Actin Dynamics and Signaling Activation of B Lymphocytes Respond to Substrate Topography

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B cells are activated by the binding of membrane-bound antigen to the B cell receptor (BCR), which induces actin dynamics, reorganization of receptors into signaling microclusters, and cell spreading. In vivo, B cells gather antigen from a variety of sources which may have different physical characteristics such as mobility, stiffness or topography. However, the effect of these physical parameters on BCR clustering and signaling activation is not understood. Here we have studied the role of topography of the stimulating surface on cell spreading, actin polymerization and signaling activation. BCR ligand coated substrates presenting ridges of variable spacing were used to probe the interaction of B cells with non-planar surfaces. Using high-resolution TIRF and confocal microscopy of live cells, we followed the movement of BCR clusters and actin dynamics. We found that small ridge separations induced actin waves that travel parallel to the ridges, resulting in protrusions and retractions of the cell edge. Large ridge separations result in global oscillations of actin intensity in the vicinity of ridges. We further investigated the temporal dynamics of calcium enrichment after antigen engagement in B cells. On flat substrates we measured periodic oscillations of calcium influx with a period of about 30 s, consistent with previously observed values. Interestingly, we found the period of calcium enrichment was dependent on ridge spacing, with increasing time intervals on smaller spacings. Drugs that inhibited actin dynamics slowed down the observed oscillations of calcium. Our results indicate that B cells are sensitive to topographical features, resulting in modulated actin dynamics and that calcium signaling is coupled to substrate-proximal actin dynamics.

Mechanics of Neutrophil Migration in Three-Dimensional Matrices

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While much research has been dedicated to the identification of the cascade of specific biochemical processes involved in the recruitment of neutrophils, much less is known about the mechanical events driving their migration; in particular, how they generate the necessary traction forces to migrate across three-dimensional (3-D) extravascular spaces is unclear. In this study, we investigated the mechanics of 3-D neutrophil motility in collagen gels using a novel Elasticographic 3D Force Microscopy (E3DFM) technique, which simultaneously determines the 3-D cellular forces and non-linear material properties of the extracellular environment. We used neutrophil-like differentiated human promyelocytic leukemia cells (dHL-60) as a model system. dHL-60 cells were embedded in collagen matrices of different porosities containing fluorescent micro-beads. The concentration of collagen was varied in order to fabricate collagen gels with different matrix porosities. Neutrophil motility was induced via the introduction of the neutrophil chemokine formyl-Methionyl-Leucyl-Phenylalanine (fMLP) in a custom build device. Both Confocal and Fluorescent microscopy techniques were used to image the movement of the embedded micro-beads as well as fluorescently labeled dHL-60 cells. Particle Image Velocimetry (PIV) and Finite Deformation Theory were used to compute displacement fields in the collagen matrices. Stress fields in the matrices were computed using a constitutive relationship with several material parameters. The over determined nature of the problem was used to estimate the aforementioned material parameters. We will present data showing that cell speed, morphological changes, and migratory patterns varied with differing matrix porosities. We will also provide data showing a clear relationship between the aforementioned migratory characteristics and computed displacement and stress fields around migrating neutrophils in these different collagen matrices. The results from our study show that neutrophils migrating in 3-D environments employ distinct mechanical mechanisms that depend on their environment’s mechanical structure.

Collective 3D Migration of Embryonic Epithelial Mesenchymal Composite Tissues are Regulated by Surface Topology

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Cells in tissues encounter a range of physical cues as they migrate. Probing single cell and collective migratory responses to physically defined three-dimensional (3D) microenvironments and the factors that modulate those responses are critical to understanding how tissue migration is regulated during development, regeneration, and cancer. One key physical factor that regulates cell migration is topology. Most studies on surface topology and cell mechanics have been carried out with single migratory cells, yet little is known about the spreading and motility response of 3D complex multicellular tissues to topological cues. Here, we examine the behaviors of microsurgically isolated tissue explants composed of epithelial and mesenchymal cell layers from naturally 3D organized embryos of the aquatic frog Xenopus laevis to complex topological cues. We control topology using fabricated micropost arrays (MPAs) with different diameters (e.g., different spacing gaps) and investigate the collective 3D migration of these multicellular systems in these MPAs. Our topographical controlled approach for cellular application enables us to achieve a high degree of control of micropost positioning and geometry via simple, accurate, and repeatable microfabrication processes. We find that the topology regulates both collective and individual cell migration and that dense MPAs reduce but do not eliminate tissue spreading. By modulating cell size through the cell cycle inhibitor Mitomycin C or the spacing within MPAs we discover a role for topology in disrupting collective enhancement of cell migration. We find 3D topological cues can direct both single cell motility and tissue spreading, altering tissue-scale processes that enable efficient conversion of single cell motility into collective movement.

