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Investigation of the use of Pulse Tube in cell cryopreservation systems ☆

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ABSTRACT

In order to obtain an acceptable rate of survival of the frozen cells, they must be first cooled in programmable freezers, while controlling the cooling rate, and then stored in maintenance freezers. Different solutions are already used to preserve cells at cryogenic temperatures without liquid nitrogen, while this is not true for controlling the cooling rate.

A Pulse Tube (PT) cryorefrigeration system type, can be used in the automatic freezing if combined with a system that monitors and "drives" the temperature generated on the cold part (cold head).

To make the Pulse Tube system a suitable one for freezing processes, the cooling curve must be "corrected" in a linear one with a slope given by requested cooling rate. The temperature regulation is obtained with the use of a power dissipator based on Joule effect.

In this study, the power to be dissipated is calculated individualizing the trend of temperature on the Pulse Tube cold head and inside the cell test tubes. The control system is not based on an historical series of data so it can be used in different operative conditions. The obtained temperature curve is in good agreement with the theoretical values, with errors within those accepted by commercial systems.

The Pulse Tube cryorefrigerator may represent a valid alternative solution to programmable liquid nitrogen freezer, especially where nitrogen's supply is difficult or extremely expensive.

Introduction

The cell cryopreservation of gametes, embryos and stem cells is a solution for preserving fertility, biodiversity, and to cure diseases such as ictus, muscular dystrophy, cardiac and hepatic disease, diabetes and Parkinson's disease [4].

The techniques of cell preservation have been optimized empirically for many types of cells. In particular, the method of "slow automated freezing", which consists of a gradual cooling of the organic material at low and controlled speed, ensures high rates of cell survival after thawing.

If cells are cooled very slowly, the increasing concentration of solutes inside the cell caused by the formation of extracellular ice, brings to cellular death due to the pH changes and dehydration; on the other side, a too fast freezing leads to the formation of nuclei of crystallization both in the solution and inside the cell, resulting in lysis of the cell membrane. The unwritten rule for cryopreservation is to cool at 1 °C/min [2]: actually the addition of cryoprotectants (DMSO, glycerol, HES and other) expands the range of the optimum speed rate, as shown in Fig. 1.

After freezing at controlled rate, the cells are stored in freezers of maintenance (at temperatures ranging from −80 to −196 °C). The temperature at which the progressive freezing is stopped affects cell survival. If the freezing is stopped at too high temperature, the cells have lost only a small amount of water and they are subjected to intracellular freezing when stored in a freezer of maintenance. If the freezing is arrested at too low temperatures, the accumulated damage during the freezing process at a controlled rate opposes the positive effects of progressive dehydration. The influence of arrest of cell freezing temperature on the survival rate is shown in Fig. 2.

Usually at temperature below −60 °C, the samples can be immersed directly in liquid nitrogen or transferred to freezer of maintenance without further loss of viability [2].

Currently, programmable freezers are based on liquid nitrogen technology, but their use is denied in areas without availability of nitrogen or during long transport [1]. Since the Pulse Tube cryorefrigerator is a closed cycle machine, it constitutes a suitable substitute. The gas circulating inside the system is never in contact with the test tubes, thus reducing the radiation exposure of the cells. The reduced vibrations [3] in correspondence of the cold surface, make the Pulse Tube system interesting for applications in the field of cryopreservation.

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Nomenclature

a, b, g, h	coefficients
C	thermal mass of liquid inside the test tube
Q	heat absorbed by the Pulse Tube
t	time
Δt	temporal shift
T	temperature
W	dissipated power
μ	ideal cooling rate
χ	thermal conductivity
ξ	thermal conductivity
φ	proportionality factor

Superscript and subscripts

∞	regime phase
a	atmospheric
i	ideal inside the test tube
tt	real inside the test tube
ch	cold head

