Maximum storage temperatures

Permanent cooling below the threshold of recrystallization

White Paper

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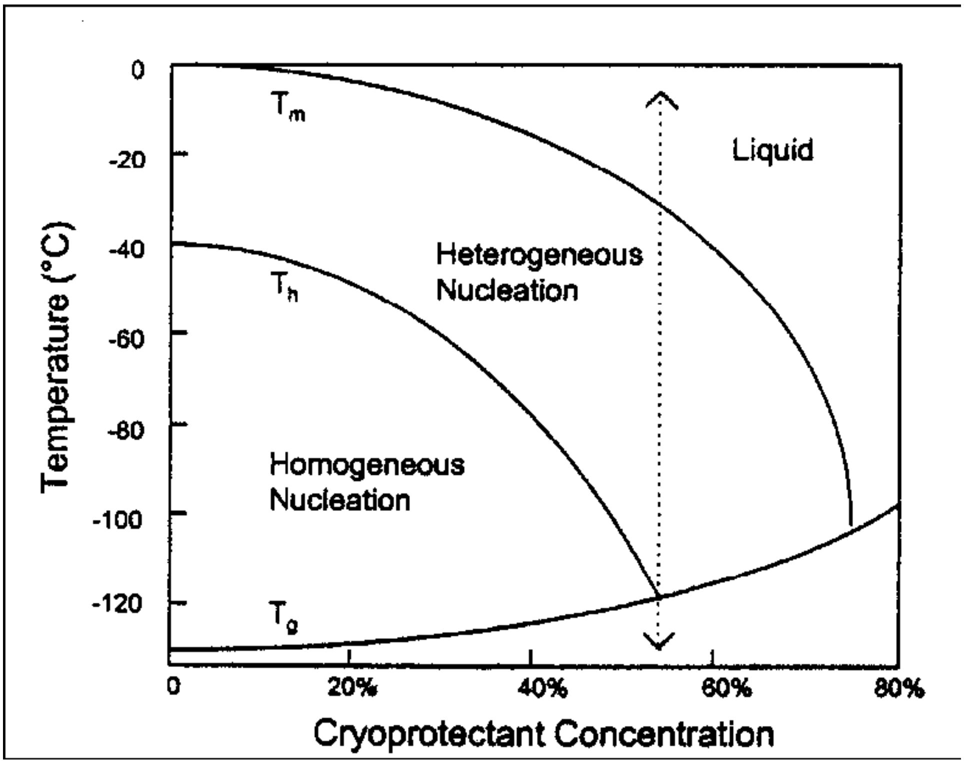
Content

1. Background

The method of cryo-preservation is used for the long-term storage of biological sample material and for the possibility to maintain its viability over decades. Therefore, specific cryoprotectants in the gas phase of liquid nitrogen are used to freeze and store the samples in.
According to the SOPs of hospitals/customers, and to the flow charts created by the Paul-Ehrlich Institute (PEI), a maximum storage temperature of ≤ -140°C is defined.
The Swiss Guidelines for cord blood banks (2) even require a storage temperature of ≤ -150°C. Askion GmbH has made it its business to develop a system that keeps the required temperatures and, as a consequence, contributes significantly to the safety of the biological samples.

2. Biological fundamentals

When freezing biological sample material, the following processes take place in the cell: At a temperature of 10°C to 0°C, only a reduced cellular metabolism is maintained and the signal transfer ceased already. Extracellular ice formation and dehydration of the cell occur at a temperature between –2°C and –15°C. At –15°C to –25°C, intracellular ice formation and the segregation of the cytoplasm take place. The migratory growth of ice crystals starts at –25°C to –130°C. Finally from < –130°C on, the cell exists as a solid body without considerable changes over a long period (9).
In order to keep living cells stable over several decades, a temperature below -130°C is mandatory. Many cells stored at higher temperatures are no longer stable possibly due to unfrozen parts of solution (4). They will die as fast as several percent per hour up to several percent per year depending on the temperature, the nature and type of the cell, and the composition of the medium they were frozen in (5). Below -130°C there are no longer any unbound water molecules. The only existing physical states of water at these temperatures are crystalline or glassy. At both states, the viscosity is so high (>10^{13}poise) that the diffusion over few geological periods is negligible (6,7).
Above -130°C, there are still measurable recrystallization processes.
In the phase diagram of water by Wowk et al. (8) it is very nicely shown at what temperature the state of aggregation of water will change depending on the concentration of the freezing media. Note that all crystallization processes are completed at -130°C, regardless of the content of cryoprotectants.



