Optimization of Cooling Protocols in Cryopreservation of Living Tissues

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Cross-link:

Dental Cell Biology

Cryopreservation is a necessary part of many medical procedures such as organ and tissue transplants, conservation of reproductive and stem cells. caesar uses a Freezer IceCube~15M (SY-LAB Geräte GmbH, Austria) which can perform controlled freezing of tissue samples (see Fig. 1). The main part of the plant is a freezing chamber containing a cooling system based on nitrogen gas, a rack for placing ampoules with samples, and two temperature sensors that measure the temperature in the chamber and in the sample, respectively. The plant is supplied with a computer that allows the user to input a cooling profile either manually or as a file prepared in off-line regime. The computer controls the cooling system such that the chamber temperature tracks the cooling profile.

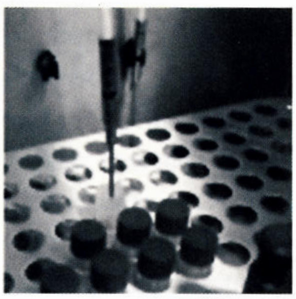

Figure 1: The outlook of the plant (left) and the freezing chamber with two temperature sensors (right).

Experiments with the IceCube show irregular behavior of the temperature near the freezing point because of the latent heat and crystallization (see Fig. 2).

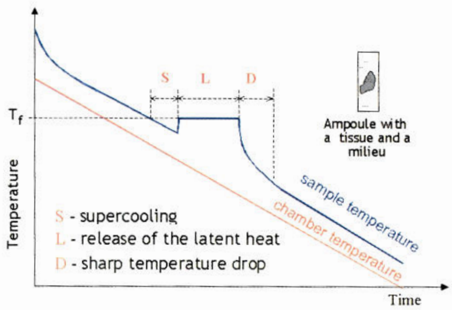

Figure 2: Typical temperature response of the sample to a simple profile of the temperature in the freezing chamber. Dangerous effects are pointed out.

The supercooling (S) and the consequent release of the latent heat (L) lead to a dendritic growth of ice crystals in the tissue. Additionally, the release of the latent heat is followed by a sharp temperature drop (D), which can cause damage to cells. Thus, a natural idea is to lead away the latent heat as rapidly as possible.

Keywords: cryopreservation, supercooling, latent heat, optimal cooling protocols
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Heuristic experimental attempts to compensate the latent heat using a sharp cooling impulse (Fig. 3a) require too many test-runs to find the right shape and intensity of the impulse, so that this approach is not promising. To apply optimization techniques, a mathematical model based on averaged values of thermodynamical parameters was developed. The main element of the model is a function T(H) that describes the dependence of the averaged temperature T of the sample on the averaged enthalpy density H. This sample-specific function is being identified from a single experimental test-run. Application of optimal control theory to the model yields optimal cooling profiles. The experimental validation shows the consistence of the model proposed and the efficiency of the optimization (Fig. 3b). The corresponding software is expected to be installed into the IceCube control system. This will be done in cooperation with the SY-LAB Geräte GmbH company.

Cooperation:

SY-LAB Geräte GmbH, Vienna, Austria

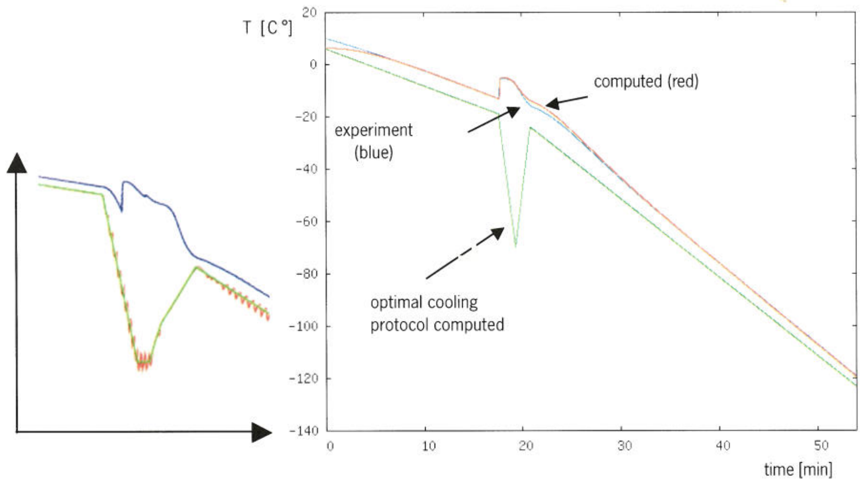

Figure 3: a) Heuristic attempt to compensate the release of the latent heat using a cooling impulse. The attempt failed because of wrong shape and intensity of the impulse.
b) Simulation results obtained by the application of optimal control theory allied to a thermodynamical model of the cooling process. The experimental test-run with the optimal cooling protocol shows the consistence of the model and a good quality of the optimization.

Additionally to the control of cooling conditions in the freezing chamber, an improvement of ampoules containing tissue samples (see Fig. 1) was proposed. The idea is to create a sharp spatial temperature gradient near to the ampoule bottom to regularize the formation of the freezing front. Certain results of the theory of phase change problems indicate the reasonability of such an idea. Simulations supporting the design of modified ampoules and estimating temperature conditions inside such an ampoule during cooling were performed. The investigation was implemented as a patent submitted to the German patent office (see reference below).

Reference: M. Thie, O. Degistirici, B. Faßbender, J. Siemonsmeier, N. Botkin. Zahn-Einfrier-Behälter. Patent submitted to Deutsches Patent- und Markenamt 07.08.2005.