Optimization of freezing and thawing protocols
for reduction of injuring effects of cryopreservation

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Postponed repair of teeth using stored stem cells


Some damaging factors of cryopreservation

**Freezing**

- Cellular dehydration and shrinkage due to the osmotic outflow through the cell membrane (slow cooling)
- Great pressure exerted on the cell membrane due to earlier freezing of the extracellular liquid
- Growth of the dendritic seeds that can cause formation of large ice crystals during subsequent thawing

**Thawing**

- Further osmotic dehydration occurring during the warming phase, if the osmotic equilibrium was violated because of the rapid cooling (rapid cooling but slow thawing)
- Rehydration and swelling of cells when as more ice melts
- Recrystallization of small ice crystals into large ones (rapid cooling but slow thawing)
Mathematical model.  
Ice formation in small pores and channels


\[
\frac{\partial e(\theta)}{\partial t} - \mathcal{K}\Delta \theta = 0, \quad e(\theta) = \rho C\theta + \rho L\beta_\ell(\theta),
\]

\[
\beta_\ell(\theta) = \phi \left( \frac{L(\theta - \theta_f)}{(T_0 + \theta_f)(T_0 + \theta)} \right) - \text{ unfrozen water content}
\]

The function \( \phi \) is recovered from data obtained in experiments with tissue samples.

\( e \) - the internal energy
\( \theta \) - the Celsius temperature,  \( \mathcal{K} \) - the heat conductivity
\( \rho \) - the density (assumed being equal for ice and water)
\( C \) - the heat capacity (assumed being equal for ice and water)
\( L \) - the latent heat of freezing
\( \theta_f \) - the freezing point in °C
Averaging of the model and reduction to ODEs

Film heat transfer condition on $\Gamma_1$ and $\Gamma_2$ is assumed

Integr. $\Omega_1$:

$$
\frac{d}{dt} \int_{\Omega_1} e_1 \, dx + \lambda \int_{\Gamma_1} (\theta_1 - \theta_E) \, ds + \alpha \int_{\Gamma_2} (\theta_1 - \theta_2) \, ds = 0
$$

Integr. $\Omega_2$:

$$
\frac{d}{dt} \int_{\Omega_2} e_2 \, dx + \alpha \int_{\Gamma_2} (\theta_2 - \theta_1) \, ds = 0
$$

Mean values:

$$
\hat{e}_i = \frac{1}{|\Omega_i|} \int_{\Omega_i} e_i \, dx; \quad \hat{\theta}_i = \frac{1}{|\Gamma_i|} \int_{\Gamma_i} \theta_i \, dx
$$

$$
\hat{\theta}_E = \frac{1}{|\Gamma_1|} \int_{\Gamma_1} \theta_E \, dx; \quad \hat{\alpha}_i = \frac{|\Gamma_2|}{|\Omega_i|} \alpha; \quad \hat{\lambda} = \frac{|\Gamma_1|}{|\Omega_1|} \lambda
$$

Resulting ODEs:

$$
\frac{d}{dt} \hat{e}_1 = -\hat{\alpha}_1 [\hat{\theta}_1 - \hat{\theta}_2] - \hat{\lambda} [\hat{\theta}_1 - \hat{\theta}_E],
$$

$$
\frac{d}{dt} \hat{e}_2 = -\hat{\alpha}_2 [\hat{\theta}_2 - \hat{\theta}_1].
$$
Comparison of two representations

\[ \frac{d}{dt} \hat{e}_1(\hat{\theta}_1) = -\alpha_1 [\hat{\theta}_1 - \hat{\theta}_2] - \lambda [\hat{\theta}_1 - \hat{\theta}_E], \]

\[ \frac{d}{dt} \hat{e}_2(\hat{\theta}_2) = -\alpha_2 [\hat{\theta}_2 - \hat{\theta}_1]. \]

\[ \hat{e}_i(\hat{\theta}_i) = \rho C \hat{\theta}_i + \rho L \beta^i_\ell(\hat{\theta}_i), \]

