BP 19: Cell Adhesion

Time: Thursday 10:45–13:15 Location: ZEU 260

BP 19.1 Thu 10:45 ZEU 260

Modelling the active mechanical response of stress fibers — •Achim Besser and Ulrich S. Schwarz — University of Karlsruhe, Theoretical Biophysics Group, Kaiserstrasse 12, 76131 Karlsruhe, Germany

Stress fibers are bundles of actin filaments held together by the crosslinker protein α-actinin and actively tensed by the molecular motor protein myosin II. We have developed a model that describes stress fiber dynamics after biochemical or mechanical perturbations. For example, our stress fiber model can be solved analytically for the contraction dynamics after severing the fiber at any point along its length. Experimentally this situation has been realized by laser cutting and our model has been applied to analyze such data. The model equations can also be solved analytically for the case of cyclic boundary forces yielding theoretical predictions for the frequency dependence of the complex modulus of stress fibers. Decomposing it into its real and imaginary parts and using model parameters determined from the laser cutting experiments, we arrive at estimates for the storage and loss moduli. These quantities could be measured in future experiments and then would provide an additional test for our model.

BP 19.2 Thu 11:00 ZEU 260

Adhesion Dynamics of Early Cell Spreading — •Pavel Ryzhkov, Christina Oettmeier, Jac-Simon Kühn, Marcus Prass, and Hans-Günther Döbereiner — Institut für Biophysik, Universität Bremen

We report on the adhesion dynamics of spreading mouse embryonic fibroblasts. Advancing membrane edges and adhesions patterns on two-dimensional substrates are characterized by reflection interference contrast microscopy. We observe spatial-temporal correlations of early spreading events. Adhesion commences via the recurring appearance and disappearance of small patches. These patches grow slowly in size and lifetime until a continuous adhesion patch has formed, which initiates fast cell spreading.

BP 19.3 Thu 11:15 ZEU 260

Influence of bilayer substrate fluidity on cell adhesion and cytoskeleton structure — Daniel Minner[1], •Philipp Rauch[2], Josef Kaes[2], and Christoph Naumann[1] — [1]Indiana University, Indianapolis, USA — [2]University of Leipzig, Germany

Contact and adhesion between cells and their environment (e.g. other cells or the extracellular matrix) play a key role in maintaining cell stability and in all cell motility processes. Transmembrane proteins of the integrin family connect to specific ligands in the extracellular matrix and establish connections e.g. via actinin between the inner cytoskeleton and the extracellular environment. Up to now tethered lipid bilayer model systems mimicking cell surfaces have found limited applications in in vitro studies since they are instable in contact with cells. The novel stacked tethered bilayer substrates developed by D. Minner and C. Naumann at the University of Indiana show good stability and reproducible diffusion properties, adjustable via linker density and number of stacked layers. We used them to investigate the influence of friction and substrate coupling on NIH 3T3 mouse fibroblasts and their cytoskeleton. We find that with increasing fluidity, a rearrangement in the actin cytoskeleton occurs, similar to that observed on gel substrates of different stiffness. This is accompanied by reduced spreading of the cells. First experiments with neuronal cell lines show a contrary effect: On more fluid substrates, dendritic growth seems to be accelerated.

BP 19.4 Thu 11:30 ZEU 260

Dissecting the Impact of Matrix Anchorage and Elasticity in Cell Adhesion — •Tilo Pompe[1], Stefan Glorius[1], Thomas Bischoff[1], Ina Uhlmann[1], Martin Kaufmann[1], Sebastian Brenner[2], and Carsten Werner[1] — [1]Leibniz-Institut für Polymerforschung, Dresden, Germany — [2]Universitätsklinik C.G. Carus, Dresden, Germany

