Evaluation of Different Cryopreservation Agents for Mesenchymal Stem Cell as Final Study Product

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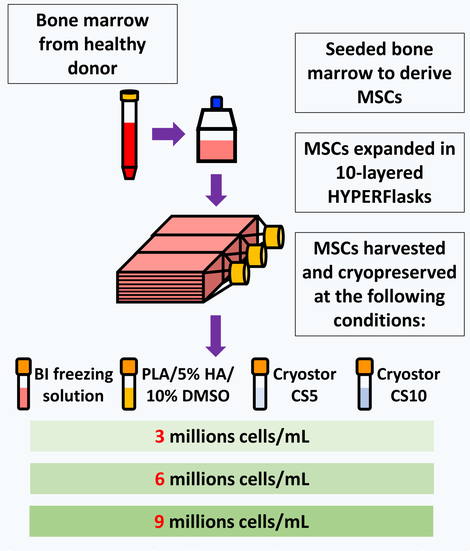
INTRODUCTION

Mesenchymal stem cells (MSCs) have been shown to exert important immunomodulatory effects in both acute and chronic diseases. In acute inflammatory conditions such as septic shock, immunomodulatory cell therapy must be administered within hours of diagnosis; therefore a cryopreserved, allogeneic cell product that can be thawed prior to infusion to the patient is best suited for this purpose. The objective of this study is to determine an optimal cryopreservant that can preserve the viability of MSCs after thawing. We systematically tested different cryopreservants and evaluated key cell product parameters to compare the relative performance merits of post-thawed MSC products.

METHODS

MSCs were cryopreserved in four different cryopreservant solutions at concentrations of 3 x 10^6, 6 x 10^6, or 9 x 10^6 cells/mL for at least 3 weeks in liquid nitrogen prior to experimentation. A final cell concentration of 3 x 10^6 cells/mL was achieved with no dilution (from 3 x 10^6 cells/mL), 1:1 dilution (from 6 x 10^6 cells/mL), or 1:2 dilution (from 9 x 10^6 cells/mL) using PLA/5% HA as diluting solution.



The size and granularity of cells post-thaw were analyzed by flow cytometry. Post-thawed cells were characterized for MSC surface markers. Cell viability was measured at 0, 2, 4, and 6 hours post thaw with Trypan blue exclusion method and Annexin V/Propidium iodide (AV/PI) analysis by flow cytometry.

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RESULTS

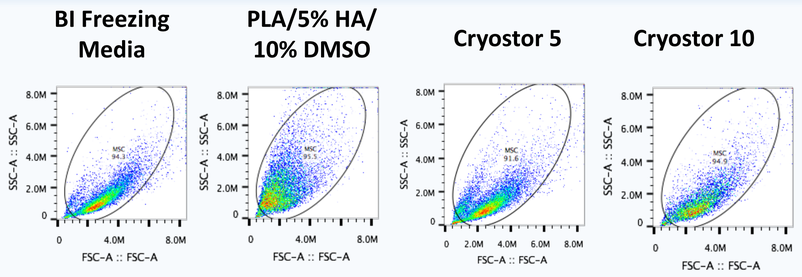

Figure 1. Comparison of size and granularity immediately post-thaw from MSCs cryopreserved in BI freezing media, PLA/5% HA/10% DMSO, CryoStor 5 or CryoStor 10 . MSCs were cryopreserved at 3 million cells/mL.

RESULTS

Table 1. Comparison of MSC surface markers post thaw from four different cryopreservation agents. N=3, shown as mean ± SEM.

	BI Freezing Media	PLA/5% HA/ 10% DMSO	CryoStor 5	CryoStor 10
CD73	98 ± 0.7	99 ± 1.06	99 ± 0.99	98 ± 1.10
CD90	98 ± 1.21	98 ± 1.68	98 ± 0.32	99 ± 0.76
CD105	89 ± 2.21	90 ± 2.48	94 ± 2.56	85 ± 2.25
CD14	0.11 ± 0.14	0.06 ± 0.08	0.08 ± 0.03	0.04 ± 0.07
CD19	0.00 ± 0.01	0.05 ±0.08	0.08 ± 0.03	0.02 ± 0.01
CD34	0.03 ± 0.03	0.10 ± 0.07	0.06 ± 0.03	0.01 ± 0.01
CD45	0.04 ± 0.07	0.03 ± 0.05	0.08 ± 0.01	0.02 ± 0.02
HLA-DR	0.08 ± 0.09	0.01 ± 0.01	0.07 ± 0.03	0.05 ± 0.08

