

The Cryopreservation of Biological Materials

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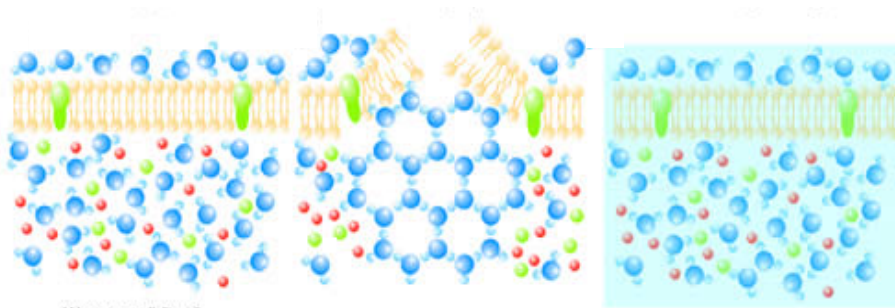
Problems

- Donor cells and tissues essential for modern medicine
 - Donor bone marrow (leukemia)
 - Regenerative medicine requires stem cells
- Finite lifetime
- Storage and transport

Limitations

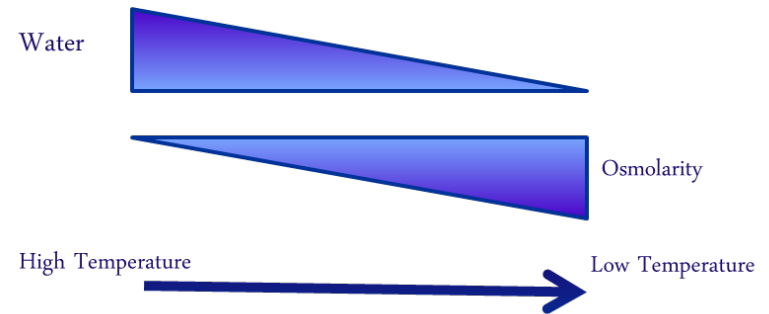
Ice

- Lipid bilayer disruption
- Internal ice is almost always lethal
- Ice recrystallization upon thawing

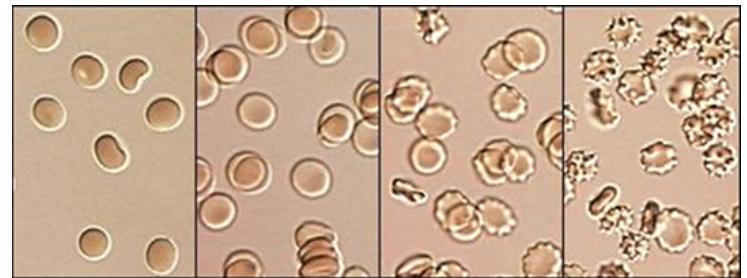


Osmolarity

- As ice forms, solute concentration changes

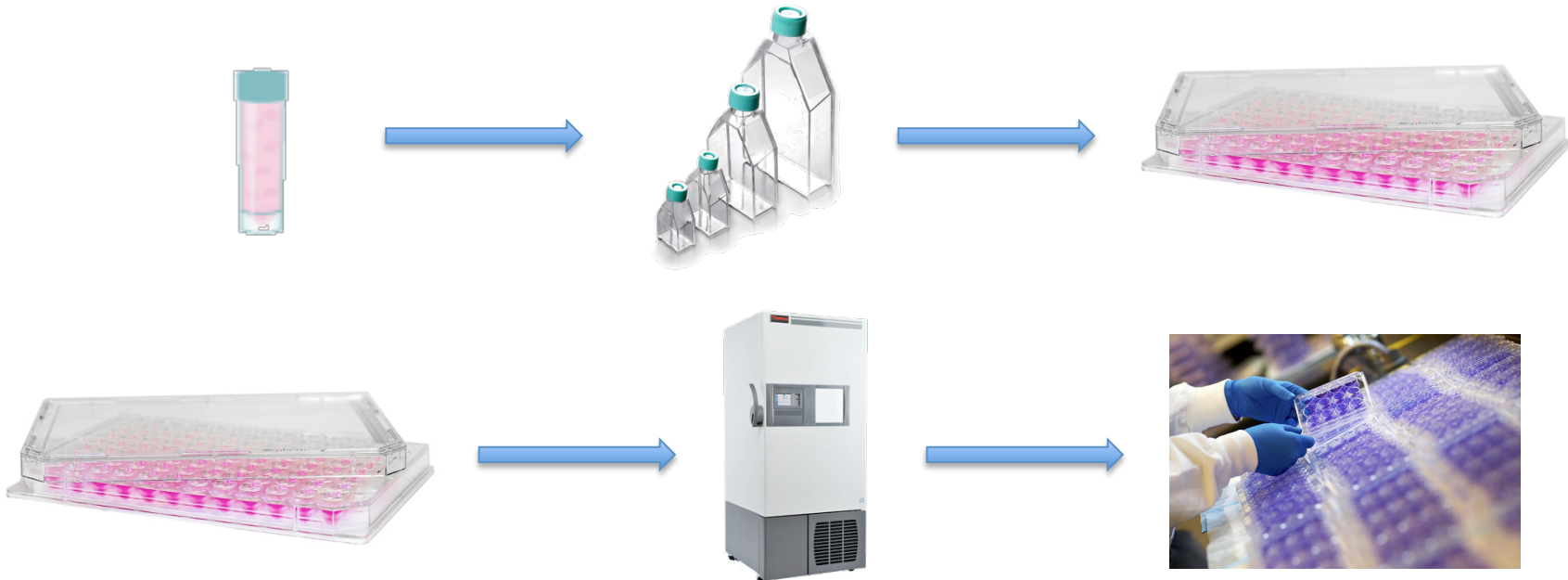
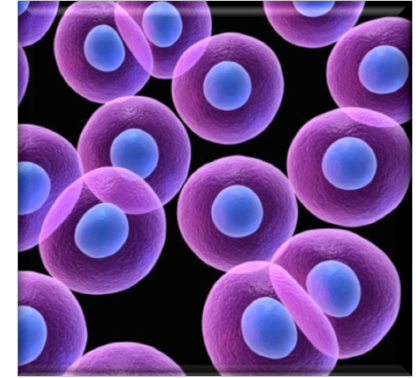


