Development of an advanced 3D-engineered neuronal cell culture as a screening tool for drugs promoting peripheral nerve regeneration.

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INTRODUCTION: Injuries associated with peripheral nerve damage effect ~1 million patients per year. Damage to the peripheral nervous system can be extremely debilitating and result in loss of end organ function, coupled with poor neuron regeneration capacity [1]. Currently the treatment option for injuries are microsurgical and there are no drug therapies available to improve recovery. Pharmacological treatments could potentially be used to maintain neuronal viability, encourage axonal growth, improve axonal specificity to end-organ targets and reduce neuropathic pain. Some drugs and targets have been identified but there are challenges associated with understanding mechanisms of action and moving therapies towards clinical translation. For successful regeneration following injury Schwann cells need to support regeneration and myelination of neurons [2]. This 3D cell-cell interaction is a key feature that needs to be recreated in order to regenerate effective in vitro models. Engineered neural tissue (EngNT) supports the regeneration of neurons within an aligned 3D Schwann cell-seeded collagen gel environment and has the potential to be used as an effective co-culture model. The aim of this study therefore was to develop an assay based on EngNT to analyse the effect of drugs on neurite growth. Initial studies tested the effect of ibuprofen, a drug that has shown some positive effects on nerve regeneration in animal models [3].

METHODS: Anisotropic 3D co-culture gels were prepared by tethering 1ml of solution containing: 80% Type I rat tail collagen, 10% 10x MEM, 5.8% neutralising solution and 4.2% Schwann cell suspension, within rectangular moulds to facilitate cellular self-alignment. After 24h incubation at 37°C, the aligned cellular gels were stabilised using plastic compression and PC12 neurons were added to the surface. Co-cultures were subjected to drug treatments for 72h before fixing. Neurites were visualised using βIII-Tubulin immunoreactivity and fluorescence microscopy. Neurite growth was quantified by measuring neurite length using ImageJ software.

RESULTS: Treatment with 100µM ibuprofen elicited an increase in neurite length of 54% compared to the control (Fig 1).

Fig. 1: A -Neurite length after 72 h in the presence or absence of 100µM ibuprofen. Representative fluorescence, micrographs show βIII-Tubulin immunoreactivity (green) and DAPI (Blue) in control (B) and ibuprofen treated co-cultures (C).

DISCUSSION & CONCLUSIONS: The results demonstrate that this engineered tissue model containing neurons and Schwann cells aligned within a collagen hydrogel mimics the pro-regenerating effects of ibuprofen seen previously in-vivo. This indicates that the 3D engineered co-culture gels can be used as an effective assay for screening drugs that have the potential to improve nerve regeneration.


ACKNOWLEDGEMENTS: This research was made possible through the support and funding of the EPSRC (Grant EP/L01646X).

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