Effect of Warming Rate On Post-Thaw Recovery Of Cryopreserved Alginate Encapsulated Liver Spheroids

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INTRODUCTION

The bioartificial liver (BAL) is used as an extra-corporeal organ designed to supplement the function of the liver in patients with acute liver failure. This would allow time to find a suitable donor or for the liver to undergo self-repair.

The BAL consists of liver cells (HepG2) encapsulated in alginate, which have formed spheroids (Alginate encapsulated liver spheroids (AELS)). To form the spheroids at suitable cell density the AELS are grown in a fluidised bed bioreactor (FBB) for 12 days.

For patients with acute liver failure an "off-the-shelf" treatment should be available. To that end we have endeavoured to develop a cryopreservation protocol to preserve a large biomass and recover it after thawing.

AIM

To test whether the rate at which the biomass is warmed affects post thaw recovery.

MATERIALS & METHODS

- Same volume of biomass frozen at different depths using cryobags and bottles, controlling the warming rate.
- HepG2 cells were encapsulated in alginate using the Jetcutter system (Genialab).
- Encapsulated cells were cultured in an FBB to a suitable cell density. 55ml samples of the resulting AELS were cryopreserved in either small 60ml polypropylene bottles or 500ml cryobags (Miltenyi Biotec).
- Bags and bottles were frozen in a controlled rate freezer (Kryo750, Planer PLC) using a non linear cooling profile. The AELS were mixed 50:50 with a cryopreservation solution including DMSO and a nucleating agent.
- Cryopreserved biomass was warmed in a 37°C waterbath until all visible ice had melted. CPS was removed using a series of DMEM washes. Thawed AELS were recovered in a small scale FBB system.
- Recovery was assessed over 4 days by measuring cell number (Nucleocounter, Chemomtec) and viability (Live/Dead staining using FDA/PI) every 24 hours.

RESULTS

- Both standards were frozen at a controlled rate. However, the bag cooled at a much faster rate at the beginning (Figure 1).
- The slow warm model (bottles) thawed in ~6 minutes, whereas the fast warm model (cryobag) thawed 10x faster in ~60 seconds

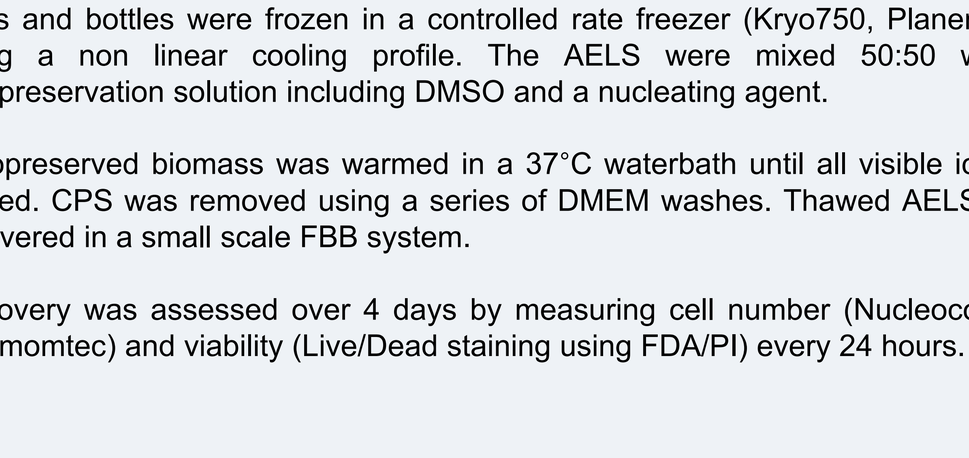

Figure 1: Cooling profile of the AELS in either cryobags or bottles. Effective cooling rate of -0.3°C/min for both.

- Viability nadir of the AELS in both conditions was at Day 1 (~50%). In neither condition did viability recover to pre-freeze levels, fast thaw recovered to 60% and slow thaw recovered to 70%.

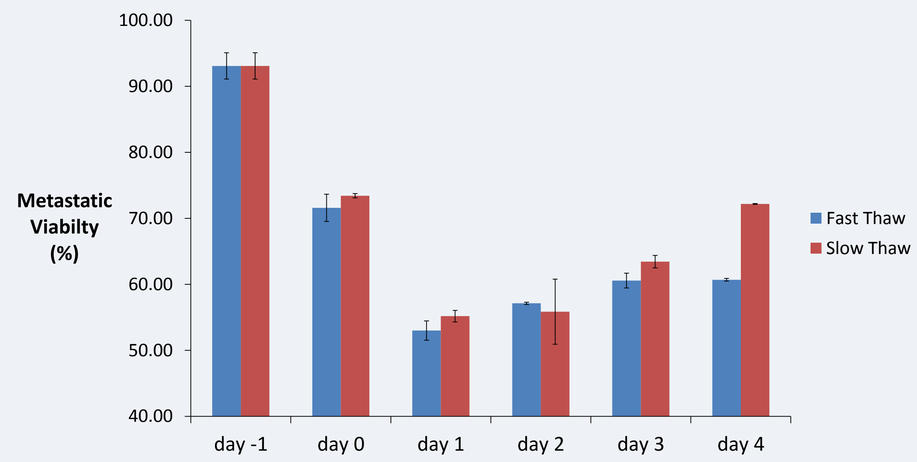

Figure 3: Viability of the AELS before freezing (day -1) and on recovery (days 0 to 4) for the cryobags vs the bottles. n=2 ± range