- Water rushes out of the cell



Current Cell Cryopreservation

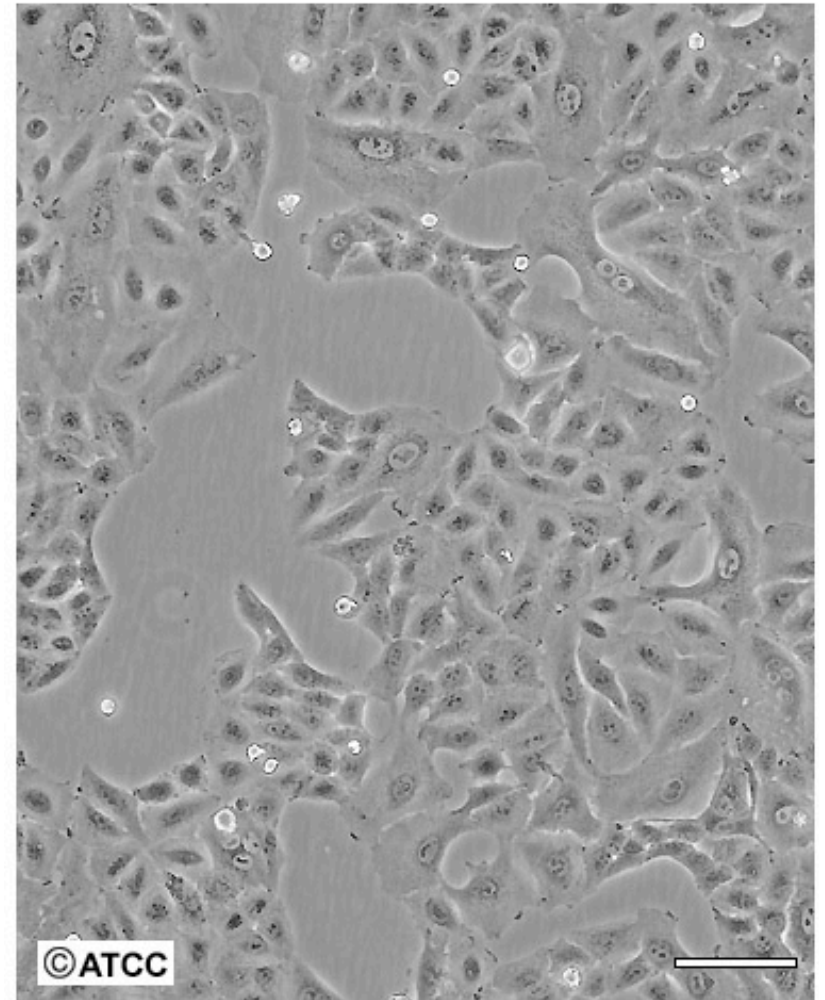
- Dimethyl Sulfoxide (DMSO)
 - Toxic at room temperature^[13]
- Frozen in solution
 - Phenotypic changes



Cell Line

A549

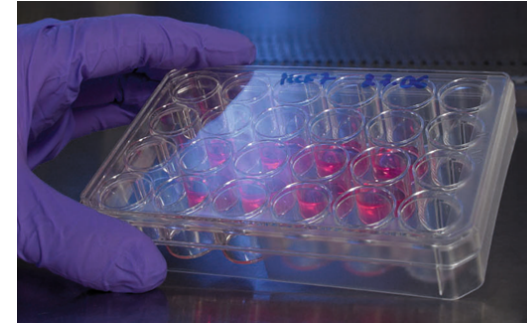
- Human lung cells
- Epithelial carcinoma



High Density

Scale Bar = 100µm

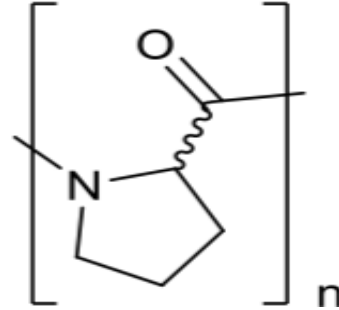
Freezing Viability



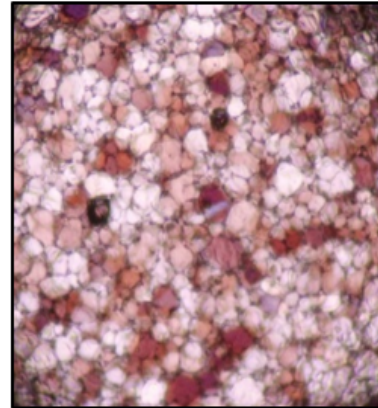
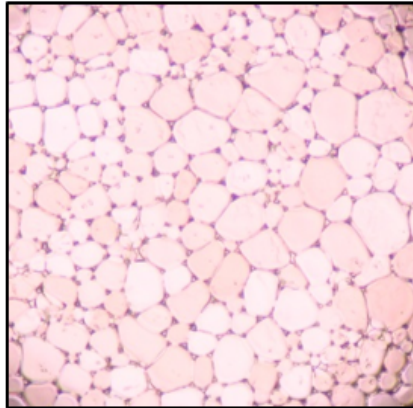
- 24 well plates
- Cells are plated for 2 hours
- Incubated with solutes for 24 hours
- CPA applied for 10 min then removed
- Placed into passive freezing device (-1 °C/min) for 24 hours
- Quickly thawed with 37 °C medium
- Incubated for 24 hours
- Counted for viability



