AN ASEPTIC CLOSED VIAL SYSTEM FOR CRYOPRESERVATION AND STORAGE OF BIOMATERIALS

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ABSTRACT

Current good manufacturing practices recommend the cryopreservation storage of cell therapy products in a closed system to prevent any possible contamination or infection during freezing, storage, thawing and shipping. We recently developed and tested a new kind of closed sterile cell cryopreservation device (CCD) for the robust prevention of microorganism and a secure control over sterility during routine cryopreservation and storage of clinically relevant cellular products at cryopreservation temperatures. The design of the CCD has two ports: One port consists of a needle septum at the end of a length of tubing integrally attached to the vial body. This needle septum port is sealed after introduction of the sample into the CCD by standard blood bag tubing sealer. The second port is a foil covered sterile septum at the bottom of the device that can be used to extract the stored products using a sterile syringe. The drawing in Figure 1 shows a concept and various components of the CCD. The design of the CCD can also be modified to accommodate a small, free-moving tube with an internal cholesterol coating to accelerate the formation of ice crystals within the CCD.

Mesenchymal stem cells from dental pulp (DPSCs) and endometrium (ERCs) were used as a test to validate of the CCD. The temperature-time history experienced by the cells in CCD during dump freezing in a -85 ºC freezer were compared with that of cells in routinely used Corning cryo-vials (Figure 3A). The thawing rates experienced by the cells in a 37 ºC water bath were also compared (Figure 3B). The data suggested that although the cells were subjected to different cooling/thawing rates at different time points in both CCD and Corning cryo-vials, the average cooling/thawing rates experienced by them were statistically similar. Furthermore, the preliminary analysis showed no significant variation in cell attachment, expansion potential and immediate post-thaw viability of DPSCs and ERCs cryopreserved in CCD when compared to Corning cryo-vials (Figure 5). For all the cryopreservation experiments, both 10%DMSO and 10%Cryostor (CS-10) (Biolife solutions, USA) were used as CPAs.

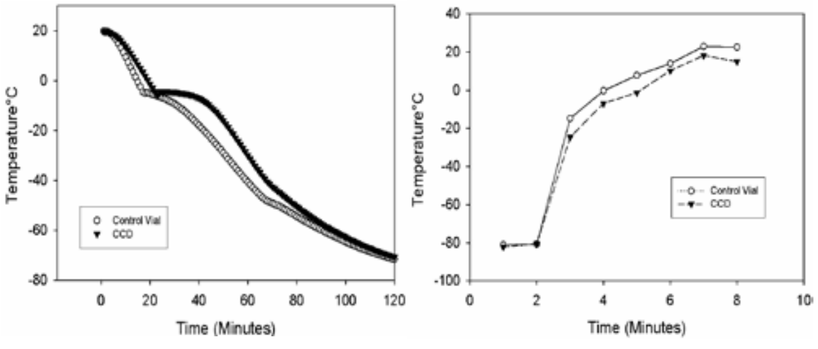

Figure 3: A: Temperature-Time history experienced by the cells in CCD and Corning™ cryo-vials when 10%DMSO was used as CPA. B: The post-thaw viability of DPSCs in CCD and control vials (Corning™ vials) when cryopreserved with 10%DMSO and Cryostor™ (CS-10) as CPAs

