

DOES DMSO ELICIT TOCIXITY IN CRYOPRESERVATION OF LIVER CELL DERIVED ORGANOID CULTURE?

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Background and Aim

- Liver disease kills >12000 people p.a. in UK alone. Deterioration from liver failure is rapid, hence any cell therapy must be readily available. A BioArtificial Liver machine (BAL), provides liver function allowing patient livers to regenerate. We developed a BAL based on alginate encapsulated liver spheroids (AELS) and cryopreservation in ~1L cryobags. The cryopreservation method will enable long term storage and fast delivery of the BAL to patients

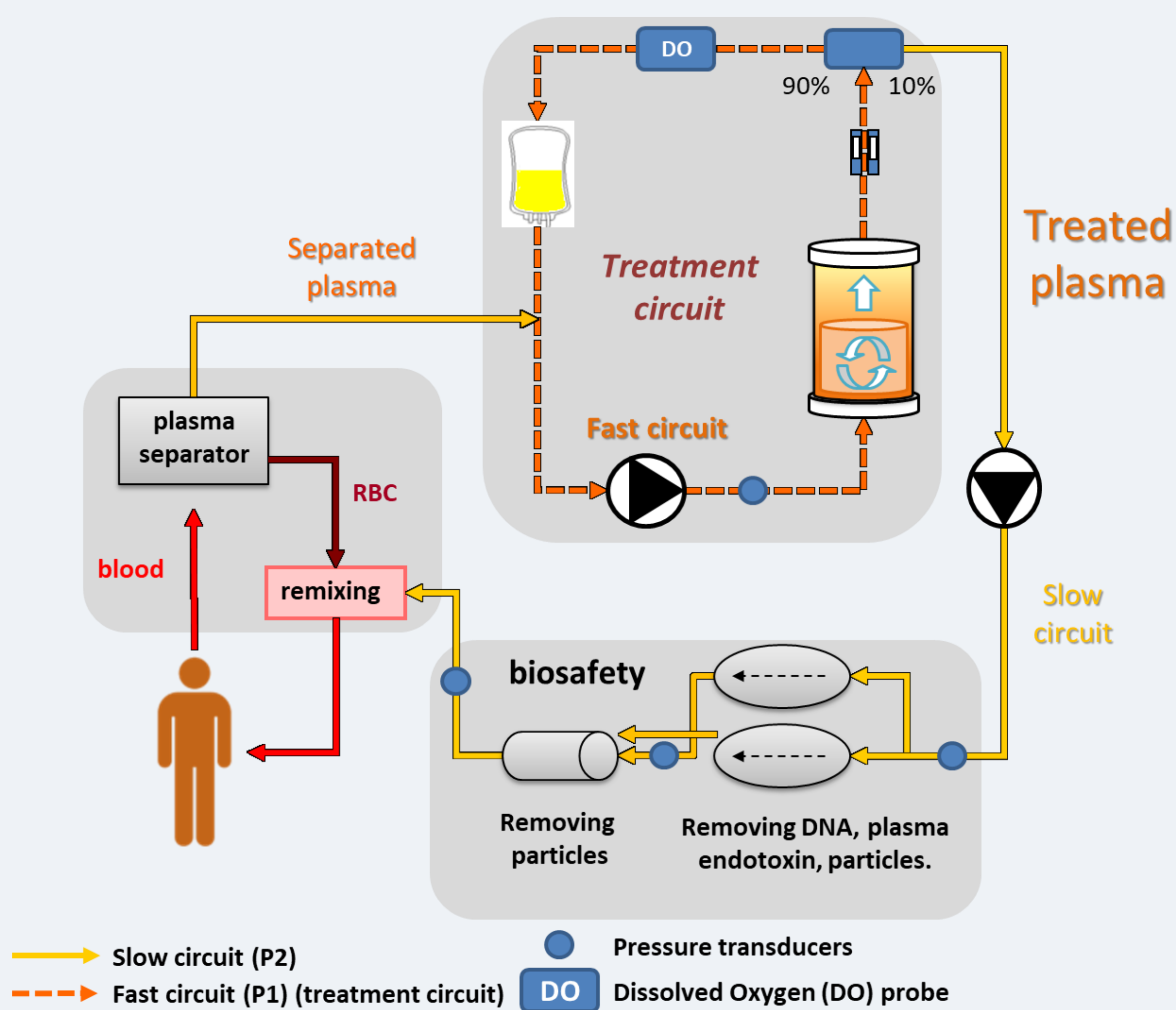


Figure 1: Bioartificial Liver (BAL) treatment diagram

- Dimethyl sulfoxide (DMSO) is widely used in cryopreservation with varying reports of cell toxicity (1, 2). However, there is little data published on its effects on cell-organoids
- Since thawing of large volume cryopreserved organoid biomass is lengthy, and leads to exposure of AELs CPS at max 24°C, we explored DMSO toxicity in organoid culture mimicking a typical thawing protocol

