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The following abstracts were published without literature citations. Herein are the abstracts as they were submitted.

Cryobiological Thermodynamics: How Math Can Save Lives

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Cryobiology is an inherently interdisciplinary research field. To solve any but the most trivial cryopreservation challenges requires cryobiology-specific knowledge from medicine, biology, thermodynamics and other engineering sciences, and this specific knowledge has been under development by cryobiologists over a period of study spanning almost 100 years. Thermodynamics, a mathematical and profoundly useful subject, is the underlying physical science of cryobiology. Thermodynamics determines at what temperature ice can form, how much ice is formed at a given temperature, how cryoprotectants supress the formation of ice or mitigate its effects, and how ice interacts with cell and tissue structures. Thermodynamics describes the transport of water across cells and tissues in response to ice formation, the permeation and efflux of cryoprotectants into and out of cells during loading and unloading, and the transport of heat in response to temperature gradients. To really leverage the understanding that thermodynamics can provide, mathematical modelling must be used in combination with

other knowledge. In interdisciplinary, collaborative research we use innovative biological experiments to measure cell- and tissue-specific parameters appearing in mathematical models. Mathematical models are then used iteratively with innovative experimental measurement of cell and tissue responses to cryopreservation protocols. Experimental results inform the development and refinement of mathematical models; mathematical models narrow the required experimental space to something feasible. In our collaborative environment engineers and mathematicians work together with biologists and surgeons, each receiving some training in the complementary discipline and even receiving joint graduate degrees in engineering and medical sciences where desired.

As well as many improvements to thermodynamic modelling in cryobiology, recent practical achievements from our group include: *i)* development of a protocol to cryopreserve hematopoietic progenitor cells (blood stem cells) with reasonable cell recovery without the need for permeating cryoprotectants [28], and *ii)* development (with NM Jomha) of a protocol to cryopreserve intact human articular cartilage on a bone base (as required for transplantation) with chondrocyte recovery (membrane intact cells after cryopreservation ÷ all cells in control) of 75.4 ± 12.1% in 10 mm dowels and 76.9 ± 6.2% in large fragments (12.5 cm^2). The cells in cryopreserved articular cartilage exhibited metabolic activity equal to control tissue after time for metabolic recovery. Cells from cryopreserved articular cartilage were pellet cultured and showed glycosaminoglycan and collagen II production similar to control [18, 19]. This success is significant because articular cartilage might be harder to vitrify than some organs due to its very slow diffusion of cryoprotectants, and no option of vascular perfusion.

A large amount of knowledge has been developed by cryobiologists over almost 100 years that is not fully appreciated outside of the cryobiology field but is critical to all aspects of

biotechnology. Even though a lot of progress has been made by our group and many others, only a small amount of available thermodynamic knowledge has yet been fully applied to understand and optimize cryopreservation. In addition, there are many other scientific subjects with available knowledge not yet fully applied in the field of cryobiology. Finally, there are some scientific questions unique to cryobiology that will require unique approaches and study that has yet to begin. We will need to put all of this together in a collaborative way to solve the harder problems we are faced with next.

Thermodynamics in Cryopreservation: Understanding Ice Formation

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Thermodynamics is the study of mathematical relationships arising from physical laws governing energy and entropy [8]. Thermodynamic equilibrium includes thermal equilibrium, mechanical equilibrium and chemical equilibrium. If one of these equilibria is not satisfied in a system, there will be a change towards equilibrium: heat will be transferred, mass will be transferred or change phase (ice will form or melt), or acceleration will occur due to a mechanical force imbalance. As such, thermodynamics (including both equilibrium and nonequilibrium thermodynamics) is the overarching physical science of cryobiology. Thermodynamics describes the freezing point of intra and extracellular solutions and how much ice is formed at a given temperature. Thermodynamics describes the flux of water and

cryoprotectants into and out of cells and across tissues. Thermodynamics describes the heat transfer that occurs during cooling and rewarming. Though vitrification is not strictly speaking a process of thermodynamic equilibrium, since vitrifiability is governed by how far the system is from its thermodynamic freezing point (vitrification is out-running thermodynamic equilibrium) and since the process of ice recrystallization of a vitrified solution is a thermodynamic one, thermodynamics plays a key role here too. Any approach to cryopreservation must be well-based in sound thermodynamic understanding. For more than fifteen years, we have worked to improve thermodynamic modelling in cryobiology [1–4,6,7,9–15,21–31]. We have introduced a multi-solute osmotic virial equation to make the most accurate predictions of multi-component extra- and intracellular solution freezing points and driving forces for osmotic transport [6,11,24–26,31]. We have described the transport of water and non-dilute components across cell membranes [3,9,12,14,15,28,29], and across complex tissues [1–3,13]. Our modelling has introduced an understanding of mechanisms of injury such as Mazur's rapid-cool and slow-cool injury for cells [28,29] or the mechanical stress of spatially uneven tissue dehydration during cryoprotectant loading in tissues [3]. We have explored curvature-induced freezing point depression and its implications for the growth of ice through cell membrane pores and tissue porosity [4,21,22]. We have investigated physical conditions for intracellular ice formation [4,7,29]. We have coupled thermodynamics to fluid mechanics to describe complicated phenomena that occur in the freezing of colloidal suspensions [10,23]. We have described vitrifiability with empirical mathematical models [30]. Even though we have made many improvements in thermodynamic modelling in cryobiology, there exists a great deal of other well-developed thermodynamics that has yet to be applied to cryopreservation challenges.

An Engineering Perspective on Toxicity

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Toxicity plays a central role in cryopreservation, whether it be toxicity of added cryoprotectants or of naturally occurring salts and other solutes concentrated in the unfrozen solution by ice formation. Toxicity may be studied from the viewpoint of biochemical mechanisms—understanding what molecule, pathway, or structure is "poisoned". However, an engineering perspective on toxicity is extremely relevant to cryopreservation. The toxicity of compounds to cells is concentration, temperature, and exposure-time dependent. Understanding the cryoprotectant exposure that a cell deep within a tissue experiences in a given loading/removal protocol requires an understanding of permeation/efflux kinetics with time. The dependence of toxicity on temperature can be used to advantage in designing cryopreservation strategies. Finally, even without knowledge of toxicity mechanisms, cryoprotectant toxicity and toxicity interactions can be modelled with empirical equations for use in protocol design. Our research group has explored these issues [1–3,5,16,17,20]. The developed engineering approaches to toxicity were critical in design of a protocol to cryopreserve intact full-thickness human articular cartilage on a bone base (as required for transplantation) with high chondrocyte viability, metabolic activity and function [18,19].

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