solution), but much higher in the presence of 5% $Me_2SO$ (85% incidence). Suppressing the formation of extracellular ice in nonpenetrating cryoformulations during the first 12-20 K of rapid cooling caused the cumulative incidence of intracellular ice formation to increase approximately 3-fold, reaching levels comparable to those observed in $Me_2SO$ solutions. This suggests that cell dehydration (driven by freeze-concentration of the extracellular solution) is significant in the absence of penetrating cryoprotectants, but not in the presence of $Me_2SO$. Preliminary results from morphometric analyses of Jurkat cells frozen without cryoprotectants were consistent with the hypothesis that cell water loss occurs during extracellular ice formation at the initial equilibration temperature and during subsequent rapid cooling. Furthermore, the observation that intracellular ice growth velocities in cells frozen in nonpenetrating cryoformulations were slower than in cells containing $Me_2SO$ also suggested that cell water content was lower in the former case. Therefore, penetrating cryoprotectants like $Me_2SO$ may promote intracellular ice formation in T cells by inhibiting cell dehydration during freezing.

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## S64
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## WATER CONFINEMENT EFFECT ON CRITICAL COOLING AND WARMING RATES IN TISSUE-CPA SYSTEM

Lakshya Gangwar [1], Zonghu Han [1], Michael Etheridge [1], John Bischof [1,2], Jeunghwan Choi [3,*]. [1] *Department of Mechanical Engineering, University of Minnesota, Minneapolis, USA;* [2] *Department of Biomedical Engineering, University of Minnesota, Minneapolis, USA;* [3] *Department of Engineering Technologies, Safety, and Constructions, Central Washington University, Ellensburg, USA*

* Corresponding Author:

*E-mail address:* Jeunghwan.Choi@cwu.edu (J. Choi).

Understanding and controlling the phase transition of water is the foundation of cryopreservation. Water is an important constituent of all biological materials and is found naturally in confined spaces inside biological materials. Water in confined domains acts differently than in bulk domain, and has been studied in many porous materials, including biological materials. Physical and calorimetric properties of water in confined spaces deviate largely from in non-confined space. Past studies have shown the effect of confinement on mechanical, physical and chemical properties of a material containing confined water. Here we aim to characterize this effect in tissues loaded with cryoprotective agents (CPAs), which is important for the design of cooling and rewarming protocols for tissue and organ cryopreservation. Phase diagrams and critical rates for dilutions of cryoprotective agent (CPA) concentrations were measured in solutions and tissue equilibrated with CPA. Specifically, we report on water confinement effects on critical cooling and warming rates inside a biological material (rodent kidney tissue) equilibrated with a common CPA (VS55). Kidney biopsies (3 mm diameter) prepared from tissue slices (1.2 mm thick) are equilibrated (>1 week) in a range of CPA (VS55) concentrations (75- 100%) and then tested for CCR, CWR and transition temperatures (Tm, Tg, Td & Th). We found that there is a measurable reduction in CCR (max ~88%) and CWR (max ~27%) inside tissue equilibrated with CPA as compared to bulk CPA solution. This knowledge is critically important for appropriately choosing CPA concentrations and cooling rates in vitrification protocols for tissues and organs. While we currently report the effects for a single model system, these methods can be expanded to a wider range of CPAs and tissues and lay the groundwork for a more mechanistic understanding of confinement.

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## S65
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## EXTREME VALUE STATISTICS FOR ESTIMATING THE FREEZING PROBABILITY OF SUPERCOOLED WATER

Anthony N. Consiglio [1,*], Yu Ouyang [1], Matthew J. Powell-Palm [2], Boris Rubinsky [1]. [1] *Department of Mechanical Engineering, University of California, Berkeley, USA;* [2] *Department of Mechanical Engineering, Texas A&M University, College Station, USA*

* Corresponding Author:

*E-mail address:* aconsiglio4@berkeley.edu (A.N. Consiglio).

The propensity of water to remain in a metastable liquid state at temperatures below its equilibrium melting point holds significant potential for cryopreserving biological material such as tissues and organs. The benefits conferred are a direct result of progressively reducing metabolic expenditure due to colder temperatures while simultaneously avoiding the irreversible damage caused by the crystallization of ice. Unfortunately, the freezing of water in bulk systems of clinical relevance is dominated by random heterogeneous nucleation, and the marked unpredictability of this behavior has prevented implementation of supercooling outside of controlled laboratory settings and in volumes larger than a few milliliters. Here, we develop a statistical model that jointly captures both the inherent stochastic nature of nucleation using conventional Poisson statistics as well as the random variability of heterogeneous nucleation catalysis through bivariate extreme value statistics. By quantifying freezing probability as a function of temperature, supercooled duration, and system volume, while accounting for nucleation site variability, this study also provides a basis for the rational design of stable supercooled biopreservation protocols.

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## S66
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## EXTRACTING ICE CRYSTAL NUCLEATION AND GROWTH RATES FROM CALORIMETRY CURVES

Joseph Kangas[*], Christopher J. Hogan, Jr.. *Department of Mechanical Engineering, University of Minnesota*

* Corresponding Author:

*E-mail address:* kanga134@umn.edu (J. Kangas).

We propose a method for determining the time, and therefore temperature, dependent nucleation and growth rates during crystallization. We do so by linking the partial differential equation governing the time dynamics of the crystal size distribution to kinetic (Avrami) parameters describing heat release. This approach is tested in-silico by nucleating and growing time-dependent diffusion limited aggregates with time varying geometry, growth rates, and nucleation rates. The associated heat release was analyzed, showing that nucleation and growth rates could be extracted with high fidelity.

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