BioCoR

Advancing the science, technology and practice of bio-preservation

Emerging issues in cryopreservation of cellular therapies

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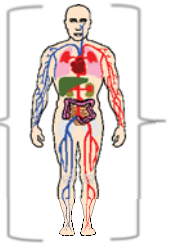
Cell Therapy

Isolated cells or aggregates administered to treat a disease

Cell types
Hematopoietic stem cells
Lymphocytes*
Dendritic cells
Mesenchymal stem cells
Skeletal myoblasts
Islets of langerhans
iPS cells
hESC cells
Placental cells
And more .



Diseases
Bone marrow transplantation
Cancer
Heart failure
Myocardial infarction
Diabetes
Urinary incontinence
Alzheimers
Crohns Disease
And more

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Role of cryopreservation

Patient access
•Permits transportation from site of collection, processing and use.
•Increases genetic diversity of cells available.

Manufacturability
•Permits coordination of therapy with patient care regimes (cells are ready when patient is)
•Helps management of staffing requirements
•Permits inventory management of therapies.

Product safety and quality
•Time for completion of safety and quality control testing prior to product release.
•Increased shelf life for product

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Current state of cell preservation

Preservation of cell therapy products

Cryopreservation paradigm:

- Cryoprotectant: 10% DMSO
- Cooling rate: 1 C/min
- Controlled rate freezing
- Storage on LN_2

Alternative:
Courier fresh product to site

Preservation of cell therapies

Each cell is unique. There is no single preservation protocol

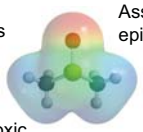
Platelets, granulocytes, hESCs, iPS cells, gametes, hepatocytes, etc. cannot be effectively preserved

Knowledge gap: we do not understand the mechanism of action for DMSO and why certain cell types survive and others do not

The DMSO 'problem'

Ubiquitous

Associated with epigentic events



Cytotoxic

Toxic upon infusion

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Preservation of immunotherapy products

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Unique challenges

Heterogeneity of products
- NK cells
- T_{regs}
- Mixed lymphocytes
- Dendritic cells
- CTL

Other factors
- Multiple infusions may be required
- Cell number varies

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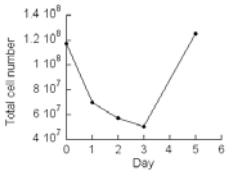
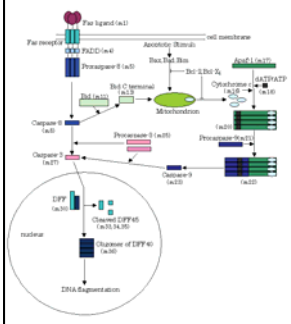
Post thaw response

- Mixed lymphocyte populations preserved
- Post thaw viability measured
- Cell losses over 48 h were significant

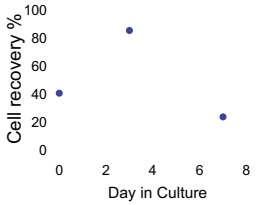




Pathways for post thaw apoptosis

- Post thaw apoptosis is mediated by the mitochondria and ac ivation of Caspase-3
  (Stroh, 2002)
- Caspase inhibition has been shown to reduce post thaw apoptosis losses.

Modifying freezing response



Mixed lymphocytes were antigen stimulated and cryopreserved using conventional methods

Post thaw recovery varied with time in culture

Summary

Each product requires a unique method of preservation

Molecular mechanisms of damage must be elucidated

Alternatives to DMSO are needed

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Biopreservation Core Resource (BioCoR)

Mission: to advance the science, technology and practice of bio-preservation

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BioCoR Resources

Research resource:
- Advance our understanding of molecular mechanisms of damage
- Develop new technologies to improve preservation

Service resource:
- Develop fit-for purpose preservation solutions
- Evaluation existing protocols

Education resource:
- Train individuals in the scientific principles of preservation
- Protocol-specific training