Materials & Methods

- **Encapsulation:** HepG2 cells encapsulated in 1% alginate solution using the Jetcutter system (GeniaLab)
- **3D cell growth:** cell beads cultured for 12 days in static culture
- **DMSO exposure:** 13.2±0.14x10⁶ cells/ml beads and 96.9% viability exposed to 12% v/v DMSO and 38% v/v Viaspan (Belzer UW, Bridge to Life) for 10min at 0°C, 24°C or 37°C. 40% DMSO v/v in Viaspan was used as a positive control for damage
- **Washes:** 3 subsequent 5minute media (AlphaMEM) washes with 1M, 0.5M and 0.025M glucose supplemented AlphaMEM
- **Recovery after DMSO exposure:** encapsulated liver spheroids (ELS) were re-cultured
- **Cell counts and viability:** NucleoCounter (Chemometec) and live/dead cell staining with FDA/PI dyes after DMSO exposure; and on re-culture for 24 hours. Image analysis quantification via NIS Elements software

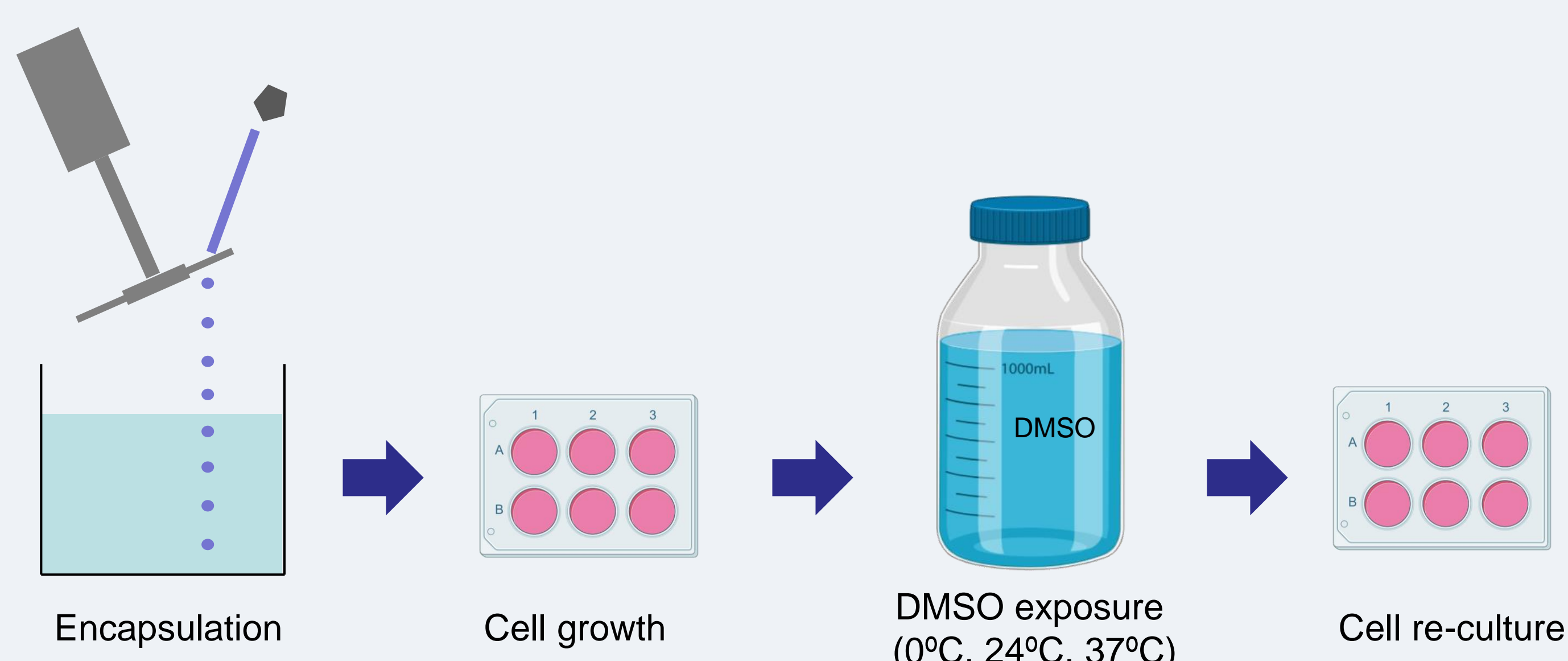


Figure 2: Diagram of the DMSO treatment experiment

Results

- The viability of AELS was not reduced after exposure to 12% DMSO either immediately or after a 24 hour re-culture period, at 0°C, 24°C or 37°C treatment (Figure 3)

