PLATEFORM AA: Cell and Bacterial Mechanics Motility

1042-Plat
Surface Protrusion of Human Umbilical Vein Endothelial Cells
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During leukocyte rolling on the endothelium, membrane tethers can be extracted simultaneously from both leukocytes and endothelial cells. Tether extraction, which has been shown to stabilize leukocyte rolling, is preceded by surface protrusion, which dictates whether tether extraction can occur. Although surface protrusion of leukocytes has been characterized, surface protrusion of endothelial cells has not. In this work, we present a detailed study of surface protrusion of human umbilical vein endothelial cells (HUVECs). Using the micropipette aspiration technique, we measured the protrusional stiffness and the crossover force during HUVEC surface protrusion. We found that, compared with leukocytes, the protrusional stiffness and the crossover force of HUVECs were both larger at similar force loading rates. The values of these two parameters depended on temperature, the cytoskeletal integrity, α-actinin1, and whether CD31 or CD29 was used as the force handle. However, they did not depend on cell attachment state or intracellular calcium. These results show that similar mechanisms govern surface protrusion, hence also tether extraction in leukocytes and endothelial cells. They will help us understand and eventually control this critical step of the immune response.

1043-Plat
Quantifying Mechanical Interactions between Cells in Small Clusters
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Tissue cells typically utilize their actomyosin contractile machinery to actively pull on their environment, which can be either the extracellular matrix or neighboring cells. The mechanical forces that a cell exerts and experiences have been shown to regulate fundamental cellular processes, including cell growth, proliferation, differentiation and migration. However, little is known about the spatial distribution of mechanical stress in tissues. In particular, the extent to which mechanical forces are communicated through cell-cell interactions across a tissue is not well understood.

Here, we present a novel method, based on high resolution traction force microscopy, to measure mechanical stresses that are transmitted through cell-cell interfaces in small cellular clusters (~10 cells). Cells are classified according to the number of neighboring cells. We find that this degree of cellular connectivity can determine many properties, including the amount of force transmitted through a particular cellular interface. In order to determine how force balance in the cell cluster is locally achieved, we compared forces transmitted through cells to forces exerted on extracellular substrates. The analysis of these forces reveals the length scale over which forces can be transmitted through the cell cluster. Furthermore, by molecular perturbations, we are identifying proteins that may be essential for long range stress communication in the cluster. The ability to quantify force communication between cells will allow us to examine how cell-cell mechanical interactions contribute to overall tissue stress and vice versa. It will also allow us to investigate the role of mechanical stresses in establishing signaling gradients. This will further our understanding of the role of mechanical stress in processes that require fine coordination between cells, such as collective migration in morphogenesis and cancer.

1044-Plat
Finite Element Modeling of Polar Growth in Walled Cells
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Walled cells such as those of plants and fungi grow by expanding their cellular surface driven by the intracellular turgor pressure. The generation of a particular cellular shape necessitates the precise spatial control of mechanical properties in the polymer-network forming the cell wall. To model protuberance formation in walled cells we established a finite element model. We aimed to identify the requirements for spatial distribution of mechanical properties in the cell wall that would allow the generation of tubular shapes that agree with experimental observations on the pollen tube, a rapidly elongating plant cell. We based our structural model on the parameterized description of a tip growing cell that allows the manipulation of cell size, shape, cell wall thickness and local mechanical properties. The mechanical load was applied in the form of hydrostatic pressure. We used two validation methods to compare different simulations based on cellular shape and the displacement of surface markers. We compared the resulting optimal distribution of cell mechanical properties with the spatial distribution of biochemical cell wall components in pollen tubes and found remarkable agreement between the gradient in mechanical properties and the distribution of de-esterified pectin.

