from neighboring cells rapidly assemble junctional complexes, self-contacting membranes do not, suggesting that cells have the ability to distinguish self from neighboring cells. Using a self-contact inducing micro-fabricated substrate, we show that self-contacts of normal epithelial cells are rapidly eliminated by membrane fusion between two opposing plasma membranes of a single cell. This membrane fusion is most frequently observed in E-cadherin expressing epithelial cells, but not in fibroblasts. The efficiency of self-contact elimination depends on extracellular calcium concentration and the level of E-cadherin, suggesting that E-cadherin, while not required, enhances membrane-fusion efficiency by bringing opposing membranes into close apposition to one another. Additionally, ROCK inhibition decreases self-contact induced membrane fusion of epithelial cells, suggesting that this fusion may be mechanically regulated through the actin-myosin network. This is the first demonstration of self-contact induced membrane fusion in mammalian cells, and that membrane fusion may be a key feature of the cell self-recognition signaling pathway.

1189-Plat
Mapping Mechanical Properties of the Extra Cellular Matrix Surrounding Cells Cultured in 3D
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There is a preponderance of evidence for roles of bulk stromal stiffness in cell regulation, but little is about the mechanical microenvironment. To investigate these roles we culture cells within 3D extracellular matrix (ECM) hydrogels. We implement automated optical tweezers active microrheology (aAMR) to probe cell elasticity and map it in the space surrounding them, where aAMR uses optical forces acting on microbeads to measure the complex response function of the ECM surrounding each bead. Hundreds of beads are probed in one hour to map the mechanical landscape and to seek correlations between local stiffness and cell properties such as contractility, signaling and differentiation. For example, we will present our study of primary human aortic smooth muscle cells (aSMCs) cultured within a collagen hydrogel. We observed a range of stiffness from over 200 Pa to less than 20 Pa, with the stiffer regions near the leading edge of the cell. Such stiffening is consistent with the non-linear stress-strain relationship of natural tissue in which stiffness increases with stretch. After treatment with inhibitors of contractility such as blebbistatin or the ROCK Inhibitor Y-27632, we observed cell relaxation coincident with a distribution of softer values ranging from 100 to ~10 Pa. Such leading edge contractile force-dependent stiffness is consistent with our earlier findings for primary mesenchymal stem cells and an invasive capillary morphogenesis model, in which only several beads could be measured in one hour due to the notable complexity of performing AMR. In contrast, our aAMR densely measures ECM stiffness, chronically, and is a new research tool for studying the interplay between viscoelastic properties, forces and signaling at the ECM-cell interface.

1190-Plat
Mechanical Extraction of Antigen from B Cell Immune Synapses: A Unique Way to Sense Ligand Affinity
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The generation of high affinity antibodies depends on the ability of B cells to acquire and internalize foreign antigens from the surface of antigen-presenting cells (APCs). This process starts by binding of antigens to the B cell receptors, which triggers intracellular signaling resulting in B cell spreading, antigen clustering and eventually antigen internalization. We show that to internalize the antigen clusters, B cells use dynamic pulling mechanisms. Particularly, ROCK inhibition decreases self-contact induced membrane fusion of epithelial cells, suggesting that this fusion may be mechanically regulated through the actin-myosin network. We present the first demonstration of self-contact induced membrane fusion in mammalian cells, and that membrane fusion is a key feature of the cell self-recognition signaling pathway.

1191-Plat
Getting the Mechanical Message Across Cell-Cell Junctions
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We present direct evidence that cadherin complexes are mechanosensors at cell-cell junctions, and we identify the rudiments of molecular cascades underlying mechanotransduction at cadherin-based adhesions. Magnetic twisting cytometry (MTC) and immunofluorescence imaging measurements demonstrate that cadherin-associated complexes are force sensors that trigger cytoskeletal remodeling and consequent mechanical reinforcement at stressed cadherin adhesions. Alpha catenin at these complexes is a postulated auto-inhibited sensor that is activated by intercellular tension to expose a vinculin binding site, which in turn recruits vinculin and actin to junctions. Bead twisting (MTC) measurements in conjunction with confocal immunofluorescence imaging directly demonstrate that alpha catenin and its vinculin binding site are obligatory for mechanosensing, and that cadherin bond shear triggers the local recruitment of vinculin and F-actin to junctions. We further demonstrate, with a FRET-based alpha catenin sensor, that force induces a conformational change in alpha catenin that coincides with force dependent changes in local protein recruitment. These results reveal a new mechanism of force sensation and force propagation in tissues that is distinct from focal adhesions.

1192-Plat
Forcing it On: Actin Dynamics During Lymphocyte Activation
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The T cell response is the adaptive immune response in the body. The binding of the T cell receptor (TCR) with antigen on the surface of antigen presenting cells leads to cell spreading and signaling activation which is accompanied by the assembly of signaling molecules into microclusters. The underlying mechanism of signaling activation is not completely understood. While cytoskeletal forces have been implicated in T cell signaling, their nature and origin is unknown. We have imaged the dynamics of actin and the spatiotemporal localization of signaling clusters during the early stages of spreading. We observed membrane waves driven by actin polymerization originating at signaling clusters. These actin-driven membrane protrusions likely play an important role in force generation at the immune synapse. We have used traction force microscopy to measure the forces exerted by Jurkat T cells during TCR activation on stimulatory elastic substrates. We find that the forces exerted are largely due to actin dynamics with myosin contractility playing a limited role. We also find that Jurkat T cells are mechanosensitive, with both the exerted forces and signaling activation being sensitive to substrate stiffness. Our results suggest that cytoskeletal forces may be important for receptor activation in Jurkat T cells and influence their signaling activity.

1193-Plat
Measuring Compressional Resistance in Large Surface Molecules
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A class of receptors on leukocytes, termed the non-catalytic tyrosine phosphorylated receptors (NTRs), play important roles in the interactions of these cells with their environment, including between lymphocytes and antigen-presenting cells. These receptors share common features, including ligands that are attached to external surfaces, and short ectodomains (i.e., the cell-external domain) compared to large surface molecules (LSMs) that regulate their function. This size difference is hypothesized to play a functional role - depending on the compressional resistance of LSMs, it could lead to emergent properties such as pressure on the NTR-ligand bond, segregation of large and small surface molecules, and coalescence of comparably-sized NTR binding partners. Although much effort has focussed on determining the flexibility of lipid bilayers, the biophysical properties of surface proteins remain poorly understood. We have developed an in situ method based on Forster resonance energy transfer (FRET) and Bayesian statistics to elucidate the mechanical properties of surface protein ectodomains. Using this method we investigated CD148, an abundantly expressed and functionally important LSM. Our initial results show that its ectodomain is stiff enough to put pressure on nearby NTR-ligand complexes and lead to segregation at cell-cell interfaces, but not stiff enough to preclude binding. These results support a functional role for LSM ectodomain mechanics. The modular nature of our FRET assay will allow it to be applied to other surface molecules. The long-term goal of this work is to manipulate structural aspects of relatively well characterised ectodomains and establish a method to relate their structural elements to biophysical properties.