OPTIMAL CONTROL IN CRYOPRESERVATION OF CELLS AND TISSUES

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Abstract. The investigation concerns the application of the theory of partial differential equations and optimal control techniques to the minimization of damaging factors in cryopreservation of living cells and tissues in order to increase the survival rate of frozen and subsequently thawed out cells. The paper is devoted to the statement of mathematical models describing the process of freezing and to the application of optimal control theory to the design of optimal cooling protocols that minimize damaging effects caused by the release of the latent heat and by delayed freezing of intracellular water.

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1 Introduction

Optimization and control are necessary because of several competitive conditions of cooling. A slow cooling causes freezing of the extracellular fluid, whereas the intracellular fluid remains unfrozen for a while. This results in an increase in the concentration of salt in the extracellular solution, which leads to the cellular dehydration and shrinkage of cells. Another effect of that are large stresses that can violate the integrity of cell membranes. If cooling is rapid, the water inside the cells forms small, irregularly-shaped ice crystals (dendrites) that are relatively unstable. If frozen cells are subsequently thawed out too slowly, dendrites will aggregate to form larger, more stable crystals that may cause damage. Maximum viability is obtained by cooling at a rate in a transition zone in which the combined effect of both these mechanisms is minimized. Thus, an optimization problem can be formulated for mathematical models describing the processes of freezing and thawing. Moreover, some general arguments and our experiments with freezing facilities show that better results can be obtained, if the ambient temperature falls not monotonically in time, especially in the temperature range where the latent heat is released. Thus, time dependent optimal controls (optimal cooling protocols) are to be designed. Our numerical simulations and experiments show that positive effects can be achieved by creating temperature gradients in the freezing area or by forcing ice nucleation through mechanical vibration or some temperature checks localized in a small area (seeding). Another control tool is cryoprotective agents which vary eutectic properties of solutions.

Additional difficulties arise when preserving structured solid tissues. Significant problems are associated with the difficulty of generating heat transfer within a large thermal mass with a complex geometry. The packing density of cells within their extracellular matrix can approach 80% whereas preservation of isolated cells becomes problematic at a cell concentration above 20%. The presence of different cell types, each with its own requirements for optimal cryopreservation, limits the recovery of each when a single thermal protocol is imposed on all of the cells. Extracellular ice can cause mechanical damage to the structural integrity of the tissue. There are mechanical stresses caused by delayed freezing of the intracellular water. Each of these is an additional source of damage, over and above those that are already known from studies of cells in suspension. Therefore, optimization and optimal control are necessary in this case even more than when preserving isolated cells.

2 Mathematical models

Usually, tissue samples are being frozen using special plants e.g. of the IceCube family developed by SY-LAB, Geräte GmbH (Austria). The main part of such plants is a freezing chamber supplied by a cooling system. A plastic ampoule with a tissue sample surrounded by a liquid (see Fig. 1) is placed into the chamber. The plant is controlled by a computer that allows the user to prescribe a cooling protocol to be tracked.

The paper is devoted to the statement of mathematical models describing the process of freezing and to the application of optimal control theory to the design of optimal cooling protocols that minimize damaging effects caused by the release of the latent heat and by
delayed freezing of intracellular water.

The first model utilizes mean values of thermodynamical parameters to describe the mean boundary temperature of the ampoule. The control here is the temperature regime in the freezing chamber. A first version of such a model including the design of improved cooling protocols is implemented and tested in experiments on freezing of dental tissues.

The second model of freezing deals with spatially distributed parameters and describes ice formation in the liquid surrounding the tissue. This approach is based on the so-called phase-field models described by partial differential equations. They have been introduced by Caginalp (see [1]) and studied by many scientists. We base the design of improved cooling protocols for freezing plants on results of [2] where an optimal control problem for a phase-field model is stated and investigated from the mathematical and algorithmic points of view. Optimal controls are computed using the gradient descent method based on adjoint equations.

The third model should describe ice formation inside the extracellular matrix of the tissue. This includes phase changes in the extracellular liquid confined inside of small pores and mechanical stresses exerted on cell boundaries due to delayed freezing of the intracellular water.

3 Mean value temperature response model

A preliminary approach to the control of global (averaged) thermodynamical parameters was elaborated and verified using a Freezer IceCube 15M (SY-LAB, Geräte GmbH, Austria). IceCube 15M is developed for controlled freezing of samples put into plastic ampoules (see Figure 2). The main part of the plant is a freezing chamber containing a cooling system based on gas nitrogen, a rack for placing ampoules, and two temperature sensors that measure the chamber and sample temperatures, respectively. The plant is equipped with a computer that allows the user to input a cooling protocol either manually or as a file prepared in off-line regime. The computer controls the cooling system and forces the chamber temperature to track the prescribed cooling protocol (see Figure 3). Freezing experiments show an irregular behavior of the temperature near the freezing point because of the release of the latent heat and the crystallization. A typical response of the object to the cooling with a constant rate is shown in Figure 4. The supercooling (S) is not too dangerous itself but in combination with the latent heat release (L). This yields the creation of injuring dendrites: the longer runs the latent heat release (L) the more dendrites appear. A sudden drop of the temperature (D) causes a temperature shock to cells. Therefore, it would be preferable to reduce both the duration of the latent heat release (L) and the temperature drop (D).

