The critical thermal range for controlled rate cooling for mammalian cell cryopreservation

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Slow, controlled cooling is essential for successful cryopreservation of somatic mammalian cells. In practice, samples are typically transferred to long-term cryogenic storage once the controlled-rate cooling process has reached an endpoint of around -80°C to -100°C. Placing samples in the liquid or vapor phase of nitrogen too early could result in rapid cooling rates, leading to intracellular ice formation and cell death. However, there is little basis in the literature for the controlled cooling endpoint to be set at -80°C or below.

The present work explored the critical temperature for the endpoint of the controlled-cooling phase in four different mammalian cell lines (Jurkat, HepG2, MG63 and CHO cells) through a combination of biological and physical measurements. Cryovials with 10^6 cells, 1ml fill and 10% DMSO as cryoprotectant were cooled at a 1°C/min in a VIA Freeze controlled-rate freezer, and samples were transferred to liquid nitrogen at different endpoint temperatures (4°C, -10°C, -25°C, -40°C, -50°C, -60°C, -80°C and -100°C). Cell viability, proliferation and functionality measurements post-thaw showed highest and comparable values for transfer temperatures of -50°C and below for the four cell lines tested. Transfers of samples performed at higher temperatures (≥-40°C) resulted in a drastic loss of viable and functional cells, while Differential Scanning Calorimetry (DSC) measurements preformed on Jurkat cells revealed an intracellular glass transition temperature (Tg_i) of -46.9 ± 1.3°C.

These results suggest that controlling cooling until Tg_i is reached is critical for a successful cryopreservation of a wide variety of mammalian systems, and no biological advantage is conferred by further cooling. Shorter controlled-rate cooling cycles could thus safely be applied, saving time and potentially allowing more cryopreservation cycles to be completed a day.

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