the cell (see figure: *cell in middle, collagen around, width 100 microns*), before and after the addition of cytochalasin. Deformations caused by cell relaxation - measured by tracking fiber network crosslinks - can then be translated into strain energy and forces by using a simple elastic beam model for each collagen fiber segment. Preliminary results on the strain energy thus measured show close agreement with previous bead-based strain measurements.

2687-Pos Board B657

Microrheology of the pericellular matrix

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A membrane-coupled pericellular matrix (PCM) is expressed by a number of cell types and is in most cases associated with cell proliferation and migration. The PCM can be micrometers thick with the glycosaminoglycan hyaluronan (HA) as its backbone. For such an extended layer, however, the presence of hyaldherins like aggrecan or versican is a prerequisite. The latter, like HA, is associated with cancer progression and metastasis. As a model for a HA producing cell we used the human prostate adenocarcinoma cell line PC3. To probe the mechanical properties of the PCM we used microrheology, based on an optical trap equipped with far-field interferometry. We were able to probe the PCM at different distances from the membrane surface and found a soft (< 1 Pa) layer with a thickness of ~ 1 μm in the absence of, and of ~ 3 μm in the presence of exogenously added aggrecan. Furthermore, in the presence of aggrecan, part of the cells expressed long (<~ 10 μm) microvilli extending from the surface. Probing in between the microvilli, we found again a soft (< 1 Pa) PCM. Both the viscoelastic PCM and the microvilli were absent on cells treated with the HA diminishing enzyme hyaluronidase, showing the structural importance of HA in the PCM.

2688-Pos Board B658

Bacterial Cell Wall Peptidoglycan at Single Molecule Resolution

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The major structural component of bacterial cell walls is the peptidoglycan sacculus, which is one of nature's strongest and largest macromolecules that maintains the large internal pressure within the cell while allowing the transport of molecules into and out of the cell and cell growth. The three-dimensional structure of this unique biopolymer is controversial, and two models have been proposed: the planar model, in which the glycan strands lie in the plane of the cell surface, and the scaffold model, in which the glycan strands lie perpendicular to the cell surface. In this study we have used atomic force microscopy (AFM) to investigate the high resolution structure of isolated, intact sacculi of Escherichia coli K12 bacteria. Atomic force microscopy (AFM)-single molecule force spectroscopy was performed on single sacculi exposed to the tAmiB enzyme which cleaves the peptide-glycan bonds. Surprisingly, the measurements revealed individual strands of up to 250 nm in length. This finding combined with high resolution AFM images recorded on hydrated sacculi provide evidence for the validity of the planar model for the peptidoglycan structure in Gram-negative bacteria.

2689-Pos Board B659

Modeling of Stability of Adhesion Clusters and Cell Reorientation under Lateral Dynamics Load

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The motivation of this study is to understand the experimental observations of different responses of cell on stretched substrate to the static and dynamic loads. In this talk, a focal adhesion model which can consider the mechanics of stress fiber, adhesion bonds, and substrate is developed at molecular level by treating the focal adhesion as an adhesion cluster. The stability of the cluster under dynamic load is studied by applying cyclic external strain on the substrate. We show that there exists a threshold value of external strain amplitude, beyond which the adhesion cluster disrupts quickly. In addition, our results show that the adhesion cluster is prone to losing stability under high-frequency loading, because the receptors and ligands can not get enough contact time to form bonds due to the high-speed deformation of the substrate, and at the same time the viscoelastic stress fiber becomes rigid at high-frequency which attributes large deformation to the bonds. Furthermore, we find that the stiffness of stress fiber takes an important role in the stability of the adhesion cluster. The essence of this work is to connect the dynamics of the adhesion bonds (molecular levels) with the behaviors of the reorientation of cell (cell level) through mechanics of stress fiber. The predictions of our cluster model are broadly consistent with the experimental results.

2690-Pos Board B660

Fiber Dynamics during Strain Stiffening in Stiff Biopolymer Networks

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Many biopolymers have the unusual mechanical property of strain stiffening. Previous work and theoretical models have focused largely on semi-flexible biopolymers such as actin. Recently, there has been increased interest in understanding stiff, athermal biopolymers. We compare the dynamics of 2 stiff biopolymer networks: fibrin, which is primarily responsible for the mechanical properties of a blood clot, and collagen, which is the main component of connective tissue. We use confocal microscopy to image these in-vitro¬ networks as they undergo a steady shear. Using image processing techniques we record information about the fiber structure as a function of strain. In particular, we quantify angle distributions and the degree of non-affine motion. This is compared to bulk rheological measurements. We also get qualitative information from reconstructed images of the network when viewed as snapshots at various strain positions.

2691-Pos Board B661

Does Substrate Stiffness Guide Neutrophils During An Inflammation Response?

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Neutrophils play a critical role in host response to infection and injury. To reach points of inflammation, they execute a series of adhesion and migration events that allow them to move from the blood stream, through the endothelial cells lining the blood vessels, and into the tissue and surrounding extracellular matrix. Within these few minutes of time, the neutrophil experiences drastically different physical environments, ranging from the viscous fluid in the blood vessel, to the elastic extracellular matrix, to the highly variable points of stiffness at sites of inflammation. Each of these physical environments offers its own unique mechanical cues which can affect a neutrophil's function and guide its behavior. Traditional studies have relied upon glass and plastic substrates, despite the fact that they are orders of magnitude stiffer than blood vessels and most tissues in the body. Turning instead to a physiologically relevant range of elasticity of 5 to 50 kPa in Young's modulus, we tested how neutrophil adhesion, spreading and migration were affected by substrate stiffness. We find that a dramatic and immediate difference is seen in the neutrophil's ability to spread on softer substrates. During migration we find that both speed and directionality are influenced by the substrate stiffness, with more efficient migration occurring on stiffer gels. We also find that these adherent neutrophils pull significantly harder on stiffer gels. These findings demonstrate that neutrophils respond and are sensitive to mechanical cues in the microenvironment, and suggest a possible novel mechanism for neutrophil guidance during injury and inflammation.

2692-Pos Board B662

Elastic Matrices that mimic normal heart are best for beating Cardiomyocytes - Beating stops on mechanical mimics of Scars

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Fibrotic rigidification following a myocardial infarct is known to impair cardiac output, and it is also known that cardiomyocytes on rigid culture substrates show a progressive loss of rhythmic beating. Here, isolated embryonic cardiomyocytes cultured on a series of flexible substrates show that matrices which mimic the elasticity of the developing myocardial microenvironment are optimal for transmitting contractile work to the matrix and for promoting actomyosin striation and 1 Hz beating. On hard matrices that mechanically mimic a post-infarct fibrotic scar, cells over-strain themselves, lack striated myofibrils and stop beating; on very soft matrices, cells preserve contractile beating for days in culture but do very little work. Optimal matrix leads to a strain match between cell and matrix and suggests dynamic differences in intracellular protein structures. A ''Cysteine Shotgun'' method of labeling the *in situ* proteome reveals differences in assembly or conformation of several abundant cytoskeletal proteins, including vimentin, filamin, and myosin. Combined with recent results that show stem cell differentiation is also highly sensitive to matrix elasticity, the results here highlight the need for greater attention to fibrosis and mechanical microenvironments in cell therapy.