Evaluation of Novel Cryopreservation Media and Methods for Cord Blood Stem Cell Banking

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Introduction

Cryopreservation protocols have remained relatively unchanged since the first cord blood banking program commenced in 1992. In general, after a cord blood unit (CBU) is red cell depleted, a concentrated leukocyte fraction is cryopreserved by gradual addition of a concentrated DMSO solution to obtain a final DMSO concentration of 10% by volume. The DMSO solutions often contain an osmotic stabilizer and serum such as dextran and human serum albumin. Current CBU cryopreservation practices typically include preparation of a concentrated cryopreservation solution (2X or 5X) followed by a slow (perhaps up to 15 minutes for each unit) gradual addition of the concentrated solution to the final CBU. A slow, gradual addition is used to protect the cells from damaging osmotic stress due to high DMSO concentrations and the high exothermic reaction which occurs as the concentrated DMSO solution mixes with the cell solution.

While traditional methods provide an "acceptable" result, new approaches and methods for cryopreservation of CBU's should be considered which could result in:

- *Decreased preparation and processing time*
- *Improved post-thaw recovery and viability*
- *Improved consistency*
- *Reduced toxic DMSO concentrations*
- *Elimination of serum and protein*

The goal of this study was to evaluate the effects of cryopreservation solution rate addition on CBU recovery and compare the results to a pre-formulated, fully defined, serum- free and protein- free, osmotically balanced biopreservation solution, CryoStor, for freezing CBUs. Results demonstrate that rapid addition of a concentrated standard cryopreservation solution reduces overall post-thaw recovery. Interestingly, post-thaw recoveries with a rapid addition of CryoStor are equivalent or better than the slow addition of a previously defined standard cryopreservation solution. Furthermore, the CryoStor provides several distinct advantages over a manually prepared standard concentrated cryopreservation solution:

Methods

Umbilical Cord Blood Collection and Processing
CBU's designated for research use only were obtained following patient consent following Puget Sound Blood Center (PSBC) standard procedure. A 0.5-mL sample was removed to perform a manual TNC count and cell viability assessment. Once processed, the leuko-enriched supernatant was then expressed into a 600-mL transfer pouch (Baxter). After centrifugation, the resulting leuko-poor supernatant was removed, and the total volume of the leuko-enriched pellet was determined. Leuko-poor supernatant was added back to the pellet to give a final volume of 25.5 mL (final product). A 0.5 mL aliquot was removed for pre-freeze analysis. Half of the remaining final product (12.5 mL) was transferred to another transfer pouch and placed on ice.

Cryopreservation
12.5 mL of the PSBC standard cryopreservation solution (20% Dextran, 20% DMSO, 48% Hetastarch, 12% ACD-A), prepared on the day of use) was added to one of the final product bags, and 12.5 mL of the CryoStor CS10 (CS10; BioLife Solutions) was added to the other final product bag. The CS10 contains 10% DMSO and having a final 5% DMSO conc. Both solutions were stored and used at 2-8°C. To evaluate the effects of rate addition of the cryopreservation solution, 10 split-samples were evaluated using a 1 mL per minute manual addition and an additional 6 split-samples were evaluated using a bolus infusion of cryopreservation solution. Immediately after addition of the cryopreservation solutions, contents were transferred to 25 ml freezing bags (Baxter), followed by standard controlled rate freezing.

Testing
TNC, viability, CD34 and CFU assays were measured post-thaw and compared to pre-freeze data. For viability, aliquots were diluted 1:10 with an acridine orange/PI solution and cells were counted microscopically. For CFU, assays were set up in duplicate by plating TNCs at a concentration of 30 x 10^3 per 1 mL of Methocult (Stem Cell Technologies) in 35-mm culture dishes and colonies were counted after 10-14 days of culture. CD34+ cell analysis was performed by flow cytometry (FACSCalibur, Becton Dickinson) using a cell enumeration kit (ProCount, Becton Dickinson) according to the manufacturer's instructions.

PSBC Solution Slow vs. Rapid

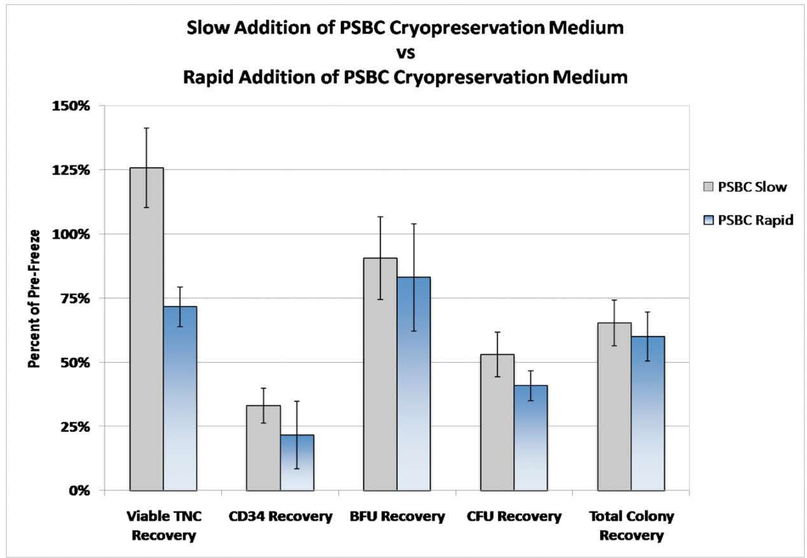

Figure 1: Cord blood cryopreservation comparing the PSBC standard slow CPA addition to a rapid addition. Post-thaw analysis of viable TNC, viable CD34+, BFU, CFU, and total colony recoveries were assessed for each of the conditions. Rapid addition of the PSBC cryopreservation solution results in a noticeable decrease in each of the parameters tested compared to the standard applied slow addition. These results support historical observations.