The following simple thermodynamical model is based on averaged values of parameters. The heat transfer in the ampoule containing a sample is described by a heat conductivity equation of the form:

$$\frac{\partial H}{\partial t} - \text{div} (k\nabla T) = 0,$$

where $H$ and $T$ are the enthalpy density and the temperature, respectively, $k$ is the heat transfer coefficient that may depend on space. The condition on the surface of the
ampoule reads:

\[-k \partial T \partial n = h(T - T_e),\]

where \( T_e \) is the chamber temperature, \( h \) is the overall heat transfer coefficient. Integration over the volume of the ampoule yields:

\[\frac{d}{dt} \int_V H dV + \int_S h(T - T_e) dS = 0,\]

which can be rewritten as follows

\[\frac{d}{dt} H = -|S||V|^{-1} h(T - T_e). \tag{1}\]

Here, \( H \) is now the enthalpy density averaged over the volume of the ampoule, \( T \) is the boundary temperature averaged over the surface area of the ampoule, \( |V| \) and \( |S| \) denote the volume and the surface area of the ampoule, respectively.

To close equation (1), a constitutive relation \( T = T(H) \) is necessary. Such a relation is obtained approximatively by recovering the mean surface temperature \( T(t) \) of the ampoule from measurements for a given temperature profile \( T_e(t) \). Substituting the functions \( T(t) \) and \( T_e(t) \) in (1) and computing \( H(t) \) yields the pair \( \{ T(t), H(t) \} \), which defines implicitly the desired function

\[ T = T(H). \tag{2}\]

Note that relation (2) is specific for the sample. Nevertheless such a relation is robust for a series of similar samples. Figure 5 shows the function \( T(H) \) obtained for a real sample consisting of a liquid milieu containing a tissue. Note that the inverse dependence, i.e. the function \( H = H(T) \) is not one-valued. That is why the material low (2) is preferable.

The chamber temperature can not change instantaneously. Therefore, equation (1) should be extended by adding the chamber temperature rate \( u \) whose maximal absolute value \( \mu \) is equal about 20 /s. Thus, denoting \( \alpha = |S||V|^{-1} \), we have:

\[\frac{d}{dt} H = -\alpha (T(H) - T_e) \tag{3}\]

\[\dot{T}_e = u, \quad |u| \leq \mu. \tag{4}\]

The aim of the control is to smooth the temperature response of the sample during the production of the latent heat (see Figure 6). Formally, this is expressed through the minimization of the following performance index:

\[ J = \int_{t_1}^{t_2} \left( \frac{d}{dt} T(H(t)) - \theta_0 \right)^2 dt \equiv \int_{t_1}^{t_2} \left( \alpha T_H(H) \cdot (T(H) - T_e) + \theta_0 \right)^2 dt, \tag{5}\]

where \( \theta_0 \) is a desirable slope of the temperature curve. Ordinary differential equations (3), (4) and functional (5) form a controlled system, where the control variable is the rate \( u \) of the chamber temperature. This model possesses the following nice property: the functional \( J \) does not depend on the choice of \( \alpha \), whenever \( H \) is found from (3) and the
constitutive law $T(H)$ from (1). Therefore, the value of $\alpha$ can be simply chosen as $\alpha = 1$, which avoids from the measurement of physical parameters $|V|, |S|$ and $h$.

Analysis based on Pontryagin’s maximum principle shows that the minimum of $J$ can be achieved using a single cooling impulse whose parameters (the start instant, finish time and the power) can be found through a direct minimization. Figure 6 shows that a heuristic (non-optimized) cooling impulse provides a bad transition regime from the latent heat release to the frozen phase. Figure 7 demonstrates the application of an optimized cooling impulse.

4 Distributed modelling of ice formation in the liquid milieu

Nowadays, phase field techniques for modelling of solidification and freezing processes become very popular. They are based on the consideration of the Gibbs free energy which depends on an order parameter that assumes values from -1 (solid) to 1 (liquid) and changes sharply but smoothly over the solidification front so that the sharp liquid/solid interface becomes smoothed. The rate of smoothing is controlled by a small parameter, which enables to reach arbitrary approximation of the sharp interface.

We use a phase field model (see [1],[2],[3], and [4]) to describe phase changes in the milieu containing a tissue. The control parameter is the temperature in the chamber. Note that the heat flux on the boundary of the ampoule is proportional to the temperature jump on the boundary. For simplicity, we do not include the solid part (i.e. the tissue and the walls of the ampoule) into the description bearing in mind that they are present and accounted for in numerical simulations. Therefore, $\Omega$ being the interior of the ampoule, $\Gamma$ the boundary of $\Omega$. The equations look as follows:

\begin{align*}
    u_t + \frac{\ell}{2} \phi_t - K \Delta u &= 0, & x &\in \Omega, \\
    \tau \phi_t - \ell^2 \Delta \phi - \frac{1}{2} (\phi - \phi^3) - 2u &= 0, & x &\in \Omega, \\
    -K \frac{\partial u}{\partial n} &= h(u - u_e(t) - g), & x &\in \Gamma, \\
    \phi_t &= 0, & x &\in \Gamma, \\
    u|_{t=0} = u_0 = \text{const} > 0, & \phi|_{t=0} = \phi_0 = 1.
\end{align*}

Here, $u$ is the scaled distribution of the temperature; $\phi$ the phase function: $\phi = 1$ for the solid state and $\phi = -1$ for the liquid state; $\ell$ the scaled latent heat; $K$ the scaled heat conductivity coefficient; $h$ the scaled overall heat conductivity; $g$ the boundary control (add-on to the nominal cooling protocol $u_e(t) = u_0 + \theta_0 t$, where $\theta_0 < 0$ is a given slope). In contrast to the work [2] we do not assume $C^2$ regularity of $\Gamma$. It is assumed that $\Gamma$ is of the class $C^{0,1}$, i.e. Lipschitz continuous. Such an assumption covers various technical designs of ampoules and permits a direct extension of the result to the case where a solid part (tissue) immersed into the fluid is present.

