

Astrocytes expressing GFP in 3D collagen gels provide an effective model for screening the glial response to potential CNS cell therapies

N Johns¹, E East¹, JP Golding¹, AJ Loughlin¹, JB Phillips¹

¹ *The Open University, Milton Keynes, UK.*

INTRODUCTION: Cell therapies hold promise for use in many CNS repair scenarios. A critical consideration in selecting cells suitable for implantation into the CNS is the extent of the host glial cell response. In particular, astrocytes can become reactive in response to the presence of non-CNS cells, undergoing proliferation and hypertrophy and contributing to the ‘glial scar’ and inhibition of neuronal regeneration [1,2]. Here we have developed a 3D culture system for screening the effect of potential therapeutic cells on astrocyte reactivity. This builds on previous work that developed 3D cultures to model reactive gliosis as assessed by immunodetection of astrocyte reactivity markers (such as glial fibrillary acidic protein, GFAP) over 15 days [3]. Here we use changes in astrocyte green fluorescent protein (GFP) volume as an early indicator of astrocyte hypertrophy, a key feature of reactive gliosis.

METHODS: Primary cortical astrocytes from rats expressing GFP were seeded at 2×10^6 cells/ml within 1.5 ml (~4 mm deep) type I collagen gels in 24-well culture plates. Schwann cells, PNS glia that induce astrocyte reactivity, were seeded onto the surface of the gels (20k cells/gel). After 5 days in culture, gels were fixed and analysed using confocal microscopy and 3D image analysis. Within each individual gel, three regions at the top of each gel (in contact with the cells under test) and three control regions at the bottom of each gel were analysed for GFP volume per cell. Gels were also immunostained for GFAP as previously [3]. For each gel, the volume of GFP and GFAP staining per cell at the top was expressed as a ratio over the equivalent control volume at the bottom.

RESULTS: Astrocyte morphology was detectable using GFP fluorescence and GFAP immunostaining (Fig 1). Astrocytes in the ‘top’ regions adjacent to the Schwann cells showed a ~5-fold greater cytoplasmic volume (hypertrophy) as determined by GFP volume analysis than control cells near the bottom of the gels. There was a corresponding increase in the volume of GFAP immunoreactivity, although this was less marked (~2-fold greater than control cells; Fig 2).

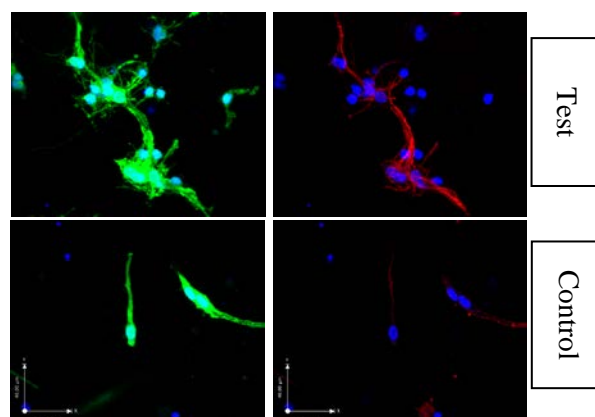


Fig. 1: Typical micrographs of GFP (green) and GFAP (red) in test (top) and control (bottom) regions of 3D astrocyte gels after 5 days.

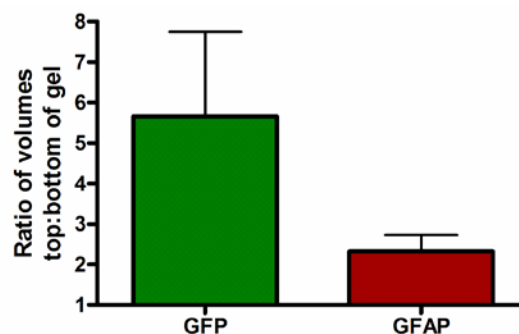


Fig 2: Astrocyte reactivity in response to Schwann cells. Data are means \pm SEM of the ratio of GFP or GFAP volume per cell compared to control regions in the same gel.

DISCUSSION & CONCLUSIONS: Astrocyte GFP volume in 3D culture provided an effective readout for detecting hypertrophy related to reactive gliosis in response to adjacent Schwann cells after 5 days. An added advantage of using GFP astrocytes is that it allows them to be distinguished from test cells, some of which might also express astrocyte reactivity markers. This approach provides a rapid and effective new tool for screening potential therapeutic cells *in vitro* in terms of their ability to elicit reactive gliosis.

REFERENCES: ¹ K.H. Adcock et al (2004) *Eur J Neurosci* **20**: 1425-35. ² A. Lakatos et al., (2003) *Exp Neurol* **184**: 237-46. ³ E. East et al (2009) *J Tiss Eng Regen Med* **3**: 634-46.