The Role of Human Platelet Lysate in the Cryopreservation of Mesenchymal Stem Cells

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Abstract

Cryopreservation is widely used to generate large stocks or cell banks that can be stored for short and long periods, ready to be recovered in a functionally potent state and used as required in a cell therapy application. Animal-based reagents are usually employed in cryopreservation media as a source of nutrients and for other perceived benefits during the post-thaw period.

Current evidence suggest that the animal serum proteins used in cryopreservation media are difficult to remove during post-thaw washing, and any residual proteins in cells may trigger adverse reactions in the patients who receive cell infusions or transplants. Therefore, demand is increasing for cryopreservation media to be devoid of any animal-derived components, especially when cells are intended for cell therapy application. Human platelet lysate (HPL) provides an option that is free of animal components and lacks the risks and regulatory challenges associated with animal-derived components.

In this study we investigated the Stemulate® culture media supplement, a commercial platelet lysate product, for its ability to recover functionally potent cells following thawing and subsequent culture in cryopreservation of human-derived mesenchymal stem cells. The results indicated that Stemulate can improve cell recovery and functionality following thawing and provides an opportunity to develop human-based regulatory compliant cryopreservation media for cell therapy applications.

Materials and Methods

Four cryopreservation media (CPA) formulations were tested in this study (Table 1). Using a Mr. Frosty freezer device, a freezing rate of 1 ˚C/min was employed for cryopreserving cells. After overnight storage at -86 ˚C, the cells were transferred to LN2 for extended storage. The rapid thawing was achieved by placing frozen vials in a 37 ˚C water bath for at least 2 min. For all experiments, the stem cells derived from adipose tissue (ASC) were used.

	DMSO concentration	Stemulate	Isolyte® solution
Stemulate-5	5%	50%	45%
Stemulate-10	10%	50%	40%
Isolyte-10	10%	-	90%
CS-10 (CryoStor®)	Preformulated 10% DMSO solution		

Table 1: Composition of the cryopreservation media tested

Viability

Cell viability was measured using a dual-fluorescence AO/PI kit on Nexcelom Cellometer®. Acridine orange (AO) and propidium iodide (PI) are nucleic acid binding dyes. AO is permeable to both live and dead cells, and stained all nucleated cells to generate green fluorescence. PI entered dead cells with compromised membranes and stained all dead nucleated cells to generate red fluorescence. Cells stained with both AO and PI fluoresced red, so all live nucleated cells fluoresced green and all dead nucleated cells fluoresced red.

Apoptosis Assay

Caspase-3/7 activities were measured using the Apo-ONE® Homogeneous Caspase-3/7 Assay kit (Promega) according to the manufacturer's protocol. For immediate post-thaw apoptosis assay, frozen-thawed ASC from each condition were plated in quintuplicate and were lysed with lysis-buffer-containing caspase substrate Z-DEVD-R100 and incubated at room temperature until being analyzed. Tor measure the delayed onset of apoptosis, the frozen-thawed cells were cultured for 24 hours and then the detached and attached cells were pooled and used for apoptosis analysis. Assays were measured by detection with a fluorescence microplate reader (BioTek Synergy™ HTX Multi-Mode Reader) and the fluorescence was measured at an excitation/emission wavelength of 485/535 nm. Results are presented as the mean ± the standard deviation of the triplicates.

Proliferation Assay

The post-thaw cells were assessed for proliferation at three time points (24, 48, and 72 hours) using CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega). Three separate plates were seeded for three time points tested, and with each time point a standard curve was created for analysis. The cells were seeded in quintuplicate. The measurement of the absorbance of the formazan was carried out using a 96 well microplate at 490 nm using a microplate reader (BioTek Synergy HTX Multi-Mode Reader).

Results

In the following figures, the error bars represent the standard error in the data. The horizontal bars denoted with asterisks (*) represent statistically significant differences (≤ 0.05) between the treatments.

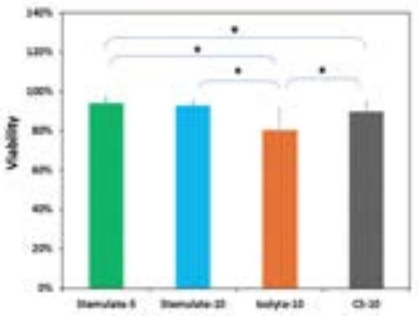

Figure 1: Immediate post-thaw viabilities of ASC in cryopreservation media with or without Stemulate. ASC cryopreserved using Stemulate showed significantly higher viabilities (92-94%) compared to Isolyte-10 (~80%) and CS-10 (~89%). The use of 5% or 10% DMSO in combination with Stemulate produced consistently similar viabilities.

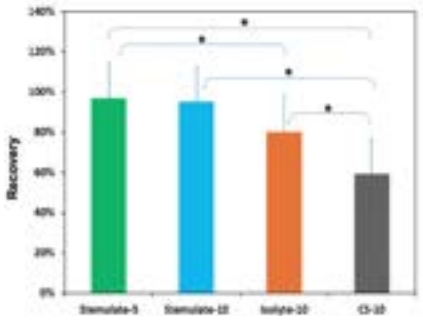

Figure 2: Immediate post-thaw recoveries of ASC in cryopreservation media with or without Stemulate. ASC cryopreserved using Stemulate showed significantly higher recoveries (~96%) compared to Isolyte-10 (~80%) and CS-10 (~60%). The use of 5% or 10% DMSO in combination with Stemulate produced similar recoveries.

