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Freezing and recovery of mesenchymal stem cells in human platelet lysate

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Introduction

Fetal bovine serum (FBS) is still used as a standard media supplement for cell expansion and freezing along with dimethyl sulfoxide (DMSO). FBS poses several regulatory and potential species cross-contamination challenges hindering its potential use for clinical application of cell expansion and freezing. In an attempt to limit these challenges, there is a need for a human-derived alternative. Human platelet lysate (HPL) is derived from human platelets and contains similar growth factors and cytokines found in FBS at comparable levels. The focus of this study is to evaluate Stemulate™ Pooled Human Platelet Lysate-NH as a freezing media, in conjunction with DMSO, and a recovery media post thaw of neonatal and adult mesenchymal stem cells (MSCs) from various tissues.

Materials and methods

Different freezing media formulations including 90% Stemulate-NH:10% DMSO, 95% Stemulate:5% DMSO, and 90% culture media containing 10% Stemulate-NH:10% DMSO were tested and compared to similar formulations substituted with FBS instead of HPL and commercially available serum-free freezing solutions on amniotic-, bone marrow-, and adipose-derived MSCs. MSCs were assessed for viability and proliferation in cultures post-thaw. Cells were either established and grown in Stemulate-NH or FBS and then frozen in different freezing formulations.

Results

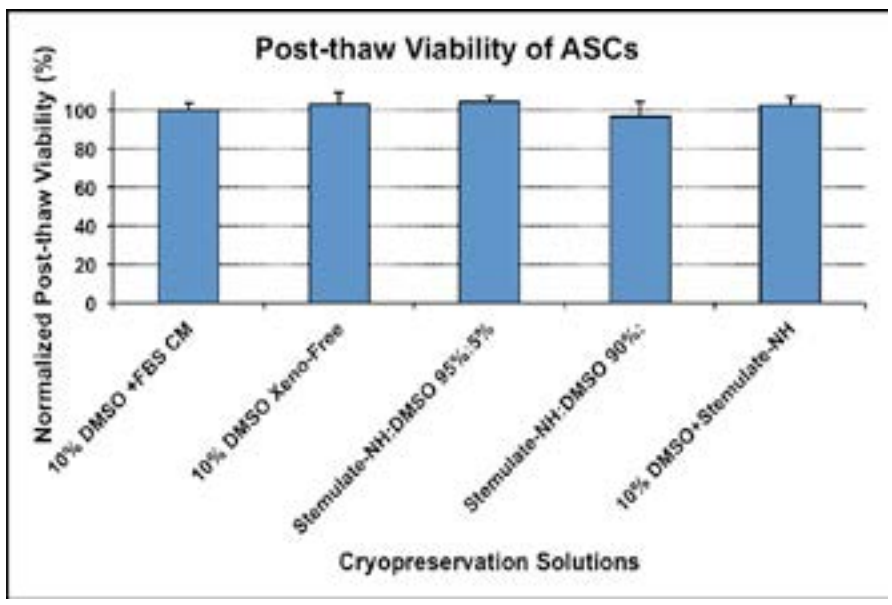

Figure 1: Post-thaw viability of adipose-derived MSCs frozen in different cryopreservation solutions. CM; culture media. Stemulate-NH does not require addition of heparin.

- Stemulate-based freezing solutions and culture media containing Stemulate maintained high viability of adipose-derived MSCs post thaw.

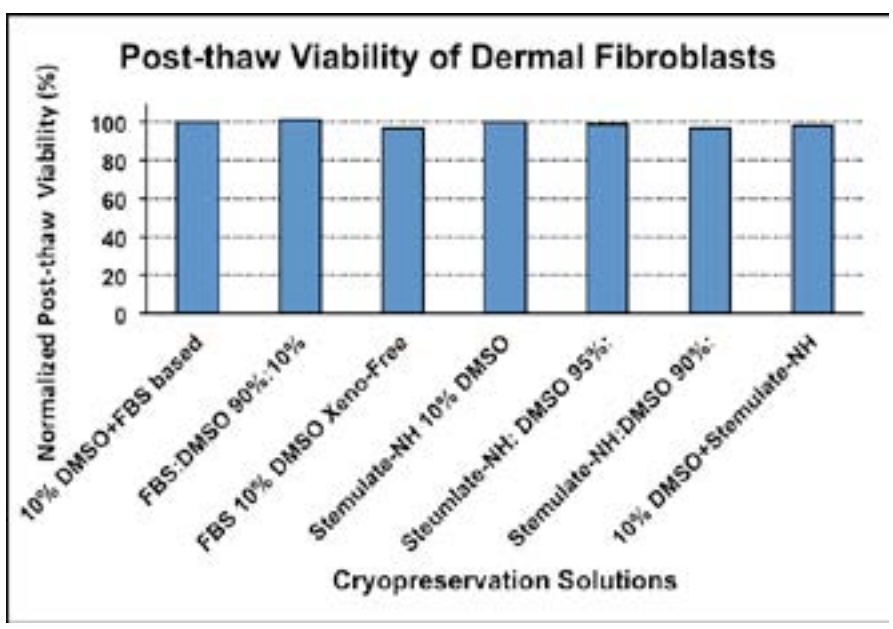

Figure 2: Post-thaw viability of dermal fibroblasts frozen in different cryopreservation solutions. CM; culture media. Stemulate-NH does not require the addition of heparin.

- Stemulate-based freezing solutions and culture media containing Stemulate maintained high viability of dermal fibroblasts post thaw.

