C-2000

Inhibition of Ice Recrystallization and Gas Hydrate Formation Using Small Molecules. M. DOSHI, C. Capicciotti, D. Tonelli, and R. Ben. University of Ottawa, 10 Marie Curie, Ottawa, ON K1N 6N5, CANADA. Email: mdosh045@ uottawa.ca

Cellular damage as a result of ice recrystallization during thawing after cryopreservation is a contributing factor to cell death (*Cryobiology* 1998,37, 171-186). Our laboratory has previously shown that simple monosaccharides can inhibit ice recrystallization at a concentration of 22mM (*J. Am. Chem. Soc*., 2008, 130, 17494-17501). Recently, we have discovered that other classes of small molecules also exhibit the ability to inhibit ice recrystallization. One class of these molecules have the pyranose ring oxygen replaced with a nitrogen atom resulting in a compound with different electronic properties. These aza-sugars are also capable of inhibiting the formation of methane gas hydrates. Gas hydrates are problematic in high-pressure environments where methane is present, such as gas pipelines (*Chem. Eur. J.*, 2010, 16, 10409-10417). Several different aza-sugars have been assessed for ice recrystallization inhibition (IRI) activity, thermal hysteresis (TH) and the ability to inhibit gas hydrate formation. Structure-function studies of these small molecules have elucidated the structural features necessary for potent IRI activity and the ability to inhibit formation of gas hydrates.

C-2001

Survival of Short-lived Desiccation Tolerant Seeds During Long-term Storage in Liquid Nitrogen: Implications for the Management and Conservation of Plant Germplasm Collections. D. BALLESTEROS and V. Pence. Cincinnati Zoo and Botanical Garden. University of Cincinnati, 3400 Vine Street, Cincinnati, OH 45220. Email: dani.ballesteros@ cincinnatizoo.org

Ex situ seed conservation is a widespread and powerful tool for the preservation of plant genetic resources. However, long-term storage of short-lived seeds is challenging in standard seed bank temperatures (4 and -20°C), and cryopreservation has been acknowledged as the only effective option for their long-term conservation. However, as seed aging has been demonstrated during cryostorage, the potential and limits of LN storage of short-lived seeds need to be evaluated. We have examined the viability and growth of seeds from species with short-lived seeds stored up to 20 years in CREW's CryoBioBank®, including *Plantago cordata*, *Populus deltoides*, several *Salix* species, and two species in the genus *Betula*. We have found survival in short-lived seeds after >14 years of LN storage (-196°C). *P. cordata* seeds did not show a significant decrease in germination over this time. However, *Salix* sp. and *P. deltoides* accessions tested showed a final germination ranging between 0 and 96% of initial germination. Seeds of *P. deltoides* stored at 4 and -20°C did not show any survival after the same storage period. Seed viability in *P. deltoides* and *Salix* sp. was overestimated when measured as total germination, rather than normal germination. Furthermore, 38-43% of seedlings from normal seedlings of *P. deltoides* did not continue normal development during early growth of the young plants. In adddition, initial seed quality was correlated with seed longevity. These results suggest that: 1) monitoring of short-lived seeds cryostored as in this study (at 8-10% moisture) should be done at 2-5 year intervals, similar to the recommendation by IPGRI for seeds stored at -18°C, and regeneration should not exceed 10-15 years; 2) in order to minimize loss of seed quality prior to cryostorage, processing of short-lived seeds after harvest must be fast and gentle; 3) based on losses during early seedling growth and development, it is very important not to exceed the time to reach 85% of initial normal germination when planning regeneration of *Populus* and *Salix* collections to avoid large genetic erosion.

C-2002

Expanding Environmental Specimen Bank Applications - Banking for Marine Mammal Health Research. J. NESS[1], P. Becker[1], R. Pugh[1], A. Moors[1], and T. Rowles[2]. [1]National Institute of Standards and Technology, Chemical Sciences Division, Hollings Marine Laboratory, 331 Fort Johnson Road, Charleston, SC 29412 and [2]National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Office of Protected Species, Silver Spring, MD. Email: jennifer.ness@noaa.gov

The National Institute of Standards and Technology (NIST) began banking specimens for contaminant trend monitoring in 1979. Based on these initial collections, NIST protocols for collection, processing, and banking have been primarily for environmental contaminant analysis. In 2010, NIST consolidated all banking operations to the Marine Environmental Specimen Bank (Marine ESB) at the Hollings Marine Laboratory, Charleston, SC. The National Oceanic and Atmospheric Administration's (NOAA's) Marine Mammal Health and Stranding Response Program began the National Marine Mammal Tissue Bank (NMMTB) in response to a bottlenose dolphin (*Tursiops truncates*) mortality event where a lack of banked specimens left questions concerning unusually high environmental contaminants unanswered. The NMMTB was designed as a resource of tissue samples for chemical analysis to determine exposure to contaminants and retrospective research on exposure history of populations to emerging contaminants. Maintained by NIST, the NMMTB tissues are collected and banked within the Marine ESB from fresh-stranded marine mammals, animals taken incidentally in fishing operations, and from Native Americans for subsistence. In 2002, NIST

began collaborating with researchers to assess the health of bottlenose dolphins. These studies involve the capture and release of live animals during which health measurements and samples are collected. This health assessment and banking approach is being expanded to other marine mammal species in the US, and has been used in studies on dolphins for natural resources damage assessment in response to the Gulf of Mexico Deepwater Horizon Oil Spill. The Marine ESB has become a chain-of-custody biorepository for samples collected as a part of the spill. NIST is working with NOAA and its collaborating partners to expand the scope of the specimen bank and develop it as a resource of samples for animal health research. This expansion will emphasize the banking of specimens for wildlife disease studies, exposure to biotoxins, and developing health biomarkers.

C-2003

A Ferromagnetic Model for the Action of Electric and Magnetic Fields in Cryopreservation. A. KOBAYASHI[1] and J. Kirschvink[2]. [1]Earth and Life Science Institute, Tokyo Institute of Technology, 2-12-1 IE-31, Ookayama, Meguro, Tokyo, 152-8550, JAPAN and [2]California Institute of Technology, 170-25, Pasadena, CA 91125. Email: kobayashi.a.an@m.titech.ac.jp

Recent discussions in the literature have questioned the ability of electromagnetic exposure to inhibit ice crystal formation in supercooled water. Here we note that strong electric fields are able to disrupt the surface boundary layer of inert air on the surface of materials, promoting higher rates of heat transport. We also note that most biological tissues contain ferromagnetic materials, both biologically precipitated magnetite (Fe_30_4) as well as environmental contaminants that get accidentally incorporated into living systems. Although present at trace levels, the number density of these particulates is high, and they have extraordinarily strong interactions with weak, low-frequency magnetic fields of the sort involved in claims of electromagnetic cryopreservation. Magnetically-induced mechanical oscillation of these particles provides a plausible mechanism for the disruption of ice-crystal nucleation in supercooled water.

C-2004

Comparison of Non-ideal Solution Theories for Multi-solute Solutions in Cryobiology and Tabulation of Required Coefficients. M. ZIELINSKI[1], L. McGann[1], J. Nychka[2], and J. A. W. Elliott[3]. University of Alberta, 3rd Floor, Canadian Blood Services Centre, Edmonton, AB T6G 2R8, CANADA and University of Alberta, 7th Floor ECRF, [2]Room W7-040 and [3]Room 7-026A 9107-116 Street, Edmonton, AB, T6G 2V4, CANADA. Email: michalz@ualberta.ca

Thermodynamic solution theories are critical components in many of the mathematical models that are used to predict the behaviour of cells and tissues during cryopreservation. Although a number of solution theories have been proposed for use in cryobiology, most either do not account for the non-ideality of cryobiological solutions or require fitting to multi-solute data. However, there are at least two non-ideal solution theories available that can make predictions in multi-solute solutions while requiring fitting to only single-solute data in order to obtain all required thermodynamic coefficients: the Elliott et al. form of the multi-solute osmotic virial equation [J Phys Chem B, 2007;111(7):1775-85] and the Kleinhans and Mazur freezing point summation model [Cryobiology, 2007;54(2):212-222]. Herein, by way of comparison to available literature multi-solute osmometric data, we have evaluated the prediction accuracy of these two non-ideal solution theories in a variety of systems of interest to cryobiology. In doing so, we have also curve-fit a single, consistent set of literature single-solute osmometric data to obtain the required coefficients for each solution theory. Our results indicate that these two non-ideal solution theories provide similar performance overall, although each model has systems where it performs better. We have also found that both non-ideal models consistently provide more accurate predictions than an ideal dilute solution theory. The thermodynamic coefficients obtained in this work can be used with the multi-solute osmotic virial equation and/or the freezing point summation model to predict thermodynamic behaviour in a wide variety of multi-solute solutions. In addition, the results of our multi-solute solution analysis can be used to help in the selection of a non-ideal solution model when working with a particular combination of solutes. Funding sources: CIHR, NSERC, AITF, University of Alberta. J.A.W. Elliott holds a Canada Research Chair in Thermodynamics. This work has been submitted for publication to the journal Cryobiology.