Slow (PSBC) vs. Rapid (CryoStor)

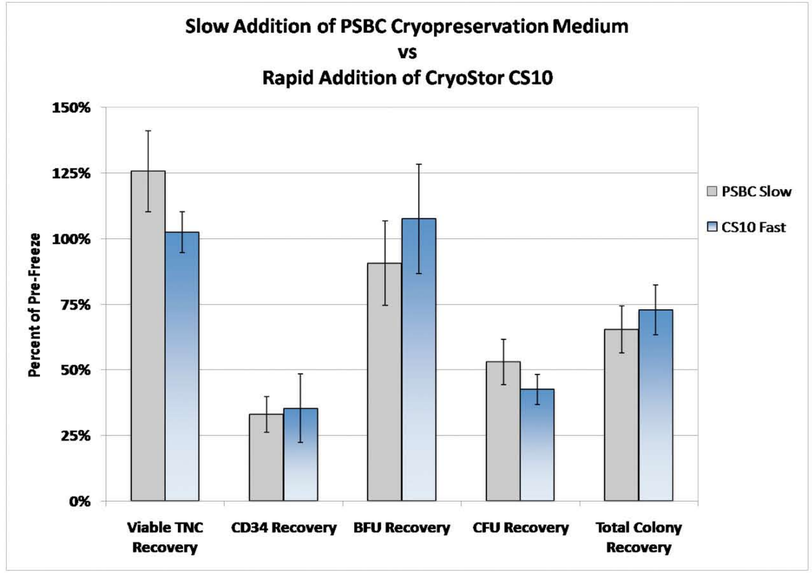

Figure 2: Cord blood cryopreservation comparing the standard PSBC slow CPA addition to that of the CS10 rapid addition. Post-thaw analysis of viable TNC, viable CD34+, BFU, CFU, and total colony recoveries were assessed for each of the conditions. Rapid addition of CS10 is comparable to slow addition of the PSBC cryopreservation cocktail in each of the parameters tested while also having half the final DMSO concentration (5%v/v).

Slow (PSBC) vs. Slow (CryoStor)

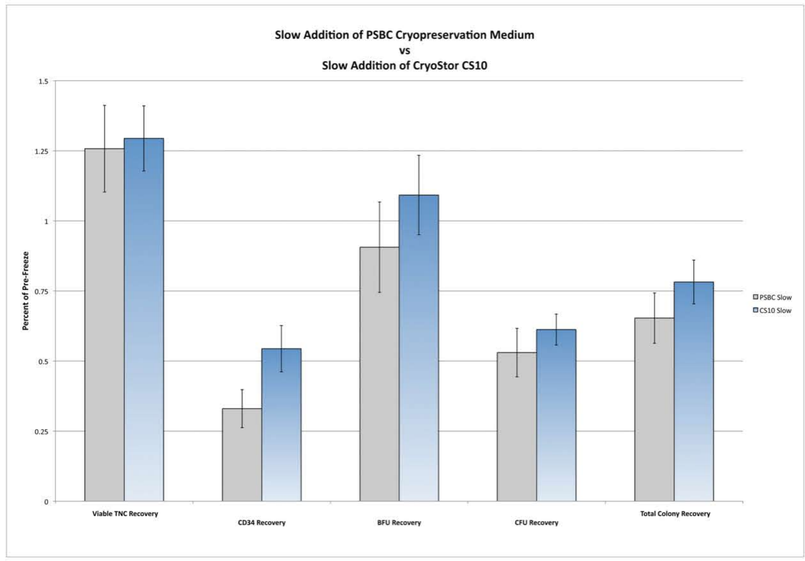

Figure 3: Cord blood cryopreservation comparing the slow addition of both the PSBC cryopreservation cocktail solution and the CS10. Post-thaw analysis of viable TNC, viable CD34+, BFU, CFU, and total colony recoveries were assessed for each of the conditions. Improved recoveries are observed for each post-thaw parameter evaluated following the slow addition of the CS10 compared to those associated with the standard slow addition of the PSBC cryopreservation cocktail.

Summary of Results

- ❑ Rapid addition of traditional cryopreservation solutions (such as PSBC) decreases overall post-thaw recovery of CBU's compared with slower rates
- ❑ Compared to slow addition, rapid addition of PSBC solution resulted in:
  - 25% loss of viable TNC
  - reductions in both $CD34^+$ cells and CFU
- ❑ Rapid addition of CryoStor CS10 resulted in similar viable TNC, $CD34^+$ cell content, and colony counts compared to slow addition of PSBC solution with half the final DMSO concentration
- ❑ Compared to either rate of PSBC addition, slow addition of CS10 resulted in:
  - 25% increase in the recovery of $CD34^+$ cells
  - Improved BFU, CFU, and total colony counts
- ❑ CryoStor CS10 offers the possibility of rapid addition to cord blood stem cell products without compromising current standards for viability and recovery
- ❑ CryoStor
  - Is a pre-formulated serum- free and protein- free cryopreservation solution and formulated for improved osmotic protection of cells during cryopreservation
  - Reduces media formulation time and improves product consistency
  - Improves post-thaw recovery
  - Can yield improved recovery with reduced toxic DMSO concentrations
  - Has a strong regulatory standing for a biopreservation media
  - Is supported by an FDA Master File