Proposition 4.1. If $\Gamma \in C^{0,1}$, $u_0$ and $\phi_0 \in L^2(\Omega)$, $g \in L^2((t_0, t_f); L^2(\Gamma))$, then exists a unique solution pair $u, \phi$ such that

\begin{align*}
    u, \phi &\in L^\infty(t_0, t_f; L^2(\Omega)) \cap L^2(t_0, t_f; H^1(\Omega)), \\
    \phi &\in L^4(t_0, t_f; L^4(\Omega)).
\end{align*}
Proof. The proof is performed using Galerkin approximation arguments. Testing the equations with \( u \) and \( \phi \), respectively, yields appropriate estimates. A trick here is that the term \( \phi^3 \) being tested with \( \phi \) yields \( \phi^4 \) with the favorable sign.

\[ \square \]

**Proposition 4.2.** If \( \Gamma \in C^{0,1} \), \( u_0 \) and \( \phi_0 \in H^1(\Omega) \), \( g \in L^2((t_0,t_f);L^2(\Gamma)) \), then the unique solution pair \( u, \phi \) satisfies:

\[
u, \phi \in L^\infty(t_0,t_f;H^1(\Omega)), \quad u_t, \phi_t \in L^2(0,t_f;L^2(\Omega)), \quad \phi \in L^\infty(t_0,t_f;L^4(\Omega)).\]

**Proof.** The proof is similar to that of the previous proposition, but testing the equations with \( u_t \) and \( \phi_t \), respectively, yields additional estimates. Here, the term \( \phi^3 \) being tested with \( \phi_t \) yields the therm \( 1/4(\phi^4) \), with the favorable sign.

Consider first the following functional that estimates the mean quadratic deviation from the nominal cooling protocol \( u_e(t) \):

\[ J = \frac{1}{2} \int_{t_0}^{t_f} \int_{\Omega} (u - u_e(t))^2 \, dx \, dt. \tag{10} \]

The adjoint system is derived as in [2] with some modifications related to the boundary method of control.

\[
- p_t - K \Delta p - 2q = h(u - u_e(t)), \quad x \in \Omega, \\
- \tau q_t - \frac{\xi}{2} p_t - \xi^2 \Delta q - \frac{1}{2} (1 - 3\phi^2) q = 0, \quad x \in \Omega, \\
- K \frac{\partial p}{\partial n} = h_p, \quad \frac{\partial q}{\partial n} = 0, \quad x \in \Gamma, \\
p(t_f) = 0, \quad q(t_f) = 0. \tag{11}
\]

Here, \( p \) is the adjoint variable corresponding to \( u \); \( q \) the adjoint variable related to \( \phi \).

**Proposition 4.3.** If \( \Gamma \in C^{0,1} \), \( u \in L^\infty((t_0,t_f);L^2(\Omega)) \), \( \phi \in L^4((t_0,t_f);L^4(\Omega)) \), then exists a unique solution pair \( p, q \) such that

\[
p, q \in L^\infty(t_0,t_f;L^2(\Omega)) \cap L^2(t_0,t_f;H^1(\Omega))
\]

and, therefore, \( p|_\Gamma \in L^2((t_0,t_f);L^2(\Gamma)). \)

**Proof.** The proof is similar to the one of proposition 4.1. Note that the therm \( \phi^2 q \) being tested with \( q \) yields \( \phi^2 q^2 \) with the favorable sign.

The derivative of the functional \( J \) with respect to the control \( g \) is defined by the formula:

\[
J'(u,\phi)(\delta g) = \int_{t_0}^{t_f} \int_{\Gamma} \delta g(t,s) p(t,s) \, ds \, dt, \quad \text{for all } \delta g \in L^2((t_0,t_f) \times \Gamma). \tag{12}
\]

Therefore, we can identify \( J'(u,\phi) \) with \( p|_{(0,t_f) \times \Gamma} \). The method of conjugate gradients looks as follows. Consider the \( n \)th step. Assume that the control \( \delta g^n \) is already known. Compute then the states \( u^n, \phi^n \) and the adjoint state \( p^n \). Compute \( \delta g^{n+1} \) as

\[
\delta g^{n+1} = \delta g^n + \alpha^n d^n,
\]

...
where \( \alpha^n \) is found as approximate solution of the line search problem \( \alpha^n \to \min_{\alpha} J(g^n + \alpha d^n) \), the conjugate direction \( d^n \) from the relation (see [5]):

\[
d^n = -p^n + \beta^n d^{n-1}, \quad \beta^n = \left[ \int_{t_1}^{t_2} \int_{\Gamma} (p^{n-1})^2 \, ds \, dt \right]^{-1} \int_{t_1}^{t_2} (p^n)^2 \, ds \, dt.
\]

Figure 8 shows the result obtained through minimizing of functional (10). It should be noted that curve (b) shows an expected behavior: oscillations about the nominal protocol \( u_e(t) \). Unfortunately these oscillations are too sharp and such a control is unusable. Thus, a modification of the functional (10) is necessary.

