Off the shelf cellular therapeutics: Factors to consider during cryopreservation and storage of human cells for clinical use

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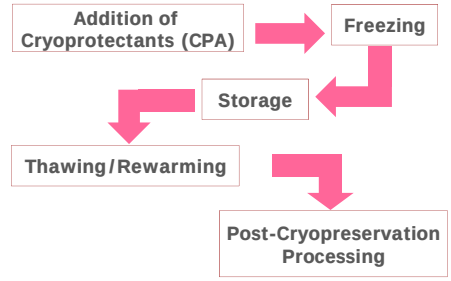
Overview :

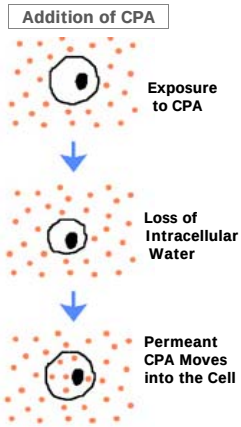
The field of cellular therapeutics has immense potential, affording an exciting array of applications in unmet medical needs. At the forefront is an emphasis on getting these therapies from bench to bedside without compromising efficacy. For a successful cellular therapy program, it is essential to extend the shelf-life of these therapies beyond shipping "fresh" at ambient or chilled temperatures for "just in time" infusion. Cryopreservation is an attractive option because of major advantages such as storing and retaining patient samples in case of a relapse, banking large quantities of allogeneic cells and retaining testing samples for leukocyte antigen typing and matching. However, cryopreservation is only useful if cells can be reanimated to physiological life with negligible loss of viability and functionality. Also critical is the logistics of storing, processing and transporting cells in clinically appropriate packaging systems and storage devices. Rationalized approaches to develop commercial-scale stem cell therapies require an efficient cryopreservation system that provides the ability to inventory standardized products for later on-demand distribution and use, as well as a method that is scientifically sound and optimized for the cell of interest. While many commercial cell therapy establishments do employ good manufacturing methods, scientific optimization of cell specific cryopreservation methods can be overlooked.

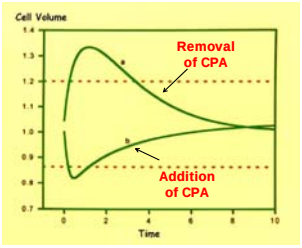
Cryopreservation Process



Damage Mechanism	Description and factors to consider
Osmotic injury or toxic injury	Injury due to the addition and removal of cryoprotective agents. Cell-specific characteristics such as biophysical parameters (size, shape, membrane permeability to water and cryoprotectants, osmotically inactive water, osmotic and volumetric tolerance limits) should be considered
Cold-shock injury	Injury due to an abrupt change in temperature. Cooling rate should be considered, with very slow cooling rates applied to cold shock–sensitive cells.
Chilling injury	Injury due to prolonged exposure to cold (but above cryogenic) temperatures. Absolute exposure time is the most critical factor to consider. If cells appear to be chilling sensitive but are tolerant of a cryoprotectant such as DMSO at warmer temperatures, strategies can be employed to perform cryoprotectant additions at or near room temperature and reduce the amount of time "chilled."
Cooling Injury	Injury associated with extracellular and intracellular ice formation. Factors to consider can be cell type–specific and include cooling rate, ice nucleation regimen, supercooling, end temperature before transfer to storage, cellular dehydration, intracellular ice formation and hypertonic solute toxicity.
Storage Injury	Injury due to unwanted thermal fluctuations (transient warming events), cosmic rays and free radical formation. Factors to consider include the glass transition temperature of the cryoprotectant and careful maintenance of the storage temperature at all times. Properly cryopreserved and stored cells are viable indefinitely. Although practically challenging, if at all possible a sample should never be removed from cryostorage until it is to be used; otherwise temperature of the sample should be monitored throughout any temporary removal (such as removing a rack of vials or frame of bags). Additional considerations should include the use of closed system containers for storage (in vapor or liquid).
Thawing/warming injury	Injury associated with warming sample from LN2 storage temperature to above phase change temperature. Potential recrystallization during warming should be considered. If slow cooling is used, a wide range of warming rates are likely acceptable; however, faster warming generally may result in less intracellular recrystallization.
Post-Cryopreservation Processing	Upon thaw, cells are in a potentially compromised state. Care must be given to appropriately prepare them for use. If a permeable cryoprotectant is used, knowledge of cell-specific osmotic characteristics is important. Cells swell and may lyse upon removal of permeable cryoprotectants and may not survive one-step dilution. If cells are administered directly from thaw without dilution or a washing step, this is effectively a one-step dilution and may result in significant cell loss *in vivo*.

OSMOTIC SHOCK INJURY: Addition /Removal of CPA



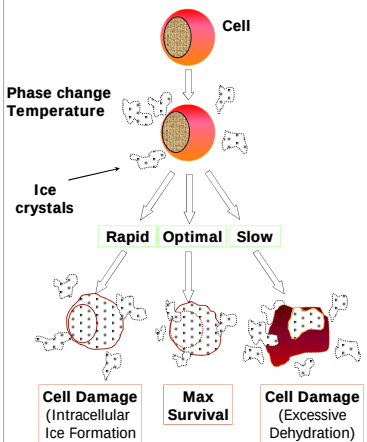


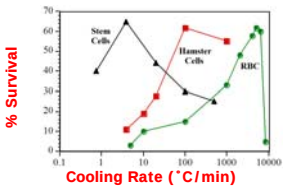
- **Minimize "Osmotic Injury"**
  - Step-wise addition and removal of CPA
- **Choice of CPA - Cell Specific**
- **Commonly used - DMSO**

THAWING /WARMING INJURY

- **Major Player: Recrystallization Injury:** This phenomenon occurs when innocuous extra- or intracellular ice formed during freezing melts and coalesces into larger, more damaging crystals during a temperature excursion or suboptimal warming procedures (typically slow warming).
- **Rapid Thawing is Optimal**

COOLING INJURY

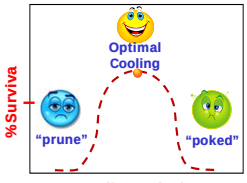




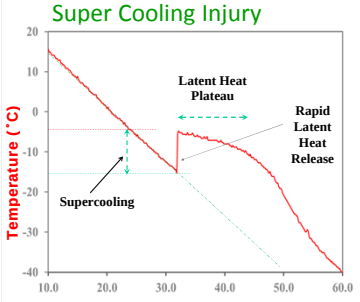
Peak survival of cells over varying cooling rates

Two Damage Mechanisms:

- "Solute Effects" Injury
  Becoming a "prune" by extended exposure to highly concentrated salt solutions – Referred as
- "Intracellular Ice Formation" Injury
  Ice crystals within the cells tend to "poke" and "rupture" them



Super Cooling Injury



This schematic indicates how sample supercooling can be observed on a time temperature profile (solid line). The equilibrium melting point of the sample is shown as a red dashed line. When the supercooled solution nucleates, the release of latent heat of crystallization will result in an increase in sample temperature toward the equilibrium melting.

Reference: Woods et al. Cytotherapy, Volume 18, Issue 6, Pages 697–711, 2016