Project A4: Interactions between bio-systems and 3D micro-structured surfaces

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Current state of the research. Biological cells react in subtle ways not only to the chemical properties of surfaces but also to topological features and the mechanical properties of the substrates. One example is the behavior of cancerous cells on post surfaces. The nuclei of these cells deform strongly (see Figure 1) and essentially wrap themselves around the posts under certain conditions. This example has been further investigated during the first funding period in an effort to understand the mechanisms behind this behavior and to develop strategies for the control of the adhesion behavior of cells on microstructured surfaces. One key result of the current project is the development of techniques and materials that allow for an independent control of the surface topology and chemistry. The use of pre-polymers which carry photochemically reactive groups together with lithographical technologies has enabled us to generate 3D microstructures in which certain regions are cell attractive while others are cell repellent (see Figure 2). Additionally, the materials that make up the posts of these microstructures are elastic and the deformation behavior of the microstructures has been successfully modelled. On the biological side it has been shown that cells react to both the topological features as well as to the surface chemistry. For the cancer cells it appears that the post height might be a critical feature in that there is a threshold height required to induce nucleus deformation. Muscle cells can be seeded on top of the elastic pillars in a way that they show their normal healthy elongation on these synthetic surfaces.

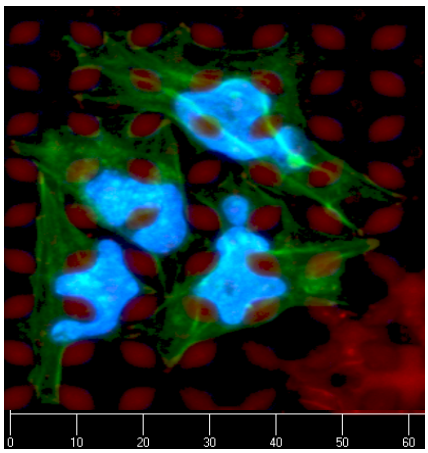
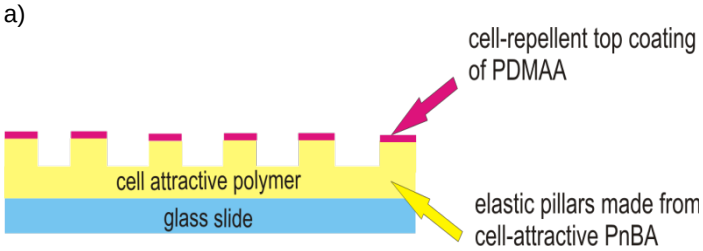


Figure 1: Cells with strongly deformed nuclei on post surface.

a)



b)

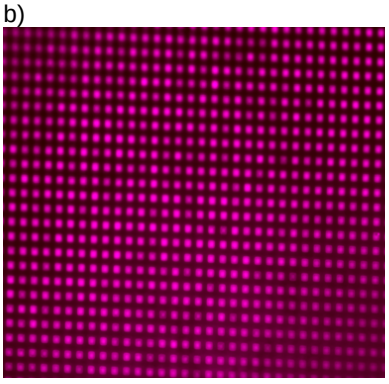


Figure 2: a) Microstructured post surface with cell attractive post covered by cell repellent top coating; PnBA: Poly-n-butylacrylate, PDMMA: Poly dimethyl acrylamide. b) Top-view of square pillars (7x6x5 microns) of PnBA, the top coated with dye labeled PDMAA (magenta).

Research project and collaboration. Our common objective is to deepen our knowledge of the mechanisms underlying the cellular and nuclear deformation of cells on micropatterned surfaces.

On the material side, and based on the previous results from the first funding period, we now want to extend these studies to carefully elucidate the interplay of the mechanical modulus of the surface and the surface chemistry on the behaviour of the cells (or its components such as the nucleus). The crucial choices are the shape of the posts, their size and spacing in addition to the post height which might turn out to be the most critical factor. The studies will then be extended to other cell types among which the myocardial cells are of special interest because anisotropically structured surfaces are interesting surface architectures to study the beating behavior of such cells.

On the biological side, we now want to elucidate the mechanotransduction pathways involved in the deformation of cells on microstructured surfaces. We will develop live cell imaging to follow cell adhesion, migration and deformation on microstructures since work on fixed cells give limited information on the mechanisms of deformation. Due to specific inhibitors of proteins involved in cell adhesion and deformation, we will elucidate the main intracellular actors of the nuclear deformation. New imaging methods will be developed in order to analyze the intracellular signals of mechanotransduction. Genomic and proteomic analysis of cells deformed on microstructures will be also performed. The knowledge acquired with this approach should allow us to determine potential targets for inhibiting deformation of cancer cells and thus reducing their metastatic (invasion) potential and/or develop new tests for invasion potential of cancer cells.

Work plan. Two PhD students will be involved in this project. Both students will work together in Freiburg on microfabrication and in Mulhouse on cell culture experiments.

First student: Advanced microfabrication for biointerfaces with controlled chemistry, topography and mechanics in Freiburg (Rühe) with regular stays for cell culture experiments in Mulhouse (Anselme).

- *Generation of 3D surface microstructures with well-defined surface chemistry, topology and mechanical properties.* Based on the previous results from the first funding period we will extend the chemistry and microstructuring techniques to allow for the generation of structures with a range of mechanical properties between a few kPa to the lower MPa region. This requires the synthesis of low T_g pre-polymers that are responsible for these mechanical properties. Their combination with the microstructuring methods will allow to tune the chemical properties of the various topographical facets of the microstructures.
- *Determination of the mechanical properties of the microstructures.* We will investigate the deformability of the post surfaces with regard to the mechanical modulus of the respective materials and the topological features of the microstructures.
- *Investigation of the influence of the surface topology on the behavior of cancer cells.* Starting from the results obtained so far we will map out the influence of the shape, the size, the spacing and especially the height of the microstructures on the behavior of the cell nuclei with regard to their deformation.
- *Behavior of myocardial cells.* As another example for cells that react to surface topologies we will study myocardial cells to show how mechanical properties of surfaces and surface topologies influence the beating behaviour of the cells. The surface properties relevant in this regard are essentially identical to those mentioned above.

Second student: Advanced biological study of mechanotransduction of cancer cells on textured surfaces in Mulhouse (Anselme) with regular stays for microfabrication of substrates in Freiburg (Rühe).

- *Generation of 3D surface microstructures with well-defined surface chemistry and topology.* Based on the previous results from the first funding period, we will define and fabricate the most pertinent post surfaces for elucidation of the intracellular mechanotransduction mechanisms.
- *Live imaging of deformed cells.* We will label by transfection approaches the different essential elements of cytoskeleton and related membrane proteins. Their movements will be analysed during cell adhesion and deformation on post surfaces before and after action of inhibitors (drugs, siRNA).
- *Development of FRET imaging of cell signalling.* This really innovative approach will allow us to visualize directly inside living cells the signal transduction (such as Rho GTPase signalling) associated with cell deformation.
- *Genomic and proteomic analysis of deformed cells.* These analyses will be correlated with live cell imaging results in order to define potential therapeutic targets against metastasis.