CPP 39: Cell adhesion, mechanics and migration I (Joint session BP, CPP)

Time: Wednesday 9:30–13:15 Location: H 1058

Invited Talk CPP 39.1 Wed 9:30 H 1058

Feeling for cell function: Mechanical phenotyping at 100 cells/sec — •Jochen Guck — Technische Universität Dresden, Germany

The mechanical properties of cells have long been heralded as a label-free, inherent marker of biological function in health and disease. Wide-spread utilization has so far been impeded by the lack of a convenient measurement technique with sufficient throughput. To address this need, we introduce real-time deformability cytometry (RT-DC) for the continuous mechanical single-cell characterization of large populations (> 100.000 cells) with analysis rates greater than 100 cells/s, approaching that of conventional fluorescence-based flow cytometers. Using RT-DC we can sensitively detect cytoskeletal alterations, distinguish cell cycle phases, track hematopoietic stem cell differentiation into distinct lineages and characterize cell*populations in whole blood by their mechanical fingerprint. Our results indicate that cell mechanics can define cell function, can be used as an inherent cell marker and could serve as target for novel therapies. Mechanical phenotyping adds a new functional, marker-free dimension to flow cytometry with diverse applications in biology, biotechnology and medicine.

CPP 39.2 Wed 10:00 H 1058

Characterizing viscoelastic properties of the cortex in mitotic cells — •Elisabeth Fischer-Friedrich[1,2], Jonne Helenius[3], Anthony Hyman[2], Daniel Müller[3], and Frank Jülicher[1] — [1]MPI PKS, Nöthnitzerstr. 38, 01187 Dresden, Germany — [2]MPI CBG, Pfotenhauerstr. 108, 01307 Dresden, Germany — [3]D-BSSE, ETHZ, Mattenstr. 26, 4058 Basel, Switzerland

Cell stiffness is a key parameter for our understanding of cell shape, cell migration and tissue organization. However, as the cell consists of several components, it is challenging to extract the force contribution and the elastic modulus of a specific component upon cell deformation. Here, we probe the stiffness of round, mitotic HeLa cells in a parallel plate compression setup, where we measure the force necessary to compress cells in between plates. An earlier study showed that in steady state, this force is due to cell surface tension. Here, we apply step strains and sinusoidal modulation of the plate distance at various frequencies allowing us to probe differential cell stiffness. We find strong indications that cell stiffness in mitosis is dominated by actomyosin and therefore by the mitotic cortex. This interpretation allows to extract an associated frequency-dependent area extension modulus. We show that myosin activity at the same time fluidizes and stiffens cells, where differential cell stiffness increases linearly in dependence of active prestress. On the other hand, the passive cross-linker α-actinin solidifies and stiffens mitotic cells. Our study shows how active and passive cross-linkers influence rheological properties of the cortical actin-network in vivo.

CPP 39.3 Wed 10:15 H 1058

Biogenic cracks in porous medium — •Arnaud Hemmerle[1], Jörn Hartung[1], Oskar Hallatschek[1,2], Lucas Goehring[1], and Stephan Herminghaus[1] — [1]Max Planck Institute for Dynamics and Self-Organization (MPIDS), 37077 Göttingen, Germany — [2]Department of Physics, University of California, Berkeley, CA, USA

Microorganisms growing on and inside porous rock may fracture it by various processes. Most of the studies have been on the chemical aspects of biofouling and bioweathering, while mechanical contributions have been neglected. However, as witnessed by the perseverance of a seed germinating and cracking up a concrete block, the turgor pressure of living organisms can be very significant. It is the effects of such mechanical forces on the weathering of porous media that will concern us here. We designed a model porous medium made of glass beads held together by polydimethylsiloxane (PDMS) capillary bridges. The rheological properties of this material can be controlled by the curing conditions and the crosslinking of the PDMS. Glass and PDMS being inert to most chemicals, we are able to focus on the mechanical processes of biodeterioration, excluding any chemical weathering.

