METHODS FOR IMPROVING CRYOPRESERVATION YIELD

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Introduction

With the rapid emergence of cell therapy and tissue engineering, the successful application of cryopreservation for cell banking and long term storage is becoming increasingly critical. To a company trying to establish a novel cell therapy product or the general researcher, the cryopreservation aspect of their potentially invaluable product is often times considered an afterthought with little consideration for the steps and components. As such, typical cryopreservation protocols have been primarily developed through imitation of others and hardly any optimization with the assumption that - what you put in is essentially what you get back. Unfortunately, conventional cryopreservation protocols often result in significant cell loss (> 50% in many cases). This loss is not often appreciated, nor is the associated loss in quality of the cell product. A loss of this scale translates to loss in product value and in the case of a cell therapy product, an impending uncertainty in therapeutic dose.

While investigations into all of the aspects of cryopreservation would be technically demanding, time-consuming, and impractical for each specific cell type, there are several basic steps regarding the cryopreservation protocol that can aid in achieving more optimal post cryopreservation yield:

- Choice of cryopreservation vehicle solution
- Concentration of DMSO
- Rate of CPA (cryoprotective agent) addition
- Sample holding time prior to freezing
- Thawing temperature/rate
- Viability assessment methods (timing / assays)

In this study, various aspects of the standard cryopreservation protocol were investigated to determine potential points where optimization can be realized. The goal of this study was to determine which steps of the cryopreservation process could aid users in developing a better protocol for achieving improved yield. Unfortunately, traditional preservation methods are not effective for all biologics and sub-optimal post-preservation yield can significantly impact the efficacy of a potential cell therapy product. For optimal development and commercialization of cellular therapies, effective biopreservation strategies are required.

Methods

Cell Cultures
Normal Human Dermal Fibroblasts (NHDF) (Lonza; Walkersville, MD), Chinese Hamster Ovary (CHO) (ATCC; Manassas, VA), and Mouse Dendritic Cells (mDC) (Stanford University) were grown and sub-cultured using standard applied technique.

Cryopreservation
Standard Cryopreservation Protocol:
To investigate the potential effects of the various cryopreservation steps, standard cryopreservation testing was performed. Briefly, cells were resuspended in 0.5ml of either cell culture media (10% serum) or CryoStor™ (BioLife Solution, Bothell, WA) with 0, 2, 5, or 10% DMSO and placed into 1.2ml cryovials. Cryopreservation studies were performed using a Nalgene Mr. Frosty. Samples were first stored at 2-8°C for 10 min, transferred to -80°C with an ice nucleation step after 20 minutes, storage at -80°C for 3 hours, and then transferred to LN_2 for 24 hours. Samples were thawed in a 37°C water bath, immediately resuspended in culture media (1:10 dilution) and plated.

Thawing Temperature/Rate:
Following the standard cryopreservation process, samples were thawed in a waterbath set to 10°C, 37°C, or 50°C. Warmer temperatures are consistent with faster rates.

Viability Assessment
Performance was tested using alamarBlue® (metabolic activity indicator; AbD Serotec) for relative cell viability. Cell cultures exposed to cryopreservation were assessed for relative cell viability 1 day post-preservation/thaw. Cell viability was determined by comparing relative fluorescence units of samples to that of non-preserved control (37°C) cultures.

Results – Vehicle Solution

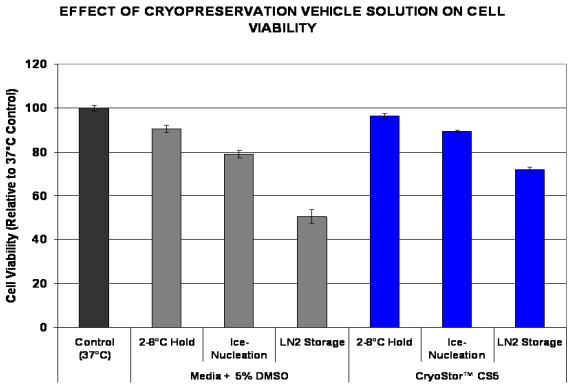

Figure 1: Viability of NHDF cells following exposure to cell culture media + 5% DMSO or CryoStor + 5% DMSO (CS5). Solution efficacy was investigated following a 10 min hold at 2-8°C, following ice-nucleation, and after LN_2 Storage. For each of the test conditions, samples were removed, thawed, diluted with media and plated. Relative cell viability was determined 1 day post-thaw and overall viability was compared to 37°C non-cryopreserved control cultures.

