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

Jordi Comelles[1], David Caballero[2], Raphael Voituriez[3], Verónica Hortigüela[4], Viktoria Wollrab[1], Amélie L. Godeau[1], Josep Samitier[5], Elena Martínez[4], Daniel Riveline[1].
[1]Laboratory of Cell Physics ISIS/IGBMC, Université de Strasbourg, Strasbourg, France, [2]Laboratory of Cell Physics ISIS/IGBMC, Strasbourg, France, [3]7 Laboratoire de Physique Théorique de la Matière Condensée, Université Pierre et Marie Curie, Paris, France, [4]Biomimetic Systems for Cell Engineering, Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain, [5]Nanobioengineering group, Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain.

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

Thomas J. Lampert, Peter N. Devreotes.
Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD, USA.

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

Chuan-Hsiang Huang[1], Ming Tang[1], Mingjie Wang[1], Changji Shi[1], Pablo A. Iglesias[2], Peter N. Devreotes[1].
[1]Cell Biology, Johns Hopkins University, Baltimore, MD, USA, [2]Electrical and Computer Engineering, Johns Hopkins University, Baltimore, MD, USA.

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

Marco Leoni, Pierre Sens.
ESPCI, Paris, France.

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

Alexus Devine, Kandice Tanner.
National Cancer Institute, Bethesda, MD, USA.

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

Ching-Fen Jiang[1], Shan-hui Hsu[2], Ka-Pei Tsai[1], Jia-Yin Li[1].
[1]Biomedical Engineering, I-Shou University, Kaohsiung, Taiwan, [2]Institute of Polymer Science and Engineering, National Taiwan University, Taipei, Taiwan.

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

improve. Cellular motility is an important index to reflect the viability and differentiation ability of stem cells during in vitro development. In order to investigate the variation of motility during this cell cultural environment, videos of cells were recorded for 72 hours by an ASTEC® CCM-1.4XZY/CO2 system with a CCD camera mounted on a time-lapse microscope with a magnification ratio of 100:1. We applied video tracking and image processing techniques to quantify cell mobility pattern in terms of moving speed and topological changes. Two types of MSCs, human adipose-derived adult stem cells (hADSCs) and human placenta derived multipotent cells (hPDMC), were investigated in this study. Each type contained two different passages: the 5th and 11th for hADSCs, and the 8th and 16th for hPDMC. The preliminary results from analysis of these 4 videos showed that the migration pattern of aggregated cellular spheroid in terms of moving speed and orientation consistency was significantly higher than those of single cells. In addition, young MSCs (with lower passage) were found to have a higher tendency to aggregate and form a spheroid than the senescent ones (with higher passage). This better spheroid-forming ability with young MSCs could result in their greater mobility than the senescent cells and thus yield their better capability in differentiation.

2298-Pos Board B435

Single Cell Biophysics Drives Wound Healing Dynamics

Dhruv K. Vig.
University of Arizona, Tucson, AZ, USA.

The ability of cells to move in collective groups or sheets is a phenomenon observed in a number of significant biological processes, such as bone remodeling, embryonic morphogenesis, wound repair and cancer invasion and metastasis. These cells are held together through cell-cell adhesion molecules and move using heterogeneous biochemical and/or environmental cues to guide force production and morphological changes. It has been observed that these cell layers exhibit non-trivial dynamics, such as vortical motion and long-range order. This suggests that the physical interactions between neighboring cells may be an important factor guiding multicellular movements. In addition, recent findings have shown that several aspects of these collective motions can be described by the biophysics of single cells, which dictates cell speed, shape, persistence of motion, traction stress and substrate adhesion. Using live-cell imaging and traction force miscopy we experimentally measured biophysical motility parameters of isolated treated and drug-treated epithelial cells. These results were then used in a mathematical model for multicellular motion to determine the role of cellular level biophysics in the collective migration of epithelial cells. The predictions of the model were tested by comparing the results to collective cell migration experiments, where we used image processing techniques to map the velocity field and force distribution of collectively migrating cells. Deviations between the experiments and the model were used to further refine the model, thereby generating new hypotheses for the biophysical mechanisms that guide epithelial cell migration.

2299-Pos Board B436

Feedback Interactions between Intracellular Contraction and Leading Edge Protrusion in Directed Cell Migration

Sangyoon J. Han, Gaudenz Danuser.
Cell Biology, UT Southwestern, Dallas, TX, USA.

