A NEW CRYOGENIC CONTAINER SYSTEM FOR CLINICAL CRYOPRESERVATION OF BIOMATERIALS

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INTRODUCTION

The use of aseptic closed systems for processing, storage and transfer of cell therapy products is recommended as per the basic principles of good manufacturing practices (GMP). We recently designed and evaluated a new closed system sterile device (CellSeal® cryogenic vials, Cook General BioTechnology LLC, IN, USA) for secure cryopreservation and storage of cell therapy products at cryogenic temperatures (Figure 1).

DURABILITY AND INTEGRITY:

The CellSeal® vials were tested for durability and integrity utilizing a 1-meter drop test. In addition, frozen, sealed vials were transported to a test laboratory in liquid nitrogen and tested using pharmaceutical packaging tests including dye ingress and microbial challenge. The results of all tests indicated container closure integrity of the vials with no failures.

PERFORMANCE:

Human umbilical cord blood hematopoietic progenitor cells (HPC-cord) and mesenchymal stem cells from dental pulp (DPSCs) and Endometrium (ERCs) were used to establish the performance of the vials. In addition, the temperature-time history experienced by the cells in CellSeal® vials during dump freezing in a -85 ºC freezer and during thawing in a 37ºC water bath were compared with that of routinely used Corning® cryo-vials (Figure 2A & 2B). For cryopreservation storage of HPC-cord, the performance was evaluated through analysis of viability of total nucleated cells/ml, CD34+ cells, CD45+ cells and total colony forming units (CFU) and was compared with cells frozen using Pall® Medical cord blood freezing bags (Table 1&2). A total of five donor cord bloods (UCB) were used in this study. For DPSCs and ERCs, the cell attachment, expansion potential and viability of cells cryopreserved in CellSeal® vials were compared with that of cells cryopreserved in Corning® cryo-vials (Figure 3 and Figure 4).

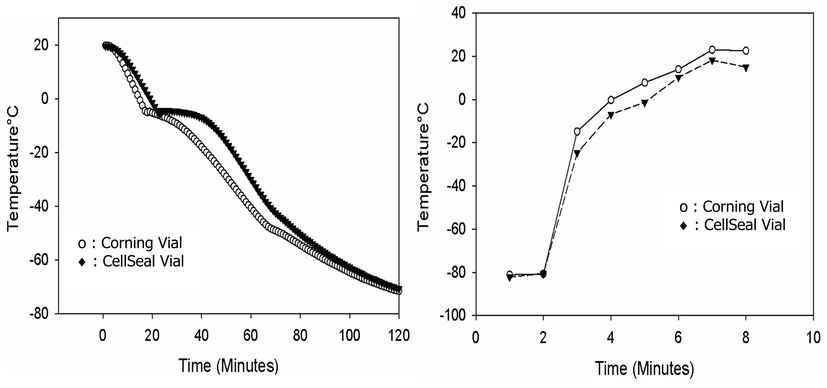

Figure 2: Temperature-Time history experienced by the cells during dump freezing in a -85 °C freezer (A) and during warming in a 37 °C water bath (B) in CellSeal® and Corning® cryo-vials when 10% DMSO was used as CPA.

CellSeal® Closed System Cryogenic Vial

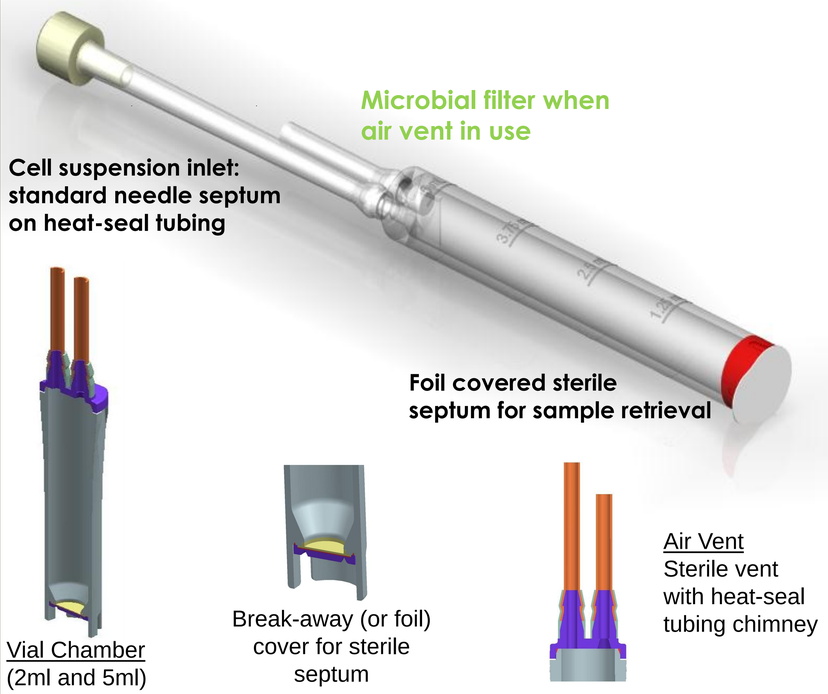

Figure 1: Concept of CellSeal® Cryogenic Vial

CellSeal® Vial Specifications:

