As stretch is applied to a model of the actin filament network, the distribution of bond angles in the network transitions from a more peaked to a flatter distribution. High variability is observed from site-to-site within the network upon an applied stretch, with a nearly uniform distribution of difference between stretched and unstretched angles (''delta angles'') at high levels of stretching. We used our approach to explore various thresholding models of how actin filament network deformations might influence rates of release of bound signaling molecules. These models allow us to project how a biochemical response might appear from a given applied mechanical stimulus. We validate these simulations using experimental data and use our model to then test different predictive capabilities of how mechanotransduction may function. Our model for the mechanotransductive release of signaling factors represents a potentially versatile mechanistic platform for examining biophysical interactions that link mechanical stimulus at the cellular level to response at the protein level.

1622-Pos Board B514
The Role of Entropy and Enthalpy of Actin Filaments in Cellular Actin-Based Processes: Two Examples
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Actin filaments play a fundamental role in cell migration and cell mechanics. Assembled by different Actin-Binding Proteins into different structures -cortex, stress fibers, filipodia, lamellipodium- actin filament is the basic unit available for the cell to apply forces or carry tensions. Actin filament is a semi-flexible polymer at the scale of the cell, which means that it is able to bend and to be bent by thermal fluctuations but it is mostly straight. This leads to a large contribution of the entropy to the dynamics of actin filaments and the networks they form. Here will be discussed our recent results regarding the link between these individual actin filament properties, semi-flexible fluctuating filaments, and their role in two examples: the actin-based force generation and the mechanics of the lamellipodium. We have recently shown that the polymerization of actin filaments that experience orientational fluctuations generates forces in a mechanism associated to the reduction of their entropy (1). In another experiments we have investigated the link between the elastic response of dense Arp2/3 branched actin networks and their microscopic architecture -in terms of branching and capping concentrations. Our results indicate that the elasticity of such dense branched networks originates more in the ''enthalpic'' deformations of the filaments themselves than in the ''entropic'' response usually probed in more diluted actin gels (2).
(1) Brangbour et al (2011) Force-Velocity Measurements of a Few Growing Actin Filaments. PLoS Biology 9 e1000613.
(2) Pujol et (2012) Impact of branching on the elasticity of actin networks. PNAS 109, p10364-9.

1623-Pos Board B515
An Elastic Actomyosin Network in Motile Fish Keratocytes
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Crawling motility of eukaryotic cells is driven by net assembly of an actin network at the leading edge and net disassembly at the cell rear, orchestrated by a combination of biochemical and mechanical processes. To understand the distribution and function of mechanical forces in motile cells, it is necessary to determine the mechanical properties and connectivities of the structural components of the cell.
We have investigated the mechanical properties of the actin network using detergent-extracted cytoskeletons from fish epithelial keratocytes. When stretched by a microneedle, the extracted lamellipodial network stretched significantly, up to several-fold its original dimensions. At moderate strains, the network was found to be highly elastic, at least over the timescales of 0.2 s to 30 s. These observations suggest that there is strong mechanical coupling within the cytoskeleton over distance scales comparable to the whole cell.
Previously we have shown that activation of myosin II in detergent-extracted cytoskeletons by addition of ATP results in contraction and disassembly of the rear network. We have found that ATP analogs (AMP-PNP and ADP-fluoroberyllate) also cause partial disintegration and inward sliding of the rear network, toward the cell body, but without observable contraction within the network. The accompanied deformation of the cell body suggests that the cell body is stretched laterally in the live cell, anchored by the flanking actomyosin networks, and that release of actomyosin bonds (triggered by binding of ATP analogs to myosin) is sufficient for the network to rupture, allowing the cell body to relax. This raises the possibility that myosin may be playing a structural role, maintaining the integrity of the rear network under tension, while simultaneously driving its contraction. The cell body may serve as a reservoir for elastic energy, stress being distributed across the rear actomyosin network.

1624-Pos Board B516
Computational and Experimental Study of the Mechanics of Embryonic Wound Healing
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The healing of embryonic wounds yields insight into mechanical forces which shape a developing organism. Here we characterize healing of wounds in epithelia of early stage chick embryos, and use a newly developed computational model to investigate underlying cellular-level mechanisms.
Early stage chick embryos, consisting of planar epithelial sheets two to three cell layers thick, were harvested and cultured ex ovo. Multicellular wounds were made and allowed to heal. We found that the closure rate of embryonic wounds displayed a two-phase behavior, with rapid constriction lasting about a minute, followed by more gradual contraction until the wound closed. Fluorescent staining revealed that soon after wounding a broad, faint ring of contractile actin and myosin-II encircles the wound. By one minute post wounding this structure gives way to a narrow actomyosin band at the wound border, consistent with a ''purse string'' healing mechanism observed in other embryonic systems. We hypothesize that contraction of the broad ring is responsible for the initial, rapid phase of wound closure, and that the narrow purse string drives the later slower phase.
To test these mechanisms, we implemented both in a finite element computational model. We found that the rapid initial phase of wound closure is consistent only with an isotropic contraction of the broad ring surrounding the wound, and that the slower phase can be accounted for by the formation and circumferential contraction of fibers at the wound margin. Together, these two mechanisms can quantitatively reproduce the observed wound healing dynamics.
The results of this integrated experimental and computational investigation suggest that a new mechanism, the isotropic contraction of cells in a broad ring around the wound, works together with an actomyosin contractile ring to close an embryonic wound.