Denote \( H = L^2(\Omega), V = H^1(\Omega) \), for brevity. Let \( \langle \cdot, \cdot \rangle \) be the inner product of \( L^2(\Omega) \), \( \Lambda : V \to V' \) the canonical isomorphism so that \( \| f \|^2_V = \langle \Lambda^{-1} f, f \rangle \), where the angle brackets denote the duality between \( V \) and \( V' \). Consider the following functional that measures a mean quadratic deviation of the time derivative of the solution from the given slope \( \theta_0 \):

\[
J = \frac{1}{2} \int_0^{t_f} \| u_t - \theta_0 \|^2_V \, dx \, dt. \tag{13}
\]

The formal derivation of the adjoint equations for this functional yields:

\[
\begin{align*}
-p_t - K \Delta p - 2q &= -h [\Lambda^{-1}(u_t - \theta_0)] t \\
-\tau q_t - \frac{\ell}{2} p_t - \xi^2 \Delta q - \frac{1}{2}(1 - 3\phi^2)q &= 0 \\
-K \frac{\partial p}{\partial n} &= h p, \quad \frac{\partial q}{\partial n} = 0 \\
p(t_f) &= h \Lambda^{-1}(u_t - \theta_0), \quad \tau q(t_f) + \frac{\ell}{2} p(t_f) = 0. \tag{14}
\end{align*}
\]

This problem does not have conventional solutions in the sense of propositions 4.1 and 4.2 because the right-hand-side of the first equation does not possess proper regularity. To formulate this problem correctly, one can use the technique of transposed isomorphisms developed in [6] and [7]. Define

\[
W_0(0, t_f) = \left\{ \eta : \eta \in L^2(0, t_f; V), \quad \eta_t, \Delta \eta \in L^2(0, t_f; V') \right\}, \quad -K \frac{\partial \eta}{\partial n} = h \eta, \quad \eta(0) = 0
\]

\[
Z_0(0, t_f) = \left\{ \mu : \mu \in L^2(0, t_f; V), \quad \mu_t, \Delta \mu \in L^2(0, t_f; V') \right\}, \quad \frac{\partial \mu}{\partial n} = 0, \quad \mu(0) = 0
\]

Then, the adjoint system is obtained by multiplying system (14) by test functions and double partial integration:

\[
\begin{align*}
\int_0^{t_f} [\langle p, \eta_t - K \Delta \eta \rangle - 2\langle q, \eta \rangle] \, dt &= \int_0^{t_f} \langle \Lambda^{-1}(u_t - \theta_0), \eta_t \rangle \, dt, \\
\int_0^{t_f} [\langle q, \tau \mu_t - \xi^2 \Delta \mu \rangle + \frac{\ell}{2} \langle p, \mu_t \rangle - \frac{1}{2} (1 - 3\phi^2)q, \mu \rangle] \, dt &= 0. \tag{15}
\end{align*}
\]
Here, \( \eta \) and \( \mu \) are the test functions running over the spaces \( W_0(0,t_f) \) and \( Z_0(0,t_f) \), respectively. The unique solvability is stated using the fact that the mappings

\[
\ell^{-1} : \eta \to \eta_t - K \Delta \eta : \quad W_0(0,t_f) \to L^2(0,t_f;V')
\]
\[
\varphi^{-1} : \mu \to \tau \mu_t - \xi^2 \Delta \mu : \quad Z_0(0,t_f) \to L^2(0,t_f;V')
\]

are isomorphisms.

**Proposition 4.4.** Let \( u_t \in L^2(0,t_f;V') \) then system (15) has a unique solution pair \( p,q \in L^2(0,t_f;V) \) and, therefore, \( p|\Gamma \in L^2((0,T);L^2(\Gamma)) \). The derivative of the functional (13) is given by (12).

**Proof.** Look for \( p \) and \( q \) in the form \( p = p_1 + p_2, \quad q = q_1 + q_2 \), where:

\[
\int_0^{t_f} [\langle p_1, \eta_t - K \Delta \eta \rangle] dt = \int_0^{t_f} \langle \Lambda^{-1}(u_t - \theta_0), \eta_t \rangle dt,
\]
\[
\int_0^{t_f} \left[ \langle q_1, \tau \mu_t - \xi^2 \Delta \mu \rangle + \frac{3}{2} \langle \phi^2 q_1, \mu \rangle \right] dt = -\int_0^{t_f} \frac{\xi}{2} \langle p_1, \mu_t \rangle.
\]

\[
\int_0^{t_f} [\langle p_2, \eta_t - K \Delta \eta \rangle - 2(\eta_t, \eta_t)] dt = \int_0^{t_f} 2(q_1, \eta_t) dt,
\]
\[
\int_0^{t_f} \left[ \langle q_2, \tau \mu_t - \xi^2 \Delta \mu \rangle + \ell \langle p_2, \mu_t \rangle - \frac{1}{2} \langle (1 - 3 \phi^2) q_2, \mu \rangle \right] dt = \int_0^{t_f} \frac{1}{2} \langle q_1, \mu \rangle dt.
\]

