BP 24: Cell Mechanics (in vivo)

Time: Thursday 10:00–12:45 Location: H44

BP 24.1 Thu 10:00 H44

Nonlinear creep measurements in living fibroblasts — •Philip Kollmannsberger, Claudia T. Mierke, and Ben Fabry — ZMPT, Biophysics Group, University of Erlangen-Nuremberg

The linear viscoelasticity of adherent cells and biological tissue is characterized by a wide distribution of relaxation times and shows a power-law creep response or a power-law viscoelastic spectrum over several decades of time or frequency. In addition, single cells and tissue exhibit a highly nonlinear stress-strain relationship. The viscoelastic behavior of cells in the non-linear regime is unknown, however, but is of particular interest to test different conflicting theories. Here we measured the viscoelastic behavior of a variety of adherent cells in the linear and non-linear regime using magnetic tweezers with real-time force feedback. We imposed a staircase-like sequence of 1 nN force steps up to a maximum force of 10 nN onto 4.5 μm fibronectin-coated magnetic beads bound to the cytoskeleton via integrins. For each stress level σ, the differential creep response of single cells followed a power law: $J(\sigma,t) = J_0(\sigma)(\frac{t}{t_0})^b$, however the differential creep modulus $J_0(\sigma)$ decreased with stress, equivalent to stress stiffening. The power-law creep exponent b showed no systematic stress dependence, although in some cells b increased at high forces, consistent with yielding and disruption events. Static stress stiffening is predicted by models of semiflexible polymers and can be modelled using Fung's theory of quasilinear viscoelasticity for biological tissues, whereas a speed-up of relaxation processes due to yielding and structural changes is consistent with soft glassy rheology.

BP 24.2 Thu 10:15 H44

Cellular pattern formation by strain-mediated active switching — •Raja Paul and Ulrich Schwarz — University of Heidelberg, Im Neuenheimer Feld 293, D-69120 Heidelberg, Germany

Using Monte Carlo simulations, we investigate the time evolution of the force generated by an ensemble of cells placed initially at random in the extracellular matrix (ECM). The ECM is modelled as a two-dimensional cable network with triangular or square lattice geometry and the cells are modelled as force contraction dipoles. We observe various patterns depending on prestrain, geometry, boundary conditions, elastic moduli, cell density, density of ECM crosslinks and temperature. Recent experiments of cellular assembly in a clamped collagen matrix show that force increases with time. This observation can be explained in our model by considering a strain mediated switching of cells into the active state and subsequent contraction from an initial quiescent state.

BP 24.3 Thu 10:30 H44

Modelling the spatially inhomogeneous contraction of stress fibers — •Achim Besser and Ulrich Schwarz — University of Heidelberg, INF 293, D-69120 Heidelberg, Germany

The contractile activity of cells is often associated with stress fibers, which are contractile bundles of actin filaments crosslinked by α-actinin and the motor protein myosin II. At their ends, they are attached to the extracellular environment through cell-matrix contacts called *focal adhesions*. Upon contraction, elastic deformations along the fibers have been observed experimentally to be inhomogeneous. We suggest that these spatial differences arise from biochemical signals originating from the focal adhesions. Stress fibers are modeled as a periodic arrangement of springs, dashpots and contractile elements. Contractile activity is coupled to the diffusible biochemical signal. We solve our model in a continuum approximation and show that it results in the experimentally observed deformation pattern.

BP 24.4 Thu 10:45 H44

High Resolution Mapping of Cell Mechanics (in Vivo) Using Digital Pulsed Force Mode — •Michael Holzwarth[1], Alexander Gigler[2], and Othmar Marti[1] — [1]Institute of Experimental Physics, Ulm University, D-89069 Ulm, Germany — [2]Present address: Section Crystallography, University of Munich, Theresienstrasse 41/II, D-80333 Munich, Germany

Local mechanical properties of living cells have been investigated by means of AFM, using Digital Pulsed Force Mode (DPFM). The DPFM images the surface while probing its mechanical properties locally. At least one force curve is recorded for each point of the scanned area. Thus, more than 500,000 curves have been recorded and completely evaluated for each single experiment.

