In vitro experiments to inform cell seeding strategies and parameterize a mathematical model for peripheral nerve repair

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INTRODUCTION: Cellular collagen constructs have been used to support peripheral nerve regeneration using stem cells that have been differentiated to a Schwann cell-like phenotype [1]. These cells are also likely to release vascular endothelial growth factor (VEGF) in response to local hypoxia, thus promoting vascularisation of the construct. However, prolonged hypoxia caused by overly dense cell seeding and insufficient vascularisation could cause cell death. To inform the cell seeding strategies of the constructs to optimise cell survival and VEGF release**,** and parameterize a mathematical model of the construct in vivo, the effect of low oxygen concentration on the cells at a range of seeding densities was investigated in a 3D collagen environment similar to that used in engineered neural tissue [1].

METHODS: Differentiated adipose-derived stem cells (dADSCs) were seeded at a range of densities in 2mg/ml type I rat tail collagen hydrogels in a 96-well plate, and stabilized using RAFT™ (TAP Biosystems, UK). The gels were maintained at one of five selected oxygen concentrations for 24h.
Viable cell density was calculated using CellTiter-Glo 3D Reagent (Promega) to measure metabolic activity and relate this to a number of viable cells, and the concentration of VEGF released into the media was measured using ELISA.

RESULTS: The analyses revealed a comprehensive pattern of cell responses to both seeding density and oxygen environment, examples of which are shown in Figures 1 and 2. There was a greater increase in viable cells at 24h with an initial cell density of 1 x 10^6 cells/ml than with the higher initial densities (Fig 1). Lower oxygen environments resulted in increased VEGF release compared to higher oxygen environments (Fig 2).

DISCUSSION & CONCLUSIONS: The results suggest a strong link between seeding density, oxygen environment and resulting viability and

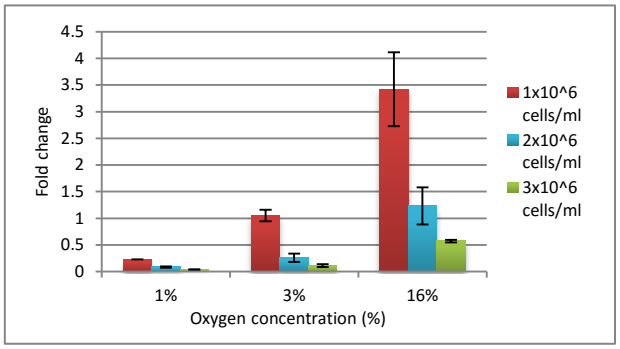

Fig. 1: Fold change in number of viable cells at 24h for initial seeding densities of 1, 2, 3 million cells/ml of gel before stabilisation.

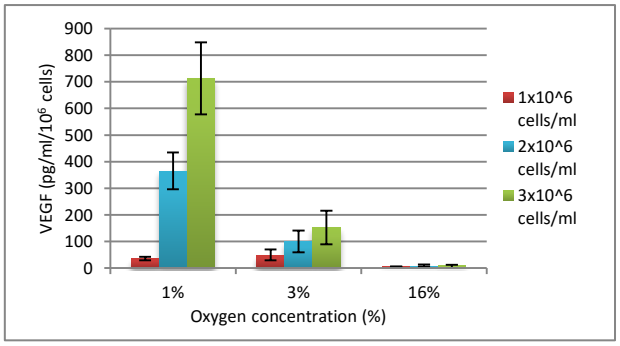

Fig. 2: VEGF present at 24h (pg/ml/10^6 cells).

VEGF release. The viability data show a strong indication that lower initial densities should be favoured for the survival of therapeutic cells, particularly for repairs in poorly vascularised tissue environments. These results further motivate the need for a more sophisticated way of optimising cell-seeding strategies in nerve repair and other regenerative medicine scenarios. Future research will use these and other data to parameterize a mathematical model to enable optimisation of cellular constructs.

REFERENCES: [1] M Georgiou et al (2014) *Biomaterials* **37:** 242-251.

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