Serum-free cryopreservation of engineered neural tissue

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INTRODUCTION: Serum is a commonly used supplement in tissue culture that provides a broad spectrum of proteins, hormones and growth factors that enrich the *in vitro* microenvironment (Gstraunthaler, 2003). Despite its prevalent use, it remains largely ill-defined, carries biosafety concerns and from an ethical standpoint it's use remains unaligned with the 3R's theme (Gstraunthaler et al., 2013). This work builds on previously published work whereby foetal bovine serum (FBS) was an integral component of a cryopreservation media for the storage and preservation of engineered neural tissue (EngNT) (Day et al., 2017). Herein we explore the use of a novel cryopreservation media to replace the requirement of FBS for the cryopreservation of EngNT.

METHODS: Cellular collagen constructs were made in 24-well plates using filter paper absorbers to stabilise 2 mg/ml bovine collagen hydrogels containing 1 million SCL 4.1/F7 cells/ml. Following hydrogel stabilisation, the cellular collagen constructs were placed into 4 different cryopreservation media. Cryogenic conditions involved controlled-rate cooling and storage at -80°C for 24 h followed by 24 h in liquid nitrogen in; (1) 2.5 M Poly(propylene glycol) (PPG); (2) 2.5 M PPG + 10% dimethylsulfoxide (DMSO); (3) Polyvinylpyrrolidone (PVP); (4) PVP + 10% DMSO. A 60% Media, 30% FBS and 10% DMSO cryopreservation media was used as a positive control. Samples were thawed after 24 h cryopreservation in a water bath at 37°C, cell viability was assessed in relation to the metabolic activity, determined by the 3D CellTiter-Glo assay.

RESULTS: Constructs preserved in PPG + 10% DMSO exhibited the highest metabolic activity that exceeded the positive control. Of the cryopreservation conditions, the second highest metabolic activity was found in those preserved in PPG alone. Constructs preserved in PVP had approximately half the number of metabolically active cells than the positive control. The addition of DMSO to PVP was seen to slightly increase the number of viable cells but not as much as seen in the PPG condition.

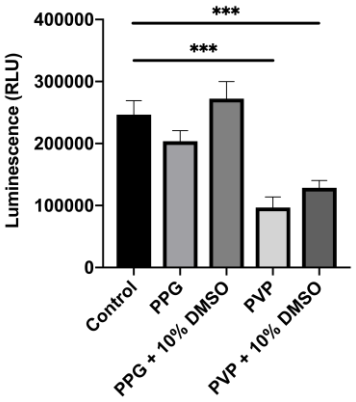

*Fig 1: Metabolic activity of SCL 4.1/F7 cells in stabilised bovine collagen hydrogels after 24 h in cryogenic preservation conditions (n=4, where the control represents samples cryogenically stored in 60% Media-30% FBS and 10% DMSO. Post-hoc Tukey test, ***P<0.01. Data are means ± SEM)*

DISCUSSION & CONCLUSIONS: This study demonstrates that EngNT can be cryogenically stored in a serum-free media with comparable metabolic activity to control media contained FBS. There was no statistical significance between the positive control and samples stored in PPG (with or without DMSO). PVP is a non-penetrating cryoprotective agent hence it's use in preserving artificial tissue is limited despite its success in preserving cells. PPG with the addition of DMSO provided a powerful penetrating cryogenic media that led to the successful serum-free preservation of EngNT. Further research is required to understand the effects of this cryopreservation media on the efficacy of EngNT following long-term storage.

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