PLATFORM 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 the underlying substrate. A correlation 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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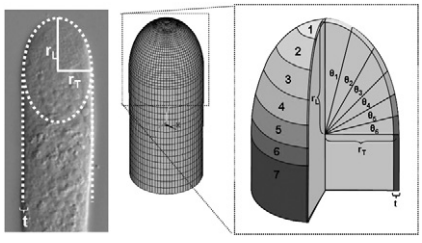
Walled cells such as those of plants and fungi grow by expanding their cellular surface driven by the intracellular turgor pressure. The generation of a particular cellular shape necessitates the precise spatial control of mechanical properties in the polymer-network forming the cell wall. To model protuberance 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 compliance 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) polyacrylamide 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 timelapse 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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