(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).

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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.

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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.

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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.

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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