BP 17: Cell Mechanics I

Time: Wednesday 9:30–13:00 Location: H 1058

BP 17.1 Wed 9:30 H 1058

Mechanical and Electrical Characterization of Cardiac and Skeletal Muscle Microtissues — •Delf Kah, Ingo Thievessen, Marina Spörrer, Wolfgang H. Goldmann, and Ben Fabry — Department of Physics, Biophysics Group, Friedrich-Alexander-University Erlangen-Nuremberg, D-91052, Erlangen, Germany

In-vitro engineered muscle tissue grafts are of growing interest for different applications including regenerative therapy, replacement of infarcted cardiac sites, or as a drug testing platform. Critical for the successful development of suitable models for engineered muscle grafts is the maturation into an in-vivo-like, highly aligned, and contractile tissue. To achieve this, we developed a stretchable and electrically paceable system consisting of an array of 4x2x2 mm microwells with two elastic pillars that serve as force sensors. Tissues can be grown from several cell sources including neonatal cardiomyocytes form rats, mice, as well as C2C12 skeletal muscle cells. Mechanical stretching with a linear stepper motor, electrical pacing with carbon electrodes, and microscopic imaging of the tissue is synchronized by a microcontroller, allowing us to study isotonic, isometric, or eccentric contractions for various pacing protocols. Cardiac tissues show remarkably uniform contraction induced by electrical pacing, which allows for imaging with a time resolution of up to 1000 Hz through heterodyning. Accordingly, contractile performance can be evaluated with high temporal precision.

BP 17.2 Wed 9:45 H 1058

Substrate stiffness affects sarcomere coherence in hESC-derived cardiomyocytes — •Daniel Härtter[1], Til Driehorst[1,2], Malte Tiburcy[2], Kengo Nishi[1], Wolfram-H. Zimmermann[2], and Christoph F. Schmidt[1] — [1]Drittes Physikalisches Institut - Biophysik, Fakultät für Physik, Georg-August-Universität Göttingen — [2]Institut für Pharmakologie, Universitätsmedizin, Georg-August-Universität Göttingen

The mechanical micro-environment affects the behavior of cells. For instance, stiff infarcted heart tissue inhibits the global contraction of cardiomyocytes. However, direct insight into how the mechanical environment influences dynamics on the sarcomere level is missing. We tracked the motion of individual sarcomeres using endogeneous z-line labeling in CRISPR/Cas9 modified hESC-derived cardiomyocytes on micro-patterned substrates with various physiologically relevant stiffnesses (7 kPa to 60 kPa). Individual sarcomere contraction is impeded for increasing substrate stiffness. Furthermore, on soft substrates sarcomeres contract coherently, whereas with increasing stiffness the sarcomere contraction gets increasingly incoherent and heterogeneous.

These findings suggest that rigid mechanical surroundings force sarcomeres into competition. Using a mechanistic muscle model, we show that z-lines elastically cross-linked to the substrate and heterogeneous elements can account for many features we observe. Theories of collective molecular motors predict emerging phenomena such as dynamic instabilities, and these experiments can provide quantitative data to understand the microscopic basis of real cardiac muscle function.

BP 17.3 Wed 10:00 H 1058

Functional analysis of larval chordotonal organ mechanics in Drosophila — •Chonglin Guan[1], Martin Göpfert[2], and Christoph F. Schmidt[1] — [1]Drittes Physikalisches Institut - Biophysik, Fakultät für Physik, Georg-August-Universität Göttingen — [2]Abteilung Zelluläre Neurobiologie, Schwann - Schleiden-Forschungszentrum, Georg-August-Universität Göttingen

In Drosophila larvae and adults, chordotonal organs (ChOs) are ubiquitous mechanoreceptors, converting a diverse range of physical forces such as sound, vibration and stretch into biological responses. The mechanoelectrical transduction, operated by an active, force-generating process, has been linked to adaptation motors. The underlying force generator, however, is poorly known, and the functional dissection of ChO mechanics in vivo has been challenging. We combine electrophysiological analyses with mechanical stimulation, and correlate mechanical properties and active manipulation with neuronal activity. We find that myosin II motors power contraction of the cap cells of ChO and regulate mechanosensation. Our in vivo model reveals larval ChOs as complex, but accessible organs to study the molecular machinery involved in the regulation and encoding of mechanical forces by primary mechanoreceptor neurons.