Extracellular matrices determine cellular fate decisions through the regulation of intracellular force and stress. It was anticipated that matrix stiffness and ligand anchorage would have distinct effects on the signalling cascades involved. We now can show how defined non-covalent anchorage of adhesion ligands onto elastic substrates allows the dissection of intracellular adhesion signalling pathways. Fourier transform traction cytometry proved the regulation of cell traction forces by the strength of the non-covalent anchorage of extracellular matrix ligands to the substrate. Using these constrained traction force levels the strain energy exerted by the cell on the substrate was quantitatively described by treating the cell as active force dipoles. Moreover matrix stiffness could be demonstrated to be the dominant exogenous signal of the global mechanical balance in cell adhesion. Besides the decoupling of biophysical signals biochemical signals like phosphorylation of the adhesion signalling protein FAK were distinctively controlled by matrix elasticity but not by varied receptor forces. Furthermore, using the net traction dipole moment of adherent cells our approach revealed a basis for a generalised biophysical treatment of extracellular mechanical signals in cell adhesion.

15 min. break

BP 19.5 Thu 12:00 ZEU 260

Vinculin lipid anchorage influences focal adhesion strength and turnover — •Lang Nadine, Gerold Diez, Thorsten Bloem, Philip Kollmannsberger, Ben Fabry, and Wolfgang Goldmann — Biophysics Group,Departmet of Physics, University of Erlangen-Nuremberg

The focal adhesion protein vinculin links the actin cytoskeleton to other proteins within the focal adhesion complex and plays an important role in cell adhesion and migration. To function properly vinculin needs to bind to the cell lipid membrane but the mechanism is currently not well understood.A lipid-membrane binding site,called lipid anchor, is located at the C-terminus of the vinculin tail. We measured the mechanical behavior of vinculin knock-out mouse embryonic fibroblast cells transfected with EGFP-linked-vinculin deficient of the lipid anchor (vinDeltaC).A magnetic tweezer was used to determine cell stiffness and binding strength.Compared to wildtype and rescue both were reduced in vinDeltaC cells suggesting that lipid binding of vinculin is important for the stability of the focal adhesion complex.Vinculin dynamics in focal adhesions measured with FRAP showed decreased turnover rates of vinDeltaC compared to wild-type vinculin. Because the lipid anchor also contains a c-SRC phosphorylation site we repeated these measurements in cells transfected with full length vinuclin in which either the c-SRC phosphorylation site or the lipid binding sites were scrambled.In both cases we found decreased adhesion strength, suggesting that lipid binding of vinculin and phosphorylation by c-SRC are important for mechanical stability of focal adhesions.

BP 19.6 Thu 12:15 ZEU 260

Correlation of Stress Fibre Pattern and Cell Morphology of Adherent Cells: Experiment and Modelling — •Jörg Meyer, Carsten Werner, and Tilo Pompe — Leibniz Institute of Polymer Research, Dresden, Germany

Cell morphology is known to play a key role in proliferation and differentiation of anchorage dependent cells. In this context the cytoskeleton acts as a mechanical signal transducer for exogenous and endogenous signals. In order to better understand the biophysical processes regulating cell morphology and intracellular stresses we cultured human endothelial cells on micropatterned surfaces. Cell elongation was tuned by adhesion promoting fibronectin stripes of 5 to 40 μm in width. Using autocorrelation image analysis the stress fibre spacing was determined to exhibit a strong discontinuity with a maximum at 15 μm of stripe width. Below this critical value the spacing of actin stress fibres, bundled near the cell edge parallel to the stripe direction, was linearly dependent on stripe width. Above the threshold actin stress fibre spacing mainly remained constant at around 2 μm. Interestingly, we found a similar dependence with a discontinuity at 15 μm of stripe width for the surface area of adherent cells using a finite element model of a liquid drop spreading on adhesive stripes. Total surface area as well as basal contact area of the cell to the stripe correlated to the stress fibre pattern and suggested membrane tension or cell adhesion receptor activation as biochemical triggers for the cytoskeletal arrangement and force distribution inside adherent cells.

