Bacteria & Motile Cells: Mechanics, Motility, & Signal Transduction

3225-Pos Board B272

Fluid Transport in the Extracellular Matrix:Kinetic Changes Induced by Metabolic Inhibitors

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Water and solutes are transported to and from cells across the extracellular matrix by osmo-mechanical forces, the fiber-mesh, glycosaminoglycans, and myofibroblasts in as yet unclear ways. Here, we analyze fluid transfer in/out of the dermal matrix as a function of pressure and time and explore the effects of glucose-metabolism inhibitors. Osmotic-stress techniques were adapted to measure transfer-kinetics in full-thickness dermal cultures at six different polyethylene glycol concentrations adjusted by membrane osmometry (range =3-211 mmHg). Influx/outflux at each pressure was followed over time by precision weighing. Progression curves were modeled using:

$Volume\ Transfer = V_{max}/[1+(time/T_{1/2})^d]$,

where V_{max} is total volume transferred; $T_{1/2}$ is the time at which volume is half-maximal; and d is proportional to the rate at $T_{1/2}$. Rates and Vmax were found to be proportional to pressures and varied with temperature, as did the pH of cultures, suggesting that flow regulation is energy-dependent. Na Azyde (0.4mg-2.5 mg/ml) increased outflow, while Iodoacetamide (1.8-4.5 mg/ml) decreased it relative to that in explants without inhibitors; at 208 mmHg, Vmax values were 0.438 $\pm$ 0.0058 (Na Azyde); 0.284 $\pm$ 0.036 (Iodoacetamide); 0.359 $\pm$ 0.029 (control) below the initial value. Inflow increased with inhibitors; at 3 mm Hg, Vmax values were 0.392 $\pm$ 0.032, 0.463 $\pm$ 0.093, and 0.265 $\pm$ 0.012 above the initial volume, respectively. With the caveat that the inhibitors' effects on transfer could be unrelated to changes in the myofibroblasts' glucose metabolism, these differences suggest that energy for flow regulation is derived primarily from anaerobic metabolism during outflow but from aerobic and anaerobic metabolism during inflow. Globally, these observations are consistent with a model where myofibroblasts respond to pressure and oxygenation changes by adjusting fiber tension to control glycosaminoglycans hydration.

3226-Pos Board B273

Untangling Hyaluronan-Protein Networks and Function

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Hyaluronan (HA), a polysaccharide present in the extracellular matrix (ECM) and attached to the plasma membrane of many cell types, is suspected to mediate a surprising number of biological functions. Researchers have puzzled over how such a relatively simple polysaccharide influences so many physiological processes. It is suspected that the wide array of hyaluronan binding proteins helps to modify the structure and activity of HA. These hyaluronan binding proteins are located in hyaluronan-rich tissues throughout the body: neurocan in the brain, versican in the skin, aggrecan in cartilage, TSG-6 in inflammatory processes. However, very little is understood about how these proteins interact with hyaluronan and lead to a restructuring of the local matrix. We employ video-based Particle Tracking Microrheology (PTMR) and Fluorescence Recovery After Photobleaching (FRAP) to characterize the mechanical and structural properties of hyaluronan-protein solutions. We have started by measuring the dependency of the viscoelastic shear moduli on frequency (1-1000 kHz) for highly-monodisperse HA solutions at four different molecular weights (160kDA, 500kDa, 1000kDa, 2500kDa). The HA concentration was varied to explore the scaling laws of the mechanical properties as predicted by polymer theory. FRAP is applied as a complimentary method to determine the mesh size and transport properties of the networks. These control studies of monodisperse HA prepare us for a detailed PTMR and FRAP study of hyaluronan-protein networks, particularly those formed with hyaluronan binding protein (HABP), the G1-domain of versican, and the link domain of TSG-6.

