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Conferences and Abstracts from the

2ND WORKSHOP IN CRYOBIOLOGY OF MEDICAL SCIENCES

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The conferences and abstracts from ***2nd Workshop in Cryobiology of Medical Sciences*** have been revised and evaluated by a scientific committee

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C.1.
FUNDAMENTAL ASPECTS OF LOW-TEMPERATURE BIOLOGY

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Cryopreservation is a core enabling technology in the application of many developments in biosciences. It is currently the only process for intermediate- and long-term preservation of viability and function for a wide variety of living cells. During cryopreservation, cells are exposed to interacting osmotic, thermal and toxic stresses which are dependent on time and temperature. Different cell types have different characteristics, resulting in differences in cellular responses and tolerance to these conditions.

As ice grows in an aqueous solution, solutes concentrate in the residual liquid, increasing the osmolality in the suspending solution. This imposed difference in osmotic pressure across the plasma membrane results in water efflux at a rate dependent the hydraulic conductivity of the plasma membrane, a characteristic of the cell describing the rate of water movement in osmotic transport. Cells with high hydraulic conductivity, such as erythrocytes, can respond rapidly to changes in extracellular osmolality, whereas cells with lower hydraulic conductivity, such as lymphocytes, respond much more slowly. If the cell suspension is being cooled at a rate where the extracellular osmolality is increasing more rapidly than the cells can respond osmotically, then the water in the cytoplasm becomes increasingly supercooled and will nucleate to form ice inside the cell. Intracellular ice is almost always lethal.

Conversely, the cell suspension can be cooled sufficiently slowly to allow osmotic water efflux and avoid the likelihood of intracellular freezing. However, exposure of cells to the high concentrations of solutes, particularly electrolytes, for longer periods of time is also damaging. Conventional approaches to cryopreservation attempt to minimize these two primary damaging conditions by using a cryoprotectant, such as dimethyl sulfoxide, to buffer the increase in electrolyte concentrations inside and outside the cells, and cooling at the highest rate that avoids intracellular freezing for that cell type.

Recent thermodynamic analyses have shown that cooling at a constant rate (a linear temperature profile) is not the most effective strategy for achieving the goals of keeping intracellular supercooling below the level where intracellular freezing becomes likely, while minimizing the time of exposure to the concentrated solutes. Using osmotic transport properties specific cell types and computer simulations, nonlinear temperature profiles are being developed that result in high cell recovery while using lower concentrations of cryoprotectants. Computer simulations allow design of custom cryoprotective strategies using the characteristics of specific cell types.

The requirement for increased understanding of cryoinjury and cryoprotection will continue to increase as applications in biosciences are being developed where preservation of diverse characteristics of biological materials is critical.

C.2.
CRYOBIOLOGY OF TISSUE SYSTEMS

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Tissue transplantation is being used increasingly as an effective and economical intervention for a wide variety of clinical conditions. Generally, viable tissues are required for optimal function after transplantation, and cryopreservation is often the only method for preserving physiological structure and function of banked tissues. However, our ability to cryopreserve tissues has lagged significantly, so interest in cryopreservation of tissues continues to expand as more tissues are considered for transplantation. Although some tissues are currently being cryopreserved for transplantation, the range of applications is limited and the viability after transplantation is often uncertain.

Engineered tissues that are being developed for a wide variety of biomedical applications have low-temperature sensitivities that are similar to natural tissues, creating additional challenges for cryopreservation as routine clinical applications emerge. The potential for toxicology testing using engineered tissues such as cornea, liver and skin has also increased the imperative for effective methods for preservation of engineered tissue constructs for distribution.

Osmotic and biophysical responses of cells at low temperatures are important for understanding the responses of individual cells within the tissue matrix, so, although cellular studies provide a starting point for understanding low-temperature responses of the cells within a tissue matrix, this is not sufficient to describe tissue responses. Heat and mass transfer within the tissue are important factors. Diffusion of water and cryoprotectants depend on time, temperature, the nature of the cryoprotectant, and on the properties and dimensions of the tissue. Different cells types within the tissue have specific characteristics relevant to cryopreservation, and cell-cell and cell-matrix interactions contribute additional dimensions to tissue responses. It is becoming increasingly clear that the amount, location, and morphology of ice in the matrix are important determinants of post-thaw function. Consequently, vitrification is currently the most promising approach tissue cryopreservation, using concentrations of cryoprotectants sufficiently high to form a glass rather than ice in the tissue at low temperatures.

