremely satisfactory. The use of PRP is still uncertain but many good results have been obtained when used in other tissues.

MUSCULOESKELETAL

P-28

APOPTOSIS MEDIATED CELL LOSS AFTER HUMAN MENISCI CRYOPRESERVATION

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Introduction:

Removal of the meniscus leads to progressive degenerative arthritis of the knee on a long term basis, therefore meniscal allograft transplantation has been proposed as an alternative to meniscectomy. Preservation methods are required to build up operational stocks and to provide living grafts of a practical size at the right time for patients.

Methods for meniscus preservation have been published and relevant literature confirm that using standard cryopreservation, the chondrocyte survival in situ is inadequate and extremely variable and the cryoinjury mechanisms are not completely established. The aim of the present study is to further investigate possible cellular injury caused by cryopreservation by analyzing apoptosis and ultrastructural damage to menisci.

Materials and Methods:

Materials used were seven human menisci which were cryopreserved by standard method. All tissue samples were processed simultaneously for routine light microscopy, scanning and transmission electron microscopy as well apoptosis assessment by the use of ISOL method.

Results:

We observed significant differences ($p < 0.05$) between the fresh (14.60 ± 3.52) (mean \pm SD) and cryopreserved meniscus (9.22 ± 2.79) (mean \pm SD). Apoptosis using ISOL method was observed in fibrochondrocytes of fresh and cryopreserved menisci. The quantitative analysis revealed significant differences ($p < 0.05$) between fresh meniscus samples, where the percentage of apoptotic cells were $0.84 \pm 2.27\%$ (mean \pm SD) and cryopreserved meniscus samples where the percentage of apoptotic cells were $50.04 \pm 18.1\%$ (mean \pm SD).

Conclusions:

Our observation suggests that apoptosis occurs during meniscus cryopreservation. Further, the use of apoptotic inhibitors during cryopreservation could result in an increase post-thaw survival.

P-29

DEVELOPMENT AND EVALUATION OF THE NEW METHOD OF GLUCOCORTICOID OSTEOPOROSIS TREATMENT

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Abstract:

The reduction of osseous tissue mineral density while administrating corticosteroids is a serious clinical problem requiring timely diagnostics and treatment. Severe secondary and, what is more serious, glucocorticoid osteoporosis requires optimization of treatment by correcting steroid therapy. The Experimental Medicine and Biology Institute of Samara State Medical University was the first in the world to develop and suggest the technology of obtaining "allogenic hydroxyapatite" as a waste-free, ecologically safe production of implants from a biological tissue. This material is related to bionanomaterials. 60-70 % of its composition consists of the particles the size of which is less than 100 nm. It contains the whole complex of osseous tissue microelements and an organic component in addition to Ca and P. At the pre-clinical stage in vivo investigations have been carried out on white lab rats (1000 animals weighing 220 gm). Morphological, biochemical, chemical, and physical scientific methods including transmission and scanning electronic microscopy have been used to evaluate the material composition and osseous tissue metabolism. The testing of the preparation has been done for hyperglucocorticoid model. The animals were injected hydrocortizone in the dose of 40 mg/kg. In 28 days they developed the processes of osseous tissue resorption, reduction of collagen biosynthesis intensity, a marked level decrease of one of the osseous remodeling markers – protein-bound oxyproline, serum free oxyproline content increase. Histologically the preparations demonstrated resorption of Haversian (central) canals osseous cell walls, thinning and destroying of spongiosa osseous beams and the appearance of numerous osteoclasts in resorption gaps. Intramuscular injection of allogenic hydroxyapatite suspension in the dose of 40 mg/ml at the background of created osteoresorption resulted in the normalization of protein-bound and free oxyproline content. Spongy substance revealed osteoblasts, compact substance demonstrated prominent periosteum microcirculation, with well seen dilated plethoric vessels in it. We have noted osseous tissue structure restoration. Immunogenesis peripheric organ investigations did not reveal any immunogenic features in this preparation. The obtained data provide evidence of osseous tissue resorption process decrease as well as its remodeling. Such medical technology presents an effective method of secondary osteoporosis treatment.

P-30

DOES THE MENISCUS TRANSPLANT PREVENT OSTEOARTHRITIS? FUNCTIONAL OUTCOME AND RADIOGRAPHIC 5-YEAR FOLLOW-UP

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Abstract / Introduction:

Partial meniscectomy is a very common procedure for treatment of meniscal injuries. The absence meniscal promoting the progression of chondral degeneration and as a result of osteoarthritis with joint space narrowing. Meniscal transplantation has been proposed for the symptomatic relief after meniscectomy, but has not yet been established whether slowing the onset of degenerative changes.

Material:

We retrospectively reviewed meniscus transplants performed in our center. Inclusion criteria included at least 5 years follow-up and no other surgical maneuvers (on anterior cruciate ligament, osteotomy or chondral injuries). Finally we analyzed the results of 10 patients with mean age 34.5 years (21-45). The technique used is frozen at -80 ° unirradiated meniscal allograft and transplantation without bone blocks.

Results:

6 were lateral and 4 medial meniscus. In all scales compared Lysholm, Tegner and VAS and joint space was measured on a preoperative radiograph charge and 5 years, and the radiological progression as Ahlbäck criteria. We obtained a satisfaction rate of 78% at 5 years, with an improvement in all scales (average preoperative Lysholm 55 to 85 to 5 years, Tegner from 3.5 to 6 and VAS from 6.8 to 2). We do not see a significant joint space narrowing (mean preoperative of 3.09 mm and 3.01 mm at 5 years) with 2 cases of improvement of the same (cases 2 and 7).

Discussion and Conclusions:

The meniscus transplant is effective for pain control in symptomatic knee after meniscectomy, with a success rate of 60-88%. Also appears to decrease the short-term joint degeneration, but still do not know its importance in the long-term chondroprotective effect. The size and graft fixation are important prognostic factors transplant. The poor results are associated with graft irradiated limb malalignment or significant chondral degeneration. We did not find deterioration of affection compartment joint space at 5 years follow-up, but these results must be confirmed in longer term studies.

P-31

ALLOGRAFT SELECTION FOR TRANSEPIPHYSEAL TUMOR RESECTION AROUND THE KNEE USING THREE-DIMENSIONAL SURFACE REGISTRATION

APONTE-TINAO ALBERTO, L.; SCHWINT, O.; RITACCO EDUARDO, L.; FARFALLI LUIS, G.; MILANO EDGARDO, F.; BOUSLEIMAN, H.; REYES, M., *Italian Hospital of Buenos Aires. Argentina.*

Abstract:

Transepiphyseal tumor resection is a common surgical procedure in patients with malignant bone tumors. The aim of this study is to develop and validate a computerassisted method for selecting the most appropriate allograft from a cadaver bone bank. Fifty tibiae and femora were 3D reconstructed from computed tomography (CT) images. A transepiphyseal resection was applied to all of them in a virtual environment. A tool was developed and evaluated that compares each metaphyseal piece against all other bones in the data bank. This is done through a template matching process, where the template is extracted from the contralateral healthy bone of the same patient. The method was validated using surface distance metrics and statistical tests comparing it against manual methods. The developed algorithm was able to accurately detect the bone segment that best matches the patient's anatomy. The automatic method showed improvement over the manual counterpart. The proposed method also substantially reduced computation time when compared to state-of-the-art methods as well as the manual selection. Our findings suggest that the accuracy, robustness, and speed of the developed method are suitable for clinical trials and that it can be readily applied for preoperative allograft selection.

P-32

THREE-DIMENSIONAL MORPHOMETRIC ANALYSIS OF THE DISTAL FEMUR: A VALIDITY METHOD FOR ALLOGRAFT SELECTION USING A VIRTUAL BONE BANK

APONTE-TINAO ALBERTO, L.; FARFALLI LUIS, G.; RITACCO EDUARDO, L.; MILANO EDGARDO, F.; SCHWINT, O.,
Italian Hospital of Buenos Aires. Argentina

Abstract:

Tumor excision is the primary treatment of aggressive or recurrent benign bone tumors and malignant bone sarcomas. This requires a surgical resection with the potential for large residual osseous defects that could be reconstructed using fresh frozen allografts. Virtual bone banks enable the creation of databases allowing a 3D pre-surgery evaluation of such allografts, based on segmentation of DICOM-CT images. This study demonstrates the usefulness of patient specific 3D models for an accurate host-donor allograft match. We describe one way to select the best match according to size and shape. The results suggest that a robust and reliable technique has been established. Since it is difficult to plan an allograft on a distal femur deformed by the tumor, we propose to plan the surgery on the contralateral side. Our results support this limb symmetry hypothesis. The use of this measurement protocol enables accurate selection of allografts from a contralateral healthy femur 3D CT model achieving the best match possible considering the geometry of available allograft candidate femur specimens.

P-33

DEEP-FROZEN FEMORAL HEADS: THE HIGHER THE WEIGHT, THE BETTER THE VIABILITY

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Abstract:

Cancellous bone is widely used in orthopedic surgery. So, femoral heads are stored in tissue banks as a source of this kind of bone. Deep freezing at -80°C, without the use of cryoprotectant or controlled cooling rate, is a common method used for their storage. Thus, the osteoconductive and osteoinductive potential is maintained, which is important for their clinical efficiency. In addition, femoral heads are usually subject to long period of ischemia, without a nutrient solution, from surgical collection until processing. All of these conditions are widely accepted to have a significant detrimental effect on cell survival and, even more, for many authors, killing any cells that are present in the tissue. However, Heyligers and Klein-Nulend, in 2005, and Simpson et al, in 2007, described the presence of osteocyte-like phenotype cells with a low proliferative capacity in culture, in deep-frozen femoral heads. In order to explain the reason for the presence of viable cells in this frozen bone, we have used an "in vitro" culture method to assess the differentiation potential of hematopoietic precursor cells from femoral head bone marrow. Once collected, the femoral head was kept in a kapton-teflon bag. No transport solution was used during ischemia (at 4°C, maximum 12h). After 1h incubation in a disinfectant solution (vancomycin, tobramycin, co-trimoxazole and amphotericin B, all 50µg/ml in Hanks balanced salt solution) the tissue was frozen by placing it in a freezer at -80°C, and stored at this temperature. We have processed 27 femoral heads from living donors undergoing hip surgery: gender, 37% female and 63% male; pathology, 67% coxarthrosis and 33% other; mean age 67.5 years (range 51-87); mean storage 133 days (range 20-442); tissue mean weight 77.4g (range 28-125). Thawing was performed by immersion in a water bath at 37°C. Afterwards, the tissue was extracted from the bag and the bone marrow was aspirated with a syringe using heparinized (50 IU/ml) medium M199. The suspension was filtered with a 100µm pore size and centrifuged (900g 10min). The pellet was resuspended in Iscove's medium, seeded in Methocult and incubated for 14 days at 37°C, 5%CO₂

in a humidified atmosphere, to assess the growth of hematopoietic colony forming units. Colonies, defined as aggregates of more than 40 cells, were counted under an inverted microscope. A thermocouple was inserted inside the femoral head, monitoring the cooling rate during freezing. Four ramps could be distinguish, which could be appropriate for cell survival in the case of heavier tissues (1.5°C/min during the transition phase from liquid to solid). Results showed CFU-GM, CFU-GEMM and/or BFU-E growth in 63% of cases. A weight of the femoral head heavier than 79g was significantly associated to the presence of viable cells.

P-34

ALLOGENIC CARTILAGE APPLICATION IN RHINO- AND GENIOPLASTY

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Abstract:

Allogenic chondral implants application in surgery has more than centenary history. It's positive properties proved by priority researches of domestic scientists in various areas of plastic surgery. Last 5 years (2006-2011) there were 125 patients under our observation with rhinoplasty operations (112 patients) and a chin surgery (17 patients), where have been applied implants from allogenic costal cartilage. allogenic implants were used at nose defects like a saddle deformations of posttraumatic and postoperative etiology, at defects of a backrest, tip part of a nose, a nasal septum. Hypognathia was the indication allogenic chondral implants application too. This method consisted in a restore of volumes and increasing of a nose and chin support. Implants getting from the preparations of 5-7 costal received from corpses on special technology were used. (The manufacturer - laboratory of CITO Tissue bank). Short technique of application: the cartilage was taken from sterile package and 30 minutes was exhibited at the sterile capacity filled of 500,0 ml of a normal saline solution. Then on a flat plastic board from rectilinear or curvilinear sites of a cartilage (under indications) by means of scalpels N.º 10, N.º 11, N.º 15 and rasps it was made implant of the settlement sizes and the form which after antiseptic processing, was entered into nose area (under periosteum, on a surface of cartilages or in soft tissues) or a chin (under periosteum). For preventive maintenance of secondary deformations chose a costal place, reinforced a cartilage threads, or PDS plates. Implants were fixed in a place in the various ways. The remote results are studied in terms from 1 till 4th years. Positive takes at a rhinoplasty are received in 96 % of observations. Secondary deformations are noticed in 3 %, a pyesis in 1 % of observations that has demanded repeated operations. Genioplasty has led to positive takes in all observations, except one, when the patient has demanded excision of implant on subjective motivation. Allogenic chondral implants application in plastic surgery of a nose and a chin – a reliable and effective way to restore of volume and increase tissue support, allowing to receive high esthetic results.

P-35

INFLUENCE OF BISPHOSPHONATES AS A PART OF A BIOCOMPOSITE MATERIAL ON AN OSTEOGENESIS

LEKISHVILI, M., RODIONOVA, S. YUROSVA, Y., TORGASHIN, A., RYABOV, A., *Priorov Central Institute of Traumatology and Orthopaedics (CITO) Tissue bank, Moscow. Russian Federation.*

Abstract:

According to some information, application bisphosphonates, simultaneously with oppression of resorbtion reduces intensity of an osteal tissue formation. The research purpose: to estimate influence of Ibandronic acid "Bonviva" as a part of a

biocomposite material on osteogenesis process. ✓ Materials and methods: experiment is spent on 20 females of white nonlinear rats who have been parted on 2 groups. In skilled group defect of a tibial bone was filled with non demineralized lyophilized bone graft, bridged to a material containing Ibandronic acid "Bonviva" 1 mg/ml, the biocomposite material didn't contain in control group bisphosphonate. Animals deduced from experiment for 90 days. The estimation of results was spent morphologically (light microscope Zeiss Axioskop 40). Preparations painted: a hematoxylin and eosine. ✓ Intensity of an osteogenesis and character of changes in area of bone graft estimated in points: 1 point – a weak osteogenesis (the area of osteal defect is filled by a quaggy fibrous tissue and fragments of implant, the presented osteal beams without osteocytes), 2 points – a moderate osteogenesis (in a defect projection there are centers of a neogenic mature osteal tissue round bone graft or a regional osteogenesis on the basis of a cartilaginous tissue with the rests of implant), 3 points – the expressed osteogenesis (the defect area is filled by a neogenic mature osteal tissue without the rests of implant). Statistical calculations carried out under program SPSS, with a significance value $p < 0,05$. Results of research: In skilled group the osteogenesis mean score has made 1.56, in control – 1.6, results had no statistically significant differences (0.05). ✓ Conclusion. Ibandronic acid at local application as a part of a biocomposite material doesn't reduce intensity of an osteogenesis

P-36

USING DEMINERALIZED BONE GRAFTS AT RECONSTRUCTION OF NASAL SEPTUM

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Abstract:

Elimination of nasal septum deformation remains to one of the most widespread and demanded methods in surgery. Existing in rhinoplasty tissue preservation techniques are referred on restoration of integrity of its skeleton. However at the expressed deformations they aren't applicable, as thus resect extensive fragments of its skeleton, and remote fragments appear not suitable for repeated implantation. For restore of deficiency of own basic local tissues in rhinoplasty wide application have received allogenic materials. It is caused by conveniences of their application, first of all that the additional trauma isn't put to the patient in connection with a material fence. Besides, the necessary form is easily given to a material. One of the most perspective allogenic materials are demineralized bone grafts which receive by extraction of a mineral component from an osteal tissue, keeping an organic matrix. Demineralized bone matrix has such valuable qualities as elasticity, possibility of modeling and manufacturing of elastic plates of the various area and a thickness, ability to hydration by various solutions, including antibiotics are inherent in it's structure. The similar modern osteoplastic material sterilized by a stream of fast electrons by a dose of absorption 25 kGr, has been developed in CITO of N.N.Priorova tissue bank under the trading name "Perfoost". The given material is safe in respect of possibility of transfer of various diseases to the recipient, we will biologically combine, stimulates restoration of own tissues, are easily modelled in the course of surgery. Preliminary, for research of reaction of tissues on implantation and studying of inductive properties developed demineralized implants, researches on experimental animals (rabbits) by whom has been made septoplasty with replacement of tissues of a nasal septum have been made. Results of the received experimentally-morphological researches have shown possibility and expediency of using demineralized implants as a plastic material on purpose tissue organotypical restoration of a facial skeleton which the area of a nasal septum concerns. Researches of application this material in clinic at septoplasty have confirmed possibility and safety of performance of high-grade reconstruction of extensive defects of a nasal septum skeleton at its expressed deformations. It has been simultaneously shown that modern technologically difficult processes of manufacturing of bioimplants where CITO technology enters, allow to receive qualitative, safe and accessible plastic materials. Thus, the experimentally-clinical researches conducted by us have allowed to make the conclusion that use demineralized bone grafts at reconstructive surgery of the expressed deformations of a nasal septum skeleton gives the chance is high-grade to restore it resected part. At reliable bracing of a plastic material in a place of defect by means of integral, equal and sufficient plates on the size, since 6 months demineralized implants are completely replaced with own

basic tissues of the recipient that promotes not only to prevention of development postoperative complications, but also restoration of physiological functions of a nose

P-37

APPLICATION OF DEMINERALIZED XENOGENIC IMPLANTS IN ORAL SURGERY

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Abstract:

Considering a difficult situation of morally-ethical character with application of allogenic materials in out-patient practice of oral surgeons, the increasing value is got by biomaterials made of a bone of animals. Using of grinded demineralized xenogenic implants (DXI), made by original licenced technology from a cortical layer of long tubular bones of a bull became one of variants of the decision of this problem. The purpose - the analysis of work with DXI during preparation and at carrying out of dental implants surgery, and also an estimation of the remote results of using DXI at patients with bone pathology in oral cavity. The technology of their manufacturing includes some stages. The fence and a choice of a donor material is carried out in full conformity with legislation demands. Cleared of soft tissues and blood elements osteal fragments freeze and then crush in the original equipment. A following stage is degreasing of an osteal crumb by an admixture of Chloroformium and ethyl alcohol. The subsequent demineralization of a crumb is spent by weak solutions of a hydrochloric acid. Demineralized material subject to additional processing for neutralization of the rests of acid with the subsequent fast cooling to -35C. Material preservation carry out by a lyophilization. As a rule, after a lyophilization a dry material by means of a set sieves part on fraction on the size of particles, pack up and pack into double plastic packages. The technology final stage is sterilization which spend in the radiative way, influencing a stream of fast electrons a dose of absorption within 20-25 kGr. The size of the grinded chips ready to clinical use varies from 0.1 to 5 mm. In our observations the materials which size has made 0.5-2 mm have been used. At an experimental investigation phase high efficiency of the given material, including in comparison with the most widespread analogs in Russia that has made necessary its introduction in clinical practice has been confirmed. At a preclinical stage the number of patients which for surgical treatment used DXI in the form of chips included 30 patients with the blasted teeth which are subject to excision, defects of alveolar ridges (including after dental implants excision), cysts, chronic generalized periodontitis. A material admixed with a blood clot of the patient and placed in bone defect separately or round inserted earlier dental implant. The age of patients from 21 till 65 years. Inspection of patients was spent with use clinical, laboratory, radiological (including a computer tomography) methods. Postoperative terms of observation have made from 6 till 12 months. At a part of patients at surgical treatment used lyophilized collagenic membranes. Using of DXI in the form of chips with the size of particles from 0.5 to 2 mm has shown their high clinical efficiency at carrying out of surgery on preparation of alveolar ridges to dental implants insertion, and also at its immediate carrying out (including it is single-step with an tooth extraction). High degree osteoinductive and osteoconductive material potention, fast terms of formation of an osteal tissue in an implantation zone (3 to 4 monthes), absence of inflammatory reaction, and also fastness formed bone regenerare to resobtion after dental implants loading becomes perceptible. Application of the xenogenic parentage implants, made on the patented technology ("Newbone") dilates possibilities of surgeons at treatment of a various pathology of an bone tissue in an oral cavity.

P-38

OSTEOGENIC EFFECT OF TETRACYCLIN IN COMPOUND XENOGENEIC BONE IMPLANTS

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Abstract:

Since about 7 years ago, we have been using -both experimentally and clinically- an antigen-free, freeze-dried bone implant from bovine source that retains the osteoinductive properties of the bone matrix.

The current knowledge about the non-antibiotic properties of low-dose topic Tetracyclines, inhibiting bone resorption (inhibition of bone matrix metalloproteinases and osteoclastic activity as well) and stimulating osteogenesis (increase of Type I collagen expression and increase of the number and activity of osteoblasts), drove us to design a compound implant expressing both the osteoinductive properties of our antigen-free bovine bone implant and the osteogenic properties of Tetracyclines.

We therein designed a double-blind experiment in rabbits, comparing the magnitude of the osteogenic response achieved by two different orthotopic bone implants placed in critical defects at the skull: bovine bone alone and Tetracyclin-embedded bovine bone.

Results demonstrated a larger, conspicuous and systematic osteogenic response within the defects filled with Tetracyclin-embedded bovine bone. Microscopically, those defects showed a significant reduction of the mononuclear infiltrate, osteoclast number and an increased rehabilitation of osteoplasts and osteoid and woven bone formation.

Keywords:

Tetracyclines, xenotransplants, osteogenesis, bone banking.

P-39

COMPARATIVE STUDIES ON BIOMECHANICAL PROPERTIES OF IRRADIATED PORCINE TENDON

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Abstract:

Collagenous tissues are employed in many reconstructive procedures. Tendon is one of them and the mechanical contribution of tendons is determined by their mechanical properties. For reconstruction following injury, these data are necessary for selecting the appropriate graft material.

