



Cryopreservation Hematopoietic Progenitor Cells

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Introduction

The science of cryobiology describes a precise and complex balance of numerous variables that contribute to the preservation of cell viability and function. These variables include the biophysical characteristics of cells, nature and concentration of the solutes in the suspension and the rate at which the cell suspension is cooled (1).

Biological Response to Freezing

Like most cells, Hematopoietic Progenitor Cells (HPCs) equilibrate osmotically with their environment by osmotic movements of water and solutes across their semi-permeable membrane (3). Membrane permeability is a function of the unique biological makeup of the cell and is temperature-dependent. When cooling a cell suspension below its freezing point, pure water forms ice, excluding any solutes present, and in turn, increasing the solute concentration and osmotic pressure in the unfrozen fraction of the suspension. If ice forms too rapidly or at too low a temperature, the cells may not have time to achieve osmotic equilibrium with the unfrozen fraction, leading to intracellular ice formation ("rapid cooling injury"). If cooled too slowly cells become increasingly dehydrated and shrink. This increases the intracellular solute concentration thereby depressing the temperature at which ice will form but will also result in increased toxicity or cell damage ("slow cooling or solution effects injury") (1).

Cryoprotectants protect cells from the damaging effects described above. The most common cryoprotectant for HPCs is dimethyl sulfoxide (DMSO). DMSO can permeate the cell membrane and significantly reduces the amount of intra and extracellular ice formed as cell suspensions are cooled (1). The natural cell process of osmosis and subsequent dehydration will still occur, but at much lower temperatures. This allows the desirable effect of dehydration, i.e. the absence of intracellular ice formation, while reducing the effect of toxicity since the cells are less sensitive at these lower temperatures. Another, less commonly used cryoprotectant is Hydroxyethyl Starch (HES). This high molecular weight polymer cannot permeate the cell but does contribute to the extracellular osmolality as the concentration increases. In practice, HES is most frequently used in conjunction with DMSO (19).

It appears that CD34+ cells exhibit higher tolerance to traditional freezing protocols over other blood cells (6, 9, 16), with the most primitive progenitors possessing the highest tolerance (9). CD34+ cells in bone marrow may be less sensitive than those collected by apheresis (12).

Cryopreservation Protocols

A universally-accepted procedure for cryopreservation of HPCs does not exist. Protocols are generally developed utilizing a blend of scientific literature, research and experience. Protocols can also be formulated mathematically (3,8). Regardless of the protocol employed, there are several critical process variables which need to be considered. Care must be taken when reviewing applicable literature as these variables are not always identified or may not be carefully controlled.

Cryoprotectant and Final Product Concentration

Acceptable CD34+ cell recoveries have been obtained with the concentration of DMSO in the final product of 5% (4, 5, 10, 14,) or 10% (2, 4, 5, 10,11, 15). There is no advantage to increasing the DMSO concentration to above 10% (13).

Addition of Cryoprotectant

Exposure of cells to cryoprotectant, especially at room temperature, should be minimized to reduce toxicity (13) however, allowing time for DMSO permeation into the cells prior to cooling is recommended (21). For example, a two step addition of DMSO at 2 °C with two 10 minute incubations (13) or simply 5 minutes incubation at ambient temperature (18).

Product Cell Concentration

Limits for both red blood cells and nucleated cells are important considerations to minimize lysis by-products (20) which may affect the viability of CD34+ cells (15) and pose risks for the recipient of the product.

Product Volume, Surface Area and Container

Product volume, surface area and container material all affect thermal conductivity and therefore the rate of cooling of the product (19).

Cooling Chamber Temperature

The temperature of the chamber in which the cells are cooled also effects the cooling rate. There may be no significant difference between the use of uncontrolled and controlled rate freezers as far as positive outcome measures are concerned (11), however, a valid advantage to controlled rate freezing is that it provides consistency plus documentation so that cooling rates can be reviewed and verified acceptable for each product processed (19).

Cooling Rate

Optimal cooling rates have been described at 1 (9,13), 2.5 (13) and 3 °C/minute (2). Poor results were reported at both 0.1 and 10 °C/minute at any DMSO concentration (13). One study found that cryoinjury was diminished below -30 °C with the majority of injury occurring between -10 °C and -20 °C (9), suggesting that this is a parameter worth defining. Some protocols (“non-linear”) employ different cooling rates at different stages throughout the process with good results (15). Steps to control when ice formation occurs and to remove the heat produced when ice crystals form (latent heat of fusion) are also utilized (20).

Storage of Frozen HPC

As with cryopreservation protocols, there is no universally-accepted standard with regards to storage temperature and time. Stability is likely best achieved by storing at temperatures below the glass transition temperature of the cryoprotectant solution used (19). For DMSO this would be below – 123 °C (22). Furthermore, a state of “suspended animation” can be achieved below – 150 °C since few biologically-significant reactions occur below this temperature (21). Storage in liquid nitrogen (-196 °C) has been associated with good outcomes for umbilical cord blood after 15 years (10). Storage at -80 °C should be limited to a shorter duration of weeks or months (22). Ultra cold storage temperatures can be achieved using either mechanical or liquid nitrogen freezers. Backup systems are required in case of equipment failure. A recently reported experience suggests that the sole use of a liquid nitrogen reservoir connected to a mechanical freezer may not be sufficient for back up, so alternate storage equipment of sufficient size and temperature is indicated.

Quality Management

Commonly-accepted standards require processes for controlling and monitoring the manufacturing of cell therapy products to ensure they meet predetermined specifications (23). Standards such as these must be the foundation of Processing Laboratory operations. In Canada certain activities are regulated. These regulations are available on the Health Canada website at www.hc-sc.gc.ca. A few examples of quality process and monitoring are described below.

Small aliquots obtained after the final processing steps then frozen and stored in parallel with the product provide a representative (pilot) sample that can be used to confirm cell recovery, viability and/or functionality. It should be noted that some of the common test methods used to assess product viability and recovery post thaw may not detect early apoptotic cells nor fully assess cell functionality (6, 7, 9, 17).

Patient outcomes (time to engraftment, adverse reactions) can be monitored so that significant trends or deviations can be investigated with regards to product quality.

Cryopreservation protocols must be validated to confirm consistent retention of clinically-significant potency in the product. Subsequent revisions to protocols should be carefully reviewed and monitored as they may require further validation.

Storage devices must be monitored to ensure deviations from defined storage temperatures can be identified. Back up storage space of adequate size and temperature should be readily available.

Summary

- Development of an optimum HPC Cryopreservation procedure requires careful consideration of several variables.
- HPC Cryopreservation procedures require validation.
- Revisions to cryopreservation procedures should be carefully reviewed to determine if validation is required.
- Long term storage of HPC is best at ≤ -150 °C.
- All processing laboratories should utilize quality standards and practice the recommended processes.
- Certain activities are regulated in Canada.

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