C-2005

Effect of Extracellular Concentration Profile on the Precision of the Experimentally Determined Cell Membrane Permeabilities Through Osmotic Shift Tests Using a Microperfusion Chamber. W. LIU and G. Zhao. Department of Information Science Technology, University of Science and Technology of China, 96# JinZhai Road, Hefei 230026, CHINA. Email: liuwei159528@163.com

The transportation of water and cryoprotective agents across the cell membrane plays an important role in cell survival during freezing and thawing of cells. And a microperfusion chamber was developed for studying cellular response to osmotic change. The cells are immobilized in the microperfusion chamber and exposed to the solution which is osmotic solution shift to hypertonic solution. The change of extracellular concentration has a certain impact on fitting cell membrane permeability parameters. The microperfusion chamber can be modeled through the mechanism of

convection diffusion, and thus the Finite Element Analysis (FEA) was applied for the simulation of extracellular concentration. The validity of the concentration shift model was confirmed by comparing the cell-free experimental results used by the optical image processing with the results of simulation model. The CuCl2 solution was used to did the cell-free experiment and NaCl solution was used to do the determined cell membrane permeabilities experiment, thus the dynamic viscosity and diffusion coefficient of the solution at the certain concentration difference were measured to ensure the accuracy of the stimulant results. The simulation results have shown that the T-pipe and Y-pipe make no difference on the concentration change, but the size of T-pipe has a significant impact on extracellular NaCl concentration. The equilibration time of the low concentration shift to high concentration is about 100s, and the simulation shows the equilibration time of concentration function affects the measurement of hydraulic conductivity.Thus using FEA to analysis the extracellular concentration profile of microperfusion chamber has a great prospect to service for the cryobiology research by improving the precision of the experimentally determined cell membrane permeabilities.

C-2006

Evaluating MicroRNA Expression in Two Cold-hardy Gall-forming Insects. P. LYONS[1], P. Morin[2], and K. Storey[3]. [1]University of Moncton, 30 North St., Moncton, NB E1C 5X6, CANADA; [2]Moncton, NB, Canada; and [3]Carleton University, Ottawa, ON, CANADA. Email: pierrelyons@gmail.com

E. solidaginis and *E. scudderiana* have been studied as insect models of cold tolerance. However, both species employ different strategies to survive deep, sub-zero temperatures. *E. solidaginis* is freeze tolerant, allowing the freezing of up to 60% of body water content, whereas *E. scudderiana* is freeze-avoiding, and prevents the formation of ice. While both enter states of reduced metabolic activity, the mechanisms by which these changes occur are not yet well understood. MicroRNAs have been shown to be capable of regulating protein expression on a vast scale, and have been involved in numerous cellular processes. This makes them ideal candidates as potential regulators of cold hardiness in gall forming insects. Our group has previously shown miR-1 to be over expressed in cold stressed *E. solidaginis* (Lyons et al., 2013). To investigate the role of microRNAs, larvae from both *E. solidaginis* and *E. scudderiana* were incubated at 5 °C for two weeks. A sub-group of larvae were gradually chilled and kept at -15 °C for two weeks. RNA extraction was accomplished via mirVana© RNA extraction kit. Both High-throughput Next Generation Sequencing and qRT-PCR were used to evaluate miRNA expression levels. Of interest, miR-92b was found to be 1,5-fold up-regulated (P=0,02) in cold-stressed vs. control larvae of *E. solidaginis*. A subsequent *in silico* approach using the PicTarFly microRNA target prediction software (Grun et al., 2005) has shown that miR-92b has the potential to target Carnitine Palmitoyl Transferase, which has been shown to have reduced activity in overwintering *E. solidaginis* larvae (Joanisse and Storey, 1996). These data represent the first steps in evaluating microRNA involvement in the cold response of gall-forming cold-hardy insects.

C-2007

Towards Understanding the Protection Afforded by Cryoprotectants Commonly Used in the Cryopreservation of Zygotic Explants of Recalcitrant Seeds. D. BALLESTEROS[1], S. Naidoo[2], B. Varghese[2], P. Berjak[2], and N. Pammenter[2]. [1]Cincinnati Zoo and Botanical Garden. University of Cincinnati, 3400 Vine Street, Cincinnati, OH 45220 and [2]University of KwaZulu-Natal. School of Biological & Conservation Sciences, South Ring Rd, Westville Campus, Durban 4001, SOUTH AFRICA. Email: dani.ballesteros@cincinnatizoo.org

Cryopreservation is the most promising option for the long-term germplasm conservation of recalcitrant-seeded species. The post-cryo success achieved with the excised zygotic explants used for cryopreservation is variable, and for some species dependent on the use of cryoprotectants and rapid drying. However, the efficacy of different cryoprotectants seems to be species dependent and there is a paucity of information on the mechanisms via which particular cryoprotectants protect zygotic embryos from recalcitrat seeds during partial drying and cryogenic cooling. The present study used Differential Scanning Calorimetry (DSC) and Cryo-Scanning Electron Microscopy (Cryo-SEM) to characterise the behaviour of selected cryoprotectants in zygotic embryos during drying and cooling to cryogenic temperatures. These studies were carried out on a range ofrecalitrant-seeded species (*Castanospermum australe, Trichilia dregeana, Amaryllis belladonna, Strychnos gerrardii*) that show differential tolerance to cryoprotectants and cryopreservation. Exposure to glycerol or sucrose did not change the behaviour of water in zygotic explants of most of the species studied. DSC revealed little to no difference between fresh samples and those cryoprotected, showing very similar enthalpy of melting transitions (~ 300 J/gH_2O) and amount of unfrozen water (~ 0.30 gH_2O/gdm); except for *A. belladonna* treated with glycerol that showed a slightly higher amount of unfrozen water (~ 0.45 gH_2O/gdm). However, Cryo-SEM images showed important changes in the mechanical properties of the cryoprotected tissue during rapid drying. In general, glycerol allowed for less destructive compaction of the zygotic tissues during desiccation, particularly in *A. belladonna*. Glycerol was also observed to help in membrane stabilization of *C. australe*, particularly during rehydration of the tissue. The results are discussed in

relation to other studies on the mechanisms of cryoprotection in shoot tips and will inform the future cryopreservation protocols for zygotic germplasm of recalcitrant-seeded species.

C-2008

Investigation of the Effects of Cryopreservation on the Histone Modification Patterns of Mesenchymal Stem Cells. A. CHATTERJEE, N. Hofmann, and B. Glasmacher. Institute of Multiphase Processes, Leibniz University Hannover, Callinstrasse 36, Hannover, 30167, GERMANY. Email: chatterjee@imp.uni-hannover.de

Mesenchymal stem cells (MSCs) are multipotent adult stem cells and have diverse potential biomedical applications such as in tissue repair, transplantation and cell-based therapies. Cryopreservation of MSCs allows long term storage of these cells and therefore plays a crucial role in their subsequent applications. The success of a cryopreservation protocol is often assessed by analyzing the survival rates of thawed cells. However this parameter does not provide information on the possible adverse cellular effects of cryopreservation, such as on gene expression regulation and cell fate decisions. Treatment with DMSO has been shown to impact the histone modification patterns and the DNA methylation patterns of various tissues. Histone modifications and DNA methylation are epigenetic mechanisms that regulate eukaryotic transcription without altering the DNA sequence. Epigenetic mechanisms play an important role in stem cell maintenance and differentiation and when deregulated may lead to the formation of diseases like cancer. Despite the widespread cryopreservation of MSCs with DMSO, the effects of cryopreservation on the epigenetic mechanisms of MSCs are yet to be fully evaluated. In the current study bone marrow derived-MSCs from the common marmoset monkey were frozen with different concentrations of DMSO using a controlled-rate freezing device. Post-thaw cell viability was examined by analyzing the membrane integrity (by trypan blue staining) and mitochondrial dehydrogenase activity (using MTT assay). The functional integrity of cryopreserved MSCs was tested by analyzing their ability to differentiate into adipogenic and osteogenic lineages. The global acetylation and methylation patterns of the different histone proteins were analyzed using immunocytochemistry and mass-spectrometry. Results indicated that the survival, differentiation ability and modification patterns of various histone proteins in mesenchymal stem cells may be altered after cryopreservation, depending on the concentration of DMSO used for cryopreservation.

