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Astrocyte alignment in 3D collagen gels increases neurite outgrowth; implications for improving spinal cord repair

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INTRODUCTION: A major impediment to tissue engineered repair of CNS damage is the glial scar that forms around implanted graft devices and creates an inhibitory environment for axon growth out of the repair site [1]. The glial scar is composed of a 3-dimensional (3D) meshwork of astrocytes which become reactive in response to damage stimuli. Previous studies have shown that longitudinal alignment of astrocytes growing in monolayer is sufficient to direct and enhance the growth of neurites over their surface [2,3]. The aim of this work therefore was to develop a 3D culture system in which the effect of astrocyte alignment on neurite growth could be modelled in a spatially relevant environment.

METHODS: Rectangular tethered collagen gels [4] (Fig 1) were made by co-culturing primary rat astrocytes (950K), neural fibroblasts (50K) and DRGs (4 dissociated) in 0.5 ml (2 mg/ml) type I rat tail collagen per mould. After setting, gels were maintained in culture to align for 3 days at 37°C. In this system the cells generate forces that contract the restrained gels, forming a central region in which cells align with the axis of principal strain. The gels have unaligned control areas (delta zones), which are stress-shielded regions with no single axis of principal strain. Gels were fixed with 4%PFA and stained for GFAP, βIII-tubulin and hoechst (astrocytes, neurons and nuclei). Astrocyte alignment (aspect ratio) and neurite length were measured using Volocity and Openlab image analysis software (Improvision) and mapped according to gel region (Fig 1C).

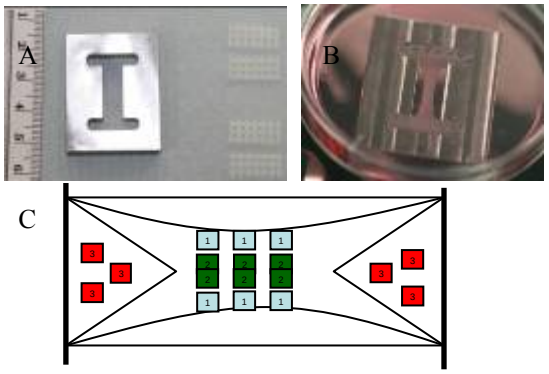

Figure 1. A. Mould and tethering bars. B. Contracted gel. C. Schematic of gel areas for analysis, 1=gel edges, 2=gel middle, 3=delta zones.

RESULTS: Astrocyte alignment was significantly greater at the edges and middle of gels ($P<0.01$ and $P<0.05$ respectively) when compared to delta zones. Neurites in aligned regions grew in an orientated manner compared to neurites growing in all directions in delta zones (Fig 2 A & B). Furthermore, neurites in aligned regions were significantly longer than those in delta zones (Fig 2C). In gels without astrocytes (fibroblast and DRG only) neurites were of similar lengths in all regions (Fig 2D), suggesting a key role of aligned astrocytes in enhancing axon outgrowth.

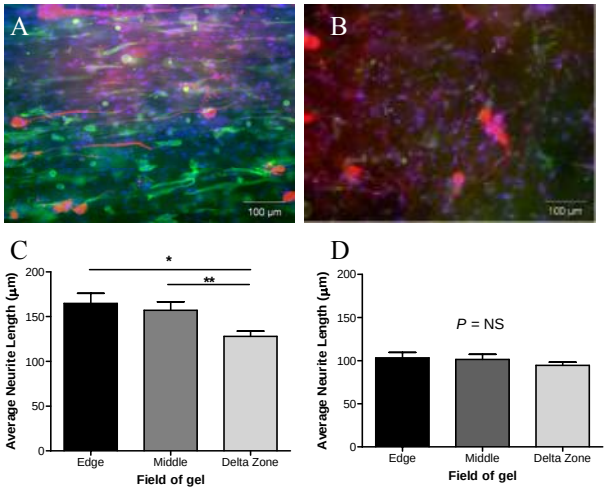

Figure 2. Staining for GFAP, βIII tubulin and hoescht in gel edge (A) and delta (B) zones. Neurite length in gels with (C) and without (D) astrocytes, n=6 gels.

DISCUSSION & CONCLUSIONS: This model provides a system whereby astrocyte/neuronal interactions can be investigated in a more realistic spatial arrangement than that offered by 2D culture. Alignment of astrocytes in a 3D matrix is sufficient to enhance the growth and directionality of neurites. This could provide a useful basis to improve the design of implantable devices for the treatment of CNS injury, and suggests reactive astrocytes per se are amenable to manipulations that may make them favourable substrates for regenerating axons.

[1] H.M. Geller & J.W. Fawcett (2002) *Exp Neurol* **174**:125-136. [2] R. Biran, M.D. Noble & P.A. Tresco (2003) *Exp Neurol* **184**:141-152. [3] J.K. Alexander, B. Fuss & R.J. Colello (2006) *Neuron Glia Biol* **2**:93-103. [4] J.B. Phillips *et al.* (2005) *Tissue Eng* **11:**1611-1617.
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