DMSO-Free Cryopreservation of Mesenchymal Stem Cells in Chemically Defined Systems

Application Note

Introduction

As stem cell-based therapies continue to be developed and make up an ever-growing role in medicine, it is essential that the final cell product being administered to the patient is able to ensure both efficacy and safety. DMSO has traditionally provided cryoprotective activity for cell-based products through the cryopreservation process. However, inclusion of DMSO can negatively impact the phenotypic and functional characteristics of stem cells [1]. Further, adverse patient reactions have been reported in multiple therapeutic stem cell transplantation applications upon infusion of DMSO-exposed cell products [2], [3].

To mitigate the potential complications associated with the inclusion of DMSO in final formulations, InVitria developed a DMSO-free cryopreservation solution, OptiFRZ, to avoid the adverse challenges introduced by DMSO and to reduce potential toxicity effects attributed to DMSO. OptiFRZ has been shown to maintain critical morphology and phenotypic characteristics of the cell product, while delivering improvements to cell product recovery yields compared to DMSO containing cryopreservation methods.

Materials

Cryopreservation Process

- OptiFRZ DMSO-Free Cryopreservation Media (InVitria 555OFR0067)
- Nalgene 1 mL Cryogenic Tubes (Thermo Fisher #5000-0012)
- 50 mL Conical Tubes
- Dulbecco's Phosphate-Buffered Saline (Thermo Fisher #14190136)
- Controlled rate freezer (ex: Mr. Frosty – Thermo Fisher #5100-0036)
- Complete MSC growth medium

Protocol

Freezing:

1. Harvest mesenchymal stem cells from flask ware and collect cell suspension in a 50 mL conical tube before taking a count.
2. Wash with DPBS and centrifuge for 5 minutes at 1600 rpm to obtain a cell pellet.
3. Remove supernatant and resuspend cell pellet in cold OptiFRZ DMSO-free cryopreservation media at a volume to achieve a density of 1e6 cells/mL.
4. Transfer 1 mL of the cell suspension directly into each cryovial.
5. Freeze cells using a controlled cooling rate protocol to achieve approximately -1°C per minute.
6. After 24 hours, transfer cryovials to liquid nitrogen (-135°C) for long-term storage.

Thawing

1. To thaw, place cryovials stationary in a 37°C water bath until there appears to be no frozen cell pellet.
2. Transfer thawed cell suspension to a 50 mL conical tube and dilute 1:50 with cold complete growth medium.
3. Centrifuge at 1600 rpm for 5 minutes.
4. Remove supernatant. Wash again by adding 50 mL of DPBS to the cell pellet and centrifuging.
5. Resuspend cells in complete growth medium for viability assessment and post-recovery functional assays.
6. Seed cells in appropriate culture ware for expansion in complete pre-warmed 37°C growth medium at a target density of 30,000 cells/cm^2 or higher for optimal results.

Results and Conclusions

Mesenchymal stem cells were expanded in chemically defined, serum-free conditions. At the time of cryopreservation, cells were placed into cryopreservation media at a density of 1e6 cells/mL. Tested cryopreservation media included OptiFRZ DMSO-free cryopreservation media, a commercial DMSO-free media, or 10% DMSO media. Frozen vials were then thawed in a water bath, washed twice with DPBS, and resuspended in complete growth media. Viable cells were determined via Calcein-AM staining through flow cytometry and percent cell recovery was calculated as the fraction of viable cells post-thaw divided by the viable cells at the start of cryopreservation (Figure 1).

