Corning® X-WASH® System for DMSO Reduction of Cryopreserved Human Mesenchymal Stem Cells

Application Note

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Introduction

With more cell-based therapies going through clinical trials, there is an increasing need for more robust tools to simplify workflows. Cryopreservation is a necessary part of workflows for both autologous and allogeneic therapies[1]. The ability to cryopreserve cells for cell therapy increases the potential range of administration, shelf life and time for safety testing to occur[2]. Cryoprotectants, such as dimethyl sulphoxide (DMSO), are often added to freezing media in order to reduce ice formation and increase cell survival post-thaw. However, DMSO itself can be cytotoxic so it is necessary to reduce its final concentration as much as possible[3]. In this article, we demonstrate how the Corning X-WASH can reduce the amount of DMSO used in cryopreserved cells through a semi-automated, closed system. Using the Corning X-WASH, we were able to achieve a significant reduction in DMSO concentration of cryopreserved bone marrow-derived human mesenchymal stem cells (hMSC), while maintaining high cell recovery, viability, and multipotency.

Materials and Methods

Bone marrow-derived hMSCs (RoosterBio Cat. No. MSC-1M-5XF) were scaled up in RoosterNourish™-MSC-XF (RoosterBio Cat. No. KT-016) per vendor recommendations. Cells were harvested from a Corning CellSTACK® 10-chamber vessel (Corning Cat. No. 3270) with TrypLE™ Express (Gibco Cat. No. 12604021) and frozen into 50 mL Corning Cryopreservation Bags (Corning Cat. No. 91-200-88). Approximately 70 million cells were processed into each bag containing 10 mL of a 90% fetal bovine serum (FBS) (Corning Cat. No. 35-010-CV) and 10% DMSO (Corning Cat. No. 25-950-CQC).

On the day of thaw, wash buffer was prepared and warmed to 37°C. Wash buffer consisted of phosphate buffered saline (PBS) (Corning Cat. No. 21-040-CM) supplemented with 2% human serum albumin (Baxter Cat. No. 2G0012) and 5% glucose (Tecknova Cat. No. G0550). hMSCs were thawed into 200 mL of wash buffer and added to an X-WASH cartridge for processing. A 1 mL sample was taken prior to centrifugation to determine the starting cell count. Cells were processed via one centrifugation at 300 xg for 5 minutes followed by a buffer exchange with 200 mL fresh wash buffer. X-WASH cartridges were then processed to harvest cells. An overview of the workflow is shown in Figure 1. One milliliter from the supernatant was collected to assess final DMSO concentration via ultra-performance liquid chromatography (ULPC). Cells were collected from harvest chamber which was then washed with an additional 4 mL of PBS to ensure complete recovery. Cells were re-plated into a T-75 flask at 10,000 cells per cm^2 to ensure typical cell morphology and multipotency.

To assess multipotency, hMSCs were harvested after 72 hours of culture with Accutase® (Corning Cat. No. 25-058-CI). Cells were stained using a Human MSC Analysis Kit (BD Biosciences Cat. No. 562245) per vendor's protocol. Once stained, marker expression was assessed using MACSQuant® Analyzer 10 (Miltenyi Biotec).

Results and Discussion

Here we demonstrated greater than 70% recovery of hMSCs following a 200 mL wash after thaw (Figure 2). Additionally, viability was maintained above 93% for all three runs (Figure 3). MSCs were re-plated in order to observe morphology and assess marker expression. Typical morphology was observed after 72 hours of growth (Figure 4). The International Society for Cellular Gene Therapy (ISCT) has defined the minimal criteria for hMSC quality as expressing >95% of CD105, CD73, and CD90, and lack of expression (<2%) of typical hematopoietic markers CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR surface molecules[3].

