Cryopreservation of Cells using Peptidomimetic Macromolecules

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Background and Challenges, the need for novel cryoprotectants

- Current techniques often use vitrifying cryoprotectants at high concentrations that require rapid freeze and thawing rates[1].
- 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[2].

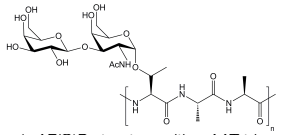
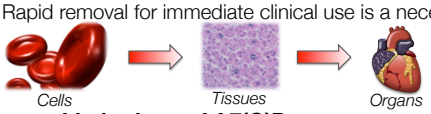


Fig. 1: AF(G)P structure with a AAT tripeptide backbone (n=4-55) with an O-linked disaccharide (β-D galactosyl-(1,3)-α-N-acetyl galactosamine).

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.



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)[3].
- AF(G)Ps display a strong recrystallisation inhibition (RI) activity and thermal hysteresis, suppressing the freezing point of a solution.

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[4].
- Their application as cryoprotectants is limited due to the secondary effect of dynamic ice shaping that increases cell damage[5].

Peptidomimetic Macromolecules

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

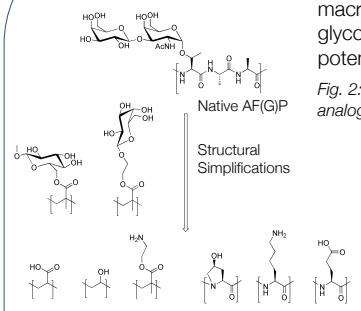


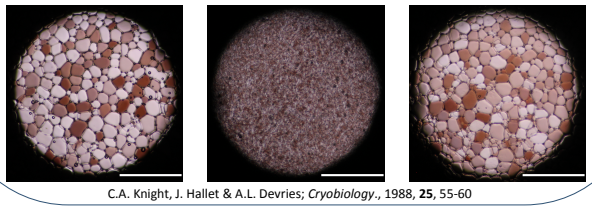
Fig. 2: Schematic showing simplified polymeric analogues of AF(G)P with varied RI activities.

M.I. Gibson *et al*; *Biomacromolecules.*, 2009, **10**, 328-333

Our aim is to further investigate RI active compounds, understand their mode of action and develop more powerful biocompatible RI macromolecules to improve the long term storage of cellular material and remove the current dependence on organic solvents.

RI activity is measured using the "splat" assay defining the mean largest grain size (MLGS) (Fig. 3).

Fig. 3: Light micrographs illustrating RI activity of PBS (left), 5 mgmL^{-1} 9 KDa PVA (centre) and 10 mgmL^{-1} 8 KDa PEG (right) Scale bars = 500 μm.



C.A. Knight, J. Hallet & A.L. Devries; *Cryobiology.*, 1988, **25**, 55-60

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).

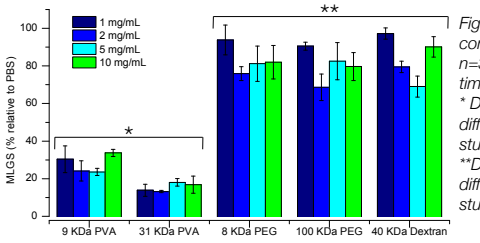
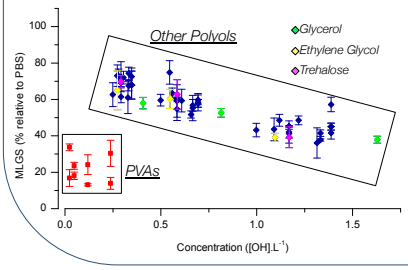


*Fig. 4: RI activity of several compounds relative to PBS. n=3; mean values ± S.D; time = 30 minutes. * Denotes statistical difference (two-tailed student's t-test $p < 0.005$) . **Denotes no statistical difference (two tailed student's t-test $p < 0.05$).*

Fig. 5: RI activity of various polyols and PVA relative to PBS against [OH].L^{-1}. n=3; mean values ± S.D; time = 30 minutes.



Numerous polyols including cryoprotectants such as glycerol, ethylene glycol and trehalose show weak RI activity with increasing [OH].L^{-1} concentration (adj $R^2 = 0.8745$) suggesting that the polymeric structure of PVA is key for RI activity rather than purely [OH].L^{-1} concentration (Fig. 5).

R. Tam *et al*; *JACS*, 2008, **130**, 17494 -17501

Biological Application

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).

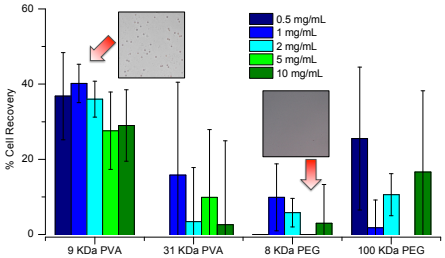


Fig. 6: Cell recovery of 1 mL ovine erythrocyte aliquots under rapid-freeze and slow-thaw conditions with indicated polymer. n=5; mean values ± S.D;

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

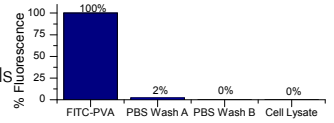


Fig. 7: FITC-tagged 9 KDa PVA uptake fluorescence values. n=3; mean values ± S.D;

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).

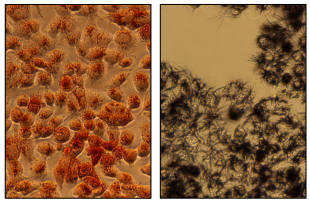


Fig. 8: Neutral red stained A549 cells post-cytotoxicity treatment (left) and post MTT treated A549 cells (right).

G. Repetto et al; *Nat.Protoc.*, 2008, **3**, 1125-1131
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Future work will explore the link between RI and cryopreservation allowing the targeted design of improved peptidomimetic molecules for eventual application with haematopoietic stem cells.

Background References

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