C-2009

Assessment of Genetic Stability of Cryopreserved Fraser Photinia via Retrotransposon-Based Molecular Markers. H. EKINCI[1], C. Öztürk[1], H. Akdemir[1], V. Süzerer[2], and Y. Özden Çiftçi[1]. Gebze Institute of Technology, Gebze Yüksek Teknoloji Enstitüsü, İstanbul Caddesi Numara 101, Moleküler Biyoloji ve Genetik Bölümü, Kocaeli, 41400, TURKEY and [2]Istanbul University, Institute of Science, Suley Maniye-Faith, Department of Botany 13434 Isatanbul, TURKEY. Email: hamitekinci@yandex.com

Fraser photinia (*Photinia* X *fraseri* Dress.) with its impressive leaf colour is an evergreen woody ornamental plant that has commercial importance. Due to the rooting problems faced in its traditional vegetative propagation of the species, biotechnological methods such as plant tissue culture is used as a complementary strategy to propagation. Moreover, medium and long-term (cryopreservation) preservation of the species also gained importance recently as photinia has economic importance. However, occurence of risk of possible genetic variation between the donor plant and cryopreserved individuals after cryopreservation, reveals the necessity of assessment of genetic stability of the long-term preserved species. Therefore, genetic stability of the plantlets proliferated from cryopreserved fraser photinia shoot tips was assessed by two retrotransposon-based molecular markers (IRAP and REMAP) in addition to the optimization of cold hardening stage of the cryopreservation. 52.1% of mean polymorphism (mean PIC value is 0.307) was found between donor plant and clones by IRAP analysis while 47.2% of mean polymorphism (mean PIC value is 0.353) was detected via REMAP. According to the results of observed with these two marker systems, applied cryopreservation protocol caused an reasonable polymorphism (PIC value

C-2010

Blood Cell Cryopreservation Under Pressure. N. GREER. Independent Researcher, Manchester, MO 63021. Email: ngreer9@gmail.com

Despite all the progress in the development of new cryoprotective agents, chemical cryoprotectants remain toxic to cells and largely inefficient for cryopreservation of tissues and organs. Difficulties of equally distributing an optimal concentration of cryoprotectants in tissues and organs have not yet been overcome. To address the above problem, a new method and apparatus for cryopreservation of live cells under hydraulic pressure has been developed. The method was tested on whole human blood. The blood cells were cooled to -80°C under hydraulic pressure with cooling rate below 90°C/min. It has been found that using high hydraulic pressure alone it is possible to obtain up to 80% recovery (20% hemolysis) of red blood cells. Cooling rates below 90°C/min make it theoretically possible to efficiently cryopreserve large biological samples, such as units of blood, tissues and organs. Hydraulic pressure can act as a cryoprotectant equally well at any point of the biological sample and has a potential to set and maintain optimal

conditions for cryopreservation not only cells, but also complex tissues and organs. For commercialization, two versions of the apparatus were developed. One version was developed for research purposes and allows precise pressure and temperature control during cryopreservation of a wide variety of cells, tissues, and organs. Another version was developed for medical applications and allows sterile cryopreservation of biological samples in individual capsules in large quantities.

C-2011

Cryotherapy and Chemotherapy of PPV-infected Apricot. B. KÖKSAL, V. Süzerer, M. Gül Şeker, and Y. Özden Çiftçi. Gebze Institute of Technology, Istanbul Str., Kocaeli 41400, TURKEY. Email: busrakoksal91@gmail.com

Plum pox virus (PPV), the cause of the Sharka disease, is considered to be the most serious virus of stone fruits. The disease can be controlled by using clean propagation material and planting naturally resistant or tolerant varieties. However, eradication of the virus is also important. In order to eradicate the virus, several biotechnological methods including meristem culture, thermotherapy, chemotherapy, electrotherapy and cryotherapy can be utilized. Among them, chemotherapy and cryotherapy techniques are used to eradicate PPV-infected apricot in this study. Different chemotherapeutic agents such as 8-azaguanine, ribavirin and quercetin (1 mg/l) were incorporated to 2 mg/l BA and 0.5 mg/l GA_3 containing MS medium for chemotherapy. 80-100% of survival was achieved in infected apricot shoot tips after 4 weeks of culture in ribavirin and quercetin containing medium, respectively, and all of the survived ones found to be virus free with RT-PCR. However, no survival was obtained in 8-azaguanine containing medium. In the case of cryotherapy, explants were pre-cultured in 1 mg/l BA, 5% dimethylsulfoxide (DMSO) and 2% mg/l proline containing MS medium at 4°C and then treated with 40 min in PVS2 solution. Slow cooling of shoot tips was achieved by transfer of the cryovials to Mr Frosty® device, which was kept in a -80°C freezer until the temperature was reduced to -40°C. Afterwards, cryovials were rapidly plunged to liquid nitrogen (LN). After storage for at least 1h in LN, explants were thawed rapidly in water bath (1 min at 40°C) and transferred to washing solution (1.2 M sucrose for 30 min) and cultured in 2 mg/l BA and 0.5 mg/l GA_3 containing MS medium for recovery. After 4 weeks of culture, 10% of survival was recorded in cryopreserved apricot shoot tips. RT-PCR analysis showed that PPV was eradicated in the survived ones after cryotherapy. Overall results showed that cryotherapy and chemotherapy could be used to eradicate PPV from infected apricot, however, survival rates should be increased by optimization and may be usage of rapid freezing techniques in the former.

C-2012

Prolonged Post-thaw Shelf Life of Red Cells Frozen Without Prefreeze Removal of Excess Glycerol. J. LAGERBERG[1] and C. Lelkens[2]. [1]Sanquin Research, Sanquin Research, Amsterdam, 1066 CX, NETHERLANDS and [2]Kortgene, The Netherlands. Email: j.lagerberg@sanquin.nl

Background: The use of a functionally closed system (ACP215, Haemonetics) for the glycerolization and deglycerolization of RBC units allows for prolonged post thaw storage. Currently, glycerolization is followed by reduction of supernatant glycerol before freezing. Aim: The aim of this study was to investigate the influence of supernatant glycerol reduction before freezing on the stability of thawed, deglycerolized RBCs during subsequent cold storage. Methods: Leukoreduced RBC units in SAGM were stored for 6 days at 2-6°C before glycerolization. The RBC units were pooled and split. After removal of additive solution, the ACP215 was used to add glycerol (final concentration 40%). Units were either frozen as such (n=4) or supernatant glycerol reduced before freezing (n=4). After storage at -80°C, the units were thawed, deglycerolized and resuspended in SAGM. An additional 4 RBC units, frozen without glycerol supernatant reduction, were resuspended in AS-3 after deglycerolization. During cold storage (2-6°C), the red cells were analyzed for their stability and in vitro quality. Results: Glycerol reduced frozen units had a volume of 212±135 mL and a Hct of 71±3%. For the non-reduced units these values were 437±32 mL and 35±1%. The FTW recovery was comparable for both groups (about 80%). During post thaw storage in SAGM, non-glycerol reduced units showed significantly less potassium leakage and hemolysis. Also ATP levels were better maintained in non-glycerol reduced units. AS-3 has been shown to be superior to SAGM in maintaining post thaw red cell integrity. Also in this study, with non-glycerol reduced units, hemolysis was strongly reduced by using AS-3 as additive solution: hemolysis remained below 0.8% for up to 28 days of storage. Conclusion: Omitting glycerol supernatant reduction before freezing simplifies the procedure and increases stability and therefore the out-dating period of thawed RBCs. This increases the practical applicability of cryopreserved RBCs in both civil (rare blood) and military blood transfusion practice.

