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# Astrocyte responses to dorsal root ganglia in 3-dimensional co-culture models

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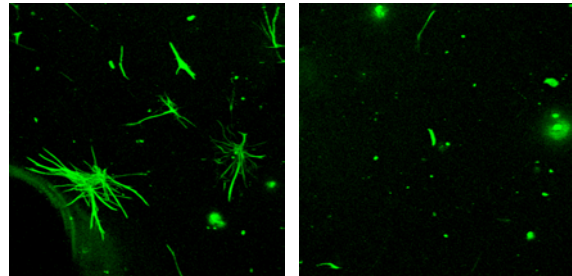
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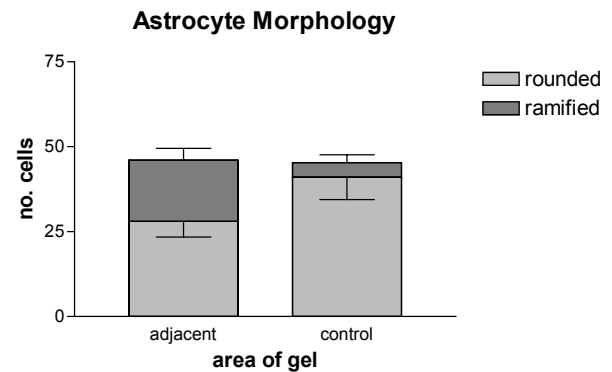
**INTRODUCTION:** A key impediment to repair following central nervous system (CNS) injury is the formation of a glial scar which inhibits neuronal growth beyond implanted tissue engineered devices [1]. Astrocytes, which support neuronal function in healthy tissue, undergo characteristic changes to form this physical/chemical barrier at the boundary of regeneration, but the precise nature of this response is poorly understood. One of the principle limitations to research in this field is the lack of an effective cell culture model; astrocytes in conventional culture support the growth of neurones despite expressing features of the reactive phenotype. However, astrocytes in 3-dimensional (3D) culture can inhibit neuronal growth [2]. The aim here is to grow astrocytes in a 3D co-culture model in order to mimic the host CNS repair environment where they encounter Schwann cells and regenerating neurones at the interface of implanted conduits [1].

**METHODS:** Primary astrocytes were prepared from neonatal rats [3] and seeded within 1 ml cylindrical collagen gels formed from type I rat tail collagen (1.92 mg/ml). Dorsal root ganglia (DRGs) were harvested from adult rats and either (i) dissociated using collagenase and injected into the centre of the astrocyte gel, or (ii) embedded as intact ganglia within the astrocyte gel during setting. These co-culture systems were maintained for 11 days, then astrocyte, neurone and Schwann cell morphologies were examined using immunofluorescence (antibodies against GFAP,  $\beta$ III-tubulin and S100 respectively) detected by fluorescence and confocal microscopy.

**RESULTS:** A halo of GFAP immunofluorescence was detected around the DRG positions. Astrocytes adjacent to DRGs were larger and displayed a more ramified phenotype than those in comparator regions of the same gels (fig 1). Classification of astrocyte morphology in the DRG-adjacent region confirmed that there were significantly more ramified cells here than in the control areas (fig 2).



*Fig. 1: Confocal micrographs showing morphology of astrocytes adjacent (left) and 2mm distal (right) to embedded DRG.*



*Fig. 2: Assessment of astrocyte morphology in adjacent and control regions.*

**DISCUSSION & CONCLUSIONS:** Astrocytes in 3D culture exhibited a localised response to the presence of co-cultured DRGs. They became larger and more ramified than comparator cells in a manner reminiscent of the reactive gliosis observed at damage sites in vivo [1]. Recapitulation of the astrocytic response in this simple model will enable triggers and therapeutic interventions to be investigated in a highly controlled environment providing a useful tool for future studies.

**REFERENCES:** <sup>1</sup> J.B. Phillips, V.R. King, Z. Ward et al. (2004) *Biomaterials* **25**:2769-79. <sup>2</sup> J.W. Fawcett, E. Housden, L. Smith-Thomas et al. (1989) *Dev Biol* **135**:449-58. <sup>3</sup> J.B. Phillips & B. Pearce (2005) *Inflammopharmacol* **13**:431-9.