A CRYOPRESERVATION SYSTEM FOR DIRECT CLINICAL USE OF MSC

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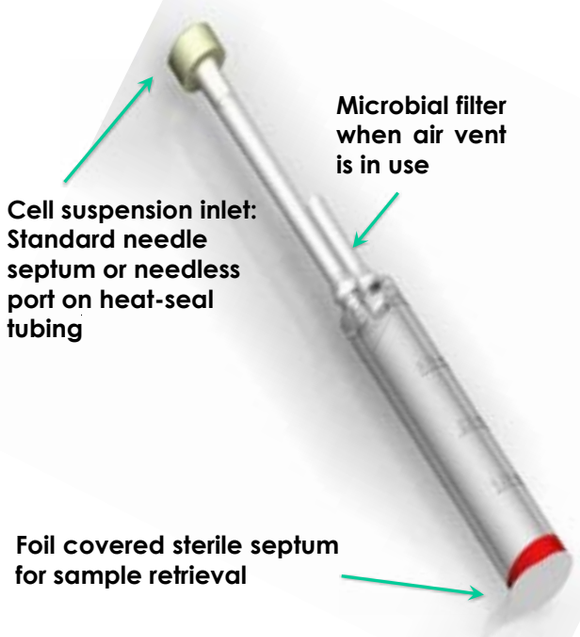
INTRODUCTION

For allogeneic "off-the-shelf" clinical transplantation or transfusion applications, a large number of frozen-stored MSC are usually required. Cryopreservation of these cells at concentrations commonly used in therapeutic procedures generates large product volumes containing undesirable quantities of DMSO. Freezing at high concentrations reduces the total amount of DMSO infused and subsequently reduces the incidence of infusion related toxicities.

The aim of the current study was to determine if cryopreserving a higher concentration of MSC at reduced DMSO concentration could have detrimental effects on the viability, stem cell characteristics and in vivo engraftment potential of adipose derived adult stem cells (ASC).

As a cryopreservation container, CellSeal® closed system cryogenic vial was used. The DMSO concentration for freezing was adjusted to a final concentration of 5% v/v. Cells at a concentration of 10 million/mL were cryopreserved to -80°C at 1 °C/min using a passive cooling system and transferred to LN2 for long term storage. The thawed cells were diluted and analyzed for viability and functionality.

CellSeal® CLOSED SYSTEM CRYOGENIC VIAL



- The CellSeal® line is a completely closed vial format device.
- CellSeal® vials are equipped with an inlet septum and microbial-barrier vent for easy fluid transfer.
- The lack of vacuum allows for complete fill capacity and easy retrieval.
- Inlet tubing can be sealed using any standard blood tubing sealer to create a closed system and also allows for multiple test segments.
- The vials are made from cyclic ollefin copolymer, are resistant to DMSO, other cryogenic materials and cryogenic temperatures.
- CellSeal® vials are specifically designed to meet the demands of the cell therapy industry.