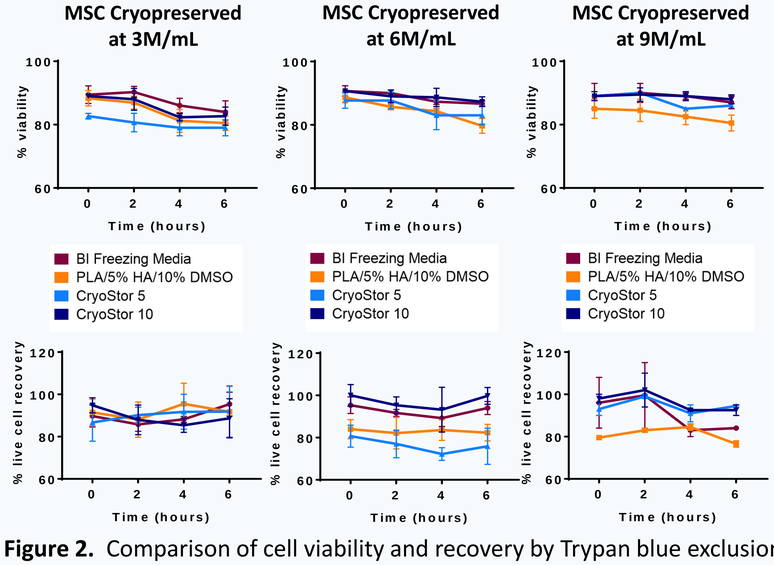

Figure 2. Comparison of cell viability and recovery by Trypan blue exclusion at 0, 2, 4 and 6 hrs post thaw from MSCs cryopreserved in BI freezing media, PLA/5% HA/10% DMSO, CryoStor 5 or CryoStor 10. N=2-3, Error bars indicate SEM.

RESULTS

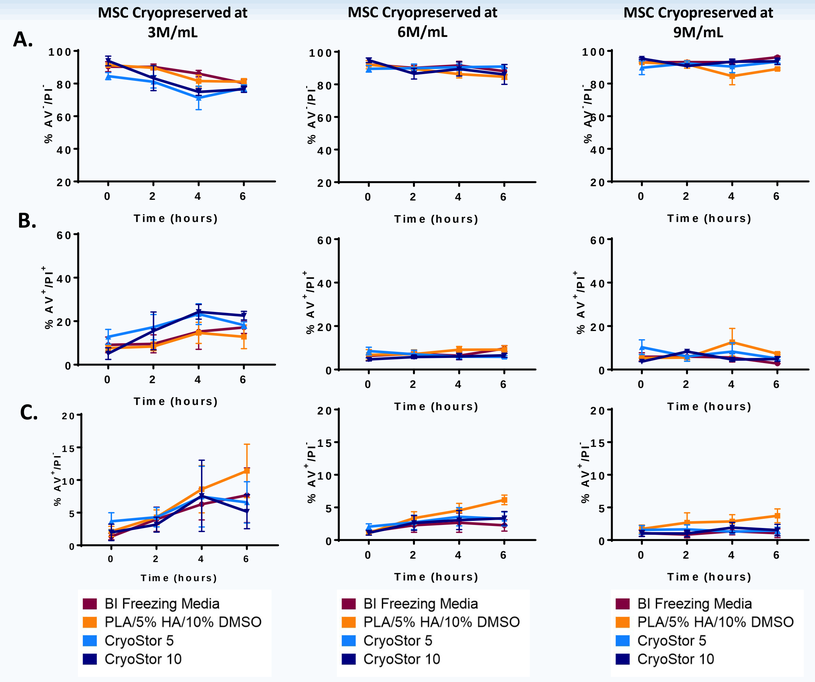

Figure 3. Comparison of (A) viable, (B) dead and (C) apoptotic cells by Annexin V/PI staining and flow cytometry analysis at 0, 2, 4 and 6 hrs post thaw from MSCs cryopreserved in BI freezing media, PLA/5% HA/10% DMSO, CryoStor 5 or CryoStor 10. N=2-3, Error bars indicated SEM.

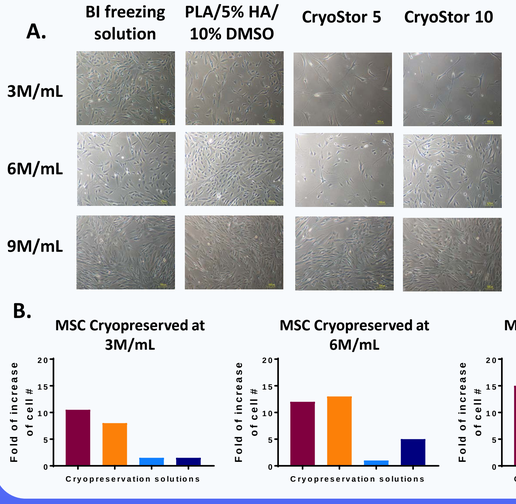

Figure 4. Comparison of (A) cell morphology and (B) recovery after 6 days of culture from MSCs cryopreserved in four different cryopreservation agents. Cell recovery is indicated by fold increase of cell number calculated from initial seeding density.

CONCLUSION

MSCs can be cryopreserved at concentrations up to 9 x 10^6 cells/mL in all four tested cryopreservant solutions. Cells cryopreserved in BI freezing media exhibit the best post-thaw viability, followed by PLA/5% HA/10% DMSO. Dilutions of total percentage of cryopreservant resulted in improved viability of MSCs post thaw up to 6 hours as evidenced by better cell recovery after 6 days of culture.