P001 Highly controlled surface presentation of protein signalling motifs to regulate cell behaviour

Elizabeth Mitchell[1], Jeremy Lakey[1], Mark Birch[1].
[1]Newcastle University, Newcastle upon Tyne, UK

Immobilised ligands have a profound affect on cell activity. In this study we used a protein engineering approach to produce monolayer surfaces that maintain consistent protein structure, orientation and hence bioactivity. Peptide motifs with the potential to influence cell behaviour (cell adhesion motif from osteopontin, OPN-Tol or BMP peptide homologue, BMP-Tol) were inserted into the protein scaffold backbone and the ability of these surfaces to control bone cell (osteoblast) activity was investigated.
Protein structure was verified by CD and the interaction of proteins with the surface was characterised by SPR. OPN-Tol dose-dependently influenced cell adhesion ($p \leq 0.05$) and significantly altered the ability of the cells to spread through vinculin adhesion sites ($p \leq 0.05$). Signalling by BMP2 (200ng/µl) was shown to be influenced by degree of cell adhesion on OPN-Tol surfaces ($p \leq 0.05$). Analysis of cells cultured on BMP-Tol demonstrated activation of the SMAD signalling cascade with significantly enhanced expression of a SMAD-binding element reporter construct ($p \leq 0.05$), indicating activity of the immobilised ligand. This technology effectively immobilises bioactive protein motifs on a surface for analysis of cell behaviour *in vitro* and may provide the basis for future tissue engineering constructs.