BP 17.4 Wed 10:15 H 1058

Magnetogenetic Manipulation of Cellular Functions — •Cornelia Monzel[1,3], Chiara Vicario[1], Domenik Lisse[2], Elie Balloul[1], Koceila Aizel[1], Mathieu Coppey[1], Jacob Piehler[2], and Maxime Dahan[1] — [1]Institut Curie, Laboratoire Physico-Chimie, CNRS UMR168, 75005 Paris — [2]University of Osnabrück, Department of Biology, 49076 Osnabrück, — [3]present address: University of Düsseldorf, Department of Physics, 40225 Düsseldorf

Many cell functions rely on the coordinated activity and spatial distribution of proteins on a subcellular scale. However, few tools are hitherto capable of probing and perturbing intracellular proteins on scales matching their natural spatio-temporal distribution. Here, we develop a novel magnetogenetic approach where intracellular proteins are specifically targeted by magnetic nanoparticles (MNPs) and manipulated with magnetic forces to remotely control individual cell functions. Among these functions are changes in organelle dynamics or the activation of a cell signaling pathway. We demonstrate that semisynthetic MNPs based on the natural iron storage protein ferritin are ideally suited for our magnetic manipulation approach. We explain the MNP design, different means of magnetic stimulation, and show the corresponding biological response.

Invited Talk BP 17.5 Wed 10:30 H 1058

Tension build-up and membrane deformations in actin-membrane biomimetic systems — •Cécile Sykes — Institut Curie/CNRS/Paris Science et Lettres — 11, rue Pierre et Marie Curie, F-75231 Paris cedex 05

In order to unveil generic mechanisms of cell movements and shape changes, we design stripped-down experimental systems that reproduce cellular behaviours in simplified conditions, using liposome membranes on which cytoskeleton dynamics are reconstituted. Such stripped-down systems allow for a controlled study of the physical mechanisms that underlie cell movements and cell shape changes. Moreover, these experimental systems are used to address biological issues within a controlled, simplified environment. We have reconstituted the actin cortex of cells at the membrane of liposomes, and characterized their mechanical properties. We will show how these cortices contract in the presence of myosin motors, and how such experiments shed light of the mechanisms of cell shape changes. We have reconstituted membrane tubules and spikes pushed or pulled by actin polymerization, and which reproduce the formation of endocytic vesicles and filopodia. We will show how membrane and actin mechanical properties govern their formation.

30 min. break

BP 17.6 Wed 11:30 H 1058

dynamic RT-DC: time-resolved mechanical single cell analysis at 100 cells / second — •Bob Fregin[1], Fabian Czerwinski[1], Konstanze Aurich[2], Doreen Biedenweg[1], and Oliver Otto[1] — [1]ZIK HIKE, Universität Greifswald, Greifswald, Germany — [2]Universitätsklinikum Greifswald, Greifswald, Germany

Real-Time Deformability Cytometry (RT-DC) is a label-free technique for single cell mechanical analysis with high throughput of up to 1,000 cells / second. By observing the shape of cells passing a narrow microfluidic channel their deformation and thus material properties can be quantified. As RT-DC is in the current implementation a single shot technique, i.e. for every single cell only one image at the end of the channel is captured, exclusively time-independent parameters like the elastic modulus can be derived.

Here, we are introducing an extension of RT-DC towards dynamic single cell measurements with the possibility to capture elastic and viscous properties of single cells for up to 100 cells / second. Measurements are carried-out in real-time and allow for monitoring cellular shape-changes along the entire length of the microfluidic channel. By varying the experimental conditions we are capable to access a dynamic range in cell response exceeding one order of magnitude in time.

Dynamic RT-DC on a precursor myeloid cell line HL60 as well as primary cells reveals two characteristic timescales which can be attributed to the non-uniform stress distribution at the inlet compared to the steady-state situation inside the channel. This approach allows to extract model-independent material properties from RT-DC assays.

BP 17.7 Wed 11:45 H 1058

Force Spectroscopy for the Investigation of Cellular Mechanotransduction — •Sandra Sindt, Steven Huth, and Christine Selhuber-Unkel — Christian-Albrechts-Universität zu Kiel, Institut für Materialwissenschaft, Biokompatible Nanomaterialien

Cells permanently explore the mechanical properties of their surroundings by applying forces. Even though some knowledge about mechanical interactions of cells have been obtained, the mechanisms are not completely understood yet. Here, we present a method based on a combination of Traction Force (TFM) and Atomic Force Microscopy (AFM) to gain deeper understanding of cellular mechanotransduction. During the detachment of a cell with a cantilever from a substrate with embedded fluorescent marker beads, the beads within the substrate are displaced. If the elastic properties of the substrate are known, the traction forces of the cell can be calculated from the displacements of the beads. In order to calculate traction forces, a precise and reliable mechanical characterization of substrates is important. The AFM is a well-established technique to measure the stiffness at the cellular scale, yet values of Young's moduli published by different authors vary significantly. Here, we present an improvement of the previously used method for measuring the stiffness of biological substrates based on indentation experiments using AFM. In this procedure, cantilevers are pressed against biocompatible soft polymer substrates using different forces and speeds. Both cantilever movements, towards and away from the substrate, are recorded and plotted as a force-distance curve. The Young's moduli are acquired from a Hertzian fit of the approach curve.

