Quick and Easy Method for Removal of DMSO from Thawed Cell Products
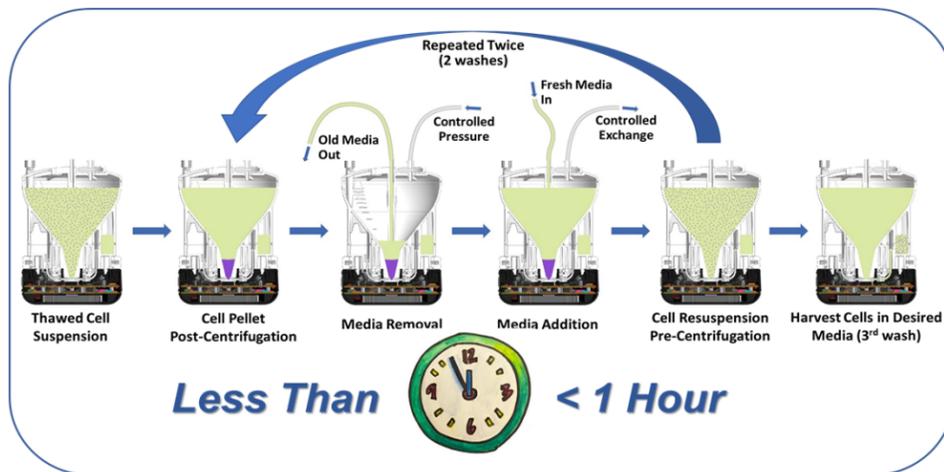
Content originally prepared for ThermoGenesis by Dalip Sethi[1], Jon Ellis[2], Zelenia Contreras[2], John Perea[2], Stephen Truong[2], Jillian Miller[1]*
[1]Cesca Therapeutics, Inc., Rancho Cordova, CA USA; [2]ThermoGenesis Corp., Rancho Cordova, CA USA

Introduction

Cell products, such as Hematopoietic Stem Cells (HSCs), have been cryopreserved and stored for decades using the cryoprotectant dimethyl sulfoxide (DMSO) at temperatures below -135°C. Although the cell products have demonstrated excellent post thaw viability, DMSO is considered a potential cause of infusion related adverse events.[1,2] Therefore, removal of DMSO and cell lysis products by washing the cell product after thawing may reduce the severity of some transplant related complications.[3,4] We have developed a quick and easy method to wash thawed apheresis products using the X-WASH™ System. Utilizing this protocol, nucleated cell recoveries were greater than 85% with no significant loss of cell viability. The entire process took less than one (1) hour.
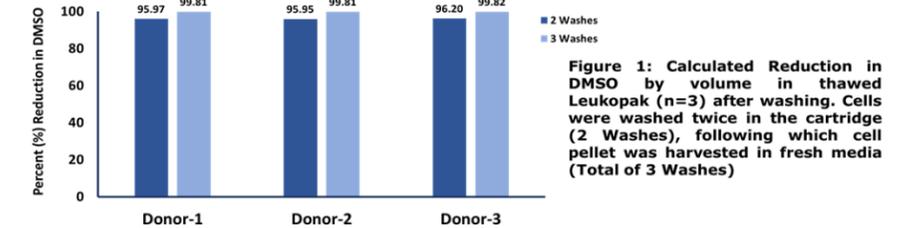
Process Outline

Materials and Methods

Control Module
Docking Station
Disposable Cartridge

Programmable Centrifuge
Thermo Scientific™
Legend™ XTR Centrifuge

Healthy adult cryopreserved Leukopak (Quarter collection) were purchased from HemaCare. Samples (n=3) were thawed as per supplier instructions. Briefly, the cryo-bag was removed from liquid nitrogen storage and immediately placed into a 37°C water bath without figure 8 motion or flicking. Thaw-wash media (2.5% HSA, 5% Dextran, and 20 µg/mL DNase I in saline) was added to the thawed products, and the sample was transferred into a transfer bag (optional step). The sample was transferred to the X-WASH Disposable Cartridge. Post-Centrifugation, waste media was removed using positive pressure, and new media was added using controlled negative pressure. The final centrifugation cycle harvested the cell pellet into the harvest chamber, which was pre-filled with media prior to centrifugation.

Results and Discussion

The reduction in DMSO in the washed product was calculated based on volume reduction. With the assumption of 10% DMSO in the starting material and even distribution of DMSO in cells and buffer. Actual DMSO quantification will be conducted in future experiments.

Figure 1: Calculated Reduction in DMSO by volume in thawed Leukopak (n=3) after washing. Cells were washed twice in the cartridge (2 Washes), following which cell pellet was harvested in fresh media (Total of 3 Washes)

$CD45^+$ cells (total and viable) and $CD3^+$ cells (total and viable) were quantified in pre-wash and post-wash fractions. The mean (SD) recoveries for the total and viable $CD45^+$ cells were 96.0% (2.6%) and 93.7% (3.7%). The mean (SD) recoveries for the total and viable $CD3^+$ cells were 96.3% (1.5%) and 92.4% (5.2%).
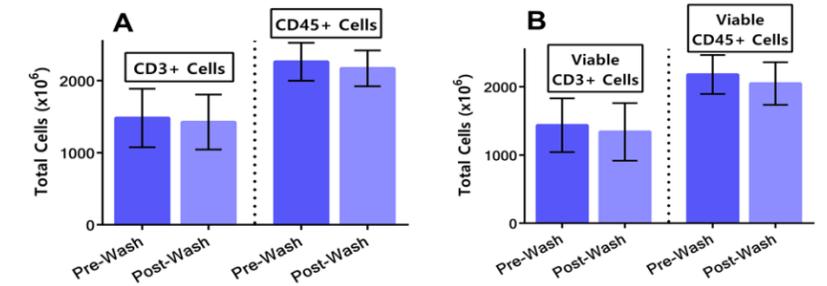

Figure 2: CD45+ and CD3+ Cells (Total (A) & Viable (B)) in Pre-Wash and Post-Wash fractions (n=3).

Conclusions

In conclusion, we have developed a fast and efficient method to remove DMSO from thawed cellular products. The method resulted in cell recoveries of greater than 90% with no significant loss of viability. The method is easy to implement in a standard laboratory.

References

1. Zambelli, et al. (1998) Clinical toxicity of cryopreserved circulating progenitor cells infusion. Anticancer Res. 18(6B); 4705-4708.
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3. Ayello, et al. (1995) A semiautomated technique for volume reduction of stem cell suspensions for auto-transplantation. J Hematother 4:545-549.
4. Del Mastro, et al. (2001) Intensified chemotherapy supported by DMSO-free peripheral blood progenitor cells in breast cancer patients. Ann Oncol 12:505-508.

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