# Cells response sensing durotaxis by varying mechanical rigidity

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**INTRODUCTION:** Cell movement plays an important role in many processes, such as immune response and wound healing[1]. Physical environment surrounding cells is a key factor in response of cells to surfaces. Recent studies have shown that cell movement is sensitive to the rigidity of the substrate, a phenomenon defined as "mechanotaxis or durotaxis"[2]. The purpose of this study is to investigate cell migration by fabricating uniform substrates imposing different mechanical stiffness with patterns of various shapes.

**METHODS:** The fabrication of the thin membrane device is illustrated in Fig. 1. Substrates with pillars of various shapes and sizes were fabricated by simply replicating a patterned SU-8 master with a mixture of PDMS 10:1, (Sylgard 184 Silicone Elastomer Kit, Dow Corning, Midland, MI), which were subsequently easily peeled from the mould. Meanwhile a 50:1 PDMS membrane, was spin-coated onto a glass cover slip on which a thin layer of Trehalose is pre-coated as a sacrificial layer. The sacrificial layer served to prevent the rupture of the thin PDMS membrane, when being peeled. Next, both the prepatterned PDMS substrate and thin PDMS membrane are treated by oxygen plasma, and brought into contact to form an irreversible bond. Finally, the resulting substrate was coated with a fibronectin..

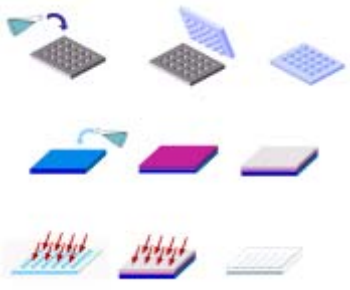

*Fig.1:Schematic steps of the fabrication of the membrane device*

The cellular response was monitored with live microscopy and determined by immunostaining.

**RESULTS:** National Institutes of Health 3T3 and hTERT (human telomerase reverse transcriptase) fibroblasts were cultured on the membrane device, and a directional locomotion to stiffer regions was clearly observed, as shown in Fig. 2. After 24 hours, it was clearly seen the accumulation of the cells following the underneath pattern, due to a preference for stiffer regions as no other physical or chemical changes had been introduced during cell migration.

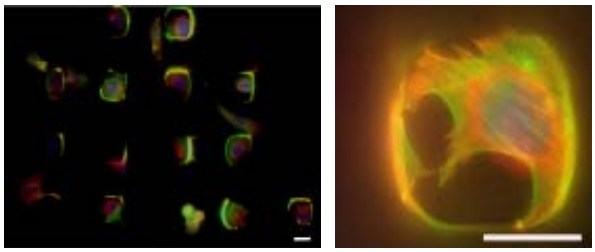

*Fig. 2: Composite immunofluorescence images of 3T3 Nih, stained with actin (red) and vinculin (green) on square pillars. Scale bar 20 μm.*

**DISCUSSION & CONCLUSIONS:** This work presents the development of a PDMS device, consisting of a thin PDMS membrane bonded on top of a stiffer patterned PDMS substrate, with a stiffness gradient, without varying chemical properties or thickness of the substrate. By culturing cells within a uniform defined environment, we have observed a migration of cells towards stiffer regions. It is obvious that this behaviour is due to rigidity gradient of the substrate. This innovative approach suggests potential applications for studies of the mechanism of cell migration due to a rigidity gradient, for biomedical and tissue-engineering purposes.

**REFERENCES:** [1] Martin, P. (1997) *Wound healing: aiming for perfect skin regeneration*. *Science*. **276**:75–81. [2]Lo, C. M., H. B. Wang, M. Dembo, and Y. L. Wang (2000) *Cell movement is guided by the rigidity of the substrate* Biophys. J. **79**:144–152.

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