Engineered neural tissue with aligned Schwann cells supports neuronal regeneration *in vivo* and can be assembled using differentiated adipose-derived stem cells

Conference or Workshop Item

Engineered neural tissue with aligned Schwann cells supports neuronal regeneration *in vivo* and can be assembled using differentiated adipose-derived stem cells.

M. Georgiou[1], J. Loughlin[1], J. P. Golding[1], P. J Kingham[2] & J. B. Phillips[1]
[1]Department of Life, Health and Chemical Sciences, The Open University, Milton Keynes, MK7 6AA.
[2]Department of Integrative Medical Biology, Umeå University, Sweden, SE 901 87

INTRODUCTION: There are many limitations associated with the current gold standard treatment for peripheral nerve injury, the nerve autograft. Another option is to use nerve guidance conduits (NGCs) to bridge the gap in injured nerves. However these are limited to repairs up to ~3cm due to their lack of neurotrophic support in long gaps. We have developed Engineered Neural Tissue (ENT) - a living biomaterial with aligned neural cell architecture that can function as the 'core' of a repair device. In this study we aimed to optimise the assembly of this material in a nerve repair device, and then to produce a construct with clinically relevant cells (adipose-derived stem cells differentiated into Schwann cells (dADSCs)).

METHODS: The rat F7 Schwann cell line was used for device optimisation. For production of ENT, cells were mixed with neutralised type I rat tail collagen (2 mg/ml) and cast into the tethering/stabilisation system[1,2], Fig 1. ENT was

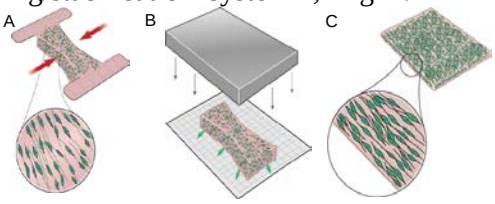

Fig. 1. Production of ENT. ***A*** *Cell activity contracts the gel, cells become elongated and form chains along the longitudinal axis.* ***B*** *Stabilisation of aligned cellular gel by removal of some of the interstitial fluid.* ***C*** *ENT: a tissue-like robust hydrogel with highly aligned cells.*

assembled to form the nerve core of repair devices, Fig 2. Various column formats were tested in the rat sciatic nerve model (5mm gap). Regeneration was assessed by immunostaining with anti-neurofilament (axons) and anti-S100 (Schwann cells (SC)) antibodies, using confocal microscopy. To investigate neuronal growth on an ENT containing dADSCs, primary adult rat neurons were cultured on the ENT surface for 3 days. Deviation of neurite growth from the direction of cell alignment was quantified in confocal micrographs following immunostaining of cultures with anti-S100 and anti-βIII tubulin (neurites).

RESULTS: *In vivo* experiments suggested regenerating axons grew preferentially between layers of cellular ENT in close contact with the aligned SCs, compared to unaligned or acellular controls (Fig 3). dADSCs maintained their alignment during preparation of ENT & over 3 days in culture. Neurons growing on ENT-dADSC were guided by the aligned cells (Fig 4).

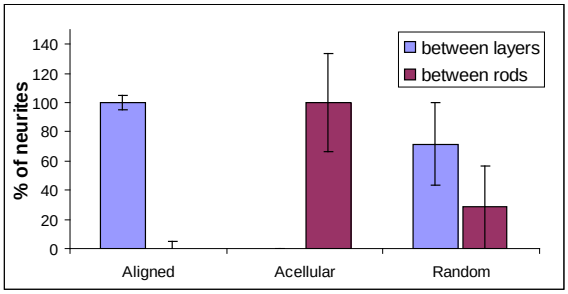

Fig. 3. Device optimisation in 5mm rat sciatic nerve model. Aligned cellular ENT promotes growth of neurites between layers (means ± SEM).

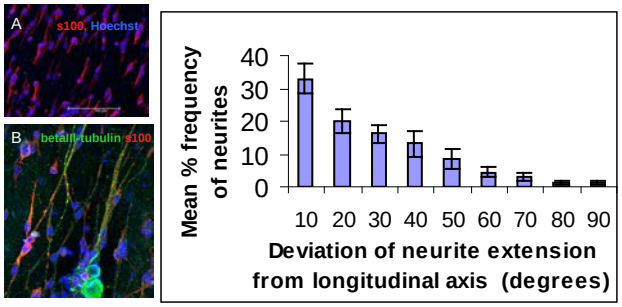

Fig. 4. ENT-dADSC guides neurite growth. ***A*** *Aligned dADSCs in ENT* ***B*** *Confocal micrograph showing neuronal growth (green) on the surface of the aligned dADSC (red) biomaterial.* ***C*** *Deviation between angle of neurite growth and dADSC alignment (means ± SEM).*

DISCUSSION & CONCLUSIONS: *In vivo* experiments showed that ENT supports axon regeneration and that this is greatest between the layers of ENT when aligned SCs are present. As a potential clinical source of SCs for development of ENT, we showed that dADSCs survive and maintain their alignment following the stabilisation process to form sheets of ENT. Neurons growing on the surface of ENT extended neurites that were guided by the orientation of the aligned dADSCs.

REFERENCES: [1] Phillips J.B. *et al.* (2005) *Tiss Eng* **11** 1611-7. [2] Brown R.A. *et al.* (2005) *Adv Funct Mater* **15** 1762-70.