Radiation sterilization after processing and packaging has been used to ensure sterility for a variety of allografts.

In this study, forty two porcine's tendons were used and the irradiation was carried out with a ⁶⁰Co source. The biomechanical properties of the tendon were measured with two universal testing machines by uniaxial loading of longitudinal fibers of collagen.

The aim of this work was to evaluate mechanical properties of irradiated tendon in an animal model.

From fresh porcine feet were obtained the tendons. The animals were male pigs of 6 month, weighted around 150 kg. The tendons were harvested and dissected, placed in polyethylene bags of 100 µm thick and immediately preserved at -80 °C (gradual freezing). The length of each tendon was 15 cm.

The irradiation was carried out at -80 °C. Fourteen samples were irradiated at 15 kGy, fifteen samples were irradiated at 30 kGy and thirteen samples were preserved for control.

The biomechanical properties of the tendon were measured with two universal testing machines with 1000 N load. In order to do these tests, the specimen must be preconditioned by repeated cycling and must respond to the similarity law. Frozen tendons were thawed in saline solution (0,9 %) during 15 minutes at room temperature. The tensile speed in all tests was 0,25 mm/sec. Each specimen was subjected to an initial preload of 25 N, then three cycles at the same speed and finally the test itself.

Statistical assessment was performed using an analysis of variance (ANOVA) to compare the three groups.

The calibrated areas or cross section areas were measure from each tendon and were used to calculate the stress, strain and the elastic modulus of Young of the material. This cross section area was around 25 mm². The Young's Modulus calculated was around 91 ± 21 Mpa.

Biomechanical testing on animal model of irradiated fresh frozen tendon through the Young's modulus of elasticity, do not shown significant differences between irradiated and non-irradiated specimens. According to these results the gamma irradiation do not produced mechanical changes in porcine tendon irradiated up to 30 kGy in frozen condition.

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OSTEOINDUCTIVE AND OSTEOGENIC PROPERTIES OF TWO XENOGENEIC BONE MATRICES

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Abstract:

Bone implants for biologic reconstruction of the skeletal system must ideally be osteoinductive, osteoconductive and antigen-free. Different Tissue Banks produce a wide variety of different bone-derived matrices, with inherent different biologic properties. Some decades ago, xenogeneic bone was used in several human trials, with controversial results. The processing methods therein used for rendering porcine and bovine bone antigen-free, destructed the matrix inductive proteins (BMPs). Since 2003, we process bovine bone preserving natural BMPs within the implants. Lacking prionic diseases (cattle and humans), the National Tissue Bank in our Country can safely produce and distribute processed bovine bone for biologic skeletal reconstruction purposes. In addition, bovine bone does not transmit human infectious diseases (HIV, Hepatitis C, etc.), is a low-cost material and has demonstrated a satisfactory biomechanic performance. Conversely, processing method for human bone at our Tissue Bank does not seem to preserve BMPs in the implants. To compare the osteoinductive potential of bovine and human processed bone, we designed a double-blind study in mice, using two processed, xenogeneic bone matrices (human and bovine), heterotopically implanted (quadriceps muscle). Results clearly demonstrate the benefits of processed bovine bone related to its osteoinductive and osteogenic properties. Keywords: Osteoinduction, Osteogenesis, Xenogeneic bone, Tissue Banking.

P-41

COMPUTER ASSISTED NAVIGATION FOR TUMOR RESECTION AND ALLOGRAFT TRANSPLANTATION

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Abstract / Introduction:

Wide-local resection with an adequate margin is a crucial step in the management of patients with a musculoskeletal malignancy. Early results in the use of image fusion for computer-assisted bone tumor surgery seem to have accurate results in bone tumor resections. The aim of this study was report our experience in preoperative planning with image fusion, tumor resection and allograft transplantation according to the desired plane using intraoperative navigation assistance.

Methods:

Thirty patients were 3D reconstructed in a virtual platform and planned determining the osteotomy position according to oncology margins in a CT-MRI image fusion. Tumor resections and allograft transplantation were performed using a computer navigation system according to previously planned. We analyzed the technical problems (crashes), time for navigation procedure during surgery, accuracy of the registration technique and surgical margins.

Results:

In four patients (13%) the navigation was not carried out due to technical problems. In two cases the crash was secondary to software problems, and in the remaining two cases the crash was secondary to hardware problems. Of the 26 cases where the navigation was performed, the mean registration error was 0.63 mm (range 0.3-1.1). The mean time for navigation procedures during surgery was 22 minutes (range 11-37). Histological examinations of all specimens showed a clear tumor margin in all patients.

Discussion and conclusion:

Our findings suggest that preoperative planning, tumor resection and allograft transplantation guided by navigation is accurate and useful method for bone tumor surgery. In our study, the navigation could not be performed in 13% of series.

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ACHILLES ALLOGRAFT AS A NEW EXTENSOR SYSTEM OF THE KNEE

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Abstract:

AIM Acute or chronic injuries of the patellar tendon are a rare complication in knee arthroplasty. Prevalence between 0.17 to 2.5% is reported in the literature. These require surgical repair to ensure proper functionality of the arthroplasty. There are several treatment options. We present our technique with Achilles allograft for the reconstruction of the extensor mechanism of the knee and the clinical results of five cases.

Material and Methods:

All cases had a deficit in active extension with passive full extension. It is strictly necessary that the patient has full passive ROM. We performed an anterior approach with eversion of the patella and a trench at the tibial tuberosity was made in order to fix the calcaneus part of the allograft in the trench with two cannulated screws. The Achilles tendon was divided into 2 bundles. It is important to avoid the patella alta in order to keep the patella in a correct position. The lateral bundle had a transtendon quadriceps position whereas the medial bundle had a medial position. Both bundles were sutured with Ethibond 5. Knees were immobilized in extension for two months. After this period gradual rehabilitation started getting a right balance, functionality and ability to walk in all cases.

Results:

With this new technique we avoid the problems with the patella resurfacing made before or the use of patello femoral arthroplasties. Our patients have a better ROM (near full extension) than preoperative situation after one year FU. The fixation with two screws help us with the arthroplasty has a tibial stem. The deep frozen Achilles tendon is helpful for this technique.

Conclusions:

This surgical technique offers a solution to a very complex problem of great concern. Our cases and the reviewed studies provide an overview of the surgical technique and short-term follow-up. Despite the promising initial results, a long-term follow-up should be made in order to reach a conclusion.

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IMPROVEMENT PLAN FOR USE OF GRAFT FEMORAL HEAD OF LIVING DONOR

VALENCIA-GARCÍA, H.; MARÍN-AGUADO M.; TORREJÓN-DE LA CAL M.; LÓPEZ-HUALDA A.; MARTÍNEZ-MARTÍN J.; VAQUERO-MATEO J.; FAHANDEZH-SADDI H., *Hospital Universitario Fundación Alcorcón. Spain.*

Abstract / Introduction:

The use of bone graft in orthopedic surgery has increased in recent years throughout the world, especially in revision surgery and prosthetic spine surgery that requires contributions of cancellous bone. That has become the bone in the second most transplanted human tissue after blood and requires health authorities and surgeons to reduce to a minimum the risk of transmitting disease. Obtaining allogeneic bone can be a living donor or cadaveric multiorgan donations. In both cases it requires informed consent from the donor, the validation of the clinical history (presence of cancer, autoimmune diseases, blood transfusions in the past 6 months, treatment with steroids or growth hormone, radiation exposure or travel to Britain 1980 to 1996, to give an example), the negativity in serological test set by regulatory agencies and the absence of pollutants. We present a review of procurement and feasibility of femoral heads obtained from living donor in the HUFA in the last 13 years. In this study we propose a possible improvement plan for the use of femoral head obtained from living donors.

Material:

We use data for our work in retrospect of the past 13 years in the HUFA on femoral heads obtained from living donor surgeries implant primary hip arthroplasty. Were analyzed that were implanted, those that were discarded and the reason thereof and which still remain in the bone bank waiting to be used.

Results:

Of the 210 femoral heads obtained in the extraction, 42% was used (90 heads without complications during follow-up), 44% were discarded and 13% in reserve is suitable for distribution. Of the Heads discarded (94), one third of them (34 pieces, 16% of total extracted) was due to contamination, 40% (38 parts, 18% of total extracted) with systemic disease (history of cancer, serology positive) or local (3 osteonecrosis) and 23% (22 parts, 10% of total extracted) fault logistics process (absence

of donor consent, absence of any serological or microbiological tests, absence of pathology). While the number of contamination is similar to the range of 5-20% in most series, we consider that 55% of parts not usable for other reasons (60 pieces, 28% of total extracted) are an important workload and cost should try to improve.

Discussion and Conclusion:

The ideal graft should be osteoinduction, osteoconductive, osteogenic and mechanical properties if the situation demands. Faced with increasing demand, tissue banks must seek new ways to obtain, but always ensuring a secure fabric. Complications of allogeneic transplantation are non-binding, bacterial or viral disease transmission and the responsible bank should try to avoid those that depend on the selection process, storage or distribution. Sometimes the removed parts do not meet all administrative requirements, serological or microbiological and should be discarded. Our improvement plan includes a demanding patient selection, surgical technique and strict aseptic packaging to ensure safe graft. The involvement of all staff involved in the process helps reduce the "failures of logistics" and therefore the costs incurred and make reasonable economic cost of the procedure.

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AUDIT OF THE FIRST 13 YEARS OF EXISTENCE OF BONE AND TISSUE BANK OF HOSPITAL UNIVERSITARIO FUNDACIÓN ALCORCÓN

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Abstract / Introduction:

The demand for bone graft has increased especially in prosthetic revision surgery, reconstructive, rachis, oncology and sports. That has become the bone in the 2nd most transplanted human tissue after blood and requires health authorities and surgeons to reduce to a minimum the risk of transmitting diseases. Bone banks are nonprofit organizations that coordinate the selection, collection, storage and distribution of bone and osteoarticular tissue donated for transplantation human and its role is essential to provide the tissue with the maximum guarantees of quality and sterility. Periodic audits allow to optimize and improve all procedures performed in the process of tissue donation / transplantation of tissue. With this work we try to evaluate the results obtained in the first 13 years of the Bone and Osteoarticular Tissue Bank in the University Hospital Foundation Alcorcón.

Material:

Retrospective review of 13 years in the HUFA in obtaining bone allograft (living donor or cadaver), under the recommendations of the Council of Europe, the American Association of Tissue Banks and the European Association of Musculoskeletal Transplant. In all cases informed consent was sought from the donor, the validation of the clinical history (presence of cancer, autoimmune diseases, blood transfusions in the past 6 months, treatment with steroids or growth hormone, or radiation exposure trip to Britain 1980 to 1996, to give an example), the negativity in serological test set by regulatory agencies (syphilis, HBV, HCV, HIV 1 and 2, HTLV I and II and Cytomegalovirus) and the absence of contaminants in aerobic and anaerobic cultures samples obtained during the extraction.

Results:

In the first 13 years of the Bank has proceeded to obtain tissue from 140 deceased donors and 210 living donors (undergoing hip replacement). Thus shows the evolution of donors per year. It also analyzes the distribution of the number of pieces obtained (minimum 18, maximum 34, average 22), the distribution of frozen tissue implanted (total 1310 pieces), the distribution of implanted freeze-dried tissue (out of 337) and as the rate of contamination, and tissue tracking provided. We analyze the profile and indications of implant recipients through the diagnosis and cause of death of deceased donors to study their profile over the years.

Discussion and Conclusion:

The ideal graft should be osteoinductive, osteoconductive, osteogenic and mechanical properties if the situation required. Faced with increasing demand, tissue banks must seek new ways to obtain, but always ensuring a secure fabric. Complications of allogeneic transplantation are non-binding, bacterial or viral disease transmission and the responsible bank should try to avoid those that depend on the selection process, storage or distribution. Any Bank should to audit bone activity to optimize their work while guaranteeing a safe bone.

P-45

THE INFLUENCE OF SINTERING TEMPERATURE ON COMPOSITION AND SURFACE MORPHOLOGY OF BOVINE BONE

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Abstract:

The use of hydroxyapatite as bone replacement has been widely discussed. The good biocompatibility, bioactivity, high osteoinductivity and osteoconductivity, noninflammatory behaviour and nonimmunogenicity properties of hydroxyapatite make it a good choice for hard tissue repair. There are various method used to synthesize these bioceramic such as ultrasonic irradiation, radio frequency (RF), thermal plasma and micromolecules. All of these processes are complicated and biologically unsafe. Recently hydroxyapatite has been extracted by normal calcinations of some biowaste such as bovine bones, pig bones and fish bones. Therefore, the aim of this study was to analyze the effect of sintering temperature on the composition and surface morphology of bovine bone. The cancellous part from the bovine femoral head were cleaned and cut into 10 mm x 10 mm x 10 mm. The bones were sintered at 200C, 400C, 600C, 800C and 1000C using sintering furnace. The non-sintered bones were used as control group. The changes of the composition and surface morphology were analyzed at every temperature stage using Field Emission Scanning Electron Microscope (FESEM). FESEM showed non-sintered bones were covered by organic substance possibly protein and collagen. However the organic materials were completely removed at 600C making the surface appeared smooth and the pore structure remained intact. Typical EDX spectrum showed the decreased in the carbon element with the increment of the temperature. At 800C calcium and phosphate were markedly higher compared to the samples sintered at 600C. At this temperature there is a possibility of crystalline hydroxyapatite formation. In addition there was evidence of ruptured areas of the bones sintered at 800C. At this high temperature it is known that damage of pore structure may occur. The result of this study showed that the suitable sintering temperature of 800C was able to remove organic compound, maintain porous structure and form hydroxyapatite of cancellous bovine bone.

P-46

SEARCH FOR METHODS TO INCREASE THE NUMBER OF PROCUREMENTS IN A TEMPORAL BONE BANK

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Abstract / Introduction:

Tympano-ossicular allografts (TOA) provide unique reconstructive capabilities, allowing reconstruction after radical removal of cholesteatoma and other middle ear pathology. To provide TOA, the University of Antwerp Temporal Bone Bank (UATB) was established in 1988. As the number of procurements has drastically decreased over the past years following the

implementation of the EU directives (e.g. exclusion of potential donors with history of malignant disease), we wanted to search methods to increase the number of procurements.

Materials and Methods:

Data of donors and potential donors reported have been captured in an Access database. Data were entered in the database on a daily basis right after procurement or after finding a contra-indication for a potential donor. The distribution of donors per age group and per sex and the distribution of contra-indications in 2009 and 2010 were analyzed.

Results:

More than half of the procured donors are over 75 years old (72.2% in 2009 and 51.9% in 2010). This correlates to the bigger death rate in this age category. Also, there is a big difference in distribution between male and female donors. In 2009, 70.9% of all donors were female, 64.8% in 2010. This is explained by males often being rejected because of not having enough hair to allow a perfect reconstruction of the incision being made during the procurement procedure. The percentage of reported deaths that lead to a procurement is very low (6.3% in 2009 and 4.3% in 2010) and is concordant with the drop between 2006 and 2009 as shown by Van Rompaey et al (Cell and Tissue 2011). The 3 most important contra-indications in 2009 and 2010 were logistical reasons (26.6% in 2009, 43.7% in 2010), malignancies (27.2% and 23.0% respectively) and risk of imperfect reconstruction (14.4% and 14.3% respectively). The main logistical reasons are: potential donors reported too late, no one available to perform the procurement, no access to pre-mortem blood samples in some hospitals and impossibility to collect a blood sample within 24 hours post-mortem, limited time slot to perform procurement because of visiting hours for family of donor.

Conclusions:

Despite the positive impact of the omission of the upper age limit of 75 years by the Superior Health Council in August 2008, the number of procurements has drastically decreased over the past years because of new regulations (exclusion of malignancies and uncontrolled systemic infections since June 2007). The search for solutions to increase the number of procurements should in the first place be focused on a maximum reduction of logistical problems and secondly in getting more reports of potential donors. Therefore ways of reporting potential donors are being evaluated and optimized where possible and new hospitals are being contacted to get approval for on-site procurements.

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THE USE OF BONE ALLOGRAFT IN REVISION TOTAL KNEE ARTHROPLASTY

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Abstract / Introduction:

Management of bone loss is a challenging problem in revision total knee arthroplasty. Options for reconstruction include: metal augments, autografts, allografts, tumor prostheses and porous metal. The purpose of the present study was to analyze the complications after revision total knee arthroplasty with bone allograft and the integration of the allograft.

Methods:

We reported a retrospective study which included revision of total knee arthroplasty with allograft, treated in our institution between 1999 and 2010. Thirteen knees were followed by clinical evaluation and periodic radiographs at a mean of 65.23 months (range 12-125 months).

Results:

Three primaries total knee arthroplasties failed because of infection and ten had an aseptic loosening. After replacement using allograft, two cases had an aseptic loosening, and required a second replacement. No case had a septic loosening.

Bone allograft was used in all cases. In small defects we used morselized allograft (73%), and massive defects (type II-II of AORI classification) were solved with structural allograft. The allograft was preserved frozen in 86% and lyophilized in 14%. Allograft incorporation was successful in 92 % of the cases, according to the knee society criterion. There was no fracture, collapse or total resorption of the allograft.

Conclusions:

Bone loss is commonly encountered at the time of revision total knee arthroplasty. Bone allograft is a safety option, with a low rate of complications and successful results. We believe that tissue bank provides a good solution for patients requiring revision total knee arthroplasty in the setting of massive bone loss.

P-48

INFLUENCE OF DONOR TYPE IN BONE AND TISSUE GRAFTS OUTCOMES IN THE QUARANTINE PERIOD

MIETH, K.; GONZÁLEZ, J.; NAVAS, J.; SOTO, C., *Fundación Cosme y Damián. Colombia.*

Abstract:

Introduction In Colombia, there are two main ways of bone and tissue donation. We have voluntary donors from the national organ and tissue transplantation net, and donors from the national forensic institute. Grafts from the later are obtain thanks to a legal figure known as donation presumption. Donor selection criteria, grafts extraction protocols and quarantine standards are the same for both groups. We would like to determine if grafts outcomes in the quarantine period are similar.

Objective:

To determine weather or not there are differences in outcomes in the quarantine period of the grafts and donors of two different types of donation at the Fundacion Cosme y Damian bone and tissue bank in Bogota, Colombia.

Material and Methods:

We did a full review of our records in the last 5 years, from 2006 to 2010. In this retrospective cohort we divided our donors in the two above mention groups and determine in each one the following variables: Donor rejection, percentage of graft losses and loss causes (i.e.: positive cultures or positive serologic tests) For continue variables we use means and standard deviations, and for qualitative variables, proportions. We present the associations between the type of donation and the outcomes in the quarantine period after a multivariate analysis.

P-49

FUNDACIÓN COSME Y DAMIÁN: A MODEL OF A PRIVATE, NON FOR PROFIT SINGLE TISSUE BANK

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Abstract:

Our institution was open in 1990 being the first in Colombia and neighbor countries to procure bone and tendons from cadaveric donors and processing them for supplying the national requirements in Orthopedic Surgery. By that time there were not public projects for tissue banking. Our structure is based in academics and research since we are orthopedic

surgeons practicing in a private medical school facility. Our institution started the medical training in allograft's reconstructive surgery programs and participated actively in the cration of legal regulations of tissue banking in our country, following the AATB and EATB standards. Therefore our institution is entirely dedicated to musculoskeletal allografts. From 1990 to date we have procured 2700 donors, have processed 120.000 allografts and distribute 90.000 for implantation.

P-50

OSSICULAR PROSTHESES FROM BANKED BONE VIA NUMERICALLY CONTROLLED MICROMILLING

RAMELLI, M.; LISCIO, M.; CARRIAGGIO, F.; BERRETTINI, S.; DANTI, S.; MANCINI, I., *Tissuelab S.p.a. Italy.*

Abstract / Intro:

In otitis media surgery the auditory ossicles often have to be replaced. The reconstruction of the ossicular chain has been performed either by autograft or homograft ossicles or by synthetic prostheses. Starting from the late 1980s, homografts and also xenografts fell progressively out of use because of the fear of transmittable infectious disease following allotransplantation. For this reasons synthetic materials were introduced. Unfortunately the extrusion, although reduced, still represents an important clinical phenomenon, which may occur in up to 20% or more of cases in long term follow-up. The current achievements in industrial processing now potentially allow the banked bone prostheses to be produced, similar in shape and weight to hydroxyapatite prostheses, thus combining the most favourable aspects of both synthetic (reproducibility, convenience and biosafety) and biological replacement (total biocompatibility).

Materials and Methods:

Cortical bone from donations was supplied by Bone Tissue Bank of Tuscany Region (Florence Italy). The bone was processed by Tissuelab to ensure complete decellularisation and biosafety according to GMP pharmaceutical standard. Cortical blocks were preliminarily measured with digital callipers and then tightened in the lock-plate of the CNC ultraprecision micromilling machine (MDX40 – Roland) to be modelled as ossicular replacement prostheses (ORPs) through a subtractive rapid prototyping method. Technical drawings and milling parameters were uploaded by means of software (Rhinoceros 4.0 and Modela player 4, respectively). The ORPs were sterilised/virus inactivated with a 50 KGy dose of gamma rays. Samples were fixed, dehydrated with alcohols, embedded and sectioned with an ultratome and stained for histological analysis.

Results:

Banked cortical bone PORPs and TORPs could be produced via CNC micromilling with high dimensional accuracy. The PORPs weights averaged $31,2 \pm 0,6$ mg, and the TORPs averaged $69,3 \pm 0,7$ mg. From a histological point of view, the ORP cortical bone was void of cells, and its extracellular matrix compactness even appeared superior to that of the auditory ossicles.

