Adapting aligned, stabilised 3D tissues for large-scale neurobiological research

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INTRODUCTION:

Recreating the 3D environment of the CNS using hydrogel matrices allows neurons and glial cells in vitro to behave similarly to their counterparts in vivo, providing a relevant tool for neurobiological studies[1]. The overall aim is to develop robust 3D CNS tissue models engineered by a process of glial cell self-alignment and subsequently stabilised. Furthermore, these models have been developed for multi-well plate format at a scale suitable for high throughput screening. CNS tissue equivalents can be used to assess numerous aspects of the CNS in a reproducible, controllable and consistent manner.

METHODS:

Characterisation studies assessed alignment and stabilisation of neurons and glia in collagen gels within a 96-well plate test rig prototype. Their potential for use in neurobiological studies involved identifying neurite growth, neuron-glial interactions and myelination following defined periods in culture. Detection and quantification analysis was conducted via immunohistochemistry and confocal microscopy.

RESULTS:

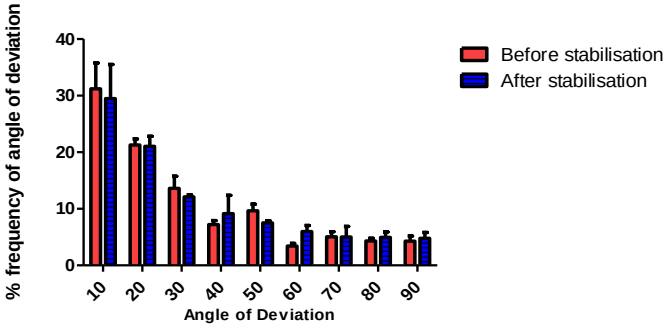

Fig 1 Alignment of neural cells persists following stabilisation of hydrogels

Hydrogels constructed within a 96-well plate rig displayed comparable cellular alignment to traditional methods using larger moulds[2], in mid and side regions, before and after stabilisation of constructs.

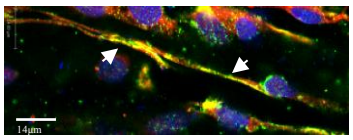

Fig 2 Confocal micrograph showing cellular alignment and neuron-glial interaction in aligned 96-well plate rig hydrogel after 14 days. Arrowheads indicate immunoreactivity for myelin basic protein adjacent to neuronal structures (Red-β-tubulin, green-MBP, blue-Hoechst).

Neurite growth was detected and measurable in the aligned tissue equivalents. Markers for myelination were identified in close proximity to neurites.

DISCUSSION & CONCLUSIONS:

Results suggest that a highly organised, stable hydrogel can be created within the dimensions of a 96-well plate. The aligned nature of the cells and extracellular matrix in this anisotropic system facilitates quantitative analysis of CNS cellular features such as neurite length and the process of myelination. This simple, consistent and physiologically relevant model system, which uses a multi-well plate format can potentially be used at a scale suitable for commercial R&D.

REFERENCES:

1. E. East et al (2012) *Tiss Eng* **16**: 3173-8.
2. M. Georgiou et al (2013) *Biomaterials* **34**: 7335-43.