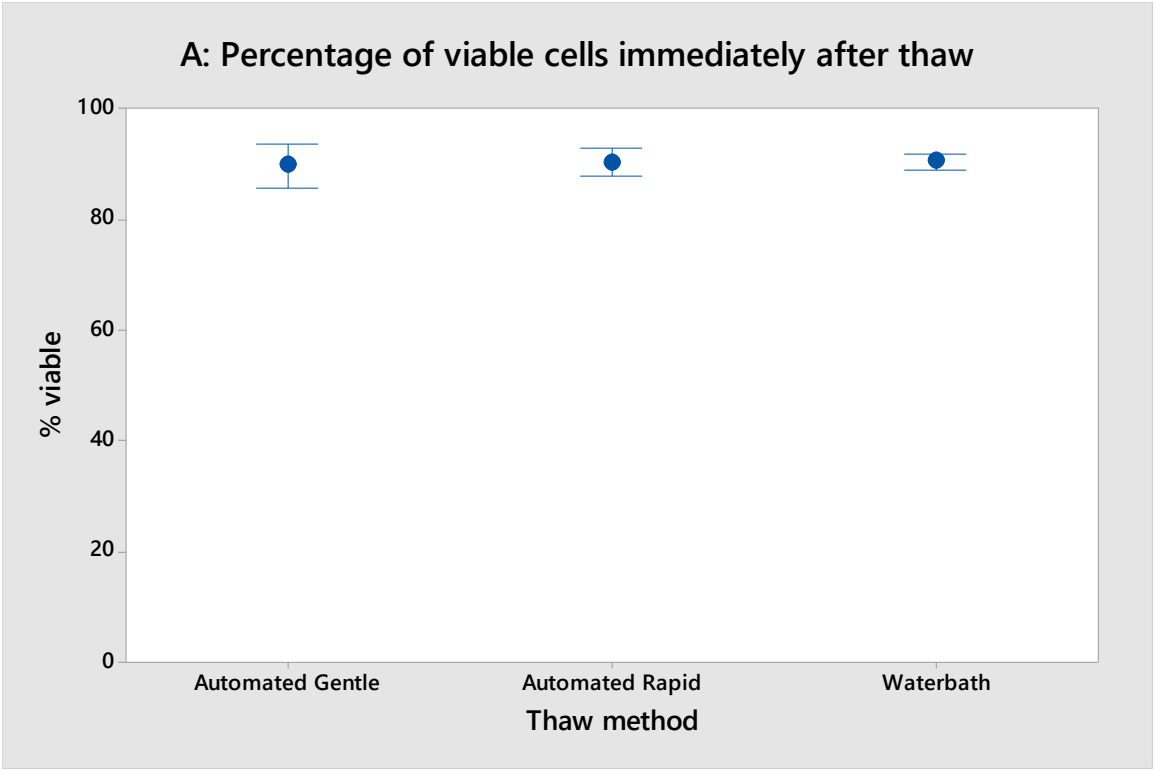
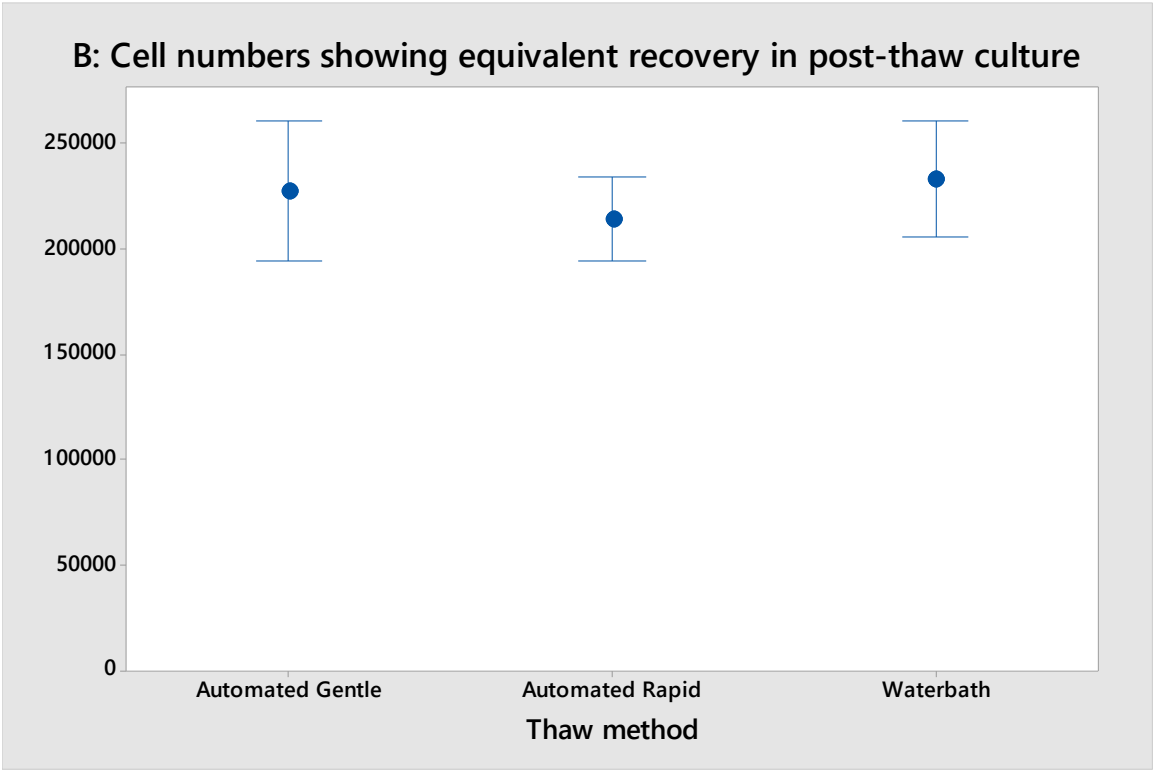
A new automated vial thawer controls the thawing of cryopreserved mesenchymal stem cells to achieve high cell viability and growth potential.

