Physical events during cryopreservation: consequences on cells' post-thaw performance and on cryobiological protocols optimisation

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Abstract

For decades, scientists have looked for the causes of cell cryoinjury so as to identify the optimum conditions for freezing and storing frozen cells. Cell dehydration and intracellular ice formation have been argued as the main mechanisms of cell damage. Maintaining the cells in a vitreous matrix below the glass transition temperature of the surrounding medium is known as a key condition for cell preservation. However, it is only in 2013 that the measurement of the intracellular glass transition of different cell types has been made possible. Moreover, the relevance of intracellular and extracellular vitrification during slow cooling of micro-organisms and mammalian cells has been the subject of recent research. The physical events taking place during freezing are reviewed here, focusing on the role of the physical state of the intracellular and extracellular environments in determining the response of cells to stresses encountered during cryopreservation. The implications on cryoprotectants selection, freezing rates, and controlled cooling endpoint to be set for cell storage are discussed.

Introduction

The widely accepted model of cells' freezing injury is the "two-factor hypothesis" (1). It has been developed with mammalian cells and exhibited in many cases good correlation with survival on thawing. Intracellular ice formation at high cooling rates and osmotic dehydration at slow cooling rates applies to eukaryotic cells, but is is only partly true for prokaryotes. At very high cooling rates, no intracellular ice is formed in bacteria, but injuries are caused by cell plasmolysis occurring during thawing (2). Characterizing the physical state of the intracellular compartment in highly dehydrated cells in which intracellular ice is absent became crucial for delivering performant controlled cooling protocols. The intracellular glass transition temperature was first detected in different unicellular organisms at high subzero temperatures (between -10°C and –26°C) (3) and vitrification was argued to provide a survival strategy. Based on this pioneering work, the recent studies here presented have focused on the characterization of the physical state of thecell

membrane, intracellular and extracellular environments and on their implications on cells' responses to cryopreservation.

Materials and Methods

The lipid phase transition temperature of cell membranes as well as the ice nucleation and ice melting temperatures were determined by Fourier Transformed InfraRed (FTIR) spectroscopy. Extracellular (Tg'e) and intracellular (Tg'i) glass transition temperatures were determined by differential canning calorimetry (DSC) on protective medium and cell pellets, respectively. The biological activity of micro-organisms and mammalian cells was quantified before and after freezing in order to relate the cell response to the freezing conditions applied and to the physical events measured.

Results

Physical events taking place during freeze-thawing bacteria and mammalian cells were characterized in the presence of reference cryoprotectants (Fig. 1). It was demonstrated that bacteria with the lowest value of intracellular Tg' survive the freezing process better than cells with a higher intracellular Tg'. Besides, cooling at a slow, controlled rate until Tg'i is reached appears critical for a successful cryopreservation of a wide variety of mammalian cells.

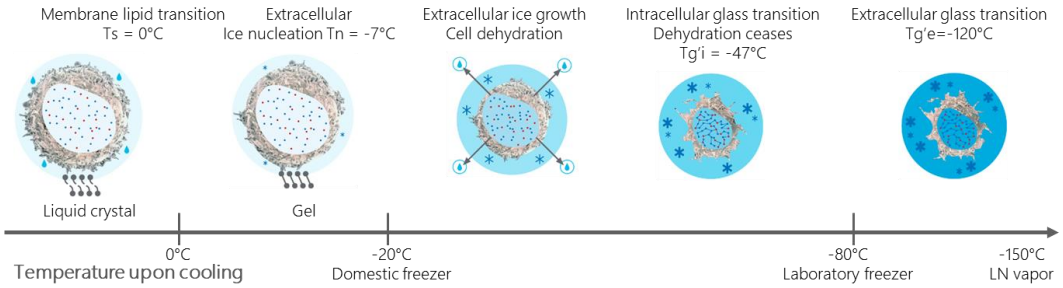

Fig. 1 *Schematic of the physical events occurring during freezing of a Jurkat cell in the presence of dimethyl sulfoxide (adapted from Meneghel et al. 2019 (4)).*

Conclusions

The vitrification of the intracellular environment has an important role in the response of cells to preservation and on the optimization of freezing protocols. However, long-term stability in the frozen state can be achieved below both the Tg'e and the Tg'i. Innovation in the field of protective molecules is required and new oligosaccharides mixtures presenting a great potential are under study (5).

References

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