Is rapid cooling step needed

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Freezing cells for cryopreservation

Successful cell cryopreservation requires a controlled cooling rate. Cooling too rapidly or too slowly will lead to a poorer outcome. This is true because of the physical environment cells experience during cryopreservation. As ice forms in the extracellular solution, pure water is locked away as ice. As a result, cells are suspended in an increasingly concentrated solution, which dehydrates them. Cooling too slowly makes this solution toxic; cooling too rapidly prevents cells from dehydrating sufficiently, and intracellular ice forms.

Controlled-rate freezers, such as the electric-powered VIA Freeze™ series or liquid nitrogen (LN_2)-based freezers, are often programmed for linear cooling. A subset of researchers using LN_2-based freezers prefer to interrupt the linear cooling to include a "rapid-cooling nucleation step," which they believe is needed for successful cryopreservation. This step is hypothesized to improve ice nucleation – an important event for good post-thaw outcomes. A typical cooling profile for linear cooling with a rapid-cooling nucleation step is shown in Figure 1.

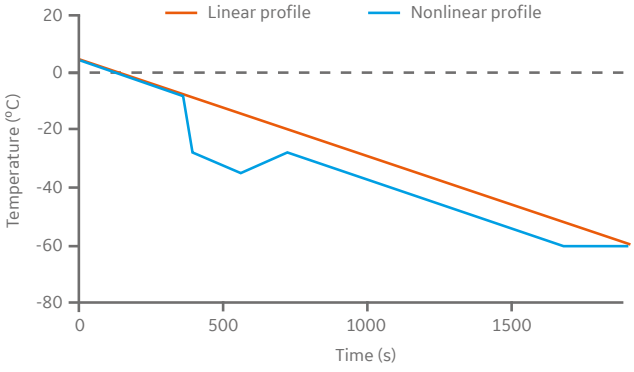

Fig 1. A linear 2°C/min cooling profile compared with a typical profile that includes a rapid-cooling nucleation step (1).

This study explores how a rapid-cooling nucleation step affects viable cell count and activity of four cell lines. Three conditions were tested: linear-rate freezing in a VIA Freeze system; linear-rate freezing in an LN_2-based freezer; and linear-rate freezing in an LN_2-based freezer, interrupted by a rapid-cooling step.

Experimental design

Cells in cryovials (1 mL fill volume) were cooled at 2°C/min from 4°C to -60°C in two controlled-rate freezers – VIA Freeze system (GE Healthcare Life Sciences) and a conventional LN_2-based freezer (Kryo 560, Planar). A third freezing method in this same LN_2-based freezer incorporated the rapid-cooling nucleation protocol from Table 1. Five cryovial samples, with 10% DMSO as cryoprotectant, were used for each condition. In all experiments, samples were plunged into LN_2 when the temperature reached -60°C. The cryovials were stored below -140°C for at least 24h, and then thawed. Thawed cells were placed into cell culture medium and allowed to grow in a standard humidified CO_2 incubator at 37°C.

Table 1 lists the corresponding cooling rate sequence that was programmed into the LN_2-based controlled-rate freezer.

Table 1. Programmed parameters for experiment that includes rapid-cooling nucleation step

Step	Cooling rate	Temperature range
1	-2°C/min	4°C to -8°C
2	-35°C/min	-8°C to -28°C
3	-2.5°C/min	-28°C to -35°C
4	+2.5°C/min	-35°C to -28°C
5	-2°C/min	-28°C to -60°C

Four cell lines were examined: Chinese hamster ovary (CHO); HepG2 (human liver); MG-63 (human bone); and Jurkat (human suspension immune) cells. Viable cell count after thawing was determined using a Cytell Cell Imaging System (GE). An alamarBlue™ bioassay was performed using a Tecan™ plate reader to evaluate metabolic function of cells after 3 h incubation. These functional tests were carried out at 24, 48, and 72 h of culture post-thaw.

