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.