

P4 – Mimicking the Cellular Environment: Effects of Elastic Nanopatterned Substrates on Integrin-Mediated Cellular Interactions

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The design of the extracellular matrix (ECM), e.g. compliance and biochemical functionality, governs a wide range of cellular properties and functions. Its influence on cell behavior such as spreading, migration and adhesion is still not conclusively evaluated by biophysical means. Therefore, an artificial substrate system, according to the biophysical and biochemical properties of the extracellular matrix in connective tissues, has been developed. The Young's moduli E_Y of poly(ethylene glycol)-diacrylate (PEG-DA) based hydrogel substrates span more than four orders of magnitude ($0.6 \text{ kPa} < E_Y < 6 \text{ MPa}$). Since PEG-DA substrates are protein repellent, they were decorated by quasi hexagonally ordered, extended gold nanoparticle arrays, manufactured by block copolymer micellar nanolithography (BCMn). To provide bioactivity in terms of cell adhesion α (RGDfK) peptide, which is specific for $\alpha_V\beta_3$ integrins, was immobilized on the nanoparticles. The interparticle spacing and, hence, spacing of integrin binding sites ΔL could be precisely tuned, independently of the substrate rigidity, between 20 nm and 160 nm. This system was used to investigate the behavior of fibroblasts as a function of changes within two-dimensional parameters space (ΔL ; E_Y). To this end, cell spreading area and cell-substrate interaction forces were determined by phase contrast microscopy and single cell force spectroscopy (SCFS), respectively.

First, the effect of variation of ligand spacing on cellular behavior was investigated on hard substrates ($E_Y > 100 \text{ kPa}$). We could demonstrate a strong increase in detachment force and spreading area on substrates featuring low ligand spacing. Then, substrate compliance was tuned whereas the ligand spacing was kept at approximately 50 nm. This reveals a significant decrease in spreading area and detachment force on soft substrates ($E_Y < 8 \text{ kPa}$).

Additionally, both environmental parameters were varied simultaneously. Results from these experiments were determined as a function of hydrogel stiffness and integrin ligand distance. They revealed two tactile set points, thresholds in cellular sensing behavior, at $E_Y \approx 8 \text{ kPa}$ and $\Delta L \approx 70 \text{ nm}$, after 6, 12, and 24 hours of adhesion, respectively. Moreover, according to the hierarchical phase model in cellular behavior, elasticity was identified to be the dominant parameter in cellular sensing processes.



Mimicking the Cellular Environment: effects of elastic nanopatterned substrates on integrin-mediated cellular interactions

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Introduction

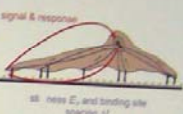
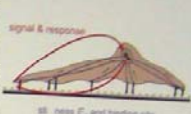


Figure 1: Cryo scanning electron micrographs of rat embryonic fibroblast on the hydrogel surface 24 h after seeding. Scale bars 10 μm (a), 2 μm (b), 500 nm (c).

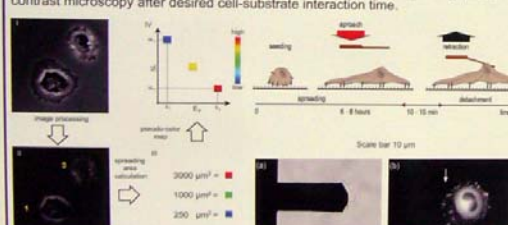
Various cells types, such as fibroblasts, leukocytes, stem cells, etc. are relied on sensing their environments, usually the extracellular matrices (ECM). Its biophysical and biochemical properties govern the intra- and extracellular organization of an organism and are related to the differential adhesion of cells. Cellular adhesion to the native ECM or an artificial substrate (e.g. implants) is a crucial event in terms of tissue formation and tissue sensing processes. Biophysical and biochemical signals originating from tissues can have dramatic effects on the regulation of cell functions. **The idea of the experiments was to alter the environmental stimuli in terms of compliance and surface chemistry of the substrates offered to the cell to quantify cell-substrate adhesion events.**



Techniques

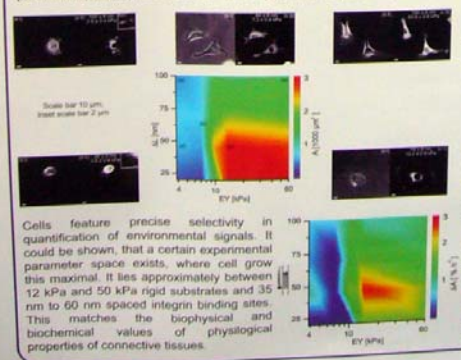
Substrates

Fibroblast spreading area or projected cell area (A) was analyzed by phase contrast microscopy after desired cell-substrate interaction time.



Cell-substrate adhesion force density ρ_{ADH} was measured by single cell force spectroscopy (SCFS) performed via atomic force microscope (AFM).

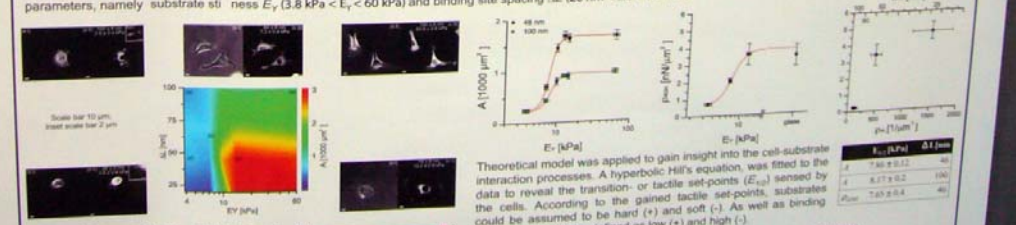
Only, due to the transfer lithography approach, the **elasticity (E_s)** of the substrates and the interparticle spacing (ΔL) of the decorating gold nanoparticles, thus spacing of the integrin binding sites, are fully independent from each other.



The elasticity of hydrogel substrates is tunable over four orders of magnitude and allows to mimic mechanical properties of nearly all significant tissues.

Results

The resulting spreading area or projected cell area A and adhesion force density ρ_{ADH} was determined as a function of the two-dimensional space of environmental parameters, namely substrate stiffness E_s ($3.8 \text{ kPa} < E_s < 60 \text{ kPa}$) and binding site spacing ΔL ($28 \text{ nm} < \Delta L < 103 \text{ nm}$).



Theoretical model was applied to gain insight into the cell-substrate interaction processes. A hyperbolic Hill's equation, was fitted to the data to reveal the transition- or tactile set-points ($E_{s,c}$) sensed by the cells. According to the gained tactile set-points, substrates could be assumed to be hard (+) and soft (-). As well as binding site spacing could be defined as low (+) and high (-).

$E_{s,c}$ [kPa]	$\Delta L_{c,1}$ [nm]
1.9 ± 0.2	48
8.7 ± 0.2	100
7.65 ± 0.4	80

Cells feature precise selectivity in quantification of environmental signals. It could be shown, that a certain experimental parameter space exists, where cell grow this maximal. It lies approximately between 12 kPa and 50 kPa rigid substrates and 35 nm to 60 nm spaced integrin binding sites. This matches the biophysical and biochemical values of physiological properties of connective tissues.

In case of alternating substrate properties and equivalent weighting, elasticity was identified to be the dominant parameter in cellular sensing processes.

