2204-Plat
Force Generation in Lamellipodia is a Discontinuous Process with Nanometer Discrete Forward and Backward Jumps
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The progressive addition of actin monomers to the existing network of actin filaments underlies force generation in lamellipodia. By using optical tweezers, we have characterized the dynamics by which lamellipodia of Dorsal Root Ganglia neurons exerted force on encountered obstacles such as silica beads. Because of the presence of adhesion forces, beads in close contact with a lamellipodium could seal on its membrane so that the standard deviation of Brownian fluctuations could be reduced by 10 times. In several experiments, the bead remained within 300 nm from the center of the optical trap where voltage sensitivity of the detector and trap stiffness is constant. Under these conditions, if the lamellipodium pushed the bead, we could detect discrete jumps possibly constituting the elementary events underlying force generation. Jumps were detected by using algorithms based on nonlinear diffusion methods and on numerical differentiation. These jumps occurred within 1 ms and had an amplitude varying from 5 to 20 nm. When the lamellipodium retracted, pulling the beads with it, no discrete events were observed. These discrete events were not observed in the presence of Latrunculin A, a blocker of actin polymerization or when neurons were fixed with paraformaldehyde. The amplitude of these jumps increased by 20-40% when cholesterol in cellular membrane was depleted by treatment with cyclodextrin. These jumps show that force generation in lamellipodia is not a smooth process but is a discontinuous process in which bursts of actin polymerization and depolymerization alternate continuously.

2205-Plat
What We Learn from Actin Comet Tails Going Awry
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We present an *in vitro* study of the actin-based movements of spherical beads coated with VCA, a partial domain of N-WASP, in comparison with *Listeria monocytogenes*. Long trajectories induced by the spherical beads show characteristic differences with those observed for bacteria, which have both an elongated shape and an asymmetric expression of the polymerization inducing enzyme. These differences in trajectory shape and curvature arise mainly from the different geometry of actin-tail inducing cargo. The experimental trajectories can be simulated using a generalized kinematic model including the rotation of the bead relative to the actin tail. These results imply that the trajectories of spherical beads are mechanically deterministic rather than random, as suggested in stochastic models found in the recent literature.

2206-Plat
Multiscale Modeling of Erythrocyte Membrane: Equilibrium Shapes and Mechanical Responses
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We develop a three-level information-passing multiscale approach to study the equilibrium shapes of erythrocytes and their quasi-static mechanical responses. In the whole cell model (Level III) of this method, both the lipid bilayer and skeleton network are modeled as continuum shells and solved by finite element method. The skeleton-bilayer interaction is depicted as a sliding-only contact. The lipid bilayer possesses a large area stiffness and negligibly small shear stiffness. The elastic properties of the skeleton network are obtained from a molecular-based model (Level II) of the junctional complex, together with a constitutive model (Level I) of the spectrin including its folding/unfolding reactions. Through multiscale simulations, we explore different equilibrium shapes and their dependences upon structural properties (e.g. spontaneous curvature). We also simulate cell deformations induced by micropipette aspiration and optical tweezer stretching, and get predictions consistent with existing studies in terms of cell shape and skeletal deformation. Furthermore, we predict the interaction force between the lipid bilayer and the skeleton that may cause their dissociation, and find that spectrin unfolding might significantly increase the loads upon individual linkage proteins between the lipid bilayer and the skeleton.

2207-Plat
Three-Dimensional Forces Exerted by Migrating Amoeboid Cells
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Cell migration results as the coordinated repetition of pseudopod protrusions and retractions and is driven by the generation of traction forces and the regulation of cell-substrate adhesions. Although traction forces generated by migrating cells are potentially important in all three directions of space, previous traction cytometry studies have neglected, for the most part, the vertical forces exerted by the cells in the direction perpendicular to the substrate. In order to study these forces, we have developed a three-dimensional (3D) force cytometry method and applied it to study chemotaxing *Dictyostelium* cells moving over flat substrates. The 3D cell traction forces produced by the cells were determined from measurements of the deformation of the substrate by solving the equation of static equilibrium for a linearly elastic medium. The 3D substrate deformation was measured from the displacements of fluorescent microbeads embedded in the substrate by applying custom correlation algorithms to time-lapse sequences of z-stacks of fluorescence images acquired with a confocal microscope. Our 3D force measurements revealed that migrating cells pull the substrate up in the vertical direction and inwards in the horizontal directions near the cell periphery, whereas they push the substrate down underneath the cell center. The ability of amoeboid cells to press on their substrate, which had not been reported in previous two-dimensional traction cytometry studies, may be important for the motion of these cells through 3D environments such as the *Dictyostelium* mound or 3D extracellular matrices. Furthermore, our 3D force measurements showed that the magnitude of the vertical forces exerted by migrating cells is similar to that of the horizontal forces, and much larger than the weight of the cell. Therefore, knowledge of the magnitude of cellular traction forces in all three directions of space is important to fully understand amoeboid motility.

2208-Plat
Integrin α5β1-Mediates Cell Invasion through Enhanced Contractile Forces
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Cell motility is a fundamental biomechanical process in tumor growth and metastasis formation. Cell migration through dense connective tissue usually requires firm adhesion to the extracellular matrix through integrins. For some tumors, increased integrin expression is associated with increased malignancy and metastasis formation. Here, we studied the invasion of cancer cells with different α5β1 integrin expression levels into dense 3-D collagen fiber matrices. Using a cell sorter, we isolated α5β1-high and α5β1-low expressing sub cell lines from parental MDA-MB-231 breast cancer cells. Cells with higher α5β1 expression showed significantly (3-fold) increased cell invasiveness, whereas knock-down of the α5 integrin subunit lead to decreased tumor cell invasion. Interestingly, knock-down of the collagen receptor integrin subunit α1 did not alter invasiveness, indicating that the effect is integrin-type specific. Fourier transform traction microscopy revealed that the α5β1-high expressing cells generated 5-fold larger contractile forces. Cell invasiveness was reduced after addition of the myosin light chain kinase inhibitor ML-7 or the myosin II inhibitor blebbistatin in α5β1-high cells, but not in α5β1-low cells, suggesting that α5β1 integrins enhance cell invasion through enhanced transmission and generation of contractile forces.

2209-Plat
Matrix Elasticity and Nuclear Physics
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Physical cues such as matrix elasticity can affect cell morphology, mechanics, and even differentiation. Human mesenchymal stem cells (MSCs) have previously been shown to change shape and specify lineage toward neurons, muscle, and bone based on tissue-like elasticity (E ~ 1 - 34 kPa) which has been mimicked with polyacrylamide gels coated with collagen. We confirm the importance of E as a material independent parameter that directs cell shape by using a wide range of cross-linked hyaluronic acid hydrogels that also demonstrate mechano-sensitivity in three dimensions. In addition, matrix elasticity is shown to impact nuclear shape, suggesting novel physical mechanisms of gene regulation - which is analyzed in initial stages of spreading by whole genome analyses.