3784-Pos
Growth Process and Mechanics of Cell Adhesion Investigated by Optical Tweezers
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Cell adhesion between extracellular matrix and integrin is one of essential structure for cell growth, differentiation and cell motility. Paxillin and vinculin reinforce strength of cell adhesion and Src controls aggregation of these proteins at initial phase of cell adhesion. Thus the relationship between molecular and physical properties is important to understand the cell adhesion mechanisms. However, we still don't fully understand the property of cell adhesion. To investigate the characteristics, we have developed a measurement system for the stiffness of cell adhesion by using a collagen coated particle (2-micron diameter), which is manipulated by optical tweezers. In this system, cell adhesion is created under the particle by attaching to mouse fibroblast (Balb-3T3) for 1 min and it is called ''initial adhesion''. After that the particle is moved back-and-forth in sinusoidal manner and analyzed the position of the particle recorded by a CCD camera. From that data we can know the actual force exerted on the particle. The minimum force sensitivity of the system is 80 fN. By using this system, we are able to measure the force of cell adhesion during growth process. This force rapidly increases for 30min from initial adhesion and gradually increases after 30 min. And we investigate the force change for 30 seconds after creation of initial adhesion. As a result, we observe the growth of cell adhesion with reinforcement and relaxation of adhesion force. And the stabilization of the reinforcement is occurred with fluctuation of the adhesion force. These phenomena are basic properties of cell adhesion and are important to reveal of molecular mechanism of cell adhesion.

3785-Pos
H2-Calponin Regulates Adhesion and Migration of Macrophages
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Calponin is an actin filament-associated regulatory protein. The h2 isoform of calponin is found in both smooth muscle and non-muscle cells including monocytes and macrophages. We previously demonstrated that h2-calponin plays an inhibitory role in the regulation of cell proliferation and migration and increases the stability of actin cytoskeleton (Hossain et al., *AJP*: 284:C156-67, 2003; *JBC* 280:42442-53, 2005; *Biochemistry* 45:15670-83, 2006). Using residential cells isolated from the intraperitoneal cavity of h2-calponin knockout mice (Huang et al., *JBC* 283:25887-99, 2008), the present study investigated the role of h2-calponin in macrophage motility. Substrate adhesion of h2-calponin-null macrophages was significantly decreased together with reduced cell spreading area in culture dish. The h2-calponin deficient macrophages further exhibited increased migration and transendothelial migration. The deficiency of h2-calponin did not affect macrophage invasion into Matrigel, suggesting that the increased transendothelial migration is based on unchanged activity of extracellular proteases. H2-calponin was co-localized with F-actin in thin spikes at all edges of stationary macrophages and the trailing edges of migrating cells while absent in the leading edge lamellipodium. Consistent with increased motility, h2-calponin-null macrophages exhibited losses of these anchoring spikes. The results suggest a role of h2-calponin in inhibiting macrophage motility and transendothelial migration through stabilization of actin cytoskeleton.

3786-Pos
Nucleation and Growth of Integrin Adhesions
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We present a model that provides a mechanistic understanding of the processes that govern the formation of the earliest integrin adhesions ex novo from an approximately planar plasma membrane. Using an analytic analysis of the free energy of a dynamically deformable membrane containing freely diffusing receptors molecules and long repeller molecules that inhibit integrins from binding with ligands on the extracellular matrix, we predict that a coalescence of polymerizing actin filaments can deform the membrane toward the extracellular matrix and facilitate integrin binding. Monte Carlo simulations of this system show that thermally induced membrane fluctuations can either zip-up and increase the radius of a nucleated adhesion or unzip and shrink an adhesion, but the fluctuations cannot bend the ventral membrane to nucleate an adhesion. To distinguish this integrin adhesion from more mature adhesions, we refer to this early adhesion as a nouveau adhesion.