Discussion and Conclusion:

The availability of ORPs based on homologous compact bone may represents the optimal solution to prevent extrusion in ossiculoplasty. The whole production process was shown to be potentially performed in accordance with the GMP guidelines. The PORP and TORP weights were comparable to those of the auditory ossicles. GMP processing of homologous bone includes controlled industrial treatment and advanced sterilization procedures that render such a biological risk almost null. Homologous bone ORPs may finally reduce to zero the extrusion-related complications that persist in ossiculoplasty. At moment 14 of the preformed bone ossicular prostheses have been implanted, within the framework of a clinical study approved by the ethics committee, with positive clinical results. References: 1) Berrettini, MD et al.; *Ann. Otol., Rhinol. & Laryngol.* 2011, 120 (1): 9-16 2) Alanay A. et al., *Spine J.* 2008 Sep-Oct;8(5):789-95.

P-51

NEW METHODS FOR CARTILAGE GRAFT EVALUATION: OPTICAL COHERENCE TOMOGRAPHY, POLARIZATION SENSITIVE OPTICAL COHERENCE TOMOGRAPHY AND THERMOGRAVIMETRIC ANALYSIS

MARTINHO JUNIOR, C., A.; FREITAS ZANARDI, A.; BROCARDI DIVA MACHADO, L.; SANTIN PLUMERI, S.; SOARES AUGUSTO NEVES, F.; MATHOR BEATRIZ, M., Nuclear and Energy Research Institute. Brazil.

Abstract:

Since some tissue banks have adopted ionizing radiation as a secure method to sterilize allografts, the basic question about the real effects of radiosterilization on tissue structure remains in mind of medical groups. For cartilage allografts, until now, the evaluation methods include mechanical and microscopic tests that are destructive methods. In this work we evaluate cartilage allografts modification induced by radiosterilization by three new methods. For the first time we have able to qualify and quantify cartilage collagen network without any previous preparation as occurs with microscopy, using a relative new technology of Optical Coherence Tomography (OCT) and Polarization Sensitive Optical Coherence Tomography (PS-OCT), which avoid unnecessary loss of allograft. Moreover, we have applied Thermogravimetric Analysis (TGA) to detect the direct effects on water flow inside cartilage before and after radiosterilization, providing new data to better understand the effects of ionizing radiation on collagen network of allografts. Human costal cartilages were obtained from 15 cadaveric donors aged 18 to 45 years old. Right costal cartilage was preserved in high concentration of glycerol and the left costal cartilage was deep-frozen at -70 °C. Each sample was divided in four fragments. One of them was kept as control and the other three were irradiated by a Co-60 source with doses of 15, 25 and 50 kGy. Before the tests were carried out, glycerolized samples were rehydrated in sterile saline solution and deep-frozen samples were thawed at room temperature. OCT images were obtained from OCP930SR (Thorlabs, USA) and a homemade software was used to determine the total optical attenuation coefficient. PS-OCT images were obtained from OCS 1300 device (Thorlabs, USA) with a PSOCT 1300 device coupled. Thermogravimetric curves were obtained from TGA-50 device (Shimadzu, Japan) set to warm the samples with a rate of 10 °C/min until 105 °C and the dehydration rate was calculated in the linear region of the curve. According our results, dose of 15 kGy promote crosslinking in collagen structure that cause an increase in total optical attenuation coefficient and a decrease in the dehydration rate of the samples for both glycerolized and deep-frozen cartilages. For 25 and 50 kGy, for glycerolized samples no significant differences to control group for total optical attenuation coefficient and dehydration rate were found. However, deep-frozen cartilages irradiated with 50 kGy showed significant differences to control group for both total optical attenuation coefficient and dehydration rate. PS-OCT images had demonstrated that birefringence of collagen network is kept in all samples after radiosterilization. Thus, the dose of ionizing radiation for cartilage sterilization depends of preservation method, once glycerolized cartilages can receive doses of up to 50 kGy, which is not recommended for deep-frozen cartilages. Acknowledgements: IAEA, FAPESP and CNEN.

P-52

THE USE OF THE OF SPONGY BONE IMPLANT IN THE CORRECTION OF THE IDIOPATIC SCOLIOSIS

JACAS TORNE F, M.; GARCÍA MESA, N.; ÁLVAREZ CAMBRA, R., Complejo Científico Ortopédico Internacional "Frank Pais". Cuba.

Abstract:

In the last three years (April 2009-March 2011) forty patients were operated on of Idiopathic Scoliosis using the technique protocol in our Service of Column Surgery: Intervention after the 30 grades of deformity, arthrodesis with Freeze-Dried bone spongy sterilized by means of Cobalt 60 Irradiation and Luke-Harrington Instrumentation. The bone autographs employment doesn't justify the risk-benefit mobility and discomfort for the patients. In our series the clinical results, mechanics and biological they were good, without bigger complications. The radiological signs of consolidation evident starting from the 12 weeks with good correction of deformity, The results showed good correction of the curves, ranging 60% of dorsal curves and 52% of lumbar curves.

P-53

BONE ALLOGRAFT USE FOR SALVAGE OF FAILED TREATMENT OF INTERTROCHANTERIC HIP FRACTURES

GARCÍA OLTRA, E.; ZUMBADO DÍJERES ALONSO, J.; CAMACHO CARRASCO, P.; MÉNDEZ GIL, A.; LÓPEZ ZABALA, I.; TORNERO DACASA, E.; SEGUR VILALTA, J.M. Hospital Clínic Barcelona. Spain

Abstract / Introduction:

Hip fractures in the elderly are frequent. Intertrochanteric hip fractures account for approximately half of all hip fractures in the elderly; of these, from 50% to 60% are classified as unstable. Unstable fracture patterns occur more commonly with increased age and low bone mineral density and they had been associated with comminution of posteromedial buttress which exceeds a simple lesser trochanteric fragment or subtrochanteric fracture lines. The most frequent mechanical complication in patients with unstable femoral fractures is migration of lag screw or blade through the femoral head that occurs more often in unstable fracture patterns, poor bone quality and suboptimal position of internal devices. The purpose of the current study was to evaluate the results of revision internal fixation and bone grafting for salvage of failed internal fixation of intertrochanteric hip fractures.

Patients and Methods:

Between may 2007 and november 2010 nine patients with intertrochanteric fractures that failed initial internal fixation were treated with revision internal fixation and bone grafting. There were five women and four men with an average age of 83.11 years (range, 76-95 years). All patients had acute failure fixation without extensive acetabular involvement and three of them associated with infection. Three patients had cut-in of the lag screw, five cut-out and one a cephalic screw disassembly. All patients were treated with implant removal, debridement, antibiotic prophylaxis, new intramedullary nail and bone allograft. In six cases a cylinder of structural frozen corticocancellous bone allograft was inserted to improve bone density and to reinforce the femoral head defect followed by morselized cancellous chips with an impactor and fluoroscopic control. In the other three cases freeze-dried morselized cancellous chips. Clinical and radiographic results were reviewed retrospectively.

Results:

Using this rescue technique after six months of follow-up seven patients had radiological consolidation of the fractures allowing them to perform activities of daily living. One patient had nonunion and the internal fixation was removed and the other one died because of massive pulmonary thromboembolism. At follow up five patients had no pain and three mild pain.

Discussion:

The treatment of failed internal fixation of intertrochanteric hip fractures is challenging because of fracture pattern, bone loss, bone quality and possibility of associated infection. Treatment options include prosthetic replacement and revision internal fixation. This retrospective study showed a high rate of union and functional improvement with revision internal fixation and bone allograft with few complications.

P-54

BIOMECHANICAL CHARACTERIZATION OF A PROCESSED, ANTIGEN-FREE BONE XENOIMPLANT FOR SKELETAL RECONSTRUCTION

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Abstract:

INDT, Tissue Bank, Hospital de Clínicas, Montevideo, Uruguay. Processed, antigen-free, freeze-dried, irradiated bone implants from bovine source have been used in our Country since 2003, for skeletal reconstruction purposes; both experimentally and clinically, as well. Results from their osteoconductive and osteoinductive properties have proven successful, both in animals and humans, and already published elsewhere. Bovine-derived processed bone is a safe and useful surgical tool for biologic skeletal reconstruction in our Country, where cattle and humans are free of prion diseases (Kuru, Jacob-Creutzfeldt, Fatal Familiar Insomnia, Gertsman- Straussler-Scheiner Disease). Nevertheless, bone processing methods for implant purposes could modify its biomechanical properties. Thus, we design an experiment to compare basic biomechanical properties (distraction and bending) of fresh bovine bone compared to processed, freeze-dried, irradiated bone for implants. According to previous similar biomechanical tests performed on human banked bone, we established maximum forces to be applied to bovine bone on an hydraulic press (Instron machine) for four-point flexion (bending) and for longitudinal traction. Density of bone probes was assessed for fresh and processed bone, before the essay. Results showed that fresh bone could bear double the four-point bending applied force compared to processed, freeze-dried, non-hydrated, irradiated bone. Besides, both fresh and processed bovine bone performed similar to traction forces, without failure, up to 250 kg. Biomechanic characteristics of processed bovine bone herein exposed -besides its osteoinductive and osteoconductive properties-, renders this material an interesting alternative to allogeneic and even autogeneic bone grafts for biologic skeletal reconstruction. Keywords: Biomechanics, Xenimplants, Bone Banking.

P-55

PROCESSING METHODS VALIDATION FOR PATELLAR TENDON ALLOGRAFT USING X-RAY DIFFRACTION

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Abstract:

AIM To validate, by X ray diffraction structural analysis, the most suitable processing method for patellar tendon allograft preservation, sterilized by gamma radiation -for clinical application. This research is in part being supported by IAEA Research Contract No: 15546/R0.

Materials and Methods:

Six patellar tendon allografts were harvested from six cadaveric donors, under informed consent. Three tendons were packed in a triple vacuum sealed sterile bag and stored in a -80°C freezer. Three tendons were previously glycerolated and stored at the same temperature. All of them were irradiated at 17 kGy dose, and preserved for 30 days until their defrosting. Previously defrosted and deglycerolized, segments of 1cm² surface and 2 mm thick, were obtained transverse to the longitudinal axis from each category tendon: Frozen-Irradiated (FI), and Glycerolized- Frozen -Irradiated (GFI). X Ray Diffraction was performed (Diffraction System Device: Rigaku Ultima IV) on the samples and average diffractometric profiles were obtained from each category. The planimetric surface under each obtained curve was calculated as indicator of the collagenic stroma molecular ordering. From the algebraic sum of both planimetric surface were obtained differential positive and negative values, to define the Differential Planimetric Surface (DPS). Ordering Planimetric Coefficient (OPC) was obtained from the cocient between Σ DPS positive values and Σ DPS negative values. OPC is the indicator of the relative molecular ordering between both categories: FI and GFI. The comparative statistical analysis was performed by "t" test.

Results:

The average measures for the positive planimetric algebraic sum were 8803,00 (relative values), and for the negative sum 8310,33 (relative values). The OPC resulting from the comparison between both tendon categories, FI and GFI, was 1,059 (n/s).

Comments:

The results obtained from the two different processing methods analysed, do not show significative differences related to molecular ordering modifications of the FI and GFI patellar tendon collagenic structures.

P-56

EVOLUTION OF THE ACL ALLOGRAFT IN THE ACL RECONSTRUCTION SURGERY. A 15 YEAR FOLLOW UP

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Abstract / Goal:

Retrospective evaluation of ACL reconstruction with allograft throughout a 15 years follow up. Materials: 120 patients underwent surgery for ACL reconstruction using freshly frozen allograft from 1991 up to 1999. 19 patients with a mean age of 39 years were evaluated and underwent physical examination and Radiographies (Kellgren). Tegner, Lysholm and IKDC indexes were used in the evaluation. The follow up mean was 15 years.

Results:

Out of 19 patients, all of them were restored back to their previous physical activities. Lysholm (78) and Tegner (5'2) tests overviewed satisfactory results. Kellgren mean punctuation of the injured knee was 2'5, while the healthy knee punctuated a 1'1 mean.

Discussion:

Frozen allograft has been widely used in the past. With the use of freshly frozen allograft in our series, stability of the injured knee was achieved and maintained.

Conclusion:

ACL reconstruction with allograft provides long term fulfilling results, which turns this procedure into an ensuring option to achieve ACL reconstruction.

P-57

TRANEXAMIC ACID AS A SUBSTITUTE OF APROTININ FOR CHONDROCYTE GRAFT PREPARATION

BURSIG, H.; SITEK, P.; WYSOCKA, A.; KURZAK, A.; KRÓL, D.; DYLAG, S.; NOZYNSKI, J., Tissue Bank. Poland.

Abstract:

Three dimensional scaffolds are becoming increasingly in treatment of cartilage defect. The fibrin graft is stabilized by antifibrinolytic agent - aprotinin. Since 2007 aprotinin was withdrew from clinical use because of a few serious adverse reactions observed. The aim of study was to prepare grafts based on fibrinogen and tranexamic acid as an antifibrinolytic agent and confirm that TA is a good substitute for aprotinin.

Materials and Methods:

Human chondrocytes were expanded in monolayer culture for 3 weeks. 450 mL blood was collected to produce the fibrinogen from Regional Blood Center voluntary donor. To prepare gel-like grafts, chondrocytes were mixed with fibrinogen and then with thrombin in CaCl₂. The dose of TA was 10mg/mL and the aprotinin was 5000KIU. There was used different concentration of fibrinogen to find which dose, together with TA, is sufficient to prepare stable graft. The quantity and quality methods were used to compare cells viability and ECM production. Grafts were cultivated for 4 weeks in vitro to evaluate and compare their disintegration.

Results:

The grafts were stable for the time of observation. No shrinkage of the grafts was observed. The examination showed that TA has no influence on cells viability and ECM production and that migration from the graft to the culture plate is observed

Conclusions:

Tranexamic acid can be used, instead of aprotinin, as a safe antifibrinolytic agent for chondrocyte graft preparation.

P-58

AUTOLOGOUS CHONDROCYTE CULTURE – 5 YEARS EXPERIENCE

BURSIG, H.; WYSOCKA, A.; SITEK, S.; KURZAK, A.; KEPSKI, F.; DYLAG, S.; GAZDZIK, T., *Tissue Bank. Poland.*

Abstract:

Tissue engineering has been shown to be a promising method for the treatment of articular cartilage defects. ACI is presently successfully adopted all over the world, and clinical and histopathological findings confirm the effectiveness of the method. Our Tissue Bank prepare autologous chondrocyte for orthopaedic clinics according the GMP principles, EU directives, and regulations applicable to Tissue Banks. Aim of study We performed chondrocyte culture for 120 patients for nine different hospitals.

Material and Methods:

Cartilage was taken from the patient's femoral condyle, as well as blood, for the purpose of preparing autologous serum. Chondrocyte were cultured about 3 weeks. 450 ml patient own blood was collected prior to transplantation to produce autologous fibrinogen, alternatively the allogenic fibrinogen was prepared from Regional Blood Center voluntary donors. Before surgery the chondrocyte suspension or gel-like fibrograft were prepared.

Results:

Within 5 years we prepared 120 autologous chondrocyte grafts for patients with cartilage defect: 42 in suspension, 32 in autologous fibrinogen, 14 in allogenic fibrinogen, 32 in commercial fibrin glue. The mean age of the patients was 31 years. The average defect size was 5,4 cm². The mean weight of harvest cartilage was 193 mg and the mean number of implanted cells were 2,8mln per cm² of cartilage defect. Sterility control cultured cells were performed at different time points. Histological evaluation of biopsy reveal round-shape chondrocytes and proteoglycan presence. Gene expression profile confirmed chondrogenic phenotype of implanted cells.

Conclusions:

Chondrocyte (fibro)graft preparation in our Tissue Bank is a promising method for treatment of cartilage defect. A novelty in our work is the application of autologous fibrinogen in combination with autologous chondrocytes.

P-59

DEVELOPMENT OF AN EFFICIENT METHOD TO DEMINERALISE BONE

CALAMARDO, M. A.¹, VITO, S.¹, FARIÑAS, O.¹, LUQUE, S.¹, VILARRODONA, A.¹, TABERA, J.¹, SEGUR, J. M.², TRÍAS, E.¹
1 Transplant Services Foundation - Hospital Clínic. Barcelona. Spain., 2 - Hospital Clínic. Barcelona. Spain.

Abstract / Introduction:

The objective of this study was to develop an easily reproducible method to obtain demineralised osteoinductive bone, including cancellous chips and cortical powder, obtained from cadaveric donors. A method of monitoring the demineralisation process was also established in order to obtain bone with a specific desired residual calcium level with the maximum osteoinductive activity (0.99). At the essays performed by pulse cycles (with the same conditions of temperature, stirring and volume of hydrochloric acid) we found that at 2% of residual calcium, the pH of the solution was near 0.9 and the time of demineralization was near 4 minutes. It has been corroborate the importance of the particles sizes in the demineralisation process, because our cancellous chips samples contained a wide range of particles sizes and some of our results do not correlate with the expected outcomes.

Conclusions:

We observe that monitoring the demineralisation process by the eluent pH is better than doing that with the time of demineralisation as it also depends on the initial quantity of bone material and on the initial % of calcium. In addition, we also conclude that it would be necessary to homogenize bone particles prior to demineralisation. Finally, if both demineralising methods are compared, we can determine that the best process to demineralise bone is the method of pulse cycles of acid because it provides a slower rate of demineralisation that allows us to control more efficiently the process.

P-60

DISTAL TIBIA FAILURE IS THE MOST COMMON COMPLICATION IN MASSIVE INTERCALARY MASSIVE BONE. ALLOGRAFT RECONSTRUCTION IN PEDIATRIC TUMOR

MARTANTO, T. W., EDWARD, M., Dept. of Orthopaedic Dr. Soetomo General Hospital/Airlangga University. Indonesia.

Abstract:

A Case Series Tri Wahyu Martanto 1, Mouli Edward 2, Ferdiansyah^{3,4}, Sjahjenny Mustokoweni⁵, Paulus Rahardjo⁶ Sri Andreani⁷ Rosy Setyawati⁸ Abstract

Background:

Pediatric Tumor had special characteristic in management. Ability of regeneration, thickness of periosteum were benefit for treatment in pediatric pathology. Allograft widely used for reconstruction of extremity cause of tumor, trauma and other bone defect. The Expensive of Expandable mega prosthesis and refusal of patients for amputation makes us use massive allograft as a substitute for bone tumors, but we had problem with distal part failure in tibia allograft reconstruction

Objective:

To evaluate of the result of this procedure since 2005 until 2010.

Subject and Method:

10 subject after resection of bone tumor has been done and substitute with massive allograft. The result was evaluate clinically, radiological, and Function base to the ISOLS criteria.

Result:

10 cases, they all are intercalary. Intercalary allograft evaluated according radiological ISOLS criteria, 6 patients were excellent, 1 patients was fracture and healed by external support, 1 patients was implant loosening and he was done by minor change of the implant, 2 Patient failure in incorporation in distal part of tibia allograft reconstruction. Function post reconstruction, 6 cases were excellent, and good in 1 cases, and 2 cases fair with external support.

Conclusion:

Massive allograft reconstruction can be considered as the method to prevent amputation and maintain the limb after bone resection of bone tumor, with excellent result, with carefully selective case and carefully preservative the graft. Keyword : massive allograft, Pediatric, Intercalary, resection bone tumor 1. Consultant in Pediatric Orthopaedic, Dept Of. Orthopaedic & Traumatology, Dr. Soetomo General Hospital/ School of Medicine, Airlangga University Surabaya 2. Consultant in Tumor Musculoskeletal, Dept Of. Orthopaedic & Traumatology, Dr. Soetomo General Hospital/ School of Medicine, Airlangga University, Surabaya 3. Head Section of Tumor Musculoskeletal, Dept Of. Orthopaedic & Traumatology, Dr. Soetomo General Hospital/ School of Medicine, Airlangga University, Surabaya 4. Director of Dr. Soetomo Tissue Bank & Biomaterial, Dr. Soetomo General Hospital, Surabaya 5. Consultant in Musculoskeletal Pathology, Dept Of. Pathology, Dr. Soetomo General Hospital/ School of Medicine, Airlangga University, Surabaya 6,7,8 Consultant in Musculoskeletal Radiology, Dept Of. Radiology, Dr. Soetomo General Hospital/ School of Medicine, Airlangga University.

P-61

INFECTION AND LOOSENING PROBLEM IN BONE ALLOGRAFT APPLICATIONS IN TUMOR CASES

MARTANTO, T.; EDWARD, M., Dept. of Orthopaedic Dr. Soetomo General Hospital/Airlangga University. Indonesia.

Abstract:

A Case Series Mouli Edward¹, Tri Wahyu Martanto², Ferdiansyah^{3,4}, Sjahjenny Mustokoweni⁵, Paulus Rahardjo⁶ Sri Andreani⁷ Rosy Setyawati⁸

Abstract Background:

Massive allograft reconstruction have been done in Surabaya in limb salving procedure to prevent amputation of the extremity in patient with bone defect post resection in bone tumor and to maintain the complete limb. As an un living bone allograft had risk for infection and loosening.

Objective:

To evaluate of the result of this procedure since 2007 until 2010.

Subject and Method:

12 subject after resection of bone tumor has been done and substitute with massive allograft. The result was evaluate clinically, radiological, and Function base to the ISOLS criteria.

Result:

12 cases, there are intercalary allograft (8 cases) and osteochondral allograft (4 cases). Intercalary allograft evaluated according radiological ISOLS criteria, 5 patients were excellent, 1 patients was infected, 2 cases fail in healed. Function post reconstruction, 5 cases were excellent, good in 1 cases, 2 fair in external support. Osteochondral allograft evaluated according radiological ISOLS criteria 4 patients were excellent (100%). Function post reconstruction, 4 cases were excellent for 2 years, and started loosening and broken in third year.

Conclusion.

