A multi-well plate model of reactive gliosis for high throughput screening of potential CNS therapies

Conference or Workshop Item

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Reactive astrogliosis is an important feature of CNS damage and disease which involves changes in astrocyte phenotype and morphology. In particular, CNS trauma can lead to the formation of a glial scar, a three dimensional (3D) mesh of astrocyte processes that can form a physical and chemical barrier to neuronal regeneration, which is a potential target for CNS drug and cell therapy. However, reactive gliosis is difficult to isolate and monitor in typical animal models of CNS damage. In monolayer culture systems astrocytes adopt a highly reactive phenotype, limiting the range of available models suitable for research in this area.

Our previous 3D cell culture systems allow astrocytes to be maintained with a relatively unreactive phenotype until stimulated, whereupon a classical reactive astrocyte response can be monitored. The aim of the current work is to adapt this approach in order to develop a multi-well plate system to provide a reliable, consistent model of reactive gliosis for high throughput screening and research. Once baseline viability and phenotype of primary rat astrocytes were investigated in these models, reactivity was triggered using treatments such as TGFβ1, as seen in Figure 1, hypoxia and low glucose. Outputs included confocal microscopy and 3D image analysis, Western blotting and RT-PCR to quantify markers such as GFAP and CSPG in test and control gels. Using GFP-labelled astrocytes permitted monitoring of cytoplasmic volume and shape, giving an additional measure of astrocyte hypertrophic response within stimulated conditions. The robust protocol that we have developed can form a basis to investigate astrocyte biology in a highly controlled environment, and to model phenotypic features of astrocytes in both damaged and undamaged CNS. A reproducible multi-well plate system will provide an experimental platform which allows potential CNS therapies to be screened at high-throughput, and the effects of potential modulators of astrocyte reactivity to be investigated simply and systematically.