

## Investigating active mechanosensing with micro- and nanostructures

M. O. Riehle<sup>1</sup>, M. Dalby<sup>1</sup>, L. Csaderova<sup>1</sup>, A. McIntosh<sup>1</sup>, M. Robertson<sup>2</sup>, & N. Gadegaard<sup>2</sup>

<sup>1</sup> *Centre for Cell Engineering, FBLs, University of Glasgow, Scotland, GB*

<sup>2</sup> *Dept of Electric and Electronic Engineering, University of Glasgow, Scotland, GB*

### INTRODUCTION:

It is widely accepted that the chemical, topographical and mechanical makeup of the environment is significantly influencing cell responses. Such environmental cues occur in a cells natural environment as well as on untreated- or surfaces specifically designed at the micro- or nanoscale. Cells react to such cues by changes in cell shape, motility, behaviour and gene expression [1]. To investigate how the responses differ between micro- and nanometric topography and mechanically yielding substrates we fabricated a variety of substrates and followed the cellular reaction by timelapse, SEM, fluorescent microscopy and gene array studies.

We hope to present evidence that a major signaling pathway relies on self-inflicted mechanotransduction.

### METHODS:

Topographies were fabricated as described earlier [2] using photo-lithography, electron-beam and colloidal lithography to create master substrates with micro- or nanotopography. For regular nanotopographic features this is followed by copying first into a nickel die and then replication into polymer substrates used for experiments. Soft substrates were fabricated according to [3]. Displacement was measured using the method developed by I Toliç-Norrelykke [4] using soft sylgard with embedded fluorescent beads (modified from [5]). Cell culture, fixation and staining of hTert fibroblasts for cytoskeletal elements and chromosome painting as well as the mRNA recovery and the hybridization on a 1718 gene microarray followed procedures published previously [1].

### RESULTS:

Substrates of increasing softness allowed cells to spread less, show less stress fibres and less organized microtubules, this is paralleled by cells on nanotopographies which reduced cell spreading and the fibroblasts also 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. mRNA isolated and

tested on a 1718 gene microarray has also highlighted a range of regulatory changes within the cell genome. Given cells a choice of increased mechanical feedback by placing them onto fabricated surface rigidity patterns cells would move from a soft to a hard substrate, and align to such features. Alternatively if the cells were plated onto patterned nanotopographies (patterned on a level >5µm) cells would preferentially spread on the non-nanopatterned parts of the substrate.

### DISCUSSION & CONCLUSIONS:

It has been hypothesised that interphase chromosomes have a consistence of position within the nucleus. Our results indicate that by altering the positions of the chromosomes, changes in gene regulation are observed. By using nano-topography to alter the spreading and adhesion of human fibroblasts, and comparing the effects observed to results obtained on flat but mechanically varied substrates we propose that the underlying principle behind the reaction are similar, in that cells try to optimize their effective mechanical feedback according to the hypothesis put forward by Bischofs & Schwarz [6]. These changes directly relate to proliferative and phenotypical responses, and thus may be relevant to the production of materials for tissue engineering.

### REFERENCES:

- <sup>1</sup>Dalby (2002) et al. *Exp. Cell Res.* **276**: 1-9.  
<sup>2</sup>Wilkinson et al. *Mater. Sci. Eng. C-Biomimetic Supramol. Syst.* **19**: 263-9. <sup>3</sup>Pelham & Wang (1997) *PNAS* **94**:13661-5. <sup>4</sup>Butler et al. (2002) *Am J Physiol* **282**: C595-605. <sup>5</sup>Balaban et al. (2001) *Nat Cell Biol* **3**: 466-72. <sup>6</sup>Bischofs & Schwarz (2003) *PNAS* **100**: 9674-9.

**ACKNOWLEDGEMENTS:** The authors would like to thank for funding by [EPSRC](#) (GR/S13415/01), [EU](#) (FP-V QLK3-CT-2000-01500), [Royal Society](#) Wolfson Foundation Laboratory Refurbishment Grant, and [SHEFC](#) (Scottish Mechanotransduction Centre). N. Gadegaard is a [Royal Society Edinburgh](#) SEELLD Fellow. M. Dalby is a [BBSRC](#) David Phillips Fellow (17yJFy20604).