Inspired by recent measurements of the high pressure ($\simeq$0.5 Mpa) exerted by a growing population of yeast trapped in a microfluidic device, we show that yeast cells can be cultured homogeneously within porous medium and investigate then the effects of such an inner pressure on the mechanical properties of the sample. We observe crack propagation for a certain range of bead sizes and cohesiveness, showing a clear interaction between biotic and abiotic processes.

CPP 39.4 Wed 10:30 H 1058

Artificial tissue, Ultra-soft elastomers for cell mechanical investigation — •Viktor Heinrichs[1,2], Sabine Dieluweit[1], Jörg Stellbrink[2], Rudolf Merkel[1], and Dieter Richter[2] — [1]ICS-7 Forschungszentrum Jülich GmbH, Jülich, Germany — [2]ICS-1 Forschungszentrum Jülich GmbH, Jülich, Germany

Most animal cells are strongly influenced by the elasticity and topography of their environment. For clear-cut investigation of cellular mechanobiology elastic model substrates are required. These materials should be biocompatible, transparent, suitable for micro structure fabrication and their elasticity should be tuneable in a wide range. However, a Young's modulus of 1 kPa (ultra-soft, necessary to model, e.g., brain or glial tissues) is difficult to achieve [1, 2]. These challenges can be tackled with cross-linked polydimethylsiloxane (PDMS) with the additional benefit of long shelf-life. We created a new PDMS material that meets all requirements in cell mechanics and examined it explicitly on viscoelastic properties with a strain controlled rheometer. The elasticity of the PDMS network was tuned via selection of the precursor polymers and their mixing ratio. Values as low as 1.5 kPa have been reliably achieved. First cell mechanical experiments on this novel material basis are underway. [1] C. M. Cesa, N. Kirchgeßner, D. Mayer, U. S. Schwarz, B. Hoffmann, R. Merkel. Rev. Sci. Instrum. 2007, 78, 034301. [2] D. T. Butcher, T. Alliston, V. M. Weaver, Nature Rev. Cancer 2009, 9, 108-122.

CPP 39.5 Wed 10:45 H 1058

Molecular stress sensors constructed from DNA — •Meenakshi Prabhune[1], Jonathan Bath[2], Andrew Turberfield[2], Florian Rehfeldt[1], and Christoph F Schmidt[1] — [1]Third Institute of Physics-Biophysics, Georg August University, Göttingen, Germany — [2]University of Oxford, Department of Physics, Clarendon Laboratory, Parks Road, Oxford OX1 3PU, UK

Molecular stress generation in cells is spatially and temporarily organized in complex patterns to drive meso-scale active processes such as intracellular transport, cell migration, or cell division. To quantitatively understand how these processes are driven, it is necessary to map local stresses inside cells, which is hard due to the lack of appropriate probes. We have designed a molecular-scale probe consisting of a self-assembled DNA hairpin with a fluorophore - quencher pair that responds to small forces applied to its ends. We demonstrate the working of this force sensor in vitro and explore possibilities for in vivo application to map local stress fields in cells.

CPP 39.6 Wed 11:00 H 1058

Shape and adhesion dynamics of amoeboid cells studied by cell-substrate impedance fluctuations — •Helmar Leonhardt — Universität Potsdam, 14476 Potsdam OT Golm

We present electrical impedance measurements of single amoeboid cells on microelectrodes. Wild type cells and mutant strains are studied that differ in their cell- substrate adhesion strength. We recorded the projected cell area by time lapse microscopy and found a correlation between kinetics of the projected area (cell shape oscillation) and the impedance long-term trend. We developed a data processing routine to extract such trends. We furthermore observed that cell-substrate attachment strength strongly affects the impedance in that the magnitude of fluctuations are enhanced in cells that effectively transmit forces to the substrate. For example, in talA- cells, which lack the actin anchoring protein talin, the fluctuations are strongly reduced. Such short-term fluctuations are extracted by high-pass filtering the original data. Typically, amoeboid motility advances via a cycle of membrane protrusion, substrate adhesion, traction of the cell body and tail retraction. This motility cycle results in the quasi-periodic oscillations of the projected cell area and the impedance. In all cell lines measured, similar periods were observed for this cycle, despite the differences in attachment strength. Based on the approach presented here, we can separate the changes in the impedance signal that are caused by the projected cell area from the fluctuations induced by the cell-substrate adhesion.