C-2013

Post-thaw Characterization of Cord Blood-derived Mesenchymal Stromal Cells Cryopreserved for up to Five Years. L. A. MARQUEZ-CURTIS[1], A. Xu[2], and A. Janowska-Wieczorek[2]. [1]University of Alberta, 8249-114 St. NW, Edmonton, AB T6G 2R8, CANADA and [2]Canadian Blood Services, 8249-114 St., Edmonton, AB, CANADA. Email: klbcurtis@gmail.com

Mesenchymal stromal cells (MSCs) are increasingly used as therapeutic agents for a variety of diseases. Because long-term banking of MSCs is critical for their clinical applications, we sought to determine whether the viability, proliferation potential, capacity for differentiation, migration ability and amenability for genetic manipulation are maintained in MSCs cryopreserved for up to five years. MSCs were established from the mononuclear cell fraction of an umbilical cord blood (CB) sample. Primary adherent MSC monolayers were sub-cultured until passage 12 and aliquots of various cell passages were frozen at -70°C in the presence of 10% dimethylsulfoxide (DMSO) and 10% fetal bovine serum. MSCs were thawed rapidly at 37°C, sub-cultured to about 80% confluency, trypsinized, and subjected to trypan blue viability test, seeded in the presence of osteogenic and chondrogenic media, analyzed by flow cytometry for expression of surface markers and by RT-PCR for gene expression of tissue markers, and assessed for migration response. We found that although the viability decreased by up to 20% post-thaw, the proliferation potential of CB-MSCs as measured by population doubling level of 7 was not affected by cryopreservation, nor was their ability to differentiate to osteoblasts (by staining of calcium deposits using Alizarin red) and to chondrocytes (by staining of proteoglycans using Alcian blue). The MSCs retained positive expression for the stromal markers CD73, CD90 and CD105 and negative expression for the hematopoietic markers CD34 and CD45. In addition, they expressed pluripotency genes and early tissue markers for muscle, bone and cartilage. Furthermore, the MSCs retained their migratory response towards the chemokine stromal cell-derived factor, which is up-regulated at sites of tissue injury. In conclusion, CB-derived MSCs cryopreserved for up to 5 years at -70°C in the presence of 10% DMSO demonstrate high-efficiency recovery of viable, proliferative and functional cells which can be employed for clinical transplantation or manipulated for gene therapy.

C-2014

Cryopreservation of *Passiflora pohlii* Mast Nodal Segments and Assessment of Genetic Stability of Recovered Plants. G. PACHECO, T. Merhy, M. Vianna, R. Garcia, and E. Mansur. Rio de Janeiro State University, Rua São Francisco Xavier 524 PHLC sl 505, Rio de Janeiro, 20550013, BRAZIL. Email: georgiappacheco@gmail.com

Passiflora pohlii Mast. (Passifloraceae) is a wild species native to Brazil, with a potential agronomic interest due to its tolerance to soil-borne pathogens that cause damage to the passion fruit culture. Because this species occurs in impacted regions, the goal of this study was the development of an efficient cryopreservation strategy for nodal segments excised from axenic plants using vitrification-based methods. The genetic stability of the recovered plants was also evaluated by RAPD and ISSR. In the encapsulation-vitrification protocol, precultured explants on medium with high sucrose concentrations (0.3 - 0.7 M) were encapsulated in calcium alginate beads, followed by incubation in liquid ½MSM medium with 0.75 M sucrose for 24h. After this, beads were exposed to PVS2 or PVS3 for different periods before immersion into LN. In the vitrification protocol, precultured explants were incubated in the cryoprotectant solutions and stored in LN using the same conditions adopted for encapsulation-vitrification. Frozen materials were rewarmed at 40°C and transferred to the recovery medium (MSM supplemented with 30.8 µM BA). Explants were kept in the dark for 30 or 60 days before transfer to the presence of light. Although plant recovery was achieved using both vitrification-based protocols, higher recovery rates were obtained with the vitrification technique, when explants were incubated in the dark. RAPD and ISSR analyses indicated that shoots derived from cryopreserved explants were genetically stable at the genomic regions tested. These results show that cryopreservation of nodal segments through the vitrification technique can be used for long-term storage of *P. pohlii* germplasm.

C-2015

Investigation of Genetic and Epigenetic Changes of Cryopreserved Mesenchymal Stem Cells. D. SAHA[1], N. Hofmann[1], T. Mueller[2], H. Niemann[3], and B. Glasmacher[1]. [1]Leibniz Universität Hannover and Hannover Medical School, Institut für Mehrphasenprozesse, Callinstrasse 36 , Hannover, 30167, GERMANY; [2]Institute for Transfusion Medicine, Carl-Neuberg strasse 1, Hannover 30625 , GERMANY; and [3]Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut Institute of Farm Animal Genetics, Höltystraße 10 31535 Neustadt. Email: saha@imp.uni-hannover.de

Cryopreservation is routinely used to store different types of cells and tissues. It is used for transportation and cell banking. However, the impact of cryopreservation on the genetic and epigenetic profile of frozen cells is still unclear. Here, we try to explore the alterations caused to cryopreserved mesenchymal stem cells and its possible implications. Our freezing protocol showed that the cells frozen with 5% dimethyl sulfoxide (DMSO) at all passages (early, mid and late) had the best post thaw survivability in terms of MTT assay and adherence capacity. 1 and 15% DMSO concentrations proved to be sub-optimal after thawing the cells. Adipogenic differentiation of MSCs showed that cells frozen in all concentrations of DMSO could differentiate to adipocytes. Cells frozen with 5, 10 and 15% DMSO showed comparable oil droplet formation. DNA methylation plays a critical role in health and disease. Changes in DNA methylation has been seen in cancer, ageing and auto immune diseases. Alterations in the 5-mC% content of DNA were observed in the frozen samples as compared to control

(unfrozen cells, never exposed to DMSO). Further studies need to be conducted so as to determine the implication of these alterations. In total, cryopreservation seemed to cause changes in the mRNA expressions, global DNA methylation which could alter the cell fate at a later time point. Therefore, there is a need for comprehensive and orderly studies to determine the possible impact of subtle genomic/epigenomic and proteomic changes resulting from cryopreservation.

C-2016

Experimental Study of Deglycerolization of Red Blood Cells with a Dilution-filtration System. L. SHEN[1], G. Zhao, and D. Gao[2]. [1]Department of Electronic Science and Technology, University of Science and Technology of China, No. 96, JinZhai Road Baohe District, Hefei, Anhui, 230026, P. R. CHINA and [2]University of Washington, Department of Mechanical Engineering, University of Washington, Box 352600, Seattle, WA 98195-2600. Email: shenlx@mail.ustc.edu.cn

Freezing of red blood cells (RBCs) is an important application of cryopreservation. As a primary cryoprotection of RBCs, glycerol must be removed from the thawed previously frozen RBCs prior to transfusion. The most commonly traditional method for deglycerolization is centrifugation, however, which has limitations in time consumption, operational complexity and high cost. Recently, a novel method with better ability to remove large molecular substances called dilution-filtration was reported in the literature. As the method involves pure ultrafiltration for separation, so it essentially differentiates from the centrifugation. In this work, an in-depth experiment was done for deglycerolization of red blood cells. In order to achieve this goal, a fully automatic instrument based on the dilution-filtration theory had been developed. Also, the experiment requests to measure some basic haematological parameters of deglycerolized red blood cells, such as red blood cells count, hematocrit, residual glycerol, supernatant hemoglobin and extracellular potassium levels. All these experimental measurement parameters had to meet the blood transfusion requirements that established by the American association of blood banking (AABB). Moreover, a high efficiency and low cost separation membrane product was determined by utilizing dialyzer, blood filter and plasma filter independently during the deglycerolization. Finally, the whole experiment process was carried out in the local blood center. Meanwhile, to ensure our experiment results could be highly persuasive, operations personnel were asked to abide a recommended quality control which was adapted from a standard operating procedure made by the Naval Blood Research Laboratory (NBRL). Taken together, the dilution-filtration system was proved to be efficient and feasible for removing glycerol from the cryopreserved red blood cells. Furthermore, with the ultimate success of industrial operations, it is expected to change centrifugation trades in the deglycerolized field in the future.