BP 19.7 Thu 12:30 ZEU 260

Stochastic dynamics and stability of adhesion sites with different bond arrangements — •Johanna von Treuenfels[1], Christian Korn[1], and Ulrich S. Schwarz[1,2] — [1]University of Heidelberg, Bioquant 0013, Im Neuenheimer Feld 267, 69120 Heidelberg, Germany — [2]University of Karlsruhe, Theoretical Biophysics Group, Kaiserstrasse 12, 76131 Karlsruhe, Germany

Adhesive contacts between cells and their environment are organized around a two-dimensional layer of transmembrane adhesion receptors that continuously dissociate and rebind to their extracellular ligands. On the cytoplasmic side, this layer of adhesion bonds is reinforced by additional layers of bonds which on their top side connect to force-generating elements in the cell, mainly the actin cytoskeleton. We introduce a master equation model for adhesion sites which includes these aspects of the spatial organization of the molecular bonds within the adhesion site. We investigate the stochastic dynamics and stability of clusters of bonds connected in series, in parallel and in combinations of these. We consider the mean rupture time as a measure for stability under the disruptive effect of force and find that different configurations are optimal depending on the level of applied force. This suggests that adhesion sites might be organized differently depending on the amount of force they are exposed to.

BP 19.8 Thu 12:45 ZEU 260

Adhesion of bacteria and adsorption of protein: influence of substrate composition — •Yvonne Schmitt, Peter Loskill, and Karin Jacobs — Universität des Saarlandes, Saarbrücken, Germany

The formation of biofilms on substrates that are exposed to a solution containing proteins, sugars, bacteria etc. is a complex process which is still not fully understood. Especially the initial adsorption of proteins and their role in the entire evolution of the biofilm is still unsettled. We focus our recent research on the characterization of the interactions between substrate materials and proteins or bacteria, respectively. Investigations of the adsorption kinetics of proteins like BSA revealed that proteins are sensitive to the composition of the offered substrate [1, 2]. Thus, a manipulation of the adsorption process by tailored substrates is conceivable. Besides, a wide range of methods such as ellipsometry, surface plasmon resonance and x-ray scattering, we use atomic force microscopy to characterize the dominant forces and parameters involved in the adsorption process and the development of the protein film. Based on the results described above, we study the influence of the substrate material and its composition to the attachment of bacteria. Elasticity measurements on bacteria adsorbed on model surfaces are performed as well as force-distance-measurements with bacteria as probes. These experiments can also be carried out on adsorbed protein films to examine the relevance of a protein layer to the attachment of bacteria.

[1] A. Quinn et al., Europhysics Lett. 81 (2008) 56003
[2] M. Bellion et al., J. Phys.: Condens. Matter 20 (2008) 404226

BP 19.9 Thu 13:00 ZEU 260

Artificial three-dimensional scaffolds for cell adhesion studies — •Thomas Striebel[1], Franziska Klein[2], Martin Wegener[3], Martin Bastmeyer[2], and Ulrich S. Schwarz[1] — [1]University of Karlsruhe, Theoretical Biophysics Group, Kaiserstrasse 12, 76131 Karlsruhe, Germany — [2]University of Karlsruhe, Institute of Zoology, Haid-und-Neu-Strasse 9, 76131 Karlsruhe, Germany — [3]University of Karlsruhe, Institute of Applied Physics, Wolfgang-Gaede-Str. 1, 76131 Karlsruhe, Germany

Adhesion of tissue cells is traditionally studied on two-dimensional culture dishes. In this way, much has been learned how environmental stimuli determine the cellular response, including migration, proliferation and fate. However, much less is known about how tissue cells behave in three-dimensional environments. We have used direct laser writing to design three-dimensional scaffolds for cell adhesion studies with feature sizes down to 100 nm. Our setup can be used to produce structures with many different geometries in a short time and gives highly reproducible results. By applying our procedure to different photoactive materials, we were able to vary the stiffness of the scaffolds and to optimize the system for imaging. Using quantitative image processing, we now can analyze shape, traction and adhesion structures of cells in three dimensions.