3227-Pos Board B274

Functional Analysis Of Intravital Two-photon Cell Motility Data

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Intravital two-photon microscopy is a powerful tool which allows to observe cell migration and interaction in living tissue. Recently this method was applied to lymphocytes in lymph nodes. The motility of lymphocytes has important implications on the functionality of immune responses. Different cell types entering and leaving lymphoid tissue have to home to their respective compartments and to find suitable interaction partners. Both processes crucially depend on chemotaxis which is mediated by corresponding chemokines and membrane bound receptors. While this knowledge was based on corresponding block- or knock-out-experiments, the aim of the recent two-photon experiments was to clarify the migration mode of lymphocytes in vivo under realistic conditions. Surprisingly two-photon experiments of lymphocyte motility in lymph nodes do not exhibit any sign of chemotactic activity. Three groups have found that the cells basically move in a random walk with directional persistence of 2 minutes (Schwickert etal Nature 2007 446:83; Allen etal Science 2007 315:528; Hauser etal Immunity 2007 26:655). These results induced a controversial discussion about functional implications that could not be resolved on the basis of the measured data alone. We show that a mathematical analysis of cell motility can clarify the interpretation of two-photon motility data for this specific case and in general. Data about tissue morphology and functionality are used together with a simulation of lymphocyte motility to understand the details of chemotaxis in lymphoid compartments. It is found that the chemotactic sensitivity of lymphocytes is tightly regulated and the simulations predict a frequent up and down regulation of chemokine receptors on the plasma membrane. It is shown that under these conditions both the motility data and lymphocyte functionality can be reconciled.

3228-Pos Board B275

Sarcomere Mechanics in the Stress Fiber

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Actin filaments inside endothelial cells assemble into tensed bundles called stress fibers. Stress fibers are observed in vivo in a variety of tissues including the aorta, the heart, the spleen, the eye, and hair follicles. Sarcomeres are the force generating units of stress fibers and are responsible for generating intracellular tension. Little is known about the mechanical behavior of individual sarcomeres in living cells. Using femtosecond laser ablation to sever individual stress fibers in living capillary endothelial cells, we are able to measure the mechanics of sarcomeres in living cells. Our results indicate that the length of a sarcomere after severing decreases in two phases- an initial elastic response, followed by slower contraction at constant speed. The latter phase, interpreted as active myosin-mediated contraction, ceases abruptly after a minimum sarcomere length is achieved, suggesting a rigid resistance that prevents further contraction. We model this with an equivalent mechanical circuit, allowing us to estimate the speed of myosin motor walking in sarcomeres for the first time. We find that this speed ranges from 0.02 to 0.1 microns/s, which compares well with in vitro measurements. Our analysis suggests a novel mechanical model of a sarcomere that includes an active force generating component in parallel with an infinite barrier and in series with a stiff elastic spring.

3229-Pos Board B276

Transport Theory For HIV Migration Through In Vivo Distributions Of Microbicide Epithelial Coating Layers

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Topical microbicide products are being developed for preventing HIV transmission. These include vaginally applied gels that deliver anti-HIV molecules. Gels may also provide partial barriers that slow virion diffusion from semen to vulnerable tissue, increasing the time during which anti-HIV molecules can act. Previously, our group developed a mathematical model for HIV transport and neutralization for a uniform layer of microbicide gel. Hindrance of HIV diffusion was found to have significant potential to impact efficacy. In vivo, however, gels do not deploy to form complete, uniform layers - not all tissue is coated, and thickness is not constant. Here, we further developed our model to assess salient parameters that determine a gel's ability to hinder HIV diffusion in vivo. We applied this model to experimental data for coating distributions of two vaginal gels in women. Time required for a threshold number of virions to reach the tissue surface was used as a metric to compare different hypothetical and experimental scenarios. We found that time-to-threshold increased with increasing gel layer thickness and with decreasing diffusion coefficient. For gel layers with average thickness $>$ 100um, fractional area coated rather than gel layer thickness was the primary determinant of time-to-threshold. For gel layers $<$ 100um, time-to-threshold was brief, regardless of fractional area coated. Application of the model to experimental data showed little difference in time-to-threshold between the gels tested. However, the protocol

(i.e., +/− simulated coitus) following gel application had a much more significant effect. This study suggests that it is important for microbicide gels to distribute in layers of thickness ≥ 100um, and that the fractional area coated is critical in determining gel ability to hinder HIV diffusion (NIH-AI077289).