The quality of cells recovered following cryopreservation is sensitive to process variation during both cooling and warming. An automated thawer (CellSeal® Automated Thawing System, CookRegentec, Indianapolis, USA) offers the potential to reduce variability in temperature and timing during the vial thaw operation. An experimental design was developed to evaluate the capability of this new system to thaw CellSeal vials (CookRegentec) containing cryopreserved human mesenchymal stem cells (RoosterBio Inc. Frederick, Maryland, USA). 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. The automated thawer demonstrated equivalent performance to a tightly controlled 37°C water bath process in both user-selectable thawing modes (Gentle, Rapid). Furthermore, a simulation of a poorly controlled water bath process with extended incubation in the bath post-thaw demonstrated significant reduction ($p=0.014$ and $p<0.001$ for 15 mins and 30 mins respectively) in cell recovery and quality, highlighting the risk reduction that could be achieved with an automated process. The absolute values for post-thaw viability and onward growth of MSCs contained in CellSeal® vials were at the upper end of the range of results we typically see for this cell type.

The figures show (A) the viability on thaw and (B) the difference in cell numbers, 4 days post-thaw, from three different thaw protocols: automated gentle, automated rapid, and water bath. 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 (water bath n=20, automated rapid n=20, automated gentle n=15). Viability and cell numbers were determined using a nucleocounter nc-3000 system using Acridine Orange and DAPI stains.

This work was conducted in collaboration with Advanced Bioprocess Services Ltd and the Centre for Biological Engineering at Loughborough University.


A: Percentage of viable cells immediately after thaw
% viable
100
80
60
40
20
0
Automated Gentle
Automated Rapid
Waterbath
Thaw method


B: Cell numbers showing equivalent recovery in post-thaw culture
250000
200000
150000
100000
50000
0
Automated Gentle
Automated Rapid
Waterbath
Thaw method