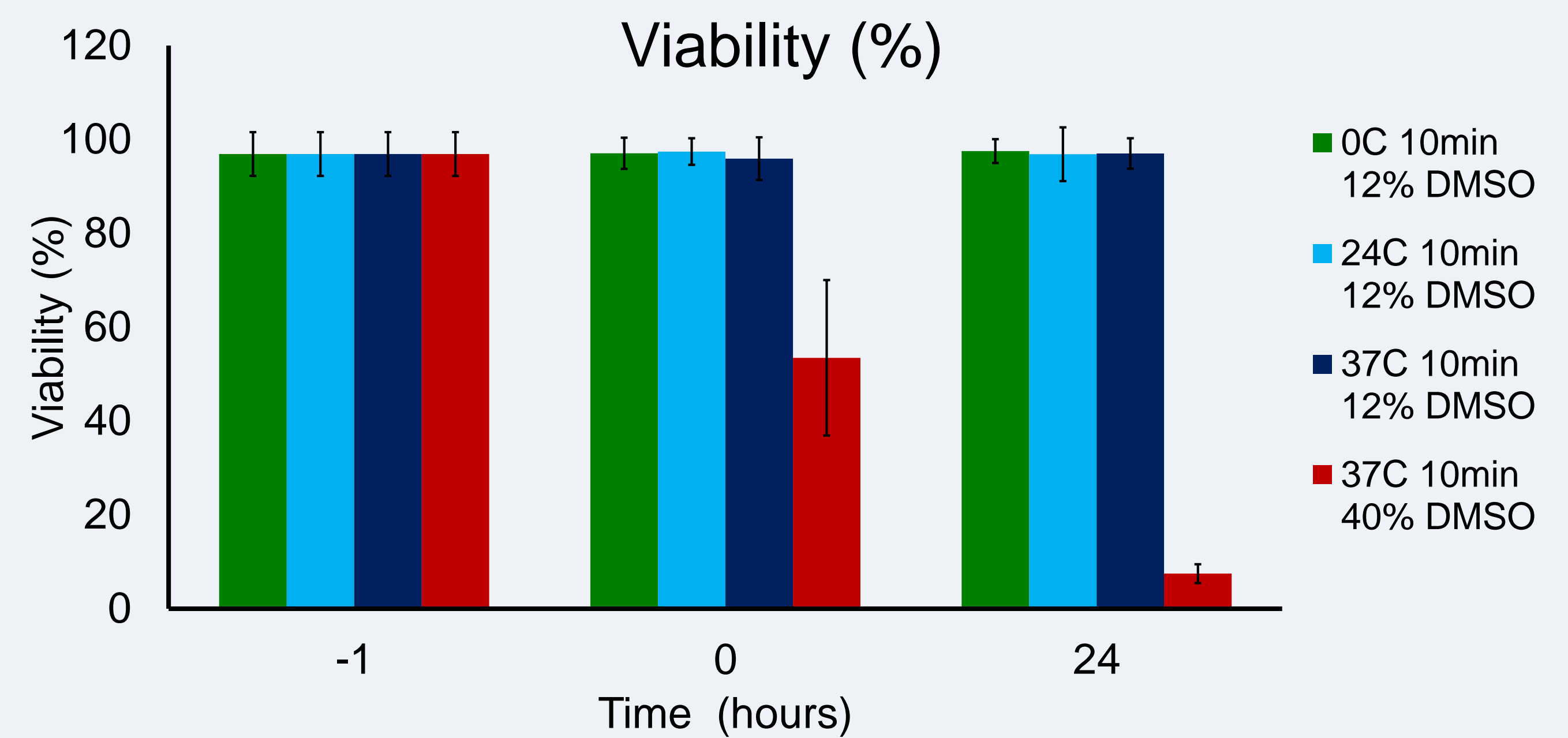


Figure 3: Viability (%) of AELS before, immediately after and 24 hours after DMSO exposure. N=3 for 0°C 10min and 24°C 10min treatments; n=4 for 37°C 10min and n=2 for 40%DMSO 37°C 10min (mean +/- range)

- 40% DMSO exposure decreased AELS cell viability. 1 hour 12% DMSO exposure did not change viability (data not shown)
- Viable cell numbers did not differ for 12% DMSO at 0°C, 24°C or 37°C treatments (12%DMSO), however was significantly reduced after 40% DMSO treatment (Figure 4)

