sample. A CMOS camera records the interference of that beam and the reference beam, resulting in a hologram. Through image processing, the refractive index change is calculated from the phase difference maps and is converted into a temperature profile. The results give accurate measurements of temperature within the sample, which correlate to the cooling and heating rates. Through these results we observe the vitrification temperature history to determine phase changes in that stage and heating conditions to optimize current warming techniques and improve biospecimen viability.

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CELL AND TISSUE CRYOPRESERVATION THROUGH PRESSURE ENHANCED SOLID SURFACE ULTRA-RAPID COOLING

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Cooling rate is critical for cell and tissue cryopreservation. By increasing the cooling rate during freezing, it is possible to decrease the cryoprotective agent (CPA) concentration by limiting or even preventing ice growth. One control parameter that can increase the likelihood of avoiding ice growth is hydrostatic pressure. Previous studies have shown that pressure can reduce the melting temperature (T_m) and the homogenous nucleation temperature (T_h), and on the other hand, increase the glass transition temperature (T_g). These alterations are encouraging indicators to improve freezing concerning eliminating ice formation. In this work, first we show that pressure also influences cooling rate outright. Our physical and mathematical modeling in this study show that increasing pressure up to 200 MPa enhances the cooling rate significantly. Second, solid-surface conductive-cooling is another approach to improve the cooling rate compared to convective cooling methods such as plunging directly into liquid nitrogen, prevalently used in cryobiology. Our numerical results demonstrate that conductive heat transfer can increase the cooling rate by one order of magnitude compared to convective heat transfer. Using this model and experimental data, we designed and manufactured a cryodevice that can apply cooling to both sides of tissue to achieve a substantial survival depth in a hermetically sealed sample container. In conclusion, understanding and insight into details from various disciplines can aid the design and development of effective cryoprotocols.

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SMALL MOLECULE ICE RECRYSTALLIZATION INHIBITORS FOR THE CRYOPRESERVATION OF TISSUE COMPONENTS AND TISSUE CONSTRUCTS

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Despite the use of cryoprotective agents (CPAs) such as dimethyl sulfoxide and glycerol, the cryopreservation of these cellular products is suboptimal and new strategies/protocols are urgently required. This is because during cryopreservation, the majority of cellular injury/death is the direct result of uncontrolled ice growth, a phenomenon known as ice recrystallization. To address this issue, our laboratory has pioneered several different classes of molecules that possess the ability to inhibit ice recrystallization, ultimately increasing post-thaw viability and/or recovery as well as the functional capacity of progenitor cells and tissues. This presentation will demonstrate the ability of IRIs to increase the post-thaw viability/recovery of dermal tissue constructs which are important for wound healing and skin regeneration where readily available tissue constructs are urgently needed to improve the patient prognosis. We hypothesized that combining innovative tissue engineering with ice recrystallization inhibitors (IRIs) would enable the cryopreservation of dermis suitable for autologous epithelial cell seeding. The self-assembled method was used to obtain the dermal constructs. Briefly, fibroblasts were cultured for 28 days to form a sheet where cells were embedded into a self-assembled matrix. The baseline viabilities of cells in suspension and dermal sheets was initially assessed using 1.5% BSA + 10% Me_2SO solution as a control. Post thaw cell viability was determined using TrypanBlue and long-term viability/functionality was observed at 96 hrs in culture after thawing. Tissue viability was assessed using metabolic and DNA-staining assays (AlamarBlue and SytoxGreen). Our results indicate that a number of different classes of IRIs decreased post-thaw fibroblast cell mortality in suspension (38-52%), and increased post-thaw tissue viability (20-27%). Production of a complete skin equivalent using cryopreserved dermis and seeded keratinocytes is currently underway. Collectively, these studies illustrate the tremendous potential of the IRI technology platform to effectively cryopreserve tissue components and tissue constructs.

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ISOCHORIC BIO-THERMODYNAMICS: RECENT PROGRESS ON EQUILIBRIUM, METASTABLE, AND NON-EQUILIBRIUM ISOCHORIC BIOPRESERVATION TECHNIQUES

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Isochoric (constant-volume) biopreservation techniques have gained significant interest in the past half-decade, with published work on preservation of human cells, tissues, and organs for medical applications; of whole marine organisms for conservation applications; of fresh food stuffs for agricultural applications; and etc. However, contrary to popular conception, these differing applications do not employ one monolithic technique, but a wide variety of different techniques that are unified only by their use of macroscopic isochoric confinement. In this talk, we will review the three principal thermodynamic variants employed in isochoric biopreservation: equilibrium isochoric freezing, metastable isochoric supercooling, and non-equilibrium isochoric vitrification. For each approach, we will discuss the differing thermodynamic and kinetic considerations at play; appropriate application domains; and specific state-of-the-art protocols. We will conclude by offering high-level perspective on the direction of the field, and identifying the most significant open questions that may help guide future research.

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Conflict of Interest: MJPP, AC, and BR have filed patents related to isochoric biopreservation, and MJPP and BR have a financial interest in a private company related to these technologies.

