

(101) Development of a 3-Dimensional *In Vitro* Model to Study Reactive Gliosis Following Nervous System Injury

East E., Golding J.P., Phillips J.B.

The Open University, Department of Biological Sciences, Walton Hall, Milton Keynes, MK7 6AA, United Kingdom

Injury to the spinal cord results in the formation of a glial scar which is associated with inhibition of axonal regeneration. One of the major limitations of research into improving repair strategies is the lack of a cell culture model that accurately recapitulates the complex *in vivo* situation. Our aim is to develop an effective model to address this need.

Astrocytes in the undamaged CNS express low levels of GFAP, but following injury exhibit a reactive phenotype exemplified by GFAP up-regulation. Primary glial cell cultures were analysed in 2D monolayers and 3D collagen gels for GFAP expression. In 2D cultures $73.4 \pm 4.0\%$ of cells were GFAP positive, whereas $40.7 \pm 3.5\%$ were immunoreactive for GFAP in 3D collagen gels. As 3D astrocyte cultures more closely modelled the *in vivo* situation we used this model to investigate the response of astrocytes to dorsal root ganglia cells (DRGs). Dissociated DRGs were labelled with CellTracker™, seeded onto astrocyte-populated collagen gels and maintained in culture for 5 days. Astrocytes near the DRG interface showed marked GFAP up-regulation and adopted a reactive morphology which was observed up to 1 mm away.

Astrocytes in 3D culture exhibit a lower basal level of reactivity than cells grown in monolayer, providing a system in which stimulation of activation can be investigated. This model provides a useful tool for investigating triggers of reactive gliosis, as demonstrated by the response observed to cells found at the inhibitory interfaces formed following damage to the spinal cord.