

A NOVEL CONTAINER SYSTEM FOR CELL THERAPY PRODUCTS

Erik J. Woods^{1,2}, Aniruddha Bagchi¹, Robert Nase³ and Vinod Vilivalam³

¹General BioTechnology, LLC, Indianapolis, IN, USA,

²Department of Microbiology and Immunology,

Indiana University School of Medicine, Indianapolis, IN, USA;

and ³West Pharmaceutical Services, Inc., Lionville, PA, USA

General
BioTechnology LLC



ABSTRACT

Purpose

This study investigated a novel plastic container system for storing and shipping cell therapy products at low temperature (-85°C) or cryopreserved (-196°C). This study investigated the suitability of Daikyo Crystal Zenith® (CZ) plastic vials, made of cyclic olefin polymers, to contain, store and transport biopharmaceutical preparations at these low temperatures.

Methods

CZ vials (0.5, 5.0 and 30mL volume) with several closure systems were filled with mesenchymal stem cell (MSC) line and stored at either -85°C or -196°C for various time intervals. Vials were tested for (a) durability and integrity of filled vial utilizing a 1-meter drop test, (b) ability to maintain viability and functionality of cryopreserved cells and (c) thermal transfer efficiency of the material upon thaw in a 37°C water bath. As controls, filled polypropylene vials were used under same conditions.

Results

(a) Drop test: No evidence of external damage was found on vial surfaces and no cracks or damage was seen on closure systems. Dye immersion studies using spectro-photometer measurements indicated container durability (break-resistant) with no failures. Temperature and time of storage had no effect on the durability (break-resistant) of vials after a drop test.

(b) Cell preservation: Post-thaw viability utilizing dye exclusion assay was >95% in all samples. Stored cells exhibited rapid recovery 2 hours post-thaw and cultures were ~70% confluent within 5-7 days, consistent with non-frozen controls and indicative of functional recovery. Doubling times were consistent over all vials. Doubling rates for CZ vials were 2.14±0.83 days (1 week), 1.84±0.68 days (1 month), and 1.79±0.71 (6 months) compared to frozen controls of 2.70±0.85 days (1 week), 1.99±0.49 days (1 month) and 1.68±0.85 (6 months) and fresh controls of 2.160±32 days. No statistically significant difference (p>0.05) was observed.

(c) Thermal transfer: The average thaw times for 0.5, 5.0 and 30mL vials were ~4, 5 and 15 mins. Control vials (1.8mL polypropylene) exhibited a thaw time of 3 mins.

Conclusions

The CZ vial container system is suitable for low-temperature frozen and cryopreserved storage of cell products. Vials were durable and allowed for preservation and maintenance of cell viability and functionality. CZ vials are optically very clear, have improved extractable profile, and lower permeability of gas and moisture compared to polypropylene.

BACKGROUND

Stem cells and CZ container system:

As research in stem cells translates into commercial products, one major obstacle in bringing cell-based products into the market is the need for quality packaging and storage systems for biological materials. A highly promising alternative to standard polypropylene cryovials is Daikyo Crystal Zenith (CZ) vials by Daikyo Seiko, Ltd. CZ is a proprietary cyclic olefin polymer material that has been developed as a packaging system for pharmaceutical and biopharmaceutical preparations and medical devices. CZ has a number of properties which make it highly suitable for packaging cell-based systems under cold and cryogenic conditions. CZ material is hydrophobic and has excellent drainability. It is compatible with a wide range of pH (2-12) and solvents such as alcohols, ketones and cellosolves. CZ also has excellent thermal characteristics. It can withstand cryogenic temperatures and be sterilized at autoclave temperatures. CZ has lower gas and moisture permeability compared with other plastics, is optically clear to allow for easy inspection of contents and has higher impact strength.

The purpose of this study was to evaluate the suitability of a CZ vial container system for storage of cell systems during cold storage and cryopreservation.

OBJECTIVES

- Determine if the CZ vial system maintained durability (break-resistant) following a drop test after frozen (-85°C) or cryopreserved (-196°C) storage.
- Determine if the CZ vial system could maintain viability and functionality of a cryopreserved cell line.
- Characterize thermal properties of vials based on visual observation of time-to-thaw.

MATERIALS AND METHODS

CZ vials and closure systems

Three CZ vial sizes (0.5, 5.0 and 30mL capacity) provided steam sterilized and each with specific closures (two closure types for the 0.5mL and 30mL and 3 closure types for the 5.0mL) were evaluated in all experiments (**FIGURE 1**). Three time points (1 week, 1 month and 6 months) at each temperature (-85°C and -196°C) were evaluated. Vials closed with stoppers were capped with 13mm aluminum crimped-caps (0.5mL vials) or 20mm polypropylene crimped caps (5.0mL vials). For comparison, standard polypropylene cryovials were used as controls. For each vial/stopper configuration, an equal number of vials were tested in standard configuration vs. overwrapped with a polyolefin membrane.

