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PSYCHROPHILES AND PSYCHROTOLERANTS: SUSTAINABLE SOURCE OF BIO-BASED ECONOMY

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Psychrophiles/cryophiles and psychrotolerants are to a great extent unexplored group of organisms with the capacity to flourish in extreme cold conditions. The disclosures of new psychrophiles and psychrotolerants and their enzymes along with their natural adaptation at low temperature will offer novel industrially significant potentials. Extremozymes such as cold-active enzymes are the enzymes produced by psychrophilic/psychrotolerant microbes, they are better at functional level over their mesophilic partners for applications at extraordinary industrial conditions. These enzymes have incredible financial potential in numerous modern procedures, including industrial and pharmaceutical applications. Psychrophiles can also synthesize antifreeze proteins with unique properties of thermal hysteresis and ice recrystallization inhibition that have one of the promising tools in industrial applications like cryobiology, food storage, and others. Cold-active enzymes/proteins along with its producing microbes are sustainable source that might be better exploited in numerous biotechnological areas towards the expansion of a bio-based economy.

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PHYSICAL DEMONSTRATION OF VITRIFICATION OF LITER SCALE CPA SYSTEMS AND CHARACTERIZATION OF 120KW RF COIL FOR NANOWARMING OF LITER SCALE SYSTEMS

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Organ cryopreservation by vitrification and rewarming have been demonstrated at size scales up to 50 mL in varied organ systems from rat organs (kidney, heart, and liver) to rabbit kidneys. The next step towards preservation of human organs requires scale-up of the vitrification and rewarming techniques (e.g. liter scale and greater). Vitrification is generally achieved through convective cooling which is size dependent and therefore cooling rates are lower internally as the size increases, which can be a major challenge for vitrification of clinical scale organs. This study demonstrates the vitrification of litre-scale volumes (0.5L, 1L and higher) of VS55, M22, and 40%EG-0.6M-sucrose. Nanowarming on the other hand is based upon volumetric heating and is size independent due to its ability to generate heat via magnetic material (iron-oxide nanoparticles) perfused throughout the organ vasculature, as opposed to convective warming where the heat flux is fixed at the boundary. The concentration of these magnetic nanoparticles can be adjusted for achieving higher and sufficient heating at large volumes. These liter volumes of CPAs can be rewarmed uniformly & rapidly using a state-of-art 120KW radiofrequency (RF) coil with magnetic field strengths variable from 0 to 34 KA/m at 360 KHz and having the capacity to uniformly heat volumes up to 2.5L. The magnetic field generated inside the coil is investigated for uniformity along the axial and radial directions along with the characterization/calibration of the field strength versus system power. This study demonstrates critical milestones for scale-up (multi-liter) vitrification and rewarming of biological material and hence moving towards preservation of human organs.

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DEEP EUTECTIC SOLVENTS FOR CRYOPRESERVATION

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Cryopreservation has had huge benefits for the world at large, including preservation of blood and stem cells, and assisted reproductive technologies. However, there are many cell types that cannot be stored using current cryopreservation methods, and no organs. In fact, 60% of all donated hearts and lungs are discarded due to inadequate storage methods, and this waste could be overcome with cryopreservation.

The main limitation in cryopreservation is the ongoing reliance on predominantly just two cryoprotective agents (CPAs), both of which are toxic: dimethylsulfoxide (Me_2SO) and glycerol. The toxicity of existing CPAs means that cells must be frozen immediately after addition of the CPA. These CPAs are inappropriate for tissues and organs because there is insufficient time to penetrate to deeper cell layers, leaving them vulnerable to freezing damage. Thus there is a need for different, non-toxic CPAs with tuneable properties.

Deep eutectic solvents (DESs) are highly tuneable solvents, many of which are non-toxic. To date, only a very few studies have examined the cryoprotective applications of DESs, but these have shown comparable viability of cells stored using DESs compared to those stored using Me_2SO. We have characterised a number of DESs for their thermal properties and interactions with mammalian cells, including toxicity and permeability. We have also investigated the interaction of these solvents with model membranes using x-ray and neutron reflectivity. One DES was then carried forward and tested for its cryoprotective effect on four distinct mammalian cell lines. It was just as effective, and in some cases more effective, than Me_2SO at protecting the cells during cryopreservation.

These results provide new avenues of cryopreservation for cell types which cannot be preserved with existing CPAs. This in turn has wide-ranging benefits, especially in the biomedical field.

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AN AUTOMATIC AND CLOSED SYSTEM FOR RAPID REWARMING OF LARGE VOLUME OF CELL CULTURE MEDIA AND CRYOPRESERVED CELL SUSPENSION

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A rapid rewarming has been shown to be beneficial to prevent potential cell cryoinjury caused by intracellular ice re-crystallization or de-vitrification. While some of the protocols or methods have been established for the rapid rewarming process of small volume of cell suspension, rapid and uniform rewarming of large volume biofluids or cell suspensions remains challenging mainly due to the heat transfer issues. An advanced system on achieving rapid rewarming of the large volume biofluids and cryopreserved cell suspensions with precise temperature control during rewarming process has been developed. To keep at the desired temperature range and precisely control the temperature of these fluids during the rewarming process, an optimal temperature control and monitoring system is required. We have designed and manufactured a system that has a compact design, low overall power consumption while maintaining the efficiency of warming performance for high flow rates. Our preliminary data has confirmed that this system could warm up the cell culture media from 0°C up to 37°C with a single pass setup and at the maximum flow rate of 50ml/min. Mathematic