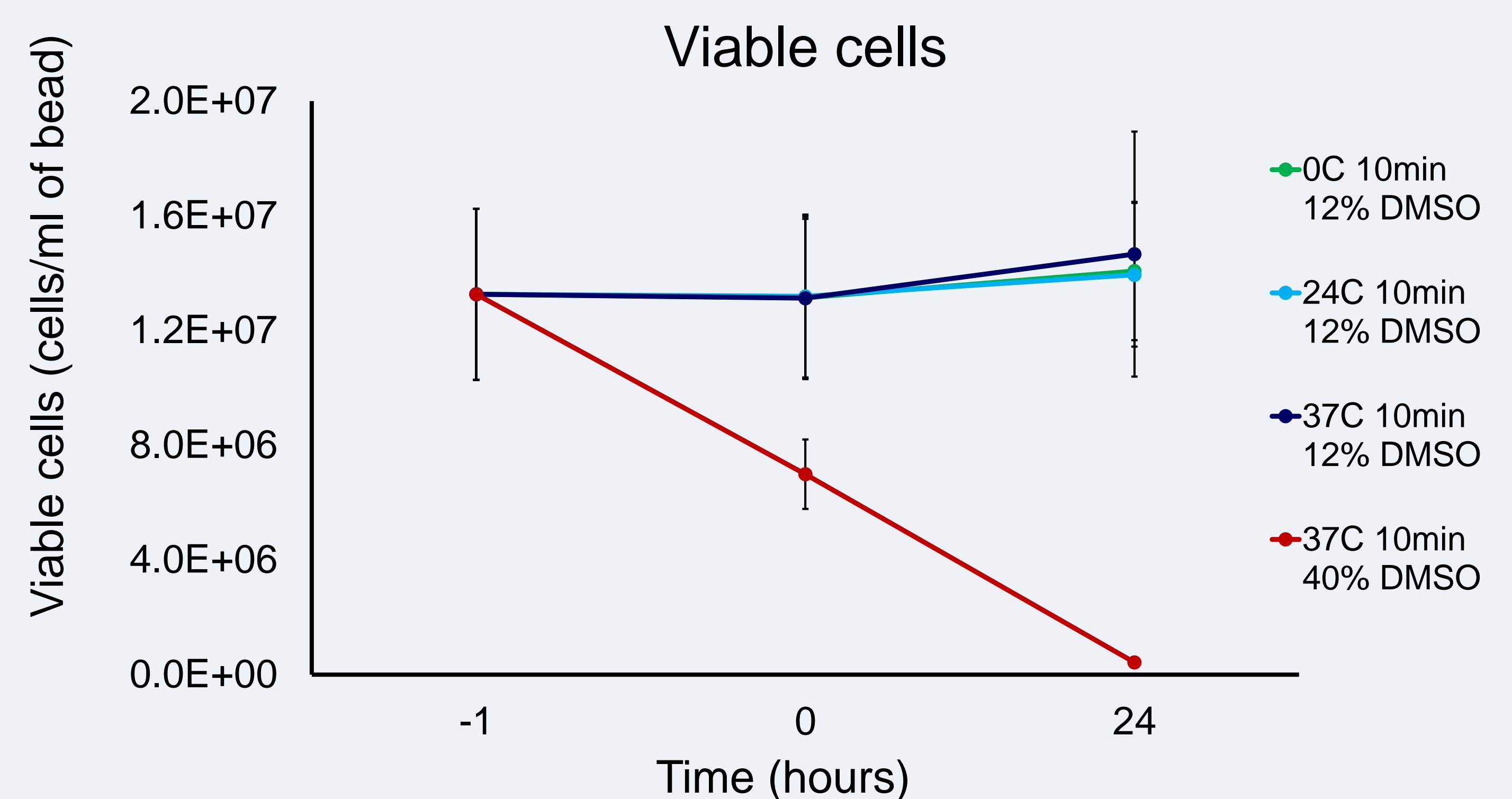


Figure 4: Viable cell numbers of AELS before, immediately after and 24 hours after DMSO exposure. N=3 for 0°C 10min and 24°C 10min treatments; n=4 for 37°C 10min and n=2 for 40%DMSO 37°C 10min (mean +/- range)

Conclusions

- AELS can be exposed to 12% DMSO for short periods of time (10min) at temperatures up to 37°C without compromise to viability or viable cell number
- High concentration DMSO (40%) is toxic for the cells and significantly affects cell viability and cell number immediately after treatment after only a short exposure
- The method described has helped to optimise a process for large scale biomass cryopreservation suitable for a clinical Bioartificial Liver machine

References

- (1) J. Galvao, B. Davis, M. Tilley, E. Normando, M.R. Duchon, M.F. Cordeiro, Unexpected low-dose toxicity of the universal solvent DMSO, FASEB J. 28 (2014) 1317–1330. doi:10.1096/fj.13-235440
- (2) Yi X Liu M Luo Q Zhuo H Cao H Wang J Han Y. Toxic effects of dimethyl sulfoxide on red blood cells, platelets, and vascular endothelial cells in vitro. FEBS open bio, 2017 vol: 7 (4) pp: 485-494