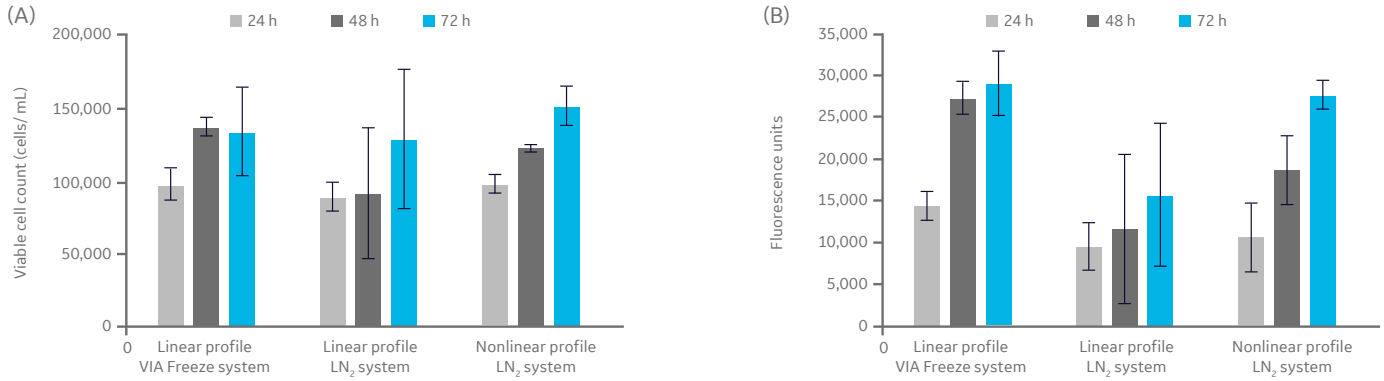

Fig 2. Post-thaw results for HepG2 cells. Cryovials were assessed 24, 48, and 72 h of culture post-thaw. (A) viable cell count, and (B) alamarBlue functionality. n = 5 ± SD.

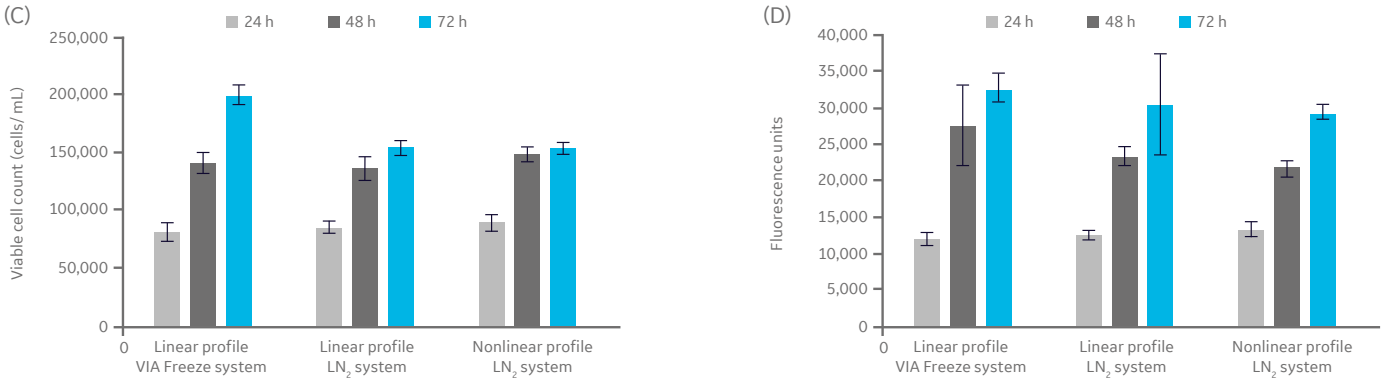

Fig 3. Post-thaw results for CHO cells. Cryovials were assessed 24, 48, and 72 h of culture post-thaw. (A) viable cell count, and (B) alamarBlue functionality. n = 5 ± SD.

Results from different freezing methods

Figure 2 shows no significant difference in post-thaw results between HepG2 cells cooled in a LN_2-based freezer with or without a rapid-cooling nucleation step included. Furthermore, there is no significant difference in results using either freezer system and a linear cooling rate. The same pattern was observed with CHO cells (Fig 3), as well as Jurkat and MG-63 cells (data not shown, to keep the article brief).

Conclusions

In this study, including a rapid-cooling nucleation step in the LN_2-based freezer did not improve post-thaw results for the four tested cell lines. No literature was found to support the hypothesis that a rapid-cooling step to induce ice nucleation is beneficial for cryopreservation.

Using VIA Freeze system for freezing cells

In addition to demonstrating that a rapid-cooling step is unnecessary, the study shows comparable results using LN_2-based and VIA Freeze systems programmed with a linear cooling rate. Although cell viability and function are expected to be similar, the VIA Freeze system offers several advantages over LN_2-based controlled-rate freezers. Liquid nitrogen is not used for cooling, which avoids the associated contamination risks and supports GMP compatibility. Furthermore, using a VIA Freeze system is expected to reduce operating costs.

Reference

1. Diener B *et al.* A method for the cryopreservation of liver parenchymal cells for studies of xenobiotics. *Cryobiology* **30**, 116–127 (1993).

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