Ca^{2+}. Because major components of the spasmoneme, the contractile organelle inside the stalk, are EF-hand Ca^{2+}-binding proteins including spasmin and centrin, the spasmonemal contraction is thought to be related to other centrin-based motility mechanisms. This study describes how stall force affects contractions of live *Vorticella*. To impede contractions, we applied hydrodynamic drag force to *Vorticella* in a microfluidic channel with Poiseuille flow of viscous PVP solution. This method enables controlling the stall force by changing flow rate and the viscosity of the solution. Cell dimension measurements show that the zooid is elongated by the flow in relaxed and contracted states keeping roughly constant volume. As the stall force increases, the end-to-end length of the contracted stalk increases while that of the relaxed stalk is almost constant, and maximum contraction speed decreases while contractions take longer time. Furthermore, the time lag in contraction commencement between the zooid and the stalk also increases. We measured time differences in movement start among polystyrene beads attached to the stalk, and they increase with increasing stall force. These increasing time lags imply that the stalk cannot contract until it develops force great enough to overcome the stall force. The stall force affects the relaxation of *Vorticella* because relaxations take longer time as the stall force increase and the extending stalk resumes its contraction after the stall force is removed. It seems that although the spasmoneme retains contractile force, the stall force extends the stalk.

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Tuning Cellular Mechano-Response Using Biomembrane-Mimicking Substrates of Adjustable Fluidity

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An important aspect of mechanobiology is that tissue cells are anchorage-dependent and respond to viscoelastic changes in their environments. The mechanosensitivity of cells is believed to play an important role in processes such as cancer cell migration and stem cell differentiation. Previously, cellular mechano-response has been mainly studied using μm-thick polymeric films of adjustable viscoelasticity. Here we report on the design and characterization of alternative cell substrates based on 8-40nm thick polymer-tethered phospholipid membranes where cellular mechano-response can be regulated by tuning bilayer fluidity. Two complementary membrane systems are employed to span a wide range from low to high bilayer fluidity. Low to medium bilayer fluidity is achieved by using a single polymer-tethered lipid bilayer of adjustable tethering concentration. Medium to high bilayer fluidity is obtained through the regulation of the number of bilayers in a stack of polymer-tethered lipid bilayers. Changes in bilayer fluidity in these substrates have been confirmed through wide-field single molecule tracking of fluorescently labeled lipids. To facilitate the adsorption and migration of cells, these biomembrane-mimicking substrates contain bilayer-cell linkages of well-defined concentrations. Phase contrast microscopy experiments on PC12 neurons show that dendritic growth can be tuned by modifying the tethering concentration in a single polymer-tethered lipid bilayer. Comparing phase contrast and epifluorescence microscopy experiments on 3T3 fibroblasts containing GFP-actin, which were plated on multi-bilayer stacks, revealed profound changes in cellular phenotype, projected cell area, cell migration, and cytoskeletal organization with the number of bilayers in the stack. For example, on very fluid substrates, neuron-like, dendritic fibroblasts were observed. The described substrates are particularly significant because, unlike in the case of polymeric films, substrate-cell linkages are free to move and matrix remodulation caused by adsorbed cells is largely suppressed.

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Cell Contact, Substrate Mechanics And Boundary Conditions In The Movement Of Epithelial Sheets

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The directed and highly coordinated movement of epithelial cells can be found in various vertebrate systems, from the separation of tissues in early development through the renewal of tissues in the adult. How the cells coordinate their movement in a sheet remains unexplained, especially in physical terms, as the movement involves a complex balance of forces generated at multiple lengthscales. The forces that stem from the cellular level, and their mediation by the physical environment that allow them to manifest a highly correlated, multicellular pattern of movement is dependent upon the relationship between local influences such as cell-cell contact and the ability for cells to deform the substrate, with longer-ranged influences, such as physical and geometric constraints placed on the population of the sheet. Therefore, in this study, we quantitatively explore the dependence between cadherin-mediated contact, substrate mechanics, and boundary conditions on the motility of two-dimensional epithelial sheets.