Polyproline

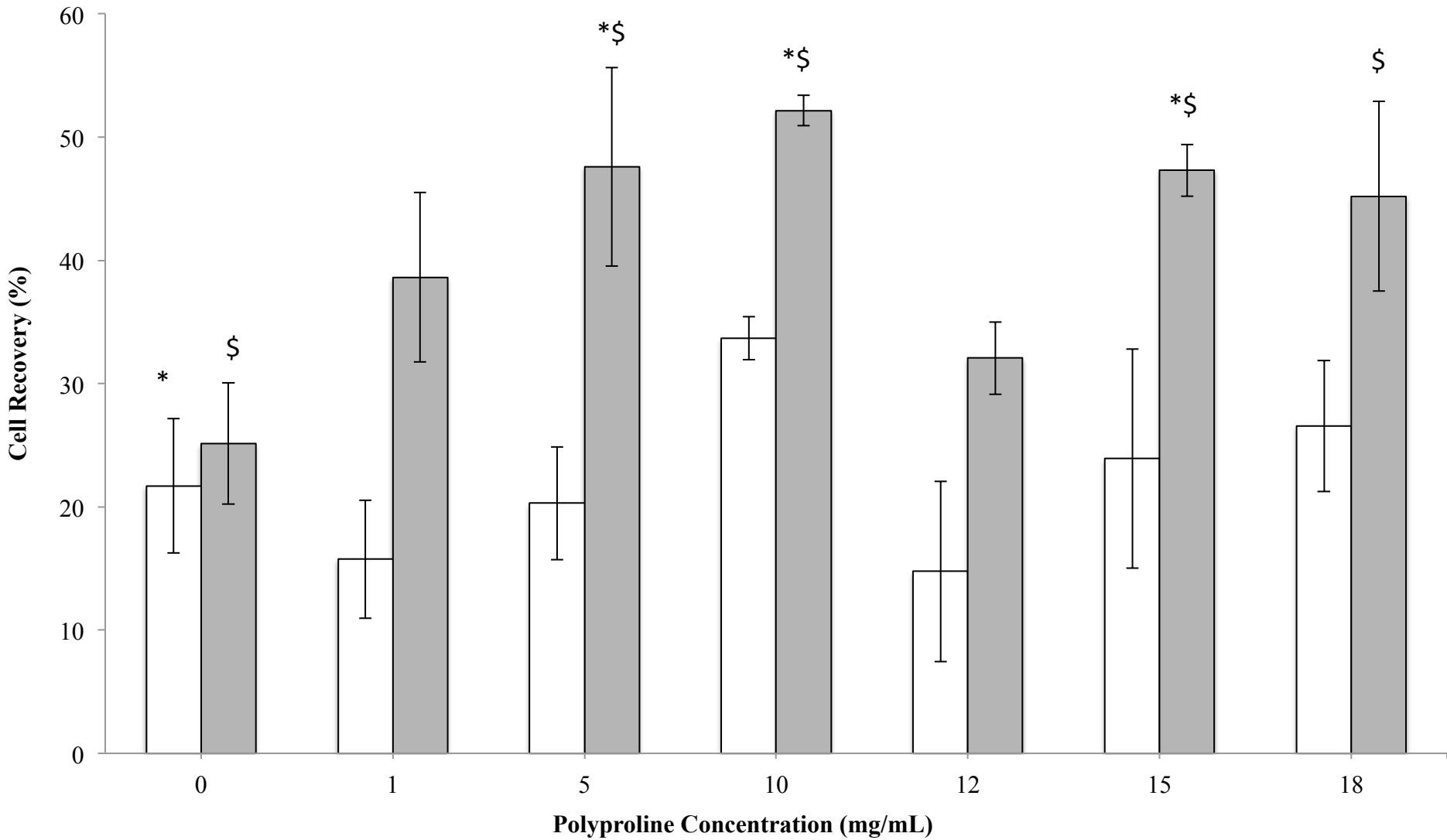


- IRI Activity

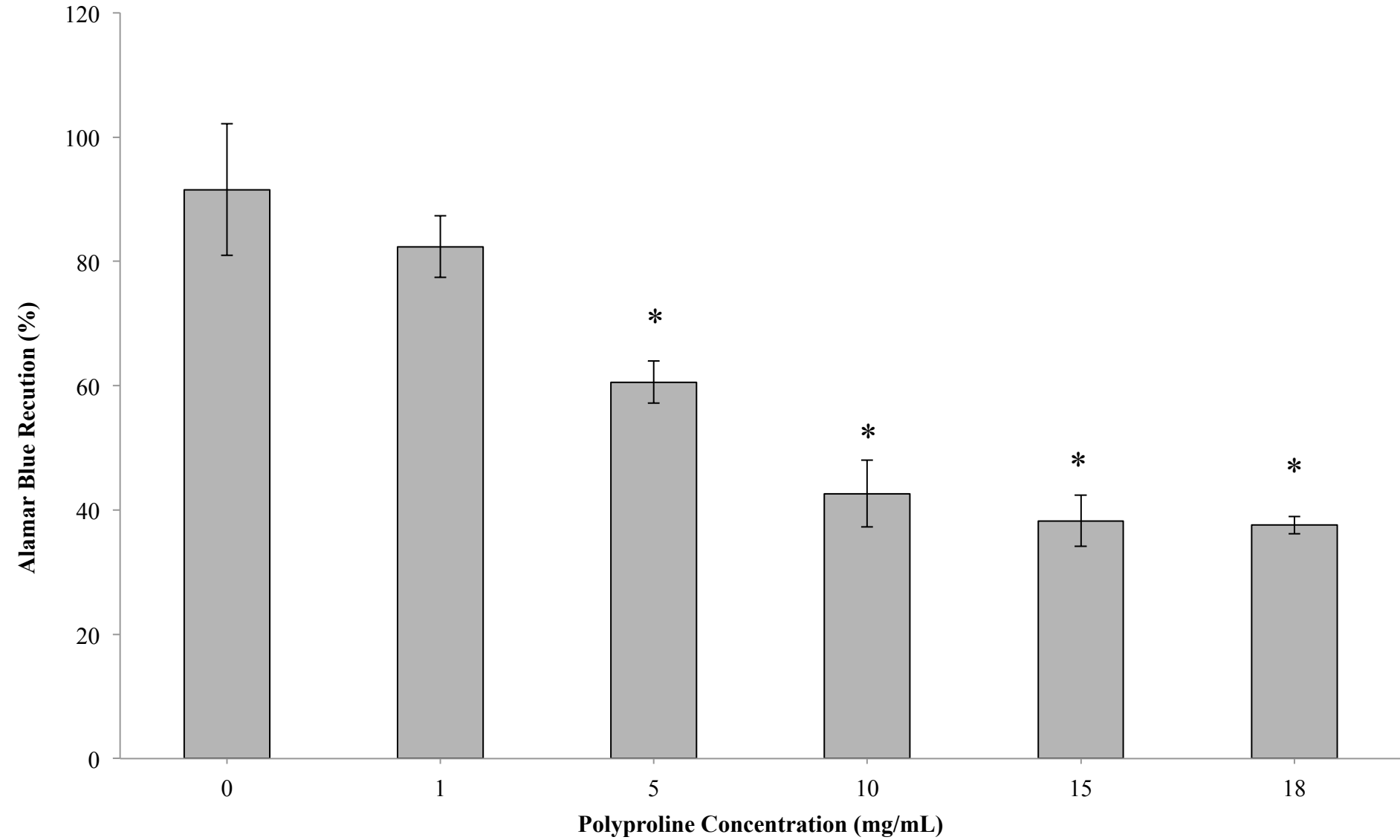


A549 Cell Cryopreservation with Polyproline

□ F-12K ■ F-12K + 23.1 mg/mL Proline



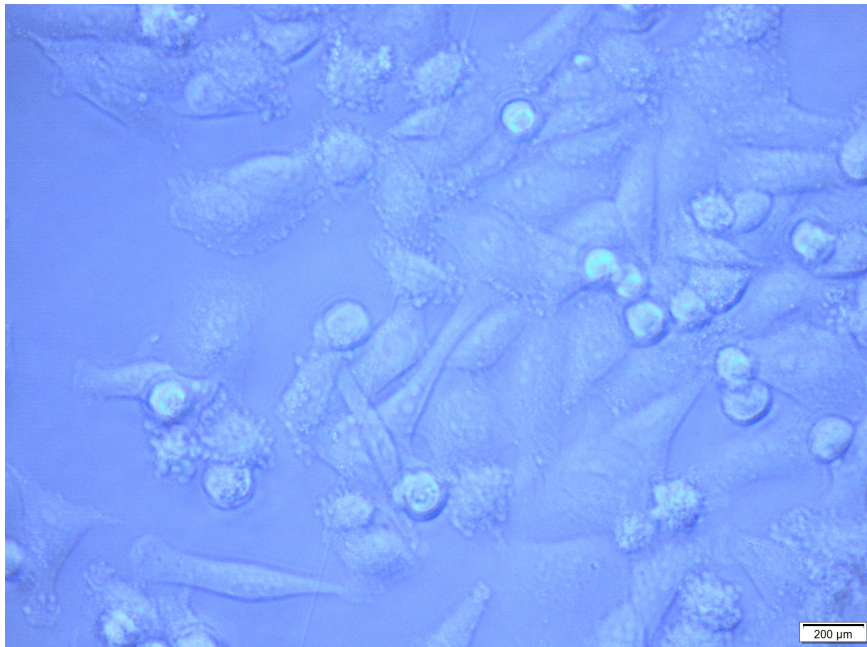
A549 Polypyrroline Cytotox



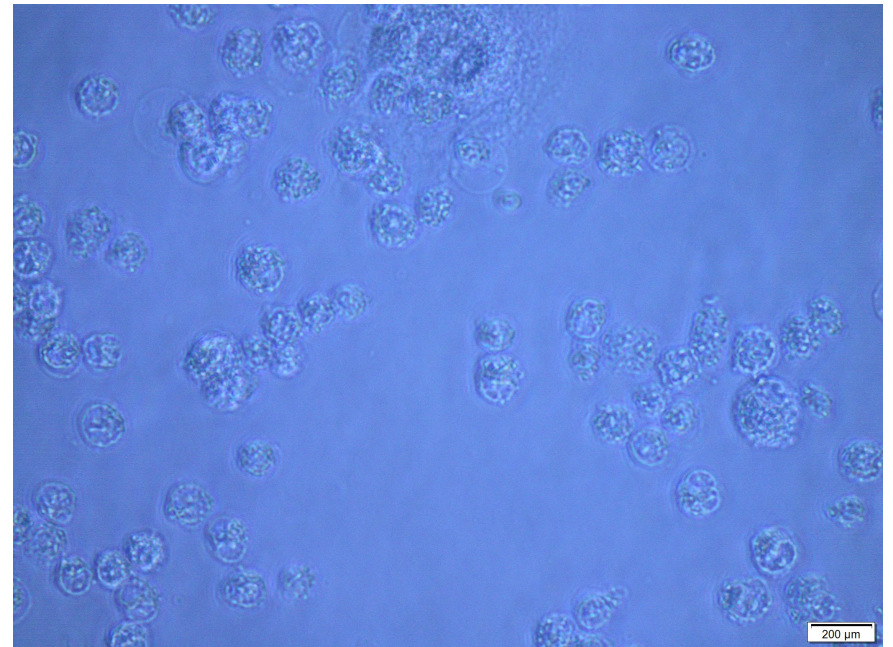
A549 Polypyrroline Cytotox

3 Hours

Control



18 mg/mL



COMMUNICATION

Polyproline is a minimal antifreeze protein mimetic and enhances the cryopreservation of cell monolayers

