Development of an in vitro model of reactive gliosis using stabilized collagen gels with defined and monitored stiffness environments.

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INTRODUCTION: Following injury or insult, failure of the injured CNS to repair is in part attributed to the inhibitory environment of the lesion site, most notably the formation of the glial scar. This consists predominantly of astrocytes, which exhibit a reactive hypertrophic phenotype exemplified by upregulation of various markers, including GFAP\(^1\). In vitro models have been employed to explore the cellular mechanisms occurring during reactive gliosis but there is little understanding of the relationship between astrocytes and their mechanical environment\(^2\). Astrocytes within soft 3D hydrogel cultures have been shown to adopt a less reactive phenotype than in stiff 2D cultures and the progression of reactivity can be monitored\(^3\), however there are difficulties in maintaining and monitoring soft gels that may discourage wider adoption. The aim of this study therefore was to investigate whether stiffer stabilised collagen gels containing astrocytes could be used as a high throughput tool for the study of reactive gliosis, and also to develop methods for measuring gel stiffness to enable the mechanical environment to be controlled and monitored.

METHODS: Primary astrocyte cultures were prepared from P2 GFP+ rat cortices. Astrocytes were dissociated and expanded for 2 weeks before seeding in 3D collagen gels\(^4\) and some gels were treated with 10ng/ml TGFβ1. Gels were stabilised using RAFT™ absorbers (TAP Biosystems, UK). GFAP gene expression was assessed using quantitative real-time PCR analysis. The mechanical properties of the collagen environment were measured using dynamic mechanical analysis (DMA; BOSE Electroforce 3200).

RESULTS: Compressive DMA enabled the mechanical properties of the astrocyte-seeded hydrogels to be characterised (Fig 1). Astrocyte-seeded hydrogel stiffness increased between 0 and 5 days in culture (Fig 1). Addition of TGFβ1, a cytokine considered to be a likely trigger of reactive gliosis in vivo, led to an increase in GFAP expression at day 10 and day 15 (Fig 2).

DISCUSSION & CONCLUSIONS: The TGFβ1-mediated upregulation of GFAP in astrocytes within stabilised gels mirrors their response during astrogliosis in vivo, indicating the utility of this approach as a research model. The stabilized gel system is more robust than previous softer hydrogel approaches and is amenable to widespread adoption. DMA permitted detailed analysis of the mechanical properties of stabilised collagen gels and provides a way to investigate the role of biomechanics in reactive gliosis.

REFERENCES:  