Quantitative Measurement and Prediction of Biophysical Response During Freezing in Tissues

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December 2, 2008

Introduction

- 1830s - Freezing of biological tissues was first introduced by Goeppert
  - During slow freezing the cells in leaves lost water, but the membranes remained intact
- Intracellular ice formation later discovered
Hypothesized Mechanism of Freezing in Tissues

- Ice formation changes the chemical equilibrium between the intra and extracellular space
- Slow freezing causes cellular dehydration as the cells attempt to achieve chemical equilibrium
- Experiments to study the response of tissue in freezing are conducted using tissues that are clear to see and uniform
  - Pancreatic islets and connective tissue
Current uses of tissue freezing

- Freezing of biological tissues
  - Cryofixation
  - Food storage
  - Cryosurgery
  - Cryopreservation

Cryopreservation

- Storing or preserving biological materials, such as tissues for a long period of time.
- Used to store:
  - Pancreatic islets
  - Skin
  - Arteries
  - Corneas
  - Artificial liver
  - Artificial skin
Cryosurgery

- Thermal therapy to destroy tissue
  - Localized diseased tissue
    - Liver, prostate, kidney
  - Surface lesions
    - Skin, gynecological, oral
    - Great success rate

Cryopreservation Limitations

- Chemical additives are needed to prevent damage to cells or tissues
- Freezing and thawing methods are complicated
- Intracellular ice formation affects cell viability
Cryosurgery Limitations

- Extracellular and intracellular ice formation
- Monitoring of temperatures in deep tissues
- Understanding tissue response to freezing in order to deduce the mechanism of destruction

Cryofixation

- Quickly freezing tissues so that the water molecules become immobilized before they crystallize.
- Commonly used for imaging of tissues/cells
Directional Solidification

- Freezing of tissue slices at constant and controlled rates

Imaged using cryoscanning electron microscopy
- A- slam freezing
- B- 100°C/min
- C- 50°C/min
- D- 5°C/min
  - Large extracellular crystals
- Followed by immersion in liquid nitrogen
Two-Step Method

- Focuses on visualizing the water transport out of cells during freezing
- Combines directional solidification with slam freezing using a copper block chilled with liquid nitrogen
- Boyle-van’t Hoff graph is constructed to find the osmolality (solid fraction) of the tissue

![Diagram](image)

- A: Control
- B: -4°C/min
- C: -8°C/min
- D: -20°C/min
### Differential Scanning Calorimetry

- Used to measure exothermic heat caused by the phase change of water in tissue samples during freezing
- Tissues are repeatedly frozen in isotonic buffered saline

![Differential Scanning Calorimetry](image)

### Dynamic Response

- Linear lines:
  - exothermic heat release during solidification in water and in phosphate-buffered saline (bottom)
- Heat release is related to volume changes in cell suspensions and tissue systems

![Dynamic Response](image)
Dynamic Response

\[ \Delta q_{dsc} = q_{initial} - q_{final} \]

\[ \frac{V_0 - V(T)}{V_0 - V_b} = \frac{\Delta q(T)_{dsc}}{\Delta q_{dsc}} \]

\[ V(T) = V_0 - \frac{\Delta q(T)_{dsc}}{\Delta q_{dsc}} \cdot (V_0 - V_b) \]

- **Unknown values**
  - Initial tissue volume
  - Non-solvent volume

Single cell water transport

\[ \frac{dV}{dT} = -\frac{L_p A_c R T}{B V_w} \left[ \ln \frac{(V - V_b)}{(V - V_b) + \varphi_s n_s V_w} - \frac{\Delta H_f}{R} \left( \frac{1}{T_R} - \frac{1}{T} \right) \right] \]

with

\[ L_p = L_{pg} \exp \left[ -\frac{E_{lp}}{R} \left( \frac{1}{T} - \frac{1}{T_R} \right) \right] \]

- Temperature-dependent representation of cell permeability
- Used to predict cellular volumetric freezing response
- Theoretical model - biophysical parameters
  - \( V \) - cell volume, \( T \) - absolute temp, \( L_{pg} \) - permeability of the cell membrane to water at the reference temperature, \( T_R \) - reference temperature (273.15 K), \( E_{lp} \) - activation energy for water transport process, \( R \) - gas constant, \( A_c \) - effective membrane surface area, \( B \) - cooling rate, \( V_w \) partial molar volume of water, \( V_b \) - osmotically inactive cell volume
Krogh Cylinder Approach

- August Krogh
  - First to model mass transfer from cellular to extracellular environment.
  - Cylinder = extracellular/vascular compartment of tissue
  - Box = cellular compartment
  - Equation from single cell transport can also be applied to Krogh cylinder

Krogh Cylinder Model

- Assumptions
  - Tissue is made up of identical Krogh cylinders
  - Each unit is made up of extracellular and intracellular space
  - Water is transported across single membrane
  - Water transport from cell to cell is ignored
  - Membrane surface area is constant
Tissue Krogh Dimensions

- Using the single cell water transport equation and Krogh model

### Table 1: Biophysical parameters of tissues

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>$L_p$ (mm/min-atm)</th>
<th>$E_p$ (kJ/mole)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat liver</td>
<td>1.90 (1.14)</td>
<td>69.5 (290.0)</td>
<td>52</td>
</tr>
<tr>
<td>DSC</td>
<td>1.01 (0.05)</td>
<td>63.6 (265.0)</td>
<td>59</td>
</tr>
<tr>
<td>Two-step</td>
<td>0.03 (0.05)</td>
<td>0.0 (0.0)</td>
<td>78</td>
</tr>
<tr>
<td>1 M DMDSO</td>
<td>0.03 (0.05)</td>
<td>8.3 (40.0)</td>
<td></td>
</tr>
<tr>
<td>Two-step</td>
<td>0.03 (0.05)</td>
<td>8.3 (40.0)</td>
<td></td>
</tr>
<tr>
<td>A7-1 tumor</td>
<td>0.36 (0.6)</td>
<td>24.0 (100.4)</td>
<td>50</td>
</tr>
<tr>
<td>DSC</td>
<td>0.36 (0.6)</td>
<td>24.0 (100.4)</td>
<td></td>
</tr>
<tr>
<td>Fetal liver</td>
<td>2.18 (5.56)</td>
<td>76.7 (321.0)</td>
<td>51</td>
</tr>
<tr>
<td>DSC</td>
<td>1.76 (2.9)</td>
<td>75.5 (316.0)</td>
<td></td>
</tr>
<tr>
<td>Two-step</td>
<td>1.18 (1.85)</td>
<td>69.0 (280.0)</td>
<td>74</td>
</tr>
</tbody>
</table>

Simulate H2O transport in rat liver

- Cellular volume decreases due to dehydration
- Radius will expand during freezing
Further Study

- Investigate the relationship between biophysical state during freezing and viability state after freezing
- Study of other tissues that are relevant to biomedical research or applications
- Study faster cooling rates to analyze the competition between intracellular ice formation and water transport

References

Questions?