3787-Pos
Adipogenic Commitment of Mesenchymal Stem Cells Regulated by ERM Proteins-Mediated Cellular Biomechanics
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Cellular mechanics plays an important role in many cell activities including, to name just a few, morphogenesis, migration, proliferation, and differentiation. Adipogenic differentiation of human mesenchymal stem cells (hMSC) is found to cause a decrease both in the cytoskeleton elasticity and membrane-cytoskeleton association and is mediated by the ERM (ezrin, radixin, moesin) family of protein linkers. Transient knockdown of ERM proteins with RNAi technique results in membrane separation from the cytoskeleton in hMSC as determined using optically extracted membrane tethers. In addition, it leads to a substantial decrease in the cell elasticity measured using AFM microindentation. This cytoskeleton biomechanics modulation is likely mediated by a partial disassembly of actin stress fibers and focal adhesions during ERM linkers knockdown. Although this kind of treatment induces changes in the stem cell mechanical properties similar to those of fully differentiated adipocytes, hMSC commitment by soluble adipogenic factors is impaired in the ERM-deficient cells. However, cell mechanics modulation by ERM knockdown following a 6-day adipogenic induction by soluble factors seems to facilitate adipogenesis. This observation is confirmed by up-regulation of lipid vacuoles formation and adipocyte-specific markers expression. Intact cytoskeleton and/or focal adhesion-mediated signaling appear to be the prerequisites for early adipogenic commitment of hMSC. However, following the initial biochemically-induced commitment, the cellular mechanics plays an increasingly important role in enhancing the stem cell differentiation efficiency. Our findings have significant implications for tissue engineering, reconstructive and cosmetic surgery, and other stem cell-based therapeutic applications.

3788-Pos
Mechanics of Molecular Bond Clusters between Elastic Media: Stochastic-Elastic Coupling in Cell-Matrix Adhesion
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Focal adhesions are clusters of specific receptor-ligand bonds that link an animal cell to an extracellular matrix. A capability to control focal adhesions, for which a quantitative description of the collective behavior of multiple molecular bonds is a critical step, is essential for tissue and cellular engineering. While the behavior of single molecular bonds is governed by statistical mechanics at small scale, continuum mechanics should be valid at large scale. How can this transition be modeled and can this tell us something about the mechanics of cell adhesion? Here we develop a stochastic-elasticity model of a periodic array of adhesion clusters between two dissimilar elastic media subjected to an inclined tensile stress, in which stochastic descriptions of molecular bonds and elastic descriptions of interfacial traction are unified in a single modeling framework. A fundamental scaling law of interfacial traction distribution is established to govern the transition between uniform and cracklike singular distributions of the interfacial traction within molecular bonds. Guided by this scaling law, we perform Monte Carlo simulations to investigate the effects of cluster size, cell/matrix modulus and loading direction on lifetime and strength of the adhesion clusters. The results show that intermediate adhesion size, stiff substrate, cytoskeleton stiffening, and low-angle pulling are factors that contribute to the stability of focal adhesions. The predictions of our model provide feasible explanations for a wide range of experimental observations and suggest possible mechanisms by which cells can modulate adhesion and deadhesion via cytoskeletal contractile machinery.

3789-Pos
Mapping Adhesion Turnover in Migrating Cells: An Image Cross-Correlation Study
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Cell migration requires the assembly and disassembly of adhesions, which provides a physical linkage between the actin cytoskeleton and the extracellular matrix (ECM). Adhesions are composed of many interacting molecules that also organize signals that regulate migration. We have previously used spatio-temporal image correlation spectroscopy (STICS) to observe the dynamics and nature of the linkage between actin and α_5-integrin as adhesions slide and disassemble. We now extend this study to probe the linkage using cross-correlation methods. STICCS analyzes the intensity fluctuations and calculates autocorrelation and cross-correlation functions in space and time to yield velocity vectors and diffusion coefficients for regions analyzed. This information is assembled into vector maps characterizing the movement of these proteins in different areas of the cell and at different times during the series acquisition. We applied a Fourier filter to remove contributions from static components to better resolve the dynamic protein populations and also carried out computer simulations to model moving boundaries and simulate edge effects to better