\[ \hat{\theta}_i = \Theta_i(\hat{e}_i) \text{ inverse of } \hat{e}_i(\hat{\theta}_i) \]

\[ \dot{\hat{e}}_1 = -\alpha_1 [\Theta_1(\hat{e}_1) - \Theta_2(\hat{e}_2)] - \lambda [\Theta_1(\hat{e}_1) - \hat{\theta}_E], \]

\[ \dot{\hat{e}}_2 = -\alpha_2 [\Theta_2(\hat{e}_2) - \Theta_1(\hat{e}_1)]. \]
Controlled system with disturbances

Notation: \( \hat{e}_1 \rightarrow x, \hat{e}_2 \rightarrow y, \hat{\theta}_E \rightarrow z, \hat{\alpha}_i \rightarrow \alpha_i, \hat{\lambda} \rightarrow \lambda \)

Controlled system:
\[
\begin{align*}
\dot{x} &= -\alpha_1 [\Theta_1(x) - \Theta_2(y)] - \lambda [\Theta_1(x) - z] + v_1, \\
\dot{y} &= -\alpha_2 [\Theta_2(y) - \Theta_1(x)] + v_2, \\
\dot{z} &= u.
\end{align*}
\]

\( z \) is the “chamber” temperature, \( u \) the cooling rate, \( v_i \) errors in data interpreted as disturbances: \( \mu_1 \leq u \leq \mu_2, \ |v_1| \leq \nu_1, \ |v_2| \leq \nu_2 \)

Phase constraint: \( \omega(x, y, z) \leq 0 \) (specifies e.g. bounds on the temperature inside and outside the cell)

Objective functional:
\[
\max \left\{ \int_0^{t_f} \sigma^2(x(\tau), y(\tau))d\tau, \max_{\tau \in [0,t_f]} \omega(x(\tau), y(\tau)), z(\tau) \right\}
\]

where \( \sigma(x, y) \) estimates e.g. the difference of the ice content in the intra- and extracellular regions
Value function and Hamilton-Jacobi equation

Value function:

\[ V(t_0, x_0, y_0, z_0) = \min_{u(t,x)} \max_{v_1(t),v_2(t)} J|_{t_0,x_0,y_0,z_0} \]

Hamiltonian:

\[ H(x, y, z, p_1, p_2, p_3) = \max_{|v_i| \leq \nu_i} \min_{|u| \leq \mu} \left( \dot{x}p_1 + \dot{y}p_2 + \dot{z}p_3 \right) + \sigma^2(x, y) \]

Isaacs-Bellman / Hamilton-Jacobi equation:

\[ V_t - H(x, y, z, V_x, V_y, V_z) = 0, \quad V(t_f, x, y, z) = \omega(x, y, z) \]

Grid function:

\[ V^n(x_i, y_j, z_k) = V(n\tau, i\Delta_x, j\Delta_y, k\Delta_z), \quad N \cdot \tau = t_f \]

Difference scheme for finding viscosity solutions:

\[ V^{n-1}(x_i, y_j, z_k) = V^n(x_i, y_j, z_k) + \tau H(x_i, y_j, z_k, V_x^n, V_y^n, V_z^n), \]

\[ V^N(x_i, y_j, z_k) = \omega(x_i, y_j, z_k) \]
Monotone “upwind” finite difference scheme