Embryonic development depends on effective cell migration whose malfunction leads to abnormalities. Migration is the integrated outcome of a cycle of interconnected component processes, namely protrusion, adhesion and contraction. Most, if not all, molecular details of these processes have been established. The major remaining challenge is to identify mechanisms that couple these processes in space and time. Compared to a relatively well-established interaction between protrusion and adhesion, however, there is no understanding as to how contraction and adhesion interact dynamically at the time scale of a single migration cycle, and whether these interactions affect protrusion through adhesion-protrusion coupling. The major hurdle that makes it challenging to investigate contraction-protrusion link is from technology: there is nearly no tool to quantify myosin II-based contraction in cytoskeletal network compared to numerous imaging approaches for characterization of protrusion - adhesion coupling. Here, we hypothesize that contraction dynamically modulates adhesion at a distance, which in turn promotes or inhibits protrusion via several redundant mechanical and signaling pathways. To test this hypothesis, we developed a continuum mechanical (CM) model to infer location and time of intracellular forces in migrating cells, which will be compared against high-resolution traction force microscopy (TFM) to obtain absolute force levels and infer material heterogeneity in the cytoskeleton. Preliminary results from Ptk1 cell wound-healing assay show that intracellular force field and traction force field are highly correlated, suggesting the feasibility of the absolute intracellular force level reconstruction. To establish the 'information flow' between contraction, adhesion and protrusion, we will use a correlation analysis of spontaneous fluctuations to show the coupling and information flow between them in unperturbed cells and in a cell where candidate molecules mediating the putative link between contraction and protrusion is slightly perturbed.

2300-Pos Board B437

Forces Behind Cell Adhesion and Migration in Microgravity

Carlos Luna, Rebecca J. Stevick, Alvin G. Yew, Adam H. Hsieh.
Fischell Department of Bioengineering, University of Maryland, College Park, MD, USA.

Cells sense and respond to their environment according to many factors, including gravity. Changes in the gravitational field during space exploration may alter cellular interactions. Our understanding of the fundamental mechanisms by which gravitational forces ultimately affect cell function, however, is limited. Based on our prior observations, human mesenchymal stem cells (hMSCs) adopt a more rounded morphology during simulated microgravity (clinorotation). We hypothesize that microgravity affects the cell-substrate forces, which in turn affects cell adhesion and motility. Therefore, we investigated the correlation between traction forces, spreading and chemotaxis. As an extension to our previously reported "clinochip" device, we developed a lab-on-a-chip device suitable for implementing traction force microscopy during clinorotation. The device contains a channel coated with an array of fluorescent beads embedded in a polyacrylamide substrate that can be processed to calculate cell-substrate traction forces. For our studies, we investigated both hMSCs and osteosarcoma cancer cells (143-B), because they represent highly regulated and deregulated cell states in the osteogenic lineage, respectively. Clinorotation speeds of 0, 30, and 75 rpm were examined, and cell shape, adhesion area, traction forces, and chemotactic migration were measured. Interestingly, results indicate that hMSCs exhibit a dose-dependent response to clinorotation speed based on a shift in the population distribution of cell shape and adhesion area, while osteosarcoma cells do not. These results suggest that a deregulated cell phenotype may possess distinct mechanosensing characteristics, which may be related to our measures of cell adhesion, traction and chemotaxis. Our results are among the first efforts to directly measure the physical interplay between the cell and its substrate during simulated microgravity. This will allow us to gain a deeper understanding of the cellular mechanisms that lead to tissue-level changes, such as atrophy and reduced bone mineral density, observed in astronauts.

2301-Pos Board B438

A Molecule Based Reaction-Transportation Model Explains the Oscillatory Migration of Zyxin-Depleted Human Fibrosarcoma Cells

Jianlei Chen, Ganhui Lan.
Physics, George Washington University, Washington, DC, USA.

Cell migration is essential in biology, and it is closely related to biological functions such as wound healing, immune responses and cancer cell metastases. Without chemical or physical gradients, cells migrate randomly. Recently, the Wirtz lab discovered the large scale periodic cell migration of Zyxin-depleted human fibrosarcoma cells with period longer than 2 hours. These cells exhibit distinct regular oscillatory migration patterns in three-dimensional ECM and along one-dimensional chambers. Here, we present a reaction-transportation model based on a coarse-grained molecular picture of the process. Migrating cells have well-defined polarity and microtubules are known to play important roles. By explicitly incorporating k, we successfully reproduced the experimentally observed periodic migrating patterns. Our results suggest that, although diffusion and motor-based active material transportation (convection) both exist in cell, the periodic switching of cell's polarity is mainly due to the motor-based convection. Surprisingly, we discovered two distinct oscillatory phases: in the first phase, the polarization factors undergo simple and fast end-to-end oscillation, which would not lead to the observed large scale periodic migration; whereas in the second phase, the polarization factors not only oscillate between two cell ends but also generate vortex-like local patterns at either ends. These vortex-like patterns greatly elongate the period of the oscillation, which effectively stabilizes the migration in either direction, leading to the large scale oscillatory migration. Based on our model, the cell length dependences of various oscillatory characteristics have been predicted for future experimental verification. The identified two oscillatory phases may provide useful insights to the general picture of how cells alter direction during rather persistent migration, and the developed reaction-transportation model provides a general framework for studying the long-range cytoplasmic translation dynamics of any molecules.

2302-Pos Board B439

Flow-Driven Cell Motility under Electrical Fields

Yizeng Li, Sean Sun.
Mechanical Engineering, Johns Hopkins University, Baltimore, MD, USA.

Cells under external electric field will migrate along electrical potential differences. The direction of migration depends on the cell type. Although cell motility on 2-D substrates is facilitated by actin and myosin, polarized cells can also migrate under confined conditions when actin polymerization is inhibited. This actin-independent migration is driven by water permeation through the cell membrane. In this work, we study flow-driven cell migration under electric fields. Our mathematical model considers 1-D cells in a confined