30 min break

CPP 39.7 Wed 11:45 H 1058

Probing the role of cytoplasmic flows in embryogenesis — •Matthäus Mittasch[1], Peter Gross[1,2], Stephan Grill[1,2], and Moritz Kreysing[1] — [1]Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany — [2]Biotechnology Center, Technische Universität Dresden, 01307 Dresden, Germany

While the genetic basis of embryogenesis is increasingly well understood, it is also clear that gene expression needs to be coupled to physical transport phenomena to account for the genesis of spatial structure. A striking example of morphogenesis is the polarization of the egg cell of the nematode worm C. elegans prior to asymmetric cell division. This process relies on the active cortical transport of morphogens (PAR proteins), and is impaired upon myosin-2 motor downregulation. However, little is known about the mechanistic role of the cytoplasmic flows that seem to stabilize cell polarization. Here, we adapt the previously described technique of light driven micro-fluidics (Weinert & Braun, J. appl. Phys. 2008), in order to now generate flows inside early stage embryos. Specifically, we report on the generation of micron-scale flow patterns confined in three dimensions, with velocities exceeding the wild type flows. By this, we aim to (i) rescue impaired embryos, (ii) manipulate wild-type cytoplasmic flow velocities, and (iii) introduce polarity multipoles through the induction of well-controlled artificial cytoplasmic flows inside C. elegans eggs. We anticipate that our findings will add to the general understanding of how biological systems utilize active transport phenomena to establish spatial structure.

CPP 39.8 Wed 12:00 H 1058

Force fluctuations in three-dimensional suspended fibroblasts — •Florian Schlosser, Christoph F. Schmidt, and Florian Rehfeldt — Drittes Physikalisches Institut - Biophysik, Georg-August-Universität Göttingen

Cells are sensitive to mechanical cues from their environment and at the same time generate and transmit forces to probe and to adapt to their surroundings. Key players in the generation of contractile forces are acto-myosin structures. To test forces and elasticity of cells not attached to a substrate, we used a dual optical trap to suspend 3T3 fibroblasts between two fibronectin-coated beads. We analyzed the correlated motions of the beads with high bandwidth. A combination of active and passive microrheology allowed us to measure the non-equilibrium force fluctuations as well as the elastic properties of the cell. We found that cortical forces deform the cell from its round shape in the frequency regime from 0.1 to 10 Hz. Biochemical perturbation experiments using blebbistatin for myosin inhibition and nocodazole for microtubule depolymerization show that cell stiffness and cortical force fluctuations highly depend on acto-myosin activity but not on microtubules. Serum-starvation also largely reduced the fluctuation amplitude. A force-clamp allowed us to observe cells under defined constant forces. Combining our optical trap with a confocal microscope allowed us to image the three-dimensional actin distribution of Life-Act transfected cells during the force measurements.

Schlosser, Rehfeldt, Schmidt, *Phil. Trans. R. Soc. B* 20140028, **2014**

CPP 39.9 Wed 12:15 H 1058

Buckling dynamics of freely diffusing single erythrocytes — •Michael Göllner, Adriana C. Toma, and Thomas Pfohl — Department of Chemistry, University of Basel, Switzerland

Containing a wealth of information, human blood is the most used sample for diagnostic purposes. Microfluidics, with its unique advantages in performing analytical functions, has been increasingly used for whole blood and cell-based analysis. However, studies on the single-cell level using microfluidic techniques often require active immobilization in order to be investigated by optical methods.

We developed a microfluidic setup for single red blood cell (RBC) assays starting with whole blood samples which permits diffusion-controlled variation of the external environment. Individual RBCs are freely diffusing inside microchambers without adhesive interactions to the glass coverslip or the use of optical tweezers. By increasing the surrounding osmotic pressure, erythrocytes are exhibiting a buckling transition which is described by means of radial Fourier analysis. Temporal evolution of the modal decomposition of cell edge movement leads to characterization of osmolarity-dependent fluctuations of freely diffusing single RBCs.