C-2017

A Study of Fundamental Cryobiological Characteristics of Human Vaginal Immune Cells for Optimizing Their Functional Cryopreservation. Z. SHU[1], S. Hughes[2], C. Fang[1], J. Huang[3], B. Fu[3], F. Hladik[2], and D. Gao[1]. [1]Department of Mechanical Engineering, University of Washington, Seattle, WA 98195; [2]Fred Hutchinson Cancer Research Center, Vaccine and Infectious Disease Division, Seattle, WA 98109; and [3]Beijing Union University, School of Information Technology, Beijing 100101, CHINA. Email: zqshu@uw.edu

Cryopreservation of collected human vaginal mucosal immune specimens is vital to the successful analysis of mucosal immunity and HIV trials. However, the mucosal immune specimens cannot be well cryopreserved with the current empirical procedures. The optimum cryopreservation protocol is determined by the cryobiological features of the cells. Thus, a cryobiological study of human female genital mucosal immune cells (FG-MIC) is crucial towards the successful specimen cryopreservation. In this work, CD3+ T cells and CD19+ macrophages were investigated. The intracellular osmotically inactive volume (V_b) was determined by exposing the cells to hypertonic saline solutions, evaluating the volume changes after equilibrium and applying the Boyle-van't Hoff plot. At room temperature, the cell membrane permeability to water (L_p) and to four different CPAs (dimethyl sulfoxide (DMSO), glycerol, propylene glycol (PG) and ethylene glycol (EG)) were measured with a microfluidic perfusion channel. Osmotic tolerance limits of the cells to CPAs and the CPA toxicities were also examined. At sub-zero temperatures (0~-40°C), the L_p (T) and the associated activation energy E_a of cell membranes were determined using a Differential Scanning Calorimetry (DSC). The "Slow-Fast-Fast-Slow" DSC scanning program, similar to the method of Devireddy and Bischof, was applied. Some preliminary cryopreservation trials were also conducted. Results showed that mucosal immune cells have relatively small V_b (14.1% for T cells and 23.8% for macrophages). T cells are more vulnerable to stresses of osmotic change and freezing compared to macrophages. Among the four CPAs, DMSO and PG have higher P_s values and lower toxicity to the cells, which indicates that DMSO and PG potentially can be better CPA options to avoid lethal IIF and severe cell dehydration with less toxicity during freezing. Based on the measured cell properties, a freezing protocol with slow cooling rate in a critical temperature range was predicted and experimentally demonstrated by preliminary cryopreservation results.

C-2018

Cryopreservation of *Mycobacterium tuberculosis* (MTB) Complex Cells and Sputum Specimens for MTB Diagnosis. Z. SHU[1], S. Inoue[1], J. Kim[1], K. Weigel[2], A. Becker[2], S. Soelberg[3], G. Cangelosi[2], K. Lee[4], J. Chung[1], and D. Gao[1]. [1]Department of Mechanical Engineering, [2]Department of Environmental and Occupational Health Services, and [3]Department of Medicine, Division of Medical Genetics and Genome Sciences, University of Washington, Seattle, WA 98195 and [4]NanoFacture, NanoFacture, Inc., Bellevue, WA 98015. Email: zqshu@uw.edu

Successful long-term preservation of *Mycobacterium tuberculosis* (MTB) cells or sputum specimens is very important for sample transport, biobanking, research and the development of new drugs, vaccines, biomarkers, and diagnostics. In this work, *M. bovis* Bacillus Calmette-Guérin (BCG) and *M. tuberculosis* H37Ra were used as models of MTB complex strains to study cryopreservation of MTB complex cells in diverse sample matrices (phosphate buffer saline (PBS), Middlebrook 7H9 medium with or without added glycerol, and human sputum). Efficacy of cryopreservation was quantified by microbiological culture. Results showed that among the variables tested, slow cooling rate was the most critical factor for the successful cryopreservation of *Mycobacterium tuberculosis* complex cells. Preincubation of frozen/thawed MTB cells in 7H9 broth before culturing did not help the cells repair cryoinjury. Cell inactivation by fast cooling was not associated with a compromised cell envelope, as indicated by the comparison results of microbiological culture and the BacLight live/dead staining. After cryopreservation, the MTB cell surface antigen can still function well for the MTB diagnosis as biomarker. For the Point-of-Care (POC) diagnosis of MTB, an occupationally safe (biosafe) sputum liquefaction protocol and a semi-automated antibody-based microtip immunofluorescence sensor were developed. Sputum was treated with a synergistic chemical-thermal protocol that included moderate concentrations of NaOH and detergent at 60 ^{o}C for 5 to 10 min. The protocol effectively liquefied sputum and inactivated microorganisms including Mycobacterium tuberculosis, while preserving the antibody-binding activity of Mycobacterium cell surface antigens. The biosafe sputum liquefaction method, cryopreservation protocol, and the microtip device make it possible to diagnose the tuberculosis with high feasibility, sensitivity and accuracy.

C-2019

Human Umbilical Vein Endothelial Cell (HUVEC) Response to Interrupted Cooling in the Presence and Absence of Dimethyl Sulfoxide. A. SULTANI, L. Ross-Rodriguez, J. A. W. Elliott, and L. McGann. University of Alberta, 8249 114 Street, Edmonton, AB T6M0B6, CANADA. Email: asultani @ualberta.ca

HUVECs were first successfully cultured in vitro in 1973, initiating the growth of modern vascular biology. Study into HUVEC cryopreservation has made it readily available for purchase, however the cryobiological response of HUVECs has not been fully investigated. Viability by membrane integrity of HUVECs as received from the supplier was 50% ± 2%, indicating that cryopreservation of HUVECs can be improved. Membrane hydraulic conductivity is an osmotic property which is necessary to simulate cryobiological response. HUVEC membrane hydraulic conductivity was measured and found to be 0.147 $\mu m^3/\mu m^2$/min/atm at 20 °C, less than fibroblasts and TF-1 cells, therefore being the slowest osmotically-responding cells studied using interrupted cooling. HUVECs were subjected to interrupted slow cooling without hold time (graded freezing) and rapid cooling interrupted with a hold time (two-step freezing) in the presence or absence of 10% Me_2SO. Preliminary results show that HUVECs have higher viabilities from graded freezing than from two-step freezing, similar to previously studied fibroblasts but different from TF-1 cells. Optimal cooling procedures cannot solely be explained by hydraulic conductivity. Graded freezing at 1 °C/min in the presence of 10% Me_2SO resulted in the best viability, with almost no damage from direct thaw after cooling to between –5 °C and –40 °C and a maximum of 66% HUVEC membrane integrity after cooling to –35 °C, plunging into liquid nitrogen and then thawing (plunge-thaw). Two-step freezing in the presence of 10% Me_2SO resulted in almost no damage from direct thaw from as low as –15 °C. Lower experimental hold temperatures resulted in damage after direct thaw and the highest membrane integrity after plunge-thaw was 42% for a hold temperature of –25 °C. Interrupted cooling in the absence of Me_2SO resulted in no HUVEC membrane integrity after plunge-thaw. The understanding gained from this investigation may lead to improved cryopreservation of HUVECs. Funding: CIHR

C-2020

Cryopreservation of Chrysanthemum Using Alminium Cryo-plate Protocol. D. TANAKA[1], A. Akimoto-Kato[2], T. Niino[3], and T. Matsumoto[4]. [1]Criobiology, 5-1, Higashiyama, Myodaiji, Okazaki, 444-8787, JAPAN; [2]National Institute of Basic Biology, Okazaki 444-8787, JAPAN; [3]University of Tsukuba, Tsukuba 305-8572, JAPAN; and [4]Shimane University, Matsue 690-1102, JAPAN. Email: dtanaka@nibb.ac.jp

Two cryopreservation procedures using an alminium cryo-plates were successfully developed using *in vitro*-grown chrysanthemum shoot tips. Shoot cultures were cold-hardened at 5°C on 1/2 MS medium for 30 days. Shoot tips with basal

plate (1.5-2.0 x 1.0 mm) were excised from shoot cultures and precultured at 5°C for 3 days on solidified 1/2 MS medium containing 0.3 M sucrose. Precultured shoot tips were placed on alminium cryo-plate with 10 wells (diameter 1 mm) and embedded in calcium alginate gel. Osmoprotection was performed by immersing the cryo-plates for 30 min at 25°C in loading solution (2 M glycerol + 1.0 M sucrose). In the D-Cryo-plate procedure, the shoot tips were dehydrated by placing the cryo-plates in the air current of a laminar flow cabinet for 30 to 150 min. In the V-Cryo-plate procedure, buds were dehydrated by immersing the cryo-plates in PVS2 vitrification solution for 40 min at 25°C. In both procedures, cooling was performed by placing the cryo-plates in uncapped cryotubes, which were immersed in liquid nitrogen. For rewarming, shoot tips attached to the cryo-plates were rewarmed by immersion in petri dish containing 1.0 M sucrose solution at 25°C. Regrowth of cryopreserved shoot tips using D-Cryo-plate and V-Cryo-plate procedures, was 90% and 80%, respectively. The two procedures were applied to 20 additional chrysanthemum lines. This protocol appears to be a promising technique for cryopreservation of chrysanthemum genetic resources.