BP 17.8 Wed 12:00 H 1058

Applications of new optical high speed cell characterization device CellMOUSE — •Daniel Geiger, Tobias Neckernuss, Jonas Pfeil, Ralf Schuster, and Othmar Marti — Institute of Experimental Physics, Ulm University

For various applications in science and medicine the identification of different cell parameters of suspended cells is essential. We developed a novel tool to assess important cellular parameters like size, shape and morphology in real time for more than 500 cells per second. This allows completely new experiments in various fields of basic research and clinical applications. After a brief introduction to the technique we present different applications of the so called CellMOUSE device. A validation for the measurement principle with well known samples will be shown. Furthermore, the determination of cellular parameters from suspended cells with superior throughput and accuracy will be presented. The samples range from simple NB4 cells to leukocytes and erythrocytes to bacteria and bacterial clusters. In addition we will present a simulation method for an easy comparison of our results with well established techniques.

BP 17.9 Wed 12:15 H 1058

Simultaneous measurement of the Young's modulus and the Poisson ratio of thin elastic layers — •Wolfgang Gross and Holger Kress — Department of Physics, University of Bayreuth, Bayreuth, Germany

The mechanical interplay between cells and their environment has become a major point of interest during the last decades. To quantify the interactions between cells and soft matrices with techniques such as traction force microscopy, precise knowledge of the elastic parameters of thin substrate layers is necessary. However, only few methods are available to simultaneously measure the elastic modulus and the Poisson ratio of thin substrate layers.

Here we describe a novel technique to measure both parameters in a single experiment. As a model system, we chose polyacrylamide and poly-N-Isopropylacrylamide layers with a thickness of 1/10th of a millimeter and a stiffness in the range of mammalian tissue. We place millimeter-sized steel spheres with different radii on the substrates which indent the surface due to gravity and visualize the indentation cap marked with fluorescent microparticles with an inverted microscope. Using a previously published model which takes finite thickness effects into account, we demonstrate experimentally for the first time that the model allows the simultaneous determination of both the elastic modulus and the Poisson ratio with high accuracy. Since the technique comes without the need of special equipment aside from an inverted microscope, we envision it to become a standard tool for the characterization of thin substrate layers.

BP 17.10 Wed 12:30 H 1058

Measurement and simulation of light scattering by deformed red blood cells (RBCs) in flow cytometry — •Jonas Gienger, Hermann Gross, Markus Bär, Volker Ost, and Jörg Neukammer — Physikalisch-Technische Bundesanstalt (PTB), Abbestraße 2–12, 10587 Berlin, Germany

Light scattering of single cells is widely applied for flow cytometric differentiation of cells. We developed a dedicated flow cytometer to simultaneously observe forward light scatter (FSC) of RBCs for orthogonal incident wave vectors $\vec{k}_1 \perp \vec{k}_2$. Bimodal distributions are observed in two-dimensional dot plots of FSC($\vec{k}_1$) vs. FSC($\vec{k}_2$) of typically 7.5×10^4 RBCs, which is a result of the RBCs' random orientation around the direction of flow, their distribution of size and optical properties.

Simulations of the light scattering by single RBCs were performed using the discrete dipole approximation (DDA). Using the axisymmetric equilibrium shape of healthy RBCs employed in most light scattering simulation studies to date, the experimentally observed bimodality cannot be reproduced. This is because this model does not account for the significant elongation due to hydrodynamic forces in the flow cytometer, involving extensional flow and Poiseuille flow at speeds of up to 5 m/s in channels of a few 100 μm width. Thus we propose a simple ad hoc model for elongated RBC shapes, which reproduces the bimodal 2D-distributions qualitatively. However, to quantitatively analyze the data and retrieve elastic parameters from such high-throughput optical measurements a detailed modeling of RBC deformation in the given flow conditions seems necessary.

BP 17.11 Wed 12:45 H 1058

Treatment of cancer cells with acoustic waves — •Maja Strugacevac[1], Nina Bartels[1], Tobias Löffler[1], Constanze Wiek[2], Marcel Glaas[2], Jörg Schipper[2], and Mathias Getzlaff[1] — [1]Heinrich-Heine-Universität Düsseldorf, Institute of Applied Physics, Düsseldorf, Germany — [2]Düsseldorf University Hospital, Department of Otorhinolaryngology, Düsseldorf, Germany

Our group is developing new, alternative, cell-selective treatment strategies for squamous cell carcinoma of the head-neck area based on the different mechanical properties of oral keratinocytes and cancer cells. As a first step we compared their cytoskeleton and observed the differences in actin filaments and microtubules, both playing a significant role in cell elasticity. Oral keratinocytes are differently formed and seem to contain more fibers then cancer cells which could lead to the conclusion that there is a difference in cytoskeleton between this cell types.

In a second step we have irradiated different cell lines with acoustic waves exhibiting frequencies from 0.5 up to 10 kHz. Using different frequencies and input power we investigated the behavior of the cells exposed to the sound waves. Our latest results will be presented and discussed.