1045-Plat
AFM Stiffness Nanotomography of Normal, Metaplastic and Dysplastic Human Esophageal Cells
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The mechanical stiffness of individual cells is important in tissue homeostasis, cell growth, division, and motility, and epithelial-mesenchymal transition in the initiation of cancer. In this work, a normal squamous cell line (EPC2) and metaplastic (CP-A) as well as dysplastic (CP-D) Barrett’s Esophagus columnar cell line are studied as a model of pre-neoplastic progression in the human esophagus. We used the combination of an atomic force microscope (AFM) with a scanning confocal fluorescence lifetime imaging microscope (FLIM) to study the mechanical properties of single adherent cells. Analyzing the force indentation curves, indentation depth dependent Young’s moduli were found for all cell lines. Stiffness tomograms demonstrate distinct differences between the mechanical properties of the studied cell lines. Comparing the stiffness for indentation forces of 1 nN, most probable Young’s moduli were calculated to 4.7 kPa for EPC2 (n=18 cells), 3.1 kPa for CP-A (n=10), and 2.6 kPa for CP-D (n=19). We also tested the influence of nuclei and nucleoli staining organic dyes on the mechanical properties of the cells. For stained EPC2 cells (n=5), significant stiffening was found (9.9 kPa), while CP-A cells (n=5) showed no clear trend (2.9 kPa) and a slight softening was observed (2.1 kPa) in the case of CP-D cells (n=16). Some force-indentation curves show non-monotonic discontinuities with segments of negative slope, resembling a sawtooth pattern. We found the incidence of these ‘breakthrough events’ to be highest in the dysplastic CP-D cells, intermediate in the metaplastic CP-A cells, and lowest in the normal EPC2 cells. This observation suggests that the microscopic explanation for the increased compli- ance of cancerous and pre-cancerous cells may lie in their susceptibility to ‘crumble and yield’ rather than their ability to ‘bend and flex’.

1046-Plat
A general Approach to Measure Three-Dimensional Forces from Cells
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We have developed a technique to quantitatively map the traction forces exerted by cells encapsulated within three-dimensional (3D) hydrogel matrices. Methods using two-dimensional (2D) polycrylamide gels, or arrays of elastic cantilevers have mapped the forces that cells generate against planar substrates and have revealed that such forces not only guide morphogenetic events, but can also feedback to regulate cellular functions including proliferation and differentiation. However, many cellular phenomena are altered or lost completely when cells are removed from their native 3D environment. Here, we use mechanically well defined synthetic hydrogels and the finite element method to measure the tractions generated by cells encapsulated within a fully three-dimensional matrix. We use this technique to investigate the role of 3D cellular tractions in both single and multicellular processes and uncover unique patterns of cellular forces that can be attributed to morphologically distinct filopodial-like extensions. Additionally, by acquiring time-lapse measurements of these forces, we have identified distinct force profiles that correspond to cellular processes which are invading into a 3D matrix. Because the hydrogels used in these studies have been shown to support a wide array of cellular and morphogenetic processes such as angiogenesis and tumor metastasis, and due to the general nature of our technical approach, we anticipate that this method will be applicable to a wide range of biological settings.

1047-Plat
Elementary Mechanisms of Force Generation
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Elementary mechanisms of force generation

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By using optical tweezers, we have characterized in details the mechanism of force generation in neuronal filopodia and lamellipodia with a nanometer sensitivity and sub-millisecond temporal resolution. Filopodia of dorsal root ganglia (DRG) neurons exert forces up to 2 pN, while those of hippocampal neurons exert forces up to 4-6 pN. When lamellipodia of DRG or hippocampal growth cones grow pushing a trapped bead, the autocorrelation of bead fluctuations decays with multiple time constants, while during Brownian fluctuations a single time constant of approximately 1 msec is observed. During push the power spectrum density of bead fluctuations is not fitted by a Lorentzian distribution and the energy content of low frequencies is higher than during Brownian fluctuations. Thus, during push the underlying dynamics is spatially and temporally correlated. Several algorithms show that during push, forward and backward jumps of 2-20 nm are detected. The net protrusion in a given time window is equal to the sum of all forward and backward jumps in the same window. These forward and backward jumps are identified as bursts of actin polymerization and depolymerization respectively and represent the elementary mechanisms of force generation.

1048-Plat
High-Throughput Cell Elastic Modulus Cytometry using Single Diode Laser Beam Optical Stretchers

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Cellular elastic properties can be measured by trapping and deforming them using optical stretchers. Dual optical traps were recently shown to be an efficient experimental method to identify cell types and detect disease states from the measured elastic modulus. However, the low throughput associated with such dual optical traps has significantly limited its utility and application due to the need to sequentially isolate and probe individual cells. We have implemented a pseudo steady-state high-throughput optical stretcher that uses single inexpensive diode laser bar to impart anisotropic forces and stretch osmotically swollen bovine erythrocytes in a continuous microfluidic flow at a rate of ~1 cell/second. This measurement rate is a factor of 10-100 higher than previous demonstrations of optical stretching. We also implemented a numerical model, dynamic ray tracing, to simulate the deformation of elastic capsules induced by the single diode laser bar optical stretching in flow conditions. Finally, we demonstrated how the numerical model is used to determine the elastic modulus of individual cells from experimental measurements of the steady-state deformation. This new optical approach has the potential to be readily integrated with other cytometric technologies, and with the capability of measuring cell populations, thus enabling true mechanical-property based cytometry.

