Cell Mechanics & Motility - II

3184-Pos Numerical Analysis of Impedance Fluctuations Measured by Electric Cell-substrate Impedance Sensing

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Board B487

For more than a decade, electric cell-substrate impedance sensing (ECIS) has been used to monitor cell behavior in tissue culture and has proven to be very sensitive to cell morphological changes and cell motility. In this method, cells are cultured on small gold electrodes carrying weak AC signals. The impedance of these electrodes changes dramatically when cells attach and spread on their surface, because the cell membranes restrict the current flow. More surprisingly, cell motion manifests itself in small-scale fluctuations, or "noise," in impedance measurements. We have taken ECIS measurements on several cultures of non-cancerous and cancerous human ovarian surface epithelial cells. By analyzing the noise in real and imaginary electrical impedance, we demonstrate that it is possible to distinguish the two cell types purely from signatures of their electrical noise. Our measures include power-spectral exponents, Hurst and detrended fluctuation analysis, and estimates of correlation time; principal-component analysis combines all the measures. The noise from both cancerous and non-cancerous cultures shows correlations on many time scales, but these correlations are stronger for the non-cancerous cells. We also applied noise analysis to detect the presence of low levels of toxins. Time-series impedance data were taken for 3T3 cells exposed to various concentrations of cytochalasin B. At the small concentrations in the impedance curves, it would be very difficult to pick out by eye features that could distinguish poisoned from healthy cells. A clear trend, however, is evident in the time of first zero crossing of the autocorrelation. The numerical methods used in this study open a new way to quantify impedance fluctuations and more thoroughly explore cell behavior and responses via the ECIS methodology.

3185-Pos Mesoscopic Modeling Of The Mechanical Behavior Of Biophysical Networks

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Board B488

Cross-linked networks of biopolymers, like those constituting the cytoskeleton, have the remarkable ability to strain-stiffen. Because of this property, F-actin, defining the actin cortex, provides the cell's mechanical stability during cell motility. About a decade ago, it was proposed that this stiffening originates from entropic effects in the behavior of semi-flexible polymers. This has evolved into the mean-field theory developed by MacKintosh and co-workers, that presumes that the network distorts in an affine manner as the sample is deformed. However, more recent numerical studies of discrete networks in two dimensions have revealed non-affine behavior at low and intermediate densities. Along those lines, we here present novel three-dimensional studies of the large-strain mechanical response of discrete, networks of, in particular, cross-linked actin filaments. We will argue that under physiological conditions, entropic effects are overwhelmed by non-affine enthalpic effects. More specifically, we will demonstrate that three-dimensional network behavior not only depends on measurable quantities such as actin concentration and cross-link density but also on network architecture, for instance through the connectivity and the filament length. This explains how small changes in the concentration of length controlling proteins in the cytosol can induce large changes in stiffness of the actin network.

3186-Pos Depolymerization-driven Flow In Nematode Sperm Relates Crawling Speed To Size And Shape

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Board B489

Cell crawling is an inherently physical process that includes protrusion of the leading edge, adhesion to the substrate, and advance of the trailing cell body. Research into advance of the cell body has focused on actomyosin contraction, with cytoskeletal disassembly regarded as incidental, rather than causative; however, extracts from nematode spermatozoa, which use Major Sperm Protein rather than actin, provide one example where cytoskeletal disassembly apparently generates force in the absence of molecular motors. To test whether depolymerization can explain force production during nematode sperm crawling, we constructed a mathematical model that simultaneously describes the dynamics of both the cytoskeleton and the cytosol. We also performed corresponding whole cell experiments using Caenorhabditis elegans spermatozoa. Our experiments reveal that crawling speed is an increasing function of both cell size and anteroposterior elongation. The quantitative, depolymerization-driven model robustly predicts that cell speed should increase with cell size and yields a cytoskeletal depolymerization rate that is consistent with previous measurements. Notably, the model requires anisotropic elasticity, with the cell being stiffer along the direction of motion, to accurately reproduce the dependence of speed on elongation. Our simulations also predict that speed should increase with cytoskeletal anisotropy and depolymerization rate.

3187-Pos Modeling Cell-Matrix Interactions During Angiogenesis: Role of Matrix Topography

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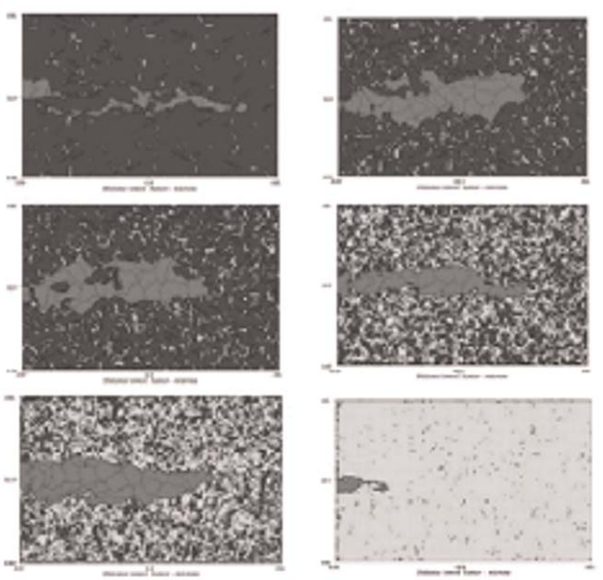
Board B490

