

ABSTRACTS

Evidence for “self-induced” mechanotransduction in human fibroblasts cultured on nanotopography.

Dalby, M.J., M.O. Riehle, N. Gadegaard, D. Sutherland, A.S.G. Curtis and C.D.W. Wilkinson.
Centre for Cell Engineering, IBLS, University of Glasgow, G12 8QQ, UK

It is becoming clear that the nano-environment is of significance when considering cell response. The use of genomics has indicated that there is a complicated interplay of signalling events that control the cell responses to nano-topographies. By using nano-topography produced by methods including electron beam lithography (producing nano-pits) and colloidal lithography (producing nano-columns) to alter the spreading and adhesion of human fibroblasts, we present evidence that a major signalling pathway relies on mechanotransduction. Different topographies can influence cell morphology and cytoskeletal formation. The nanotopographies tested reduced cell spreading and the fibroblasts had less organised microtubules, microfilaments and intermediate filaments. As a result of these factors, the nuclear morphology was altered in to a more spherical shape, and the positioning of chromosome 3 was altered during interphase. 1718 gene microarray has also been used to elucidate the range of regulatory changes within the cell genome. It has been hypothesised that interphase chromosomes have a consistence of position within the nucleus. We are presently looking at other chromosomes. Our results indicate that by altering the positions of the chromosomes, changes in gene regulation are observed. These changes directly relate to proliferative and phenotypical responses, and thus may be significant in producing nano-materials for tissue engineering.

Acknowledgements: Matthew Dalby is a BBSRC David Phillips Fellow and is supported through that route (17/JF/20604). H. Agheli.

The effects of competitive guidance cues on fibroblast cell alignment: electric fields vs. contact guidance

Ian Gibson and Colin McCaig

When bovine ligament fibroblast cells were cultured on parallel micro-grooved surfaces, they aligned their long axes parallel to the groove direction. This alignment was dependent on the groove depth, with increasing groove depth (62, 347, 547 and 1024nm) resulting in increased guided cell alignment. When fibroblast cells were cultured in a physiological electric field (EF) on non-grooved, flat surfaces, the cells aligned in response to the electric field, with their long axes aligning perpendicular to the electric field vector. This response was field strength dependent, with increasing field strength (20, 50, 100 and 200mV/mm) resulting in increased guided cell alignment, perpendicular to the EF vector. These two guidance cues were applied simultaneously, so that the EF vector was parallel to the groove direction. For high field strengths (200mV/mm) cells ignored the topography and were guided by the EF alone, with similar alignment, perpendicular to the EF vector, to cells on non-grooved surfaces. Low field strengths (20mV/mm) resulted in cells responding only to the topography as a guidance cue, with cells aligning parallel to the groove direction and the EF vector. Intermediate field strengths (50 to 100mV/mm) produced a mixed response, with cells appearing to be responding to both guidance cues, with cell alignment relative to the field vector ranging from approximately 30-55°. The effect of removing serum from the culture medium on the EF and topographical guidance of fibroblast cells was studied using grooves of 347 and 1024nm depth, and an EF strength of 100mV/mm, and the results were compared to cells on non-grooved surfaces. Removal of serum produced a small decrease in the angle of cell alignment for cells on non-grooved surfaces, from 78 to 63°, relative to the EF vector, but did not completely suppress the EF guidance cue. In contrast, the EF guidance of cells on both grooved substrates was completely suppressed by the absence of serum, with cells responding only to the grooved topography, aligning their long axis parallel to the groove direction/EF vector. These results imply that alignment of fibroblasts by topography is serum-independent, but alignment by EFs is serum-dependent. An initial attempt has been made to try to identify which component of the serum is critical in EF-guided cell alignment by adding different concentrations (10 and 100ng/mL) of basic fibroblast growth factor (b-FGF) to serum-free culture medium. These additions did not restore the EF-guided alignment of cells on grooved surfaces observed in experiments containing serum. These results demonstrate that the alignment of fibroblast cells can be tailored by the dual guidance cues of topography and electric fields.