The first equation of (16) has a unique solution \( p_1 \in L^2(0,t_f;V) \) because the right-hand side is a continuous functional on \( W_0(0,t_f) \). The left-hand-side \( \mathcal{L}(q_1,\mu) \) of the second equation of (16) is coercive, i.e. \( \mathcal{L}(q_1,q_1) \geq \nu \|q_1\|^2_{L^2(0,t_f;V)} \) if \( q_1 \in Z_0(0,t_f) \). Therefore, \( \sup \{ \mathcal{L}(q_1,\mu)/\|\mu\|_{Z_0(0,t_f;V)} : \mu \in Z_0(0,t_f) \} \geq \nu \|q_1\|_{L^2(0,t_f;V)} \) for all functions \( q_1 \in L^2(0,t_f;V) \) because \( Z_0(0,t_f) \) is dense in \( L^2(0,t_f;V) \) in the norm of the last space. Therefore, the form \( \mathcal{L}(q_1,\ell^{-1} \zeta) \) defined on \( L^2(0,t_f;V) \times L^2(0,t_f;V') \) satisfies the inf sup condition. Taking into account that the right-hand-side \( \mathcal{R}(\mu) \) of the second equation of (16) is a continuous functional on \( Z_0(0,t_f) \) and, therefore, \( \mathcal{R}(\ell^{-1} \zeta) \) is a continuous linear form on \( L^2(0,t_f;V') \) proves the solvability of the second equation of (16), and \( q_1 \in L^2(0,t_f;V) \).

Note that the right-hand-sides of system (17) are regular so that the system admits the conventional formulation. Therefore, the arguments of proposition 4.3 prove the solvability of (17) and the corresponding regularity of \( p_2 \) and \( q_2 \).

Consider now the following functional that seems to be more natural then (13):

\[
J = \frac{1}{2} \int_0^{t_f} \|u_t - \theta_0\|^2_{H_t} dt.
\]
To formulate adjoint equations, we need now the spaces:

\[
W_0(0,t_f) = \left\{ \eta : \eta \in L^\infty(0,t_f; V), \; \eta_t, \Delta \eta \in L^2(0,t_f; H), \; -K \frac{\partial \eta}{\partial n} = h\eta, \; \eta(0) = 0 \right\},
\]

\[
Z_0(0,t_f) = \left\{ \mu : \mu \in L^\infty(0,t_f; V), \; \mu_t, \Delta \mu \in L^2(0,t_f; H), \; \frac{\partial \mu}{\partial n} = 0, \; \mu(0) = 0 \right\}.
\]

The adjoint equations are:

\[
\int_0^{t_f} \left[ (p, \eta_t - K \Delta \eta) - 2(q, \eta) \right] dt = \int_0^{t_f} (u_t - \theta_0, \eta_t) dt,
\]

\[
\int_0^{t_f} \left[ (q, \tau \mu_t - \xi^2 \Delta \mu) + \frac{\ell^2}{2} (p, \mu_t) - \frac{1}{2} ((1 - 3\phi^2)q, \mu) \right] dt = 0.
\]

The following can be proved in analog to proposition 4.4.

**Proposition 4.5.** Let \( u_t \in L^2(0,t_f; H) \) then system (19) has a unique solution pair \( p, q \in L^2(0,t_f; H) \).

The trouble here is that the derivative of the functional (18) should be defined by (12), but the trace \( p_\Gamma \) can not be defined properly because of insufficient regularity of \( p \). To overcome this problem, consider a functional that estimates the deviation of the slope of the mean temperature from a given slope:

\[
J = \frac{1}{2} \int_0^{t_f} \left( [u]_t - \theta_0 \right)^2 dt,
\]

where \( [u] = \frac{1}{|\Omega|} \int_\Omega u dx \).

The idea is to express \([u]_t \) through values that do not contain time derivatives. Integrating the model equations (6) and (7) over \( \Omega \) yields:

\[
[u]_t - \theta_0 = -\frac{1}{|\Omega|} \left[ h \int_{\Gamma} [u - u_e(t) - g] ds + \frac{1}{4\tau} \int_\Omega (\phi - \phi^3) dx + \frac{1}{\tau} \int_\Omega u dx \right] - \theta_0 =: \gamma_{u,\phi,g}(t)
\]

The adjoint system and the derivative of the functional are given by:

\[
\begin{align*}
- p_t - K \Delta p - 2q &= \frac{1}{\tau} \gamma_{u,\phi,g}(t), & x &\in \Omega, \\
- \tau q_t - \frac{\ell}{2} p_t - \xi^2 \Delta q - \frac{1}{2} (1 - 3\phi^2)q &= \frac{1}{4\tau} \gamma_{u,\phi,g}(t)(1 - 3\phi^2), & x &\in \Omega, \\
- K \frac{\partial p}{\partial n} &= hp + h \gamma_{u,\phi,g}(t), & \frac{\partial q}{\partial n} &= 0, & x &\in \Gamma, \\
p(t_2) = 0, & q(t_2) = 0, \\
J'(u, \phi, g)(\delta g) &= \frac{h}{|\Omega|} \int_0^{t_f} \int_{\Gamma} \delta g(t,s)[p(t,s) - \gamma_{u,\phi,g}(t)] ds dt, & \forall \; \delta g &\in L^2((t_0,t_f) \times \Gamma).
\end{align*}
\]

System (21) is well posed because \( \gamma_{u,\phi,g}(t) \in L^2(0,t_f) \) and \( \phi \in L^\infty(0,t_f; L^4(\Omega)) \) (see Proposition (4.2)). Thus, the same regularity of \( p \) as in Proposition (4.2) holds and,
therefore, formula (22) is well defined. The result of the application of such a technique is shown in Figure 9.