Massive allograft reconstruction can be considered as the method to prevent amputation and maintain the limb after bone resection of bone tumor, with excellent result, with carefully selective case and carefully preservative the graft. Keyword : massive allograft, Intercalary, Osteochondral, resection , bone tumor 1. Consultant in tumor musculoskeletal, Dept Of. Orthopaedic & Traumatology, Dr. Soetomo General Hospital/ School of Medicine, Airlangga University 2. Consultant in Pediatric Orthopaedic, Dept Of. Orthopaedic & Traumatology, Dr. Soetomo General Hospital/ School of Medicine, Airlangga University 3. Head Section of tumor musculoskeletal, Dept Of. Orthopaedic & Traumatology, Dr. Soetomo General Hospital/ School of Medicine, Airlangga University 4. Director of Dr. Soetomo Tissue Bank & Biomaterial, Dr. Soetomo General Hospital, Surabaya 5. Consultant in Musculoskeletal Pathology, Dept Of. Pathology, Dr. Soetomo General Hospital/ School of Medicine, Airlangga University 6,7,8 Consultant in Musculoskeletal Radiology, Dept Of. Radiology, Dr. Soetomo General Hospital/ School of Medicine, Airlangga University.

P-62

TENDON ALLOGRAFT FOR MULTILIGAMENT KNEE INSTABILITY

MORALES, J., MORALES, J. D., POPESCU, D., SEGUR, J. M., Hospital Clínic. Barcelona. Spain.

Abstract / Introduction:

High energy trauma, like knee dislocation cause varus or valgus forced moments on the knee and produce multiligament disruption (anterior cruciate, posterior cruciate, lateral and medial sided capsuloligamentous). In multiligament injuries of the knee, several techniques for anatomic reconstruction have been used. The optimum management of these injuries remains controversial including timing, technique (repair versus reconstruction) and rehabilitation protocol. Variables to consider for repairing are: Anatomical individual variations Number of damaged structures Number of previous surgeries Image exams findings Allografts are a suitable resource for multiple ligaments reconstruction. We present 3 cases using multiple allografts for these injuries. Case 1 36 years old woman.

Accident:

Knee dislocation after doing sports with multiple ligament disruption. Picture 1 Surgical procedure: Picture 2,3 Table 1 Surgery Procedure Allograft type First PCL* + PLC* reconstruction. Laprade technique Achilles tendon + Anterior tibialis tendon Second (6 months later) ACL reconstruction Anterior tibialis tendon

Results:

Balance 0-120. No knee fails or instability complaining. Case 2 27 years old man Problem: Chronic ACL + PLC disruption. Picture 4 Surgical Procedure: Picture 5,6 Table 2 Surgery Procedure Allograft type First time ACL reconstruction Anterior tibialis tendon Second time PLC reconstruction Fanellis technique Anterior tibialis tendon Results: No knee instability complains. Patient keep on rehabilitation for muscle strengthening Case 3 42 years old man Problem: Knee dislocation after car accident. Multiple ligament disruption. Picture 6 Surgical Procedure: Picture 8,9 Table 3 Surgery Procedure Allograft type First PCL* + PLC* reconstruction. Larson technique Achilles tendon + Anterior tibialis tendon Second (4 months later) ACL reconstruction Achilles tendon Results: No knee instability complains. Returned to daily activities. Not sportive activities.

Discussion:

Allograft has proved to be a good replacement for anatomic reconstruction of damaged ligaments in several studies 1,2 Ligament reconstruction results in short and long term studies using either allografts or autografts are similar. 3,4,5 Postoperative care is mainly determined by the type of reconstruction, but essentially allograft and autografts are treated using the same protocol. 6,7 The main goal of the surgical technique is recreation of the central knee axis through anterior cruciate ligament and posterior cruciate ligament allograft reconstruction. This involves complete release and excision of scar tissue, use of the most reliable surgical technique and maintenance of postoperatively stability and functional motion with a functional brace.

Conclusion:

Allograft tendons provide a valuable alternative to host tissue in cases of multiple ligament disruption. Its low immunogenicity, low donor site morbidity, availability and biological safety make them reliable for surgery. Bibliography 1 Anatomic posterior cruciate ligament reconstruction with allograft. Stannard JP J. Knee Surg 2010 Jun 23(2):81-7 2 Surgical Technique: Medial Collateral Ligament Reconstruction Using Achilles Allograft for Combined Knee Ligament Injury. Marx RG. Clin Orthop Relat Res 2011 Jun 10 3 Anterior cruciate ligament reconstruction: a multicenter prospective cohort study evaluating 3 different grafts using same bone drilling method. Leal Blanquet J. Alentorn Geli E, Tuneu J. Clin J Sports Med 2011 Jul;21(4):294-300 4 Posterior cruciate ligament reconstruction using single-bundle patella tendon graft with tibial inlay fixation: 2- to 10-year follow-up Cooper DE, Stewart D. Am J Sports Med. 2004 Mar;32(2):346-60. 5 Comparison between hamstring autograft and free tendon Achilles allograft: minimum 2-year follow-up after anterior cruciate ligament reconstruction using EndoButton and Intrafix Noh JH, Yi SR, Song SJ, Kim SW, Kim W. Knee Surg Sports Traumatol Arthrosc. 2011 May;19(5):816-22. Epub 2011 Feb 3. 6 Rehabilitation following knee dislocation with lateral side injury: implementation of the knee symmetry model. Kinzer A, Jenkins W, Urch SE, Shelbourne KD. N Am J Sports Phys Ther. 2010 Sep;5(3):155-65. 7 Rehabilitation after multiple-ligament reconstruction of the knee. Edson CJ, Fanelli GC, Beck JD. Sports Med Arthrosc. 2011 Jun;19(2):162-6.

P-63

EFFECTS OF CHONDROPROTECTIVE AGENTS: GLUCOSAMINE, CHONDROITIN SULFATE, AND HYALURONIC ACID ON CHONDROCYTES CULTURED FOR TRANSPLANTATION

OLENDER, E.; UHRYNOWSKA-TYSZKIEWICZ, I.; KAMINSKI, A., Medical University of Warsaw; National Centre for Tissue and Cell Banking. Poland.

Abstract / Background:

In the conservative treatment of articular cartilage defects chondroprotective agents (also natural components of extracellular matrix) are administered. Autologous chondrocytes transplantation (ACI) is a modern method of surgical treatment of isolated cartilage defects. Chondrocytes are retrieved from a cartilage biopsy and propagated in culture. The success of the treatment depends on the number of chondrocytes transplanted and their ability to synthesize collagen type II, which is characteristic for healthy articular cartilage. Chondrocytes propagate poorly in culture and dedifferentiate during the culture which manifests itself in cessation of the synthesis of collagen type II and switching to the synthesis of collagen type I typical for scar tissue. In the experiment culture medium was supplemented with chondroprotective agents applied in the conservative treatment. The aim of the experiment was assessment of the effects of such supplementation on the propagation and collagen gene expression, and in practice, developing of new more effective culture media for chondrocytes cultured for ACI purposes.

Methods:

Cartilage fragments were digested enzymatically in order to isolate chondrocytes. Chondrocyte from each donor were divided into several groups and next cultured in a medium supplemented differently. Supplements used were: glucosamine, chondroitin sulfate, hyaluronic acid. Osmosity of supplemented media was assessed. Positive control was culture in medium supplemented with insulin growth factor-1 (stimulating chondrocytes to synthesize collagen type II). The results were compared with a culture in standard medium. After each passage (3) the number of chondrocytes was assessed as well as the expression of genes for collagen I and II (real time PCR).

Results:

In the experiment positive effects of the supplementation of culture media with chondroprotective agents on the proliferation and gene expression were observed.

Conclusions:

Chondroprotective agents applied in conservative treatment can be applied with positive effects as components of media for culturing of chondrocytes for transplantation.

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STRUCTURAL BONE ALLOGRAFT FOR RECONSTRUCTION OF THE ACETABULAR COMPONENT IN SEVERE HIP ARTHROPLASTY LOOSENING

FERNÁNDEZ-VALENCIA, J.; MEDRANO, C.; TORNERO, E.; TOMÁS, X.; BORI, G.; SEGUR, J.M.; GALLART, X. Hospital Clínic. Barcelona. Spain.

Abstract:

Acetabular revision of total hip replacement is challenging when bone stock is severely deficient. In these cases, bulk structural allografts can be indicated. Although it is a well-known technical option, there are few series evaluating the results in the medium and long term. The present series describes the results of this technique for a 10 years period in our institution, with a follow-up of 4.2 years (range 2-11). 13 hips in 12 patients were identified. Two patients died at 2 years-follow-up due to causes not related with the surgery. The indication was aseptic loosening in 9 cases and the second stage treatment of a septic loosening in 4 cases. The arthroplasty mean duration was 10.8 years (range 1-30). Acetabular deficiency was defined as type IIIA in 9 cases, IIIB in 2 cases and IIC in 3 cases, according to Paprosky's Classification. Radiographic analysis included a detailed study of implant migration, screw breakage and radiolucencies; 2 cases were considered as possibly loose according to the criteria by Gill et al. modified by Van der Linde. A minor resorption of the graft was observed in 4 cases. Graft integration was evaluated by CT-Scan: a complete or more than 50% of integration of the graft was observed only in 4 cases; 4 cases were integrated less than 50% and in 3 cases no integration was present. No patients required revision of the allograft for problems related to the acetabular reconstruction. The functional result at the final follow-up visit was 14.63 (range 10-18) according to Merle d'Aubigné, and 65.19 (range 41-94.9) according to Harris Hip Score. The results showed in the present series, outline structural bone allograft as a satisfactory option to treat sever acetabular deficiencies. However, an incomplete integration was the most common finding in the CT-Scan. We consider that this technique should evolve in order to increase the integration rate, to guaranty a high survival rate of the reconstruction.

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HOW OPTICAL COHERENCE TOMOGRAPHY CAN BE USED TO EVALUATE QUANTITATIVELY GRAFTS BEFORE ITS USE

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Abstract:

For a long time, in order to evaluate graft safety before its use, tissue banks have been used destructive methods, as mechanical tests and microscopy, reducing the amount of tissue available for transplanted. Optical Coherence Tomography (OCT) is a non-destructive technique that provide high resolution images in real time. Because it is a contactless technique, it is possible evaluate grafts after radiosterilization process, once the light penetrate trough plastic bags. Analogous to ultrasound, OCT measure the backscattering intensity of infrared laser rather than sound. Once backscattering intensity cannot be measured directly due to the high speed of the light, the OCT use a technique known as low coherence interferometry. Basically, in an OCT system the broadband light is coupled into a fiber-optic Michelson interferometer and is divided in two light beams by a beam splitter. One beam is directed to a reference mirror that is precisely controlled by a computer and another light beam is directed to the sample. Light backscattered by the sample and the light reflected by the mirror are recombined at the beam splitter generating an interference pattern which is collected by the detector. OCT does not only generate images and the data contained in each pixel of the digital image may be used to evaluate

quantitatively the tissue. Indeed, our group have been used the "total optical attenuation coefficient" (TOAC) to determine mathematically the modifications induced by ionizing radiation in tissues as cartilage and bones after radiosterilization. TOAC is calculated according with exponential decay of the light when it penetrates in tissue. With a homemade software we can calculate the average value of pixel for each column and a final average for all columns of the interest region selected by user. The software also perform an exponential fit using the function $f(x) = a \exp(-b x) + c$, where "a" is the amplitude of backscattering signal in the surface, "b" is the total optical attenuation coefficient inside the sample and "c" is a background level due to signal noise. Our first results shown that TOAC can be used to stipulate the mechanical tension property of cartilages, suggesting that TOAC is an indirect measure of the real state of internal structures in the tissue. Thus, OCT associated with TOAC can be very useful for tissue banks to determine the graft safety before its use in surgeries. Acknowledgements: International Atomic Energy Agency (IAEA - project RC16119), Foundation for Research Support of the State of São Paulo (FAPESP – project 2008/10437-9) and National Nuclear Energy Commission (CNEN).

P-66

ENDOGENOUS AND EXOGENOUS RESISTANCE FACTORS OF CONNECTIVE TISSUE TRANSPLANTS TO THE EXPOSURE OF THE RADIATIVE EMISSION

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Abstract:

The scientific development of the radiation sterilization technologies which on the one hand would guarantee full sterility of donor tissues and on the other hand the preservation of their biological properties is one of the most topical problems of modern transplantology and restorative surgery. Allografts made of tendons, derma, fibrous capsule of the kidney, subcutaneous fat, hyaline cartilage have different degree of resistance to the radiative effect. Changes in the structure of the allograft fibrous framework following the radiation sterilization depend first of all upon the donor tissue fibroarchitectonics. The least resistant to the radiation effect are tendon allografts, the fibroarchitectonics of which are determined by a dense pouch of collagen fibre (CF) bundles oriented strictly in one direction. High radiation resistance of derma allograft is determined by two endogenous factors, the first being a complicated spatial fibroarchitectonics of the fibrous framework in which CF bundles have a spiral tract passing from one layer into another, one and the second factor being the presence of the hyaluronic acid. The inhomogeneity of layers are characteristic for the allograft of the kidney fibrous capsule. The external dense layer is of the maximum radiation resistance since it is presented by complicatedly twisted CF whereas the internal layer fibroarchitectonics has a maximum radiation resistance since it is presented by thin isolated collagen fibre bundles. High degree of the hyaline cartilage allograft radiation resistance is determined by the heterogeneity of its structure; i.e. the prevalence of II type collagen and high concentration of sulfated glycosaminoglycans which are thought to be natural radiation protectors. The allograft structure of subcutaneous fat is connective tissue bands consisting of packed unidirectional collagen fibres which, in the isolated form, present a sufficiently radioresistant structure and fat cells containing neutral fats (triglycerides) which play a role of radioprotectors and increase radioresistance of the given allografts. Thus the allograft resistance to the radiation effect depends upon the peculiarities of their fibroarchitectonics and composition of noncollagenous components defining by us as endogenous factors of the allograft radiation resistance. There is still a number of exogenous factors that have an influence upon the allograft radiation resistance, i.e. their chemical and physical treatment. The radiation resistance of allografts, which underwent the lyophilization process, for example, substantially decreases compared with the allografts preserved in the liquid media. It is necessary to assume that the destruction process in lyophilized allografts takes place as a result of the ionizing radiation direct effect whereas the allografts preserved in liquid media are subjected to the indirect radiation effect. To exogenous radiation factors of allografts it is necessary to refer types (gamma radiation or high-speed electrons) and doses of the radiation effect. Thus allograft radioresistance is determined by a set of endogenous and exogenous factors. Endogenous factors are the so-called constant reflecting the initial structure of the donor tissue whereas exogenous factors may be modified depending upon the aims of the allograft practical use.

P-66 Bis

LABRAL TRANSPLANTATION IN HIP INSTABILITY

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Introduction:

Acetabular labrum is important to maintain hip sealing and shock absorption. There is an increasing interest in hip-joint related procedures to preserve the labrum. Labral debridement can lead to increased joint degeneration when compared to reattachment. We present a 2 year follow up case report of a sportsman with hip pain and instability secondary to subtotal labral debridement due to a traumatic rupture. It was treated by allogenic labral transplantation.

Case report:

A 27 years old amateur basket ball player was operated in 2005 due to hip pain related to sport trauma. A hip arthroscopy was carried on, an anterior extensive labrum lesion was identified and regularized. Five months later, new hip arthroscopy was carried out for partial labrectomy.

The patient could not return to sports due to mechanical symptoms. In 2006 a CAM lesion was identified and another hip arthroscopy was undertaken for femoral osteoplasty and subtotal labrectomy through indirect hip access technique. The patient was first visited at our clinic because of onsetting hip instability and pain. He was able to deal with daily live activities, but he couldn't perform heavy works or even sports activities. X Ray showed still CAM lesion with grade 0 jointspace according to Tönnis hip degenerative classification. MR showed absence of anterior acetabular labrum from 10 to 1 o'clock. In 2007, after obtaining patient consent, allogenic labral transplantation and femoral re-osteoplasty was performed. A 50 mms long labrum allograft was procured from a living donor 6 weeks before this procedure and preserved at -80° C. EATB protocol was used to manage all allografting process (standards in musculoskeletal tissue banking, EATB).

A mini open anterior approach was used. Degenerative labrum around the defect was eliminated with a 35 mm final defect in the anterolateral area. Acetabular host bed at the rim was refreshed with a round burr. Labral defect was measured and transplant was fixed with 5 high fix bone anchors. Weight bearing was protected with crutches during 3 weeks. Rotations and flexion over 80° were avoided for 6 weeks. At 3rd month flat running was allowed and high demand sports was restricted for 8 months. At 18th month follow up the patient was pain free (WOMAC score 90,6% and NAHS 95% without any hip instability. The patient was able to return to sports at an inferior competitive league (UCLA score level 10).

Discussion:

Labral debridement has been in the past the gold standard technique for labrum pathology for many years. It was assumed, that labral resection did not significantly increase pressure on acetabular surface. Published results of labral debridement demonstrated initial pain relief. However pain control and physiological labral function are compromised in the midterm. New strategies have been developed in the recent years to treat labral lesions, like sutures with bone anchors to reconstruct and preserve labral function.

In case of non reparable labral tears and subsequent labrectomy, hip instability has been reported. Such a condition has lead to look for another surgical alternatives like reconstruction with autologous Fascia Lata grafts.

Labral allogenic transplantation can be a good option to treat defects or non reparable labrum tears. It can be managed with similar routine as for meniscal transplantation in a musculoskeletal tissue bank.

P-66 Bis 2

INVETERATE SERIOUS DEFECTS OF ACHILLES TENDON. RECONSTRUCTION PROCEDURES

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Introduction and Objectives:

We see with low frequency, patients with severe lost of substance in the Achilles tendon that they can not be repaired with termino-terminal suture or basic procedures.

The origins of these lesions are repeated failures surgical treatment, misdiagnosis, or degenerative cystic tendinosis treated with multiple steroids infiltrations.

This situation produce progressive elongation of the gap and dysfunction of own Achilles tendon with affection of achilleous calcaneous plantar system

Over therapist view, we present our experience in these cases by Christensen surgical technique for defects less than 8 cms. And the cryopreserved massive tendon bone transplant from donor for cases with lost of substance with more than 8 cms.

Material and methods:

Because there are very small serious diseases, we present a number of cases operated on by us in that we have used the techniques described in the previous section.

Results:

All patients have at least a following of one year. The clinical and functional results have been optimal, valued in individual way.

Comments and Conclusions:

We think that these techniques solve the problems posed by these serious injuries. Massive Transplantation Tendon, consider it an excellent solution for very specific cases, being the world bibliography very scarce for the latter procedure.

SKIN & AMNIOTIC MEMBRANE

P-67

IN VITRO RECONSTRUCTION OF AN ARTIFICIAL BILAMINAR MULTICELLULAR SKIN

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Abstract / Background:

In recent years, the reconstruction of human skin by tissue engineering represents a clinical challenge and has offered a therapeutic alternative. Avascular engineered skin equivalents have been available for several years and used to treat wounds due to burns, non-healing ulcers and surgical excisions. These artificial skin equivalents are constituted by many types of cells and a three-dimensional structure that permits cultured cells to proliferate and to create three-dimensional tissues or tissue substitutes. The problem is that this artificial skin is the lack of blood supply, since the survival and proliferation of cells depends on an adequate supply of oxygen and nutrients and the removal of toxins. These functions are served by the vascular system, whenever is not present, the graft is rejected. We have produced a new model of endothelialized reconstructed dermis that promotes the formation of capillary-like structures. We have seeded human umbilical vein endothelial cells (HUVECs) with dermal fibroblasts and adipose derived mesenchymal stem cells (ADMSC) in a fibrin matrix. Dermal fibroblasts and ADMSC have been able to produce extracellular matrix and permit cells to proliferate and grow. ADMSC secrete significant quantities of angiogenic and antiapoptotic factors (VEGF, HGF). They are able to differentiate into endothelial cells in vitro and to maintain the optimal microenvironment to promote angiogenesis and participate in tissue repair and skin regeneration processes.

Objectives:

To describe a novel strategy to obtain vascularized skin substitutes in vitro. Methods. Vascularized artificial skin substitutes were prepared by seeding $4,5 \times 10^5$ - 1×10^6 dermal fibroblasts and ADMSC, and 2 - 3×10^6 HUVECs in a fibrin matrix along with other extracellular components secreted by the ADMSC. 1×10^6 keratinocytes were seeded on the dermis to make a complete skin. Artificial skin was maintained in culture for up to 15 days. The culture medium was changed three times a week. Afterwards, the artificial skin was collected for histological and immunohistochemical analysis.

Results:

We obtained an artificial skin with similar histological structure to normal skin, including dermis and epidermis. By immunohistochemistry, we demonstrated that endothelial cells (CD31 positive cells) grew, proliferated and organized themselves into capillary-like structures in the fibrin matrix. The epidermis showed a complete epithelization (cytokeratin positive cells) with keratohyalin granules, hyperkeratosis and parakeratosis.

Conclusions:

In this study, we have established a novel model of artificial complete skin based on the culture of keratinocytes, HUVECs, ADMSC and dermal fibroblasts. We have observed that this model has the capability of promoting a spontaneous formation of a capillary-like network. Our artificial skin model would provide a novel therapeutic approach to different pathologies and could be a useful tool for regenerative medicine.

P-68

HUMAN AMNIOTIC MEMBRANE AS SCAFFOLD FOR KERATINOCYTES AND FIBROBLAST CULTURE

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Abstract / Objective:

To determine that the amniotic membrane can be used as support for the culture of fibroblasts and keratinocytes and that this will allow for the development of a model of artificial total skin for use in patients.

Material and Methods:

The placentas are obtained from cesarean sections. The amnio was separated from the chorion and was incubated in 199 medium with antibiotic solution during 24h at 4°C and after this was cryopreserved. From 5 biopsies of skin of patients the fibroblasts and the keratinocytes were obtained after a double digestion with trypsin and collagenase, respectively. The amnio was placed with the chorionic side or with the epithelial side upwards in a Cell Crown insert so that it was immobilized. The following models were performed: chorionic side without treatment, epithelial side without treatment, and epithelial side with treatment to eliminate the epithelium. For the elimination of the epithelium, the membrane was treated with the following treatments: trypsin 1%, 0.25% (10 or 30 minutes) and EDTA 0.1% during 30 min at 37°C. The same number of fibroblasts and keratinocytes obtained for each biopsy were sowed in each model for its study. Histologic studies (hematoxylin-eosin) and immunohistochemic studies (AE1/AE3) were carried out.

