(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.

3231-Pos Board B278

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

structure, we find that despite the chirality of the ciliary structure, cilia can in principle generate clockwise as well as anticlockwise twirling beat patterns. However, our results show that the axoneme's chirality leads to one sense of rotation being selected dynamically for given parameter values and properties of dynein motors. This dynamic selection of asymmetric states is analogous to how the direction of motion of a motor protein moving along a filament.

3236-Pos Board B283

Cellular Potts Modeling of Matrix-Dependent Endothelial Cell Networking

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Networking of endothelial cells during fetal and post-natal development relies upon dynamic remodeling and subsequent stabilization of cell-cell and cell-extracellular matrix (ECM) interactions. Herein, we investigate lung endothelial cells network dynamics on thin films of engineered ECM. These systems adhere to the Differential Adhesion Hypothesis and their behaviors are well reproduced with a Cellular Potts Model. In order to gain insight on the profound effect external environment has on cell behavior, our model explicitly includes the ECM, as we explore particular changes in networking dynamics with changes in substrate parameters. Further, we describe specifics of the system Hamiltonian governing the Monte Carlo methods with addition of experimentally derived rules that can simulate both normal and non-networking cells systems. The biological significance of derived cell-cell and cell-matrix adhesion and cohesion energies that most appropriately model our experimental data is discussed.

3237-Pos Board B284

Accelerated Proliferation and Migration of Keratinocytes, Fibroblasts and Macrophages Isolated from H2-Calponin Knockout Mice

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Calponin is a family of actin-associated regulatory proteins that play a role in modulating smooth muscle contractility and actin cytoskeleton functions. The h2 isoform of calponin is found in smooth muscle and certain non-muscle cells. Keratinocytes, fibroblasts, and macrophages express h2-calponin at significant levels. To investigate the function of h2-calponin in these cell types that are key players in wound healing, we studied primary cultures of epidermal keratinocytes, dermal fibroblasts and peripheral macrophages isolated from h2-calponin knockout mice recently developed in our laboratory (Huang et al., *J. Biol. Chem.* 283:25887-99, 2008). Cell proliferation studies revealed faster growth rates of all of the three cell types from h2-calponin knockout mice as compared with that of wild type control cells. Similarly, the three types of cells exhibited faster migration in in vitro wound healing experiments when h2-calponin is absent. The results suggest that h2-calponin may be a regulatory factor in the balance of cell proliferation and migration during wound healing. We have previously observed that mechanical tension built in the cytoskeleton regulates h2-calponin expression and degradation in cells including keratinocytes and fibroblasts (Hossain et al., *J. Biol. Chem.* 280:42442-53, 2005; *Biochemistry* 45:15670-83, 2006). Therefore, experiments are underway to investigate the role of h2-calponin in the effect of mechanical tension on keratinocyte differentiation and skin wound healing.

3238-Pos Board B285

How deep cells feel: Mean-field Computations and Experiments

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Most cells in solid tissues exert contractile forces that mechanically couple them to elastic surroundings and that significantly influence cell adhesion, cytoskeletal organization, and even cell differentiation. However, strains within the depths of matrices are often unclear and are likely relevant not only to the fact that some matrices such as so-called basement membranes are thin relative to cell dimensions but also to defining how far cells can 'feel'. Here we present experimental results for cell spreading on thin, ligand-coated gels and for prestress in stem cells in relation to gel stiffness. Spread area on thin and soft gels was found to resemble cells on thick and stiff gels. Matrix thickness also affects focal adhesions and cytoskeleton organization in stem cells, which we will compare to differentiated cells. We introduce a finite element computation in which a cell is placed on an elastic matrix, while matrix elasticity and thickness are varied in order to compute and compare elastostatic deformations within the matrix. Average interfacial strains between cell and matrix show large deviations only when soft matrices are a fraction of the height and width of a cell, proving consistent with experiments. Three-dimensional (3D) cell morphologies that model stem cell-derived neurons, myoblasts, and osteoblasts show that a cylinder-shaped myoblast induces the highest strains, consistent with the prominent contractility of muscle. Groups of such cells show a weak crosstalk in matrix strains, but the cells must be much closer than a cell-width - experimental tests of this are emerging. Cells thus feel on length scales closer to that of adhesions than on cellular scales or larger.

3239-Pos Board B286

Interactions Between Lipid Bilayer And Protein Skeleton In Erythrocyte Deformations

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We study mechanics of a red blood cell in large deformations by using a multi-scale modeling approach, in which the interactions between the lipid bilayer and the protein skeleton are considered as two parts: vertical contact and lateral sliding. The sliding is caused by the mobility of the transmembrane proteins (e.g. band 3 and glycophorin C). Our model consists of a complete-cell model which depicts the cell membrane as two continuous shells, and a molecular-detailed model of a junctional complex (JC) that provides the constitutive properties of the inner layer (the skeleton). The folding/unfolding reactions of the spectrin are also considered and incorporated into the JC model. This multi-scale model is validated by comparisons with other modeling approaches and experiments about micropipette aspirations and optical tweezer stretching. Applying this method, we numerically duplicated the boundary-value problem associated with cell deformation in a flow channel. The critical contact force, i.e. the maximum contact force that can exist between the bilayer and the skeleton without inducing skeleton-bilayer disassociation, is extracted. This critical force is then applied to predict conditions of vesiculation in other mechanically-induced cell deformations.

3240-Pos Board B287

Measurement of Adhesion Force between a Human Neutrophil and a *Candida albicans* Hyphae Using a Micromanipulation Technique

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Yeast infection (candidiasis) is a common and persistent threat to human health. Normally harmless, the fungus *Candida albicans* is present in 40-80% of normal human beings, but in immune-compromised individuals, it can proliferate and cause a variety of health problems including pneumonia, septicemia, or endo[[Unsupported Character - Codename -]]carditis. The fundamental mechanism towards the control of candidiasis and other fungal infections involves understanding how human neutrophils interact with β-Glucan, a polysaccharide present in fungal cell walls, at the single cellu[[Unsupported Character - Codename -]]lar level. We hypothesize that the complement receptor 3 (CR3), a member of the integrin family, can recog[[Unsupported Character - Codename -]]nize the β-Glucan on the *C. albicans* hyphae, initiating neutrophil adhesion and caus[[Unsupported Character - Codename -]]ing a respiratory burst. We test this hypothesis using a two-pipette micromanipulation technique to measure the adhesion force between a single neutrophil and a *C. albicans* hyphae. A micromanipulator attached to a suction pipette is used to trap a single *C. albicans* hyphae that is attached by a single neutrophil to a second, flexible pipette. The micromanipulator slowly pulls up on the hyphae, exerting an increasing force and causing the flexible pipette to bend until the hyphae detaches from the neutrophil. By measuring the deflection of the flexible pipette at the instant the hyphae detaches, Hooke's law can be used to calculate the adhesion force between the hyphae and the neutrophil. By measuring the average adhesion force of neutrophils from knock-out mice missing CR3 and compare with that from the wild type animals expressing CR3, we can determine the mechanical role the receptor plays in neutrophil adhesion.

3241-Pos Board B288

Mechanical Computation in Neurons

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Motility is a major function of cells, playing a fundamental role in development and embryogenesis. Growth cones are the major motile structures usually located at the tip of neurites and are composed of a lamellipodium from which thin filopodia emerge. We have analyzed the kinetics and dynamics of growth cones from a computational point of view with the aim to understand two major issues: firstly, the strategy used by filopodia and lamellipodia during their exploration and navigation; secondly, which kind of mechanical problems neurons need to solve during their operation. Filopodia grow and retract following