Ben Graham^[a], Trisha L. Bailey^[a], Joseph R. J. Healey^[b], Moreno Marcellini^[c], Sylvain Deville^[c] and Matthew I. Gibson^{*[a]}

Abstract: Tissue engineering, gene therapy, drug screening and emerging regenerative medicine therapies are fundamentally reliant on high-quality adherent cell culture, but current methods to cryopreserve cells in this format can give low cell yields and requires large volumes of solvent 'antifreezes'. Herein we report polyproline is a minimum (bio)synthetic mimic of antifreeze proteins, which is accessible by solution, solid phase and recombinant methods. We demonstrate that polyproline has ice recrystallization inhibition activity linked to its amphipathic helix and that it enhances the DMSO-cryopreservation of adherent cell lines. Polyproline may be a versatile additive in the emerging field of macromolecular cryoprotectants.

Tissue engineering, gene therapy, therapeutic protein production and transplantation rely on the successful storage and transport of donor cells.¹ For example, in the production of therapeutic proteins, a specific cell line for each protein must be developed.² Given that any *in vitro* culture will undergo phenotypic and genotypic changes when propagated for long periods of time, it is

cell membranes.¹² Synthetic polymers with potent IRI have emerged as a new paradigm for controlling ice growth.¹³ The most studied is poly(vinyl alcohol) (PVA), which can inhibit ice growth at less than 0.1 mg.mL⁻¹ and enhances the cryopreservation of cells in suspension.^{14,15,16} It is hypothesized that PVA's activity is related to its regularly spaced hydroxyl groups.¹⁷ Matsumura *et al.* have developed polyampholytes¹⁸ which are cryoprotective but have moderate IRI activity.^{19,20} Wang *et al.* have demonstrated the significant IRI activity of graphene oxide.²¹ Ben *et al.* have developed low molecular weight surfactants which also inhibit ice growth.²² A major setback is that the above synthetic IRI's are not biodegradable nor bioresorbable and have not been applied to significant challenge of cell monolayer storage.

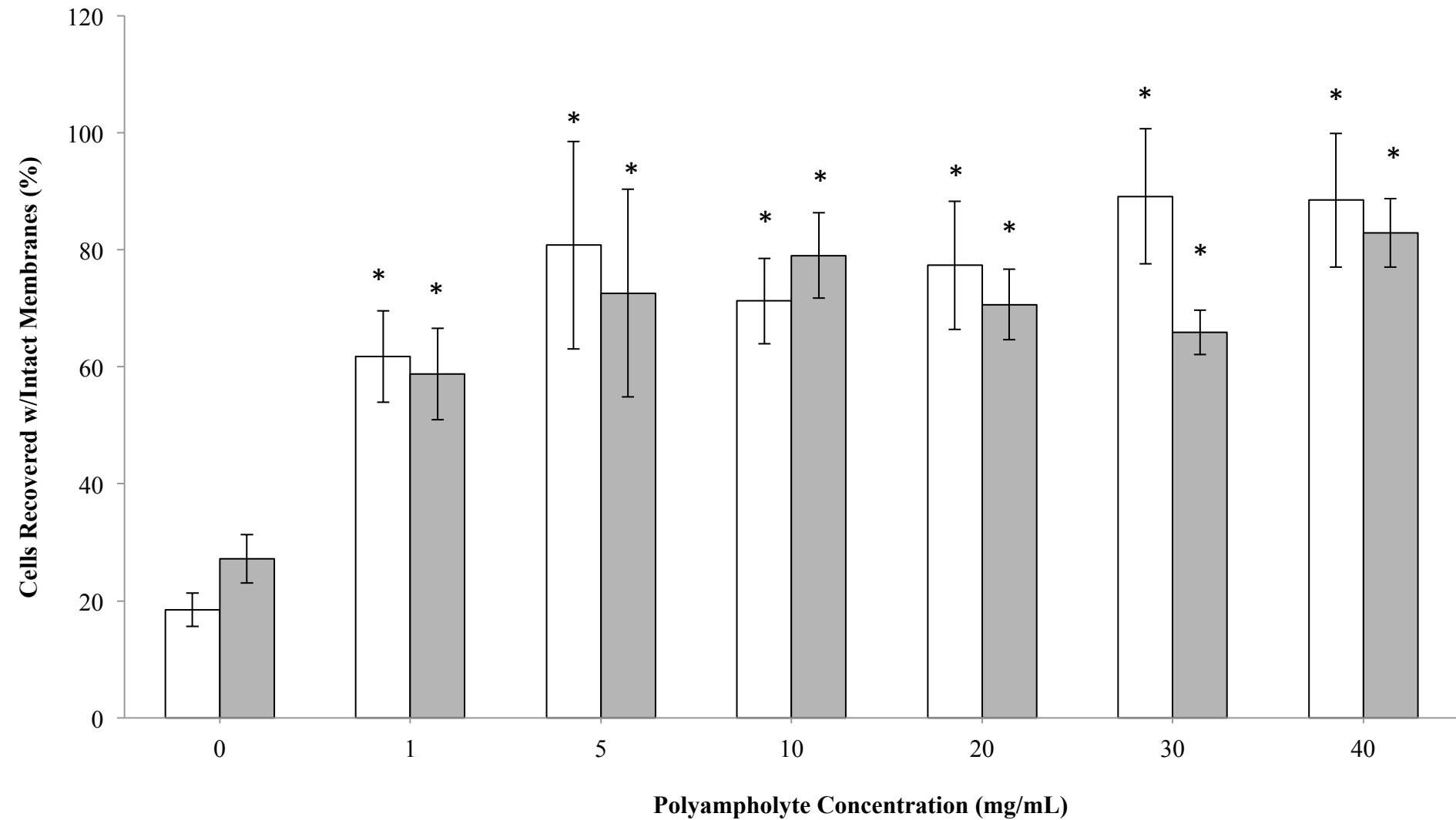
There are no crystal structures for AFGPs but solution state NMR and CD (circular dichroism) spectroscopy suggest a polyproline II type helix.²³ Polyproline is unique amongst the canonical amino acids in that it has no amide N-H, meaning it cannot form intramolecular hydrogen bonds. Due to this, it is water soluble whilst being quite hydrophobic, as is the case for