Experience over the past few decades has demonstrated that low-temperature responses of tissues are too complex for purely empirical development of effective cryopreservation protocols, as has been done for many cells in suspension. Increased understanding of the complex interactions in tissues is required to develop effective strategies for tissue preservation.

C.3.

HYPOTHERMIC ORGAN STORAGE FOR TRANSPLANTATION, AND THE HUMAN TISSUE ACT - THE 'DEVIL IN THE DETAIL

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A series of news headlines in the UK between 1997 and 2000 uncovered what until then had been a very 'grey' area in British medicine – who had the right to decide about access and storage of human tissues for *post mortem* pathological assessment, what information patients or relatives were entitled to receive, and who should be allowed to use the tissues for clinical research or teaching. Transplantation services, which had always operated on a basis of informed consent from deceased patients' relatives for organ donation, were suddenly caught up in a 'bystander' effect – public confidence collapsed in the organ donation process. The British Government decided that the only way out of the confusion was to enact a new legislation by 2004 – the Human Tissue Act. Their goal was to provide transparent regulations surrounding access and storage of human body parts or tissues for any reason, including organ and cell transplantation, pathology, teaching, research, whilst enhancing and facilitating the solid organ transplant services which had developed over 20 years. This paper will discuss how the implementation of the Human Tissue Act has worked within traditional cadaveric organ procurement in the UK, and some unexpected outcomes.

C.4.

NEW ASPECTS IN CLINICAL AND EXPERIMENTAL ORGAN PRESERVATION FOR TRANSPLANTATION

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Organ preservation in clinical services across Europe over the past 20 years has developed around cold hypoxic flush. The growing pressures from organ shortages has led recently to several new approaches, including use of organs from older or less physiologically-fit donors (marginal donors) and non heart-beating donors (NHBD) where variable periods of warm hypoxia have preceded cold flush. These new approaches have necessitated the development of preservation methods capable of repairing at least some of the prior hypoxic damage in the NHBD donor, or identifying irreversibly-damaged organs. Continuous hypothermic perfusion preservation (HPP) was the method of organ preservation favoured during the early development of transplantation services, but was gradually replaced on the basis of cost and logistics. Recently, new technological developments have re-awakened interest in HPP, and the growing evidence indicate that HPP can be of benefit in the NHBD situation. This review will discuss the early development of HPP, the basic principles underlying its' application, and recent outcomes where HPP has been used.

1.

LIPID-PROTEIN INTERACTIONS GOVERNING COLD SENSING

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Bacillus subtilis, a gram-positive bacterium, frequently encounters stress conditions in its natural environment, the soil. In order to detect and respond accordingly to these stressful conditions, it employs two-component signal transduction systems. Changes in the growth temperature or nutrient availability alter the membrane fluidity, and B. subtilis overcomes this situation by regulating the proportion of unsaturated fatty acids. The B. subtilis Des pathway has been identified as the system that mediates this response, and is the first described system that senses changes in membrane lipid fluidity. It is composed of a membrane histidin-kinase, DesK, a soluble response regulator, DesR, and the effector enzyme, Δ5-desaturase.
This study aims to unveil the mechanistic details by which changes in the membrane fluidity due to temperature variations regulate the signaling state of the sensor protein DesK. The dissection of this molecular mechanism will provide important insights into the basic question of how the DesK transmembrane segments sense the lipid environment. This information might also be used to understand the role of membrane fluidity in bacterial pathogenesis.

2.

CRYOPRESERVATION ALTERNATIVE METHOD OF NERVOUS TISSUE FOR IMMUNOHISTOCHEMICAL STUDIES ON FREE FLOATING SECTIONS

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In CNS histological studies, free floating sections of perfusion fixed samples are frequently used. Samples storage may be performed freezing either the entire brain at -80ºC or sections at -20ºC. When studying hypothalamic Tyrosine Hydroxylase enzyme (dopaminergic neurons marker) by immunohistochemistry in Wistar rats exposed to 2,4 -dichlorophenoxyacetic acid (2,4D), entire brains were stored at -80ºC. Due to an abrupt freezer technical fail, samples should be thawed to -20ºC with the resulting samples damage. To avoid this situation, subsequent brains were stored in 30% sucrose in saline phosphate buffer (PBS) with 0.01% sodium azide and kept at 4ºC for different periods (weeks to months) until they were frozen with chlorofluorade gas and cut. These brains showed no morphological alterations of tissue structure. This preservation method appeared to be an alternative valid option to laboratories with no -80ºC freezing equipment.

