Current techniques often use vitrifying cryoprotectants at high concentrations that require rapid freeze and thawing rates. There is a real need for improvements in the cryopreservation of biological materials. Ice recrystallisation during freeze/thawing of cells is a major contributor to cell damage during cryopreservation.

Nature’s antifreezes, Antifreeze(glyco) proteins

Antifreeze(glyco) proteins (AF(G)Ps) are a naturally occurring class of proteins found in cold-acclimatised species that have a simple polymeric structure (Fig. 1). AF(G)Ps display a strong recrystallisation inhibition (RI) activity and thermal hysteresis, suppressing the freezing point of a solution.

Previous work (Fig. 2) explored the RI activity of several peptidomimetic macromolecules and synthetic glycopolymers highlighting their potential as cryoprotectives.

We have demonstrated that PVA can improve the cryopreservation of ovine erythrocytes comparable to existing methodologies but at less than 1% equivalent molar concentrations (Fig. 6).

FITC-tagged 9 KDa PVA is unable to enter lung adenocarcinoma (A549) cells (Fig. 7).

9 KDa PVA does not alter cell integrity or cell metabolism at concentrations with RI activity in human choriocarcinoma (BeWo), lung adenocarcinoma (A549) or mouse monocyte (J774) cells (Fig. 8).

Future work will explore the link between RI and cryopreservation by targeting the selected design of improved peptidomimetic molecules for eventual application with haematopoietic stem cells.

Peptidomimetic Macromolecules

Physiochemical Properties

PVA shows significant RI activity at both low and high molecular weights. Other common cryoprotectants such as dextran and PEG showed no effect over a wide concentration range (Fig. 4).

Numerous polyols including cryoprotectants such as glycerol, ethylene glycol and trehalose show weak RI activity with increasing [OH]-L1 concentration (adj R² = 0.8745) suggesting that the polymeric structure of PVA is key for RI activity rather than purely [OH]-L1 concentration (Fig. 5).

Background and Challenges, the need for novel cryoprotectants

Demand for Cryoprotectants

Rising demand for blood transfusions and tissue/organ donations that have increasing size and complexity.

Need to preserve biological functionality between procurement and transplantation.

Rapid removal for immediate clinical use is a necessity.

Limitations of AF(G)Ps

The isolation of AF(G)Ps in significant quantities is highly demanding and financially unviable. Transgenic and synthetic approaches have had limited success to date.

Their application as cryoprotectants is limited due to the secondary effect of dynamic ice shaping that increases cell damage.

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Cryopreservation of Cells using Peptidomimetic Macromolecules

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References