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TβRIII Restores Normal Cytoskeleton Mechanics In Ovarian Cancer Cells

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Changes in cellular phenotypes in cancer are characterized by alterations of the cytoskeleton and several important signaling pathways in the cell. One of the signaling pathways implicated in controlling proliferation, angiogenesis and apoptosis is the TGF-β signaling pathway. Loss of expression of the TGF- β superfamily co-receptor, TβRIII/betaglycan, occurs in a broad spectrum of cancers, including those of the breast, ovary and prostate. Recent studies have shown that restoration of TβRIII to metastatic populations of ovarian and breast cancer cells suppresses migration via alteration of the cytoskeleton. Using our 3 dimensional force microscope system (3DFM) for passive and active microrheology, we compared the response of normal ovarian surface epithelial cells, ovarian cancer cells with reduced TβRIII expression, and ovarian cancer cells with restored expression of TβRIII. We found that cancer cells lacking TβRIII were at least 3× more compliant (less stiff) than either normal epithelial or TβRIII-expressing cancer cells. Our results, are consistent with potential invasiveness being correlated with increased cell compliance, and both being regulated by the TβRIII pathway. Compliance measurements using the 3DFM could be a useful tool to measure invasiveness in the future.

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Three Dimensional Superresolution Fluorescence Microscopy Reveals Protein Stratification in Focal Adhesions

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Focal Adhesions (FA) are dynamic structures consisting of large numbers (>150) of different proteins that mechanically link the actin cytoskeleton to the extracellular matrix (ECM). Despite the central role of FA in cell migration and the wealth of biochemical and cell biological data on FA proteins, it remains virtually unknown how these proteins are organized within FA. Based on the differential dynamics of distinct FA proteins we previously observed using fluorescent speckle microscopy, we hypothesized that FA proteins may be organized into stratified layers within FA that serve as dissipative elements in a ''molecular clutch'' to form a regulatable, force-transducing link between the actin cytoskeleton and the ECM. To test this hypothesis, we employed a 3-dimensional superresolution fluorescence microscopy technique, interferometric photoactivated localization microscopy (iPALM), to determine sub-20 nm z-axis localizations of several key structural components of FA labeled with photoactivatable fluorescent proteins and expressed in U2OS cells plated on a fibronectin-coated substrate. Within FA, we found that the cytoplasmic face of the plasma membrane, marked by Farnesylated tdEosFP, was localized at ~20-30 nm from the substrate, in agreement with previous electron miocroscopic analyses. Talin and vinculin, putative force transducing elements of FA, were observed within distinct planes parallel to the substrate, with the highest densities at ~35, and ~50 nm above the substrate, respectively. Actin appeared with the highest density at ~90 nm above the substrate plane, and was largely excluded from area adjacent to plasma membrane up to a height of ~50 nm. iPALM reveals for the first time the organization of specific proteins within the nanoscale core of the FA. The protein stratifications in FA provide a structural context for the mechanosensing and mechanotransducing functions of FA.

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Role of Mechanotransduction in Cellular Processes

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The role of mechanical properties of cells is gaining increasing attention due to the regulatory role that it plays in cellular processes. In particular the transduction of an applied force on the cell membrane through the cellular components can have significant influence on such phenomena as stochasticity in gene expression and cancer metastasis. We are currently investigating the effect of mechanical forces on the stochasticity of gene expression in E.coli cells and

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.

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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.

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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.

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

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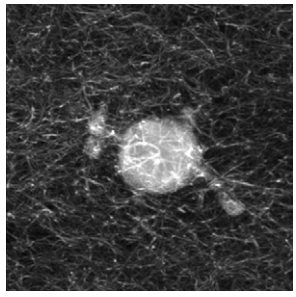
Mechanical interactions between cells and the extracellular matrix play an important role in determining essential cell behaviors such as cell migration, proliferation, wound healing and metastasis. While creative techniques have been recently devised and successfully implemented to measure the forces 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