C-2021

Determination of Membrane Permeability of Porcine Adipose Derived-Stem Cell. J. WANG and G. Zhao. Electronic Science and Technology Program,University of Science and Technology of China, No.96, Jinzhai Road, Hefei, 230027, P. R. CHINA. Email: jywong@mail.ustc.edu.cn

Adipose-derived stem cells (ADSCs), as an abundant and accessible source of adult stem cell, self-renew and differentiate into various cell lines and have promising potential in tissue engineering and an alternative to the use of regenerative medicine strategies. In order to meet clinically urgent demand of ADSCs, the cell cryopreservation was applied to the ADSCs long-term preservation. To optimize the cryoprotective agents (CPAs) addition, removal and the freezing as well as thawing processes, we measured the transport properties of the porcine adipose-derived stem cells (pADSCs). In this study, the microperfusion system was used to measure the transport properties of porcine adipose-derived stem cells (pADSCs) membrane. The membrane permeability coefficient to water (L_p), the membrane permeability coefficient to DMSO, EG and glycerol (P_s), and the activation energies and reference membrane coefficient have been investigated at 28, 18, 8 and -2°C, respectively. We determined the inactive cell (V_b), the hydraulic conductivity (L_p) and the CPAs permeability (*Ps*) at various temperature in 2-paramerter (*2-p*) model. No significant difference in the DMSO and EG permeability was obtained at experimental temperature and the effect of the temperature on the transport properties of the pADSC membrane was analyzed. However, the glycerol permeability of pADSCs was too small to be precisely measured. We obtained the parameter of pADSCs with 1.5 M glycerol at 32°C and determined the accurate activation energies of glycerol permeability and the referent of glycerol permeability.Membrane Permeability of porcine Adipose Derived-Stem cellquantitatively measuredwas used to minimize the excessive volume shrinkage and the toxicity with CPAs addition.

C-2022

Effects of Different Cryoprotectants During Cryopreservation of Fish Eggs. A. REICHEL, S. Rakers, and M. Gebert. Fraunhofer EMB, Paul-Ehrlich Strasse 1-3, Luebeck, 23562, GERMANY. Email: aileen.reichel@emb.fraunhofer.de

While cryopreservation of mammalian gametes made great progress within the last decades, a successful storage of cryopreserved fertilized or unfertilized eggs from fish was not described yet. The storage of fish gametes in liquid nitrogen would be a great accomplishment though for several reasons: it could help in preserving rare species from extinction, it would allow larvae production independent from spawning seasons and therefore improve breeding capacities and would provide constant sample supply for research. Two main problems arise with fish cryopreservation: First the big size of fish eggs (e.g. ~1 mm for *Danio rerio*) that results in a disadvantageous volume-surface-ratio, and second the egg chorion impedes an easy uptake of CPs, which would minimize the formation of ice crystals. Likewise, the protection of the yolk sack, which is basically composed of different nutrients like proteins and fatty acids, is very difficult, because the yolk has no self-repairing mechanisms like cells have. Initially, it is important to investigate the detailed cryoprotective effects of different CPs on eggs and how the permeability of such CPs can be increased. Therefore, the cryoprotectants DMSO, ethylene glycol and propylene glycol were tested in combination with sugar-based extenders such as sucrose and trehalose using eggs from *Danio rerio*. Hatching and survival rates were determined and light microscopy images were taken during incubation with CPs to exclude toxic effects and to evaluate dehydration processes due to the CP treatment. In addition it was investigated, which structures and parts in the eggs are destroyed during cryopreservation and how these structures could be better protected. Transmission-electron microscopy (TEM) images have been taken to evaluate the yolk-cell connection before and after cryopreservation.

C-2023

Monitoring of the Residual Cryoprotectant Concentration with Electrical Conductivity Measurements During Cryoprotectant Removal. Z. SHU[1], C. Fang[1], X. Zhou[2], D. Liao[1], F. Hladik[3], and D. Gao[1]. [1]Department of Mechanical Engineering, University of Washington, Seattle, WA 98195; [2]University of Electronic Science and Technology of China,

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Cryoprotective agents (CPA) should be added to cell suspension before freezing and removed after thawing. Nowadays people mainly apply centrifugation for CPA removal. This method may cause many problems. Meanwhile, a fast, easy and cheap method to monitor the residual CPA concentration remains an unfilled need. In this work, real-time monitoring of residual CPA concentration with electrical conductivity (EC) measurements was proposed and tested. It was found that CPA concentration is correlated with the EC of the solution. The standard data of "CPA concentration—EC of the solution" for a few widely used CPA solutions were obtained. A "dilution-filtration" system based on a hollow fiber dialyzer, three peristaltic pumps and some well-designed tubing connections was applied to remove CPA. DMSO solutions (DMSO-NaCl-H_2O solutions with or without 5% BSA) were selected as representative examples. The results showed that CPA can be removed effectively by the "dilution-filtration" system. The ECs of waste solution (filtration product) and "cell suspension" were almost identical after short period of processing, which were validated by osmolality measurements with osmometer. EC readings of waste solution can convey the instant residual CPA concentrations in cell suspension. This validates the feasibility of a safe (no contamination), easy and cheap way to real-time monitoring of CPA concentration in cell suspension by measuring the EC of the waste solution. Compared to other CPA removal methods that are applied traditionally (centrifugation) or proposed recently (microfluidic device), the "dilution-filtration" method may offer advantages of low time and labor consumption, low injury to the cells, high effectiveness, ease to control the final suspension volume and low risk of contamination. The configuration of the "dilution-filtration" system can also be modified easily to achieve CPA addition and volume control of cell suspension (concentration or dilution of the cell suspension).

C-2024

Freezing Response Characterization of Pancreatic Cancer Cell Lines BxPC-3 and PANC-1.

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Pancreatic cancer (PCC) accounts for 11% of US cancer deaths annually. Radiation and chemotherapy have been used to treat PCC with limited success. Despite its relative low incidence rate, PCC has one of the highest lethality rates, mainly attributed to inadequate early diagnosis and therapy efficacy issues, calling for the development of alternative treatment options. Cryoablation is an underutilized avenue for PCC treatment. This study evaluated post-freeze responses of PCC and identification of the minimum lethal temperature needed to completely ablate PCC *in vitro*. Human PCC lines, BxPC-3 and PANC-1, underwent a 5 min freeze to temperatures from -10 to -25°C at 5°C intervals before a passive thaw at RT for 10 min and return to standard culture conditions. Microfluidic flow cytometry (PI/YO-PRO-1) and fluorescence microscopy (PI/Calcein-AM) provided measures of viability, necrosis and apoptosis at 1, 4, 8, and 24hrs after freezing exposure. Analysis of cellular metabolic activity (AlamarBlue) was performed on days 1, 3, 5, and 7 of recovery. Viability data on day 1 showed both cell lines were minimally impacted by freezing to -10°C (PANC-1=116.9%; BxPC-3=92.8%) compared to 0hr controls. Exposure to -15°C showed decreased viability (PANC-1=62.6%; BxPC-3=23.7%) after 24hrs, with an apoptotic peak in BxPC-3 samples at 8hrs (18.3%). The -20°C isotherm resulted in decreased survival (PANC-1=9.7%; BxPC-3=1.7%) following 1 day of recovery with minimal repopulation over 7 days. Flow cytometry live cell populations corroborated metabolic analysis at 24hrs; -15°C (PANC-1=48.5%; BxPC-3=16.6%) and -20°C (PANC-1=7.4%; BxPC-3=3.8%). Microscopy revealed increasing amounts of necrosis, the primary mode of cell death over 24hrs, at -15 and -20°C. Overall, BxPC-3 were found to be more susceptible to cryoablation than PANC-1. The minimal lethal temperature for complete destruction of either cell population was determined to be -25°C. These results provide the foundation for developing improved methods to treat PCC using cryoablation.