The glass-like substrate served as an online reference material for calibration purposes. First, the force trajectories were corrected for the viscous drag force in the liquid environment. Secondly, the curves within the region of the substrate were phase corrected to compensate for the time lag of the signal in the setup assuming a purely elastic response of the reference material. Finally, all the force traces have been corrected by using this calibration and evaluated according to common continuum-elastic models.

The resulting images allow the assignment of values of Young's modulus, local adhesion and hysteretic behaviour at a high lateral resolution all over the cell body. The procedure of our measurement and the corresponding signal correction strategy of the automated data evaluation will be shown.

BP 24.5 Thu 11:00 H44

Rate of stress increase during cell spreading on substrates with different matrix rigidity — •Daniel Paranhos Zitterbart, Claudia T. Mierke, Thorsten M. Koch, and Ben Fabry — ZMPT, Biophysics Group, Universität Erlangen

For most cell types, adhesion, spreading and tension generation are crucial for cell survival. These processes are strongly influenced by the rigidity of the extracellular matrix: Cells spread more and faster, and generate higher tension on more rigid substrates. We report simultaneous measurements of cell spreading and traction generation during adhesion of MDA-MB-231 breast carcinoma cells onto collagen coated polyacrylamid gels. The Youngs modulus of the gels was tuned between 1500 ('soft') and 6000 ('hard') Pa. The evolution of cell tractions was computed from the gel deformation measured every 30 sec by tracking the displacements of fluorescent beads ($\o 0.5\mu m$) embedded at the gel surface. As a robust estimate of total force generation, we computed for each cell the elastic strain energy U stored within the gel. As expected, cells generated a higher maximum strain energy $U = 1.01pJ$) and spread more ($A = 6002 \pm 961\mu m^2$) on harder gels compared to softer gels ($U = 0.20pJ, A = 3012 \pm 492\mu m^2$). When the strain energy vs. time data of individual cells were normalized by spreading area, they collapsed onto a single relationship, regardless of gel stiffness. These data extend earlier findings of a proportionality between cell spreading and tension generation (Reinhard-King, Biophys J 2005) and show that individual cells exhibit a constant rate of stress increase during early adhesion events regardless of the substrate rigidity.

15 min. break.

BP 24.6 Thu 11:30 H44

Cell elasticity as a function of actin expression — •Carsten Stüber and Josef Käs — Institute of Experimental Physics I, University of Leipzig, Germany

The deformation response to an external force of an eurkaryotic cell mainly depends on its cytoskeletal composition. Theoretical models have been introduced to quantify the concentration dependence of the different cytoskeletal components to the elastic strength of cells. Verifying the models experimentally, the optical stretcher, a two beam optical trap, is used to elongate fibroblast cells. These fibroblasts are transfected with GFP-actin, which leads to an overexpression of actin within the cell and allows to determine the actin concentration using fluorescence image analysis. The dependence of the elasticity on the actin concentration of fibroblasts shows a softening of the cell with increasing number of actin filaments.

BP 24.7 Thu 11:45 H44

Active mechanical stabilization of the viscoplastic intracellular space of Dictyostelia cells by microtubule-actin crosstalk — •Doris Heinrich and Erich Sackmann — Department für Physik, Ludwig-Maximilians-Universität, Geschwister-Scholl-Platz 1, 80539 München, Germany

We investigated the micro-viscoelasticity of the intracellular space of Dictyostelium discoideum cells by evaluating the intracellular trans-

port of magnetic force probes and their viscoelastic responses to force pulses of 20-700 pN. The role of the actin cortex, the microtubule (MT) aster and their crosstalk is explored by comparing the behaviour of wild type cells, myosin II null mutants, latrunculin A and benomyl treated cells. The MT coupled beads perform irregular local and long range directed motions which are characterized by measuring their velocity distributions (P(v)). The correlated motion of the MT and the centrosome are evaluated by microfluorescence of GFP-labeled MTs. P(v) can be represented by log-normal distributions with long tails and it is determined by random sweeping motions of the MTs and by intermittent bead transports parallel to the MTs. The viscoelastic responses are strongly non-linear and are mostly directed opposite or perpendicular to the force, showing that the cytoplasm behaves as an active viscoplastic body with time and force dependent drag coefficients. Force-balance is established by the mechanical coupling between the soft microtubules and the viscoelastic actin cortex, providing cells with high mechanical stability despite the softness of the cytoplasm.

