Assessment of SmartThaw™, a novel dry thawing system for cryopreserved cell products

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Abstract

Thawing cryopreserved cell products in a 37°C water bath is standard practice in most laboratories and clinical settings. Water baths are also a major source of contamination given their access to open air and warm, humid environment. The utilization of a device that provides a clean, safe, and reproducible thaw for a variety of container types would be optimal. We compared the viability of multiple cell types thawed in either a standard water bath or novel dry thawing system (SmartThaw™). Multiple cell types were tested, including Chinese hamster ovary (CHO) cells, umbilical artery smooth muscle (UASMC) cells, and human umbilical vein endothelial (HUVEC) cells. The data demonstrate that SmartThaw yielded similar or better results upon post-thaw assessments in standard cryovials as well as 25ml cryostore (CS25) bags. These results support the use of the SmartThaw™ system as an alternative to water baths.

Introduction

Standard cryopreservation practices dictate the use of CPA’s in supportive media solution with a controlled slow freeze process and a rapid rate of thaw to avoid intracellular ice and obtain viable samples1,2. Ongoing efforts are being made to reduce and remove open water sources in clinical and cellular manufacturing settings. Thawing CP samples currently requires the use of a water bath and user manipulation. Additionally, the requirement of continual sample agitation within a water bath is subject to subtle differences between users. As such, SmartThaw™ was designed to provide a clean, safe, and reproducible thaw every time, for a variety of commonly utilized container types3. A user friendly interface gives step by step instructions while still allowing for individualized programming flexibility. The ability to track and record the thermal profile of a sample throughout the thawing process gives a level of security to users who require documentation. SmartThaw’s gentle oscillation ensures complete sample mixing in a consistent manner, without the need for constant user manipulation.

Results

Table 1. Day 1 viability data of various cell lines thawed in cryovials using water bath or SmartThaw. alamarBlue data is presented as % of control values ± SD converted from raw fluorescent units.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Water Bath (%)</th>
<th>SmartThaw (%)</th>
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<tbody>
<tr>
<td>CHO</td>
<td>82.7 ± 5.7</td>
<td>85.4 ± 6.2</td>
</tr>
<tr>
<td>HUVEC</td>
<td>67.8 ± 12.9</td>
<td>65.6 ± 16.4</td>
</tr>
<tr>
<td>UASMC</td>
<td>75.1 ± 5.4</td>
<td>72.3 ± 3.9</td>
</tr>
<tr>
<td>hMSC</td>
<td>60.4 ± 10.0</td>
<td>58.8 ± 6.6</td>
</tr>
</tbody>
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Figure 1. SmartThaw device and tablet controller (A), SmartThaw pillow view with CS25 bag and thermocouple (B).

Figure 2. Recovery of UASMC and CHO cells thawed in 2ml cryovials. Data obtained with alamarBlue for 3 days post-thaw revealed similar viability between samples thawed with SmartThaw (pillows set to 45°C) or 37°C water bath.

Figure 3. Recovery of HUVEC cells thawed in 2ml cryovials or CS25 bag. Data obtained with alamarBlue for 3 days post-thaw revealed similar viability between samples thawed with SmartThaw (pillows set to 45°C for vials and 40°C for bags) or 37°C water bath.

Figure 4. Comparison of thawing profiles between SmartThaw and standard water bath. A) 2ml cryovials containing 1ml media with 10% DMSO were thawed in a 37°C water bath or in SmartThaw with pillows set to 45°C

Materials and Methods

Cell Culture: Chinese hamster ovary (CHO) cells were maintained in RMP1 1640 (Caisson) with 10% fetal bovine serum (FBS, Atlanta Biologicals) and 1% penicillin/streptomycin (Caisson); human umbilical vein endothelial cells (HUVEC) were maintained in Endothelial cell growth medium (EBM Bulletkit, Lonza); umbilical artery smooth muscle (UASMC) cells were cultured in smooth muscle cell growth medium (SmGm-2 Bulletkit, Lonza). All cultures were maintained in a standard 37°C/5% CO2 incubator.

Cryopreservation: All cell samples were cryopreserved in their respective media with 10% DMSO as a cryoprotective agent (CPA). Cell pellets were resuspended at 1 x 106 cells/ml in CP solutions at 10°C. Cell suspensions were then placed into cryovials (1 ml/vial, VWR) or CS25 freeze bags (18 ml/bag, GrilGen Biomedical) and frozen under a standard controlled rate protocol of ~1°C min-1 to ~80°C, and stored at liquid nitrogen (LN2) temperatures (~196°C). Following storage, cells were rapidly thawed in a 37°C water bath or SmartThaw™ until ice dissipated and samples were cold (~0-4°C). A one-step dilution (1:10) in growth medium was performed, and then 100μl of cell suspension was plated onto individual wells of a 96-well tissue culture plate (TrueLine) and placed into standard culture conditions (37°C, 5% CO2) for recovery and assessment. Growth medium was replenished in the cultures at 24-h intervals for cell survival assays. Viable assay: The metabolic activity assay alamarBlue™ (Invitrogen) was utilized to assess cell viability. Cell culture medium was aspirated from the 96-well plates and 100 μl/well of the working alamarBlue™ solution (1:20 dilution in HBSS) was applied. Samples were then incubated for 60 minutes (± 1 min) at 37°C in the dark. The fluorescence levels were analyzed using a Tecan SPECTRAFluorPlus plate reader (TECAN, Austria GmbH). Relative fluorescence units were converted to a percentage compared to nonthawed controls set at 100%. Viability measurements were taken at 1, 2 and 3 days of recovery.

Data Analysis: Viability experiments were repeated a minimum of three times with an intra-experiment repeat of seven replicates (N=23, n=21 per condition). Thermal profile evaluations were conducted on a minimum of three separate experiments for each container type using an OmegaHH806AU reader. Standard errors were calculated for viability values and single-factor analysis of variance (ANOVA) was utilized to determine statistical significance (Microsoft Excel).

Summary of Results

• In most cell types and container formats tested, no significant difference in post thaw viability was observed between SmartThaw or water bath thawed samples (P>0.05)
• 10% improvement in viability was observed in CHO cells thawed in SmartThaw compared to water bath in cryovials (P<0.4)
• A number of cell types have been utilized, including some with known sensitivities to CP including mesenchymal stem cells (hMSC) and smooth muscle cells (UASMC).
• Cell repopulation dynamics following thawing were similar between the water bath and SmartThaw samples.
• Thermal profiles showed a slightly slower rate of thawing in SmartThaw, yet this did not affect post-thaw viability.
• SmartThaw represents an effective method of thawing with significant advantages over conventional water baths.

Conclusions

The SmartThaw dry thawing device is designed to deliver an effective, reproducible thaw without compromising sample integrity. The closed-loop nature of the device resulted in a slight reduction in heat transfer capacity; therefore, thaw pillow temperatures of 40°C and 45°C for CS25 bags and 2ml cryovials, respectively, were utilized to provide effective thawing without risk of sample overheating. Thermal traces of internal sample temperatures and experimental results support these set temperatures. Thaw pillows were found to consistently maintain the set temperature (±1°C) throughout the thaw duration (data not shown). This study demonstrates that post-thaw viability attained with SmartThaw does not significantly differ from that of the conventional water bath in UASMC, PC-3 or hMSC cells in cryovials or in HUVEC and CHO cells in cryovials or CS25 bags (CHO CS25 bag data not shown). Additionally, SmartThaw reduces the risk of sample contamination due to the elimination of an open water source. SmartThaw’s compliant thaw pillow design allows for the utilization of multiple container types to fit the needs of any laboratory or clinical setting.

References