anionic PL, cardiolipin (CL), in membranes. A model describing the packing of NAO with CL provided a framework for explaining observations of the distribution of CL in bacterial and mitochondrial membranes, and the relationship between the localization of lipids and proteins. In this work, we extend the spectroscopic characterization the interaction of NAO with CL and other anionic PLs, present a revised mechanism that describes the binding, and highlight both the limitations and the expanded utility of NAO for studies in vivo and in vitro.

Platform: Cell Mechanics & Motility II

1914-Plat
Crosslinked Collagen Films Stiffen the Nucleus of Marrow Stromal Cells and Promote Osteogenesis
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Commitment of stem cells to different lineages is regulated by many cues in the microenvironment, and pluripotent marrow stromal cells (MSCs) prove particularly sensitive to matrix mechanical cues. MSCs were cultured on nano-thin highly ordered collagen films in which we tune the mechanical properties with enzymatic crosslinking by transglutaminase while the films maintain the same nanostructure. Cells pull on the collagen films, and visibly deform the fibrils when not cross-linked, and cells cultured on cross-linked films initially respond by altering their morphology, acto-myosin organization and nuclear shape. Mechanically anisotropic, native collagen films promote strong polarization and orientation along the highly aligned fibrils, whereas cross-linked films cause cells to spread isotropically and stiffen their nucleus with increased lamin-A/C expression. The morphology on cross-linked films appears osteoblastic and the early osteogenic transcription factor RUNX2 and the later marker osteonectin are indeed upregulated in comparison to cells on pristine collagen films. On other hand stiffening the nucleus by Lamin-A overexpression enhances osteogenesis. We also found positive correlation between matrix and nucleus mechanics and that they have a synergistic effect on MSCs differentiation potential. The results demonstrate that stem cells and their nuclei are highly sensitive to the detailed nanomechanics of their matrix.

1915-Plat
Force-Induced In Vitro Cancer Metastasis
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Cancer deaths are primarily caused by metastases, not by the parent tumor. During metastasis, malignant cells detach from the parent tumor, and spread through the circulatory system to invade new tissues. The physical-chemical mechanisms and parameters within the cellular microenvironment that initiate the onset of metastasis, however, are not understood. Such a gap in the understanding and prediction of the onset of metastasis is of particular concern in colon cancer, since at the time of diagnosis, most colon cancer patients already have advanced disease. In this talk, we will present our recent discoveries that human colon carcinoma (HCT-8, HCT116 and SW480) cells can be induced to undergo a phenotypic, genetic and proteomic transition similar to the early stage of metastasis simply by being grown on a substrate with appropriate mechanical stiffness. This force-induced transition is observed as a change from a flattened, epithelial to a rounded, detached morphology, which we have termed an ''E-to-R transition''. The post-transition cancer cells express a remarkable number of in vivo biophysical and biochemical metastasis hallmarks, such as loss of cell-cell and cell-substrate adhesion, reduction of cell-substrate traction force and cell-cell tugging force, gain of anchorage-independence capability, decrease of cell elasticity, and increase of cell migratory behaviors, etc. Furthermore, the post-transition cancer cells are verified to be significantly more invasive than the original cancer cells that were never exposed to soft substrates. Our novel finding suggests that the onset of metastasis may, in part, be linked to the intracellular forces and the mechanical, biophysical microenvironment of the parent tumor.

1916-Plat
Mutations in Lamin A/C Gene Causes Mechanosensing Defects in Human Myoblasts
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The mechanisms underlying mechanosensitivity are critical for muscle development and functionality. The presence of lamin A/C is essential for intact nucleoskeleton and mechanical transmission between the nucleus and the extracellular matrix. Therefore, we tested the hypothesis that mutation in the LMNA gene (encoding for lamin A/C) cause mechanosensing defects in human myoblasts.
Myoblasts with LMNA p.Lys32del mutation (Lmna) and normal human myoblasts (WT) were immortalized and cultured in a linear 3D fibrin matrix. At day 1, WT have spontaneously aligned along the gel axis whereas Lmna cells exhibited random orientation ($p<0.001$). Mutated myoblasts had larger and more abundant actin bundles, and vinculin adhesion sites were longer and thicker compared with WT myoblasts (each $p<0.05$). This was associated with higher protein and mRNA expression of vinculin in Lmna cells ($p<0.01$). In WT, uni-axial cyclic stretch of the gel (0.5 Hz, 10% for 4 hours) induced cytoskeletal reorganization characterized by thickening of actin stress fibers and elongation of vinculin adhesion sites. These changes were absent in Lmna and in blebbistatin- or Y-27632-treated WT. Similar results were obtained in primary myoblasts from another patient with similar mutation and in myoblasts with LMNA p.Arg249Trp mutation. In conclusion, these data provide first evidence that lamin A/C mutation causes defects in myoblast mechanosensing that may contribute to mechanical damage in striated muscles from L-CMD.

1917-Plat
Different Function of α5β1 and αVβ3 Integrins: Elucidating their Interactions and Spatial Requirements for Adhesion and Migration
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The coordination of specific functions of α5β1 and αvβ3 integrins is crucial for cell spreading and migration. For the spatio-temporal transduction of signals, cells interacting with their environment regulate the localization of α5β1 and αvβ3 integrins at adhesive contacts. The understanding of integrin function has progressed because of new techniques and analytical tools; however, the analysis of integrin specific contribution and crosstalk during adhesion remains a challenge.
To determine αvβ3 and α5β1 integrin specific functions we used nanoarrays of gold particles presenting immobilized integrin-selective peptidomimetics. For measuring forces generated upon adhesion-mediated by these integrins, we produced pillar arrays decorated with gold particles presenting the peptidomimetics. Finally, to characterize integrin crosstalk, we achieved the controlled sumultaneous presentation of αvβ3 and α5β1 integrin peptidomimetics by using binary nanoarrays of alternating gold and iron oxide particles.
We show that integrin binding to the peptidomimetics is highly selective and cells spread on both ligands. However, spreading is faster and cell area and intracellular forces are increased on the α5β1 mimetic. Analysis of integrin clusters indicates that αvβ3 clusters are more pronounced on αvβ3 ligand and are present in cells adhering to α5β1 ligand. Quantitative analysis of adhesion plaques shows that focal adhesion size is increased in cells adhering to αvβ3 ligand. Integrin α5β1clusters are present in cells adhering to α5β1 ligand and colocalize with αvβ3 clusters. There are numerous fibrillar structures in cells adhering to α5β1 ligand, while clusters are mostly localized at the cell margins in cells adhering to αvβ3 ligand.
These findings suggest that the activation by ligand binding of αvβ3 integrin doesn't play an active role in promoting intial adhesion and spreading, but it is essential for the formation of stable focal adhesions.

1918-Plat
Motor and Track Systems for Navigating the Cytoskeleton
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An emerging paradigm in motility is the notion of ''specialized'' motors, or motors that are fine-tuned to perform a specific function. Rather than merely traveling anywhere and everywhere, such motors are programmed to select certain tracks, to respond to forces in a defined way, or to actively remodel their tracks. Here, we further develop the ex vivo motility assay to determine how cells remodel their actin tracks and redirect myosin V traffic in response to Rho GTPase signalling. We transfected 3T3 cells with constitutively active or dominant negative forms of Rac1, RhoA, or CDC42, triton extracted the cells to expose the cytoskeletons, and applied labeled myosin V for single molecule tracking. We find that all Rho constructs increase myosin V activity. Remarkably, only a small fraction of actin filaments are used by myosin V, as we find that motors repeatedly travel in limited zones while ignoring nearby regions of high actin density.