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Defining glial cell self-alignment parameters for 3D CNS tissue models.

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INTRODUCTION: Recreating the 3D spatial environment of the CNS allows neural cells *in vitro* to behave more like their counterparts *in vivo*, providing robust and controllable model systems that mimic key aspects of the cell biology of the nervous system¹. The overall aim is to develop engineered neural tissue models to resemble functional CNS tissue, with anisotropic tracts of neurons and glia arranged within a robust collagen hydrogel, at a scale suitable for drug screening. To establish viable production technology for the manufacture of CNS tissue models, the parameters, cell density and contraction, that govern glial cell self-alignment have been optimised.

METHODS To achieve consistent predictable glial cell alignment, an assay system was developed for determining optimal glial cell seeding density. 24-well and 96-well plate contraction profiles were conducted using C6 glioma cells in free-floating round collagen gels at 0.1 to 6 million cells/ml. Specific seeding densities were then used in alignment assays using tethered rectangular collagen gels². Tethered gels were fixed and stained using Haematoxylin and Eosin and micrographs were analyzed to assess cellular alignment in 2 regions with characteristic alignment patterns (side and middle) and a control region, in which cells are randomly oriented².

RESULTS: The two contraction profiles (Fig 1) follow a similar pattern, illustrating that a satisfactory profile can be constructed in either a 96-well or a 24-well plate. Contraction reached a plateau at 3×10^6 cells/ml.

A significant degree of alignment was measured using cell densities of 2, 3 and 4×10^6 cells/ml in both middle and side regions, whereas 0.5 and 1×10^6 cells/ml only produced significant alignment in the side region (Table 1).

DISCUSSION & CONCLUSIONS: A two-stage approach was developed to determine the optimal glial cell seeding density to achieve consistent, predictable alignment. The establishment of the relationship between contraction and alignment will allow the optimal seeding density for alignment to be predicted using a small number of cells, regardless of the cell source.

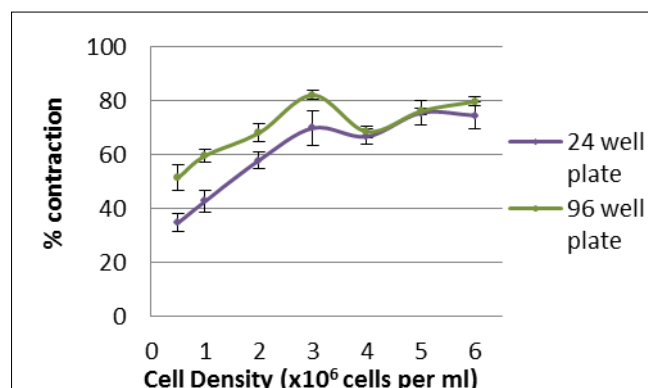


Fig. 1: **Contraction Profiles** show the extent of gel contraction after 24 h.

Seeding density (cells/ml gel)	Control v Mid	Control v Side
0.5×10^6	ns	***
1×10^6	ns	***
2×10^6	***	***
3×10^6	***	***
4×10^6	***	***

Table 1. Table shows whether statistically significant alignment was present in test (middle and side) vs. control zones of tethered gels. (ANOVA with Dunnett's post test to compare each test zone to the unaligned control zone, ns = no significance, *** $P < 0.001$).

This is important for the development of viable production technology to generate anisotropic CNS tissue models; since the contraction profile of a small sample of cells can now be used to indicate the optimal seeding density required to give alignment throughout the collagen hydrogel. Thereby, the optimal cell density, regardless of type and source of cell, which will produce reliable alignment is one that achieves between 60 and 80% contraction, information which can be easily calculated using a simple 96-well-plate assay

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