3.

MECHANICAL TESTING OF CRYOPRESERVATED TISSUES

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The Laboratory of Biomechanics of the Testing of Materials Institute of the Uruguayan Engineering School has ongoing biomechanical research for the last 25 years. First about fixators employed in osteosynthesis and now also on the characterization of biological tissues like bones, tendons and vascular tissues. A multidisciplinary group with physicians, chemists and statistical and mechanical engineers was integrated for that purpose. Research of biological tissues is carried out together with the National Organs and Tissues Bank (Instituto Nacional de Donación y Trasplante de Células, Tejidos y Órganos). All materials are provided from cadaver donors. The objective is the biomechanical evaluation of tissues to be used as allografts and the improvement of preservation methods. Elastic properties are determined for example in compression, tensile and bending tests. Sample extraction and preparation, equipments, testing procedures and some results for tendons and bones are detailed. Evaluation of fresh and cryopreserved vascular tissues is described and conclusions about their biomechanical difference between them are drawn.

4.

PROTECTIVE EFFECT OF A CARBON MONOXIDE-RELEASING COMPOUND (CORM3) IN COLD STORED RAT LIVERS. A STUDY IN THE ISOLATED PERFUSED LIVER MODEL

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A direct role of carbon monoxide (CO), an effector-signaling molecule during heme oxygenase-1 (HO-1) catalysis of heme, in the protection against hepatic cold ischemia/reperfusion (I/R) injury needs to be established. We design a study to analyze the role of CO in the maintenance of hepatocyte functionality and integrity. We use an isolated perfusion rat liver system (IPRL) in a clinically relevant model of *ex vivo* 48 hour cold ischemia in a modified UW solution to determine the impact of exogenous administration of CO on hepatocyte injury, regulation of vascular resistance, and liver function. These findings suggest that CORM3 could be included therapeutically in UW solution as an efficacious strategy to prevent the injury sustained by organs (Heart, Kidney, livers, etc.) during Cold Storage prior to transplantation.

5.

CRYOPRESERVED ALLOGRAFT VASCULAR TISSUES: A STRUCTURAL ANALYSIS BY X-RAY DIFFRACTION

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To determine any variation that could arise due to the cryopreservation processes at molecular level by x-ray diffraction analyses on human samples blood vessels specimens, to check the fibre organization. Difractometric profiles was performed on cryopreserved-thawed (C) vs fresh (F) aorta and carotid blood vessels from 17 informed consent cadaveric donors with Seifert Scintag PAD-II powder diffractometer. (CuKα radiation; λ = 1.5418 Å). Scans in the 5-60º range in 2-theta, with steps 0.1º and 10 second per step have been performed. 10 aortic and 8 carotid diffractometric profiles were analized, with differential planimetric patterns measured under x-ray diffraction curve. Ordering Pattern Coefficient (OPC) was made by the quotient between the planimetric surface of (F) vessel ordering diffraction, and the (C) one. Clears peaks at d-spacings 2.86 Å and 2.15 Å (2-theta = 31.3º and 42.0º, respectively) are always confirmed in spite of the different profiles of samples. OPC shows a higher ordering profile in (C) vs (F): 70% Aortas; 62,5% Carotids. Cryopreserver-Thawed procedure process employed has no damaging effect on the fibrillar organization of the vessels.

6.