understand how they perturb STICCS. We imaged migrating cells expressing fluorescent paxillin and integrin using two-color, total internal reflection fluorescence (TIRF) microscopy. By applying two-color spatio-temporal image cross-correlation spectroscopy (STICCS), we detected a robust interaction in retracting regions where adhesions are sliding and eventually disassembling; this shows that they move as a complex. We also detected transient, dynamic interactions of $\alpha_6\beta_1$- or $\alpha_L\beta_2$-integrins with paxillin in CHO cells plated on a laminin-5 or a CD54 matrix respectively only in adhesions that were visibly dynamic. That is, the integrin and paxillin moved, in contrast to the behavior of the a_5-integrin, which was fixed and uncoupled from the movement of the paxillin. We did not detect any co-fluxing in static adhesions.

3790-Pos

Nanoscale Protein Architecture of Focal Adhesions

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Focal adhesions (FAs) mediate cell interactions with their extracellular matrices (ECMs) and consist of integrin ECM receptors linked to the actin cytoskeleton via plasma-membrane-associated protein plaques. Despite their fundamental importance in multicellular organisms, the three-dimensional organization of proteins within FAs is unknown. Here we determine FA molecular architecture by using 3D superresolution microscopy (interferometric Photo-Activated Localization Microscopy) to map nanoscale protein organization. We find that the FAs consist of partially overlapping proteinspecific vertical layers of 15-50 nm thickness, with integrins and actin separated by a 30-50 nm FA core which is spanned by talin tethers. This reveals a structural basis for FA function whereby a multilaminar core architecture mediates the interdependent cell processes of adhesion, signaling, force transduction, and actin cytoskeletal regulation.

3791-Pos

Mechanical Coupling through Endothelial Cell Adhesions Determined Using a Novel Live-Cell Strain Device

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Mechanotransduction is mediated by cell-matrix adhesion sites in response to extracellular mechanical forces. Cytoskeletal dynamics in single cells have been measured during migration on substrates of varying elastic modulus, but live-cell measurement of structural dynamics during substrate stretch in confluent cell monolayers has been difficult to achieve. We developed a novel stretch device optimized for high-resolution live-cell imaging. The unit assembles onto standard inverted microscopes and applies static or cyclic stretch at physiological magnitudes to cultured cells on elastic membranes. Interchangeable modular indenters enable rapid switching between equibiaxial and uniaxial stretch profiles. In endothelial cell monolayers expressing EGFP-vimentin and paxillin-DsRed2 and subjected to constant equibiaxial or uniaxial stretch, the 2-D strain tensor demonstrated efficient transmission through the extracellular matrix and focal adhesions. Strain transmission to the intermediate filament network was decreased in magnitude, as demonstrated by spatial correlation of vimentin and paxillin displacement vectors, and cells did not align perpendicular to constant uniaxial stretch. During cyclical uniaxial stretch at 1 Hz, strain focusing was increased relative to constant stretch and peaked at 10-15 min after stretch onset. Strain focusing recovered more slowly over a time scale of ~1 hr, and cells aligned perpendicular to the stretch direction in 6 hr. These observations using the live-cell stretch device demonstrate that sustained strain focusing and mechanical coupling through adhesion sites may be required for endothelial cell morphological adaptation to cyclical uniaxial stretch.

3792-Pos

Mimicking the Cellular Environment: Effects of Elastic Nanopatterned Substrates on Integrin-Mediated Cellular Interactions