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Research resource projects

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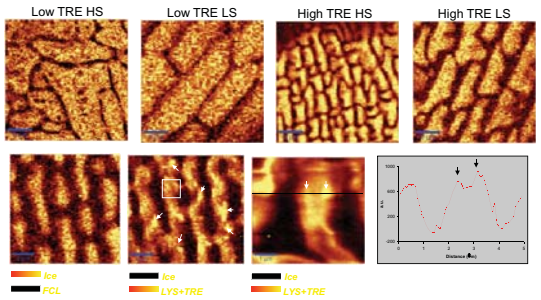
Molecular Mechanisms of Protein Damage

Microcompartmentalization in Frozen Protein Solutions



Dong, Hubel, Bischof, Aksan
"Freezing-Induced Phase Separation and Spatial Microheterogeneity in Protein Solutions"
J. Phys. Chem. B *published online on 07/02/09* DOI: 10.1021/jp8097404

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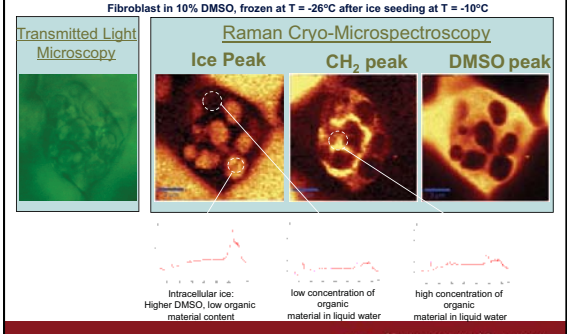
Molecular Mechanisms of Cell Damage

Fibroblast in 10% DMSO, frozen at T = -26°C after ice seeding at T = -10°C



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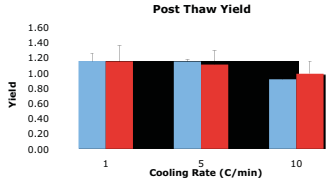
Alternatives to DMSO

Objective: develop a DMSO-free solution for human MSCs

Blue: 10% DMSO

Red: replacement formulation



All components approved for human infusion

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DMSO alternatives, cont.

Post thaw viability very good. Attachment and proliferation poor.

Raman spectroscopy gives insight into mechanisms of protection

We are screening additional compounds based on our imaging studies

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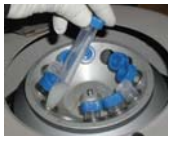
Technology development

DMSO used to preserve cells is associated with adverse reactions.

DMSO has also been associated with epigenetic effects and protein denaturation



Conventional methods of DMSO removal using centrifugation is labor intensive, time consuming and results in cell losses of 25-30%.

Objective to develop a microfluidic device capable of removing DMSO with minimum cell losses.

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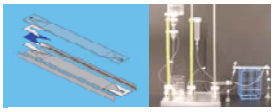
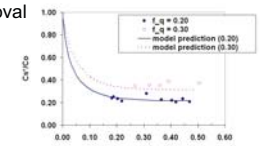
Microfluidic technology development



95% DMSO removal
~2.5 ml/min



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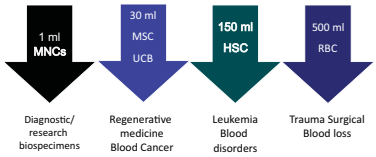
Microfluidics as a technology platform



Reduce cell losses, semi-automate process, reduce processing time, remove CPAs

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Education

Preservation is not a 'cold black box'

Understanding current scientific principals is critical to:
- *Maintaining a 'quality approach' to preservation of cell therapies*
- *Rationally designing protocols for new products*

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Service resource projects

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SRP projects

Fit-for-purpose solutions to preservation problems
- Cryopreservation
- Hypothermic storage

Provide methodological expertise

Perform scientific review and assessments

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Education

Preservation of molecular, cellular and tissue biospecimens

May 2011 Minneapolis, MN

Topics covered:
Liquid storage of biospecimens.
Fundamentals of cryopreservation
Protocol development
Quality systems
Clinical cell cryopreservation
Repository design
Tissue preservation
Preservation of biomarkers
Regulatory issues for cell/tissues

Lecturer
Allison Hubel, University of Minnesota
Charles Lee, U of North Carolina, Charlotte
Ian Pope, CoreCryolab, Toronto
Amy Skubitz, University of Minnesota
Fran Rabe, University of Minnesota
Alptekin Aksan, University of Minnesota
David McKenna, University of Minnesota
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