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DEVELOPMENT OF HIGH CONCENTRATION CELL BANKING PROCEDURE

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Mammalian cell culture that can be produced on an industrial scale has become the dominant production system for recombinant proteins. A typical mammalian cell culture includes the steps of thawing a vial from the relevant cell bank, bringing the amount of biomass to a sufficient size for production, and production steps. The seed train is a critical part of the bioprocess, producing enough biomass to inoculate the production bioreactor and start protein production in an optimized manner. In a conventional seed train, this is achieved by passing cells through a working cell bank flask through increasingly larger cultivation systems including shaker flasks, rocking motion bioreactors, and stirred tank bioreactors. Efforts are being made to create a highconcentration cell bank to avoid these intermediate culture steps and to allow direct inoculation of a production bioreactor with cells from cryovials or cryobags. The common cell banking concentration for mammalian cells is 10-15x10^6 cells/ml. In this study, cell banks with different concentrations up to 200x10^6 cells/mL were created and these cell banks were inoculated directly into production to test the growth profiles of the cells and the amount of monoclonal antibodies produced. The ultimate goal of this study is to reduce time, labor, cost and operational risks by using high concentration cell banks.
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APPLICATION OF PORTABLE CRYODEVICE AS A SOLUTION FOR CENTRALIZED CRYOBANKING

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Ovarian tissue cryopreservation (OTC) is the best option for fertility preservation (FP) in prepubertal girls and women with cancer. Thus, interest in adopting the OTC has been growing all around the world. However, the cost to establish and maintain one cryopreservation center for thirty years is more than €5 million. Therefore, the establishment of central cryobanks would overcome these difficulties. For such central cryobanks, the ovarian tissue transportation (OTT) network is necessary to increase the patient's access to the OTC technique. However, OTT is considered an empirical procedure and is associated with the negative effect of ischemia. Hence, shortening the pre-cryopreservation time could be a key strategy to reduce ischemia-induced damage and improve graft longevity. An ovarian tissue cryopreservation network (OTCN) would address the shortages of OTT and expand the FP options available to patients who are in geographic areas that don't have an OTC cryobank. OTCN can be called "The patient stays- the staff comes". One approach to achieve this goal is employing portable cryo-devices such as Asymptote and Mr. Frosty. Therefore, we examined the feasibility of portable cryopreservation devices, including a controlled rate asymptote freezer and Mr. Frosty against the programmable slow freezer for the establishment of an OTCN. To determine the application of the different cooling machines, the viability, reactive oxygen species (ROS) levels, total antioxidant capacity (TAC), and morphological characteristics of bovine ovaries were evaluated in frozen groups and results were compared with the fresh group. Our results indicate that Asymptote can freeze the ovarian cortex similar to a programmable slow freezer; however, we observed lower cell viability and TAC and higher ROS, and degenerative follicle when we used Mr. Frosty device. In conclusion, a portable liquid nitrogen-free device like Asymptote can be considered as a possible solution for the establishment of an OTCN.
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THE IMPACT OF CRYOPRESERVATION ON THE FUNCTIONAL RECOVERY OF LUCIFERASE REPORTER CELLS

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Though in vivo animal models are commonly used in cancer biology and drug research, their imprecision and lack of physiological relevance pose a major hurdle for clinical translation. ATCC's luciferase-labeled cell lines provide a simple, sensitive, and versatile means to measure specific biological processes through bioluminescence imaging. Because these engineered cell lines are a powerful tool for oncology and immuno-oncology research, it is important to consider the impact that low-temperature storage will have on the luciferase reporter functionality. Cryopreservation using a controlled-rate freezer followed by storage in the vapor phase of liquid nitrogen is the gold standard for long-term preservation of mammalian cells at ATCC. It is a well-known phenomenon that cryopreservation and subsequent thawing are stressful processes that generally require a recovery period in culture for growth, metabolism, and functionality to return to normal. Because of this, it is important to understand how these processes impact the resulting recovery time required for our luciferase reporter cells to be able to provide the accurate and repeatable results expected. In this study, we compared the immediate post-thaw viability, growth, and functionality characteristics of THP-1 luciferase reporter cells (ATCC® TIB-202-NFkB-LUC2™) to those of non-frozen controls. As expected, the cells immediately recovering from cryopreservation had different characteristics than those in fresh culture. Specifically, we identified an atypical cellular response to immediate post-thaw lipopolysaccharide (LPS) stimulation. Observing the impact of preservation on the cell characteristics and understanding the mechanisms that lead to these phenotypic changes provide us with an opportunity to optimize our cryopreservation formulations and procedures toward reducing stress and enhancing recovery. Minimizing this recovery time will enable our customers to start their research faster.
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BANKING CORAL LARVAE AT SCALE WITH CRYOMESH: OPPORTUNITIES AND CHALLENGES