It should be noted that the implementation of such a control method is not simple. First, the computation of an optimized cooling protocol demands a pair hours. Second, an optimized control obtained has a complex boundary distribution, which can hardly be implemented technically. Therefore, it would be reasonable to look for a control that is constant on the boundary so that $g$ is a function of $t$ only. Compute such a $g(t)$ from the condition $\gamma_{u, \phi,g}(t) = 0$, i.e. $[u]_t - \theta_0 = 0$ (see relation (20)). This yields the following feedback rule:

$$g(t) = \frac{1}{h_{\Omega}||\Gamma||} \left[ h \int_{\Gamma} |u - u_e(t)| ds + \frac{1}{4\tau} \int_{\Omega} (\phi - \phi^3) dx + \frac{1}{7} \int_{\Omega} u dx \right] + \frac{1}{h_{\Omega}||\Gamma||} \theta_0. \quad (23)$$

Figure 10 demonstrate the application of such a heuristic rule. The result seems to be comparable with that obtained using techniques based on (21) and (22), but the run time is sufficiently shorter.

5 Ice formation in the extracellular matrix

The description of ice formation in the extracellular matrix of a tissue is based on the thermodynamics of porous media (see e.g. [8]). The main feature of porous or soil media is that the unfrozen water content is a function of the temperature only. Such a function can be considered as material property that depends on the pore size distribution and the material of the solid matrix. The interaction of the liquid with the solid matrix causes as a rule the homogenous nucleation of the liquid and, therefore, avoids the it from supercooling. Thus, the unfrozen water content is not defined from any equation but is a given function of the temperature. In freezing soil science, it is measured directly by NMR (Nuclear Magnetic Resonance). There are a lot of theoretical works on derivation of this function (see e.g. [8] and [9]).

Introduce some notation. Let $\beta_\ell$ be the liquid water volume fraction (unfrozen water content), $\beta_i$ the ice volume fraction, $\beta_m$ the volume fraction of the solid matrix, $\rho$ the density (assume that the densities of the liquid and ice are equal), $L$ the phase change latent heat, $C$ the specific heat capacity (assume they are equal for the liquid and ice), $T_0$ is the freezing temperature. Obviously, $\beta_\ell + \beta_i = 1 - \beta_m$. Assuming that $\beta_m = \text{const}$ and scaling the last equation yields: $\beta_\ell + \beta_i = 1$.

According to [8]), the density of the free energy of the liquid and ice are given by:

$$\Psi_\ell = \rho_\ell \beta_\ell \left\{ -\frac{L}{T_0} (T - T_0) + Th_\ell(\beta_\ell, \beta_i) - CT \log T \right\},$$  

$$\Psi_i = \rho_\ell \beta_i \left\{ Th_i(\beta_\ell, \beta_i) - CT \log T \right\}, \quad (24)$$

where $h_\ell(\beta_\ell, \beta_i)$ and $h_i(\beta_\ell, \beta_i)$ are functions that take into account the interaction of the liquid and ice, respectively, with the solid matrix. The total free energy density is:

$$\Psi = \rho_\ell \beta_\ell \left\{ -\frac{L}{T_0} (T - T_0) \right\} + \rho T \left\{ \beta_\ell h_\ell(\beta_\ell, \beta_i) + \beta_i h_i(\beta_\ell, \beta_i) \right\} - \rho CT \log T.$$
The second law of thermodynamics gives the model equations:

\[
\frac{de}{dt} = -\text{div} q + r, \quad q = -K \nabla T, \quad \text{with} \quad e = \Psi - T \frac{\partial \Psi}{\partial T}.
\]  

(25)

To determine the function defining the unfrozen water content, assume (see Chapter 11.3 of ([8])) that the phase transition itself is not dissipative, and the dissipation is caused by thermal conditions only. This results in the equilibrium of the driving forces of the phase change:

\[
\frac{\partial \Psi}{\partial \beta} = \frac{\partial \Psi}{\partial \beta_i} \quad \text{if} \quad 0 < \beta < 1,
\]

which is equivalent to:

\[
\frac{L}{T_0} (T - T_0) = T \frac{\partial \tilde{h}(\beta)}{\partial \beta}, \quad \text{if} \quad 0 < \beta < 1,
\]

(26)

where

\[
\tilde{h}(\beta) = \beta h(\beta, \beta_i) + \beta_i h_i(\beta, \beta_i)|_{\beta_i=1-\beta}. \]

Using (25), (26), and introducing the Celsius temperature \(\theta := T - T_0\) yields the complete model:

\[
\rho C \frac{\partial \theta}{\partial t} + \rho L \frac{\partial \beta}{\partial t} - K \Delta \theta = 0,
\]

\[
-\frac{\partial \theta}{\partial n} = \lambda (\theta - \theta_0 t - g),
\]

\[
\beta = \phi \left( \frac{L \theta}{T_0 (T_0 + \theta)} \right), \quad \text{where} \quad \phi(y) = \left[ \frac{\partial \tilde{h}(\beta)}{\partial \beta} \right]^{-1}(y).
\]

(28)

Here, \(g\) is the boundary control (add-on to the nominal cooling protocol \(\theta_0 t\), where \(\theta_0 < 0\) is a given slope). The function \(\beta\) (see Figure 11) was recovered from data obtained in experiments with tissue samples on an IceCube plant (see section 3). Figure 12 shows the mean temperature response when \(g = 0\). Figure 13 shows a time snapshot of the spatial distribution of the temperature and unfrozen water content for this case.

