Why do we need research in Preservation Science?

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Biospecimens

Biofluids
- Plasma/serum
- BALF
- Ascites
- Urine
- Etc.

Cells

Tissues

Biomarkers

DNA
- Genome

RNA
- Transcriptome

Proteins
- Proteome

Sugars

Nucleotides

Amino Acids

Lipids

Metabolome

Candidates for stabilization

Isolated Biomarker
Intact biospecimen

University of Minnesota
Driven to Discover™
Myths about preservation
Myth #1: Preservation is a ‘solved problem’. We have effective methods of preservation for all the biospecimens of interest
Stabilization of biomolecules

Single phase solution
- Primary solvent (water)
- Macromolecule (protein, enzyme, lipid, etc.)
- Small solutes (salts, etc.)
- Cryoprotectant(s) (glycerol, DMSO, trehalose, etc.)

FCL: Freeze-concentrated liquid
- Ice (water) crystals
- Solute crystals

Multi-phase mixture
- Water: Mainly frozen
- Small solutes: Mainly crystalline
- Macromolecule: ???
- Cryoprotectant(s): ???
Stabilization of biomolecules

DNA, proteins
- Most highly studied
- Basic stability strategies have been developed

RNA
- Much less stable than DNA
- Commercial product: RNA later

Lipids, amino acids, sugars, nucleotides
- Lipid stability studied only in association with cell membrane

Common issues
- Cold denaturation
- Molecule/ice interactions
- Molecule/molecule interactions
- Effects of co-solutes
- Effect of cooling rate
- Influence of pH shifts
Stabilization of cells

![Graph showing the survival of different cell types at various cooling rates.]

- **Bone Marrow**
- **Yeast**
- **Hamster Ova**
- **RBC**

The graph indicates the survival percentage of these cell types across different cooling rates (°C/min).
Stabilization of cells

Many cells cannot be preserved effectively: platelets, granulocytes, hESCs, iPS cells, gametes, etc.

We do not understand why certain cells can be effectively preserved and others not

We do not understand why survival of the same cell type varies from species to species

→ We cannot develop scientific protocols to preserve cells refractive to current approaches
Stabilization of tissues

Viability:
• We can preserve islets of langerhans and achieve post thaw function
• Preserving other tissue involves preservation of scaffold with little post thaw cellular function
• Cells isolated from a tissue survive much more readily than cells embedded in a tissue

Biomarkers:
Genes and proteins can change significantly within 30 mins of ischemia

We simply cannot preserve tissues and organs and we do not understand why
Myth #1: We have effective methods of preservation for all the biospecimens of interest
Myth #2: Conventional methods of preserving biospecimens are suitable for new and emerging applications
DMSO as a stabilization agent

Most cellular biospecimens are preserved with DMSO

DMSO has well documented toxicity (cellular and infusion)

DMSO is also associated with epigenetic events and may irreversibly denature macromolecules

→ Alternative stabilizing agents are needed
Myth #2: Conventional methods of preserving biospecimens that are successful are suitable for new and emerging applications.
Myth #3: All biospecimens are equal*

*well some are more equal than others....
Rapid changes in biomarkers

- Whole blood stored at RT for 2 h
- 35 different factors differed between the two samples
- Protease inhibitors had little effect
- Effects were cell mediated

By 15 min: 10%–15%
By 30 min: 20%
Detectable genes and proteins differed significantly from the baseline values.


Spruessel, BioTechniques, 36:1030, 2004
Controlling and specifying pre-analytical variables is important.

But is it sufficient??

Other elements of the protocol are also important (cooling rate, storage conditions, etc).

Conundrum: setting the bar too high has its downsides.
Myth #1: Preservation is a “solved problem”. We have effective methods of preservation for all the biospecimens of interest.

Myth #2: Conventional methods of preserving biospecimens are suitable for new and emerging applications.

Myth #3: All biospecimens are equal.
Freezer Farms

Millions of samples are in storage (with more added every day)

What (if any) usefulness do these specimens have?

Advancing preservation science
Advancing preservation science: driving forces

- Cell biology
- Spectroscopy
- Nanotechnology
- Imaging
- Single molecule sequencing
- Biospecimens
- Biomarker discovery
- Personalized medicine

Quantum Leap Forward
How do we advance preservation science?

BioCoR
Advancing the science, technology and practice of bio-preservation
Education

Preservation is not a ‘cold black box’

**Understanding current scientific principals is critical to:**

- Improving/harmonizing biospecimen quality and best practices
- Filling in the gaps in our knowledge
Education

Preservation of molecular, cellular and tissue biospecimens

May 18-20, 2010 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
- Diane Kadidlo, University of Minnesota

This course has been endorsed by ISBER
Service

A series of ‘little things’ may make the difference

⇒ Ask the BioCoR expert

⇒ Have BioCoR develop a protocol for your biospecimen
Research

Bronchoalveolar Lavage Fluid
• In contact with affected organ
• Potential biomarkers identified: proteins, cells, lipids
• Banked but not used for diagnosis monitoring treatment

Integrated team: preservation scientists, biomedical researchers searching for biomarkers and clinicians collecting biospecimens

Alveolar macrophages
Influence of freezing on protein structure

BALF samples were analyzed using FTIR

Condition: fresh, frozen, repeated FT cycles

Data: height of $\beta$-peak

Stabilizers: trehalose and glycerol

- Proteins in frozen samples exhibit higher levels of $\beta$-sheet
- Trehalose/glycerol reduced changes in proteins
- Repeated freeze thaw cycles influenced protein in sample
Molecular Mechanisms of Protein Damage
Microcompartmentalization in Frozen Protein Solutions

Low TRE HS  |  Low TRE LS  |  High TRE HS  |  High TRE LS

- Ice
- FCL
- LYS+TRE

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/jp809710d
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