1625-Pos Board B517
Single Piconewton Forces at Individual Integrins Support Robust Cell Adhesion
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Mechanical interactions between cells and the extracellular matrix (ECM) exert a profound influence on cell migration, proliferation, and stem cell differentiation. However, fundamental aspects of how cells detect and generate mechanical forces at the cell-ECM interface remain poorly understood. Here we describe a new technique, termed Molecular Force Microscopy (MFM) that visualizes the forces experienced by single cellular adhesion molecules with nanometer, piconewton, and sub-second resolutions. MFM uses a new class of FRET-based molecular tension sensors that bind to an avidin-coated glass coverslip at one end and present an integrin binding site at the other. Cellular integrins transmit force to the FRET pair, resulting in decreased FRET with increasing load. Unlike previously reported force sensors, MFM sensor molecules allow quantitative FRET imaging at the single molecule level. We found that human foreskin fibroblasts (HFFs) adhered to and spread on surfaces functionalized with the MFM probes, and developed mature focal adhesions as evidenced by paxillin localization and actin stress fiber formation. We observed a bimodal distribution of FRET efficiency values for MFM sensor molecules beneath HFFs, with one peak corresponding to zero load and the other indicating a distribution of forces between 1 and 4 pN. Despite evidence of robust adhesion, the forces we measured were ~10-fold lower than the force necessary to break individual integrin-ECM bonds. Our data provide the first direct measurement of the tension per integrin molecule necessary to form stable contacts with the ECM. The relatively narrow range of forces that we observed suggests that mechanical tension at individual adhesion molecules is subject to exquisite feedback and control. Ongoing work uses the unique capabilities of MFM to elucidate the mechanical signal transduction mechanisms that underlie cell migration and adhesion.

1626-Pos Board B518
The Role of Mechanical and Biochemical Interactions in Focal Adhesion and Cellular Shapes
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We present findings from a two-dimensional mathematical model of a biological cell interacting with a soft substrate and focus on the role of the interaction

of intracellular mechanical stresses and plaque protein concentrations on cell and focal adhesion shapes. The cell is treated as a hypoelastic actively-deforming continuum and the substrate is modeled as a linearly elastic continuum. The active deformation, captured by the addition of an active rate of deformation tensor, models local cytoskeletal reorganization. Focal adhesions connecting the cell and the substrate are modeled as a collection of discrete elastic springs. The model allows for the focal adhesion complexes to grow and shrink depending on mechanical forces that are acting on them and local concentrations of plaque protein. A model of stress-induced plaque protein dynamics, which is based on earlier work of Besser and Safran (2006), is coupled to the model of cell-substrate mechanics. Finite element simulations of the model allow us to explore the effects of original focal adhesion configuration, cytoskeletal dynamics, and focal adhesion strength on the shape evolution of individual focal adhesions and on overall cell shape.

1627-Pos Board B519

Dynamics of Focal Adhesions

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Focal adhesions(FA) are the cells machinery to sense the environment and help in migration. They are macromolecular complexes which link the actin to the integrins and are extremely dynamic in nature. The mechanism of FA movement in the cell is unclear. Treadmilling and sliding are the two modes in discussion. Recent experimental results for force velocity relationship in focal adhesions show a biphasic relationship between F-actin speed and traction force.
In our study, an FA complex is modelled as a one dimensional array of point masses connected laterally to each other and to the substrate by springs. Binding and unbinding of molecules to the substrate corresponds to sliding and adding/deleting from the ends corresponds to treadmilling in the system. Through gillespie simulations, we sought to understand the mechanism of FA movement by comparing the force velocity curves generated to the experimental data. From our preliminary results, for a one layer model in a small range of forces, we see that there is a range of possible velocities for a given force depending on the ratio of rate constants for treadmilling and sliding mechanisms. Another observation is that on increasing the stiffness of the substrate, the slope of these curves decreases significantly only in the sliding dominated mechanisms.

1628-Pos Board B520

Focal Adhesion Size Uniquely Predicts Cell Migration

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Focal adhesions are large protein complexes organized at the basal surface of cells, which physically connect the extracellular matrix to the cytoskeleton and have long been speculated to mediate cell migration. However, whether clustering of these molecular components into focal adhesions per se is actually required for these proteins to regulate cell motility is unclear. Here, we use quantitative microscopy to characterize large families of focal adhesion and cell motility descriptors across a wide range of matrix compliance, following genetic manipulations of focal adhesion proteins. This analysis revealed a tight, biphasic relationship between mean size of focal adhesions - not their number, surface density, or shape - and cell speed. The predictive power of this relationship was comprehensively validated by disrupting non-focal adhesion proteins and subcellular organelles (mitochondria, etc.) not known to affect either focal adhesions or cell migration. This study suggests that mean size of focal adhesions robustly and precisely predicts cell speed independently of focal adhesion surface density and molecular composition.

