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
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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
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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
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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
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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
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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

microenvironment. The fluid flux through the membrane is governed by the difference of chemical potential across the membrane. The osmotic pressure is obtained from the ion diffusion and flux and the hydrostatic pressure is obtained from the fluid dynamics inside the cell. The flux of cations and anions across the cell membrane is determined by the properties of the ion channels as well as the external electric field. Results show that without the contribution from actin network and myosin contraction, water permeation can also drive non-polarized cells with the presence of an external electric field. The direction of migration is affected by the properties of ion channels which are cell-type dependent. The results suggest that external voltages can be used to sort cells.

2303-Pos Board B440

Circular Dorsal Ruffles Increase Directional Persistence of Cell Migration by Actin Diffusion from Ruffles to Lamellipodia

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Circular dorsal ruffles (CDRs) are transient actin structures which have been linked to cell motility but the exact mechanism is still unclear. CDRs appear and grow in size after cells are stimulated with growth factors, such as the platelet-derived growth factor, eventually disappearing tens of minutes after stimulation. The role of CDR formation in cell motility is investigated for NIH 3T3 fibroblasts seeded on compliant polyacrylamide substrates. We found that CDR formation increases cell migration directional persistency but did not affect the migration speed. Furthermore, an increase in the localization of lamellipodial protrusion at the cell edge in the vicinity of the CDRs was observed. Relocalization of lamellipodia occured 1 to 6 min after CDR formation at the cell edge closest to the site of CDR formation. The time lag between peak CDR formation and the peak lamellipodial protrusion is then correlated with the spatial distance between the CDR and the lamellipodia; this time scale is consistent with the diffusive time scale of cytosolic globular actin (G actin). Using green to red photoswitchable Dendra2-conjugated actin, we photoconverted CDR actin from green to red and observed the subsequent appearance of red fluorescent actin in the lamellipodia at the cell leading edge. These findings help shed light on the interconversions between mesoscopic actin structures in cell behavior.

2304-Pos Board B441

Modeling Transmigration of Malaria Infected Red Blood Cells through Inter-Endothelial Slits in Human Spleens using Dissipative Particle Dynamics

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We simulate the transmigration of malaria-infected red blood cells (RBCs) through the inter-endothelial slits in the human spleen by using Dissipative Particle Dynamics based two-component RBC model. We modeled the spectrin-actin network and the lipid bilayer separately and considered the real number of the structural proteins in the model. The mechanical properties of the bilayer-cytoskeletal interactions, such as stiffness and friction, are calibrated by comparing with membrane fluctuations and tank-treading experiments. First, we further validated our numerical model by comparing the predicted retention rates of healthy and pathological cells in an 'artificial spleen' consisting of micro beads with the experimental measurements. To explore the possibility of the bilayer-cytoskeletal detachment during this transmigration process, which is strongly related to RBC aging and hereditary spherocytosis, we predicted the maximum interaction force between the spectrin-actin cytoskeleton and the lipid bilayer and compared the value with the previously predicted bilayer-cytoskeletal bond strength. Furthermore, we systematically studied the effects of cell rigidity, cell shape and inter-endothelial slit dimensions on the critical pressure gradient for RBCs to pass through the spleen. We found that the cell shape plays a much more important role than the cell rigidity in the transmigration process, which may guide the future experiments and the drug design for eradicating malaria and treating anemia such as hereditary spherocytosis.

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Restricted Exchange Environment Chambers for Creating and Manipulating Diffusive Gradients in 2D Cell Culture

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Diffusive exchange between capillaries and the tissues they serve generates concentration gradients of a significant number of soluble molecules, creating heterogeneous microenvironments on the length scale of single cells. This heterogeneity is not well captured in 2D cell culture models, and it is not easily controlled or studied in spheroid models. Here we describe a simple chamber in which diffusive gradients similar to those found in vivo can be formed and manipulated. In restricted exchange environment chambers (REECs), cells are grown in a narrow gap formed by two coverslips, and diffusive exchange occurs via one or more small openings machined into one coverslip – through which the cells exchange nutrients and metabolic waste with the bulk medium. Based on a concept similar to the sandwich assay (Hlatky and Alpen, Cell Tissue Kinet., 18:597, 1985) and compatible with multiwell plate formats used in high-throughput investigations, REECs improve experimental control of gradient structure in cell culture. Because diffusive concentration gradients vary as a function of distance from a source or sink, the dimensions of the chamber (e.g. height of the gap) and the number, shape, and size of openings create the gradient structure. For example, in REECs with a single round opening (order 200 μm diameter), concentration gradients form radially. Only cells within a several hundred micrometers of the opening exchange sufficient metabolites to survive - similar to diffusive exchange near a capillary in a tissue. Alternatively, cells cultured in REECs with two parallel bar-shaped openings produce the 1-dimensional equivalent of a spheroid. Using these restricted exchange environment chambers, we find that fibroblasts align themselves along the axis of diffusion while MDCK cells do not. MDCK cells do, though, exhibit morphological variations along the diffusive gradient.

2306-Pos Board B443

Coupling a Mechanosensitive Channel with a Vesicle under Shear Flow

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Mechanosensitive channels enable cells to respond to their local environment. Continuum mechanical models have been proposed to describe how bilayer deformation induced by the transmembrane protein and the membrane tension influence the free energy of channel gating under static conditions. The dynamics of mechanosensitive channels under flow conditions however remains largely unexplored. Cells under flow display interesting features not observed under static environments. Here we present a model coupling a mechanosensitive channel with the dynamics of a vesicle under shear flow to investigate how the channel gating responds to hydrodynamic stress. The model could be used to investigate the release of signaling molecules, transport of ions or drugs across cell membranes under flow in biological systems, as well as the design and control of channel gating in synthetic cells.

2307-Pos Board B444

Perturbing the Active Process of Hair Cells: Self Recovery of Spontaneous Oscillations Following Overstimulation

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In the inner ear, hair cells perform the transduction of mechanical input into electrical output. An energy-consuming process enhances their sensitivity to incoming auditory and vestibular stimuli. One manifestation of this active process is spontaneous oscillation of the mechano-sensitive organelle, the hair bundle, which is at the apical surface of each hair cell. To attain this increased sensitivity, the hair bundle is postulated to operate near a bifurcation, where an internal control parameter vital to the active process determines whether the bundle shows limit cycle oscillations or is quiescent. This control parameter may be linked to adaptation in vertebrate hair cells and could help explain how prolonged high-level sounds cause a temporary threshold shift in mammalian hearing. High amplitude, prolonged deflection of bullfrog sacculus bundles have been shown to temporarily suppress spontaneous oscillations, suggesting a readjustment of the control parameter through a bifurcation. The transition back from quiescence to limit cycle oscillations has been shown to depend on the duration of the imposed deflection and on calcium ion concentration around the mechanically gated transduction channels.

Here, we present experiments where we identify other environmental factors that affect this control of the active process. We introduce various pharmacological agents to manipulate the mechano-sensitive transduction channels and the myosin motors inside the hair bundles. We compare how these agents affect particular components of the internal control parameter by measuring the duration of the induced quiescent intervals and the time scales associated with the return of the bundle's position to equilibrium. Additionally, we attach magnetic bead particles to the hair bundles and deflect with a strong magnetic field. Thus, hair bundles avoid physical contact with the stimulus probe, and experience no external hydrodynamic effects. Selected results are discussed.