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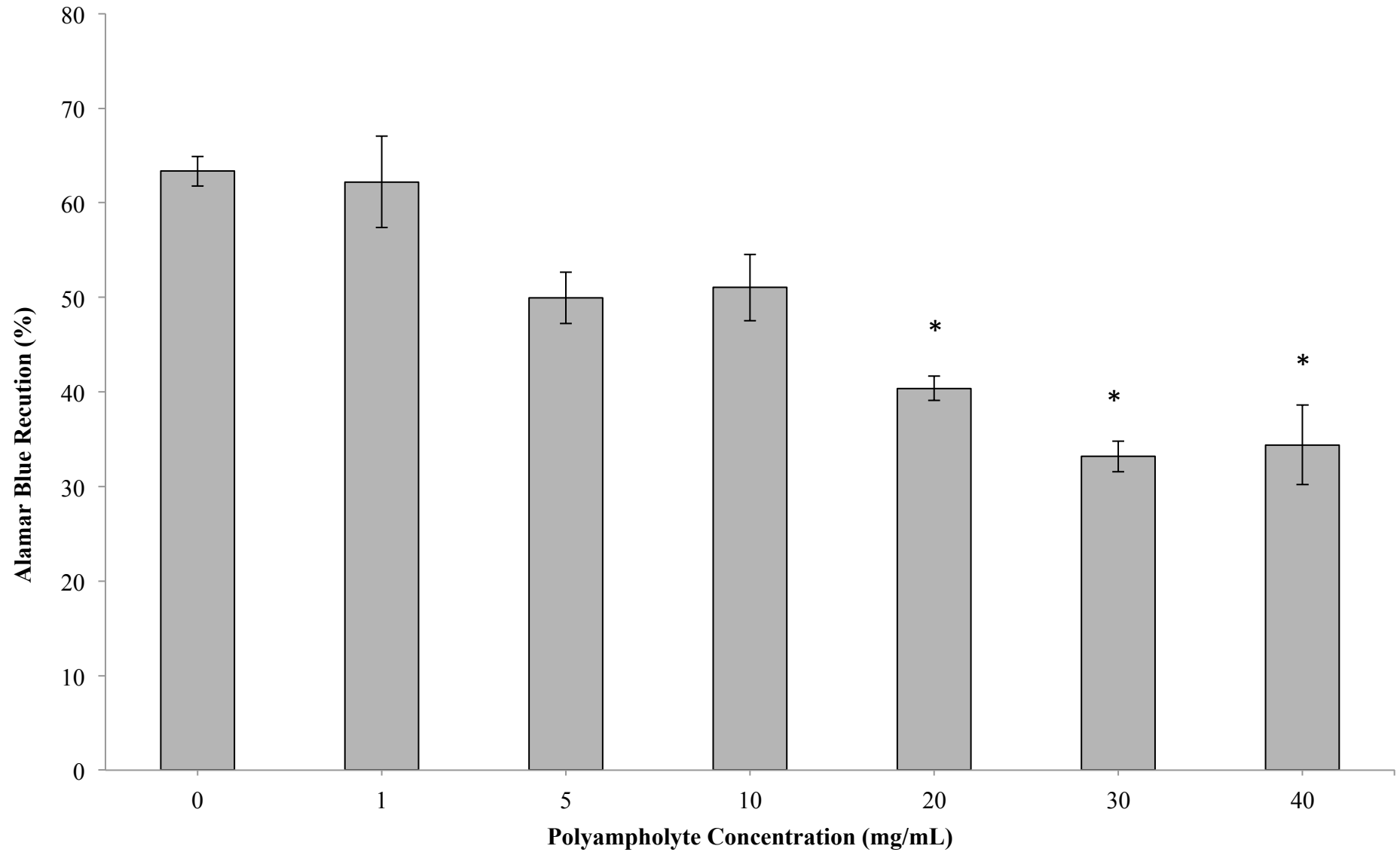
- Poly-D-proline cytox

