2287-Pos Board B424
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.

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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 investigate the mechanics of 3-D neutrophil motility in collagen gels using a novel Elastographic 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.

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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 over 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.

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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 0.12mm^2 to 2.63mm^2. The average spreading rate increases with explant size, from 22 ± 5μm/hr for the smallest to 45 ± 5μm/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.

2291-Pos Board B428
Key Effects of Confinement on Cell Motility
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Cell migration is an important process in Biology. So far, the phenomenon has been characterised on flat surfaces, usually in Petri dishes. This environment is far from physiological conditions encountered in vivo, and it can generate artifacts. We therefore study cell migration in a 3D environment synthesized by cells - the Cell Derived Matrix (CDM) - and in microfabricated channels.

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.

2292-Pos Board B429

Cells as Active Particles in Asymmetric Potentials: Motility under External Gradients

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Cells undergo motion and this phenomenon is known to be important during development and in diseases such as cancer. In particular, cells can migrate *directionally*: this phenomenon drives tissue rearrangements that shape organs in embryos. Mechanical constraints and chemical gradients can contribute to set cell directions, but their respective roles remain poorly understood. Here we report a new assay where we tested the effects of external cues on single cell motion. We show, by using microfabricated topographical ratchet, that the nucleus dictates the directions of cell through mechanical guidance with its environment. We demonstrate that this direction can be tuned by combining this ratchet with a gradient of fibronectin adhesion. We report competitions and cooperations between both external cues depending on their relative orientations. We also quantitatively compare the measurements to a model treating cells as fluctuating particles trapped in a periodic asymmetric potential. We show that the nucleus is contributing to the strength of the trap whereas protrusions guided by the adhesive gradients add a constant tunable bias to the motion.

2293-Pos Board B430

Characterizing New Genes Regulating Cell-Substrate Adhesion to Discover Novel Regulatory Mechanisms of Cell Motility

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The model organism Dictyostelium 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 and many of the phenotypes are similar to those of PTEN knockout cells. The extent of increased adhesion, cell motility, directed migration, cell shape, and new filamentous actin at the periphery are all parameters that have been examined in these new overly adhesive cell lines. The degree in which these parameters have been effected and the correlations between these changes is providing novel insights into the networks controlling cell motility. Many of these genes have human homologs with unknown functions. Therefore, the future study of this new group of regulators of adhesion and motility genes in Dictyostelium will not only advance the knowledge of cell migration in amoeboid cells but elucidate the functions of novel human genes with potential disease relevance.

2294-Pos Board B431

Evolutionarily Conserved Coupling of Adaptive and Excitable Networks Mediates Eukaryotic Chemotaxis

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Numerous models have been proposed to explain the remarkable ability of chemotactic cells to sense and migrate toward extremely shallow chemoattractant gradients independently of the ambient concentration. We carried out experiments to distinguish the various models of gradient sensing in migrating cells. First, signaling activity was strongly suppressed toward the low side of cells in a gradient or following sudden removal of uniform chemoattractant. Second, signaling activities displayed a rapid shut off and, with stimulation of increasing duration, a slower adaptation during which responsiveness to subsequent test stimuli declined. Simulations of existing classes of models indicated that these observations can only be explained by the coupling between an adaptive module and an excitable network. Moreover, stimulation of cells lacking G-protein function suppresses downstream activities, while constitutive G-protein activation induced persistent responses. This indicates that chemoattractant sensing is mediated by a G-protein-dependent excitor and a G-protein-independent inhibitor forming an incoherent feedforward loop. The salient features of the coupling between adaptive and excitable networks were observed for the chemoattractants cAMP and folic acid in Dictyostelium as well as fMLP in human neutrophils, suggesting an evolutionarily conserved mechanism for eukaryotic chemotaxis.

2295-Pos Board B432

Cell Polarisation Driven by Substrate-Mediated Intracellular Interactions - Consequences for Migration and Chemotaxis

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We study a generic model for the polarisation and motility of cells and biomimetic systems interacting with a viscous substrate, where traction forces generated by the cell are modelled by means of oscillating force multipoles at the cell/substrate interface. We find that symmetry breaking and cell polarisation naturally "emerge" from long-range mechanical interactions between oscillating units, mediated both by the intracellular medium and the substrate. However, the harnessing of cell polarisation for motility requires substrate-mediated interactions. Motility can be optimised by adapting the oscillation frequency to the relaxation time of the system, and maximal velocity is found when the substrate and cell viscosities match. Cellular noise can destroy mechanical coordination between force-generating elements within the cell, resulting in sudden changes of polarisation. The persistence of the cell's motion is found to depend on the substrate viscosity. Within such a model, chemotactic guidance of cell motion is obtained by directionally modulating the persistence of motion, rather than by modulating cell motility, in a way that resemble the run and tumble chemotaxis of bacteria.

2296-Pos Board B433

The Interplay between Cell Motility and Proteolysis in the Establishment of Brain Metastasis

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Cells actively respond to the mechanical signals received from the extracellular matrix (ECM) milieu. Reciprocally, cells can also modify the chemical and physical composition of the ECM via coordinated motility and proteolysis. Tumor cells actively remodel their microenvironment during colonization of distant organs. Here, we sought to understand the mechanisms that allow for successful brain metastasis. Using 3D in vitro models, we determined that there are phenotypic differences between brain tropic cells and those that metastasize to other organs. We visualized the morphogenetic program of the cells to determine if a specific type of cell motility is necessary for successful colonization.

2297-Pos Board B434

Comparsion of Migration Pattern between Young and Senescent Mesenchymal Stem Cells in Time Lapse Microscopy

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Mesenchymal stem cells (MSCs) can differentiate into a variety of cell types, and thus are fundamental players in modern regenerative medicine. To maintain the viability and the potentials for self-renewal and multilineage differentiation of MSCs in vitro development remains a big challenge. Previous approaches found that when MSCs were cultured on chitosan membranes, they tended to aggregate and form a 3D spheroid; meanwhile, their differentiation efficiency was likely to