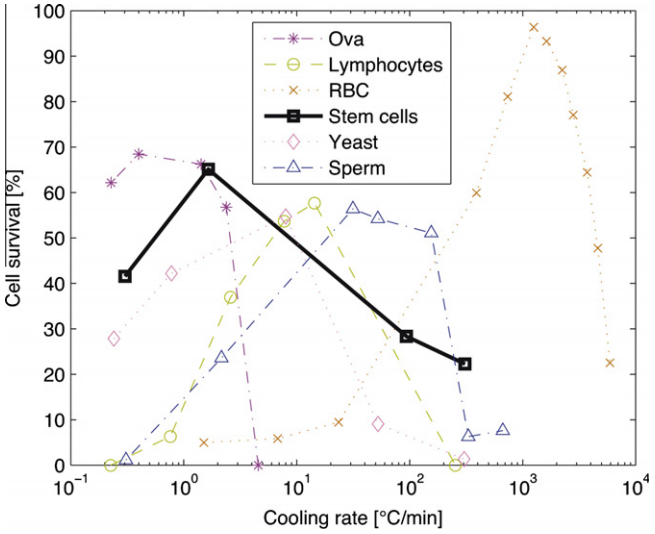

Fig. 1. Cell survival percentage after the unfreezing vs cooling rate for different cells.

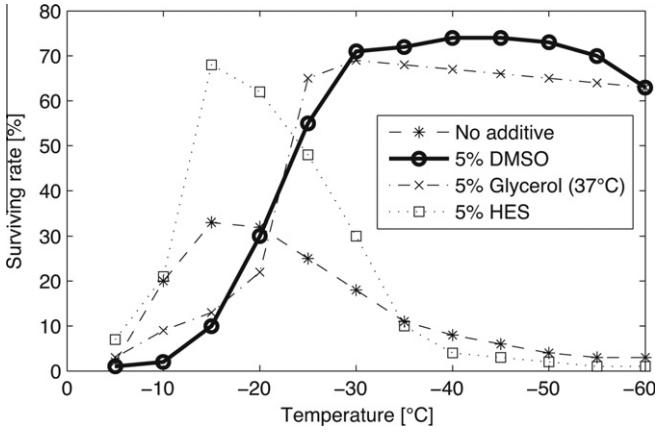

Fig. 2. Cell survival percentage after unfreezing vs freezing temperature arrest, at a constant rate for different samples with the addition of several cryoprotectants.

Materials and methods

In Fig. 3a, a scheme of the experimental apparatus is shown.

As it will be evident in the graphics below, the cooling rate of the PT, when it works without thermal loads applied, is higher than those required by cellular methods of freezing. The cooling slow-down can be achieved by providing an amount of heat, variable with the time, that will be able to raise the temperature of the PT cold head to the desired value.

In order to make the Pulse Tube suitable for the progressive freezing, a test tubes holder, with square base which can accommodate two tubes and in a central position an heat dissipator for Joule effect, has been manufactured (Fig. 3b). The dissipator can provide a power between 0 and 100 W.

The surface of the base of the test tubes box has been put in contact with the cool head of the Pulse Tube. To reduce the heat exchange with the atmosphere, the system has been insulated with glass wool and aluminum foil.

A Labview software has been developed in order to control and monitor the system. It records and displays the value of three thermocouples situated in the two test tubes and in contact with the cold surface of the PT. Moreover, the unit controls the power that must be dissipated to align the temperature of the test tubes to the theoretical values, taking into account thermal inertia due to the test tube box and the liquid itself.

The aim of this study is to investigate the possibility of "driving" the temperature curve independently of the operating conditions of the system. For this reason the optimal condition of Pulse Tube operation, which requires a thermal insulation using a cryostat and the reduction of the thermal inertia in the test tube box, has not been taken into account.

Theory

In a first analysis a negative feedback control scheme, shown in Fig. 4, may be considered, where T_i is the expected temperature according to the theory of the cell freezing and T_{tt} the actual measured temperature inside the test tube.

The dissipated heat W will be proportional to the algebraic sum $(T_i - T_{tt})$ and to the amount of heat subtracted by the Pulse Tube. This simplified model does not take into account the heat transfer for adduction in atmosphere and the thermal inertia of the materials. A more accurate model of heat transfer must be introduced. The new model bases on the following simplifying hypotheses:

- the heat exchange between test tube box and atmosphere is ignored,
- the dissipated power by Joule effect is absorbed completely by the test tube,
- the temperature inside the test tube is homogeneous,
- the heat transfer between test tube and atmosphere is for adduction.

Starting from the previous hypotheses, it is possible to introduce a new model for the heat exchange as shown in the electric circuit in Fig. 5, where W is the heat provided by the dissipator, T_a is the ambient temperature, T_{ch} the temperature of the cold head of the Pulse Tube and T_{tt} the temperature measured inside the test tube. Moreover, C indicates the heat capacity of the solution contained inside the test tube and ξ and χ the thermal conductivities.