SUBZERO NONFREEZING STORAGE OF RAT HEPATOCYTES USING UW AND 1,4-BUTANEDIOL.
I- EFFECTS ON CELLULAR METABOLITES DURING COLD STORAGE

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Various cryopreservation techniques have been investigated to extend the storage of isolated hepatocytes; however, most have a reduced viability after rewarming due to ice crystal formation. Subzero nonfreezing conditions could theoretically reduce organ metabolism without damage due to ice crystal formation. In the present work we evaluated the viability and metabolic parameters of isolated rat hepatocytes preserved in subzero nonfreezing condition. Cell suspensions were maintained in modified University of Wisconsin (UW) solution using 8% - 1,4-butanediol as cryoprotectant, up to 120 h at -4ºC. The time course evolution of hepatocytes viability were measured by LDH release and propidium iodide assay. The cellular concentrations of glutathione, ATP, glycogen and the lactate production during cold storage were also determined. Finally, results were compared with conventional hypothermic storage at 0ºC in modified UW solution without cryoprotectant. After 5 days of subzero storage, we found an improvement in the ability of rat hepatocytes to maintain the metabolic resources in comparison with the cold preserved group.

7.

SUBZERO NONFREEZING STORAGE OF RAT HEPATOCYTES USING UW AND 1,4-BUTANEDIOL.
II- FUNCTIONAL TESTING ON REWARMING AND GENE EXPRESSION OF UREA CYCLE ENZYMES

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In the present study we have analyzed the viability and metabolic competence of isolated rat hepatocytes subjected to subzero nonfreezing storage (up to 120 h at -4ºC) in modified University of Wisconsin (UW) solution with 8% 1,4-butanediol during the normothermic rewarming step (KHR media, 37ºC, up to 120 min, carbogen atmosphere). Results were compared with hepatocytes stored up to 120 h at 0ºC in modified UW solution and with freshly isolated cells. We have found that only cell suspensions stored in subzero nonfreezing conditions were able to finish the rewarming period with a viability comparable with the control group. Also, we have investigated the activities and the relative expression levels of two of the Urea cycle (UC) enzymes: Carbamyl phosphate synthetase I (CPSI) and ornithine transcarbamylase (OTC), during 60 min of rewarming. Results were compared with the ammonium removal efficiency of the three groups. These data indicated that hepatocytes preserved under cold or subzero conditions up to 120 h followed by 60 min of rewarming, maintain UC enzymes at levels similar to freshly isolated hepatocytes, allowing their use in bioartificial liver.

8.

HISTOLOGICAL FEATURES OF HUMAN VASCULAR TISSUES AFTER TEMPERATURE CHANGES DURING CRYOSTORAGE

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Cryopreservation temperature changes could induce morphological alterations on vascular tissues. We examined histological features of human aortic and pulmonary arteries comparing three groups: fresh, conventionally cryopreserved and cryopreserved which suffered temperature changes from –130ºC up to –47ºC and back to –130ºC. Aortic and Pulmonary arteries were histological processed to embedded them into paraffin, cut and stained with orcein dye. Fresh tissues showed numerous parallel elastic lamellas which appeared continuous without interruptions. Cryopreserved groups showed less amount of elastic fibers and the disorganized lamellas had interruptions. There were no histological differences between cryopreserved groups in both type of arteries. However, in these last groups we found that pulmonary arteries had more alteration in their elastic fibrous composition and organization than aortic ones. In conclusion, cryopreservation temperature changes has not induced extra damages to vascular tissue compared with the ones conventionally cryopreserved; but, pulmonary arteries seems to be more susceptible to cryopreservation process than aortic ones.

9.

VIABILITY AND FUNCIONALITY TEST IN COLD STORED CELLS AND TISSUES

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The development and application of hypothermic protocols to living cells and tissues are now widely used in areas of biotechnology and medicine, where the cells or tissues are from mammalian origin (i.e., hepatocytes. Therefore it is very important to know the functional cell responses to low temperature preservation protocols and how these may affect the overall outcome. From this requirement has arisen the need to assess "cell viability" which, because it is rather general term that should express the quality of the preserved tissue. Viability, as was defined by David Pegg, "is the ability of a treated sample to exhibit a specific function or functions, expressed as a proportion of the same function exhibited by the same sample before treatment or an identical fresh sample". In the field of cell/tissue control quality the ideal assay for cell viability is one which would be easy and rapid to perform, which can be used in the several situations described above, which reflects a vital cell function and which would be sensitive to even minor alterations of such function. As it has been pointed out there is no single assay that fulfils all these criteria. The use of a combination of viability assays can facilitate to evaluate how the preservation protocol affects viability. In the light of new findings into the cellular and molecular mechanisms of cold storage injury, since cold induces cellular programmed death, usually called apoptosis, it is necessary to include studies to asses apoptosis in protocols to determine viability and functionality of cells.