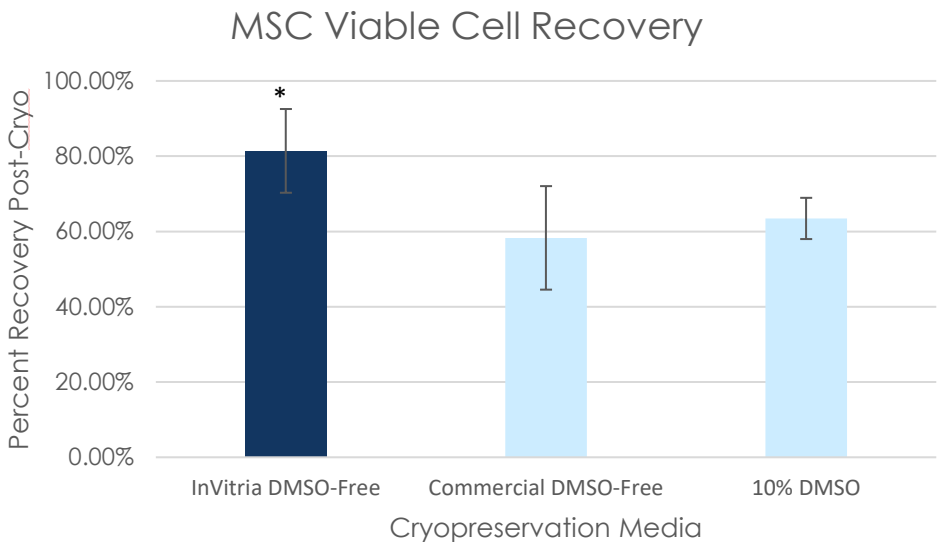

Figure 1. Percent recovery of mesenchymal stem cells after cryopreservation.
**, p = 0.01 by One Way ANOVA.*

Mesenchymal stem cells cryopreserved in OptiFRZ DMSO-free media demonstrated a statistically significant increase in viable cell recovery coming out of the cryopreservation process. Cells frozen in OptiFRZ had an average of 81.42 ± 11.12% recovery whereas cells frozen in the commercial DMSO-free media and 10% DMSO media exhibited an average of 58.30 ± 13.75% or 63.46 ± 5.48% recovery, respectively.

To further characterize the stability of the cryopreserved MSC, sentinel viability was also analyzed at thaw through Calcein-AM based flow cytometry. Viability percent was calculated as the fraction of viable cells over total cells post-thaw (Figure 2). As shown, the average viability of the cells coming out of the cryopreservation process was consistently above the targeted 80% across all media conditions. OptiFRZ DMSO-free media demonstrated 94.14 ± 1.52% viability, comparable to that of the commercial DMSO-free media with 93.61 ± 1.07 % viability and 10% DMSO media with 94.38 ± 0.10% viability.

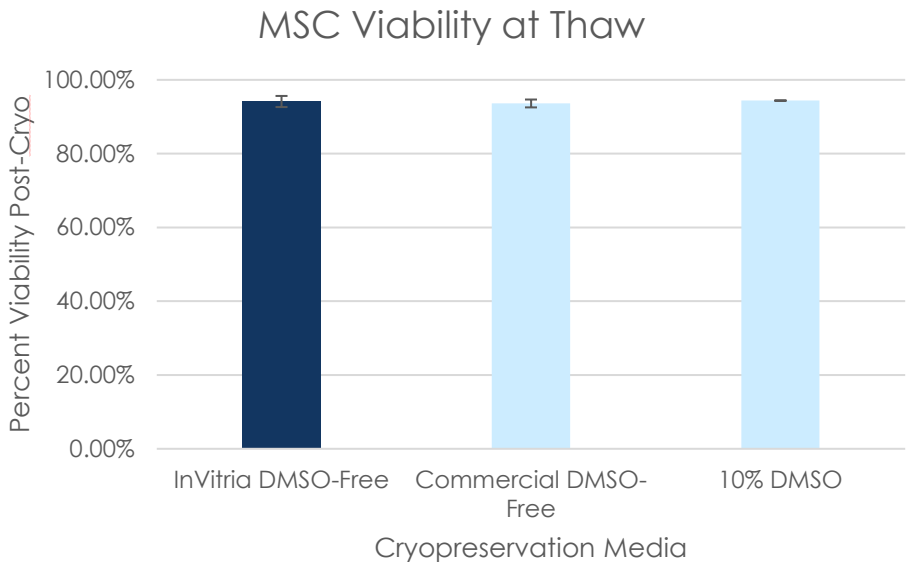

Figure 2. Viability of mesenchymal stem cells post-thaw after cryopreservation.

References

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3. Z. Shu, S. Heimfeld, and D. Gao, “Hematopoietic Stem Cell Transplantation with Cryopreserved Grafts: Adverse Reactions after Transplantation and Cryoprotectant Removal Prior to Infusion,” Bone Marrow Transplant., vol. 49, no. 4, pp. 469–476, Apr. 2014, doi: 10.1038/bmt.2013.152.

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