Results continued

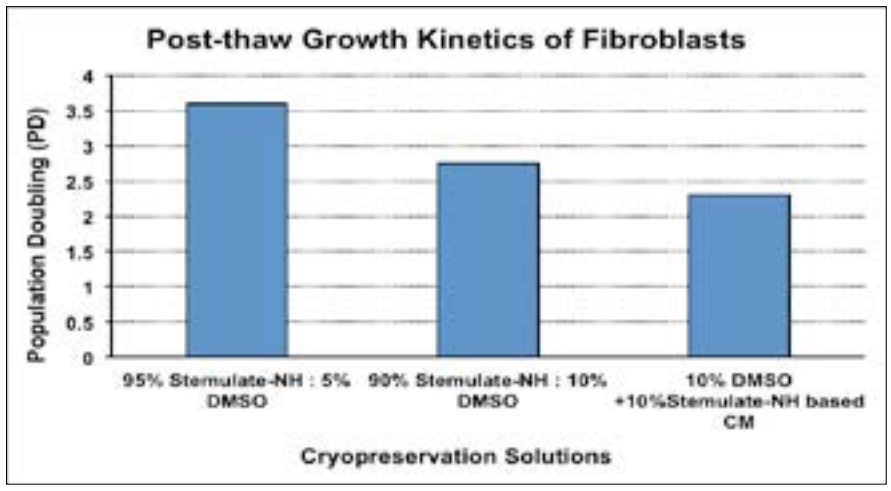

Figure 3: Growth kinetics of fibroblasts post-thaw. CM; culture media. Stemulate-NH does not require the addition of heparin to the media

- Stemulate-based freezing solutions and culture media containing Stemulate maintained growth kinetics activity of fibroblasts for several passages post thaw.

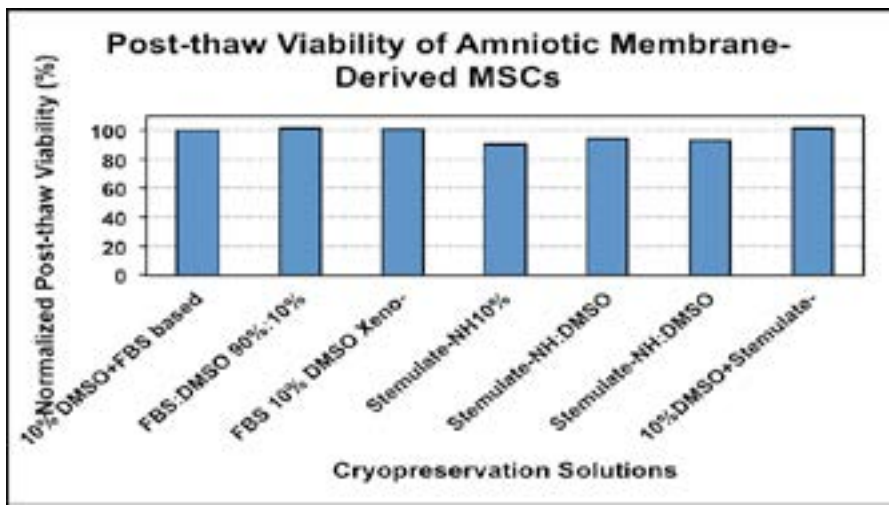

Figure 4: Post-thaw viability of amniotic membrane-derived MSCs frozen in different cryopreservation solutions. CM; culture media. Stemulate-NH does not require the addition of heparin.

- Stemulate-based freezing solutions and culture media containing Stemulate maintained high viability of amniotic membrane-derived MSCs post thaw.

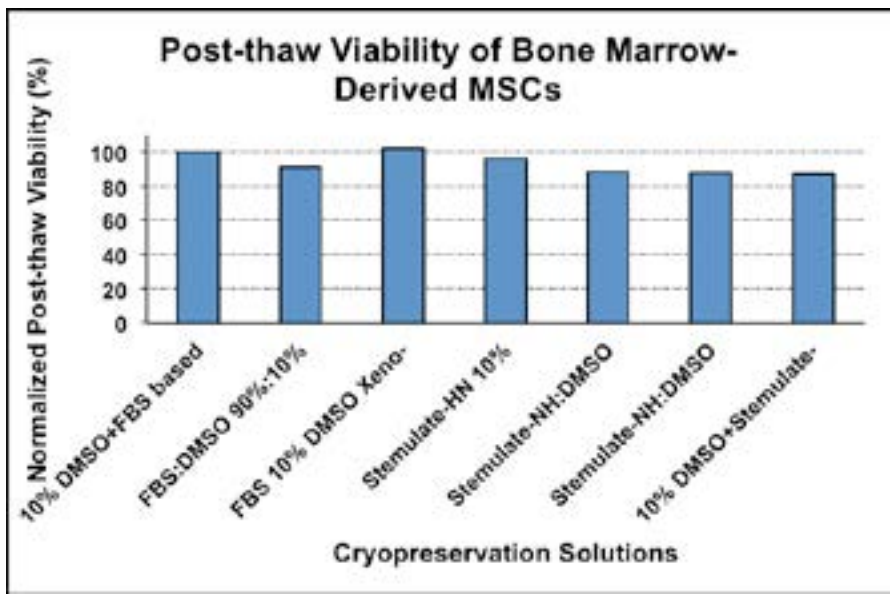

Figure 5: Post-thaw viability of bone marrow-derived MSCs frozen in different cryopreservation solutions. CM; culture media. Stemulate-NH does not require the addition of heparin.

- Stemulate-based freezing solutions and culture media containing Stemulate maintained high viability of bone marrow-derived MSCs post thaw.

Conclusions

- Stemulate-based cryopreservation solutions maintain high viability of adult and neonatal stem cells and primary cells post thaw.
- Stemulate can substitute for FBS in freezing solutions and serum-free freezing solution and offer xeno-free cryopreservation of cells for clinical applications.
- Growth kinetic of cells cryopreserved in Stemulate-based cryopreservation solution was maintained post thaw.

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