C-2025

Numerical Simulation of a Hybrid Cryotherm Probe with the Influence of the Vessels and the Nanoparticles.

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Vessels around the tumors play an important role in the cryosurgery or microwave ablation for the treatment of tumors. To analysis the influence of vessels around the tumors, the bio-heat transfer equations are modified since the biological blood perfusion is changed due to the consideration of the vessel network. Thermal properties of liver tissue, tumor and blood are given as the function of the temperature. Furthermore, the phase change is considered so that the computational domain around the cryoprobe is divided into three regions, i.e. the frozen region, the mushy region and the unfrozen region. The temperature of the tumor was changed

on the interval of [-80,60] ^{o}C cycles by the function of the cryoprobe and the RF antenna, respectively. And the mathematical models are simulated by numerical method of Finite Element Method(FEM). The 2-D temperature distribution at the axial symmetry plane of the tumor without large blood vessel is compared with that the tumor with large blood vessel. After the tumor is embedded with nanoparticles, the thermal conductivities and thermal capacities of the tumor are changed and the tumor then is considered as a two-component biomaterial system. The 2-D temperature distribution at the axial symmetry plane of the tumor with large blood vessel without nanoparticles is compared with that the tumor with nanoparticles. Moreover, the temperature gradient and thermal stress at that plane are given numerically. In our opinion, the hybrid treatment of tumors utilizing cryosurgery and microwave ablation will damage the tumor cells more effectively after the tumor is embedded with nanoparticles. Therefore, more experiments should be done for specific and irregular vessels of tumor to achieve a better result. Acknowledgements: This work was supported by the National Natural Science Foundation of China (Nos. 51076149, 51276179), and the Fundamental Research Funds for the Central Universities.

C-2026

The Trial in Vitro to Evaluate a New Blood Purification Equipment - The Portable Hemodialysis Instrument. Y. XU, Z. Jiang, and G. Zhao. University of Science and Technology of China, Road JinZhai 96#, Hefei 230027, P. R. CHINA. Email: xyp@mail.ustc.edu.cn

A new blood purification equipment, the portable hemodialysis instrument (PHI), is designed and developed. With many advantages like small size, portability and simple operability, this machine can be used for continuous blood purification, especially daily hemodialysis. To investigate the effect of the PHI on urea and creatinine clearance in hemodialysis, a rigorous pig blood trial in vitro is carried out. Traditional citrate phosphate dextrose adenine (CPDA) nutrition anticoagulant solution is used to prevent blood clots. After adding quantitative reagent of urea and creatinine, 1000ml fresh blood of pig is prepared for each trial. Different arterial blood pump rates are set as follows: 60ml/min, 100ml/min and 150 ml/min to evaluate the effect of pump rate, while the ultrafiltration pump rate is set as a constant of 30ml/min. In the same hemodialysis conditions, the blood samples are dialyzed for 60 minutes, and the trial of each rate is repeated 3 times. The fresh pig blood are sampled per 5 minutes to obtain concentrations of urea and creatinine, and the corresponding reduction ratios are calculated, respectively. All the trial consequences demonstrate a sufficient dialysis according to the urea and creatinine clearance standards. In addition, the arterial blood pump rate is proven to be a promotion for the clearance, approximate 10% clearance increase from 60ml/min to 150ml/min in arterial blood pump rates.

C-2027

Observations of Damage to Stage V Zebrafish Oocytes in Contact with Extracellular Ice. F. JALALI, A. DiBenedetto, and J. Karlsson. Villanova University, Villanova, PA 19085. Email: fjalali@ villanova.edu

The successful banking of zebrafish germplasm by embryo or oocyte cryopreservation remains elusive, due to the rapid onset of cryoinjury following extracellular ice formation. The present study used high-speed imaging to investigate the causes of damage to Stage V zebrafish oocytes following contact with extracellular ice and subsequent cooling. During cryomicroscopy experiments in the absence of cryoprotectant additives, extracellular ice formation was initiated approximately 8 mm away from the oocyte; the resulting crystals were then allowed to envelop the oocyte during an isothermal hold at -0.9°C. When oocytes were exposed to the external ice for up to 20 min at this temperature, only 13% exhibited any evidence of injury (rupture or darkening). In contrast, our previous studies have shown that when zebrafish embryos are maintained in contact with external ice for 20 min at -0.4°C, mechanical damage occurred in as many as 47% of embryos. Controlled-cooling experiments with oocytes were performed at rates of 0.5, 1, and 2°C/min, all of which were preceded by a 5-min isothermal hold at -0.9°C, to allow equilibration with the extracellular ice. In all 68 oocytes observed, cryoinjury manifested as a sudden increase in oocyte opacity (darkening) during the cooling ramp. The average darkening temperature decreased slightly with increasing cooling rate, but this effect was not statistically significant (ANOVA, $p > 0.25$). However, we found a statistically significant effect of oocyte age (time since collection) on the darkening temperature (ANOVA, $p < 0.001$). Because it was only possible to observe one oocyte at a time in the cryomicroscopy experiments, the average oocyte age in the first, second and third trials were 19 ± 2, 72 ± 2, and 121 ± 3 min, respectively. The darkening temperature in the first run (-6.4 ± 0.5°C) was significantly lower (Tukey, $p < 0.05$) than in the second (-3.9 ± 0.5°C) and third runs (-3.7 ± 0.8°C), which suggests that oocytes became more sensitive to cryoinjury with time in culture.

C-2028

Enhancement of Survival Rate of Cells with the Active Control of the Nucleation Temperature. L. LAUTER-BOECK[1], N. Hofmann[1], T. Mueller[2], and B. Glasmacher[1]. [1]Institute for Multiphase Processes, Callinstraße 36, Hannover 30167, GERMANY and [2]Institute for Transfusion Medicine, Carl-Neuberg Strasse 1, Hannover 30625, GERMANY. Email: lauterboeck@imp.uni-hannover.de

For the field of regenerative medicine the preservation of cells and tissue in liquid nitrogen is the state of the art method for their long term storage. There are problems in conservation of complex tissues and organs, because mechanisms behind tissue damage are still not fully understood. At the moment only single cell suspensions and small tissue pieces are possible to preserve with high viability. The viability of cryopreserved cells can be enhanced by optimizing the freezing parameters. These parameters include cooling/ thawing rate, cryoprotective agent (CPA) concentration and osmotic response of the cell membrane [Mazur, 1984]. In the last years a new parameter, nucleation, went into focus in contrast to cell survival. Nucleation is the process accumulation of water molecules to form a nucleation embryo. If the critical size is passed a phase change from liquid to solid occurs. There are many ways to induce the nucleation such as seeding, ultra sound, bacteria, pressure shift and electrofreezing. In this work we used electrofreezing and seeding to induce the nucleation. In literature there are few studies to optimize the nucleation temperature. In this study we investigated the influence of different nucleation temperatures on mesenchymal stem cells derived from the common marmoset, *Callithrix jacchus* (cjMSC) concerning of membrane activity, recultivation efficiency and metabolic activity. Cells were frozen under optimal conditions using a controlled rate freezer and a nucleation device (electro freezing) using different concentrations of dimethyl sulfoxide (Me_2SO). The effect of the nucleation temperature on cell viability and proliferation of cjMSC were studied by analyzing the membrane integrity with trypan blue staining, efficiency of recultivation and metabolic activity with Alamar blue assay. Our results showed that electro freezing with active controlled induced nucleation at the desired temperature has a positive effect on the survival rate of cryopreserved cells.