Results:

A great number of fibroblasts and keratinocytes were obtained after double digestion. The fibroblasts adhere to and proliferate better on the chorionic side without treatment of the amnio and the keratinocytes on the basal side without epithelium. To remove the epithelium from the amnio the best treatment was with trypsin at 1% for 30 min. The amnio with keratinocytes and fibroblasts, showed a similar structure histologically to that of normal skin after seven days of culture.

Conclusion:

The amniotic membrane is a good support for the adhesion and expansion of fibroblasts and keratinocytes.

P-69

PRODUCTION OF RADIOSTERILIZED PIG SKIN AS BIOLOGICAL DRESSING

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Abstract:

Production of pig skin as a biological dressing in the Radiosterilized Tissue Bank at the National Institute of Nuclear Research of Mexico began in 2001. Pigs considered for skin retrieval are selected from two slaughterhouses. All pig specimens undergo veterinarian control prior to procurement, which is carried out at the slaughterhouse. In each harvest, skin from 2 pigs is collected. In the Tissue Bank, porcine xenografts are processed under controlled conditions: the subcutaneous fat is removed, the skin is shaved, washed, disinfected and obtained using an electric dermatome. The grafts are double packed, labeled and frozen by placing into a freezer or they are freeze dried during 24 hours and then double packed and labeled. Both, frozen and freeze dried xenografts are sent to the Irradiator Department of the National Institute of Nuclear Research, where the grafts are sterilized with gamma radiation. Sterilized pig skin grafts are preserved at -80 °C or under ambient

conditions. Quality control is performed in all the steps of pig skin dressings production. From 2001 to 2010, the production of pig skin dressings reached a total of 301446 cm². Pig skin xenografts have been provided by the Radiosterilized Tissue Bank to several hospitals located in different places of Mexico. The main user is the Burned Children Unit of the "Dr. Nicolás San Juan" Hospital. Processing of human skin in the Radiosterilized Tissue Bank began in 2007. Due to the number of donors is limited, the use of xenografts is an excellent alternative for many patients.

P-70

MORPHOMETRICS ANALYSIS OF IRRADIATED SKIN FROM TRANSMISSION ELECTRON MICROSCOPE IMAGES

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Abstract:

In the treatment of burns or accidental loss of skin, cadaveric skin allograft provides an alternative to cover temporarily the wounded area. The skin is a highly contaminated tissue, so is necessary to reduce it to safe level in order to avoid infection. Radiation is an excellent alternative and Skin Banks of Argentina are using this method of sterilization for around two decades and frozen irradiated skin allografts are successfully being applied in repair surgery. The aim of this work was to study, from images of transmission electron microscope, the effect of gamma rays on the ultrastructure of frozen and glycerolized human skin by measuring the thickness of the collagen fibers. Cadaveric human skin was processed for preservation by two methods. One of them, treated with glycerol 10 % was frozen at - 70 °C, and the other was glycerolized to 85 % and stored, at 4 °C. The processed skin allografts were treated with a ⁶⁰Co source, at 25,0 kGy and 50,0 kGy; non irradiated samples were kept as control. The dose of 25,0 kGy is habitually used in tissue radiation sterilization and 50 kGy was chosen in order to evaluate a maximum tolerated dose. Histological evaluation was carried out by observing the collagen structure under a transmission electron microscope, Zeiss EM 109T. The thickness of collagen fibers was analyzed by a morphometric technique. The software used for measuring the thickness was Image J 1.43u. Statistical assessment was performed using a factorial analysis of variance (Factorial ANOVA) and Tukey-Kramer Multiple- Comparison Test. Glycerolized (85%) skin samples showed no significant differences ($p=0,05$) in the thickness of collagen fibers between samples unirradiated and irradiated with 25 kGy, perhaps due to the protector effect of glycerol, a well-known scavenger of reactive species produced during the irradiation process. But differences were found between unirradiated and 50 kGy as well as between 25 and 50 kGy. The stored frozen skin samples showed differences between unirradiated and irradiated with 25 and 50 kGy, but no significant differences were found between irradiated samples with 25 kGy and with 50 kGy. In spite of morphometric differences observed between controls and irradiated frozen samples, evidences of excellent results in clinical application of irradiated frozen skin allografts have been obtained in Argentina. In the usual sterilization dose of 25 kGy no significant differences in the thickness of collagen fibers were found between both conditions, glycerolized and frozen, thus these preservation methods would be suitable.

P-71

DONOR PARAMETERS AFFECTING SKIN TISSUE RECOVERY

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Introduction:

Nowadays advances in critical care and burn resuscitation have led to the improvement in survival with large complex burns. The increase in the population of survivors among these patients has forced to widen the sources to obtain products able of covering the body surface area burned. Lack of autologous split-thickness skin graft requires the use of temporary skin substitutes, being the skin from human deceased donors a good choice.

Aim:

To establish relation between the amount of square cm of suitable recovered skin from each donor and several factors related to skin recovery process, in order to assure the quantity and quality of the skin allograft available in our tissue bank.

Methods:

This prospective and retrospective study was conducted on 344 skin donors available in Transplant Services Foundation tissue bank during the period of January 2009-June 2011.

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 18.0 for Windows software package (SPSS Inc., Chicago, Illinois, USA).

The statistical analysis involved the Spearman's correlation coefficient for continuous variables (significance at the 0.05 level) and for categorical ones the chi-squared test with significance at $p < 0.05$.

Results:

The amount of recovered tissue was influenced by some parameters such as weight, body surface area, body mass index (significance at 0.01), height, body cooling time before retrieval (significance at 0.05) recovery team leader ($p < 0.001$) and type of donor ($p < 0.05$). The best tissue procurement was achieved in donors with the greatest weight, body surface area, body mass index, height, body cooling time before retrieval and recovery team member's experience (experienced team members obtained more suitable skin than less trained ones) and was lower in brain death donors compared to exitus and non-heart- beating donors.

Conclusions:

The evidence thus obtained leads us to conclude that body surface area, body mass index, weight, and recovery team leader could be closely related to the amount of suitable removed skin allograft. Also older donors could be good skin donors. These factors must be taken into account to assure the quantity and quality of the retrieved skin.

P-72

TIME-KILL STUDIES OF BASE.128 AGAINST S. EPIDERMIDIS II, S. CAPITIS AND P. ACNES ISOLATED FROM DONOR SKIN

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Abstract:

Gatto C. and Pianigiani E. contributed equally to this work Purpose To investigate the decontamination efficacy of the BASE.128 medical device against clinical isolates of the most frequent skin allograft contaminants by in vitro time-kill study. Methods S Epidermidis II, S. Capitis and P Acnes were isolated from the skin of non heart beating (NHB) and

heart-beating (HB) donors, which was retrieved by the Skin bank of Siena. Microorganisms resistance to the antibiotics was determined by Kirby-Bauer method. Microorganisms were cryopreserved in growth broth with addition of 15% glycerol. For time-kill studies, microorganisms were cultivated under optimal growth conditions (Thioglycollate broth) at 37°C overnight and inoculum concentration was determined by Mc Farland method. BASE.128 RED and BASE (growth control) were inoculated with 10(4) CFU/ml of the selected microorganisms, each assessed in triplicate, and then incubated at +4°C for 72h and +37°C for 24h. The number of living microorganisms was determined by dilution and spread plate technique at 0h, 3h, 6h, 9h, 12h (24h, 48h, 72h only for incubation at +4°C) spreading 1 ml of three successive dilutions on a solid surface (Fastidious agar and TSA). Each sample was assessed in triplicate, and colonies were counted after 24h.

Results:

All isolated strains were resistant to one or more antibiotics, mainly to Penicillin, Gentamicin, Cefoxitin, and Oxacillin. Time-kill curves and growth curves of the three bacterial strains exhibited significantly different kinetics depending on the incubation temperature. All microorganisms showed a lag phase of approximately 6 h at +4°C, followed by a killing phase at 24h of 1.93, 1.23 and 3 log for *S. Epidermidis*, *S. Capitis* and *P. Acnes*, respectively. The killing curve of *P. Acnes* was linear and reached complete elimination (4 log killing) at 48h. The curves of the other two strains showed a plateau after 24h. The growth curves at 4°C of all strains showed an initial lag phase of 6h, followed by 0,4 - 1 log growth. A plateau was reached at 24h for *S. Epidermidis*, *S. Capitis* and at 48h for *P. Acnes*, indicating overlapping between growth and killing phases. All microorganisms showed an exponential growth at +37°C, which reached approximately 4-6 Log at 12h. After a 3h lag phase for *S. Epidermidis* and *S. Capitis* and a 6h lag phase for *P. Acnes*, killing of 2.7, 3 and 4 log was observed at 12h for *S. Epidermidis*, *S. Capitis* and *P. Acnes*, respectively. When incubated at +37°C, complete elimination of all three strains (4 log killing) was observed within 24 hours.

Conclusions

Our results showed that BASE.128 eliminated effectively all investigated microorganisms and that the decontamination efficacy varied as a function of the incubation temperature. All investigated microorganisms were completely eliminated within 24 hours, when incubated at +37°C. Only *P. Acnes* was completely eliminated after incubation at +4°C. On the contrary, complete elimination of *S. Epidermidis* II and *S. Capitis* was not obtained even after 72h of incubation. Our results suggest that an exponential growth phase is required for efficient killing with BASE.128. Incubation at 37° might be considered as a part of the decontamination procedure in order to increase the skin decontamination effect.

P-73

UV LIGHT STIMULATES CELL PROLIFERATION BY EGR-1 ACTIVATION IN HUMAN PRIMARY DERMAL FIBROBLASTS OBTAINED FROM MULTIORGAN DONORS

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Abstract / Background:

The EGR-1 protein is a finger zinc transcriptional factor with transcriptional activity on collagen type I and TGF-B gene in skin. This transcriptional activity could be induced by UV, serum, hormones and drugs through serum response elements (SRE), and CAMP response-like elements (CRE). Hereby we demonstrate conserved transcriptional regulation of Egr-1 in human dermal fibroblasts obtained from skinfoil allografts. Human cell culture: Human Primary Fibroblasts (HPF) were obtained from skingrafts procured on multiorgan donors. Samples of skin foil (0.5 cm²) were enzymatically digested isolated and cultured in standard conditions. Viral transduction efficiency was determined on HPF at 25, 50, 75, 100, 125 MOI's with an adenovector control (Ad-GFP), and using Laser Scanning Confocal Microscopy at 488 nm. Cells were starved by serum for 24 h and exposed to UV light at 254nm. Induction assay was performed in 1)FBS(+); 2)FBS(-); 3)FBS(-)/betamethasone(+). All conditions were grouped in A) UV(-) and; B). UV(+). The reporter activation was measured by luciferase activity assay at 3 and 6 hours and quantified by luminometry in a DTX50 Multilector.

Results:

Optimal MOI of Ad-Egr-1-Luc7 in HFP was 50. The optimal dose of exposition to UV for induction of promotes transcriptional activity of AdEgr-1-Luc without cell death was 120". In presence of UV light Ad-Egr-1-Luc7 (4.1X106LC), Luciferase maximum activity was observed at 6 hours (10 fold times more than Ad-CMV-Luc (4.5 X 105LC). Interestingly, the CMV activity is 2 fold times more over than Egr-1 in presence of SFB. For induction assays in the presence of betamethasone, luciferase activity shows a lag time to 3 hours.

Conclusion:

Hereby we demonstrate the functional activation of egr-1 promoter and correlates with cell proliferation, presumably induced by UV light in human primary fibroblast cultured from skin allografts. Additionally, UV light promotes cell proliferation at lower dose (15 seconds of exposure). Possible applications could be considered for preconditioning of tissues before preservation or therapeutically implantation on burned patients. Research granted by the Ministry of Health of Mexico and de ICYT-DF (PIFUT-08169).

P-74

MICROBIOLOGICAL ANALYSIS OF SKINGRAFTS PROCESSED BY THE SKIN & TISSUE BANK OF THE INR IN MÉXICO

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Abstract / Background:

The viable skin grafts is considered the best biological coverage in the treatment of severe burns. Unfortunately, common sterilization methods are not applicable for skin without compromise physiological, biological and structural properties. Previous reports about microbiological analysis have been shown contamination over more of 50% of skin banked samples. Contaminant microorganism includes: E. coli, K. pneumoniae, S. aureus, C. albicans and also the presence of microorganisms of slow growth. Hereby we present the analysis of microbiological screening of human skin foils allografts obtained and processed according to the implemented process in the skin banking of the INR in México. Patients, tissues and methods. We analyzed 14 batches of skin foils procured from multiorgan donor under aseptic conditions into the surgical room. All procured tissues (0.5mm thick) were ablated from the back body regions (Shoulder, gluteus and legs). Sequential and timed pre-surgical washing was used for decontamination process, based on chlorhexidine, iodopovidone and isopropanol. Screening for microbiological control was performed for: 1) transport media, 2) washing solutions and 3) tissue samples for the detection of bacteria and fungi based on 14 days of culture in rich media for growth of aerobes, anaerobes and fungi (Thioglycollate broth, Sabouraud and blood agar). Visual test was used to report bacterial growth 1, 7 and 14 days.

Results:

1 of 14 batches (7.14 %) was positive for bacterial on blood agar plates after 24h of incubation. The resting 13 batches (92.86%) were negative for bacterial growth pattern (turbidity) at 7 and 14 days. However, the retrospective analysis to determine the possible infective focus, shows evidence of human field of a packaging process. A sample of a positive bacterial culture reports E coli as contaminant agent only in the washing solution. Positive batches were subjected in a second new microbiological assay with negative results.

Conclusion:

E. coli growth was due to contamination in the process of manipulation of the striatum of the plate and all tests were performed in triplicate and only one plaque was presented that colonial development, the sample was subjected to a second analysis without positive results. 99.7% of sample analyzed presented negative microbiological growth at 14 days. This preliminary analysis, shows a very low rate of contamination, and is possible due to: a) a carefully selection process for donors; 2) ablation of skin grafts like a surgical process in a surgical facilities and aseptic technique; 3) Effective decontamination process based on antibiotics and washing process may help to eradicate prolific bacterial load. Finally, the possible contamination in the handling of tissue during the process of cryopreservation is part of the learning curve process. Project granted by Ministry of Health of Mexico and the ICYTDF-PIFUT08-169.

P-75

THE EXPERIENCE OF THE OPERATIVE MODEL THE SKIN AND TISSUE BANK OF INR IN MÉXICO

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Abstract / Background:

The tissue banking is a medical practice developed with scientific bases since 1960. However the international standards of quality for the operation on skin banks have been seriously modified in the last decade due to the technological and scientific advance in cell biology, cryopreservation, molecular biology and biotechnology. In México the skin and tissue bank of National Institute of Rehabilitation (INR) founded in 2009 is the only one tissue bank that has the corresponding health license and the authorization for dispose organs, tissues and cells with therapeutic purpose in the extraction, preservation and release mode. Also being the only one in Latin America to implement a process focused to preserve cell viability of skin foil allograft from multiorgan donor, (less than 6 hours of death) using washing solutions, transport and preservation medium formulated by the own skin and tissue bank core facilities.

Methods:

The skin ablation process, like other tissues is developed under aseptic conditions in a surgery room. The ablation zone is meticulously clean and disinfected before releasing the ablation. Then the obtained tissues are rinsed in washing solution for 25 minutes (a set of buffers, antibiotics and antimicrobials) and set in transport medium (high glucose iso-osmolar media and PH buffer agents) at 4°C until transported to the skin and tissue bank of the INR. Also blood samples are taken to be analyzed in the quality control process. In the bank, the tissues are processed and subjected to quality control by different testings that includes: The 14 days microbiological culture for tissue and transport medium to determine presence of bacterial load and fungus. Biological safety test includes: a.) Hemoculture from the donor for aerobic and anaerobic microorganism, b) molecular probes based on Real Time-PCR (to discard the presence of HIV, HBV, HCV, Cytomegalovirus and Treponema pallidum) and c). Histopathological analysis. Once the tests are all negative and the biological security is guaranteed, the tissues are processed and cryopreserved (Medium with glucose, osmolytes, buffers and antibiotics) at -80°C until required. If not, the infected tissues are discarded. The cell viability assay is performed by the DCF method and analyzed by Laser Scanning Confocal Microscopy.

Results:

Viability assays recently performed to skin samples cryopreserved on 2009 shows more than 80% of alive tissue (n= 12). By other way there is not microbiological growth in the tissues as well as in the washing solutions.

Conclusions:

The correct selection of the donor and the ablation of the tissue within 6 hours after death guarantee high percentage of tissue's viability in cryopreservation for a longer time. Low microbiological load in the tissues, has been reported with this operative model. Project granted by Ministry of Health of Mexico and the ICYTDF-PIFUT08-169.

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ACTIVITY IN THE RECENTLY ESTABLISH SKIN BANK OF TSF

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Abstract / Introduction:

Donor skin transplant is a valid therapy for skin loss due to extensive severe burns, trauma, surgery and several diseases such as bullous epidermolysis, diabetic and pressure ulcers. Deceased donor allograft skin provides temporary cover of the burn wound when autograft is not available. Lack of autologous split-thickness skin graft requires the use of temporary skin substitutes, being the human skin from deceased donors a good choice. Two preservation methods, glycerol and cryopreservation, are commonly used by tissue bankers for long-term storage, showing both to be effective. To evaluate our results this study has been carried out with the data available so far, composed by the returned follow ups forms of the skin distributed directly by our bank and part of the data collected from the intermediary banks. Aim: On one hand to know the general features of the skin donors, on the other hand to determinate if we are able to have available skin in stock to provide hospitals. For the last, we have considered the difference between the amount of recovered skin square centimeters (cm²) with the amount of the distributed one during 2009-2010. The last aim is to report the results obtained after transplantation of the skin delivered in the period 2009-2010.

Methods:

This retrospective study evaluates the amount of skin obtained and shipped to several hospitals in Spain and overseas. The general features of the shipped donors such as age, sex, type of donor, and mean body cooling time before retrieval have been reported. The clinical results from the recipients after transplantation were reviewed. The analysis was conducted in our tissue bank during the period of January 2009 -December 2010.

Results:

During the study period we have dispatched 590570 cm² of skin from 229 donors, of which, 255700 were cryopreserved and 334870 were glycerol-preserved. At the time we have stored others 877666.40 (cm²) of suitable skin from 279 donors, of which, 363637.00 were cryopreserved and 514029.40 were glycerol-preserved. The viability rate obtained with these 279 donors was 84%. Nowadays we have in stock more than 100000 cm² of viable skin graft from deceased donor. Until now, we have never received any report of complications that could be related to the skin after transplantation.

Conclusions:

Skin recovered from human deceased donors, both, cryopreserved and glycerol-preserved, is a safe and effective choice to skin loss due to extensive severe burns and other diseases. Our institution is a high-volume skin graft provider and it is able to have in stock suitable skin graft to respond to the routine demand.

CORD BLOOD

P-77

USE OF AMNION IN THIRD DEGREE BURNS AND NON-HEALING WOUNDS

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Abstract / Introduction:

The use of freeze-dried, irradiated amnion in the management of second degree burns is well established. The present study assesses its possible role as a non-surgical option for the treatment of third degree burns and non-healing infected wounds.

Materials and Method:

Between June 2008 – 2011, 15 patients with third degree burns or infected, non healing wounds of various sizes were treated with freeze-dried, irradiated amnion obtained from the Tata Memorial Hospital Tissue Bank.

Results:

12 of the 15 patients managed with amnion dressings had complete wound healing. In the remaining 3 the wound size reduced. The dressing was painless, therefore avoided anesthesia. Reduced pain post dressing preempted the use of pain killers and allowed the patient to perform daily activities comfortably. One patient was hospitalized during the early treatment period. All the others were treated on an outpatient basis. Good healing was achieved within 28 to 60 days depending on the depth and extent of the wound. Hypertrophic scars were observed in 5 patients. Standard care of these patients would have involved surgical intervention entailing skin grafting with associated risks and costs. This was avoided.

Conclusion:

Freeze-dried, irradiated amnion dressing may provide an effective option to surgical intervention in wound healing, especially in patients who are either reluctant to or not fit for surgery.

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EVOLUTION FROM 2007-2010 IN MALAGA CORD BLOOD BANK

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Background and objective:

Over the years, in relation to the existence of Cord Blood Banks (CBB), quality parameters that measure the work system has been established, and they improve the own banks.

The aim of this work is to define these parameters and apply them to CBB of Malaga to assess their current situation and optimize the available units for transplant.

Material and methods:

We have analyzed a retrospective study about the total sent units to Malaga Cord Blood Bank from 3 autonomous communities in Spain.

In this study, we have analyzed several clinical parameters:

- Received units.
- Average of initial volume.

- Total Nucleated Cells (TNC).
- Percentage recovery of TNC.
- Shipped units for transplantation and their TNC.

They settled on an annual average during 2007 to 2010.

Results:

Our results showed that the total received units were 27.822 and processed 13.286. The average of initial volume was 94.71 ml in 2007, 93.89 ml in 2008, 11.79 ml in 2009 and finally, 13.30 ml in 2010. The percentage recovery of TNC was 88.13% in 2007, 86.76% in 2008, 87.59% in 2009 and 87.79 in 2010. The shipped units for transplantation and their TNC were 18 units (2007), 23 units (2008), 34 units (2009) and 52 (2010) and average cellular were 13.3×10^8 (2007), 15.7×10^8 (2008), 17.7×10^8 (2009) 18.7×10^8 (2010).

Conclusion:

- Malaga Cord Blood Bank has evolved positively.
- The transplant unit demand is keeping with more content units of TNC.
- It is necessary to continue optimizing the umbilical cord blood for transplantation.

