Advancing Key Processes of the MSC Therapy Manufacturing Workflow

Yosuke Kurokawa*, Anastassia A. Tselikova, Katerina Villafuerte, Ashly Campbell, Shay Fazilat, Vivian Tovar, Shahram Shahabi, David T. Ho, and Chandana Sharma
Department of Research and Development, FUJIFILM Irvine Scientific, 1830 E. Warner Ave., Santa Ana, CA 92705, USA
*yosuke.kurokawa@fujifilm.com

INTRODUCTION

Human mesenchymal stromal/stem cells (hMSCs) hold incredible potential for use in cell therapies. Due to their immunomodulatory properties and multipotent potential to support cartilage, bone, and other cell types, hMSCs are being investigated for use in a wide range of indications. With the recent FDA approval of remestemcel-L-rknd (Ryoncil) for steroid-refractory acute graft-versus-host disease (SR-aGvHD), therapy developers are increasingly focused on bringing MSC therapies to the market.

Like most cell therapies, MSC therapies require the expansion of hMSCs *ex vivo* to generate a clinically relevant number of cells. Such a manufacturing process must be reproducible and minimize cell stress in order to maximize the therapeutic efficacy of the cells. There are numerous challenges in the manufacturing workflow for MSC therapies that need to be addressed to realize the full potential of this therapeutic modality. Specifically, these manufacturing challenges include achieving high levels of MSC expansion while maintaining stemness and maintaining cell quality throughout the cryopreservation process.

FUJIFILM Irvine Scientific offers a wide range of cell culture reagents that support the manufacturing workflow of MSC-based therapies **(Figure 1)**. Here we demonstrate how these reagents can be utilized for large-scale expansion and cryopreservation of hMSCs from various sources.