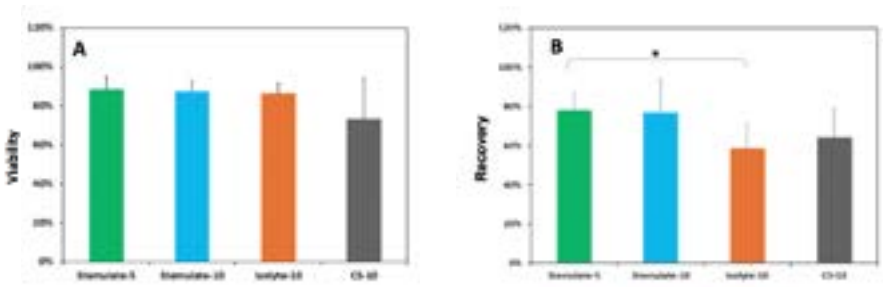

Figure 3: 24 hour post-thaw viabilities (A) and recoveries (B) of ASC in cryopreservation media with or without Stemulate. The use of 5% or 10% DMSO in combination with Stemulate produced similar viabilities and recoveries. The data show that the Stemulate based cryopreservation media provides > 90% cell survival following 24 hour post-thaw culture plating. Higher recoveries are observed with Stemulate-5 (~78%) and Stemulate-10 (76%) compared to Isolyte-10 (~58%) and CS-10 (64%).

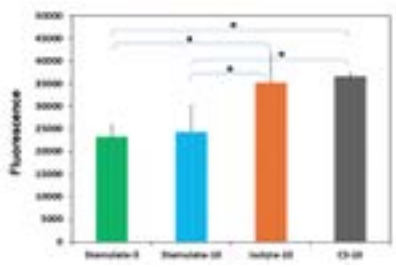

Figure 4: Assessment of immediate post-thaw apoptosis in ASC treated with cryopreservation media with or without Stemulate. The use of 5% or 10% DMSO in combination with Stemulate produced significantly lower apoptosis compared to Isolyte-10 and CS-10. Caspase-3/7 activity—immediately after thawing and following CPA removal—was carried out using Apo-ONE Homogeneous Caspase-3/7 Assay kit from Promega.

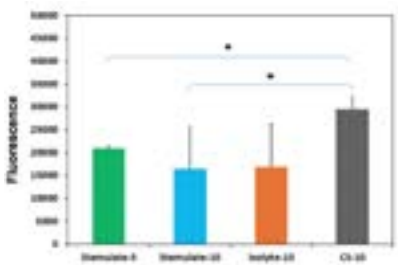

Figure 5: Assessment of 24 hour post-thaw apoptosis in ASC treated with cryopreservation media with or without Stemulate. The cells were cultured immediately after thawing and following CPA removal in standard culture media for 24 hours. After 24 hours, the floating cells, along with attached cells, were collected and analyzed for apoptosis using the Apo-ONE Homogeneous Caspase-3/7 Assay kit from Promega. The use of 5% or 10% DMSO in combination with Stemulate produced similar apoptosis but significantly lower than CS-10.

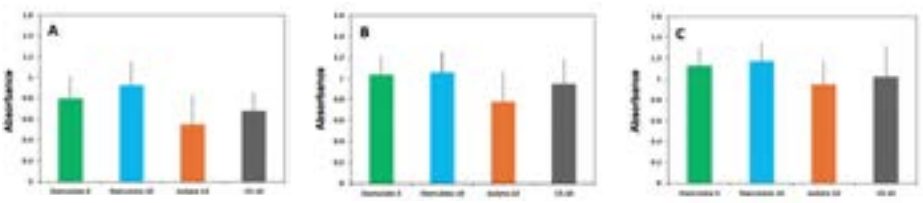

Figure 6: Post-thaw proliferation analysis of ASC treated with cryopreservation media with or without Stemulate. The cells were seeded in a 96 well microplate in standard cell culture media at 5000 cells/well immediately after thawing and following CPA removal. Proliferation of the cells at 24 hours (A), 48 hours (B) and 72 hours (C) was assessed using CellTiter 96 AQueous One Solution Cell Proliferation Assay. The use of 5% or 10% DMSO in combination with Stemulate produced a similar proliferation response compared to Isolyte-10 and CS-10.

Conclusions

1. In this study, a human platelet lysate product, Stemulate, was assessed in relation to its performance as a cryopreservation media additive in the presence of 5% and 10% DMSO.
2. The results indicate that the ASC cryopreserved in a medium supplemented with Stemulate show better viability and recovery than those preserved in the standard medium.
3. The presence of Stemulate during cryopreservation showed a significantly improved post-thaw performance of ASC compared to other standard media.
4. The current results show an alternative avenue to develop human-based regulatory compliant cryopreservation media for cell therapy applications.

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