Corning® X-WASH® System for Closed, Sterile PBMC Washing After Thaw

Application Note

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Hilary Sherman and John Shyu
Corning Incorporated, Life Sciences
Kennebunk, ME USA

Introduction

In recent years, cell therapies relying on immune cells have become more prominent. Many of the workflows for this type of therapy involve isolating PMBCs from whole blood. To simplify the process, initial work is performed in acquiring a large population of PBMCs which are cryopreserved for future processing. Unfortunately, the cryopreservation process can impact cell viability due to ice formation during freezing[1]. Cryoprotective agents (CPAs), such as dimethyl sulfoxide (DMSO), are often added to freezing media in order to reduce ice formation and increase cell survival. However, DMSO itself can be cytotoxic, therefore, its removal post-thaw is often recommended[2]. In this article, we demonstrate how the Corning X-WASH system can be utilized to reduce DMSO concentrations, exchange buffer, and concentrate cells in a sterile, closed system. Additionally, the X-WASH system provides flexibility in that it allows the user to balance DMSO reduction with cell recovery, which can be negatively impacted by washing cells[3]. Here, we compare peripheral blood mononuclear cell (PBMC) recovery and viability from two commonly used cryopreservation formulations in association with two DMSO reduction protocols.

Materials and Methods

PBMCs were isolated from fresh, whole blood containing Anticoagulant Citrate Dextrose Solution A from healthy donors using the Corning X-LAB® System as previously described in Corning X-LAB System for Closed, Sterile PBMC Recovery (Corning App Note CLS-AN-632). PBMCs were equally divided between 90% fetal bovine serum (FBS) (Corning Cat. No. 35-010-CV) containing 10% DMSO (Corning Cat. No. 25-950-CQC), or a protein-free freeze media with 5% DMSO (BioLife Solutions Cat. No. 205102). Cells were frozen overnight at -70°C using a Corning CoolCell® FTS30 freezing container (Corning Cat. No. 432006) and then transferred to the vapor phase of liquid nitrogen for at least 24 hours.

On the day of thaw, RPMI 1640 (Corning Cat. No. 15-040-CM) supplemented with 10% FBS and 2 mM L-glutamine (Corning Cat. No. 25-005-CI) was pre-warmed to 37°C. PBMCs were thawed into 200 mL of pre-warmed media and added to an X-WASH cartridge for processing. A 1 mL sample was taken prior to centrifugation to determine the initial cell count using a Beckman Coulter DxH 520 analyzer. Cells from each CPA formulation were processed via one of two protocols; centrifugation and immediate harvest in a volume of approximately 4 mL (dilution) or centrifugation followed by a 200 mL media exchange prior to harvest in a volume of approximately 4 mL (1X wash). Cells were collected and the harvest chamber was washed with 4 mL of phosphate buffered saline (Corning Cat. No. 21-040-CV). PBMCs were assessed for recovery and viability. Additionally, the supernatant was collected and DMSO concentration was quantified via ultra-performance liquid chromatography (ULPC).

Results and Discussion

Two different DMSO reduction protocols were utilized to compare PBMC recovery from both CPA formulations tested. Figure 1 shows cell recovery of conditions tested from 3 separate donors. A two-way ANOVA found statistically significant, higher recovery with 10% DMSO versus 5% DMSO ($p<0.05$). This is not surprising due to the cryoprotective role that DMSO plays. Additionally, we also saw higher cell recovery when the dilution wash protocol was utilized as compared to the 1X wash protocol ($p<0.01$). The results showed a statistically significant difference in cell viability between CPAs ($p<0.05$) but not wash protocols (Figure 2). The lower viability with the 10% DMSO solution could be attributed to the cytotoxic effect of DMSO itself. Finally, Figure 3 shows DMSO concentrations in final cell product. Dilution of the frozen PBMCs resulted in DMSO concentrations of less than 500 parts per million (ppm) when a 10% DMSO concentration was used and averaged 230 ppm when a 5% DMSO concentration was used. The addition of a 200 mL buffer exchange further diluted the DMSO concentration to less than 3 ppm for 10% DMSO and less than 2 ppm for 5% DMSO (with several of the samples being below the 1 ppm detection limit of the instrument).