BP 24.8 Thu 12:00 H44

Single fibroblast viscoplasticity: elastic stiffening and kinematic hardening — •Pablo Fernandez[1,2], Pramod Pullarkat[1], and Albrecht Ott[1] — [1]Universität Bayreuth, Germany — [2]Present address: Technische Universität München, Germany

The deep biological relevance of mechanics is well illustrated by features such as cell locomotion, contractility, and mechanotransduction. The advent of single-cell rheology brings hope of a physical understanding of these phenomena. We report that the mechanical response of single 3T3 fibroblasts to uniaxial extension in the 1–100% range obeys a remarkably simple and robust phenomenology. Below 10% deformation cells exhibit a previously reported, stress-stiffening master relation probed with sinusoidal oscillations. Beyond 10% stretch, deformations at a constant rate in a 0.03–3 μm/s range always exhibit pure plastic flow. The plastic deformation translates the elastic region, a behaviour known as kinematic hardening. Fixing the cells abolishes the plastic response. Then the force-length relation shows dramatic stiffening, the integral of the previously described master-relation. Thus 2 key features summarise fibroblast mechanical behaviour: exponential elastic stiffening and viscoplastic kinematic hardening.

BP 24.9 Thu 12:15 H44

Physical description of mitotic spindle orientation during cell division — •Andrea Jiménez-Dalmaroni[1], Manuel Théry[2], Victor Racine[2], Michel Bornens[2], and Frank Jülicher[1] — [1]Max Planck Institute for the Physics of Complex Systems, Nöthnitzer Str. 38, 01187 Dresden, Germany — [2]Institut Curie, CNRS UMR144, Compart. et Dynamique Cellulaire, 26 rue d'Ulm 75248, Paris, France

During cell division, the duplicated chromosomes are physically separated by the action of the mitotic spindle. The mitotic spindle is a dynamic structure of the cytoskeleton, which consists of two microtubule asters. Its orientation defines the axis along which the cell divides. Recent experiments on dividing cells, which adhere to patterned substrates, show that the spindle orientation depends on the spatial distribution of cell adhesion sites. Here we show that the experimentally observed spindle orientation can be understood as the result of the action of cortical force generators acting on the spindle microtubules. We assume that the local activity of force generators is controlled by the spatial distribution of cell adhesion sites determined by the particular geometry of the adhesive substrate. We develop a simple physical description of the spindle mechanics, which allows us to calculate the torque acting on the spindle, as well as the energy profile and the angular distribution of spindle orientation. Our model accounts for the preferred spindle orientation, as well as the full shape of the angular distributions of spindle orientation observed in a wide variety of patterns. We conclude that, based on a few simple assumptions, we can provide a quantitative description of the spindle orientation.

BP 24.10 Thu 12:30 H44

Beyond the Rim: The Viscoelastic Pericellular Coat — •Heike Boehm, Joachim Spatz, and Jennifer Curtis — Max-Planck-Institute for Metals Research, Department New Materials & Biosystems & University of Heidelberg, Department of Biophysical Chemistry

Most mammalian cells are surrounded by an optically-transparent layer of highly hydrated polysaccharides and proteins, the pericellular coat (PCC). The most vital component is a linear, flexible polyelectrolyte: hyaluronan, which is synthesized directly on the outer cell membrane. Different proteins can bind to the hyaluronan and thus anchor it to the cell membrane, stiffen and/or crosslink it. The resulting viscoelastic coat plays a vital role in cell migration, proliferation and various diseases like cancer and arthritis.

Few studies quantitatively examine the PCC's mechanical properties induced by structural or compositional reorganization. We perform microrheology studies to determine the viscoelasticity and define the impact of proteins and glycosaminoglycans on the structure of the PCC. Information about the structure of the polymer matrix can thus be gained by observing the diffusion of a particle embedded in the PCC of living cells (passive microrheology). With our holographic optical tweezers (HOT) setup we can create a dynamic array of traps, that enable us to carefully place several beads at different sides of a living cell simultaneously and to measure or apply forces ranging from femtonewtons to 10's of piconewtons (active microrheology).