

## **CONTROL OVER CYTOSKELETON OF ADHERENT CELLS BY GUIDANCE OF SUBCELLULAR COMPONENTS**

JW. Lussi<sup>1</sup>, R. Michel<sup>2</sup>, A. Goessl<sup>1</sup>, [M. Textor<sup>2</sup>](#) and [JA. Hubbell<sup>1</sup>](#)

<sup>1</sup>*Biomed. Eng., ETH, Zurich, CH;* <sup>2</sup>*Surface Science (LSST), ETH, Zurich, CH*

**INTRODUCTION:** Restricted cell size and shape achieved by adhesive/non-adhesive patterning have been shown to affect growth, differentiation and death of cells. In contrast to earlier studies we want to study not how the overall cell shape, but how spatial organization of the cell components responsible for contact formation (which also participate in signaling) affects cell physiology. Patterns with feature sizes down to 1  $\mu\text{m}$  in width were chosen to target subcellular components with sizes in the same range (e.g. focal contacts). These patterns were specifically designed to study the effect of different geometries on cell attachment, focal adhesion formation and stress fiber orientation via guidance of subcellular components.

**METHODS:** 10x10 mm Si-or glass wafers exposing hydrophobic features in a non-fouling background were produced by a novel technique, termed SMAP, described elsewhere. Briefly, structures of  $\text{TiO}_2$  in a  $\text{SiO}_2$  matrix were created by standard photolithographic techniques on these substrates. Selective adsorption of dodecylphosphate to the  $\text{TiO}_2$  rendered these structures hydrophobic, while the subsequent adsorption of poly(lysine)-graft-poly(ethylene glycol) to  $\text{SiO}_2$  lead to the formation of a non-fouling background. Upon exposure to the medium, adhesion proteins present in serum, particularly fibronectin and vitronectin, strongly adsorb onto the hydrophobic areas and mediate cell-surface contact formation. These samples were then placed in 24-well plates and seeded with human foreskin fibroblasts (HFF) at a density of 5000 cells/ $\text{cm}^2$  in serum containing medium. After an incubation for typically 15 hours the cells were fixed and immunostained for f-actin and the focal adhesion protein vinculin to visualize stress fibers and focal contact sites, respectively. Stained samples were imaged on a Zeiss 510 confocal laser scanning microscope.

**RESULTS:** The chemical contrast created by SMAP is well recognized by the cells who show clear discrimination between cell adhesive and non-adhesive areas. This contrast is maintained for at least 10 days. Experiments with oxide patterns not subjected to SMAP treatment showed no pattern recognition by the cells, and cells spread freely over the whole substrate. The topography present on SMAP substrates (10-20 nm) by itself has no visible effect. Chemical patterning is therefore needed for spatial cell organization.

Focal contacts represented by vinculin only form on the adhesive features, as can be seen in Figure 1. No adhesion plaques can be observed in the surrounding non-fouling areas. Stress fibers themselves originate in and terminate on these vinculin-rich areas. This shows that SMAP allows us to spatially control focal contact distribution, and therefore dictate cytoskeleton distribution. We have looked at different specific pattern geometries, two of which can be found as insets in Figure 1. We wanted to test whether it is possible to restrict focal contact formation such that these generally elongated structures could only be formed when oriented in a particular direction, either parallel to the main axes of the cell or perpendicular to them. And does it affect the distribution of stress fibers in the cell body?

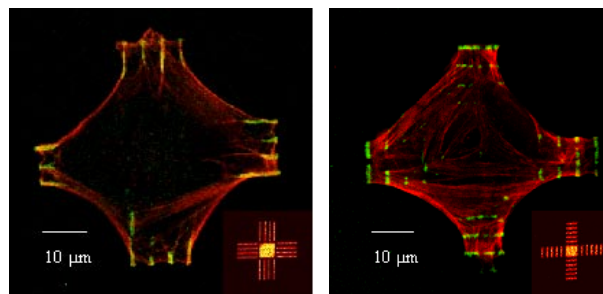


Fig. 1: Single cells, stained for f-actin (red) and vinculin (green), on two types of patterns (insets).

While no distinct correlation between stress fiber distribution and substrate pattern have been observed on the patterns it was found that stress fiber density along the periphery of the cell is higher on patterns with lines perpendicular to the main axes. We speculate that this could be a direct consequence of the fact that our patterns allow formation of larger focal contacts ( $>1\mu\text{m}^2$ ) only when elongated in the direction of the lines. We will test this further using pattern sizes down to 200 nm created by electron beam lithography.

**CONCLUSIONS:** We have shown that by using SMAP it is possible to control distribution of focal contacts and therefore to dictate where stress fibers can be formed. With the availability of nanometer patterns we will investigate whether also orientation of stress fibers can be controlled and study a possible effect of different pattern geometries on physiological functions of the cell.

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