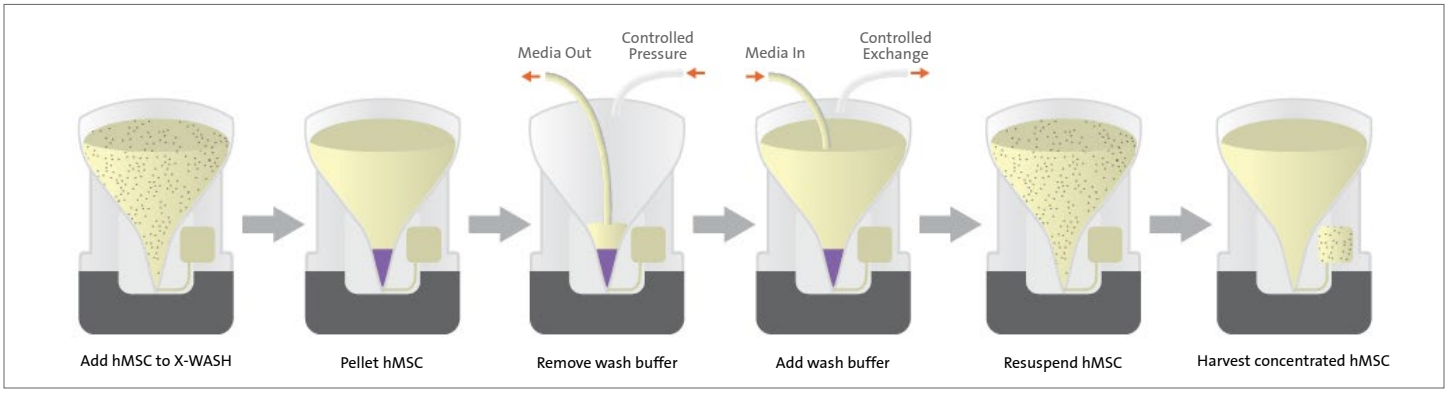

Figure 1. hMSC Corning X-WASH system workflow.

Figure 5 shows greater than 99% expression for positive markers CD105, CD73, and CD90, and less than 0.5% expression of negative markers CD45, CD34, CD11b, CD19, and HLA-DR. Lastly, ULPC analysis showed that the final DMSO concentration present was reduced by at least 40-fold, when a 200 mL dilution followed by an additional 200 mL wash was utilized (Figure 6).

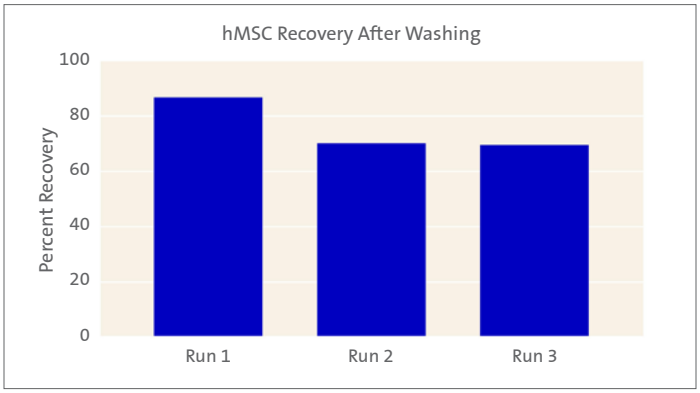

Figure 2. hMSC recovery after washing. hMSC recovery after washing using the Corning X-WASH system. Data is from 3 independent runs.

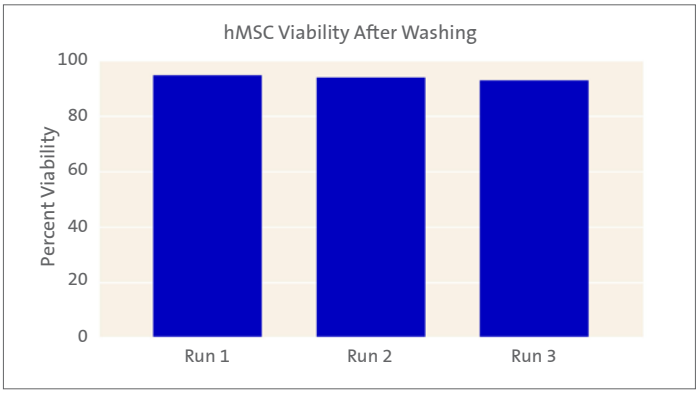

Figure 3. hMSC viability after washing. hMSC viability after washing using the Corning X-WASH system. Data is from 3 independent runs.