Note that supercooling does not occur when freezing a porous medium. Therefore it would be reasonable to use the following simple functional to minimize effects of the latent heat:

\[
J = \frac{1}{2} \int_0^{t_f} \int_{\Omega} (\theta - \theta_0 t)^2 dx dt.
\]

The adjoint equation looks as follows:

\[
- \left[ \rho C + \phi_\gamma \left( \frac{L \theta}{T_0 (\theta + T_0)} \right) \right] \frac{\partial p}{\partial t} - K \Delta p = \lambda (\theta - \theta_0 t), \quad x \in \Omega,
\]

\[
-\frac{\partial p}{\partial n} = \lambda p, \quad x \in \partial \Omega,
\]

(29)

The derivative of the functional is given (up to notations) by (12). Figure 14 shows the mean temperature response when applying optimization based on (29). Figure 15 shows a time snapshot of the spatial distribution of the temperature and unfrozen water content in this case (compare with Figure 13).
6 Stresses in a frozen porous medium

It is well known that the increase of the volume during the water to ice phase change is rather large. If the phase change occurs in a pore of a porous material, a very large stress can be exerted on the pore walls (see Figure 16).

Let $\Omega_{\text{ice}}$ and $\Omega_{\text{mat}}$ be domains occupied by the ice-water mixture and the solid matrix, respectively. It is easy to show that the displacement vector $\vec{u}$ is governed by the following equation given in a weak formulation:

$$
\int_{\Omega_{\text{ice}}} C_{ijkl}^{\text{ice}} \varepsilon_{ij}(\vec{u}) \epsilon_{kl}(\vec{\varphi}) + \int_{\Omega_{\text{mat}}} C_{ijkl}^{\text{mat}} \varepsilon_{ij}(\vec{u}) \epsilon_{kl}(\vec{\varphi}) = 0.
$$

(30)

Here, $C_{ijkl}^{\text{ice}}$ and $C_{ijkl}^{\text{mat}}$ are the elastic stiffness tensors of the ice and the solid matrix material, respectively, $\vec{\varphi}(x)$ the test functions,

$$
\varepsilon_{ij}(\vec{u}) = \frac{1}{2} \left( \partial u_i / \partial x_j + \partial u_j / \partial x_i \right), \quad \varepsilon_{ij}^{tr}(\vec{u}) = \varepsilon_{ij}(\vec{u}) - \varepsilon_{ij}^{tr}(\vec{\beta}), \quad \varepsilon_{ij}(\vec{\beta}) = \frac{1}{3} \alpha (1 - \beta) \delta_{ij},
$$

and $\alpha$ the expansion coefficient (the relative volume increase during the phase change).

Obviously, these equations are not appropriate for numerical implementations because of a fine structure of the domains $\Omega_{\text{ice}}$ and $\Omega_{\text{mat}}$. To state a computable model, homogenization techniques is used (see [10, 11, 12, 13]). A porous medium is a probabilistic composite material, which demands the use of stochastic homogenization techniques (see e.g. [12]). Nevertheless, the assumption of periodicity provides a reasonable first approximation. Suppose the structural cell of the porous material is scaled to the unit cube $Y = [0,1]^3$.

Let $Y_{\text{ice}} \subset Y$ be the part occupied by ice-water mixture, $Y_{\text{mat}} = Y \setminus Y_{\text{ice}}$ the part occupied by the solid matrix, $I_{Y_{\text{mat}}}(y)$ and $I_{Y_{\text{ice}}}(y)$ their $Y$-periodically continued indicator functions, respectively (see Figure 17). Let $\varepsilon$ be the parameter of homogenization. Then the system (30) can be rewritten as follows

$$
\int_{\Omega} \left[ C_{ijkl}(x/\varepsilon) \varepsilon_{ij}(\vec{u}) - \alpha (1 - \beta) I_{Y_{\text{ice}}}(x/\varepsilon) \delta_{ij} \right] \epsilon_{kl}(\vec{\varphi}) = 0,
$$

(31)

where

$$
C_{ijkl}(y) = I_{Y_{\text{ice}}}(y) C_{ijkl}^{\text{ice}} + I_{Y_{\text{mat}}}(y) C_{ijkl}^{\text{mat}}.
$$

Using techniques of [13], it is easy to show that the limiting equation, as $\varepsilon \to 0$, read:

$$
\int_{\Omega} \left[ D_{ijkl} \varepsilon_{ij}(\vec{u}) - \alpha (1 - \beta) d_{kl} \right] \epsilon_{kl}(\vec{\varphi}) = 0,
$$

(32)

where the constant tensors $D$ and $d$ are given by the formulæ:

$$
D_{klmn} = \int_{Y} C_{ijkl}(y) \left[ \delta_{im} \delta_{jn} + \varepsilon_{ij}(\vec{N}_{mn}(y)) \right] dy,
$$

$$
d_{kl} = \int_{Y} C_{ijkl}(y) \left[ \varepsilon_{ij}(\vec{M}(y)) - \delta_{ij} I_{Y_{\text{ice}}}(y) \right] dy.
$$

(33)

Here the auxiliary functions $\vec{N}_{mn}(y)$ and $\vec{M}(y)$ satisfy the following cell equations:

$$
\int_{Y} C_{ijkl}(y) \left[ \delta_{im} \delta_{jn} + \varepsilon_{ij}(\vec{N}_{mn}(y)) \right] \epsilon_{kl}(\vec{\varphi}(y)) dy = 0, \quad n = 1, 2, 3, \quad m \leq n,
$$

$$
\int_{Y} C_{ijkl}(y) \left[ \varepsilon_{ij}(\vec{M}(y)) - \delta_{ij} I_{Y_{\text{ice}}}(y) \right] \epsilon_{kl}(\vec{\varphi}(y)) dy = 0,
$$

(34)
where \( \vec{N}_{mn}(y) = \vec{N}_{nm}(y) \), if \( m > n \), \( \vec{N}_{mn} \) and \( \vec{M} \) are Y-periodic, and \( \vec{\psi}(y) \) are Y-periodic test functions.

