A NEW AUTOMATED VIAL THAWING SYSTEM CONTROLS THE RESUSCITATION OF CRYOPRESERVED MESENCHYMAL STEM CELLS TO ACHIEVE HIGH CELL VIABILITY AND GROWTH POTENTIAL

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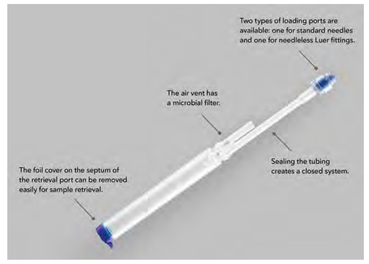
Cell quality following cryopreservation is sensitive to process variation during both cooling and warming. The current standard practice of thawing relies on a manual process using a water bath. The CellSeal Automated Thawing System (CATS) (Cook Regentec) works through dry conduction with no need of water, a potential source of contamination, and provides an effective, reliable and scalable solution which supports Good Manufacturing Practice (GMP) requirements. The CellSeal Automated Thawing System offers a way to reduce variability in temperature and timing during vial thawing by automatically detecting the precise thaw end-point - extremely difficult to achieve using the standard water bath process.

An experiment was designed to evaluate the capability of this new system to thaw CellSeal vials (Cook Regentec) containing cryopreserved human mesenchymal stem cells (RoosterBio). Post-thaw cell numbers, viability and onward growth capacity from vials of various sizes (2ml, 5ml), with different fill volumes (1ml, 2ml, 4.7ml), warmed from different start temperatures (-196°C or -80°C) were determined.

METHODOLOGY

Culture of hMSCs (RoosterBio): Bone marrow-derived hMSCs were expanded to the required density in tissue culture flasks using RoosterBio high performance medium.

Cryopreservation: Cells were harvested and pooled in CryoStor® CS10 at a density of $1x10^6$ cells/mL. 2mL and 5mL CellSeal vials (Cook Regentec) were filled with different fill volumes (1mL, 2mL, 4.7mL) and frozen at a rate of -1°C/min to -80°C using a VIA Freeze™ (Asymptote Ltd.). Vials were then stored at -80°C or -196°C for a minimum of 48hrs.



Overview of CellSeal vial

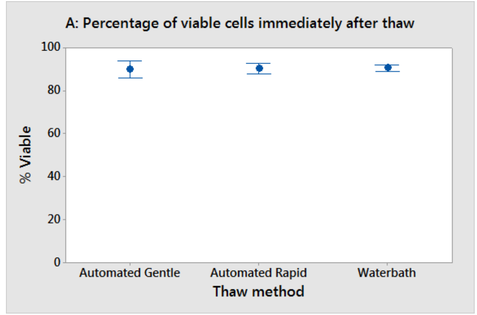
Thaw: Vials were warmed from -80°C or -196°C using either a waterbath set at 37°C or one of two modes (Gentle, Rapid) on the CellSeal Automated Thawing System. Extended exposures of 15 and 30 minutes in the waterbath were used to demonstrate uncontrolled thaws.

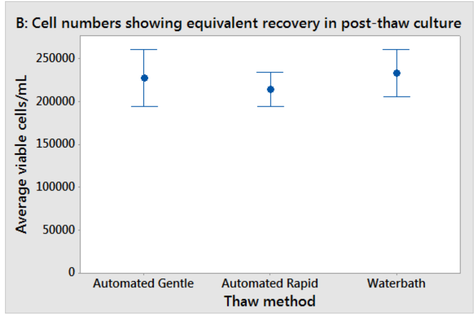
Analysis: Thawed vial contents were analysed for cell number and membrane integrity using a Nucleocounter-3000 with Acridine Orange and DAPI stains (Chemometec) then seeded at $5x10^4$ viable cells per cm^2 and cultured for 4 days. Flasks were then harvested and cell number and membrane integrity analysed.

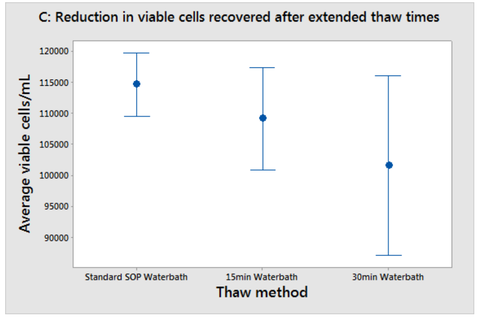
The CellSeal Automated Thawing System in use

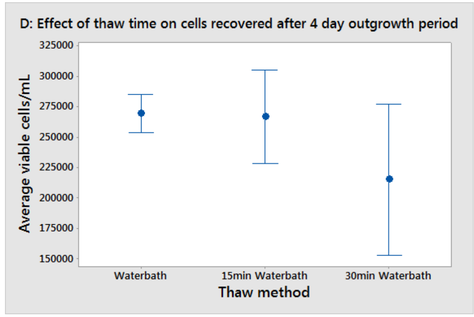
RESULTS

Use of the CellSeal Automated Thawing System in both gentle and rapid thawing modes demonstrated no statistical difference in viability on thaw (A) and viable cell numbers 4 days post-thaw (B) to a tightly controlled 37°C water bath process. Extended incubation in the waterbath post-thaw demonstrated significant reduction in viable cell yield immediately post-thaw (C) and after 4 days post-thaw culture (D).









Data is grouped for all vial sizes, fills and storage temperatures and shows mean ± 95% confidence interval based on individual standard deviation for each group ($n \geqslant 15$ for all other conditions other than extended thaw where n=5).

DISCUSSION

The results of this work demonstrate equivalent performance of both automated thaw methods (gentle and rapid) to a tightly controlled 37°C water bath process. The values of post-thaw viability and onward growth of MSCs contained in CellSeal vials were at the upper end of the range of results typically seen for this cell type. Furthermore, a simulation of a poorly controlled water bath process with extended incubation in the bath post-thaw demonstrated both a reduction in cell recovery and increased variation in quality, highlighting the risk reduction that could be achieved with automation.

Summary: The CellSeal Automated Thawing System offers an effective, scalable and reliable alternative to the use of a waterbath for the resuscitation of cryopreserved human cells.