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Modelling the injured spinal cord using 3-dimensional cell cultures; strategies for improving tissue engineered repair

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INTRODUCTION: Traumatic injuries to the spinal cord are debilitating and often lead to paralysis and loss of sensation. CNS injury results in the formation of a glial scar that is largely composed of reactive, hypertrophic astrocytes which upregulate various markers including glial fibrillary acidic protein (GFAP) and sulphated proteoglycans. The glial scar microenvironment is inhibitory for axon growth and regeneration. A common finding of strategies aimed at bridging CNS lesions [1], in particular recent tissue engineered approaches using fibronectin [2], is that whilst axons readily enter and traverse the bridging graft they generally fail to exit the graft and re-enter the parenchyma due to the inhibitory glial scar at the graft-CNS interface. The aim of this work was to create a 3D cell culture model of CNS reactive gliosis, in which improved tissue-engineered repair strategies can be developed.

METHODS: Primary rat astrocytes were seeded onto collagen coated coverslips (2D) or into 3D collagen gels and the expression of markers associated with glial scar formation were analysed by immunocytochemistry (GFAP, chondroitin sulphate proteoglycan (CSPG), vimentin, aquaporin 4 (AqP4) and S100β). To stimulate reactive gliosis, 3D astrocyte cultures were treated with TGFβ1 (10ng/ml) every other day for 15 days [3]. Cultures without TGFβ1 were used as controls. Expression of glial scar markers was assessed and measured using Volocity image analysis software (Improvision). Cell perimeter was measured as an indicator of astrocyte activation. Area of immunoreactivity was measured for CSPGs released from astrocytes.

RESULTS: Astrocytes in 3D cultures had significantly less immunoreactivity for GFAP, CSPG, vimentin, AqP4 and S100β, than astrocytes in 2D cultures indicating a lower level of activation in 3D (fig 1). However immunoreactivity for β-actin did not differ in 2D or 3D. Following treatment with TGFβ1, astrocytes in 3D became hypertrophic and reactive, with changes in the expression of gliosis markers over time relative to control cultures (fig 2).

DISCUSSION & CONCLUSIONS: Astrocytes in 3D are less reactive than their 2D counterparts, thus resembling the physiological in vivo situation and providing a useful system in which activation can be investigated. TGFβ1 treated astrocytes over 15 days take on characteristics of the mature glial scar, including their morphology and protein expression profile. This model will provide a useful test-bed for tissue engineered strategies to improve axonal growth through the glial scar environment.


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