THE EFFECT OF SUBSTRATE STIFFNESS ON ASTROCYTES AND LEPTOMENINGEAL CELLS

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INTRODUCTION

The meninges cover and protect the brain and spinal cord. The meninges are connected to brain tissue by the pia mater and arachnoid layers, also known as the leptomeninges. The cells that cover the leptomeninges and trabeculae of the subarachnoid space are known as leptomeningeal cells (LMC's). The LMC's interact with cortical astrocytes within the brain tissue – connecting the brain to the meninges on a cellular level.

As the structure and stiffness of the tissue's extracellular matrix (ECM) regulates cellular function, changes in its stiffness with ageing and neuropathology can alter the mechanosensitivity of these resident cells. This may contribute to the progression of neuro-degenerative diseases [1]. From research it has been shown that as we age our cerebral ECM softens [2, 3, 4]. Moreover, research has found adult brain tissue to have a mean Young's Modulus value of ~3kPa. Although, no research has been carried out in relation to the age dependency of leptomeningeal mechanics, the Youngs Modulus of the adult leptomeninges was found to be ~8MPa [5, 6]. This difference in stiffness between these two tissues in contact needs to be studied further to aid in understanding the neurological environment more in-depth to be able to diagnose disease and offer better treatments.

Therefore, the aim of this research is to investigate the effect of different substrates and substrate stiffnesses on cortical cells such as astrocytes and LMC's.

MATERIALS AND METHODS

Immortalized DiTNC1 (rat cortical astrocytes) were seeded on substrates at different concentrations. The substrates chosen for this study were collagen, gelatin, and poly-L-lysine (PLL). Immunofluorescent staining and confocal microscopy were performed, as well as western blotting.

For immunofluorescence, three identical 12-well plates were incubated at 37ºC, and one plate was fixed every day for three days. The three plates were then stained with primary antibody (GFAP 1:500, G3893) and secondary antibody (Anti-mouse 1:1500, Ab150113) as well as DAPI and phalloidin. Plates were then imaged using the confocal microscope at a magnification of 40X.

For western blotting, T75 flasks were coated with substrates, seeded with $5x10^5$ cells and incubated at 37ºC for three days. Cells were then lysed, and electrophoresis and transfer was performed. The membrane was incubated in primary antibodies (GFAP 1:1000, G3893, GAPDH 1:5000, PA1987) at 4ºC overnight, and then in secondary antibodies (Goat anti-mouse 1:10,000, A11375, Donkey anti-rabbit 1:10,000, A32802) for ~2hrs at room temperature.

RESULTS

The main findings of the experiment will be cellular response and morphology (Figure C-F). Immuno-fluorescence and western blotting will be used to analyse and interpret the most prevalent biomarker identified in astrocytes, GFAP, in order to determine the frequency of expression. The data obtained will include quantitative protein expressions and qualitative descriptions of the morphology of the cells seeded on different substrates at various concentrations. The cells seeded on plastic culture ware will serve as the control.

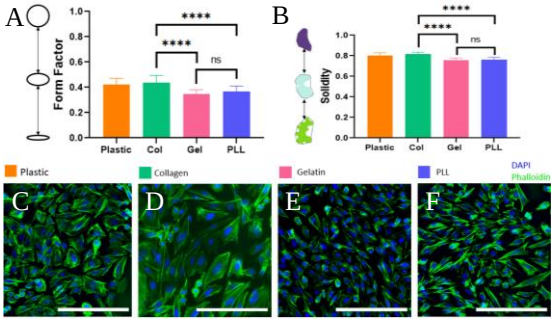

Figure 1 (A) Bar chart of form factor (circularity) of cells at confluency on plastic and substrates at recommended concentrations. (B) Bar chart of solidity (density) of cells at confluency on plastic and substrates at recommended concentrations. (C-F) Immunofluorescent images of astrocytes seeded on Plastic, Collagen, Gelatin and PLL, respectively, staining for phalloidin (green) and DAPI (blue). Scale bar = 200µm.

DISCUSSION

For this study, by examining the morphological changes of astrocytes on different substrates and stiffnesses, the response of astrocytes varied across substrates. As the concentrations used for this experiment were that of the recommendations by the manufacturer, future experiments will aim to mimic the in vivo stiffnesses. Additionally, substrates which mimic the in vivo environment of the leptomeningeal and cerebral ECM's will be examined. Moreover, future experiments will use LMC's, as well as both cells in contact to establish a better understanding of their response to stiffness.

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