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C-2029

Rare and Endangered Bird of Northeast Eurasia, the Siberian White Crane Grus Leucogeranus: Habitats, Ecology, Migration, Wintering. I. BYSYKATOVA, G. Nickolai, S. Nikita, S. Sleptcov, M. Vladimirtseva, N. Egorov, and V. Okoneshnikov. IBPC SD RAS, Lenina 41, Ave, Yakutsk 677000, RUSSIA. Email: ipbysykatova@gmail.com

Siberian white crane occupies an extensive area in the eastern part of Eurasia. North East of Yakutia is a region of its reproduction, Southeast China - wintering area . Until the 50s of the twentieth century the study of Siberian Cranes were sporadic. In the early 60s, there appeared the first information on the distribution, number and biology (Vorobyev, 1963). Derailed study of the eastern population of the Siberian Crane, in Russia and in China began in the 70s - 80s. In order to preserve the species, in Russia (Oka Reserve) and Baraboo (USA, Wisconsin) Russian- American scientists created nursery of the Siberian crane , and for what on the breeding grounds in Yakutia conducted numerous egg collection . Was collected the new data on the contours and size of the breeding range, number, and clutch size, etc. (Egorov, 1971; Vshivtsev et al, 1979 ; Flint, Sorokin, 1979, 1982). Until the early 90s the number of Siberian Crane in the breeding grounds was assessed by avia – counting in 300 - 800 individuals (Vorobyev, 1963; Degtyarev Labutin, 1991), on the wintering grounds in China by ground-based observations - in 100-2500 individuals (Harris et al., 1995). Since 90s Yakutian, American, Japanese and Chinese scientists have been monitoring the state of the Siberian Crane and its habitats within the entire species population range in East Asia. In Yakutia, from 1990 to 2008 banded 71 birds - adults and chicks, from 1992 to 2005 for study their movements and migrations was attached 19 satellite transmitters (Germogenov et al., 2007). Currently, these studies established migratory corridor of the population, timing and intensity of migration, the place of their major transit stopovers in Russia and China, which are equipped with observation points. The main breeding area, winter-ing grounds, intensive flight and stopover places during migration of Siberian Cranes in North-east Asia are taken under protection.

C-2030

Winter Nesting of the Birds Near North Pole in the Northern Hemisphere-Yakutia/Russia. I. BYSYKATOVA, N. Germogenov, N. Egorov, and S. Sleptcov. IBPC SD RAS, Lenina 41, Ave, Yakutsk 677000, RUSSIA. Email: ipbysykatova@gmail.com

In winter in Yakutian taiga nesting 10 species of the birds (3.7% breeding fauna), when the night temperatures reach -20 ÷ 30C°. In March nests Bubo bubo (4 nests), in April Surnia ulula (4). In March-May begins the egg-laying Loxia leucoptera (50.0-66.7% clutches; n = 10), at the end of March to early May, Corvus corax (93.2-100.0; 47) and Perisoreus infaustus (75.0 -87.6, 93). In April and May start egg-laying Strix nebulosa (52.9; 17), Nucifraga caryocatactes (33.3-100.0; 13), Accipiter gentiles (0-33.3; 4), Haliaeetus albicilla (33.3; 3) , up to July it stretched for Acanthis flammea (1.0-27.3; 637). In the middle taiga in winter nest in earlier calendar and phenological periods than in other parts of their range, 87.6 % pairs of the Siberian jay (n = 89). Also here amount of their clutches are larger (2-5, average 3.99 ± 0.06 eggs; n = 90). In the woods of valleys species begins egg-laying earlier than in the watersheds - the end of March (22.7%; n = 44) versus the beginning of April (38.5, 26), has a larger clutches – 4.13 ± 0.09 (n = 40) versus 3.75 ± 0.1 (28), and nesting mostly on the trees (84.5%; n = 73), and watersheds - on the willows (67.2, 70) . There are other mechanisms that allow to species to breed at winter

temperatures and in various landscapes. Pairs are constant and strictly territorial, nest on the trees and shrubs (n = 192) at a height of 0.5-8.0, on average, 1.6 (willow) -2.9 (pine) m, mainly on south side (50.0 %). Feathers, down and rag compose up to 22.8 % weight of the nest (n = 5), a supporting part (dry twigs) - 63%. The number of the population in the site of the watershed taiga (10.6 km^2 in spring and autumn are, respectively, 59-84 and 55-87 individuals. In November 2012 residents amounted up to 71.9%, immigrants – 18.7 (n = 64). In the autumn of 2009, the ratio ♀ and ♂ of ad (n = 25) was equal, and for juv (29) prevailed ♂ (1:1.5), but for the year it aligned for them also. On eggs ♀ spend an average of 96.7 % of the day. Chicks hatch for 1.5 days on average, they are fed in the end of April, from 5:25 to 19:45 hours, after 1.5 hours.

C-2031

Investigation of Anti-freeze Proteins Effect on Vitrification-Devitrificartion Processes in a Micro-scale View. L. BAHARI and I. Braslavsky. Robert H. Smith Faculty of Agriculture, Food, and Environment, The Hebrew University of Jerusalem, Rehovot, ISRAEL. Email: bahari.liat@gmail.com

Ice crystal can greatly damage biological matter which was kept in low temperature for preservation. This problem can be avoided using vitrification, a process by which a liquid is solidified into a non-crystalline (glassy) state by lowering the temperature in the presence of solutes and greatly increasing the viscosity. This process is also beneficiary since it avoids the formation of concentration gradients introduced during crystallization and thereby avoids an osmotic injuries result in loss of sample viability. Since the late thirties, when Luyet raised the idea of cells cryopreservation by vitrification, a lot of progress was made in understanding this process and developing this technique to suit the biological needs. However, there is still main difficulty which has not come into a successful solution- the phenomena called 'devitrification', which means ice crystals formation during the rewarming step of partially vitrified biological material. We suggest a natural alternative to face this challenge by using antifreeze proteins (AFPs) found in cold-adapted organisms. These proteins are known to inhibit ice growth in slightly suppercooled conditions, and inhibit recrystalization of ice in frozen tissues. In this study we investigate the influence of AFPs on the vitrification and de-vitrification processes under microscope equipped with controlled cold stage and by differential scanning calorimetry (DSC). Observation on vitrification and de-vitrification of DMSO solutions at different volumes, temperatures and cooling and warming rates have demonstrated these processes in a micro scale. Preliminary results show that addition of the hyperactive AFP from *Tenibrio molitor* in various concentrations appear to have less ice nucleation centers during warming. Moreover, ice crystals, at post vitrification temperatures (i.e~-105˚C), display slower growth in solutions containing AFPs. These novel effects of *Tm*AFP will be farther characterized and developed in a course of utilizing these findings into biological matter cryopreservation applications.

C-2032

Effect of Temperature Storage on Motility Parameters of Cryopreserved Blackbelly Hair Ram (*Ovis Aries*) Spermatozoa. Á. E. DOMÍNGUEZ-REBOLLEDO[1], J.Cantón[1], H. Loeza[2], A. Alcaraz[1], and J. Ramón[2]. [1]INIFAP, Calle 23, No. 208 x 30 y 32, La florida, Mérida 97138, MEXICO and [2]Instituto Tecnológico de Conkal, Km. 16.3 Antigua carretera Mérida-Motul, MEXICO. Email: alvaroedr@gmail.com

The aim of this study was to assess the use of two ultra-low temperature freezers to store ram semen comparing the efficiency of these techniques versus the use of liquid nitrogen (NL_2) to freeze and store semen samples. Semen from 5 Blackbelly hair ram were used in this study. Ejaculates were pooled, diluted in Triladyl® + 20% egg yolk to a final concentration of 400 x 10^6 spz./mL, loaded into 0.25 ml straws and cooled at 5 ºC for 3 h. Then, three protocols were tested to evaluate the cryosurvival of sperm at 10 days after freezing: (I) semen was frozen and stored at -196 in NL_2; (II) semen was frozen in NL_2 and stored in the ultra-low freezers at -50 ºC; (III) semen was frozen in NL_2 and stored in the ultra-low freezers at -70 ºC; (IV) semen was frozen in NL_2, stored in the ultra-low freezers at -70 ºC and frozen again in NL_2. Mean values of percentage of total motility (MT, %) and progressive motility spermatozoa (PM, %), curvilinear velocity (VCL, µm/s), average path velocity (VAP, µm/s), straight-line velocity (VSL, µm/s), linearity coefficient (LIN, %), straightness coefficient (STR, %), wobble coefficient (WOB, %), beat cross frequency (BCF, beat/s), and amplitude of lateral head displacement (ALH, µm) were assessed by the CASA (ISAS, Proiser; Valencia, Spain). Data were analysed by linear mixed-effects models. Protocol (I) yielded the highest ($P<0.05$) of all parameters motility to respect of the others protocols. However, spermatozoa can be stored at -70 ºC with a slight loss of motility compared to protocol (I). Results showed that protocol (I) is the most adequate to cryopreserve Blackbelly ram semen.

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