Combinatorial Approach to Assess Neuronal Cell Interactions on Topographically and Chemically Defined Surfaces

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Introduction:

Cell-surface interactions are key in terms of many cellular characteristics including attachment, migration and proliferation. Scaffold materials used for tissue engineering applications have excellent bulk properties but often require coatings to impart desired cell responses. Surface cues currently recognised to direct cell responses are chemistry, topography and rigidity, although particular characteristics presented at the surface may vary between cell types. A detailed understanding of cell-surface interactions requires methods to control and investigate these interactions to allow further optimisation of biomaterials.

Our approach is to design and fabricate platforms that present surface chemistry varied in the form of a gradient, overlaid orthogonally onto a gradient of topography. Such surfaces allow a wide range of surface properties to be evaluated simultaneously offering high throughput advantages. The current project explores neuronal cell behaviour on chemically and topographically engineered surfaces. Single culture of neurons as well as co-culturing of neurons on a pre-seeded bed of radial glia are of interest as the latter naturally guide neuronal growth in the developing brain.

Methods:

Surface Preparation

PMMA platforms having ranging groove widths from 5-100 µm were fabricated via hot embossing. Gradient chemical modification was afforded by plasma polymer deposition of allyl amine (ppAAm) followed by a subsequent layer of plasma polymerised hexane (ppHex) deposited to have a varying thickness across the surface orthogonal to the groove direction.

Cell Culture

C6 glial tumour cell line was used; along with primary material collected from embryonic stage 14 wistar rat cerebella and postnatal day 1 cortex, for radial glia and neuronal cultures respectively. Enrichment of glial and neuronal fractions was achieved via differential adhesion followed by FACS cell sorting. Cells were then seeded onto platforms and fixed after allotted time points. Fluorescence microscopy allowed visualisation of cells in relation to surface cues.

Results and Discussion:

A protocol was developed for the enrichment of glial and neuronal fractions, proving successful via identification of nestin/3CB2 radial glial markers and neurofilament respectively.

Cell alignment onto grooved structures was observed with specific areas of higher cell density. Cell alignment with groove direction was also assessed, showing areas of higher alignment compared to others.

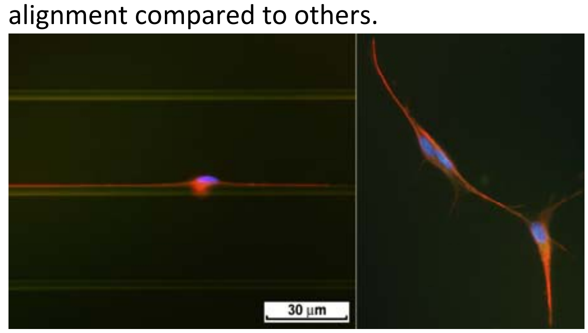

Figure: Radial glia alignment on ppAAm grooves (Left) and culture on a surface with the same chemistry but not patterned (Right).
Red – 3CB2 radial glial marker, Blue – DAPI nuclear stain, Green – autofluorescence of PMMA substrate.