3230-Pos Board B277

Detection and Characterization of elementary events underlying force generation in lamellipodia of Dorsal Root Ganglia Neurons

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Force generation in lamellipodia of growth cones originates from the progressive addition of small polymers to the existing network of actin molecules pushing the cellular membrane forward. Using optical tweezers we have characterized with high temporal resolution and sensitivity the molecular mechanisms by which lamellipodia generate force on encountered obstacles such as silica beads. When beads are positioned in close contact to the lamellipodium, because of adhesion forces, beads can seal on the membrane decreasing the standard deviation σ of Brownian fluctuations to less than 10 nm. Under these conditions, when the lamellipodium leading edge pushes the bead it is possible to detect discrete jumps with a variable shape and amplitude. The amplitude of these jumps varies from 5 to 40 nm. The summation of these jumps leads to a plateau level, during which an almost constant force can be measured for several seconds. During this plateau, asymmetric brief transients are observed, ultimately leading to the collapse of the generated force. These transients have amplitude up to 150 nm and last some hundreds of msec. These jumps and transients constitute different phases of the polymerization and depolymerisation cycles of the actin filament network and constitute also the elementary events underlying force generation in lamellipodia.

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Stick-Slip Motion of a Red Blood Cell in a Capillary

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The collective vaso-occlusive event in sickle cell disease induced by multiple red blood cells (RBC's) has recently been evoked and controlled in vitro using a microfluidic platform [1]. The interplay between the cells tunable stiffness and its interaction with the endothelium is believed to be a predominant factor at the onset of the event. We report here the stick-slip motion of a RBC in a capillary. We use a tapered glass capillary with inner diameter from 8 to 4 microns, and track the squeezed cell driven by a variable pressure drop. This allows us to scan the variations of the RBC velocity as a function of the pressure gradient and of the capillary local diameter in a single experiment. We analyze our findings in terms of a Stokes flow lubrication model. The adhesion force of the red blood cell to the inner wall can thus be computed to refine a model of dynamical cell-wall bonds activation.

[1] Higgins et al., Proc. Natl. Acad. Sci. U. S. A. 104: 20496 (2007).

3232-Pos Board B279

Examining Integrated Cell Structural Responses: Probing Cytoskeleton Behavior through a Coupled Dual-Mode Mechanical Stimulation Approach

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Mechanical stimulation of cells has been shown to affect various cellular functions through the actin cytoskeleton such as cell motility, apoptosis, and proliferation. The influence of mechanics on cells is evident whether the stimulation is in the form of tension, compression, or even shear stress. In this there is a need to influence cellular function through its extracellular matrix connections with multiple integrated mechanical approaches to gain a better understanding in the field of mechanotransduction. In this study we developed a device that when utilized with an elastomeric material allows us to stimulate cells with uniaxial strip stretching, shear fluid flow or both simultaneously. This device uses a pressure regulator to induce uniaxial strip stress along the basal surface of cells and a peristaltic flow pump to induce shear stress across the apical surface. We exposed NIH/3T3 fibroblasts to uniaxial strip stretching, shear fluid flow and both simultaneously to examine the question of how the integrated inputs of mechanical stimulation are processed by the cell in terms of its structural response. We used fluorescence microscopy to examine the orientation of F-actin and G-actin structures and found alignment along the direction of force for both uniaxial strip stretching and shear fluid flow in comparison to cells exposed to both mechanical modes, which revealed an alignment out of phase between both axes of applied force. This integrated response is helping to discern the influence of the modes of stimulation in terms of overall cell behavior. These intriguing results have potential implications in a variety of fields including biophysics, mechanotransduction, and cell structure.