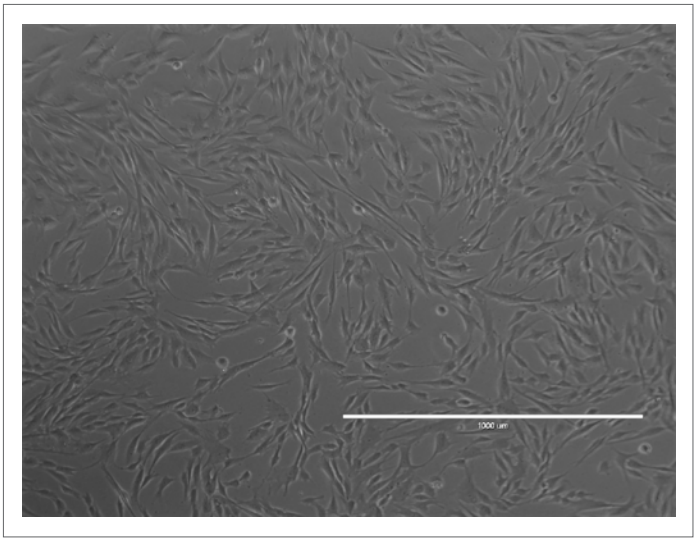

Figure 4. Typical hMSC morphology. Representative photomicrograph of hMSCs 72 hours after washing with the Corning X-WASH system. 4X objective.

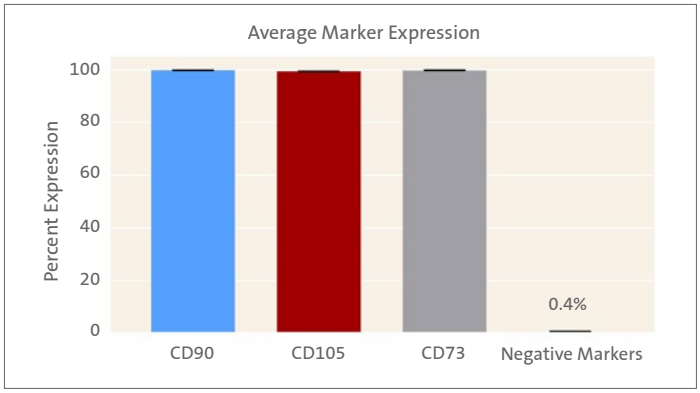

Figure 5. hMSC multipotency. Average marker expression of hMSC after Corning X-WASH system processing with standard deviation. N=3.

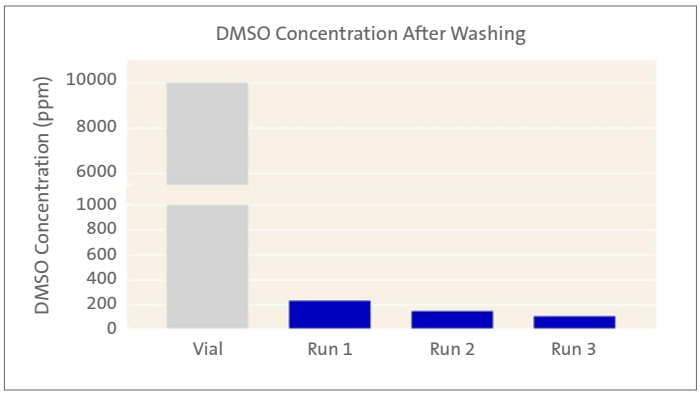

Figure 6. Final DMSO concentration after washing. DMSO concentration in the final product after 200 mL dilution followed by 200 mL wash. Data is from 3 independent runs.

Conclusions

In order to address the growing demand for cell-based therapies, optimization of cryopreservation and recovery is essential. With some hMSC therapies projecting as many as 1 billion cells per dose, it will be essential to have high recovery and viability post-cryopreservation[4]. High hMSC recovery and viability must be maintained while minimizing any undesired components from manufacturing. The Corning X-WASH system allows for reduction of DMSO and other reagents from cell products. More importantly, the Corning X-WASH allows cell processing and collection in a sterile and closed system.

References

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