P-79

VIABILITY AND FORMATION OF ERYTHROID, GRANULOCYTE/MACROPHAGE AND MEGAKARYOCYTIC COLONIES IN BLOOD HEMATOPOIETIC PROGENITORS AFTER 20 YEARS STORED IN LIQUID NITROGEN

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Introduction:

Blood hematopoietic progenitor cells destined for allogenic transplantation, often are stored in liquid nitrogen (LN) for long periods of time.

It is considered that in LN (-196°C), any activity that would induce the degradation of these cells is paralyzed, remaining therefore intact their properties.

At the same time, there has been demonstrated degradation of certain proteins at very low temperatures.

Objectives:

Study the viability and the capacity of proliferation of blood hematopoietic progenitors (BHP) stored for 20 years in LN.

Materials and Method:

We have tested ten samples of BHP cryopreserved apheresis with DMSO to 10 % in the year 1990. In all samples were studied the viability (v) for two different methods, tripan blue and orange acridine; also erythroid (CFU-E), granulocyte/macrophage (CFU-GM) and megakaryocytic (CFU-MK) colonies that were obtained cultivating for duplicate 3×10^4 nucleate cells in MethoCult[®] and MegaCult[™]-C[®] mediums.

In 2011, after more than 20 years stored, we have repeated the same studies, using the same methods.

Results: Tables 1, 2 and 3

Table 1. Results year 1990

Sample	V	CFU-E	CFU-GM	CFU-MK
1	78	36	25	14
2	87	26	18	18
3	79	25	17	22
4	85	20	24	15
5	74	42	23	16
6	78	35	18	18
7	82	37	18	14
8	68	35	23	21
9	76	38	21	17
10	86	26	23	18
	9.3	32	21	17.3

Table 2. Results year 2011

Sample	V	CFU-E	CFU-GM	CFU-MK
1	67	12	16	8
2	82	18	13	13
3	76	21	16	12
4	86	22	18	5
5	70	26	15	14
6	79	25	13	16
7	79	24	15	12
8	67	22	3	16
9	72	23	19	11
10	76	21	10	9
	75.7	21.5	13.8	11.6

Results table 3

	Average value 1990	Average value 2011	Difference	p	IC
V	79.3	75.7	-3.6	<0.01	-1.28 a -5.92
CGU-E	32	21.5	-10.5	<0.05	-5.24 a -15.70
CGU-GM	21	13.8	-7.2	<0.05	-3.09 a -11.30
CGU-MK	17.3	11.6	-5.7	<0.001	-0.17 a -11.5

Conclusions:

It's observed a general decrease of all the studied parameters,. The decrease of the CFU-E is more marked. There is significance, $p < 0.05$, recounted to the difference of CFU-E, CFU-GM and CFU-MK. Nevertheless, in all the studied parameters, the obtained values would be sufficient to use the BHP in transplants.

P-80

NEW GMP METHOD FOR CORD BLOOD CRYOPROCESSING WITH CLOSED TUBING SYSTEM AND PRE-COOLED GEL SLEEVES

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Abstract / Introduction:

Precise proceeding of cryoconservation is fundamental for obtaining maximal numbers of thawed cells and it is very important especially in hematopoietic stem cell (HSC) transplantations. According to the actual European legislative new method of HSC processing is needed. We developed close system with pre-cooled gel sleeves for HSC manufacturing which is regardful of cells and in compliance with GMP standards. No clean rooms are required then.

Material and Methods:

For the process validation we used umbilical cord blood from eight healthy donors after the sign of informed consent. During the process we used only our designed system of bags and tubes, sterile tubing welder and gel sleeves for cooling the cryopreservative solution during the mixing with cell suspension.

Results:

We compared two methods of cord blood processing before freezing. In the first case, we used system of bags and tubes joined with three-way cocks. For cooling the cell suspension before adding the cryoprotectant (DMSO) we used standard water bath; the temperature inside the bag was 3.6-3.8°C, median 3.7°C). Via the temperature probe inside the bag, we noted significant increase in temperature during the addition of DMSO (12.4 – 12.7 °C, median 12.5°C). In the second case we used close system of bags and tubes joined with sterile welds. For cooling the cord blood (starting temperature: 3.5-3.8°C, median 3.6°C), we used pre-cooled gel sleeves. At the time of DMSO adding we logged out only slight temperature variation (4.2 – 4.8°C, median 4.7°C).

Conclusions:

In Department of Hematopoietic Stem Cells in Tissue Bank of UH Brno, new method of HSC proceeding before cryopreservation was developed. In this technique, specially designed gel sleeves for bags were used. Previous cooling of these cases minimizes the risk of warmth damage of the cells during the process of their mixing with cryoprotectant containing (DMSO). This method was approved with Czech national authority (SUKL).

P-81

INVENTORIES: MALAGA CORD BLOOD BANKING

RIZO ALFARO, P.; PONCE VERDUGO, L.; GÓMEZ MALDONADO, P.; FALCÓN MORALES, F.; MAYORGA PASTRANA, C.; HERNÁNDEZ LAMAS, C. M.; PRAT ARROJO, I., Málaga Cord Blood Bank. Spain.

Background and objective:

Umbilical cord blood (UCB) is a source of hematopoietic precursors for transplantation. The creation of UCB banks (UCBB) in 1992 led to the storage of units for unrelated transplant. The distribution of the historical inventories is not homogenous concerning the cell content of the units and many units are not therefore suitable for adults. The aim of this study was to analyze the UCBB inventory and calculate the economic impact of the current process per unit of UCB stored, in order to determine future expectations.

Material and Methods:

Three study periods were defined: the first, from 23 January 1996 to 9 January 2006 with TNC (Total Nuclear Cell) acceptance levels for processing of $4-6 \times 10^8$ and a manual processing system; the second, from 1 October 2006 to 30 July 2010, with automated processing and variation in the number of TNC from $8-10 \times 10^8$; and the third, from 1 January 2009 to 30 June 2010, with an automated Sepax-BioArchive procedure and starting TNC $>10 \times 10^8$. Within each period various ranges of cryopreserved TNC units were established: A, $>16.2 \times 10^8$; B1, from $12.5-16.1 \times 10^8$; B2, from $5.2-12.4 \times 10^8$; and C, $<5.1 \times 10^8$. For this analysis, we divided B2 into different subcategories: B2.1, from $10-12.4 \times 10^8$; B2.2., from $8-9.9 \times 10^8$; and B2.3, from $5.2-7.9 \times 10^8$.

Results:

The third period was the most representative of the results, with homogenous acceptance criteria and automated processing. Group A contained 15.7% of the units and group B contained 25.5%. The mean TNC for transplant was 14×10^8 (4.6×10^8 to 36.5×10^8). The cost of the processed UCB was 720.41 euros per unit.

Conclusion:

An UCBB should nowadays have high-quality units in relation with the TNC sent for transplant, possess a training program to optimize and select donations before birth, use similar volume reduction systems and homogenous recovery indexes, express its indicators in the same units, use validated analytical techniques, and bear in mind ethnic minorities.

P-82

DONOR SELECTION OF UMBILICAL CORD BLOOD

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Background and objective:

Umbilical Cord Blood Banks contribute at the international records introducing their stored units to facilitate the localization of the best available unit. The main aim was to determine the influence of the maternal factors in the quality parameters before cryopreservation and how to improve the extraction procedures.

Material and Methods:

A total of 502 units were analyzed in Malaga Cord Blood Bank, coming from 46 hospitals. In this study, we have analyzed several clinical parameters: mothers and neonates clinical data (age, mother nationality, number of pregnancies, duration and type of delivery, neonates gender, histocompatibility, blood group) and this variables have been related with the feasibility, number of CD34+ cells, nuclear total cells, volume and weight units.

Results:

The descriptive analysis of the population studied, showed the following mean values $30,7 \pm 0,2$ years, $39,8 \pm 0,05$ gestation weeks, 74,8% eutocic delivery and the proportion of male neonate was 52,8%. In relation to the maternal factors that could influence in the units quality, we detected a negative correlation between gestation weeks and number of CD34+ cells (Pearson coefficient -0,141 and -0,096) and a positive correlation between the quality parameters analyzed and the following variables: longer duration of delivery, non eutocic ($p < 0,05$), multiparity and newborn male (OR 0,836).

Conclusion:

The feasibility and proportion of CD34+ cells are influence by the factors studied: age of the donor over 35 years, multiparity, no eutocic delivery, longer delivery time extended beyond 10 h and newborn male. The protocols for obtaining it should be optimized and involve careful selection of donors in order to reduce the number of units discarded.

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INFLUENCE ON TIME TO CRYOPRESERVATION

PONCE VERDUGO, L.; GÓMEZ MALDONADO, P.; RIZO ALFARO, P.; GALEOTE PADILLA, A.; MARTÍN BEJAR, T. M.; HERNÁNDEZ LAMAS, C. M.; PRAT ARROJO, I., Málaga Cord Blood Bank. Spain.

Abstract / Background and objective:

It is accepted that the Umbilical Cord Blood (UCB) units can be cryopreserved to 48 h after their harvest and the delay in this procedure influences the final quality. The aim of this study was to determine the influence on time to cryopreservation in Umbilical Cord Blood Units.

Material and Methods:

A selection of the 2134 samples arrived to the UCB Bank from 36 Andalusian linked hospitals was analyzed for a period of six months. The date and hour of collection, hospital of origin, time in the hospital, time of transport, time of arrival to the bank to cryopreservation, viability and CD34+ cells were determined.

Results:

Our results showed that the median time from harvest to cryopreservation was 27.9 h. However, a great variability was found (range: 5.3 to 47.6 h), which was dependent on transport variables: time from collection to leave the hospital (median 12.6 h, range: 0.1-28.8), time of transport from hospital to UCB Bank (median 1.8 h, range: 0.1-20.6) and time kept in the bank before freezing. Viability of cells were reduced directly proportional in samples that took longer to be cryopreserved (93.9 vs 96.6%, $p=0.001$), by contrast, CD34+ count did not differ significant at shorter or longer times of cryopreservation.

Conclusion:

The delay in transport time and in processing reduce the umbilical cord units quality, consequently, the time of transport and storage previous to cryopreservation must be reduced less than 36 h.

P-84

UMBILICAL CORD BLOOD:ECONOMIC STUDY

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Abstract / Background and objective:

Malaga Cord Blood Bank started its activity in 1996, and this bank now receives UCB from 61 maternity units. The aim of this study was to analyze the cord blood inventory, calculate the economic impact of the current process per unit of stored UCB in order to establish future expectations.

Material and Methods:

A total of 20,762 units processed between 1996 and 2010 were studied. In 2006 a volume reduction system was initiated with HES, automated separation (Sepax®), programmed freezing and automated storage (BioArchive®). The cost of the UCB units processed by Sepax® was 720.41 euros per unit.

Results:

The cost of the UCB units processed was 720.41 euros per unit, the collection cost was 17.1 euros, which included a Macopharma® collection bag, tubes, containers, forms and various collection material. The processing cost was 310.15 euros, which included validation, separation by Sepax Biosafe®, cryopreservation and storage using BioArchive®, as well as administrative expenses. This heading also included sample storage (fetal red blood cells, fetal and maternal plasma bank, and tissue fragment). The quality control included HLA typing, flow cytometry (CD34+), viability, cell count blood cultures, ABO and Rh grouping, and testing for transmissible diseases (HCV, HBV, Lues, HIV, Chagas, Malaria). The data for the personnel were studied for each person involved throughout the process: midwife, laboratory technician, administrative staff, professional staff. The analysis did not include units that were not processed.

Conclusion:

The implantation of quality systems, economic impact and their legislation have all contributed to the continual improvement in the quality of stored UCB units. Umbilical cord blood is scarce and expensive, and the protocols for obtaining it should be optimized and involve careful selection of donors in order to reduce the number of units discarded.

P-85

MALAGA CORD BLOOD BANK: EVALUATION THE UNITS SENT FOR TRANSPLANT

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Abstract / Background and objective:

Umbilical cord blood (UCB) is a source for the transplant of hematopoietic precursors. The creation of UCB banks (UCBB) in 1992 led to the storage of units for unrelated transplant. Malaga Cord Blood Bank started its activity in 1966 and this bank now receives UCB from 61 maternity units. The aim of this study was to analyze the cord blood inventory, evaluate the units sent for transplant to establish future expectations.

Material and Methods:

A total of 20,762 units processed between 1996 and 2010 were studied and a total of 172 umbilical cord blood units were sent to transplant and distributed for all over the world.

Results:

The number of units sent for transplant has increased in proportion to the number of units stored and the increase in the number of TNC. The units were transplanted in Spain (23.7%), the rest of Europe (42.7%), USA (25.4%) and elsewhere (8.2%). The mean TNC of the units sent for transplant was 14×10^8 (4.6×10^8 to 36.5×10^8).

Conclusion:

The implantation of quality systems, their legislation and the use of practices based on scientific evidence have all contributed to the continual improvement in the quality of stored UCB units to transplant.

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CONTINUING EDUCATION MALAGA CORD BLOOD BANK

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Abstract / Background and objective:

Malaga Cord Blood Bank received 9684 UCB (umbilical cord blood) units in 2010 and were accepted 40%. They were obtained in 59 linked maternities of three Spanish geographical areas (0-600 km). It is the responsibility of the Bank, within a system of quality management, initial and continuing training of the collection facilities depending on it. The training program began in 2006 and is developed by the responsible external quality. Since that time the program has continued to grow in scope and currently performs in 59 maternities. It consists of classroom training, developed in maternity and aimed at all health staff involved in donations of UCB. The main aim of this study was to analyze the period 2009-2010 and compared with the previous year. In 2009-2010 more than 600 people attended these courses.

Material and Methods:

The aim of this program is to prepare and recycle healthcare professionals involved in donations and have an update list of people trained according to standards of quality NETCORD and Joint Committee on Accreditation. The specific objectives are: • To provide participants with the knowledge and skills necessary to obtain UCB. • To promote and encourage donations. • Get UCB under the procedures of Cord Blood Bank. • Improve the quality of the units received. • Implement a methodology of work under a quality system. - Audiovisual material: slides. - Support for material donation. Results: There was a tendency to increase in 11.3 ml volume of the units in 2010, increase the number of samples received (in 2007: 4922 units, in 2008: 5068 units, 2009: 8148) and decreases the percentage of UCB discarded for reasons related to the hospital of origin. Conclusion: • Improving the quality of the units received. • Consistency of documentation. • Awareness of teamwork. • Promotion of communication links: -Audiovisual material: slides. -Support material donation.

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THE CORD BLOOD BANK OF THE RED CROSS BLOOD TRANSFUSION SERVICE OF UPPER AUSTRIA

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Abstract:

The Cord Blood Bank of the Red Cross Blood Transfusion Service of Upper Austria is the first public cord blood bank in Austria. It was founded in 2002 as part of the blood bank. The Red Cross Blood Transfusion Service of Upper Austria is certified according to the EC Directives 2004/23/EC, 2006/17/EC, and 2006/86/EC for cord blood and several tissues, as well as GMP- and ISO9001:2008- approved. At the beginning cord blood units were obtained from a single hospital next to the cord blood bank. In the meantime donations are already sent to the cord blood bank from eight hospitals located in Upper Austria. Initially, processing of the donations was done manually according to the method by Rubinstein et al. (1998) but since 2004 cord blood units are processed automatically on the Sepax System (Biosafe, Switzerland). Freezing and storage is performed with the BioArchive System (Thermogenesis, USA). In 2010 a total of 911 donations arrived at the cord blood bank. From these obtained donations 211 (23.16%) fulfilled the quality criteria for processing which resulted in 183 cord blood units (20,09%) that were finally stored in the BioArchive. Since 2002 about 1,400 units were stored in our cord blood bank. Based on our participation in the Austrian Stem Cell Register "Geben für Leben" (www.stammzellspende.at) since 2007, three products were already released and shipped for transplantation. Furthermore we are currently completing our participation in Cytolon (www.cytolon.de).

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SEMI-AUTOMATED HIGH-THROUGHPUT NEXT GENERATION SEQUENCING BASED HLA-TYPING FOR CORD BLOOD SAMPLES

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Abstract:

To overcome some of the intrinsic HLA-typing problems, we established a new, sequence-based method using Roche / 454 Life Science's sequencing technology. Applying this high-throughput technology offers the possibility for ambiguity-free typing and high level automation. The use of the clonal pyrosequencing approach with approximately 450 base pairs long sequence reads covering the whole exons made a straightforward amplicon strategy feasible. For exon 1-7, where appropriate, we developed HLA-A, -B, -C, -DP, -DQ and -DR locus specific primers. For amplicon generation target specific primers and GS-FLX specific adaptors were elongated with sample-identifying barcode sequences to trace each of the 40 samples which represent the current maximum loading capacity of one single GS-FLX sequencing run. Sequencing protocols were performed according to supplier's instructions without modifications. But, we set up protocols for Hamilton robotics based amplicon production and post emulsion-PCR bead preparation and present correspondent HLA-typing results. Although the great number of samples was identified and typed correctly we currently work to establish improved protocols for problematic allele combinations and automated HLA allele assignment with less manual editing needed and to deepen our data pool consequently. The presented system can be used for low or high allele resolution in dependence of the used amplicons for high-throughput typing of cord blood samples.

GLIMPSE INTO THE FUTURE; ADVANCES THERAPIES

P-89

HEPATIC CELLULAR THERAPY UNIT, IIS-HOSPITAL LA FE: ESTABLISHMENT OF HEPATIC CELL BANK FOR CELLULAR TRANSPLANTATION

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Abstract:

The creation of a regulatory-compliant cell bank is an essential element in the production of uniform biological products. Cell banking systems assure that a uniform population of cells is preserved, that their integrity is maintained and that a sufficient supply of material is readily available for the life of the product. Of equal importance is the maintenance of these banks in a secure, controlled and monitored storage environment. Hepatocyte transplantation is an alternative therapy to orthotopic liver transplantation for the treatment of liver diseases. The application of hepatocytes for transplantation purposes demands high-quality cell preparations, which depend to a large extent on the nature of the tissue used for cell harvesting. However, isolation, cryopreservation, and thawing processes can seriously impair hepatocyte viability and functionality. The Hepatic Cellular Therapy Unit-IIS La Fe (UTCH) began its activity in 2007, and since then it has processed more than forty livers under ISO standards. The main aim of this unit is to offer excellent batches of cells that allow restoring hepatic function loss or maintaining hepatic function while the patient is on the waiting list. In the present work, we summarize the recent effort of the UTCH at our hospital for the purpose of establishing a cell bank of cryopreserved human hepatocytes for clinical application. To achieve this goal we focused on: a) expanding the liver donor pool by exploring the suitability

of livers from non-heart-beating donors (NHBDs) and from neonates as a potential source of hepatocytes for transplantation; b) analyzing the influence of the length of the cold ischemia time on the outcome of isolated hepatocytes; c) improving the cryopreservation protocol; and finally, d) assessing the functionality of hepatocyte preparations with a view to promoting customized cell preparation for each receptor. For this purpose, viability, attachment efficiency and metabolic functionality (ureogenic capability, cytochrome P450 and phase II activities) were assayed prior to clinical cell infusion to determine the quality of hepatocytes. Moreover, the evaluation of urea synthesis from ammonia and UDP-glucuronosyltransferase 1A1 activity, a newly developed assay using beta-estradiol as substrate, allows the possibility of customizing cell preparation for receptors with urea cycle disorders or Crigler-Najjar Syndrome type I. Sources of human liver and factors derived from the procurement of the liver sample (warm and cold ischemia) have also been investigated. The results show that grafts with a cold ischemia time exceeding 15 h and steatosis should not be accepted for hepatocyte transplantation. Finally, livers from NHBDs and neonates are a potential suitable source of hepatocytes which could enlarge the liver donor pool.

P-90

CLINICAL TRIAL COMPARING PLATELET-RICH PLASMA TO BETAMETASONA AND BUPIVACAIN SUBACROMIAL INJECTION IN ROTATOR CUFF TENDYNOISIS. PRELIMINARY RESULTS

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Abstract:

The platelet-rich plasma (PRP) is an ultraconcentrate of peripheral blood containing growth factors that, as some studies demonstrate, favor the healing process of soft tissues. The PRP has been used to treat tendinitis in some areas of the human body, epicondylitis, patellar tendinitis, aquilean tendinitis ... The chronic tendinitis of the rotator cuff, pathology very prevalent among the active population, also could to be treated with PRP. The present prospective, randomized and double blind clinical trial, tries to compare the therapeutic effect of two alternative treatments in a group of patients with subacromial syndrome due to chronic tendinitis without rotator cuff tear. The new treatment will consist on subacromial injection of autologous PRP and the other one will consist on subacromial injection of betametasona acetate and bupivacaina, as it is used to do in the clinical practice while its reported little effect. PRP is prepared following the Anitua method. The injection is guided with ultrasounds. We show the preliminary results which support PRP usage with the first 30 patients.

P-91

ESTABLISHING THE SNBTS ISLET ISOLATION LABORATORY

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Abstract:

SNBTS Tissues and Cells in conjunction with NHS Lothian recently received funding from the Scottish Government to establish a pancreatic islet cell transplantation service for Type I diabetic patients presenting with poor glycaemic control and/or awareness, often associated with numerous hypoglycaemic events. Over the last 18 months SNBTS Tissues and Cells have installed and commissioned a new Grade B/C processing facility and established the islet isolation service (based on the Edmonton Protocol), to the appropriate GMP and legislative standards, creating in excess of 150 controlled documents and

completing over 20 individual validations. The process of islet cell isolation (as described in the 'Edmonton' protocol, Shapiro et al. NEJM. 2000) is laborious and requires several members of staff working concurrently. Once the organ is received it is decontaminated before being cannulated and perfused with collagenase enzyme until visibly distended. The organ is then cut into pieces and placed within a chamber in the presence of warm (active) collagenase, which is recirculated through the chamber and associated circuit. The combination of mechanical and enzymatic digestion results in a crude islet preparation that can then be purified on the basis of density using a COBE 2991 cell processor. Purified islets are quantified before being placed in culture for a period of 24-48 hours, enabling additional tests/checks to be completed (endotoxin, viability), prior to clinical/QA release. Islets are then transplanted into the portal vein of the patients' liver under local anaesthetic. The clinical service was established in December 2010 and the first transplant took place in February 2011, the first of its kind in Scotland and one of just over 20 in the UK. Early indications suggest the graft was extremely successful as evidenced by a reduction in insulin requirements, reduced hypoglycaemic events, restoration of glycaemic awareness and production of c-peptide, a marker of endogenous insulin production. A second transplant in the same patient 6 weeks later has led to insulin independence.