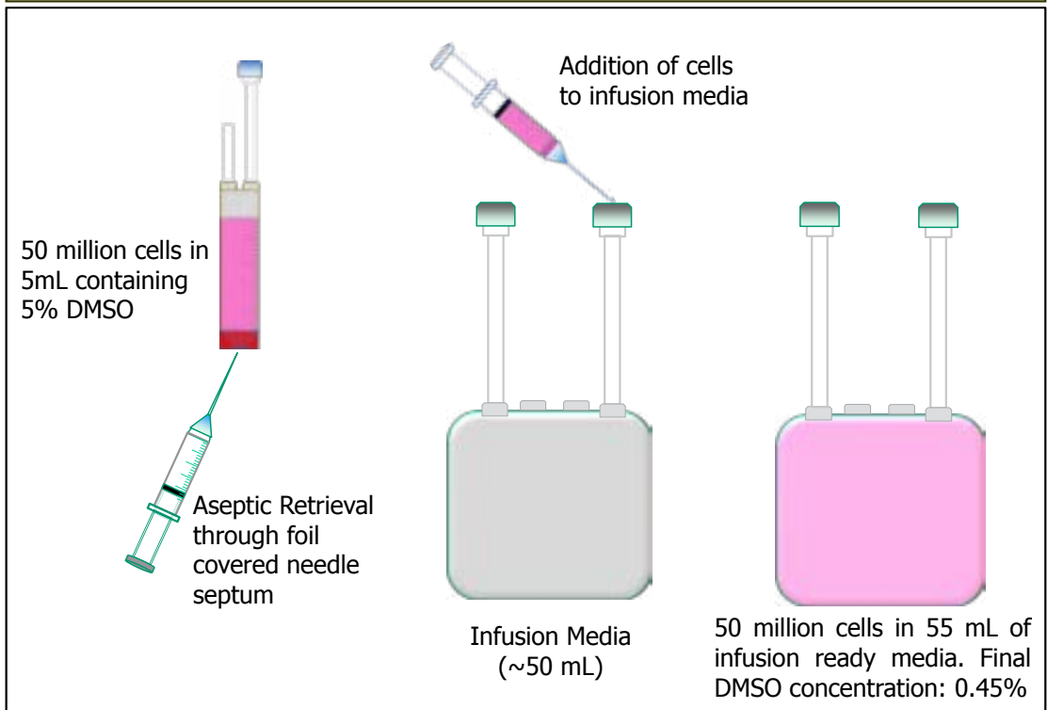


Figure 1: Proposed clinical protocol for use of cryopreserved ASC. Cells are stored in CellSeal® vials with 5% DMSO. Post thaw, cells can be diluted 1-step into isotonic infusion media without wash, resulting in a final DMSO concentration of 0.45% with no cell loss from centrifugation or washing.

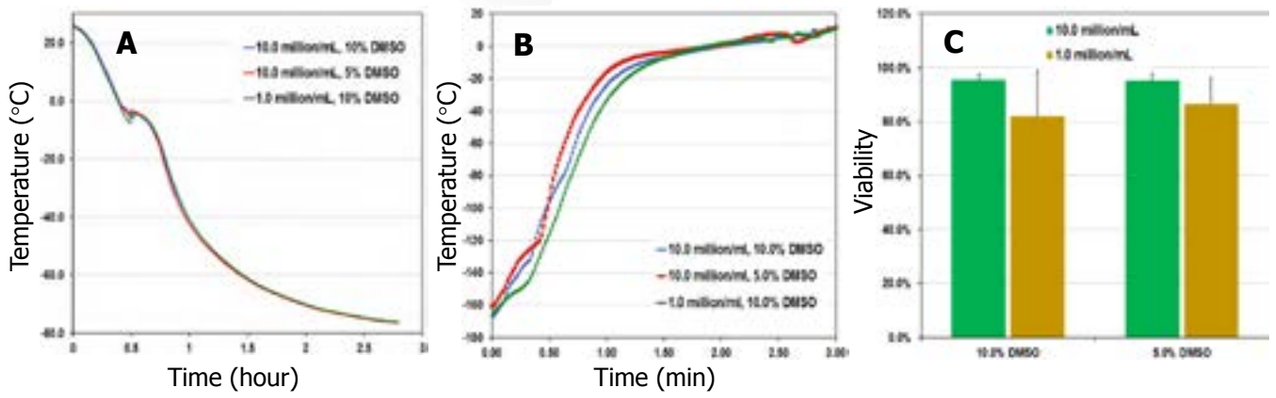


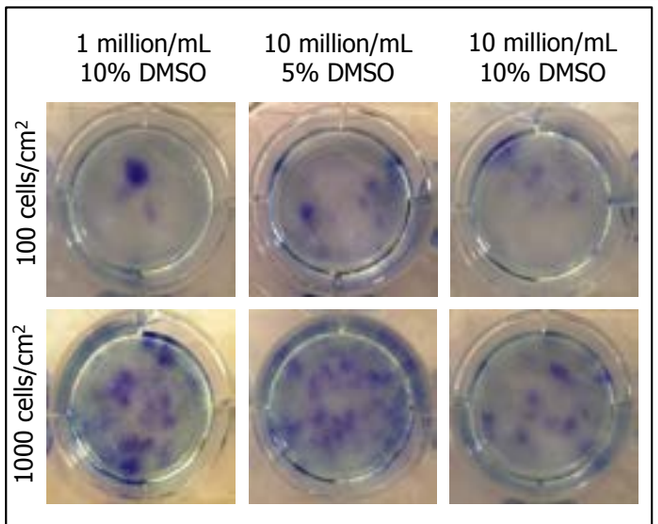
Figure 2: A & B: Temperature-Time history experienced by the cells during passive freezing in a -85 °C freezer (A) and during warming in a 37 °C water bath (B) in CellSeal® cryogenic vials when cryopreserved at different cell and CPA (DMSO) concentrations. **C:** The immediate post-thaw viability of ASC in CellSeal® vials when cryopreserved at different cell and CPA concentrations after a one-week long storage at LN2 temperatures.