10.

IMPACT OF CRYOPRESERVATION TEMPERATURE CHANGES ON HUMAN AORTIC VALVULAR AND VASCULAR ALLOGRAFTS

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Cryopreservation of valvular and vascular allograft (VA) conserved its integrity, maintaining cell viability. Lethal damages could occur if the cryopreservation temperature is elevated. We compared three groups of aortic VAs: fresh (F), cryopreserved (C,-130ºC) and cryopreserved with temperature changes from –130º up to –47ºC and back to –130ºC (T). The three valve layers and fibroblasts but not endothelial or smooth muscle cells were seen. Collagen fibers were in the fibrosa, spongiosa and between spongiosa and ventricularis. Elastic fibers were abundant in the ventricularis. Glycosaminoglycans were only in the spongiosa, which had a great amount of fibroblasts. There were no differences between groups in cell composition or in fibrous content. VAFs showed organized fibers and no interstitial edema. There were no histological differences between VACs amd VATs. Sinotubular junction of VAFs showed continued and abundant elastic fibers. The other groups had disorganized and fenestrated elastic lamellas. In conclusion, temperature changes during cryopreservation period of human VAs induced no morphological differences compared with VACs.

11.

CRYOPRESERVATION OF PLANT GERMPLASM: THE CUBAN EXPERIENCE

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Cryopreservation is currently the only safe and cost-effective method for long-term storage of plant germplasm. In Cuba, the research on cryopreservation begun in 1994 at the Bioplantas Centre University of Ciego de Avila with the general objective to evaluate and develop feasible cryopreservation techniques for crops of great commercial importance of the province. In this sense, sugarcane and pineapple are considered examples. It has been possible by the scientific and financial support from the International Plant Genetic Resources Institute and the International Foundation for Science. In the case of sugarcane, a cryopreservation methodology for embryogenic calluses using a simplified procedure of slow cooling was established. The effect of cryopreservation on the structural and functional integrity of cell membranes and the field performance of plants both derived from cryopreserved sugarcane calluses has been studied. Recently, a droplet-vitrification procedure applicable to sugarcane somatic embryos clusters was developed too. In the case of pineapple, a vitrification procedure was used to cryopreserve apices sampled from in vitro plantlets and ovule excised from ovary of inflorescence, and simplified freezing process was applied to calluses. Moreover, the application of cryopreservation methodology for different accessions and related species has been tested successfully for pineapple apices.

12.

PARAMETERS OF QUALITY IN THE PROCESSING OF HUMAN VALVULAR TISSUES CRYOPRESERVATES

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We evaluate different quality parameters in the processing of the homografts heart valves (HHV) in the Heart Valve Bank of CRAI Sur CUCAIBA by a retrospective analysis of the processing of 212 hearts obtained by dissection in six periods (P), Aug-Dec1999(1), Jan-Dec2000(2), Jan-Dec 2001(3), Jan-Dec2002(4), Jan-Dec2003(5) and May-Dec.2006(6). The bacteriologic results at previous dissection (StageA) and the processing previous cryopreservation (StageB), efficacy of sterilization with antibiotics solution (anfotericine, cefuroxime, piperaciline and gentamicine) during 24 Hs and dissections defects (DD) were assessed. Processing technique was carried out in a restricted area with environmental flow class 10000, laminar flow vertical class 100 and registers of technician- processing.

P	Hearts	Stage A		Stage B	
	N	N	%	N	%
1	15	6	40	1	6.6
2	45	16	35.5	0	---
3	43	13	30.2	0	---
4	38	17	44.7	0	---
5	39	20	51.2	0	---
6	32	13	40.6	1	3.1

Table 1: Bacteriologic isolation in hearts processed.

P	HHV	D D	
	n:	n:	%
1	30	0	---
2	90	0	---
3	86	4	4,6
4	76	2	2,6
5	78	0	---
6	64	1	1,5

Table 2. Dissections defects on HHV

Results suggest that analysed variables are quality indicators as they are capable to determinate the efficacy of sterilization by antibiotics solution, the absence of contamination in the stage previous of cryopreservation in an aseptic area and as they allow to evaluate the dissection process.