*Approximation of the spatial derivatives:

\[ V^n_{x} f_1 = p_1^R f_1^+ + p_1^L f_1^−, \quad V^n_{y} f_2 = p_2^R f_2^+ + p_2^L f_2^−, \quad V^n_{z} f_3 = p_3^R f_3^+ + p_3^L f_3^− \]

\[ f_1^+, \ f_2^+, \ f_3^+ \] are the positive and \[ f_1^−, \ f_2^−, \ f_3^− \] the negatives parts of the RHSs of the controlled system: \[ a^+ = \max(a,0), \ a^- = \min(a,0) \]

\[ \hat{V}^{n−1}(x_i, y_j, z_k) = V^n(x_i, y_j, z_k) + \tau \max_{|v_i| \leq \nu_i} \min_{\mu_1 \leq u \leq \mu_2} \sum_{m=1}^{3} (p^R_m \cdot f^+_m + p^L_m \cdot f^-_m) \]

\[ V^{n−1}(x_i, y_j, z_k) = \max \{ \hat{V}^{n−1}(x_i, y_j, z_k), \omega(x_i, y_j, z_k) \} \]

**Theorem (convergence)**

The finite difference scheme is monotone. The grid function converges point-wise to the value function as \( \tau \rightarrow 0 \). The convergence rate is \( \sqrt{\tau} \).


Optimal feedback control

Current time $t_n$, current position $(x(t_n), y(t_n), z(t_n))$

$$\mathcal{U}_\varepsilon = \{(x_i, y_j, z_k) \in \mathbb{R}^3 : |x_i - x(t_n)| \leq \varepsilon, |y_j - y(t_n)| \leq \varepsilon, |z_k - z(t_n)| \leq \varepsilon\}$$

$$(x_*, y_*, z_*)$$

$$V^n(x_*, y_*, z_*) = \min_{(x_i, y_j, z_k) \in \mathcal{U}_\varepsilon} V^n(x_i, y_j, z_k)$$

* Extremal aiming

$$u(t_n) = \arg \max_{\mu_1 \leq u \leq \mu_2} \left( (x_* - x(t_n)) f_1 + (y_* - y(t_n)) f_2 + (z_* - z(t_n)) f_3 \right)$$

$f_i$ are the right hand sides of the controlled system computed at

$x(t_n), y(t_n), z(t_n), u$, and $v$

Simulations.
1. Cell freezing with accounting for supercooling effects

The functional expresses the balance of ice formation inside and outside the cell:

\[
J = \int_{0}^{t_f} \left| \beta_1^1(\Theta_1(x(\tau))) - \beta_2^2(\Theta_2(y(\tau))) \right|^2 d\tau, \quad \beta_{i(ce)}^j = 1 - \beta_{i}^j, \quad j = 1, 2
\]

Simultaneously, this functional expresses the water outflow from the cell:

\[
c_{in} - c_{out} = \frac{c_{in}^0}{\beta_1^2} - \frac{c_{out}^0}{\beta_1^1} \quad | \quad c_{in}^0 = c_{out}^0
\]
\[ \theta^1_f - \theta^2_f = -13^\circ C, \text{ with phase constraint: } z \leq -2^\circ : \omega(x, y, z) = z - 2 \]

2.7 less liquid outflow with optimal control!
2. Cell thawing with optimization of the osmotic inflow

$\beta_{\ell}$ - freezing

$\theta$ - thawing

$m_0$ - salt amount in the cell

$W_0$ - initial cell volume

$W_0 \beta_{\ell}^2$ - water volume in the cell

$g\left(\frac{m_0}{W_0 \beta_{\ell}^2}\right)$ - salt concentration in the cell

$c_0$ - concentration of physiologic salt solution

$c_*$ - some limiting concentration

Functional to minimize expresses amount of water inflow into the cell

$$J = \alpha \int_0^{t_f} \left| c_0 - g\left(\frac{m_0}{W_0 \beta_{\ell}^2(\Theta_2(y(\tau)))}\right)\right| d\tau$$
Phase constraints: \( z \leq 40^\circ, \ z \geq -50^\circ, \ \Theta_2(y) \leq 20^\circ \)

65 % less water inflow with optimal control!
Conclusions

Using these techniques, some other injuring effects of cryopreservation like generation of the dendrite seeds during freezing and subsequent dendrite growth during thawing can also be accounted for.

Implementation of optimal temperature profiles for freezing devices like e.g. IceCube (SY-LAB Geräte GmbH, Austria) is possible.

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