Cell Freezing Concentration	Post-Thaw Viability (%)	
	10.0% DMSO	5.0% DMSO
10.0 million/mL	95.5 (2.0)	95.5 (±2.6)
1.0 million/mL	82.0 (±17.0)	86.7 (±9.4)

Table 1: Immediate post-thaw viabilities

Cell Freezing Concentration	Post-thaw cell number recovery (million/mL)	
	10.0% DMSO	5.0% DMSO
10.0 million/mL	9.3 (±0.51)	9.2 (±1.6)
1.0 million/mL	0.92 (±0.1)	0.96 (±0.08)

Table 2: Immediate post-thaw cell number recoveries



Seeding Concentration (cells/cm²)	1.0 Million/mL, 10% DMSO	10.0 Million/mL 5% DMSO	10.0 Million/mL 10% DMSO
100	4.2 (±2.4)	3.9 (±1.8)	4.7 (±1.5)
1000	32.2 (±8)	35 (±13)	29.8 (±10.1)

Figure 3 & Table 3: Clonogenic (CFU-F) capacities of post-thawed ASC cryopreserved at different cell number and CPA concentrations.

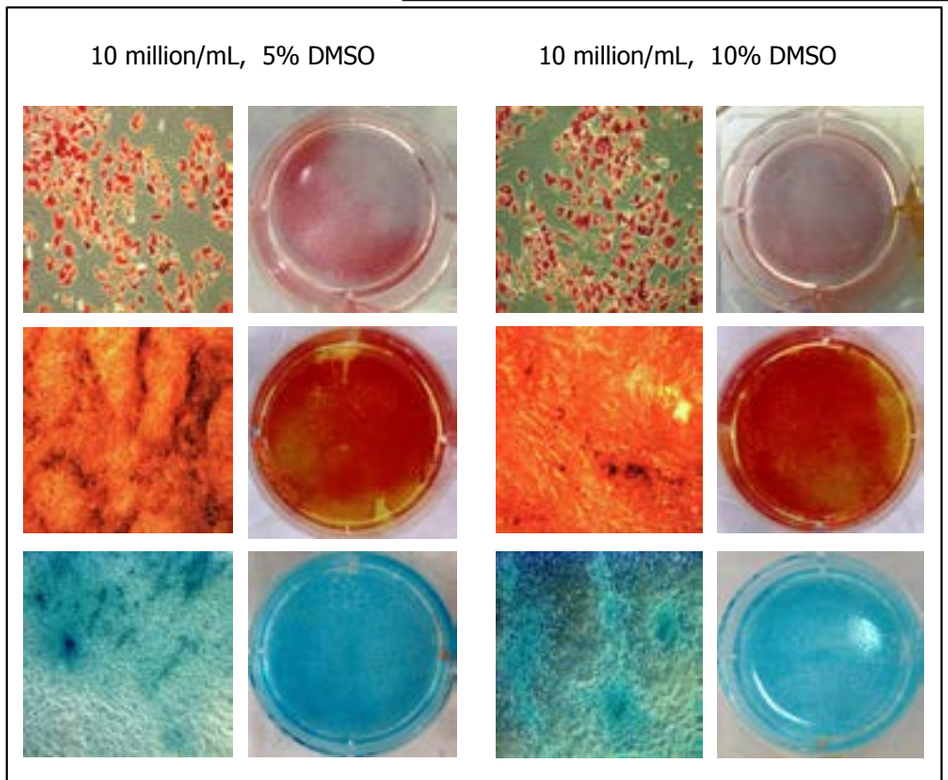


Figure 4: Differentiation potential of post-thawed ASC cryopreserved at different cell number and CPA concentrations. Adipogenesis (top row), Osteogenesis (middle row) and Chondrogenesis (bottom row).

Freezing Condition	CD34+	CD45+	CD73+	CD90+	CD105+
5% DMSO	3.1	0.0	94.3	98.8	98.8
10%DMSO	2.5	0.0	92.1	98.9	99.4

Table 4: Surface phenotypic expression (percent positive by flow cytometry) of frozen-thawed ASC cryopreserved at 10 million/mL concentration.

CONCLUSIONS:

- ASC cryopreserved and recovered at concentrations of 10 million cells/mL in a 5% DMSO cryopreservative exhibited high functional viability
- A system has been described that would allow for easy bedside thaw and administration of doses of therapeutic cell doses with minimal residual DMSO without the need for cell washing
- Further studies include examining the engraftment potential of post-thaw ASC *in vivo*