A joint theoretical-experimental approach to investigate the effects of low oxygen environments upon therapeutic cell viability and VEGF production

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INTRODUCTION: The speed and efficiency of progress in tissue engineering is hindered by the need to investigate experimentally a huge number of different parameters, such as initial seeded cell types and densities, which can impact the clinical efficacy of designs. A careful balance, dependent on oxygen levels, must be achieved to obtain sufficient levels of VEGF for vascularisation of engineered tissue, whilst maintaining a population of viable therapeutic cells. Mathematical modelling, a powerful tool able to make predictions based upon *in vitro* data which can be used alongside experimental work, has the potential to accelerate and direct tissue engineering design. The aim of this work was to investigate the effect of oxygen conditions upon the production of VEGF and the viability of stem-cell derived therapeutic cells, via a symbiotic theoretical-experimental method. The results of the work can be used to inform the design of nerve regeneration constructs and demonstrate the benefits of this interdisciplinary approach.

METHODS: Differentiated adipose-derived stem cells (dASCs) were maintained in plastic compressed collagen gels with various initial seeding densities and oxygen levels. Viability was assessed using CellTiter-Glo (Promega) and VEGF release measured using ELISA after 24h. A mathematical model able to simulate the interactions between oxygen and VEGF concentrations and cell density within both the *in vitro* gel and peripheral nerve construct scenarios was created. The theoretical framework consists of three coupled differential equations, including terms representing the processes of diffusion, cell proliferation and death, VEGF production and oxygen consumption. Parameters within the model were assigned using values from the literature and parameter fitting based upon the experimental results [1-3].

RESULTS: Initial cell density and atmospheric oxygen concentration both appear to impact viability over time (Figure 1). Model simulations show that initial conditions have a large effect upon subsequent conditions within a construct.
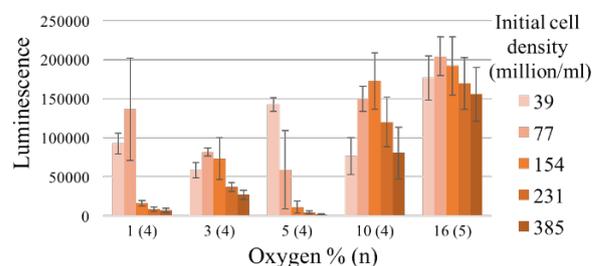

Fig. 1: dADSC viability in collagen gels after 24h incubation with different concentrations of controlled atmospheric oxygen (mean $\pm$ SEM).
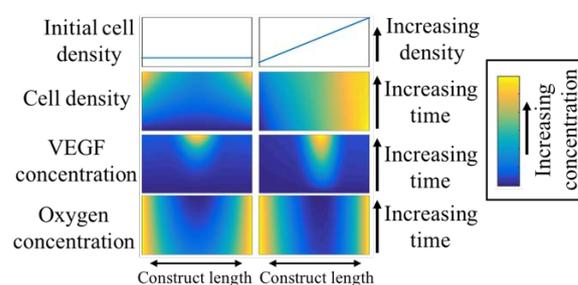

Fig. 2: Results of model simulation. Initial cell density has an effect upon spatial distributions of solutes within a peripheral nerve construct.

DISCUSSION & CONCLUSIONS: The experimental results suggest that there is an optimal initial seeding cell density that will ensure good continued cell viability within engineered tissue, under low oxygen conditions. The theoretical framework can be used to investigate in more detail which values and spatial distributions of cell density and oxygen provide a good balance between levels of cell viability and VEGF.

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