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Coral populations around the world are threatened by human activity and warming oceans. Novel and scalable approaches to cryopreserve coral germplasm, algal symbionts, and adult tissues can improve access to banked material for biological research and reef restoration efforts, thus supporting biodiversity and increasing population resilience to climate change. We recently vitrified and rewarmed larvae of the solitary Hawaiian stony coral species *Lobactis scutaria* (mushroom coral) with one-step loading of a 3.5-M multi-component cryoprotective agent (CPA) solution, removal of excess CPA, liquid nitrogen plunge cooling, warming on a novel cryomesh substrate, and two-step CPA unloading. With optimization of substrate geometry and experimental methods, estimated cooling rate within the larvae was 9.4×10^4 °C/min, warming rate was 1.2×10^5 °C/min, and post-thaw larval recovery reached 85%. This result improves on prior vitrification work with the same species using 1-μL droplet plunge cooling of the same CPA solution on a plastic film followed by nanoparticle-mediated infrared laser pulse warming (plunge cooling rate < 6.9×10^4 °C/min, laser warming 4.5×10^6 °C/min, 43% recovery). Here we present our cryomesh results, propose technical directions and collaborative efforts that will allow deployment of this and related technologies at a scale that will support research and restoration activities, and describe the remaining challenges in cryopreservation of larvae of a wider range of coral species.

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IMMUNOMODULATING RESPONSE OF AUTOLOGOUS CRYO-HEMATOLOGICAL TREATMENT IN PATIENTS WITH MALIGNANT MANIFESTATIONS

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Cryoinduced antigen is one of the main terms of autologous and allogeneic cryo-immunological medicine. The newly formed cryomodified protein components, coupled with cryocell detritus / cryocell debris formed extracorporeally in living biological tissue—specifically, human blood after exposure to ultralow temperatures—are crucial in cryo-immunology from the perspective of the formation of immunological substances at ultralow temperatures. The aim of the presented study was to evaluate the immunomodulating response of autologous cryo-hematological treatment with the objective of beneficial antitumor effect. In this study, the native blood was frozen to −180°C rapidly, at a temperature stabilization of ±1%, to achieve complete blood component desolation. A one-minute rapid freezing followed by complete inactive thawing of the blood sample was used only once as a freeze−thaw cycle. Parameters of immunophenotyping with cellular immune status were laboratory-determined in blood in patients with malignant diseases on pre- and post-treatment days after extracorporeal blood procedures applying ultralow temperature in vivo and *in vitro*. In response to mitogen treatment *in vitro*, the absolute count of CD69+ circulating B-cells progressively increasing during the post-procedure period, indicating the positive effect of subcutaneous injection of extracorporeal freeze−thawed autologous blood on naive B-cell proliferative capacity. A sharp progressive increase of both the absolute and relative number of CD56+CD45+CD69+ lymphocytes in response to the treatment of blood sample with pokeweed mitogen *in vitro*, was registered during the post-procedure period. We hypothesized that the extracorporeal frozen-thawed blood produces new cryoinduced specific immunogenic substances in human blood (e.g. cryomodified protein components, cryoantigen, cryocell detritus) and generates cell-mediated anti-tumor responses (e.g. cryoinduced anticancer antigen), changing of phenotypes in peripheral blood of patients and triggering specific and non-specific cellular immune responses, which are important to initiate tumor rejection. Equally, we assume that the generated after the injection of post-frozen blood antibodies could be an important protagonist in antibody-dependent antitumor immune responses, suggesting a potential systemic benefit to treatment malignant manifestations.

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NERVE PROTECTION DURING PROSTATE CRYOSURGERY

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Cryosurgery is a minimally invasive approach to the treatment of focal prostate cancer (PCa). A major complication is the cryoinjury to the cavernous nerve in the neurovascular bundle (NVB). This nerve cryoinjury halts conduction of action potentials (APs) and can eventually result in erectile dysfunction and therefore diminished quality of life for the patient. Here, we propose the application of cryoprotective agents (CPA) to the regions of the nerves in the NVB, prior to prostate cryosurgery, to minimize non-recoverable loss of AP conduction. We modeled a cryosurgical procedure based on data taken during a clinical case and applied ex-vivo porcine phrenic nerves and rat sciatic nerve with temperature profile of NVB. The APs were measured before and after the CPA exposures and during 3 h of recovery. Comparisons of AP amplitude recovery with various CPA compositions reveal that certain CPAs (e.g., 5% Me_2SO + 7.5% Trehalose and 5% M22 for porcine and rat nerves, respectively) showed little or no toxicity and effective cryoprotection from freezing (on average 48% and 30% of recovered AP, respectively). In summary, we demonstrate that neural conduction can be preserved after exposure to freezing conditions if CPAs are properly selected and deployed onto the nerve.

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COLD-RESPONSIVE NANOPARTICLES-POTENTIATED CRYOIMMUNOTHERAPY FOR COMBATING LOCALIZED AND METASTATIC BREAST CANCER

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Cancer immunotherapy is a revolutionary treatment strategy that utilizes the host's immune system to identify and eliminate tumors. However, the effectiveness of this approach is greatly limited by the immunosuppressive (i.e., immunologically cold) tumor microenvironment (TME), which prevents the immune system from properly recognizing and attacking cancer cells. Cryosurgery, a minimally invasive surgical technique that uses low temperatures to kill cancer, has been shown to impact the TME and generate a cryoimmunotherapy effect to improve treatment of localized tumor. Unfortunately, the capability of cryoimmunotherapy against distant/metastatic tumor, the major cause of mortality in cancer, is not shown yet. In this study, we developed an in-situ cryo-immune engineering (ICIE) strategy that can reprogram the immunologically "cold" TME into a "hot" one with increased immunoactive cells and decreased immunosuppressive cells, to potentiate the immune system's attack on