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An artificial substrate system, according to the biophysical and biochemical properties of the extracellular matrix in connective tissues, has been developed. The Young's moduli E_Y of poly(ethylene glycol)-diacrylate (PEG-DA) based hydrogel substrates span more than four orders of magnitude between 0,6kPa and 6MPa. Since PEG-DA substrates are protein repellent, they were decorated by extended gold nanoparticle arrays, manufactured by block copolymer micellar nanolithography. To provide bioactivity in terms of cell adhesion c(RGDfK) peptide, which is specific for $\alpha_V\beta_3$ integrins, was immobilized on the nanoparticles. The interparticle spacing and, hence, spacing of integrin binding sites ΔL could be precisely tuned, independently of the substrate rigidity between 20nm and 160nm. This system was used to investigate the behavior of fibroblasts as a function of changes within two-dimensional parameters space $\Delta L;E_Y$). To this end, cell spreading area and cell-substrate interaction forces were determined by phase contrast microscopy and single cell force spectroscopy (SCFS), respectively. First, the effect of variation of ligand spacing on cellular behavior was investigated on hard substrates ($E_Y>100$kPa). We could demonstrate a strong increase in detachment force and spreading area on substrates featuring low ligand spacing. Than, substrate compliance was tuned whereas the ligand spacing was kept at approximately 50nm. This reveals a significant decrease in spreading area and detachment force on soft substrates ($E_Y<8$kPa).
Additionally, both environmental parameters were varied simultaneously. Results from these experiments were determined as a function of hydrogel stiffness and integrin ligand distance. They revealed two tactile set points, thresholds in cellular sensing behavior, at $E_Y=8$kPa and $\Delta L=70$nm, after 6, 12, and 24 hours of adhesion. Moreover, according to the hierarchical phase model in cellular behavior, elasticity was identified to be the dominant parameter in cellular sensing processes.

3793-Pos

Development of Micropatterned Elastic Gelatinous gels to Control Cell Mechanotaxis

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Cell motility plays the essential roles in various physiological and pathological processes such as morphogenesis, wound healing, inflammation, and tumor metastasis etc. Appropriate control of such biological processes is a longstanding task in the development of high-functional biomaterials. Establishment of the surface engineering of biomaterials to manipulate the cell motility has been strongly required as well as the understandings for its mechanism. In relation to this issue, we are focusing on the understanding and control of directional cell movement towards a harder region of a cell culture substrate surface, so-called mechanotaxis, which might provide a solid basis for designing mechanobio-materials to manipulate cell motility. We have developed the photolithographic surface microelasticity patterning method for fabricating a cell-adhesive hydrogel with a microelasticity-gradient (MEG) surface using photocurable styrenated gelatin to investigate the condition of surface elasticity to induce mechanotaxis. Patterned MEG gels consisting of different absolute surface elasticities and elasticity jumps have been prepared. From analyses of trajectories of fibroblast movement on each prepared MEG gel, two critical criteria of the elasticity jump and the absolute elasticity to induce mechanotaxis were identified: 1) a high elasticity ratio and sharpness of the elasticity boundary between the hard region and the soft one, and 2) elasticity of the softer region to provide medium motility. Design of these conditions was found to be necessary for fabricating an artificial extracellular matrix to manipulate cell motility based on mechanotaxis behaviors.

3794-Pos

A Novel Multiscale Model of Cell Adhesion and Migration on Defined Extracellular Matrices

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While many of the molecules and signaling pathways that govern biophysical interactions between cells and the extracellular matrix (ECM) have been identified, the mechanisms through which these components cooperate to control whole-cell shape, adhesion, and motility remain incompletely understood. This is due in part to the absence of multiscale models that integrate local activation of biochemical signals with adhesion and force generation. In this study, we present a novel computational model of cell migration on ECMs of defined biophysical properties that incorporates adhesion growth & rupture dynamics, stress fiber contractility, and a protrusive machinery. Critically, our model makes use of a reduced one-dimensional geometry, which enables us to systematically probe multiple intracellular and extracellular parameters in a computationally tractable framework. Using this model, we examine effects of substrate stiffness, ligand density, and Rho family GTPase activation on cellular adhesion, contractility, and migration speed and dynamics. Our simulations yield results that are in qualitative agreement with previously observed experimental observations, including a biphasic relationship between migration speed and ligand density, enhanced cell adhesion and faster random migration on stiffer ECMs, and recently-reported transitions from filopodial to "stick-slip" to gliding motility on ECMs of increasing stiffness (Ulrich et al., Cancer Research 2009). Our model enables us to investigate experimentally-inaccessible microscale relationships between mechanotransductive signaling, adhesion, and motility and offers novel insight into how these factors interact with one another to produce complex migration patterns across a variety of cell types and ECM conditions.