13.

WHAT HAVE WE LERNT CRYOPRESERVING 1800 HEART VALVES DURING 12 YEARS

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Cryopreservation of cardiovascular tissues is mandatory to prolong their lifespan. We began to cryopreseve heart valves in 1995 and since then we have processed more than 1.800. Adverse effects related to cryogenic procedures that determine the lost of valves during this period is presented. From 305 valves discarded, only 19 (1%) were relate to cryopreservation process itself. Taking in account related procedures as packaging and transport, another 17 valves were discarded reaching 2% of all valves. For each adverse event a corrective action was taken. It is very important to develop a quality control system to avoid unnecessary lost of heart valves that are unique, due to this process.

14.

SEARCHING A "FUNCTIONAL VIABILITY" CRITERION IN A TISSUE BANKING MANAGEMENT: 9 YEARS OF EXPERIENCE

Pérez Campos H[1], Saldias M[1], Silva W[1], Álvarez O[1], Machin D[1], Wodowoz O[1], Pérez N[1], Ferrin S[1], Acosta M[1], Do Santos T[1], Álvarez I[1], Faccio R[2], Suescum L[2], Mombru A[2], Melissari B[3], Deri E[3], Lantero J[3], Bia D[4], Armentano R[4,5], Zócalo Y[4], Pessana F[5], Graf S[5].
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Validation of hemodinamical vascular functions and structural scaffolds by comparison between cryopreserved/defrosted (C) and human fresh (F) arterial vessels produced for therapeutic purpose, from 17 cadaveric donors. Three sets of variables groups was analyzed: a) 8 structural destructive parameters by uniaxial transversal loading deformation test, in annular segments of descending aorta; b) 15 Physiological and physio pathological in vitro functional patterns, by hemodinamic, behavior vs in vivo correlated tests from normo tensive and hypertensive subjects. c) 18 test of Diffractometric x – Ray profile looking for fiver organization level by Ordering Pattern Coefficient (OPC) through the quitient between the diferential planimetric surface of (F) vessel ordering diffraction and the (C) one. Results and Discussion: a) No difference was seen between (F) and (C) thoracic descending aorta in structural destructive load-deformation tests; b) Similar hemodinamic behavior was shown the in vitro test for (F) and (C) arterial vessels. The in vivo comparison showed hemodinamic significative differences between hypertensive patients and both (F) and (C) cadaveric vessels; c) (C) arterial vessels had higher fiver organization than (F) measuring by (OPC). This results validate the quality of (C) cadaveric vessels for allogenic transplant.

15.

TREATMENT WITH DANAZOL FOR CIRRHOSIS-INDUCED THROMBOCYTOPENIA

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Hepatitis-virus related cirrhosis is a condition that carries along many complications, among them thrombocytopenia due to disturbances in platelet membrane function, portal hypertension and splenic platelet pooling. It has been also shown that a decreased thrombopoietin production might also promote the development of thrombocytopenia. Several therapeutic options have been employed for this condition but a standard treatment has not been established. Total and partial splenic embolization with the later being more likely to have fewer complications than splenic artery embolization does (complication rate of 50% vs 100%, respectively).
Danazol is an inhibitor of pituitary gonadotropin, derivative of C17 ethinyltestosterone with weak androgenic effects, currently used in the treatment of endometriosis, fibrocystic disease of the breast, idiopathic thrombocytopenic purpura and hereditary angioneurotic edema.

16.

HYDROCARBON BIODEGRADATION IN CONTAMINATED ANTARCTIC SOILS: AN EXAMPLE OF BIOTECHNOLOGICAL APPLICATION OF COLD-ADAPTED BACTERIA

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Antarctica is one of the most pristine sites of the world. However, the use of crude-oil derivative fuels caused the contamination of some restricted areas. Because the restriction imposed by the Antarctic treaty, only Antarctic bacteria can be used to develop *in situ* bioremediation processes. Here we proposed to isolate hydrocarbon-degrading psychrotolerant bacteria, characterise them at laboratory scale and apply them in bioremediation processes with Antarctic soils. Several *Rhodococcus* and *Pseudomonas* sp (having high aliphatic and PAHs degrading activity respectively) were isolated. A polyphasic (biochemical and molecular) approach showed that *Pseudomonas* and *Stenotrophomonas* were the dominant taxa in a bacterial consortium (M10). *In situ* bioremediation assays (using micro and mesocosms) showed that biostimulation is essential for improving hydrocarbon degradation. Bioaugmentation showed to be useful for treatment of acutely contaminated soils but not for chronically contaminated ones, where the natural microbiota are closely adapted to the pollutants. In conclusion, results confirmed that removal of hydrocarbons from Antarctic soils is feasible using "on site" bioremediation. However, the strategy of choice closely depends on the previous history of the polluted soil.