- Slow warmed AELS recovered to pre-freeze cell number by day 2 (2.6e^{7}cells/ml) and the fast warmed AELS by day 3. By day 4 slow thaw cell densities were 4e^{7}cells/ml and fast thaw 3.2e^{7}cells/ml.

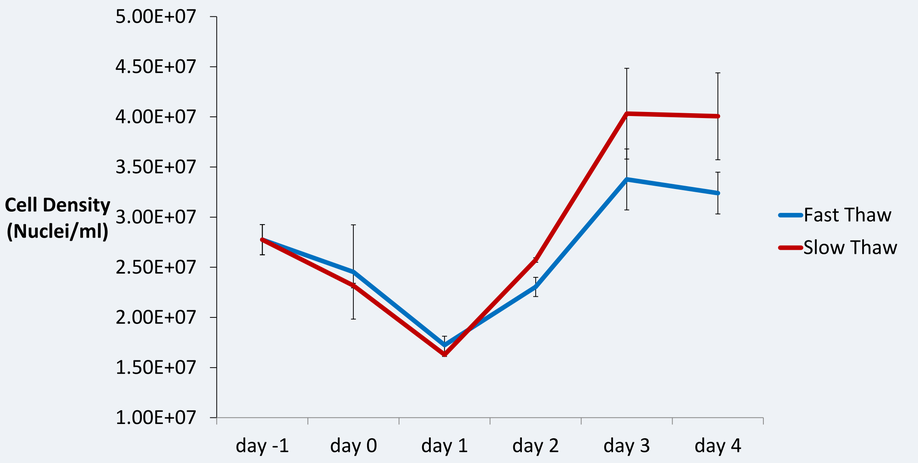

Figure 2: Cell number before freezing (day -1) and on recovery (days 0 to 4) for the cryobags vs the bottles. n=2 ± range

- Viable cell number recovered to pre-freeze levels by day 3 (2.6e^{7}cell/ml) in the slow warm condition. In contrast, fast warmed AELS did not recover to pre-freeze levels (2e^{7}cells/ml).

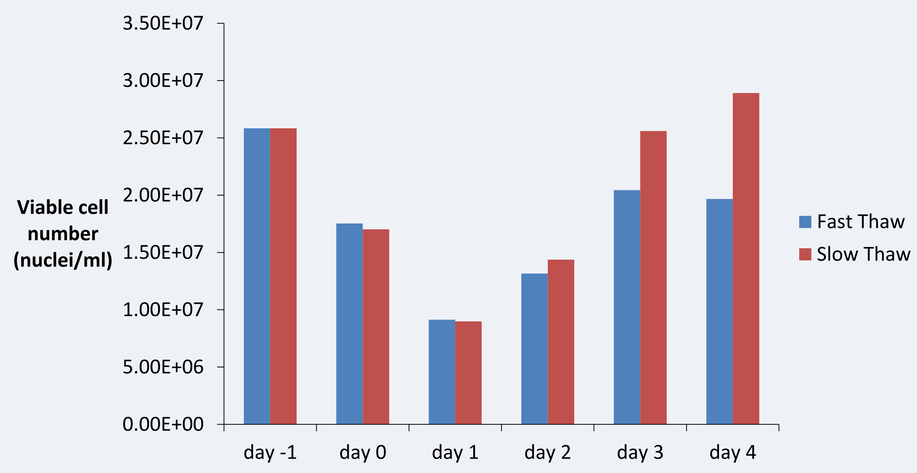

Figure 4: Viable cell number of AELS frozen in either cryobags or bottles, from before freezing (day -1) and during recovery (days 0 to 4). n=2

CONCLUSIONS

- Results suggest slow thawing produces better post thaw recovery than when the same volume biomass is fast thawed. This would infer that slow thawing aids recovery of cryopreserved AELS post thaw. The 24 hr nadir is in agreement with other studies
- However, the cooling rate for each condition was not equal. During the initial cooling the bag cooled faster than the bottle and remained at -50°C for longer. This likely resulted from better heat transfer in bags, also allowing dissipation of latent heat of ice nucleation. This may potentially alter ice crystal format and have a negative effect on the biomass. Slower warming may also allow less stressful water re-equilibriation in the spheroids as ice melts.

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