## P.08 Induced guidance of NIH 3T3 fibroblasts on Polydimethylsiloxane (PDMS) ridge-groove substrates: a time-lapse live-cell study

C-K Huang and A Donald

Biological and Soft Systems Sector, Cavendish Laboratory, University of Cambridge, UK

Adherent mammalian cells and micro-organisms typically exhibit distinctive responses towards substrates with particular textures; the textures can be topographical, chemical, or both. Textured surfaces are readily observed to influence a cell's migration direction, alignment, adhesion, morphology, and even differentiation. Although the phenomenon has been extensively surveyed on different types of cells and on a variety of surface textures, the exact dynamical picture of how the cells are influenced by its underlying substrate has yet to be revealed. By creating thin film ridge-groove structures from polydimethylsiloxane (PDMS), we were able to probe, using live-cell methods, the dynamic interactions between cells and surface topographies at greater resolutions. Our investigations on NIH 3T3 murine fibroblasts revealed that the cellular protrusions lamellipodia and filopodia readily bend over the 90 degree angle at the ridge edge. Nevertheless, despite the versatility of these cellular protrusions, the 3T3s rarely trafficked from the ridge into the groove, or vice versa. In fact, if using the cell nucleus as the determinant in identifying ridge or groove cells, out of the 51 cases of 3T3s tracked over 10 hours on average, only 3 instances were observed where the cell trafficked across the ridge edge. Intuitively, this suggests possible contributions from the cell nucleus towards cell-topography interaction. Contemplating that nucleus' stiffness would potentially impose enough mechanical resistance when incident upon the edge, the nucleus stiffness of our 3T3s were modified using the histone deacetylases inhibitor, trichostatin A (TSA). It has been shown that this drug loosens the chromatin and subsequently induces a reduction in nuclear stiffness by as much as 60%. However, our results do not show an improved migration across the ridge edge, even with this much modulation in nucleus stiffness. Specifically, 37 3T3s incubated with TSA were tracked, but only one event showed the nucleus crossing the ridge edge. This result can, by no means, discount influences from nuclear stiffness though, but merely that a greater reduction in the nucleus' stiffness maybe required before the influence is revealed. There may also be possibilities of other determinants towards this topographical restriction. And to further complement our results we will also investigate into the contributions from cytoskeletal components; in particular the filamentous actin (F-actin). We plan to adopt live-cell time-lapse imaging of F-actin through the transfection of the Lifeact plasmid; this plasmid DNA, when expressed in the cell, produces green fluorescent protein (GFP) tagged markers that stain F-actins. The purpose of this endeavor is to identify any cytoplasmic actin structures that may be induced by the edge restrictions, which in turn restricts the cell nucleuses.