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TEMPERATURE-DEPENDENT THERMOPHYSICAL PROPERTIES OF CPAS AND TISSUES RELEVANT TO CRYOBIOLOGY

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The objective of this study is to create a comprehensive living database of thermophysical properties for the benefits of the cryobiology community, in the range of -150°C to 0°C (https://www.atp-bio.org/)). Thermophysical properties are essential for accurately modeling the behavior of cryoprotective agent (CPA) cocktails and tissues, ultimately improving the design and outcomes of cryopreservation protocols and cryotherapy procedures. The focus of this study is compiling temperature-dependent thermal conductivity, density and thermal expansion coefficients for various materials, while future efforts will target incorporating specific heat and elastic material properties to this database. The property of thermal conductivity is related to the ability of a material to transfer heat across the domain. Density and thermal expansion also affect the material response to changes in temperature in various ways. In fluids, density differences can drive fluid flow, while in solids, expansion and contraction can lead to mechanical deformation. This study further reviews common measurement techniques, ranging from molecular-scale to cellular-level and macro-scale. In turn, this can inform the user on the appropriate measurement method for a specified sample type, application, and temperature range.

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DELAYED INJURY AND CELL-TYPE-SPECIFIC RESPONSES DURING CRYOPRESERVATION OF ENDOTHELIAL MONOLAYER

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The cryopreservation of endothelial cell monolayer is an important stage that bridges the cryopreservation of cells in suspension to tissues. Previous studies have identified clear distinctions in freezing mechanisms between monolayers and cells in suspension, as well as developed novel protocols for monolayer cryopreservation. Recently our group has shown that human umbilical vein endothelial cell (HUVEC) and porcine corneal endothelial cell (PCEC) monolayers grown on Rinzl plastic substrate can be cryopreserved in 5% dimethyl sulfoxide, 6% hydroxyethyl starch, and 2% chondroitin sulfate, following a slow cooling protocol with rapid plunge to liquid nitrogen from −40 °C. However, viability assessments were done immediately post thaw, which may have resulted in an overestimation of cell viability due to possible delayed injury responses. Here, we show that for the optimal protocol condition of plunge at −40 °C, HUVEC and PCEC monolayers exhibited no immediate post-thaw injuries and no delayed injury response after overnight culture. HUVEC monolayers experienced reduced proliferation during the post-thaw culture, while PCEC monolayers maintained a proliferation rate comparable to non-treated controls. The difference in the low-temperature responses between HUVEC and PCEC monolayers is enhanced under high temperature plunge conditions that would result in intracellular ice formation. HUVEC monolayers exhibited intermediate immediate membrane injury but a pronounced delayed injury response, while PCEC monolayers showed significant immediate membrane injury but no delayed injury response. Therefore, we confirm that our previously designed endothelial monolayer cryopreservation protocol is successful for HUVEC and PCEC monolayers, and we identified several cell-type-specific responses during the freezing process.

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FREEZE-DRIED DECELLULARIZED HEART VALVES AS STARTER IMPLANTS FOR HEART VALVE REPLACEMENT THERAPIES

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Decellularized heart valves have the potential to replace a malfunctioning valve. Such tissue engineered heart valve scaffolds not only have regenerative capacity, but also exhibit growth capacity when implanted in children. We have shown that heart valves can be freeze-dried after decellularization processing ensuring off-the-shelf availability. Sucrose is needed as lyoprotective agent to mitigate pore formation in the tissue caused by ice crystals and to preserve biomechanical characteristics after freeze-drying. Exposure to highly concentrated sucrose solutions is needed to completely diminish pore formation. In vivo trials in animals have shown that freeze-drying does not alter the long-term durability and repopulation potential of decellularized grafts. Here we aimed to study oxidative damage in freeze-dried heart valves during storage. Decellularized porcine aortic heart valves were analyzed by nitroblue tetrazolium (NBT) staining and Fourier transform infrared spectroscopy (FTIR) to identify oxidative damage during freeze-drying and subsequent storage as well as after treatment with H_2O_2 and $FeCl_3$. NBT staining revealed that sucrose at a concentration of at least 40% (w/v) is needed to prevent oxidative damage during freeze-drying. Dried specimens that were stored at 4 °C depict little to no oxidative damage during storage for up to several months. FTIR analysis combined with a feed forward artificial neural network model showed that fresh control, freeze-dried and stored heart valve specimens cannot be distinguished from one another, whereas H_2O_2 and $FeCl_3$ treated samples can be classified based on differences in characteristic molecular group vibrations. This implies that freeze-drying and storage have little effects on the overall biomolecular fingerprint of the scaffolds. Taken together, we conclude that sucrose can minimize oxidative damage caused by freeze-drying, and that subsequent storage has little effects on the biochemical composition of heart valve scaffolds.

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COMPRESSION-MEDIATED CRYOPROTECTANT DELIVERY ENABLES CRYOPRESERVATION OF INTACT INTERVERTEBRAL DISC

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In the US, over 1.3M people annually undergo invasive surgery to alleviate back pain associated with intervertebral disc (IVD) degeneration. These surgeries do not treat IVD degeneration, and often lead to further health complications. These problems have driven the research community to seek new solutions, such as IVD allografting, tissue engineering and stem cell therapies. In all of these cases, the lack of readily available human IVDs is a significant barrier. While it is currently possible to obtain fresh IVDs from