1629-Pos Board B521

Mechanosensing of Cells in Laminin-Fuctionalized Biomembrane-Mimicking Substrates

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Adherent cells actively probe and respond to the mechanical properties of their environment. Studies of cellular mechanosensing are mostly conducted on polymeric substrates of adjustable elasticity with immobilized cell-substrate linkers that neglect the dynamic and viscoelastic nature of tissues. Here, we study cell migration on a polymer-tethered multi-lipid bilayer substrate with mobile linkers and dissipative material properties. The substrates are functionalized with laminin, which replicates the linkages of cells to the extracellular matrix. The polymer-tethered lipids connecting the bilayer stacks do not hinder the lateral mobility of individual cell linker molecules in the fluid lipid bilayer matrix, but obstruct or even prevent the free lateral diffusion of clusters of cell linkers. These biomembrane-mimicking substrates therefore exhibit a viscous response with respect to individual linkers, and an elasto-plastic response in the presence of linker clusters, allowing the cells to rearrange and recruit ligands. Moreover, the mechanical properties of the substrate can be tuned by stacking multiple bilayers. Experiments with mouse embryonic fibroblasts confirm that cells can sense linker mobility and dissipative mechanical properties of the substrate. Cells respond to decreasing linker mobility, decreasing substrate elasticity, and increasing substrate plasticity with a reduction in spreading area, cell stiffness, traction magnitude, and focal adhesion size, but with an increase in focal adhesion number density. In conclusion, the results illustrate that cells change their morphological and mechanical properties in response to changes in substrate elasto-plasticity and linker mobility.

1630-Pos Board B522

Probing Mechanosensitivity of Myoblasts on Cadherin-Functionalized Biomembrane-Mimicking Substrates

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There is a growing recognition that cells not only respond to biological signals, but also to those of mechanical nature. Cellular mechanosensing is exemplified by the cell's ability to respond to changes in substrate viscoelasticity. Traditionally, experiments about cellular substrate sensitivity were conducted on polymeric substrates with immobilized linkers. Here we present an alternative strategy based on biomembrane-mimicking cell substrates with E-cadherin cell linkers. This substrate design allows a systematic variation of viscoelastic properties by changing the number of lipid bilayers in a polymer-tethered multi-bilayer system. Importantly, individual cell linkers within this type of cell substrate are laterally mobile, thus leading to a viscous substrate behavior. In contrast, clusters of linkers, which are immobilized, cause an elasto-plastic substrate response. By using E-cadherin linkers, multi-bilayer substrates can be seen as a mimetic of a cell-cell linkage. The functionality of the multi-bilayer substrate for cellular mechnosensing studies is illustrated in terms of specific cell parameters, including cell morphology, migration speed, cytoskeletal organization, and size of cell adhesions. Furthermore, the influence of linker density on cell adhesion and spreading is discussed.

1631-Pos Board B523

Role of Integrin in Cellular Mechanotransduction

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Chemical and physical cues from the extracellular matrix (ECM) are used as input for an integrated mechanochemical sensory system that controls cell behavior. Forces derived from the ECM have been implicated in various biological systems, including mesenchymal stem cell differentiation and tumor growth. Integrin transmembrane receptors connect the ECM network to the intracellular actomyosin network and are expected to play an important role in force sensing. Our group has previously shown that switching between two different integrin heterodimers ([alpha]5[beta]1 vs [alpha]v[beta]3), that bind the same ECM protein (fibronectin), causes remarkably distinct actin cytoskeletal organization. To investigate the role of these integrins in cellular mechanotransduction, we used fibronectin coated polyacrylamide substrates with varying rigidities and micropillar arrays. Data obtained thus far point to distinct rigidity thresholds for spreading and different distribution of ECM pulling forces for cells expressing either of these integrins. In order to further unravel the role of these integrins in cellular mechanotransduction we developed cyclic substrate stretcher technology coupled to real time confocal imaging.

1632-Pos Board B524

Viscoelastic Cell Adhesion Model (VECAM)

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Circulating leukocytes and tumor cells deform during their interactions with vascular endothelium and other adhesive substrates under physiological flow conditions. We studied the effect of cytoplasmic viscosity on these interactions using our novel three-dimensional computational algorithm that treats the cell as a compound droplet in which the core phase (nucleus) and the shell phase (cytoplasm) are viscoelastic fluids. This algorithm, known as viscoelastic cell adhesion model (VECAM), includes the mechanical properties of the cell cortex by cortical tension and considers cell microvilli that deform viscoelastically and form viscous tethers at supercritical force. Stochastic binding kinetics describes binding of cell adhesion molecules. VECAM predicts that the cytoplasmic viscosity plays a critical role in cell rolling on an adhesive substrate. High-viscosity cells are characterized by high mean rolling velocities, increased temporal fluctuations in the instantaneous velocity, and a high probability for detachment from the substrate. A decrease in the cell velocity, drag