P-92

HUMAN AMNIOTIC MEMBRANE AS A SOURCE OF PROGENITOR CELLS FOR HUMAN ARTICULAR CARTILAGE REPAIR

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Abstract / Purpose:

There is an increasing interest about human amniotic membrane (HAM) for its use in the field of regenerative medicine. Two kinds of progenitor cells can be removed from the amniotic membrane, the amniotic mesenchymal stromal cells (hAMSC) and amniotic epithelial cells (hAEC). Both cell types are capable of differentiating towards three germinal cell lines. It has large advantages over the other sources of progenitor cells. The aim of this study is to determinate the usefulness of the hAMSC and the hAEC for regenerating human joint cartilage in an in vitro model.

Methods:

HAM was used as support for the culture of hAMSCs or hAECs. Focal injuries in human joint cartilage biopsies were done. Later, a pellet of cells (hAMSCs or hAECs, depending on the repair model developed) was implanted into the focal defects of cartilage. HAM, with the cells grown on it, was placed in direct contact with the cartilage surface to be repaired. These implants were cultured in DMEM+10% FBS for 8 weeks. The repair tissues were analyzed by histological and histochemistry analysis considering the ICRS macroscopic evaluation of cartilage repair.

Results:

hAMSCs and hAECs cultured on HAM and transplanted onto focal injuries of cartilage penetrated into the nearby surface of the chondral defect. The Hematoxylin and Eosin staining showed that the hAMSC or hAECs pellet filled the chondral defect. There was a good integration between the repair tissue and native cartilage. Type II collagen and aggrecan stainings of repair tissue were slightly positive on the extracellular matrix, and positive inside the cytoplasm of the cells. The safranin O staining expressed the presence of proteoglycans. Finally, type I collagen stainings were weak or totally negative. We realized an ICRS macroscopic evaluation of cartilage repair to compare both kinds of progenitor cells in the in vitro model. The hAMSCs displayed better degree of defect repair, greatest integration to border zone and, in general, higher repair assessment.

Conclusion:

We get a reduction of the area of the defect with quality integration. The morphology of the repair tissue showed a fibrocartilaginous appearance and a high cellularity. The hAMSCs showed better results considering the ICRS macroscopic evaluation of cartilage repair.

P-93

COMPARISON OF DIFFERENT METHODS OF GENOMIC DNA EXTRACTION AND PURIFICATION FROM TISSUE

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Abstract / Introduction:

In a Tissue Biobank, genomic DNA (gDNA) extraction and purification from tissue are indispensable. A method's optimization for this extraction and purification to find a reproducible procedure are necessary to obtain high performance and elevated concentration and hereby improve the samples quality. OBJECTE Evaluate the amount and quality of gDNA obtained by different extraction and purification methods from frozen lung tissue.

Material and Methods:

In this study, five gDNA extraction and purification methods were compared: three methods based on silica membrane columns: gDNA from Tissue by MACHEREY-NAGEL (method 1), ReliaPrep™ gDNA Miniprep System by PROMEGA (method 2), QIAAMP® DNA Mini and Blood Mini Kit by QIAGEN (method 3); and two methods based on alcohol precipitation: Gentra® Pueregene® by QIAGEN (method 4) and ArchivePure DNA Purification by 5 PRIME (method 5). We tested 10 samples for method and each sample was adjusted to 30 mg wet weight before storage at -80°C. All samples were extracted from an only donor's surgery. Chemical (Proteinase K digestion) and mechanical (shaking at 56°C for 24 hours) pre-treatment are used to facilitate an efficient homogenizing and lysis. gDNA obtained are eluted or dissolved in 100 µL of Buffer Solution and shaking for 2 days at room temperature. To evaluate the amount and quality we use spectrophotometer Nanodrop® by ThermoFisher. Express gDNA concentration on ng/µL. The absorbance ratios at 260 and 280nm (A260/280) and at 260 and 230nm (A260/230) are used to assess the purity of nucleic acids. For pure gDNA, A260/280 and A260/230 are larger than 1.8. Continuous variables are described by medians and non-parametric tests are used in order to compare them.

Results:

Concentration: methods 1 and 2 are homogeneous (P-value 0.123 and medians 160.60 and 199.27 ng/µL); methods 3, 4 y 5 are homogeneous too (P-value 0.285 and medians 48.12, 48.02 and 54.80 ng/µL). Medians of methods 1 and 2 are clearly larger than medians of methods 3, 4 and 5. A260/230 ratio: similar than Concentration; methods 1 and 2 homogeneous (P-value 0.165 and medians 2.17 and 2.11) and methods 3, 4 and 5 homogeneous too (P-value 0.295 and medians 0.85, 0.78 and 0.85). A280/230 ratio: for this criterion, methods 1 and 3 are homogeneous (P-value 0.739 and medians 1.86 and 1.84) and methods 4 and 5 too (P-value 0.909 and medians 1.99 and 1.98). The method 2 obtained the higher median (2.06).

Conclusion:

Due to the observed medians of the ratio A260/280 were bigger than 1.8 (usual cut-off value), all considered methods yield High Quality gDNA. However, methods 1 and 2 obtained concentrations and A260/230 -ratios larger than the ones derived from the methods 3, 4 and 5. Therefore, it can be concluded that the best method among the five studied ones was the 2. The results get for this method are clearly the best ones for all considered criteria.

P-94

HUMAN MESENCHYMAL CELLS OF ADIPOSE TISSUE INHIBITS THE PRODUCTION OF PGE2 IN HUMAN MONOCYTES

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Objective:

It is known that mesenchymal stem cells have immunomodulatory capabilities. However there is very little documentation about what mechanisms may be involved. Prostaglandin E2 (PGE2) is a lipid mediator that acts by enhancing the inflammatory response. Numerous cell types produce PGE2 in response to other inflammatory mediators. In this work we studied the effect of mesenchymal stem cells from human adipose tissue (CMTAh) on PGE2 production in human monocytes from peripheral blood.

Methods:

CMTAh were obtained from 3 healthy individuals lipectomy by digestion with collagenase I. The resulting cell suspension was incubated until the confluence with growth medium (GM: DMEM/F12 containing 15% human serum and antibiotics). Phenotypic characterization of the cell population was performed by flow cytometry using the following markers: CD45, CD34, CD90, CD105 and CD13. A different time, the culture medium (MA) was collected, centrifuged and frozen at - 80 °C. Human monocytes were isolated from 5 buffy-coats by Ficoll density gradient. The suspension of nucleated cells obtained was seeded at a density of 106 cells / ml with medium DMEM/F12 containing 15% human serum and antibiotics. Monocytes were selected for their ability to adhere to culture support. Phenotypic characterization was performed using markers CD11c and CD14. After 12 h of incubation, monocytes were stimulated with LPS (1 ng/ml) with GM or MA for 24 hours. PGE2 production was measured by radioimmunoassay.

Results:

Phenotypic characterization showed that CMTAh are CD13, CD90 and CD 105 positive cells, being variable in the case of CD34 (which decreases with time in culture), and lack CD45. Monocytes were characterized as cells positive for CD45, CD11c and CD14. In monocytes incubated with DMEM/F12 and treated with LPS there was a significant increase in PGE2 production compared to the basal cells (23 ± 2 ng/ml vs. 1.3 ± 0.7 ng/ml, $p < 0.05$). However, in monocytes incubated with LPS and treated with MA, the production of PGE2 was significantly lower compared to cells incubated with DMEM/F12 and LPS (12.5 ± 1 ng/ml vs. 23 ± 2 ng/ml, $p < 0.05$).

Conclusions:

Our results indicate that CMTAh can modulate the inflammatory process by inhibiting PGE2 production in human monocytes.

P-95

HEPATOCTE ISOLATION FROM LIVERS NOT SUITABLE FOR WHOLE ORGAN TRANSPLANTATION. LIVER CELL THERAPY

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Abstract / Introduction:

The development and use of new clinical cell therapies from livers not suitable for whole organ transplantation could improve the problem of scarcity of donor livers. Urea Cycle Disorders (UCD) in children is a rare group of inherited metabolic diseases that have a poor prognosis despite optimal conservative treatment. Early liver transplantation before the onset of neurological damage may cure the disease. However, the obtainment of livers compatible for this kind of recipient is difficult due to the small size of the recipients. Liver cell transplantation (LCT) may be a good alternative to early liver transplantation, as it is less invasive and can be performed during the first weeks of life.

Material and Methods:

A liver network was created with defined rules and criteria for organ selection. Collaborative centers were coordinated and logistic and communication processes were established. Two steps were defined to link hospitals to the network. LCT was performed on four patients with severe UCD with neonatal onset and poor prognosis.

Results:

The network started in 2003 and linked hospitals in Spain, Germany, Italy and Portugal. So far, 99 livers have been obtained and a total of 250,3 billion cells with an average cell viability of 77% have been isolated. The Cryopreserved human liver cells were transplanted in the four children through the portal vein after thawing. Metabolic stabilisation was shown in all patients. However, one patient died after four months from a fatal decompensation triggered by infection and poor compliance regarding immunosuppression. In the remaining three children, metabolic crises vanished after LCT, and ammonia levels remained in the normal range. One girl is doing well on conservative therapy 28 months after LCT. In the two other boys, subsequent liver transplantation was performed 10 and 15 months after LCT. Both host organs were investigated after retrieval. Whereas the activities of the affected enzymes were around 0% prior to LCT, total enzyme activities of 4.5% and 15.6% of healthy controls were found in the explanted livers. These enzyme activities are in the range of mildly affected heterozygotes.

Discussion:

The promising results of the first clinical series of liver cell transplantation show that this innovative method may be a suitable option for the treatment of various hepatic-based diseases. It is possible to obtain viable cells from livers rejected for transplantation. Because of the implication involved in using organs for research or other uses other than transplant, it is necessary to clearly identify and define the steps involved in organ procurement and distribution.

P-96

DIFFERENCES IN SURFACE MARKERS EXPRESSION AND CHONDROGENIC POTENTIAL AMONG DIFFERENT TISSUE-DERIVED MESENCHYMAL CELLS FROM ELDERLY PATIENTS WITH OSTEOARTHRITIS

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Abstract:

Mesenchymal stem cells (MSCs) are self-renewing cells with multipotential capacity that could be used to repair or regenerate cartilage, which is damaged by some diseases such as osteoarthritis (OA). These cells are present in some adult and fetal tissues, including bone marrow [Prockop, 1997], adipose tissue [Zuk et al., 2002], synovium [De Bari et al., 2001], peripheral blood [Zvaifler et al., 2000], umbilical cord blood [Erices et al., 2000; Mareschi et al., 2001] and term placenta [Fukuchi et al., 2004]. In this study, we have used bone marrow, adipose tissue (from articular and subcutaneous locations) and synovial fluid samples from 18 patients with knee OA in order to find an alternative source of bone marrow for the isolation of MSCs with a high chondrogenic potential. MSCs from all the tissues showed a fibroblastic morphology, but their rate of proliferation was different: while subcutaneous fat-derived MSCs proliferated faster than bone marrow and Hoffa's fat pad-derived MSCs, the growth rate of synovial fluid-derived MSCs was slower. The phenotype of the MSCs from the four different analyzed tissues was similar, with some isolated differences such as CD36 and CD54 expression. The highest expression for these surface markers was in subcutaneous fat-derived MSCs, which differentiated poorly towards hyaline cartilage. Synovial fluid-derived MSCs presented only a medium chondrogenic differentiation capacity, while Hoffa's fat pad-derived MSCs showed a great chondrogenic potential. In conclusion, MSCs from elderly patients with osteoarthritis still showed a suitable chondrogenic potential, but it depended strongly on their tissue of origin. In conclusion, from all our results, bone marrow-derived MSCs showed a proliferation pattern and chondrogenic potential similar to that of Hoffa's fat pad-derived MSCs in elderly patients with osteoarthritis, while subcutaneous fat-derived MSCs differentiated poorly toward hyaline cartilage and synovial fluid-derived MSCs proliferated slower.

P-97

ADIPOSE TISSUE BANKING FUTURE PROSPECTS: FIRST STEP, SET UP OF CRYOPRESERVATION METHODOLOGY

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Abstract / Background:

Nowadays, adipose aspirates are widely used in plastic surgery for conducting reconstructive interventions. Fat transfer is defined as harvesting fat from parts of the body where it accumulates for transferring into other body locations in the same patient. Unfortunately, inflammation and congestion of the treated area often do not allow using all lipo-aspirated tissue. In addition, some part of the transferred material is reabsorbed and more interventions are needed for achieving the expected results. The leftover fat has to be thrown away if no preservative method is used. There are several published studies of adipose tissue potential applications like the obtainment and culture of adipose-derived stem cells (ADSC) and the isolation of extra cellular matrix (ECM) for tissue engineering.

Objectives:

The aim of this study was to set up an optimal, safe, reliable and cost effective lipoplasty-derived adipose aspirates cryoprotective methodology that ensures the viability of the tissue after cryopreservation.

Methods

The study was mainly conducted in female patients undergoing reconstructive surgery after suffering breast cancer. Raw adipose material obtained from conventional lipoplasty was transported to the laboratories at room temperature. When it arrived, a microbiological control was done. The intermediate phase of adipose aspirates was collected after centrifugation and each specimen was randomized into 4 groups: control group (fresh adipose aspirates without preservation); simple cryopreservation group (adipose aspirates preserved with our established cooling and thawing protocol); complex preservation group 1 (adipose aspirates preserved with our protocol using 0,35M Trehalose as cryoprotective agent (CPA)); complex preservation group 2 (adipose aspirates preserved with our protocol using 0,25M Trehalose + 0,5M DMSO as CPA solution). Before next step, another microbiological control was done to assess the processing methodology. Cryopreservation of adipose aspirates was conducted with controlled slow cooling and fast rewarming rates. Fresh or cryopreserved adipose aspirates in each group were evaluated by viable adipocyte counts, glycerol-3-phosphate dehydrogenase (G3PDH) assay, and routine histology.

Results:

At this moment the project are in an embryonic stage. Since we have started, we have been setting up the optimal controlled slow cooling program for two kinds of cryopreserving containers (cryovials and cryobags), counting viable adipocytes from fresh samples and making microbiological controls for the assessment of the arriving samples.

Conclusion:

More samples are needed to achieve statistically reliable results. However, our first experiences are encouraging. In terms of cellular viability, results show that room temperature is the best condition to transport samples; also we can say that our established cooling and thawing protocol seems to treat with care adipose tissue.

P-98

INTRA-ARTICULAR KNEE INJECTION OF PLATELETS RICH PLASMA TO TREAT OSTEOARTHRITIS

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Abstract / Introduction:

Platelets rich plasma (PRP) is autologous plasma containing thrombocytes in a concentration above normal blood counts. Due to its high platelets concentration and therefore of growth factors, cytokines and plasmatic bioactive proteins, PRP is considered a promising and useful biological product in wound healing and tissue regeneration. In orthopedic surgery, PRP has been largely used for tendon or ligament injury treatments as well as to medicate lesions of muscular tissue. Nevertheless, the benefit of PRP in cartilage injuries, specifically those with a degenerative origin, has not been clearly determined. Hypothesis PRP intra-articular injections in osteoarthritic knees reduce pain and improve knee function.

Objective:

To determine the clinical usefulness of PRP in the knee osteoarthritis treatment regarding pain, joint function, and quality of life improvement. Methodology Thirty patients with painful knee osteoarthritis were treated with three intra-articular PRP injections. Quality life score (SF36), knee function score (KOOS) and visual analogue pain score (VAS) were used for clinical evaluation. Complications, adverse events and patient satisfaction were also recorded.

Results:

We evaluated 30 patients, 10 men and 20 women, with mean age of 62,63 years. The mean BMI (body mass index) was 29, most of the patients presented degenerative chondral lesion Kellgren 3. All of the evaluated parameters improved at 1 and 3 months follow-up visits. Quantitatively, VAS improved from 7,13 points pre-treatment to 4 and 3,7 at 1 and 3 months post-treatment respectively. SF36 improved from 55 points to 60 and 59 for the same time points of study. Additionally all the 5 items evaluated in KOOS also improved.

Conclusions:

Our results indicate that administration of PRP injections can reduce pain and improve knee function and quality of life at short-term. Further studies are needed to find out which platelet concentration and which frequency of infiltrations provides better and more durable results.

P-99

COLD STORAGE OF PRIMARY HEPATOCYTES IN TIPROTEC VARIANTS

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Abstract:

For the use of primary hepatocytes in liver cell transplantation or extracorporeal liver support, short term storage options are required. However, diverse cells are very vulnerable towards cold-induced cell injury. Previous research in adherent rat hepatocytes and other cell types showed that cold-induced injury is iron dependent and that rat hepatocytes in addition suffer a chloride-dependent injury. Based on studies in adherent cells and isolated vessels we recently developed the tissue preservation solution TiProtec®, which is chloride-rich, contains several amino acids and two iron chelators. Here, we tested whether this solution or variants thereof can be used for the cold storage of adherent human hepatocytes and whether it might also be useful for the cold storage of hepatocyte suspensions. Adherent rat and human hepatocyte cultures and rat hepatocyte suspensions were stored in diverse variants of TiProtec®, cell culture medium or organ preservation solutions (UW, HTK) at 4° C, rewarmed/seeded in cell culture medium, and cell injury, cell attachment and metabolic function (resazurin conversion, gluconeogenesis) were assessed. Survival after cold storage and rewarming was significantly improved in adherent rat and human hepatocytes in variants of the solution TiProtec®. However, while rat hepatocytes survived best in a chloride-poor variant of TiProtec®, human hepatocytes survived best in the chloride-rich TiProtec®, both with an increased deferoxamine concentration compared to TiProtec®. In this solution, human adherent hepatocytes maintained > 60 % of their viability (LDH retention) after 3 weeks of storage, largely exceeding results in UW solution or cell culture medium (< 10 % viability after 2 weeks of storage). After 1 week of storage in TiProtec with the higher deferoxamine concentration, metabolic activities were similar to unstored control cells, after 2 weeks of storage 60-80% of control cells. Rat hepatocytes were then used to test the potential of these solutions for the storage of cell suspensions. The chloride-poor variant also proved best in suspensions of these cells. After one week of cold storage in this solution cell attachment rate was 76 ± 24% of control cells, far better than after storage in cell culture medium (1 ± 2%), UW solution (4 ± 2%) or HTK solution (2 ± 6%). The main protective effects could be allotted to glycine/alanine which inhibited injury during cold storage, and iron chelators and absence of chloride which improved cell attachment. Attached cells displayed normal morphology and metabolic function was similar to unstored adherent control cells. In summary, it is possible to significantly improve viability of adherent human hepatocytes and attachment ability and post-attachment function in rat hepatocyte suspensions using variants of TiProtec®. Current studies assess the use of TiProtec® or variants for the storage of human hepatocyte suspensions.

P-100

BIOLOGICAL PROPERTIES OF POROUS BIOMATERIALS OF THE CONNECTIVE TISSUE ORIGIN AND POTENTIALS OF THEIR USE IN SURGERY

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Abstract:

One of the possible ways to expand the allogeneic biomaterial spectrum for restorative surgery is a structural modification of donor tissues. We have developed a technology of the physico-chemical treatment which allows to obtain porous biomaterials as well as to increase the period of their biodegradation following the implantation thanks to the structure modification of the initial tissues. Aim of the investigation. To study the biological properties of allogeneic porous biomaterials and outline the prospects for their use in restorative surgery.

Materials and Methods:

Two series of the experiments were carried out to achieve this goal. In the first series two types of the biomaterials varying in resistance to collagenolytic enzymes were subcutaneously implanted to 48 Vistar rats. In the second series the reconstruction of the eye anterior chamber angle drainage zone was performed with the aid of the porous biomaterial on 36 grey rats with modeled glaucoma. To study the dynamics of the morphological changes in the operated on zone, the rats were being sacrificed after 14, 30, 60, 90, 180 and 360 days following the surgery. The histological sections were hematoxylin-eosin stained as per Mallory and Van Gieson. There were also used transmission and scanning electron microscopy methods. In the second series tonographic methods of the investigation were as well used.

Results:

In case of the subcutaneous implantation during the early period after the surgery, the biomaterials were infiltrated by macrophages and in the following the collagenous fibres were subjected to a gradual enzymatic lysis and synchronically were substituted by the newly-formed ones. There is established a biomaterial degradation period dependence of the forming regenerate structure. In the first group of the animals a full substitution of the biomaterial was observed after 90 days following the implantation which led to the formation of the regenerate with a relatively loose collagen fibrous framework. In the second group the regenerate was being formed in later periods (120 days) and had a dense fibroarchitectonics. In the second series of the experiments the porous biomaterial in the reconstructed drainage zone of the eye anterior chamber angle was being impregnated in the early period by the aqueous humour which led to the normalization of the intraocular pressure. Thereafter the collagen fibres of that part of the biomaterial which faced the eye anterior chamber were not subjected to biodegradation. The walls of the biomaterial cells were being gradually covered by the regenerating endothelium and thus a drainage zone of full value was being formed. At the back part of the biomaterial there were taking place a degradation of collagen fibres and the substitution by the newly-formed tissue with a well-developed venous channel. The afore-said morphological changes led to the ophthalmotonus normalization and restoration of the optic nerve structure.

Conclusion:

The use of porous biomaterials exhibits promise for the development of new high effective restorative surgeries in different fields of surgery and ophthalmology.