CPP 39.10 Wed 12:30 H 1058

Biomechanics of the Spinal Cord — •David E. Koser and Kristian Franze — Department of Physiology, Development and Neuroscience, University of Cambridge, United Kingdom.

In cell physiology and pathology mechanical signaling plays an important role. Many cell types, including central nervous system cells, respond to the mechanical cues in their environment. Yet, in spinal cord, data on tissue stiffness are sparse and therefore the mechanical environment is unknown. To fill this gap, we conducted atomic force microscopy indentation and tensile measurements on acutely isolated mouse spinal cord tissue sectioned along the three major anatomical planes (transverse, coronal and sagittal planes), and correlated local mechanical properties with the underlying cellular structures. Our measurements revealed that gray matter is significantly stiffer than white matter irrespective of directionality and force direction. While white matter behaved like a transverse isotropic material on all length scales, gray matter was isotropic at the tissue and anisotropic at the cellular scale. Most importantly, tissue stiffness correlated with axon orientation, cell body size, and cellular in plane proximity, which we combined into a linear model to estimate local central nervous system tissue stiffness. Our study may thus lay the foundation to predicting local tissue stiffness based on histological data, and hence contribute to the understanding of cell behavior in response to mechanical signaling under physiological and pathological conditions.

CPP 39.11 Wed 12:45 H 1058

Properties of Single Squamous Cell Carcinoma Cells — •Susanne Steeger[1], Tanja Schreyer[1], Stefan Hansen[2], Jörg Schipper[2], and Mathias Getzlaff[1] — [1]Heinrich-Heine-Universität Düsseldorf, Deutschland — [2]Univ.-HNO-Klinik Düsseldorf, Deutschland

In this contribution we report on measurements of the mechanoelastic properties of ENT squamous cell carcinoma cells. The study of these single cancer cells in culture medium is carried out by Atomic Force Microscopy. Our main interest is the determination oft the Youngs Modulus calculated by the Hertzian Model. We identify the elasticity of cancer cells in order to compare it with that of similar benign cells. Because Live Cell Imaging is a challenging task we first focus on testing different cantilevers and various strategies to treat the cells carefully. In order to determine the individual properties of the cancer cells we additionally analyse their cytoskeleton (actin and tubulin) by using a confocal fluorescence microscope. Cancer cells are known for their modified cytosekelton which is reflected in the different elasticities of both cancer and comparable benign cells.

CPP 39.12 Wed 13:00 H 1058

PAR polarity pattern in C. elegans zygotes establishes via a mechanochemical feedback module — •Peter Gross[1,2], K.Vijay Kumar[2,3], Nathan W. Goehring[4], Justin S. Bois[5], Frank Jülicher[3], and Stephan W. Grill[1,2,3] — [1]MPI-CBG, Dresden — [2]BIOTEC, TU Dresden — [3]MPI-PKS, Dresden — [4]London Research Institute, UK — [5]UCLA, Los Angeles, CA

The interplay between biochemistry and cell mechanics is critical for a broad range of morphogenetic changes. A prominent example hereof is the emergence of cell polarity during the embryogenesis of C. elegans, resulting in a patterned state of the membrane-associated PAR polarity proteins. Crucial for the emergence of the patterned state are large-scale flows in the membrane-associated actomyosin cortex, which are observed concomitantly with the emergence of PAR polarization. The coupling of biochemistry and cortical flows, driving this mechanochemical patterning processes, remain poorly understood. Here we establish that PAR polarization of C. elegans zygotes represents a coupled mechanochemical feedback system. We demonstrate that the biochemistry in form of the PAR domains controls mechanics by establishing a myosin gradient. We measure the spatiotemporal profile of the anterior and posterior PAR concentration, the myosin concentration and the induced flow-field. Furthermore we present a theoretical description of this process in the framework of active fluids combined with PAR biochemistry in a coupled reaction-diffusion active-fluids approach. We show that this mechanochemical feedback description quantitatively recapitulates the spatiotemporal profile of PAR polarity emergence.