Results – DMSO Concentration

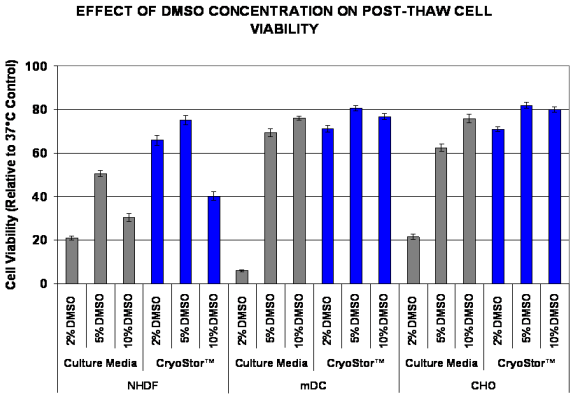

Figure 2: Post-thaw viability of cells following cryopreservation with varying concentrations of DMSO in either cell culture media or CryoStor. Relative cell viability was determined 1 day post-thaw as described in the methods and overall viability was compared to 37°C non-cryopreserved control cultures. DMSO concentration and vehicle solution impact cell viability post-thaw.

Results – Thawing Temperature

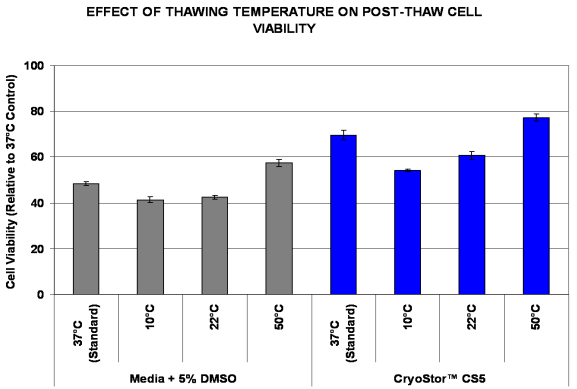

Figure 3: Effect of thawing temperature/rate on Post-thaw viability of NHDF cells following cryopreservation in either cell culture media with 5% DMSO or CryoStor CS5. Relative cell viability was determined 1 day post-thaw as described in the methods and overall viability was compared to 37°C non-cryopreserved control cultures. Increasing thawing rates may improve cell viability post-thaw.

Vehicle Solution (No DMSO)

Table 1: Post-thaw recovery of cells following cryopreservation in either cell culture media or CryoStor without the presence of DMSO.

	NHDF	mDC	CHO
Cell Culture Media (No DMSO)	1% (± 1)	2% (± 1)	3.5% (± 1)
CryoStor™ (No DMSO)	19% (± 3)	22% (± 2)	21% (± 2)

Summary of Results

- ❑ Enhancing the cells environment prior to cryopreservation through vehicle solution optimization can improve post-thaw viability and yield
- ❑ Use of the optimized CryoStor vehicle solution significantly improves recovery of cell viability post-thaw when compared with standard cell culture media
- ❑ DMSO concentration may impact post-thaw cell recovery/yield
- ❑ Use of an optimized vehicle solution can permit use of lower DMSO concentrations
- ❑ Thawing temperature/rate can impact post-thaw recovery
  - Cooler temperatures (slower rates) may reduce cell viability and recovery
  - Warmer temperatures (faster rates) may improve cell viability and recovery
- ❑ The combination of an optimized vehicle solution (CryoStor), DMSO concentration, and thawing temp may result in significant improvements to overall cryopreservation yield