Coupled equations (32) and (27) have been implemented in a two dimensional rectangular domain \( \Omega \). Figure 18 shows a time snapshot of the stress in a porous medium under freezing.

Consider now stresses exerted on a sole cell located in a pore with freezing water (see Figure 19). The cell membrane is a complicated structure that provides a good heat isolation of the cell. Therefore, liquids inside and outside the cell freeze not simultaneously, which results in the development of high stresses. The displacement vector is described by the following equation:

\[
\int_{\Omega_{\text{ice}}} C_{ijkl}^{\text{ice}} \vec{\varepsilon}_{ij}(\vec{u}, \beta \ell)(\vec{\varphi}) + \int_{\Omega_{\text{cell}}} C_{ijkl}^{\text{cell}} \vec{\varepsilon}_{ij}(\vec{u}) \vec{\varepsilon}_{kl}(\vec{\varphi}) = 0,
\]

where \( C_{ijkl}^{\text{cell}} \) is the stiffness tensor describing averaged material of the cell. Properties of this tensor are similar to these established in [14] for a limiting tensor obtained when averaging a liquid-fiber structure under oscillation. The main feature of such a tensor is the absence of shear stresses. Two dimensional simulations of equation (35) coupled with equation (27) are shown on Figures 20 and 21. The first figure shows the temperature and phase jumps on the cell boundary. The second one represents the jump of the pressure.

Figure 22 explains the idea of balancing the pressures on the cell boundary: liquids inside and outside the cell must freeze simultaneously. This can be achieved through reducing the freezing point of the outside liquid, i.e. the relation \( T_{0}^{\text{ins}} > T_{0}^{\text{out}} \) should hold. An appropriate choice of the control parameter \( \delta T_{0} = T_{0}^{\text{ins}} - T_{0}^{\text{out}} \) provides a good result.

\section{Conclusion}

The further investigation should be aimed to the coupling of the presented techniques with conventional descriptions of cell dehydration, water nucleation, and ice crystal growth (see e.g. [15] and [16]). The aim of the control should be the minimization of the intracellular ice volume because the cell damage correlates positively with it. The modelling should account for the effect of cryoprotective agents.

\section{References}


Figure 1: Ampoule with a tissue sample and its milieu

Figure 2: Outlook of the IceCube plant (to the left) and its freezing chamber with two temperature sensors and ampoules containing tissue samples (to the right).
Figure 3: Input and output data of the IceCube: (a) prescribed cooling protocol; (b) measured chamber temperature (oscillating line tracking the cooling protocol); (c) measured temperature of the sample.

Figure 4: Idealized typical temperature response of the sample when the chamber temperature falls monotonically. Three dangerous processes are pointed out.
Figure 5: Function $T(H)$ obtained for a real sample using equation (1) and measured sample and chamber temperatures.

Figure 6: Temperature response (a) of the sample to the cooling protocol (b). The idea to suppress the effect of the latent heat and to smooth the transition to the frozen phase using a cooling impulse fails because of wrong parameters of the impulse.
Figure 7: Temperature response when applying an optimized cooling protocol: (a) real temperature response measured with the sample sensor; (b) computed temperature response; (c) optimized cooling impulse.

Figure 8: Tracking the nominal linear cooling profile: a) without control; b) with control.
Figure 9: Tracking the nominal slope: a) without control; b) with control. Vertical and horizontal axes measure the mean temperature and the time respectively.

Figure 10: Application of the feed-back control (23): a) without control; b) with control; c) control temperature protocol. Vertical and horizontal axes measure the mean temperature and the time respectively.
Figure 11: Functions defining the unfrozen water content for a porous medium.

Figure 12: Mean temperature response of a porous medium: a) chamber temperature; b) the response.

Figure 13: Time snapshot of the spatial distribution of the temperature and the unfrozen water content in a porous medium.
Figure 14: Application of optimization: a) chamber temperature with a control impulse; b) mean temperature response.

Figure 15: Time snapshot of the spatial distribution of the controlled temperature (left) and the unfrozen water content (right).
Figure 16: a) porous medium filled with a liquid; b) pressure in the pore.

Figure 17: Scaled structural cell $Y$.

Figure 18: Time snapshot of stresses in a porous medium under freezing: a) the pressure; b) the shear stress.
Figure 19: Pore with a cell inside (at the upper left), finite element model (at the upper right), structure of the cell membrane (below).

Figure 20: Snapshot of the temperature (left) and the unfrozen water content (right). Presence of jumps of them on the cell boundary under cooling with a relative slow rate comparable with that on Figure 7.
Figure 21: Pressure exerted on the cell boundary

Figure 22: Balancing the pressures on the cell boundary through the reduction of the freezing point of the outside liquid. Time snapshot of the maximal jump of the pressure on the cell boundary (right) and the unfrozen liquid content (left).