

## Cell Response to Microfabricated Surface Rigidity Patterns

<sup>1</sup>L. Csaderova, <sup>1</sup>M. Riehle, <sup>1</sup>A. McIntosh, <sup>2</sup>M. Robertson

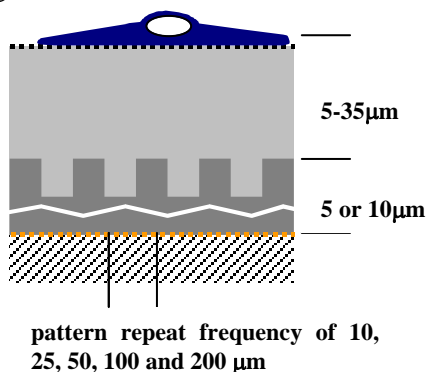
<sup>1</sup> *Centre for Cell Engineering, IBLs, Glasgow University, Scotland, GB*

<sup>2</sup> *Department of Electronics and Electrical Engineering, Glasgow University, Scotland, GB*

**INTRODUCTION:** Lo et al. [1] demonstrated the ability of cells to react to a gradient in surface rigidity by orientation and migration towards a stiffer substrate. This reaction was termed durotaxis. To explore this phenomenon further a better control over rigidity gradient and position of rigidity defining features is needed. We developed a method to fabricate substrates with micrometric rigidity landscape and investigate cellular sensing mechanism at a single cell level.

**METHODS:** Rat calvaria bone cells isolated from neonatal rats by serial digestion were used.

Polyacrylamide gel substrates were created using a modification of Pelham and Wang [2] by incorporating a microfabricated grooved surface underneath the top gel to achieve a surface rigidity pattern. When a curved structure (CSEM, Neuchatel, Switzerland) is used to create a bottom gel, the resulting double gel provides a defined rigidity gradient.



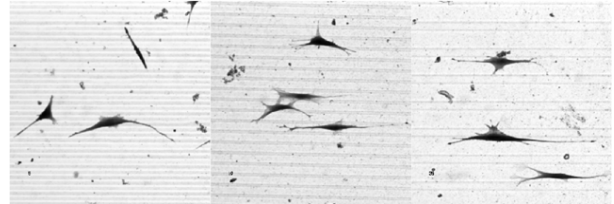
*Fig.1: Fibronectin coated polyacrylamide “double gel”, with a softer top layer, polymerized over a groove patterned stiffer bottom layer.*

Force measurements were performed by tracking the changes of positions of beads incorporated into top gel of substrates and using a Fourier transform traction cytometry [3].

**RESULTS:** Polyacrylamide double gels provide a surface with defined linear patterns of differences in surface rigidity, with various repeat frequencies (10-200 $\mu\text{m}$ ) depending on the width of an underlining grooved pattern. The rigidity step value corresponds with a depth of bottom gel grooves.

Cells align in parallel with step changes in substrate rigidity and elongate. This response was observed for burrowed grooves of both 10 and 5 $\mu\text{m}$  depth and for repeat frequency of 25 $\mu\text{m}$  and higher. After 24h culture, approx. 80% of total cell area is in touch

with a ‘stiffer’ substrate. These results are confirmed with live video microscopy, tracking the cell movements over 24h. Repeated alignment with burrowed steps was observed. Differences in cell area and elongation seen in case of 10% elasticity gradients further support cell preference for a stiffer substrate and its ability to discriminate it.



*Fig.2: Reaction of cells to spatial frequency of 10, 25 and 50 $\mu\text{m}$  pattern repeat. Surface rigidity difference < 5%.*

Fluorescence microscopy of vinculin shows that cells prefer to form focal adhesions on ‘stiffer’ substrates even in case of cells spread over several features of a rigidity pattern.

Force measurements also show that the direction of maximum force is parallel with a rigidity step, corresponding with a position of underlying grooves. Beads displacements seem to be bigger on ‘stiffer’ parts of the substrate.

**DISCUSSION & CONCLUSIONS:** We were able to demonstrate a new method to create substrate with rigidity patterns of micrometric features and to provide an otherwise uniform environment for cell adhesion. Results show that cells are sufficiently sensitive to discriminate 5% step changes in surface rigidity and react by alignment and elongation. This is consistent with cells applying maximum forces in parallel with linear rigidity features, in the direction of maximum effective stiffness. There seems to be a spatial sensitivity limit of a pattern repeat frequency 25 $\mu\text{m}$ .

**REFERENCES:** <sup>1</sup>C.M. Lo, et al. (2000) *Biophys. J.* **79**:144-52. <sup>2</sup>R.J. Pelham and Y.L. Wang (1997) *Proc. Natl. Acad. Sci. USA* **94**: 13661-5. <sup>3</sup>J.P. Butler, et al. (2002) *Am. J. Phys. Cell Phys.* **282**:C595-605.

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