Drop test

Each vial was filled to 60% capacity with a 10% solution of DMSO in PBS (Sigma-Aldrich, St. Louis, MO). Half the vials of each type were overwrapped as described above; three replicates were prepared for each storage condition. Vials were then cryopreserved as described above. At appropriate time points, vials were dropped, while still frozen, from 1 meter on a standard laboratory floor at an angle of about 15° to the vertical. After the drop, the vials were thawed, visually inspected and subjected to a dye penetration test by immersion in a 10% solution (v/v) of FD&C Red No. 40 dye for 1 hour, and the contents checked using a spectrophotometer.

Cell preservation

A multipotent mesenchymal stromal cell line derived from human teeth was used for these experiments. Cells were frozen using GBT's standard cryopreservation protocol for this cell type, which consisted of a two-step equilibration to

a final concentration of 10% dimethyl sulfoxide (DMSO; Cryoserv, Edwards Lifesciences, Irvine, CA) prepared in complete Mesencult™ medium (Stem Cell Technologies, Vancouver, Canada). Next, all vials were cooled at -1°C/min within a -85°C mechanical freezer (VIP Series Ultra-Low Temperature Freezer, Sanyo Scientific, Bensenville, IL). Once frozen, half of the vials were removed from the -85°C mechanical freezer and plunged into a liquid nitrogen (LN2) storage tank for storage at -196°C (Cryomed CryoPlus II, Thermo Fisher Scientific, Waltham, MA). Vials then remained in either -85°C or -196°C storage for the specified time points.

At the appropriate time points (1 week, 1 month or 6 months) samples were retrieved from -85°C or -196°C storage and thawed in a 37°C water bath. The cells were then carefully washed to remove the DMSO. Because it was initially unknown how the cells would survive freezing in the CZ vials, for the initial 1-week time point, all of the cells from a vial were put into culture in a T-25 flask. For the subsequent 1-month and 6-month evaluations, cell count was normalized per flask from each vial size. Initial viability post-thaw was measured using a standard trypan blue dye exclusion assay. To measure functionality, time to confluence and doubling rates were measured using standard cell culture technique.

Thermal transfer

To gain an estimate of thermal transfer characteristics of CZ, time until thaw was measured for each vial type. For this, time was recorded from the point at which the frozen vials were first placed into a 37°C water bath until the last ice just melted.



Figure 1. Vial configurations for (A) non-wrapped (left) and wrapped (right) 0.5mL, (B) non-wrapped (left) and wrapped (right) 5.0mL, and (C) non-wrapped (left) and wrapped (right) 30mL (Note: the 30mL overwrap was translucent while the overwrap for the smaller vials was opaque.)

RESULTS

Drop test

Drop tests indicated that the freezing process did not affect the mechanical strength and durability (break-resistant) of the CZ vials or their closures (**TABLE 1**). After the initial drops, no evidence of any gross external damage was found on the vial surface. Neither was any noticeable cracking or damage seen on the caps. Also, no differences were seen between wrapped and unwrapped vials. The temperature or time of storage also did not seem to have any effect on the strength and durability (break-resistant) of the vials and their closures. The only noticeable observation was that the caps for the 5.0mL vials were prone to breaking off during drops.

Using dye ingress tests, following immersion in the dye, no noticeable evidence of dye ingress was seen. The solution inside the vials was optically clear, and no absorbance was detected by the spectrophotometer compared with control. These observations were consistent through all vials under all storage conditions, indicating temperature and time of storage did not affect the durability (break-resistant) of the vials.

Thermal transfer

Visually observed thaw times ranged from 4 to 15 minutes depending on the vial size and were comparable to polypropylene controls.

Cell preservation: Viability and functionality tests

Post-thaw viability based on trypan blue dye exclusion assay was $98.5 \pm 1.31\%$ (mean \pm SD) over all samples. There were no significant differences among vial type, closure type and temperature of storage or time of storage. Frozen-thawed cells exhibited rapid recovery, with cells beginning to stick and regain morphology in as little as 2 hours post-thaw. All cultures went on to become ~70% confluence within 5-7 days, consistent with non-frozen controls, and indicative of functional recovery (**Figure 2**), and no contamination was evident in any of the cultures. Doubling times were consistent over all vials and all closure types. Comparisons among vial size, temperature of storage and duration of storage revealed no significant difference (**Figure 3**) with all results very similar to the control frozen-thawed cells.

Storage temperature: -85°C

Vial Size	1 Week	1 Month	6 Months
0.5mL	No damage	No damage	No damage
5.0mL	No damage to vials, some caps popped off on unwrapped vials. Dye penetration negative confirmed by spectrophotometry.	No damage to vials, some caps popped off on unwrapped vials. Dye penetration negative confirmed by spectrophotometry.	No damage to vials, some caps popped off on unwrapped vials. Dye penetration negative confirmed by spectrophotometry.
30mL	No damage	No damage	No damage

Storage temperature: -196°C

Vial Size	1 Week	1 Month	6 Months
0.5mL	No damage	No damage	No damage
5.0mL	No damage to vials, some caps popped off on unwrapped vials. Dye penetration negative confirmed by spectrophotometry.	No damage to vials, some caps popped off on unwrapped vials. Dye penetration negative confirmed by spectrophotometry.	No damage to vials, some caps popped off on unwrapped vials. Dye penetration negative confirmed by spectrophotometry.
30mL	No damage	No damage	No damage

Table 1. Drop test results for vials stored at (A) -85°C and (B) -196°C. Results were determined by direct observation and through spectrophotometric analysis of the solution after the test for all samples at week one and only for samples with apparent damage for other time points.