- Equipped with an inlet septum and microbial-barrier vent for easy fluid transfer.
- The ability to transfer fluids/cells from any device with a sterile syringe.
- Lack of vacuum allows for complete fill capacity.
- Inlet tubing can be sealed using any standard blood tubing sealer to create closed system.
- Inlet tubing allows for multiple test segments.
- Retrieval septum is conical in shape allowing complete easy removal of sample.
- No vacuum so retrieval is easy and causes no damage to cells because of vacuum compression.
- Completely submergible.
- Made from state of the art materials resistant to DMSO, other cryogenic materials and cryogenic temperatures.
- 2 mL and 5 mL vials in clinical and research grade.
- Septum or Needle-free fill port available.
- Storage in boxes or on canes is convenient and readily achieved for all dewar style storage units.
- Specifically designed and patented to meet the demands of cell therapy industry.

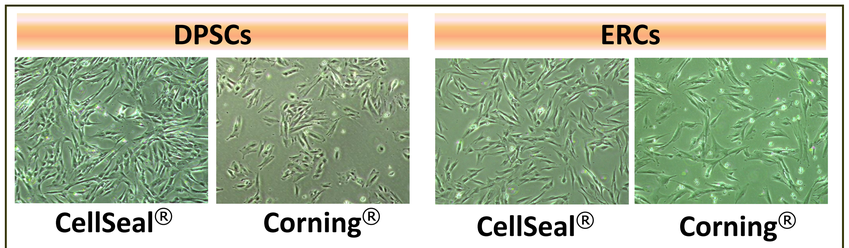

Figure 3: Photomicrographs showing the attachment and expansion of DPSCs and ERCs cryopreserved in CellSeal® and Corning® cryovials.

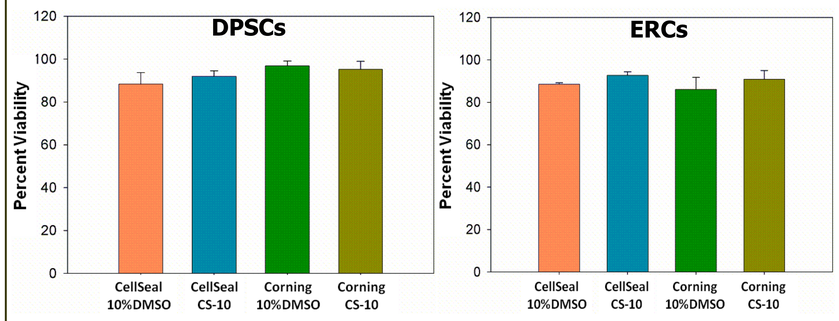

Figure 4: The post-thaw viability of DPSCs and ERCs in CellSeal® & Corning® vials when cryopreserved with 10% DMSO and Cryostor® (CS-10) as CPAs.

	%Viability	%CD34+	%CD45	TNC/ml
Pre-Freeze	90.5 (±4.8)	0.6 (±0.4)	68.1 (±15.9)	2.4×10^7 (± 4.5×10^6)
Pall Bag	90.6 (±4.8)	1.0 (±0.2)	67.2 (±16.9)	1.1×10^7 (3.2×10^6)
CellSeal	89.1 (±3.2)	1.2 (±0.7)	71.3 (±10.7)	1.1×10^7 (2.5×10^6)

Table 1: Cellular viabilities of post-thawed HPC-cord cryopreserved in CellSeal™ vial and Pall® Medical freezing bag.

	Pre-Freeze	Post-Freeze	
	UCB	Pall Bag	CellSeal
CFU-E	5.4 (±3.6)	6.4 (±1.8)	5.4 (±1.8)
CFU-GM	70.8 (±9.3)	49.6 (±11.7)	61 (±14.9)
BFU-E	69.8 (±20.6)	47.2 (±15.3)	54.4 (±15.7)
CFU-GEMM	6.2 (±1.1)	3.2 (±2.2)	3.4 (±0.5)
Total	152.2 (±28.7)	106.4 (±27.4)	124.2 (±28.8)

Table 2: Clonogenic capacities of post-thawed HPC-cord cryopreserved in CellSeal® vial and Pall® Medical freezing bag.

CONCLUSIONS

- Development of a closed system cryogenic device for storage and shipping of biomaterials to clinical applications.
- No significant difference was observed in terms of cooling and thawing rates experienced by the cells in CellSeal® and viability post-thaw when compared to control vials (Corning® vials).
- No significant difference in the performance of CellSeal® when compared to Pall® Medical Freezing bag.