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Development of DMSO-Free Chemically Defined Cryopreservation Solution for Human-Derived Mesenchymal Stem/Stromal Cells

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Introduction

Cryoprotective agents (CPA) such as dimethyl sulfoxide (DMSO), glycerol, and propylene glycol have been used for the cryopreservation of cells and tissues. Amid growing concerns that DMSO may compromise the potency of mesenchymal stem/stromal cells (MSCs) and its differentiation potential, a DMSO-Free cryopreservation solution is highly desired. To address this concern, a novel chemically defined, protein free, and animal component free DMSO cryopreservation solution has been developed.

In this study, we demonstrate the effectiveness of using a novel DMSO-Free cryopreservation solution on human adipose-derived, bone marrow-derived, and umbilical cord-derived mesenchymal stem/stromal cells (AD-MSC, BM-MSC, and UC-MSC) as compared to traditional DMSO cryopreservation solutions (10% DMSO).

Methods

All three primary MSCs were expanded in PRIME-XV® MSC Expansion XSFM before and after cryopreservation. MSCs were frozen at equal cell density in PRIME-XV FreezIS (10% DMSO) and in PRIME-XV FreezIS DMSO-Free solution for 2 to 5 days in liquid nitrogen. After storage, the MSCs were thawed and cultured through two passages and assessed for morphology, cell viability, cell marker expression, and differentiation potential. Cell marker expression was assessed by flow cytometry with PerCP-Cy5.5 conjugated mouse anti-human CD45, APC-conjugated mouse anti-human CD90, and PE conjugated mouse anti-human CD105 (BioLegend, San Diego, CA).

Differentiation potential was determined by culturing the MSCs for 2 to 4 weeks in PRIME-XV Adipogenic SFM, PRIME-XV Osteogenic Differentiation SFM, and PRIME-XV Chondrogenic Differentiation XSFM prior to staining.

Results

Initial differences in attachment was observed within 24 hours of thaw with superior quality found in cells cryopreserved in DMSO-Free solution. Three days post thaw, comparable attachment to plastic and similar fibroblastic, spindle-shaped morphology as expected of MSCs was observed prior to passaging (Figure 1).

Viable cell counts were comparable between MSCs frozen in 10% DMSO and DMSO-Free post-thaw and through two passages until 80% confluent (Figure 2). All MSCs were able to proliferate on tissue culture plastic coated with fibronectin (Irvine Scientific, Santa Ana, CA).

No differences in marker expressions were observed. Multiple passaged cells retained positive MSC marker expression for CD105 and CD90 while negative for CD45 (Figure 3). No affect of cryopreservation solution was seen.

All three types of MSCs retained multi-lineage differentiation potential. The cells formed visible lipid vacuoles typical of adipogenic differentiation. The cells also had positive expression of transcription factor RUNX2 in conjunction with Osteocalcin as expected for osteogenic differentiation. Cryosection and staining of the chondroblast spheriod demonstrated the presence of Aggrecan and Collagen Type II which is typical of chondrogenic differentiation (Figure 4).

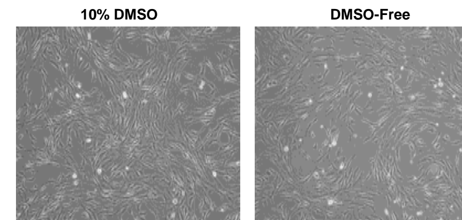

Figure 1 Morphology comparison of MSCs cryopreserved in 10% DMSO and DMSO-Free cryopreservation solution. Human adipose- derived MSCs were frozen in 10% DMSO containing and DMSO-free cryopreservation solution prior to thaw. Post-thaw, cells were expanded in PRIME-XV MSC. Attachment and morphology was observed. Images were taken at 10X magnification.

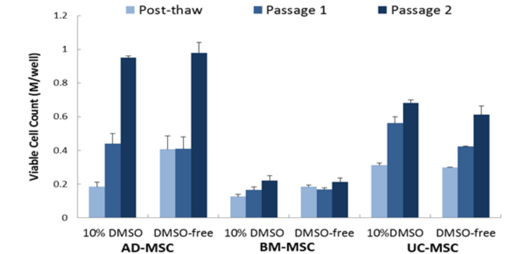

Figure 2 Expansion comparison of MSCs cryopreserved in 10% DMSO and DMSO-Free cryopreservation solution. Human adipose-derived (AD-MSC), bone marrow-derived (BM-MSC), and umbilical cord-derived (UC-MSC) cell count post-thaw and through two passages.

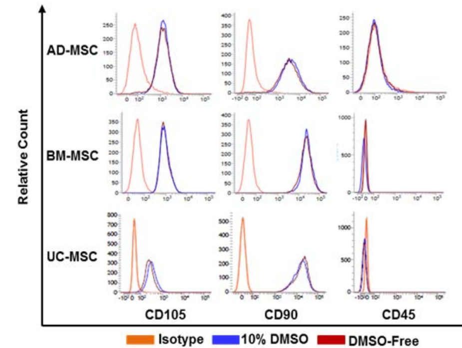

Figure 3 Multiple passaged cells retained MSC markers: positive for CD105 and CD90 and negative for CD45. Human adipose-derived MSCs were cultured in PRIME-XV MSC Expansion XSFM. Marker expression was assessed by flow cytometry.

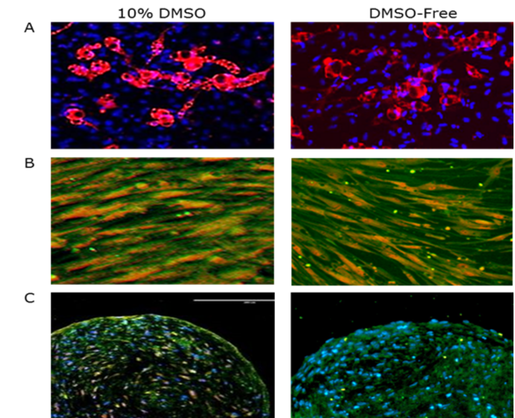

Figure 4 MSCs retains multi-lineage differentiation potential. Human adipose-derived MSCs were cultured in PRIME-XV MSC Expansion XSFM after thaw. MSCs were differentiated for into (A) adipocytes, (B) osteocytes, and (C) chondrocytes. The cells were stained with (A) FABP-4 (red), (B) OSTEOCALCIN (green) and RUNX2 (red), and (C) AGGRECAN (red) and COLLAGEN TYPE II (green). Nuclei were counterstained with DAPI (A and C).

Conclusion

This study found comparable before and after cryopreservation performance characteristics. No significant difference was noted for cell viability and the expression of CD105 and CD90. MSCs frozen in DMSO-Free solution retained its differentiation potential. Overall, this study demonstrated a novel alternative to the traditional DMSO cryopreservation solution that can be used on human-derived mesenchymal stem/stromal cells.