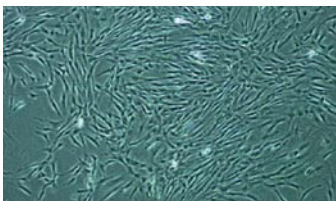


Figure 2. Cultures from frozen-thawed dental pulp derived MSCs. Cells were frozen in 5mL CZ vials after 7 days of culture, and stored for 1 month at -196°C. Cells began to stick after 2 hours, and all cultures became ~70% confluent in 5-7 days, consistent with the frozen-thawed and fresh controls. Additionally, no cultures showed any evidence of contamination.

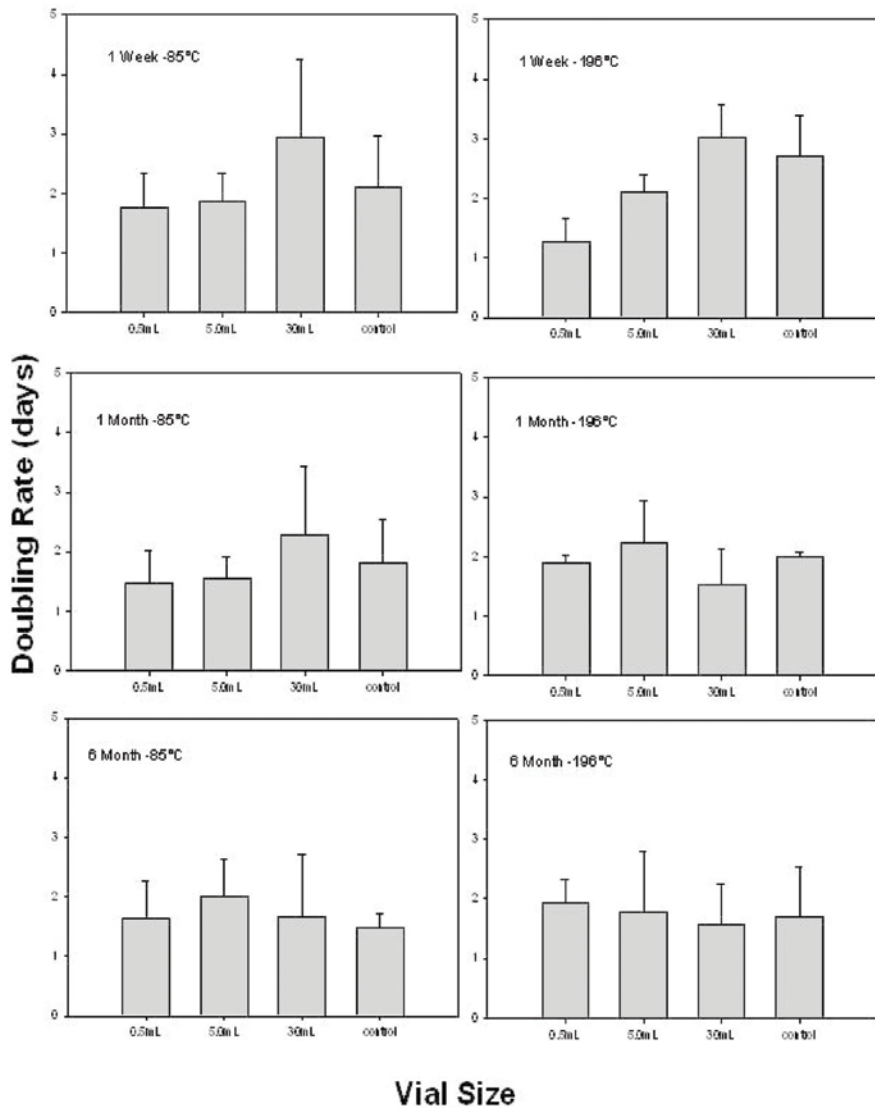


Figure 3. Doubling times for cells frozen in each vial/each condition after 1 week, 1 month or 6 months of storage. Values are expressed as means \pm SD. Controls were cells frozen in standard 2mL polypropylene vials. No significant differences between groups were observed.

CONCLUSIONS

- CZ vial container systems are highly suitable as cell-preservation systems either at frozen (-85°C) or cryopreserved (-196°C) storage.
- CZ vials are durable (break resistant) after storage at frozen or cryogenic temperatures, determined by the drop test.
- Time to thaw for cells stored in CZ vials ranged from 4 to 15 minutes, depending on vial size and were comparable to controls.
- Dental pulp derived MSCs can be stored in CZ vials at these temperatures for at least 6 months with successful viability and functionality over the time points tested.

Contact West's Technical Customer Support
TCS.Americas@westpharma.com
+1 800-231-3000
TCS.Europe-AsiaPacific@westpharma.com
+49 (2403) 7960