High Local Curvature Reduces Migration Rate in Spreading Multi-Layer Tissues

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Development requires the concerted movement of complex tissues, composed of multiple cell types. The in vivo mechanics of tissue migration remains largely unknown, despite significant progress in understanding collective migration of confluent monolayers. To bridge this gap, we study migration in Xenopus laevis animal cap tissue explants, which are composed of two cell types spreading in concert: a multi-layer mesenchymal layer covered by an epithelial layer. To probe the mechanics of spreading, we investigated differences in spreading rate in explants ranging in initial area from 0.12mm2 to 2.63mm2. The average spreading rate increases with explant size, from 22±5 m/hr for the smallest to 54±5m/hr for the largest explants. Since both edge curvature and the number of cells change with explant size, either could lead to the increase in spreading rate. Changes in curvature could affect the distribution of forces along the explant’s edge. E.g., these forces in regions of low curvature would work more cooperatively, leading to a faster spreading rate. However, the difference in spreading rate can also be explained by intercalation between layers and programmed height change in the cells. Larger explants have more cells, thus these changes would lead to a faster spreading rate than in smaller explants. To distinguish between these models, we investigated the local spreading rates in explants with multiple curvatures, such as triangular explants. In these explants, spreading rate is lowest for regions of high curvature, even when the distance, and therefore number of cells, is greater between the edge and the center of the explant. Thus, our results indicate that the local rate of tissue migration depends on the initial curvature of the explant.
We successfully engineered the CDM network labeled with fluorescent markers highlighting fibronectin - a protein of the extracellular matrix, and we also observed the dynamics of key components driving cell migration, such as the actin cytoskeleton and focal contacts. Our results reveal key differences between 2D and 3D cell migration. (i) We report new types of protrusions distinct from filopodia/lamellipodia reported on planar surfaces, which are driven by pressure. (ii) Our 3D network is deformed reversibly during migration and this allows the extraction of forces locally applied by cells. We correlate these local forces to the focal contacts dynamics, and our measures indicate a local pulling mechanism for forward cell motion and nucleus translocation. (iii) During migration, the nucleus local deformation by the cytoskeleton is needed to facilitate motion. These three phenomena - pressure-driven protrusions, local forces correlated to local focal contacts, and nucleus deformation driven by the cytoskeleton - are reproduced in microchannels matching cell dimensions. Altogether, our results show that mechanical confinement of cell and nucleus is the main cause for differences between 3D and 2D motions.

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Cells as Active Particles in Asymmetric Potentials: Motility under External Gradients
Jordi Comelles1, David Caballero2, Rafael Voituriez2, Verónica Hortigüela4, Viktória Wollrab1, Amélie L. Godeau1, Josep Samitier5, Elena Martínez4, Daniel Riveline1, Vero´nica Hortigu¨ela4, Viktoria Wollrab1, Ame´lie L. Godeau1, France, 37 Laboratoire de Physique The´orique de la Matie`re Condense´e, 1Laboratory of Cell Physics ISIS/IGBMC, Universite´ de Strasbourg, Jordi Comelles External Gradients and nucleus deformation driven by the cytoskeleton - are reproduced in micro-pressure-driven protrusions, local forces correlated to local focal contacts, and cytoskeleton is needed to facilitate motion. These three phenomena - pressure-driven protrusions, local forces correlated to local focal contacts, and nucleus deformation driven by the cytoskeleton - are reproduced in micro-channels matching cell dimensions. Altogether, our results show that mechanical confinement of cell and nucleus is the main cause for differences between 3D and 2D motions.

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Characterizing New Genes Regulating Cell-Substrate Adhesion to Discover Novel Regulatory Mechanisms of Cell Motility
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The model organism Dictostelium has greatly facilitated our understanding of the signal transduction and cytoskeletal pathways that govern cell motility. Cell-substrate adhesion is a target of many chemotaxis signaling events and it can be used to screen for cells that have defects in cell migration. In fact, cells lacking PTEN, a negative regulator of cellular extensions, is flatter and adheres strongly to the surface. This leads to reasoning that other regulators of migration would also effect adhesion, a screening method was devised and isolated overly adherent mutants from a pool of mutagenized cells. Restriction enzyme mediated insertion (REMI) mutagenized cells, comprising more than 50000 insertions, yielded about 100 mutated cell lines with the desired phenotypes. The mutation sites in 20 of the strains have been mapped by cloning and sequencing of revertants, yielding about 100 mutated cell lines with the desired phenotypes. The mutation sites in 20 of the strains have been mapped by cloning and sequencing of revertants, providing novel insights into the networks controlling cell motility. Many of these genes have human homologs with unknown functions. Therefore, the future studies of the new group of regulators of adhesion and motility genes in Dictostelium will not only advance the knowledge of cell migration in amoeboid cells but elucidate the functions of novel human genes with potential disease relevance.