P-101

A DECELLULARIZATION PROCESS IN TISSUE BANKING

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Abstract:

Decellularization is an important tool when we consider the tissue engineering area but it can also plays a significant role to improve the service offered in a tissue bank. This process is based in the complete elimination of the cellular component although maintaining the extracellular matrix as intact as possible. Most of the decellularization processes are linked to the use of a detergent, as a chemical method to ensure cell death, and they are followed by a washing step to remove the cellular material. It has been described that putative remains of the detergent in the decellularized scaffold could have an adverse effect in cellular recolonization. We have optimized a decellularization protocol replacing the use of a detergent component by a combination of hypotonic and hypertonic solutions. Histological techniques and viability assays have shown that it could be possible to get a complete decellularization avoiding the use of detergents. This modified protocol has been applied in several tissues such as cornea, muscle, heart valve, tendon, pericardium, bone, sclera... According to the sample size, the time in the decellularization solutions has varied between short times for smaller and longer times for bigger ones. This protocol has kept the same decellularization results on the different tissue sources. In the next step, it's needed to evaluate the recellularization potencial of these acellular scaffold obtained. The main goal is that the implanted decellularized tissue has biological activity and mechanical integrity to support the cell migration from the surrounding tissues. An additional option it's to maintain the acellular scaffold "in vitro" with a cell culture obtained from the own tissue receptor. A tissue bank has to adequate the new science tools to improve his service in order to ensure the quality of his products. We have found that this decellularization process may possibly be the key to the most immediate future.

CORNEA

P-102

ASSESSMENT OF SUBJECTIVE PARAMETERS OF PATIENTS WITH AUTOLOGOUS VS HETEROLOGOUS EYE DROPS TREATMENT IN OCULAR PATHOLOGY

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Introduction:

Autologous serum is an effective method to stimulate viability of corneal-conjunctival epithelial cells, due to its content in growth factors, which are shortfall in dry eye syndrome associated with ocular surface diseases (OSD). It's considered the last therapeutic stage after numerous pharmacological options. Because of the impossibility of extraction in some patients, it might be done with donor's heterologous serum.

Objective:

Evaluation of the effectiveness of the autologous Vs heterologous eye drops in patients suffering OSD.

Material and Method:

Retrospective observational study using a validated OSD status self-assessment questionnaire, completed for the each patient and comparing before and after the treatment score, of 18 different items (red eyes, lid inflammation, flakes in lashes, sticky

eyes in the morning, discharge, dry eye, sandy and foreign body sensations, burning, itching, discomfort, pricking, tearing, teary eyes, photophobia, transitory blurring vision, lid reddening and heavy sensation).

Results:

Study sample has two groups: heterologous serum eye drops (28 patients: Sjögren syndrome 16 and neurotrophic Keratopathy 12) and autologous serum eye drops (33 patients: Sjögren syndrome 23 and neurotrophic Keratopathy 10) Mean age is 68,36 (SD= 14,66) years in heterologous group and 61,67 (SD= 15,94) in autologous group. Proportion according to sex is 6/28 men and 22/28 women in heterologous group and 5/33 men and 28/33 women in autologous group. Mean questionnaire scoring before the treatment for each group is 35,18 (SD= 11,33) in heterologous group and 41,24 (SD= 16,95) in autologous group. There are not statistical significant differences between groups for these variables. Mean administrated vials is 21,86 (SD= 22,35) in heterologous group and 55,68 (SD= 58,79) in autologous group, (p= 0.0058).

Exclusion causes for autologous serum eyes drops elaboration are: 15 cases of impossibility for extraction (53,57 %); 7 HCV+ (25 %); 3 HBV+ (10,71%), 1 HIV+ (3,57%) and 2 cases of hematologic pathology (7,14%)

Subjective improvement defined as the difference between the mean punctuation before and after the treatment results statistically significant with $p < 0.0001$ in each group, without differences between them ($p = 0.52$)

Conclusions:

According to the results found in our study, the heterologous serum eye drops is as beneficial as the autologous serum eye drops for symptomatic improvement in OSD patients. Thus we can consider it as an effective treatment option in patients with impossibility for autologous serum eye drops elaboration.

P-103

PROSPECTIVE DEVELOPMENT OF EYE BANKING IN THE RUSSIAN FEDERATION

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Abstract:

In connection with existing problems of sampling and procurement of cadaver human corneas all over the Russian Federation less than 3000 keratoplasties are performed annually, however a real demand in transplantation treatment of patients with corneal pathology is more than 15 thousand operations per year. Absence of Eye Tissue Banking Association in the Russian Federation the same like EBAA and EEBA, absence of united standards for algorithm of donor corneas procurement, unsettled informative, normative, legal, methodological and medical technological aspects of the Russian Eye Tissue Banks activity in provision of transplantation operations with cadaver human corneas do not allow to use possibilities of ophthalmic transplantation in a complete volume. The Eye Tissue Bank of the S. Fyodorov Eye Microsurgery Federal State Institution is established in 1988, has a 23-year experience of the work, fundamental investigations in the field of most problematic divisions of ophthalmic transplantation and serves as an organizational methodical center at the Russian Society of Ophthalmologists, renders a practical assistance to existing and newly creating Eye Tissue Banks in the Russian Federation. Since 1995 the Eye Tissue Bank of the S. Fyodorov Eye Microsurgery Federal State Institution is a Full Member of the European Eye Bank Association. The Section of Eye Tissue Banks and Cell Technology was established by the decision of the Board of the Russian Society of Ophthalmologists in 2009. In the Executive Committee of the Section 14 leading specialists in the field of ophthalmology and transplantation were included from different institutions and cities of the Russian Federation. The Chairman of the Executive Committee (Professor S.A. Borzenok, M.D., Ph.D.) and Executive Secretary (Dr. Y.A. Komakh, M.D., Ph.D.) were approved by the decision of the RSO Board as well. Main trends of efficient activity of the Section are an exchange of information and coordination of scientific and treatment institutions activities for an increase of level in research, educational and organizational methodic work, developments and improvement of medical technologies and a

normative legal base of cadaver tissue donor regulations and cell technologies, an ophthalmic care in the field of surgical treatment of corneal pathology and anterior segment of the eye. The Section is organized with the purpose to realize basic trends of development for the Russian ophthalmic and transplantation science and practice in the field of tissue donor selection and transplantation, cell technologies and surgical treatment of corneal pathology and anterior segment of the eye.

P-104

MARKERS TO IDENTIFY LIMBAL STEM CELLS ON HUMAN AMNIOTIC MEMBRANE

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Abstract / Objectives:

To evaluate the culture of a limbal biopsy on human amniotic membrane (HAM): directly on the chorionic side and on intact epithelium, and the expression of the stem cell associated markers: ABCG2, p63.

Material and Methods:

Eyes are collected within 24 hours post-mortem from human cadavers, with permission. The limbal epithelial fraction is scraped of and collected and sent to the unit of cryobiology in antibiotic solution (DMEM, 1% Penicillin and Streptomycin). Human placentas are obtained from Caesarean Section. The amnio is separated from the chorion of the placenta and then incubated in 199 medium with antibiotic solution for 24h at 4°C and cryopreserved. Two biopsies are carried out directly in fresh after the extraction of the limbal biopsy. In 13 biopsies, the biopsy of the limbus is placed in a culture dish and exposed for 5-10 minutes to Dispase (1.2U/ml) at 37°C. The amnio is placed on a Cell Crown insert that permits immobilization of the sample. Then the biopsy is placed in the middle of the amnio directly on the epithelial side (9 biopsies) or on the chorionic side (4 biopsies). Histological and Immunohistochemical studies are performed to evaluate certain markers: p63 and ABCG2, directly in fresh limbal biopsies and in limbal biopsies after expansion on HAM.

Results:

Fresh limbal samples show that the limbus had a multilayered epithelium with the basal layer arranged in a palisade pattern and that the palisades of Vogt in the superior limbus are structured with papilla-like columns. Histological studies show that both the chorionic and epithelial sides of the HAM showed a characteristic pattern with 1-2 layers of cell growth. However, on the chorionic side the monolayer of cells is sometimes not attached directly to the membrane causing the release of limbal epithelial cells cultured upon it. In our study, in fresh and after 3-4 weeks, the p63 protein was immunodetected in the nuclei of the limbal epithelial basal layer, but not in most limbal suprabasal layers and the ABCG2 transporter protein is primarily immunodetected in the cell membrane and cytoplasm of certain limbal basal epithelial cells, but not in most limbal suprabasal cells.

Discussion:

Our analysis confirmed that a normal limbal epithelium is formed on the HAM. This epithelium is 1-2 cell layers thick. The basal layer of cells shows high expression of the putative limbal stem cells markers p63, ABCG2 and intact HAM is a good substrate to culture limbal stem cells but on the epithelial side preferentially and not on the chorionic side.

P-105

CIRCUIT FOR IMPLEMENTING A DONATION OF EYE TISSUE

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Abstract / Introduction:

To avoid possible loss of donor corneas in our hospital developed a system for detecting all exitus. This defined a channel circuit to the warnings of the patients who died in asystole and the family does not know the donation.

Objectives:

Increase cornea donation in a tertiary hospital. - Identify potential loss of organ donors **METHODOLOGY** We designed a circuit to give notice to the coordination of transplant nurses, in charge of the program on a schedule (Monday to Friday from 8h to 15h) and providing the number of seeks. Also designed a brochure that was included in the folder of the patient for more knowledge. The circuit was circulated at the meeting to supervisory and emergency room attendants who provide death certificates in our hospital. The supervisors explained the circuit to staff their units both physicians and nurses. The nurse transplant coordination to notice, assess the case and if there is no contraindication to the interview should be familiar to the application of the will of corneal donation. If the family agrees the documentation is completed, blood samples are obtained and alerts the ophthalmologist for removal.

Results:

In one year (June 2010 - June 2011) of operation of the circuit is detected 40 warnings, 37.5% accepted the donation and 62.5% did not become a donor for several reasons: a refusal rate of 35% and 27.5% according to our protocol of contraindications for donor selection of fabrics: 2 older than 80 years and multimorbidity, 6 hematology diseases, 1 pseudomonas colonized by HIV and MDR-1. Notice I made the flight attendants 77%, 10% nurses, medical staff 7.5% and 5% nursing supervision. For slots, from 9am to 11am, 57%, from 11h to 13h 12% 13h to 15h 30% of the ads. Were obtained from 15 donors and 30 corneas taken real.

Conclusion:

Activation of a multidisciplinary program detection alerts the patient died in-hospital avoids the loss of potential cases and increase the donor pool because many families unaware of the possibility of donation by various criteria such as age, underlying disease or other.

P-106

EVOLUTION OF OCULAR TISSUE COLLECTION IN AN ACUTE CARE HOSPITAL FROM 1995 TO PRESENT DAY

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Introduction:

Social workers meet with the families after a death that has occurred at our centre. This means that other of their responsibilities is to ask about eye tissue donation when there are not medical contradictions.

There have been changes in the criteria for acceptability of eyes tissue causing more rejections of the potential donors.

Objective:

To analyse differences between 4 periods based on changes in the acceptance criteria of eye tissue and socioeconomic and cultural changes that may affect the donation, with the hypothesis that despite using more restrictive criteria have achieved more donations.

Material and Method:

nine months of 1995, 1999, from April 2005 to March 2006, and the first half of 2011 (daytime only) make up the four periods mentioned. Age and sex from the prospective donor, effective donation, and type of ceremony (burial vs. cremation) were collected. We used descriptive statistics, Chi2 and ANOVA.

Results:

There have been collected data from 1277 requests for corneas of 1946 deaths, which were obtained in 4 periods totalling 16 years. The mean age of potential donors was 73 years (SD 15), with differences between the fourth quarter with the previous periods (74 vs. 71, $p = 0.001$). Tendency to increase the number of women 40% in the first period to 48% in the last ($p = ns$). By changes in selection criteria, they asked 100% in the first period, 78% in the second, and 33% and 42% in the third and fourth respectively ($p < 0.001$). The donation rate increased from 10% in the first period, 15% in the second, 20% in the third, and up to 37% in the fourth ($p < 0.001$). The cremation increased significantly along the 4 periods, from 10% in the first three periods to 37% in the fourth ($p < 0.001$). The donation was significantly higher in the cremation group than in the burial group (39% vs. 12%, $p < 0.001$).

Conclusions:

Despite more stringent criteria and fewer requests, the donation rate has increased significantly. The people who were cremated were more likely to donate than the buried people and this may be a major cause of increased donations.

P-107

DONOR AGE AND CORNEA ENDOTHELIAL CELL DENSITY, INFLUENCE ON TRANSPLANT OUTCOME

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Abstract:

More than 5000 corneas are retrieved annually in Spain. In the Basque Country are performed approximately 200 cornea transplants per year. Whether donor age should be used to determine suitability of a cornea for transplantation has been a subject of international controversy among corneal surgeons. In our environment more than half of the cornea donors are older than 60 years. The cornea transplant requirements will increase in coming years due to population aging. According to national and international standards each tissue bank may establish its own criteria on the age limits of donor corneas. These age ranges depend on the therapeutic purpose it is intended for and the assessment methods available in each bank.

Objective:

To determine whether cornea endothelial cell density (ECD) and/or donor age are predictive of graft failure, and to analyze distribution of endothelial cell densities among several cornea donor age subgroups in our population.

Materials and Methods:

Donor corneas were procured and evaluated according to standards procedures of our eye bank, including assessment of ECD by specular microscopy. Corneas were retrieved from 2005 to 2010, the corneoscleral buttons were stored at 4°C or by organ culture at 31°C. The ECD of all analyzable images was independently determined at least by 2 readers using the

variable frame analysis method. The final ECD was the mean of all ECDs. We made a retrospective analysis of keratoplasty procedures performed in one hospital by three surgeons from the years 2008 to 2010. Statistical evaluation of baseline ECD among donor age groups was performed using Pearson correlation test.

Results:

Between January 2005 and December 2010, 840 corneas were received and evaluated in our eye bank. Eligible corneas were from donors 2 to 80 years old with a measured endothelial cell density from 1235 to 4505 cells/mm². Corneas from 2- to 60-years old donors had a median baseline cell density (2731 cells/mm²) higher than that of corneas from donors 66 to 80 (2404 cells/mm²). Median ECD was 2516 cells/mm² for the graft failure cases and 2641 cells/mm² for the nonfailure cases (p=0.17). Although donor age correlated significantly with endothelial cell density (r=- 0.56), this association appears to have no effect statistically significant on the transplant outcome.

Conclusions:

The ECD declines with age in the normal cornea, however cornea ECD and donor age are unrelated to graft failure. Our results indicate that older donor corneas could be considered suitable for transplant.

P-108

ANALYSIS OF FACTORS AFFECTING THE VIABILITY OF CORNEAL TISSUE

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Abstract / Introduction:

Allogenic corneal tissue transplantation is an effective therapy for restoring vision in patients affected for inherited or acquired cornea diseases. Medical, technical and scientific activity of tissue banks can be considered, after donation, the main actor to provide corneas for this medical treatment. Major concern in tissues bank is to make better use of donated tissues minimizing the proportion of grafts rejected for transplantation. Banc Teixits is the division responsible for the procurement, processing and distribution of tissues of the Banc de Sang i Teixits (BST). Purpose Examine exclusion causes which determined corneal graft rejection for transplantation in the BST. Identify critical points to increase the number of viable tissues for clinical use.

Material and Methods:

We performed a retrospective review of the corneas received in BST from January 2009 until June 2011. We have evaluated the following parameters: age, ischemia time, whole-globe enucleating versus cornea retrieval and causes of rejection. The causes of rejection were divided into: medical history, serologies, microbiology, technical incidence and tissue quality control.

Results.

The number of corneal tissue donors were: 202 in 2009 (394 total corneas obtained with a 26.9% rejection), 245 in 2010 (472 corneas with a 30.7%) and 134 donors during the period January-June 2011 (266 corneas with a 25.1% of rejection). The median age of cornea donors was 67 years (2-81) and total ischemia time was 6 hours (1-8) in average. Whole-globe enucleating versus cornea has varied from 49 % in 2009, 62% in 2010 to 76% in 2011. Regarding the causes of rejection, which has suffered major variation is tissue quality control (QC), representing the 48 % of rejections in 2009 (51 corneas), 36% on 2010 (53 corneas) and 33% in 2011 (22 corneas). In the period studied during the 2011, QC rejection causes were: 40% stromal edema, 26% endothelial damage, 22% epithelium defects, 7% inadequate free diameter and 5% injury during retrieval.

Conclusion:

During the period 2009-2011 our organization has been able to increase the number of transplanted corneas. Analyzing this improvement we identified the decrease of rejection by QC as a major cause although not having a significant higher

number of cornea donors. Among all the variables studied; age and ischemia time variables were constant parameters. Increased whole globe enucleation in front of cornea button excision seemed to directly influence the enhancement of viable corneas registered. Finally, protocolized cornea culture, optimized cornea allocation avoiding caducity as well as the advantage offered by new surgical techniques has permitted the transplant of a higher number of corneas. We believe that improvements at any step of the process including screening of the donor, retrieval, processing and client services can reduce the number of corneas rejected and thus increase the number of transplants.

P-109

**COMMUNICATION BETWEEN TISSUE BANK AND IMPLANTING SURGEON:
SHORT-TERM FOLLOW-UP OF TISSUES FOR OCULAR SURGERIES**

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Abstract / Introduction:

Traceability of tissue grafts in recipients is a crucial tool to assess the quality of distributed tissues by tissue banks. Follow up forms result extremely useful to know the evolution of patients and if possible incidents such as adverse effects or reactions occur in a short term basis. Through follow up forms the tissue bank is also informed about other parameters such as clinical diagnosis, characteristics of the recipients and microbiological issues which are also of a great importance for both traceability and for the tissue bank.

Purpose:

This study is focused on a short term monitoring of ocular tissue transplanted. To conduct this study, specific follow up forms have been sent together with the distributed graft. Follow up forms have been reported back by transplant centers to the tissue bank and data has been collected for analysis between April 2010 and April 2011.

Results:

During this period, 46 scleral grafts, 312 amniotic membrane grafts and 644 sclero-corneal buttons have been distributed. Response through follow up forms was 21.7% for scleral tissue, 15.7 % for amniotic membrane and 40.4 % for corneal buttons. All scleral grafts forms reported refer to patients with glaucoma to which grafts have been used to covering drainage tube surgery devices. Regarding amniotic membrane grafts, main indications were ulcer, chemical burns and corneal perforation. In corneal buttons, Penetrating Keratoplasty was the main use reported. However, during 2011, Anterior Lamellar Keratoplasty has significantly increased. Transplant indications were mainly corneal edema, different type of corneal dystrophies, corneal opacities and keratoconus. Microbiological control was varied depending on the reported tissue: no evidence of microbiological tests regarding scleral grafts, in amniotic membrane tissue 32 % of the centers reported negative microbiological tests while 52 % did not conduct any test or did not answer. For corneal buttons, 67 % reported negative results, 5.7 % were positive and 27.4 % did not conduct any test or did not answer. Postsurgical information about the evolution of the patients was very similar for the three studied tissues resulting in 98.46 % of reported satisfying evolution.

Conclusions:

The analysis of follow-up forms is very helpful to improve traceability of transplanted grafts. Unfortunately, it has been observed that only 31.5% of the follow-up forms have been reported. This fact indicates that communication between transplant centers and tissue banks need to improve in order to achieve a complete feedback of transplanted tissues and, therefore, permit tissue banks to offer adequate, safer and high quality tissues.

P-110

A COMPARISON OF DIFFERENT CULTURE MEDIA FOR THE STORAGE OF HUMAN DONOR CORNEAS FOR GRAFTING

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Abstract / Purpose:

To compare the influence of different culture media on the qualitative and quantitative endothelial parameters of human donor corneas stored in organ culture for grafting.

Methods:

Thirty-one paired human corneas obtained from the Ocular Tissue Bank Prague were stored in organ culture for 28 days at 31°C in the following media: Minimal Essential Medium with Earle's salts and 2% Foetal Bovine Serum (EMEM)/EMEM with 5% dextran prepared by the bank staff, commercial media adhering to Directive 93/42/EEC: a) TissueC/CarryC (Alchimia, Italy) and CorneaMax/CorneaJet (Eurobio, France). Comparisons of EMEM vs. Alchimia (G1) and EMEM vs. Eurobio (G2) were performed on paired corneas. Corneal transparency, endothelial cell density (ECD), live endothelial cell density (LECD), the percentage of dead endothelial cells (% DC), their hexagonality (6A) and coefficient of variation (CV) were assessed. The assessment was performed before storage (1st assessment), at day 28, i.e. before transfer to deswelling medium (2nd assessment), and after 24 hours in deswelling medium (3rd assessment). The evaluations were performed from phase contrast and bright field photographs using a semiautomatic computer analysis system. A two-tailed Student's t-test was used for the statistical analysis. P values <0.05 were regarded as significant.

Results:

The mean ECD in the 1st assessment for corneas in G1 was 2844±572 (EMEM) and 2787±531 (Alchimia) and in G2 2823±392 (EMEM) and 2821±444 (Eurobio) cells/mm². The transparency of corneas stored in EMEM and TissueC allowed microscopic assessment before transfer to deswelling media. The corneas stored in CorneaMax were too edematous for microscopic evaluation. In the 3rd assessment, no statistically significant differences (p=0.45 for G1 and 0.37 for G2) in mean ECD were observed (2538±484 vs. 2559±442 cells/mm² in G1 and 2545±409 vs. 2592±380 cells/mm² in G2). In the 3rd assessment, there was a difference in % DC (0.62 vs. 1.72 in G1 and 0.22 vs. 1.20 in G2). No significant differences in mean 6A and CV were found in either comparison.

Conclusion:

The relatively high % of DC in both commercial media may be explained by the presence of persisting remnant dead cells due to the slower reparative capacity of the corneal endothelium under these conditions. However, the obtained results indicate that both commercially available media are comparable to standard EMEM in the main qualitative and quantitative parameters of stored corneal endothelium and can be used for organ culture storage instead of media lacking the appropriate certification required by European Directives.