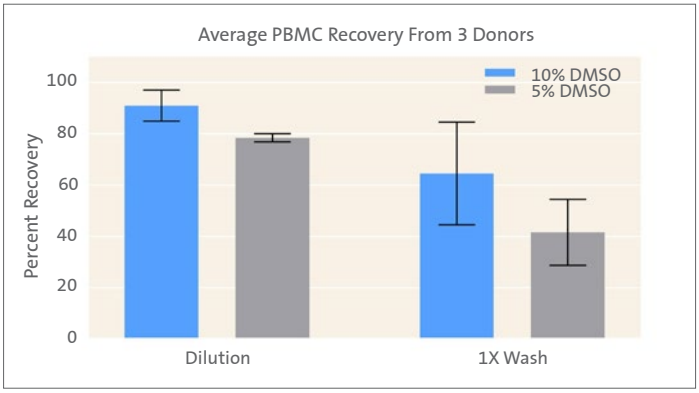

Figure 1. Average PBMC recovery from 3 donors. PBMC recovery from the Corning X-WASH system with dilution or 1X wash protocol. Data is average with standard deviation from 3 donors.

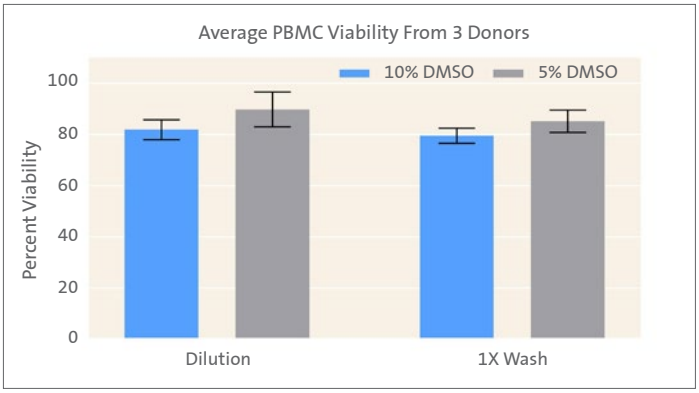

Figure 2. Average PBMC viability after Corning X-WASH system recovery. Average PBMC viability using X-WASH after dilution or 1X wash protocol. Data is average with standard deviation from 3 donors.

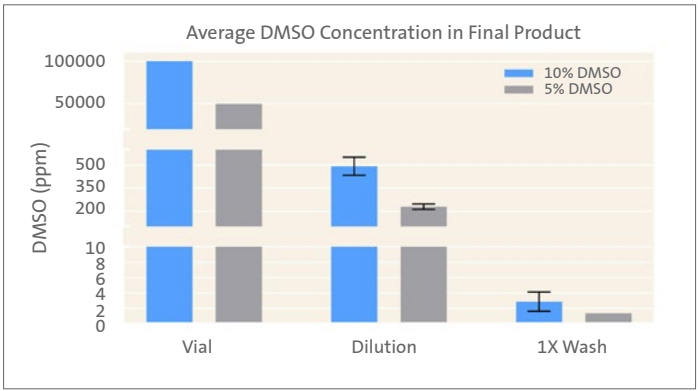

Figure 3. Final DMSO concentration. Average DMSO concentration in final product after dilution or 1X wash protocol. Data is average from 3 independent experiments with the exception of 1X wash in which multiple samples were below the 1 ppm detection limits of the instrument.

Conclusions

In order to enable PBMC cryopreservation in a research or cell therapy workflow, process optimization is essential. It is necessary to reduce unwanted components such as DMSO as much as possible, while at the same time maximizing cell recovery. Importantly, DMSO reduction and maximizing cell recovery may not be independent. If cell recovery is the priority, a singular, large volume dilution might be ideal, as increased washing can result in less recovery. Alternatively, if reducing the cryoprotectant as much as possible is the priority, further dilution can be achieved by adding more washes or starting with a lower cryoprotectant concentration. With the Corning X-WASH system, the user can determine what criteria are more pertinent for their application.

References

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EUROPE
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t +31 (0) 206 59 60 51

LATIN AMERICA
grupoLA@corning.com
Brazil
t 55 (11) 3089-7400
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