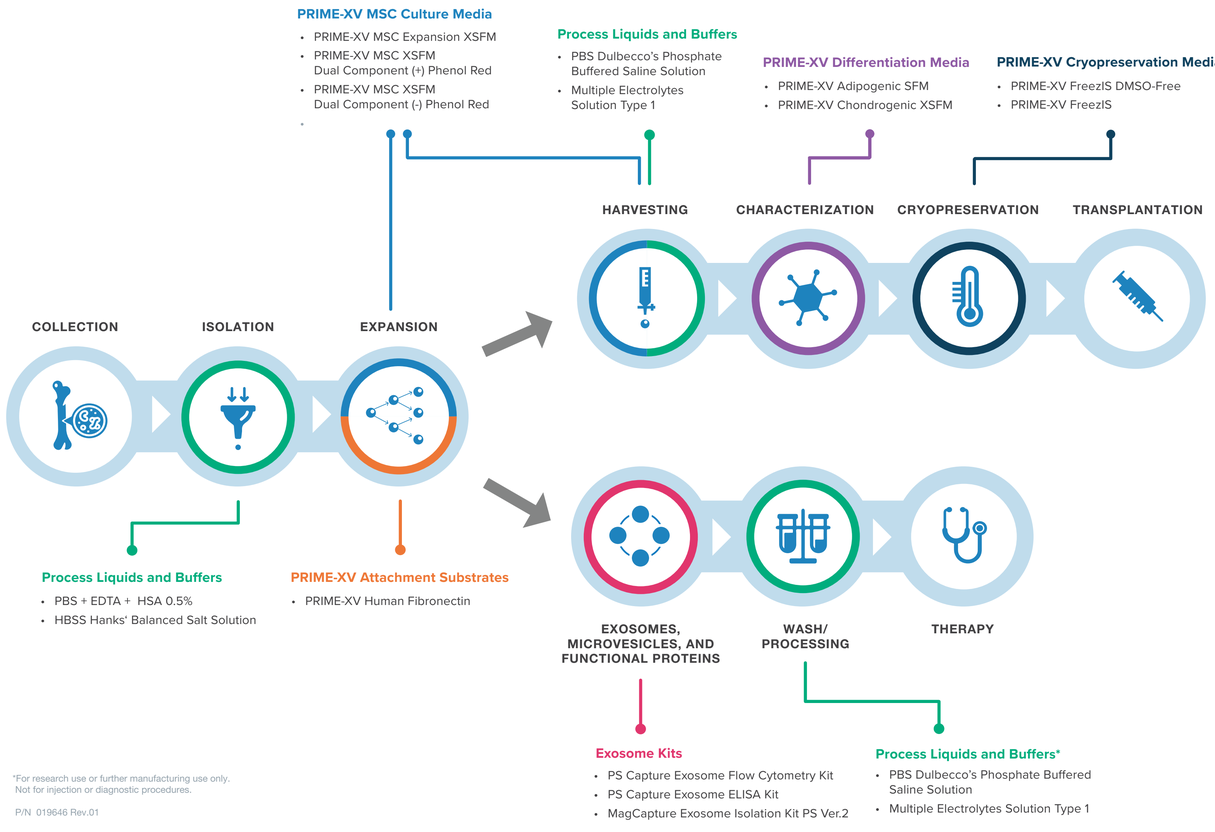

Figure 1. Reagents for supporting MSC workflow. FUJIFILM Irvine Scientific provides GMP compliant reagents to support research and clinical use of hMSCs.

METHODS

Cell source: Commercially-available, human adipose-derived MSCs (AD-MSCs), bone marrow-derived MSCs (BM-MSCs), or umbilical cord-derived MSCs (UC-MSCs) were used to generate the data presented here.

Quantum bioreactor: hMSCs were initially expanded in flasks to a seeding amount of 14–25 x 10^6 cells per bioreactor. RUO bioreactors were coated with 5 mg/unit of human fibronectin, and the seeded hMSCs were expanded for 7 days following an MSC expansion protocol provided by Terumo Blood and Cell Technologies (BCT).

Flow cytometry: Cell samples were taken at Day 0 and Day 7 of culture in the Quantum bioreactor, prepared in a PBS + 0.5% BSA flow buffer solution, and analyzed using a standard MSC marker panel. Analysis was performed using a 6-color panel for 3 positive markers (CD73, CD90, and CD105), 2 negative controls (CD34 and CD45), and an amine-reactive viability dye.

Differentiation: hMSCs were harvested, seeded, and differentiated following the protocol for PRIME-XV Adipogenic SFM and PRIME-XV Chondrogenic XSFM for adipogenic and chondrogenic differentiation, respectively. Osteogenic differentiation was performed following a commercially-available kit.

Cryopreservation: hMSCs were harvested and resuspended in cryopreservation solution and filled into a commercially-available cryopreservation bag. The bags were frozen using a controlled rate freezer and stored in a liquid nitrogen dewar. The cells were thawed in a water bath and immediately assessed for viable cell density and viability. The thawed cells were also cultured for 3 days to assess post-thaw expansion.

SUMMARY

Choosing the right reagents is critical in the successful development and manufacture of MSC-based therapies. Here, we have highlighted the capability of PRIME-XV MSC Expansion XSFM to support robust expansion of hMSCs of various sources. Furthermore, we demonstrate the use of PRIME-XV MSC XSFM Dual Component Kit in a closed-system bioreactor to achieve large-scale expansion of hMSCs, yielding 200 x 10^6 hMSCs in 7 days of expansion while maintaining stemness of hMSCs post-expansion. Finally, we show that PRIME-XV FreezIS DMSO-Free performs similarly to a commercially-available, DMSO-containing cryopreservation solution when cryopreserving hMSCs in a clinically relevant format using a cryopreservation bag. Together, these reagents from FUJIFILM Irvine Scientific address some of the most critical challenges that currently exist in MSC cell therapy workflows.