A549 Cell Cryopreservation with Polyampholyte

□ F-12K ■ 23.1 mg/mL Proline



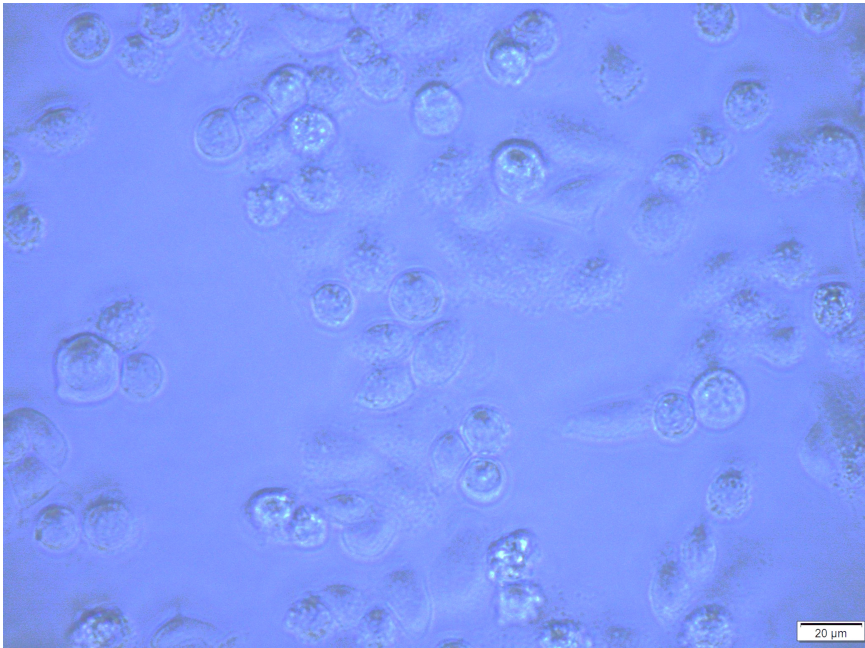
A549 Polyampholyte Cytotoxicity



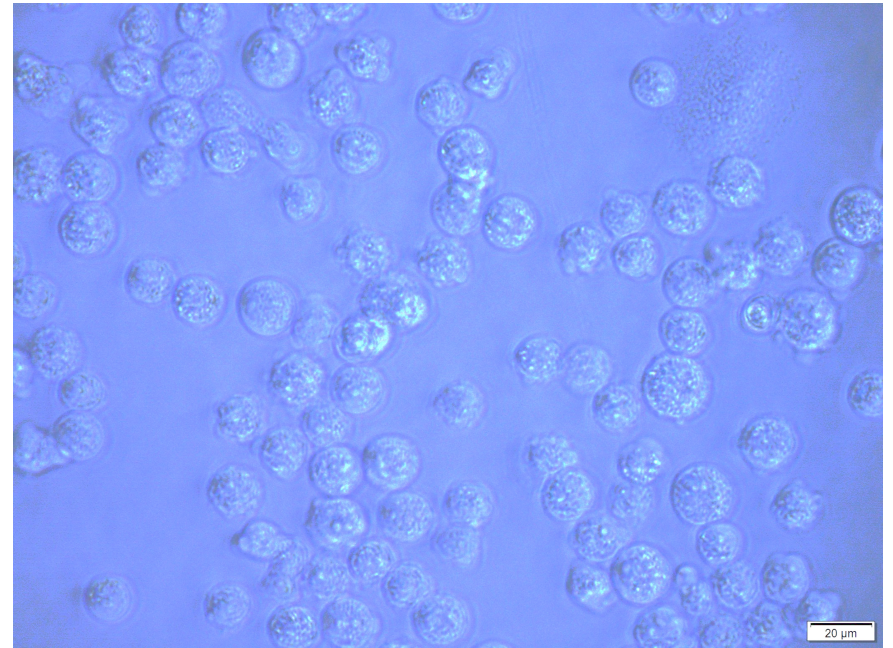
A549 Polyproline Cytotox

1 Hour

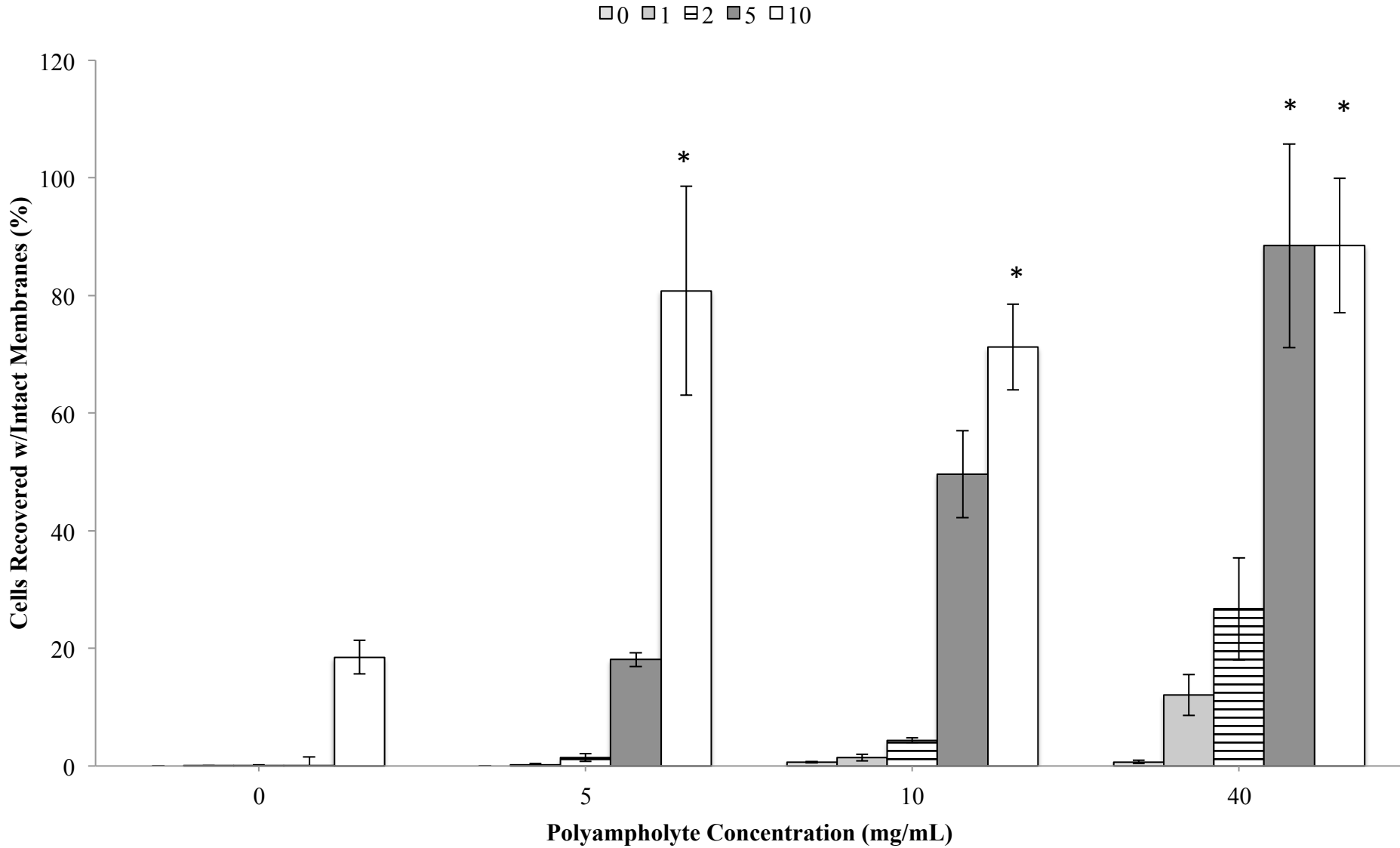
Control



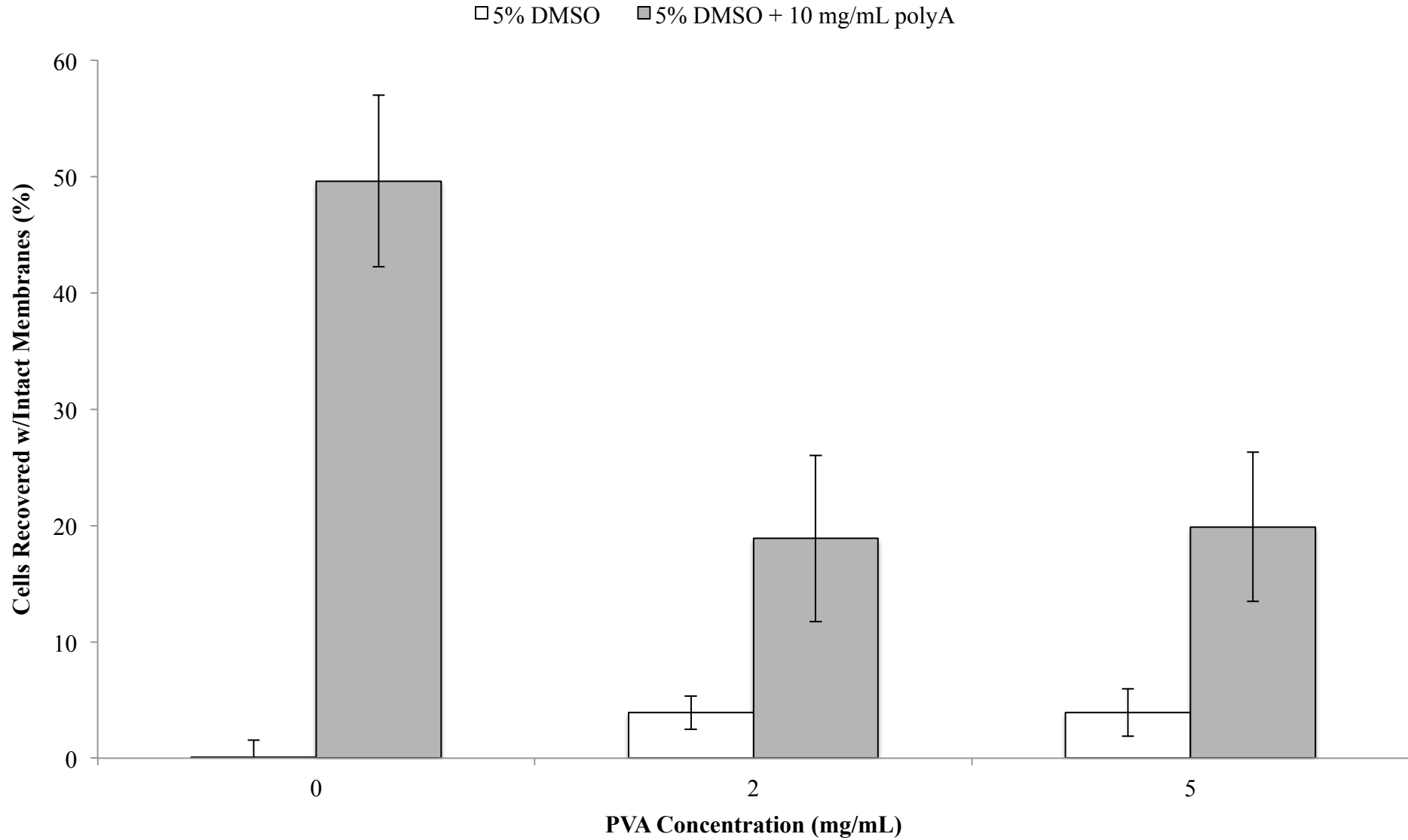
40 mg/mL



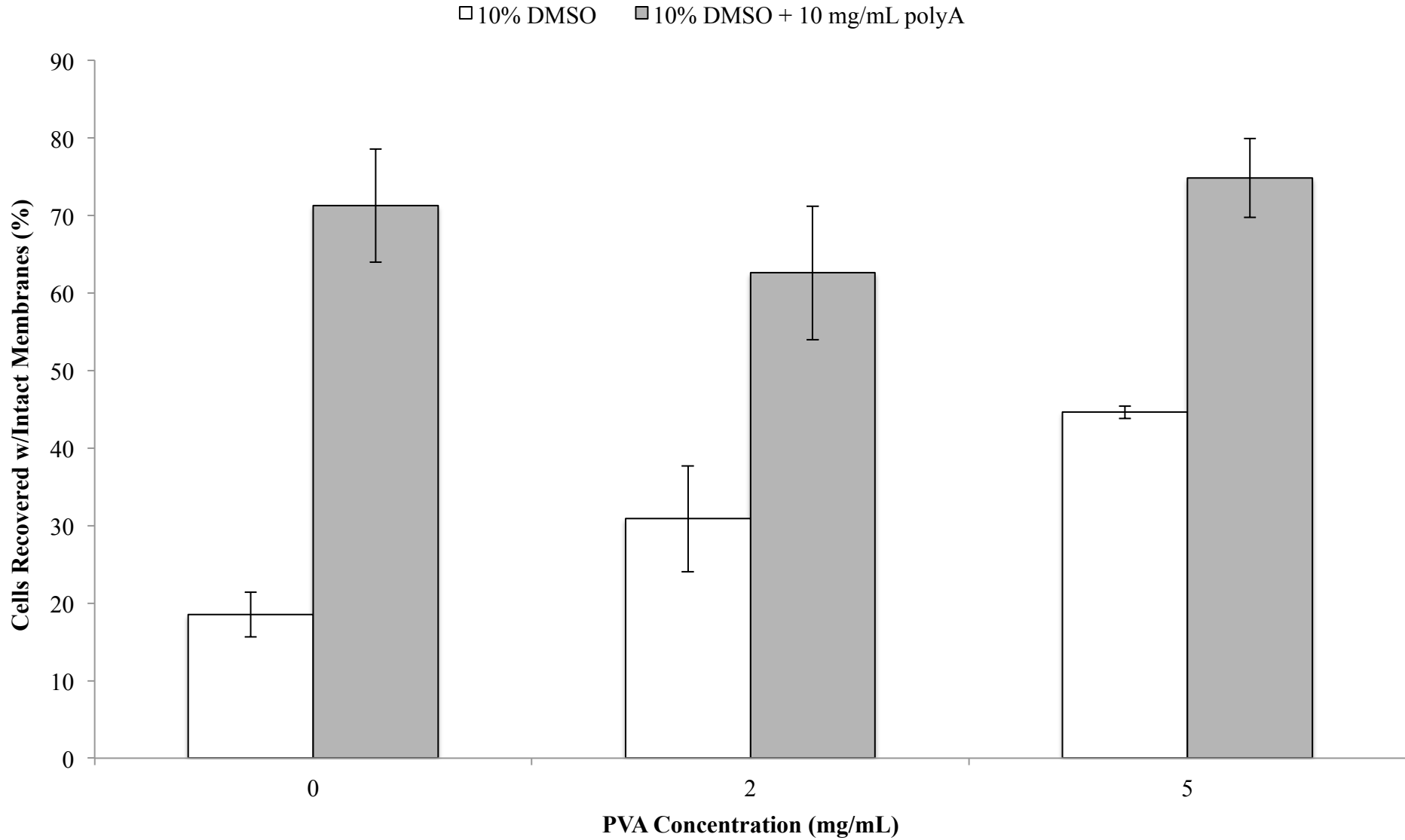