1049-Plat
Force Dependent Enzymatic Activity of Non-Erythroid Spectrin and Cell Proliferation

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Mechanotransduction in cells describes the process by which external physical stimuli are converted into biochemical activity and plays an important role in many biological functions on both the cell and tissue level. However, the specific mechanisms by which mechanical forces lead to particular molecular and cellular responses are much less understood. We investigate the functional changes of non-erythroid z-II and β-II spectrins as a result of equi-biaxial strain application to cells in culture. Specifically, we focus on the spectrins’ role in the ubiquitination process of spectrin associated ankyrin, a vital process in the regulation of its degradation as well as its interactions with other membrane proteins. We utilize epifluorescence, FRET and TIRF microscopy, immunofluorescence staining and fluorescent fusion proteins for quantitative fluorescence imaging of spectrin, ankyrin and ubiquitin in live and fixed cells. Protein expression levels and localization between cells exposed to mechanical stimuli of different temporal and spatial profiles are compared. In addition, the threshold behavior of cell proliferation - as measured by number densities - of CHO, 10T1/2 and 3T3 cells as a function of mechanostimulation with different frequencies and durations is investigated in depth.

Key words: Ankyrin, FRET, Proliferation, Spectrin, Ubiquitination.

PLATFORM AB: Protein-Nucleic Acid Interaction

1050-Plat

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Abstract
We have developed tethered particle motion (TPM) experiments to investigate the details of E. coli RecA-mediated pairing and strand exchange at the single-molecule level. Experiments with beads labeled on either the invasion strand or the outgoing strand, both showed that DNA pairing and strand exchange occurred essentially in ATP or its non-hydrolyzable analog, ATPγS. RecA-mediated strand exchange efficiency is similar for ATP and ATPγS, suggesting that ATP hydrolysis by RecA is not required to complete strand exchange in these substrates. In addition, the RecA-mediated strand exchange is shown to progress from 5 ’ end to 3 ‘ end within a small synopsis segment while the exchanged strand is temporally bound to heteroduplex DNA and eventually released without ATP-hydrolysis. One the other hand, experiments with beads labeled on the invasion strand either homologous or heterogeneous to surface anchored duplex DNA showed that there were a lot of transient synapse events existing only from ms to seconds long. The lifetime of these synaptic related to synapse states failing to pass on strand exchange states represents the stability of synaptic intermediate which is correspondence with the strand exchange efficiency. The lifetime values of synaptic state are 1.960±0.10s and 2.740±0.38s for 229mer and 427mer single strand DNA respectively. Moreover, the initial Brownian motion amplitude comes from the length contribution of the RecA nucleoprotein filament and interacted surface anchored duplex DNA. A sum of two-dice model is adapted to simulate the initial pairing event and shows that synaptic-complex formation follows a random collision initial step without invasion end preference for unsuccessful strand exchange events. One the contrary, 5 ‘ end invasion preference is required to drive synaptic state efficiently pass on the strand exchange process.

1051-Plat
Type III Restriction Enzymes Use 1D Diffusion to Communicate the Relative Orientation of their Distant Target Sites

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Type III restriction enzymes sense the relative orientation of their distant target sites and cleave DNA only if at least two of them are situated in an inverted repeat. The communication process is strictly dependent on ATP hydrolysis catalyzed by their superfamily 2 helicase domains. Given the similarity to Type I restriction enzymes, which couple ATP hydrolysis to directed motion on DNA, unidirectional loop translocation that may partially be accompanied by 3D diffusive looping has been the suggested communication mechanism for Type III enzymes. Based on magnetic tweezers single-molecule cleavage experiments and ATPase measurements we suggest an alternative inter-site communication mechanism using 1D diffusion along the DNA contour (1). In order to verify this hypothesis we directly visualize the motion of quantum-dot labeled Type III restriction enzymes along DNA. For this we use a setup that combines magnetic tweezers with total internal reflection fluorescence microscopy. The enzymes undergo a fast diffusive motion along DNA capable of scanning kbp distances per second. We also find that the affinity of the enzymes to non-specific and specific DNA is regulated by the presence of ATP suggesting that ATP hydrolysis acts as a trigger for diffusion. Thus Type III restriction enzymes are the first DNA-modifying enzymes which communicate the target site orientations over long distances via 1D diffusion. (1) van Aelst K, Töth J, Ramanathan SP, Schwarz FW, Seidel R, Szczelkun MD. Proc Natl Acad Sci U S A. 2010 May 18;107(20):9123-8.