RESULTS

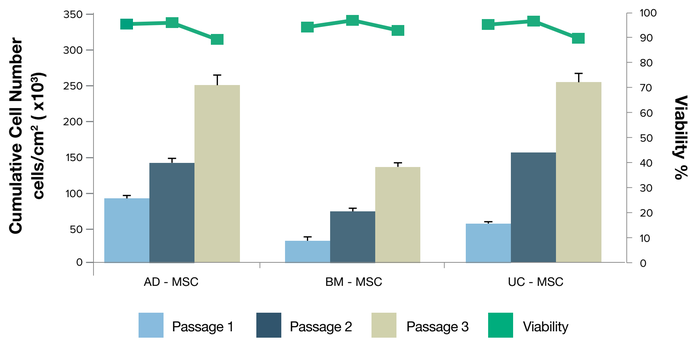

Figure 2. PRIME-XV MSC Expansion XSFM supports robust expansion of hMSCs derived from bone marrow, adipose tissue, and umbilical cord. AD-MSCs, BM-MSCs, and UC-MSCs were cultured in PRIME-XV MSC Expansion XSFM through 3 passages. Each passage was harvested at 80% confluence and assessed for cell count and viability.

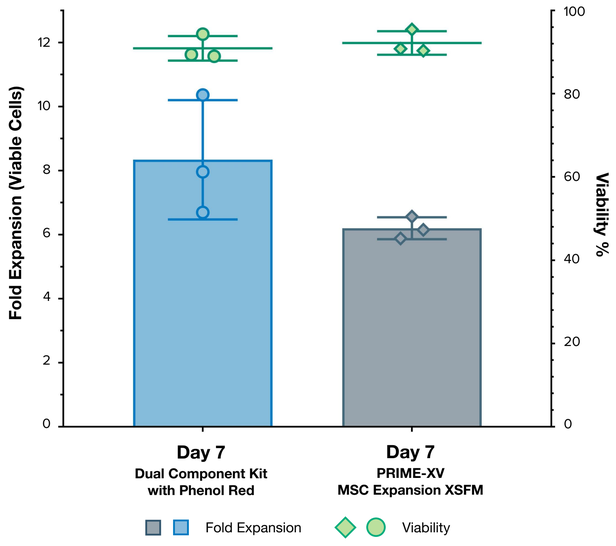

Figure 3. Successful large-scale expansion of hMSCs in a closed-system format. Results from 7-day expansion of hMSCs using PRIME-XV MSC XSFM Dual Component Kit with Phenol Red shows an average of 8-fold expansion while maintaining viability above 90%. Using the Quantum bioreactor, a total cell yield of greater than 200 million cells can be achieved.

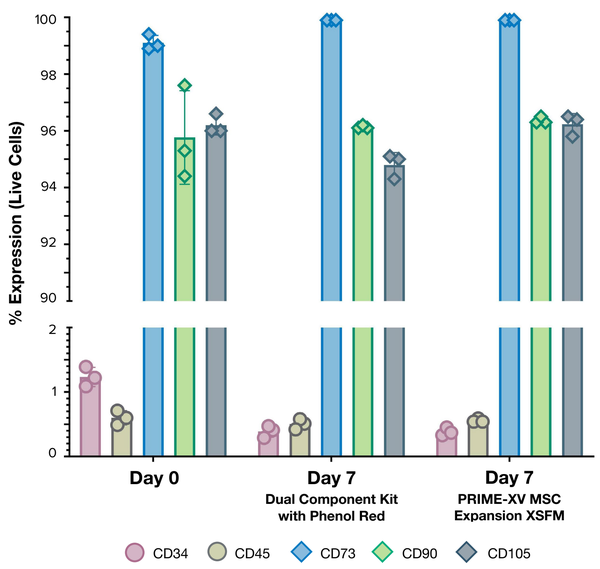

Figure 4. hMSCs maintain their stem cell phenotype at Day 7 post-expansion in PRIME-XV MSC XSFM Dual Component Kit with Phenol Red. The expression of negative markers CD34 and CD45 remained below 2% over the course of the culture. The expression of the positive markers CD73, CD90, and CD105 ranged from 94–100%, indicating the maintenance of MSC differentiation potential over the course of the culture, similar to the PRIME-XV MSC Expansion XSFM.