A549 Cell Cryopreservation with Polyampholyte



A549 Cell Cryopreservation with Polyampholyte



A549 Cell Cryopreservation with Polyampholyte

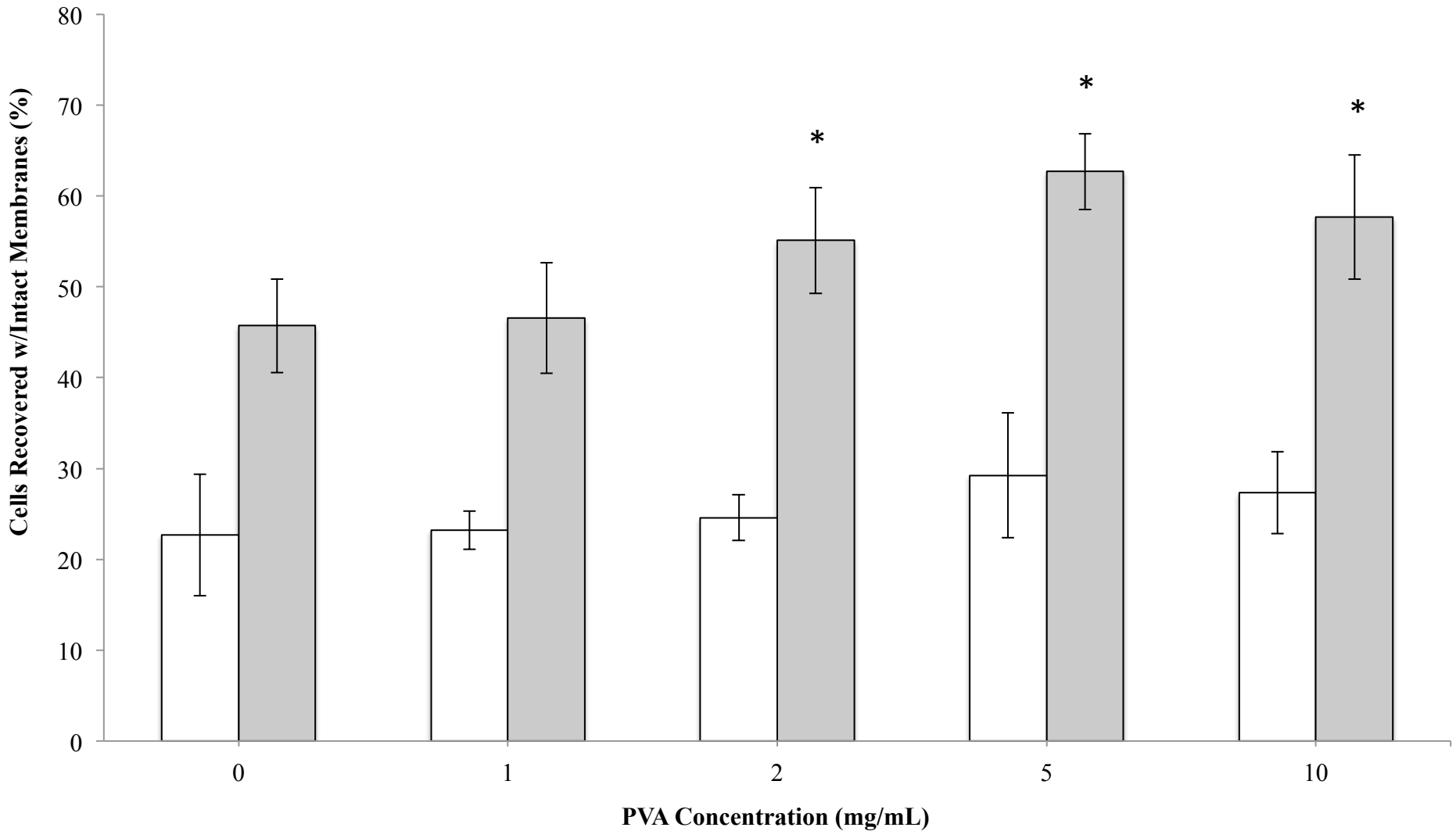


Next

- Neuro-2a polyampholyte freezing
- DSC polyampholyte solution
- DMSO cytox (24 hr)
- DMSO and polyampholyte cytox (10 min)
- Viability after freezing