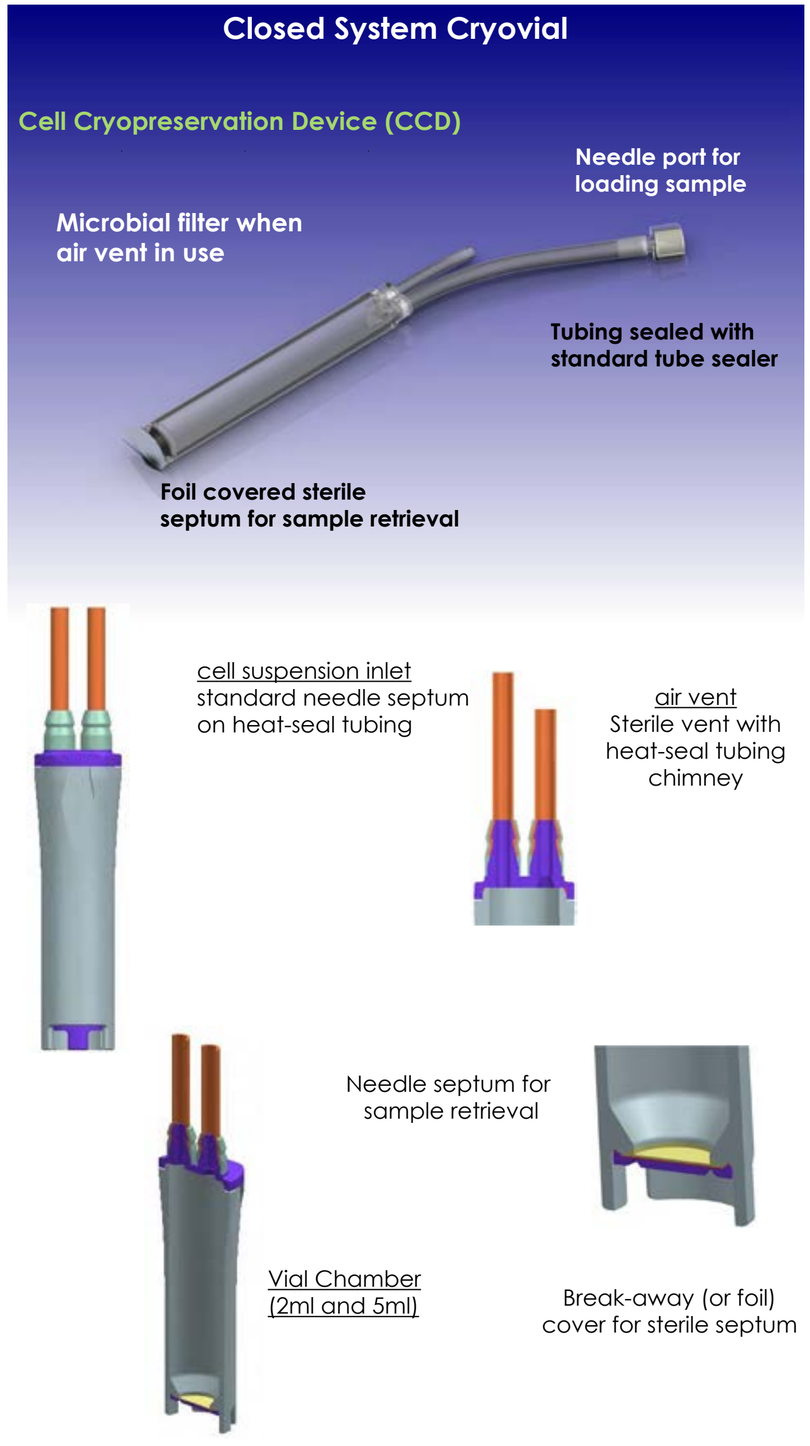

Figure 1: Concept of Cell Cryopreservation Device (CCD)

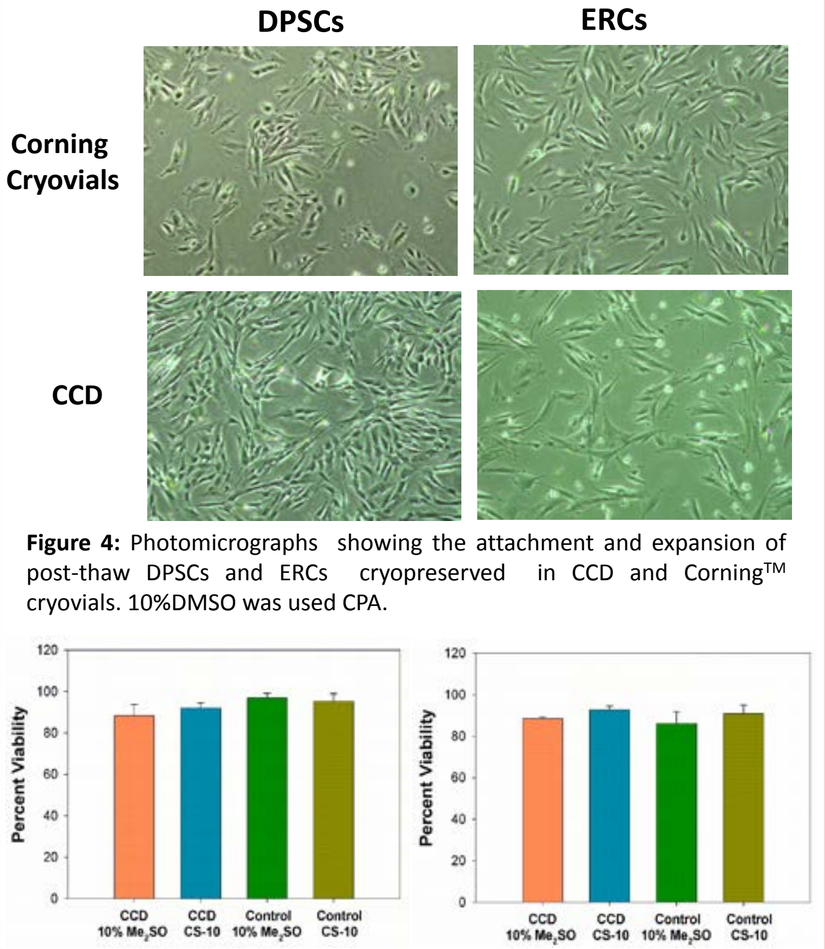

Figure 4: Photomicrographs showing the attachment and expansion of post-thaw DPSCs and ERCs cryopreserved in CCD and Corning™ cryovials. 10%DMSO was used CPA.

Figure 5: The immediate post-thaw viability of DPSCs and ERCs in CCD and control vials (CorningTM vials) when cryopreserved with 10%DMSO and CryostorTM (CS-10) as CPAs

CONCLUSIONS

- Development of a closed cryopreservation 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 CCD and viability post-thaw when compared to control vials (Corning™ vials)
- Ongoing studies on the CCD include mechanical evaluation through drop tests, sterility maintenance tests, dye ingress and microbial challenge tests to further validate the suitability of CCD for clinical banking of cell therapy products
- Further studies also include the gene marker analysis of the MSCs derived from several tissue sources and cryopreserved in CCD to several time points