the adhesion and migration of prostate cells in various stages of metastasis. The effect of external forces on a synthetic gene network in E.coli is being studied to determine if they impact intrinsic or extrinsic stochasticity. The stochasticity is monitored through the expression of three different fluorescent proteins CFP, YFP, and RFP. The emission intensities as a function of applied force are monitored to discern the effect of applied force on gene stochasticity. The influence of mechanical stress on cancer metastasis is being investigated by determining the expression levels of membrane and cytoplasmic proteins as a function of applied force. Additionally the cell-cell adhesion, cell-matrix adhesion, cell stiffness and elasticity, and expression levels of membrane proteins are determined by AFM. The AFM cantilever is employed to exert a local force and measure the response of the force in terms of the expression of adhesion proteins, and cell-cell and cell-matrix adhesion. Significant results of these studies will be presented.

2682-Pos Board B652

Strain Stiffening And Soft Glassy Rheology In A Generalized Sliding Filament Model

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Despite their enormous complexity and structural diversity, most biological materials show a remarkably similar viscoelastic phenomenology: nonlinear elasticity, power-law or logarithmic stress relaxation, and plastic length adaptation. Here we present a simple model based on Huxley's sliding filament model to demonstrate that such behavior can arise from generic structural properties, independent of the actual molecular constituents of the system. We compare the model predictions to data from active and passive microrheological experiments on epithelial cells and fibroblasts, smooth muscle tissue, and extracellular matrix protein networks.

The material is represented by an uniaxial arrangement of infinitely stiff filaments crosslinked with parallel elastic elements that have a distribution of attachment angles. When the system is sheared or stretched, elements start to align, leading to strain stiffening due to a geometric recruitment of springs. The elastic elements have force-dependent average lifetimes described by energy traps with a broad distribution of energy trap depths. Broken links can reattach at random positions and attachment angles after unbinding. Such nanoscale structural rearrangements lead to viscous flow and plastic length adaptation on a macroscopic scale. Due to a broad distribution of energy trap depths, the system displays power law stress relaxation and soft glassy rheology.

The model is capable of qualitatively reproducing experiments, and gives quantitative agreement for creep compliance, stress stiffening and plasticity in the case of cell microrheology. These results suggest that recruitment and dynamic unbinding of elastic elements are the common mechanism underlying the mechanical behavior of many complex biological materials from single cells to whole tissues.

2683-Pos Board B653

Mechanical perturbation of T cell actin retrograde flow

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The interplay between the plasma membane morphology and the actin network at cell-cell interfaces is believed to play an important role in various signaling pathways. Here, we manipulate the curvature of the membrane and the conforming actin at hybrid cell-supported membrane junctions. We demonstrate that the micron scale protein patterns in the T cell immunological synapse are altered merely by the curvature imposed by the supporting substrate. The radial symmetry of actin and other signaling proteins breaks, and the shape of the cell junction elongates up to three fold across one-dimensional (1-D) grooves. Cell aspect ratio is dependent on groove frequency and curvature. Our observations show that geometrical perturbations at membrane junctions can remodel actin retrograde flow.

2684-Pos Board B654

Non-linear Rheology Of Collagen Type I Gels Probed On The Length Scale Of A Migrating Cell

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Recent investigations showed that the dimensionality of the environment in which living cells are cultured - flat 2D culture wells versus 3D biopolymer networks - has a strong effect on cell morphology, metabolism and migration. The reason for these differences are unclear. What is lacking is a fundamental understanding of the mechanical and morphological properties of 3D matrices at varying length scales.

We probe the local microrheology of a series of reconstituted collagen gels with different concentrations (1.2 - 2.4 mg/ml) by applying a calibrated force on embedded magnetic particles (Ø4.5μm) using magnetic tweezers. The resulting strain field within the matrix is visualized by tracking the positions of polystyrene spheres (Ø1μm) embedded in the collagen gels. This strain field is compared to expectations from continuum theory. In addition, the local microrheology is compared to bulk rheological properties measured in a cone-plate rheometer. At low forces and strains below 3%, local and bulk rheological properties agree closely, and the strain field follows that of a continuum linear elastic material. At higher strains, marked non-linear strain stiffening occurs, showing an increase in modulus of nearly 20-fold until the material eventually yields. Because of the non-uniform shear conditions around magnetic beads in the local microrheology experiments, the non-linear stiffening appeared to be less pronounced, but the strain field spread much farther out than expected from continuum theory. These data suggest that the strain stiffening behavior of collagen gels, together with the well-documented ability of cells to sense the stiffness of their surroundings, could account for the differences in cell behavior seen in 2D versus 3D culture conditions.

2685-Pos Board B655

The Role of Quaternary Structure in the Signaling Mechanisms of PAS Sensor Domains

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The modular nature of proteins containing PAS (Per-ARNT-Sim) or other sensor domains enables signaling networks to be diverse and poses an interesting question: how can sensor domains with largely conserved tertiary structures regulate effector domains with very diverse structures and functions? We address this question by examining signal processing by the PAS sensor domain, which can regulate the activity of covalently linked effector domains such as a kinase, phosphodiesterase or DNA binding domains. In many cases oligomerization of sensor proteins is essential for signal transduction. We present the structure of a heme-PAS domain dimer from *Bradyrhizobium japonicum* (*bj*FixLH) in a new space group (P1) and at higher resolutions (1.5-1.8 Å) than those previously obtained. Interestingly, *bj*FixLH can form two different dimers in the same crystallization solution, where the monomers in one dimer are rotated ~175° relative to the second. This suggests that PAS monomers are plastic and that two quite distinct quaternary structures are closely similar in free energy. Comparison of PAS domain dimers using screw rotation analysis reveals that PAS monomers adopt a discrete range of monomer orientations. Similar to the light-sensitive PAS domain YtvA-LOV from *Bacillus subtilis*, *bj*FixLH undergoes signal-induced quaternary structural changes where monomers rotate ~2° relative to each other. Signal-induced quaternary structural changes accommodate the ability of PAS sensor domains to regulate a wide variety of effector domains since PAS and effector domains would not be required to interact with each other in a structure-specific manner. Our results will guide the rational design of novel PAS signaling proteins.

2686-Pos Board B656

A 3D Cell Traction Force Measurement Technique Based on Collagen Fiber Tracking

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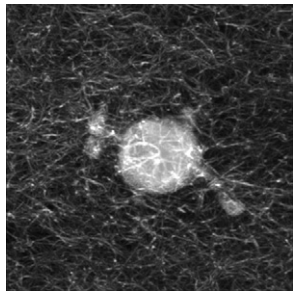
Mechanical interactions between cells and the extracellular matrix play an important role in determining essential cell behaviors such as cell migration, proliferation, wound healing and metastasis. While creative techniques have been recently devised and successfully implemented to measure the forces a cell can generate on a two-dimensional substrate, three-dimensional measurements have yet to be validated. Because many cells, in their physiological environment, live in a 3D matrix rather than on a 2D surface, a true understanding of cell-matrix interactions requires robust 3D force measurements.



We describe a new experimental technique and image analysis tools to measure forces generated by cells in a 3D reconstituted collagen matrix. This technique is based on confocal imaging of fluorescently-labeled collagen fiber networks around

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.