A549 Cryopreservation with Proline & PVA

□ F-12K □ 23.1 mg/mL Proline

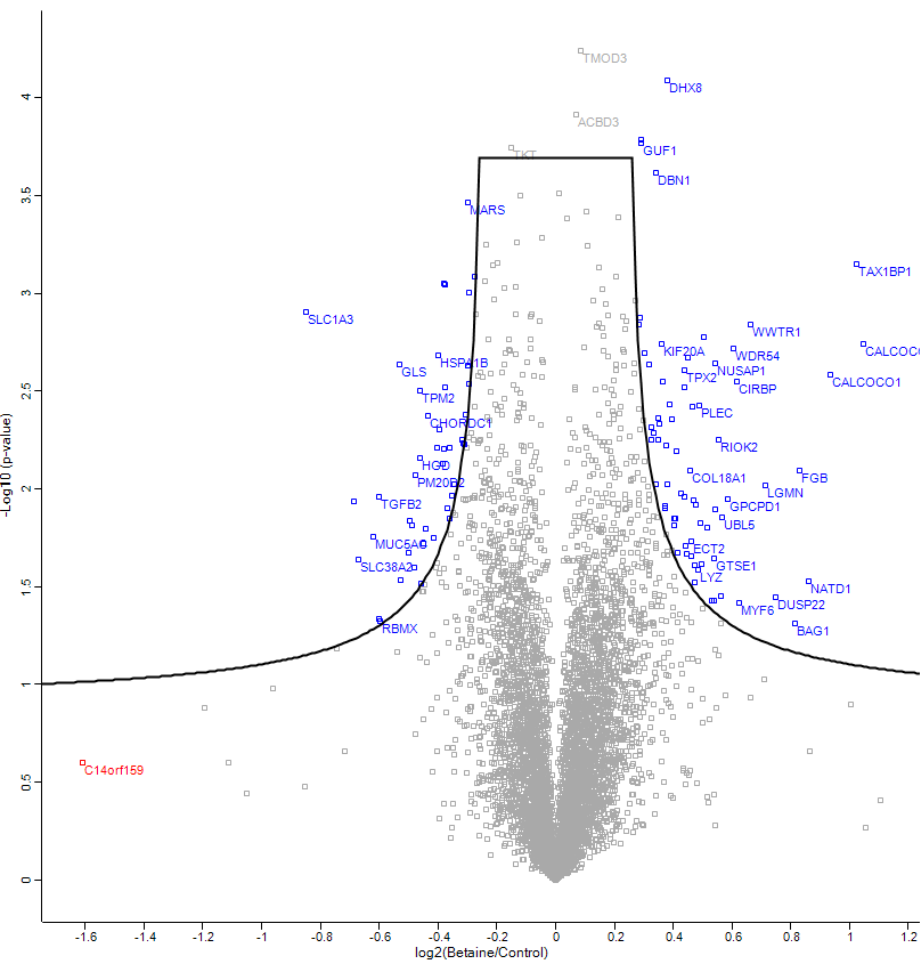


Next

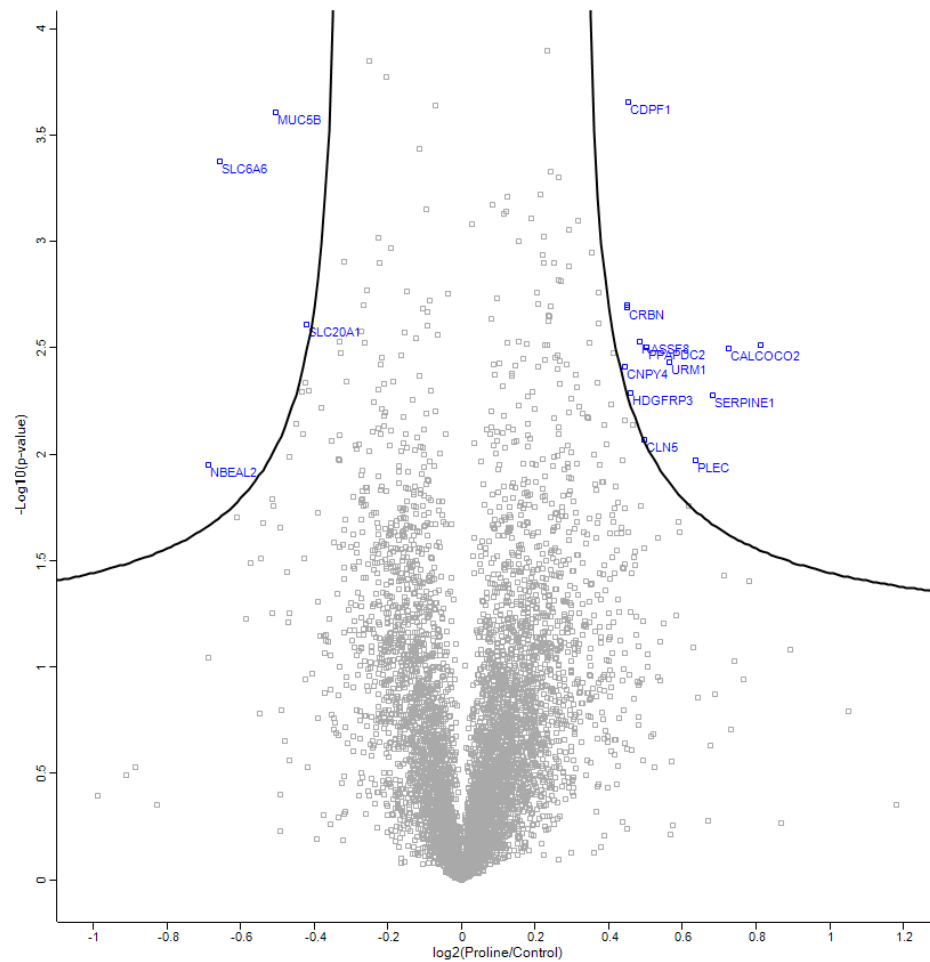
- Live/Dead staining for PVA/proline
- DSC PVA/proline solutions
- SPLAT solutions

A549 Proline and Betaine Incubation Proteomics

Betaine



Proline

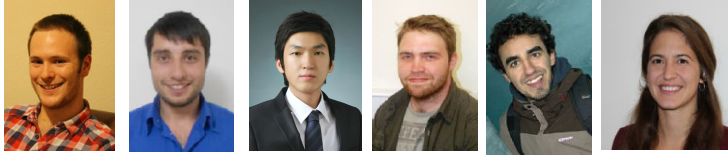


Next

- Betaine freezing
- Meet with Juan to discuss proteomics results
 - Downstream/upstream effectors

Acknowledgements

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Extra bits in case you need them.