3233-Pos Board B280

Matrix Elasticity Dictates Cytoskeletal Polarization In Mesenchymal Stem Cells

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It is now generally accepted that cells are as responsive to their mechanical environment as they are to biochemical stimuli. As reported recently, human mesenchymal stem cells (hMSCs) plated on collagen-coated gels with a Young's modulus E = 1, 11, and 34 kPa, differentiate towards the neurogenic, myogenic, and osteogenic lineage, respectively [Engler AJ et al. Cell 126(4):677-89 2006]. This mechano-sensing is non-muscle myosin II (NMM II) dependent as shown with the potent inhibitor blebbistatin. While up-regulation of specific proteins occurs on the time scale of several days, the MSCs already show significantly different morphologies several hours after initial cell adhesion. We present experimental data and a theoretical model to explain the non-monotonic dependence of stress-fiber polarization in MSCs on matrix elasticity. The cytoskeletal organization is analyzed with immunofluorescence images of NMM IIa and actin in the cells at various time points using an automated segmentation algorithm. The theory generalizes the treatment of elastic inclusions in solids to ''living'' inclusions (cells) whose active polarizability, analogous to the polarizability of non-living matter, results in the feedback of cellular forces that develop in response to matrix stresses. This study demonstrates that matrix rigidity dictates cytoskeletal organization - a bio-mechanical process that results in different cell shapes and finally leads to differentiation.

3234-Pos Board B281

Membrane Mechanics of B Lymphocyte Activation

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B lymphocytes are a critical component of the immunological machinery whose primary role is to produce and secrete antibodies that detect foreign antigens. When stimulated by their corresponding antigen, B lymphocytes are triggered to differentiate into antibody secreting Plasma cells. It has been shown that this differentiation requires the transcription factor XBP-1. The mechanism by which XBP-1 deficiency deters Plasma cell differentiation is not understood. XBP-1 regulates lipid synthesis in B lymphocytes, and hence in activated cells, the lipid composition of the cell membrane in XBP-1 deficient cells is different. The aim of this work is to mechanically characterize the B lymphocyte membrane and quantify the consequences of XBP-1 deficiency on the membrane mechanical properties of activated B lymphocytes. We probed the mechanical properties of the cell membrane using optical tweezers. Membrane tethering experiments were performed by locally dissociating the lipid membrane from the underlying cytoskeleton and extending a tube of lipid bilayer from the cell surface while measuring the force of extension, and then the relaxation of the force after extension. Wild type B lymphocytes exhibit three stages of tethering: 1) a linearly increasing force due to local cell stiffness 2) an approximately constant force (plateau force) regime after the membrane locally dissociates from the cytoskeleton, and 3) force relaxation after the tether extension is stopped. Tethering experiments were performed on wildtype (WT) and XBP-1 deficient B lymphocytes activated by bacteria derived lipopolysaccharide. Experimental results show that activated XBP-1 deficient cells have a lower membrane viscosity indicated by a lower plateau force and a faster tether force relaxation. Additionally, a micromechanical model is developed to describe the force of tether extension.

3235-Pos Board B282

Cilia And Embryonic Handedness - On Which Side Lies Your Heart?

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Although the superficial appearance of the vertebrate body plan is left-right symmetric, the inner organs of vertebrates exhibit a strikingly asymmetric arrangement. It has been shown that this left-right asymmetry is induced early during embryonic development and the result of a fluid flow generated by the clockwise rotation of cilia, which are are motile, hair-like cellular appendages. What determines the specific handedness of these ciliary rotations is the subject of ongoing debate. Based on a three-dimensional theoretical description of the ciliary geometry we discuss the bending modes generated by the cooperativity of force generating dynein motors working against elastic microtubules within cilia. Taking into account both bending and twisting of the ciliary