Effects of nanotopography on cell adhesion, morphology and differentiation

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INTRODUCTION: Topography and its effects on cell adhesion, apoptosis and differentiation have been well documented. Current advances with the use of nanotopography provided us with promising results in the field of regenerative medicine (Dalby et al 2007). Examining closely the effects of nanotopography on cell adhesion and morphology and the consequences of cell shape changes in the nucleus and gene expression we will be a step closer to understand and even control stem cell differentiation. In doing so, a molecular approach was used in combination with immunostaining studies and data will be presented.

METHODS: Stro-1 selected skeletal stem cells were used to study early time-point (3day) events in mechanotransduction. To study this, nanopits (120 nm diam, 100 nm depth) that were ordered (300 nm centre-centre square) and also with a controlled degree of nanodisorder ($\pm$50 nm from centre of square) were used and compared to planar controls. These surfaces are known to change stem cell fate and to examine mechanotransductive events, cell, nucleus and adhesion morphology has been quantified and microarray analysis performed. Transcriptional changes were analysed with Ingenuity Pathway Analysis (IPA). Several inhibition studies have also been performed. Furthermore, the organization of the interphase nucleus has also been considered by lamin nucleoskeletal staining and chromosome territory analysis using FISH.

RESULTS: The results clearly show large changes in cell adhesion, nucleus and lamin morphologies in response to the different surfaces. Furthermore, these changes relate to changes in packing of chromosome territories within the interphase nucleus. IPA shows a wide range of signalling pathway regulatory changes hinging around hub signalling effectors such as ERK (extracellular receptor kinase). This, in turn, leads to changes in transcription factor activity and functional (phenotypical) signalling.

DISCUSSION & CONCLUSIONS: Nanotopography is a very useful non-invasive tool for studying cellular mechanotransduction, gene and protein expression patterns, through its effects on cell morphology. The different nanotopographies resulted in different morphological changes in the cyto- and nucleo-skeleton as well as the chromosomes. Consequences of these changes have possibly contributed to the genomic changes observed. We propose that both indirect (biochemical) and direct (mechanical) signaling is important in these early stages of tuning stem cell fate. The work presented here provides us with a better understanding of cell-surface interaction and possibly new insights of how to control cell differentiation with future applications in areas like regenerative medicine.

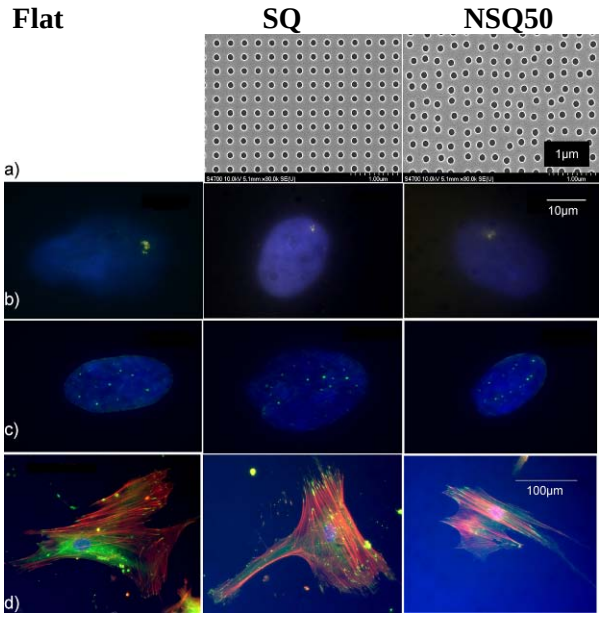

Fig. 1: Effect of different nanotopography patterns (a) on Chromosome 1 territory (FISH) (b) Lamin A staining shows differences in the organisation of the interphase nucleus (c) cell attachment /morphology using Actin(red filaments)/Vinculin (green adhesions) /DAPI (nucleus) staining

REFERENCES:

[1]Dalby MJ, Gadegaard N., Tare R, Andar A., Riehle M.O., Herzyk P., Wilkinson C.D.W., Oreffo R.O.C. The Control of Mesenchymal Stem Cell Differentiation Using Nanoscale Symmetry and Disorder. (2007) Nature Mat.

ACKNOWLEDGEMENTS: This project is funded by BBSRC grant BBG0088681.