Wowk et al. 2000

3. Reasons for the temperature requirements – current state of the art

The Paul-Ehrlich Institute obtains the requirements for the storage temperature from the European Pharmacopoeia, which demands the storage at ≤ -140°C. In case the samples are stored at other temperatures, the functionality and the duration of the storage are to validate (1).

In principle, storage in the gas phase of liquid nitrogen (according to PEI) is to be guaranteed as well as temperatures of -140 to -150°C in the storage tanks should be maintained. The Swiss Guidelines for cord blood banks specify a storage temperature of -150°C that must not be exceeded. The reason given is that, at -135°C, the sample comes too close to the glass transition (2). The risk of the ambient temperature of the samples exceeds these -130°C during the storage, especially in the event of further storage and retrieval processes would be too big.

It has to be considered that for cryostorage in conventional tanks, the racks have to be lifted from deep-cold temperatures into room temperature. During the storage and retrieval process of single samples, all samples are exposed to a temperature leap of about 170K (from -150°C in the tank up to the room temperature). Thereby, heat is inserted into the system and the storage temperature increases for a short time. This temperature increase depends on the duration and frequency of the storage and retrieval processes. In particular, small sample volumes such as straws as well as such samples positioned at the edge of the rack, are strongly affected.

4. Summary

According to the temperatures specified by the Paul-Ehrlich Institute for the permanent storage of stem cell products in the gas phase of liquid nitrogen at maximum temperatures of -140°C, Askion has set itself the task not to exceed the glass transition temperature of water during the storage and retrieval processes. Conventional systems have to compensate a temperature difference of about 170K for the period of the storage and retrieval processes because the storage racks have to be brought to room temperature for a short time. Askion, on the other hand, has developed a system in which the racks are lifted into a working space at temperatures of max. -100°C. Therefore in the storage tank, the samples themselves no longer get to temperatures above the glass transition.

5. Sources

[1] European Pharmacopoeia 5.6 01/2007 Human haematopoietic stem cells

[2] Cord Blood Accreditation Manual
International Standards for Cord Blood Collection, Banking and Release for Administration Accreditation Manual

[3] PEI
Fließschema der Herstellung von Stammzellzubereitungen aus Nabelschnurblut
110207-Fließschema-NSBSC.doc
(flow sheet of the production of stem cells out of cord blood)

Fließschema der Herstellung von Stammzellzubereitungen aus peripherem Blut
110207-Fließschema-PBSC-autolog+allogen.doc
(flow sheet of the production of stem cells out of peripheral blood)

Fließschema der Herstellung von Stammzellzubereitungen aus Knochenmark
110207-Fließschema-KMSC.doc
(flow sheet of the production of stem cells out of bone marrow)

[4] Mazur 1970, Cryobiology: the freezing of biological systems, Science

[5] Mazur 1984, Freezing of living cells: mechanisms and implications

[6] Mazur 1985, Basic concepts in freezing cells; aus Deep Freezing of Boar Semen, Uppsala, Sweden, Proceedings First International Conference on Deep Freezing of Boar Seemen, 1985

[7] Özkavukcu et al. 2002, Cryopreservation: basic knowledge and biophysical effects

[8] Wowk et al. 2000, Vitrification Enhancement by Synthetic Ice Blocking Agents, Cryobiology 40, 228-236.

[9] IBMT Kryobankbroschüre, Februar 2005