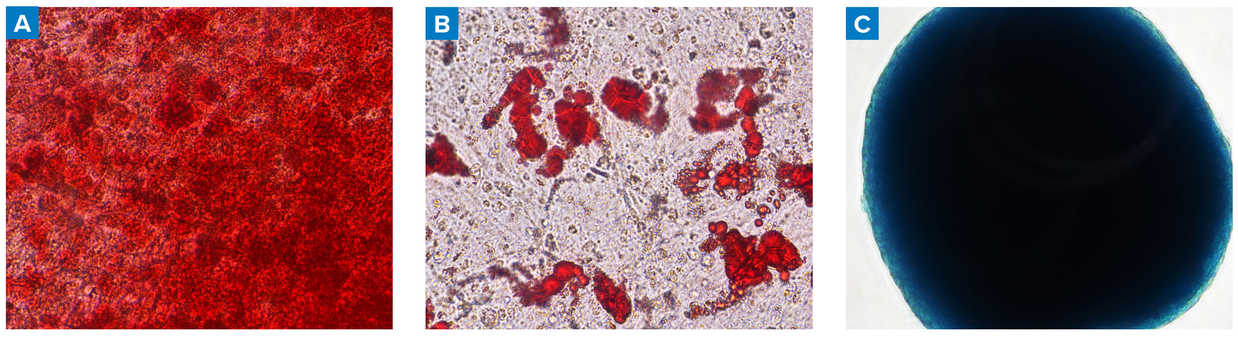

Figure 5. hMSCs expanded in PRIME-XV MSC Expansion XSFM in the Quantum bioreactor remain capable of trilineage differentiation. BM-MSCs were harvested from the Quantum bioreactor post-expansion and seeded into differentiation media in tissue culture plates (adipogenic and osteogenic) or conical tubes (chondrogenic). After differentiation, the cells were fixed and stained as follows: **(A)** Alizarin Red stains for calcium deposits in osteocytes, **(B)** Oil Red O stains for lipid droplets in adipocytes, **(C)** Alcian blue stains for cartilage.

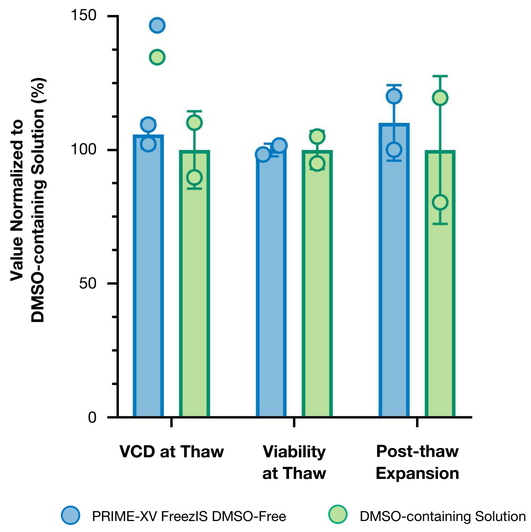

Figure 6. Cryopreservation of hMSCs in bags using PRIME-XV FreezIS DMSO-Free achieves similar performance to a commercially-available, DMSO-containing solution. BM-MSCs were frozen using a 15 mL fill volume in cryopreservation bags following a custom freezing protocol using a controlled-rate freezer. When compared against a commercially-available, DMSO-containing cryopreservation solution, the DMSO-free cryopreservation solution achieved similar cell recovery as assessed by viable cell density (VCD) and cell viability at thaw. Furthermore, the hMSCs that were cultured post-thaw showed no loss in proliferative capacity compared to the DMSO-containing solution. All values are normalized to the average of the values from the DMSO-containing solution.