17.

ADAPTATIONS AND BIOTECHNOLOGICAL APPLICATIONS OF PSYCHROPHILIC BACTERIA

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Bacteria can proliferate all around the world under a broad range of environmental conditions. Understandings of the adaptation mechanisms that allow bacteria to grow under extreme conditions are very interesting from the biotechnological point of view. Among extremophiles, psychrophilic bacteria are those able to colonize cold environments. These environments challenge psychrophilic bacteria in different ways, being the most important the low thermal energy and the high viscosity of the aqueous phase. A decrease in temperature results in changes of the of fatty acids composition of the bacterial membrane (increase of the fraction of mono and poly unsaturated as well as methyl branched fatty acids). Cold-adapted enzymes are able of catalysis at low temperatures by destabilization of the active site or the entire protein structure. This adaptation also turns enzymes highly thermo-labile. Most of psychrophiles organisms use antifreeze proteins (AFPs) as a shield against the damage produced by large-size ice crystals. The cold adaptations mentioned above have several potential biotechnological applications. Enzymatic catalysis at low temperatures is one of the main advantages obtained from psychrophiles. The broad diversity of microorganisms in cold regions, including Antarctic and Arctic ecosystems, promises new developments in the biotechnological field for the near future.

18.

CRYOPRESERVATION OF PARATHYROIDS FOR CLINICAL USE

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The maintenance of both structural and functional characteristics of different cell types forming a tissue is a main objective of cryopreservation. The cryopreservation of parathyroids is carried out for the deferred transplantation of patients suffering from hypoparathyroidism following the total/partial resection of thyroid and/or parathyroid glands. The cryopreservation of parathyroids was set up at the Hospital Italiano de Buenos Aires for treatment of patients suffering from primary, secondary or tertiary hyperparathyroidism. The cryopreservation is performed with controlled-rate freezing equipment (60%DMEM, 10%DMSO, 30%autologous serum), and the storage is in gaseous phase of nitrogen. Histological and functional (Cl_2Ca stimulation and measure of PTH levels) analysis were carried out on cryopreserved (6 months) tissue. It was observed that the structure was well maintained after defrost, and PTH was found for more than 2h. However the Cl_2Ca stimulation produced a deleterous effects (alterations of principal and oxyphil cells, trabecular lost and apoptosis). The parathyroid cryopreservation seems to be a useful resource for patients suffering from hypoparathyroidism after thyroid and/or parathyroid resections. The establishment of proper conditions for the parathyroid cryopreservation is necessary in order to optimize the maintenance of both viability and functionality for the tissue to be transplanted.

19.

CRYOPRESERVATION APPLIED AT HUMAN HEART VALVES

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The clinical use of Cryopreserved human heart valves in liquid nitrogen at -143°C allows storage of prostheses for years maintaining graft viability. The principles of freezing drying applied to cell and tissue preservation are the same. The freezing drying require of penetrating cryoprotective agents. Their effect is change or stabilise ph-values, replace water molecules, reduce salt concentration. The cryoprotectant agent is present during the freezing process inside as outside the cells. In general in the cryopreservation of heart valves dimehylsulphoxide (DMSO) has been the preferred. Improved sterilization and preservation techniques could preserve maintain cellular and structural viability of heart valves. The cryopreservation techniques plays an important role in the prolonged functional durability. In adults patients especially these beyond 40 years of age have more prolonged functional durability with actuarial freedom from reoperation of 98% at 10 years. The results are less satisfactory in padiatric and under 40 years of age. In imunologically high risk patients under 40 years of age might need pulmonary autograft (non-immunogenic tissue) for aortic replacement. Cryopreserved allografts are antigenic and induce immune response. Presently allografts heart valve is preferred for aortic valve endocarditis.