The extracellular matrix plays a critical role in orchestrating the events necessary for wound healing, muscle repair, morphogenesis, angiogenesis, and cancer invasion. We investigate the influence of extracellular matrix topography on the coordination of multi-cellular interactions in angiogenesis. We validate our spatiotemporal cellular model of angiogenesis against empirical data, focus on the effects of extracellular matrix topography on capillary sprout morphology and average extension speeds. We vary the density of the matrix fibers to simulate different tissue environments and to explore the possibility of manipulating the extracellular matrix to achieve pro- and anti-angiogenic effects. The model predicts specific ranges of matrix fiber densities that maximize sprout extension speed, induce branching, or that interrupt normal angiogenesis. We then explore matrix fiber alignment as a key factor contributing to peak sprout velocities, and in mediating cell shape and orientation. We also quantify the effects of proteolyticmatrix degradation by the tip cell on sprout velocity and conjecture that degradation promotes sprout growth at high densities, but has an inhibitory effect at lower densities. Our results are discussed in the context of ECM targeted pro- and anti-angiogenic therapies that can be tested empirically.



3188-Pos Viscous Regularization And R-adaptive Remeshing For Finite Element Analysis Of Lipid Membrane Mechanics

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Board B491

Lipid bilayer membranes are a critical part of life because they serve as a barrier to separate the contents of the cell from the external world. As two-dimensional fluids, these membranes are a thin shell structures that resist bending and stretching but are unable to sustain shear stresses. This property gives membranes the ability to adopt dramatic shape changes. In this talk, we describe a finite element model to study equilibrium mechanics of membranes. In particular, we propose an artificial viscosity method to improve the convergence rate and to stabilize the mesh performance. The Augmented Lagrangian method is used to enforce area and volume constraints during membrane deformations. As a validation of our method, equilibrium shapes for a shape-phase diagram of lipid bilayer vesicle are calculated. These numerical techniques are also shown to be critical for simulations of large deformation problems. For further demonstrations of the methods usefulness, the formation of tethers (long tube-like extensions) is simulated in three dimensions.

Lastly, using Ginzburg-Landau theory, we further extend our model to treat the phase separation of a two-lipid-component vesicle. To deal with the large mesh distortions of the two-phase model, we explore modification of the artificial viscosity method to achieve r-adaptive mesh optimization.

3189-Pos Modeling Cell Movement: Mechanics, Forces, and Cell Shape Maintenance

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Board B492

Cell movement is an important process during development of organs, cancer metastasis, and wound healing. Many different eukaryotic cells show amoeboid movements when they crawl. This amoeboid movement is thought to involve protrusion of the "foot" or lamellipod, adhesion, and contraction of the cytoskeletal network. Different cells exhibit different shapes during movement, presumably utilizing a common set of mechanisms such as polymerization/depolymerization of filaments, bundling/debundling cytoskeleton, adhesions and molecular motors. In order to quantitatively investigate the roles of these mechanisms, we present a continuum model of cell movement. We mathematically incorporate the growth of cell cytoskeleton, forces from molecular motors and formation of adhesions in a unified framework. Elastic stress in the cell during movement is computed. Conditions at which different cell shapes are achieved are investigated. Commonly observed motions such as cell ruffles are described. The model depends on the mechanics of the cytoskeletal network and our formulation can incorporate experimentally measured constitutive laws. Finally, forces exerted by a moving cell on the substrate are also computed.

3190-Pos Understanding The Molecular Architecture Of The Budding Yeast Kinetochore By Live Cell K-SHREC

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Board B493

The kinetochore is a complex protein machine that drives chromosome movement during mitosis along the plus-ends of attached microtubules. The core structure of the kinetochore, predicted to be ~ 60 nm in length, consists of more than 40 different proteins. A mechanistic understanding of how these proteins assemble and interact with the microtubule requires the localization of each complex within the kinetochore. We have adapted the technique for "Single molecule High Resolution Colocalization" (SHREC) to determine the separation between selected pairs of kinetochore proteins *in vivo*. Cells expressing the selected pair of kinetochore proteins, one tagged with GFP and the other with tdTomato at the C- or N-terminus, are imaged simultaneously in two colors. During metaphase, the 16 kinetochores in each spindle half of a budding yeast cell are oriented with their axis approximately parallel to the spindle axis, and appear as a diffraction-limited spot. Therefore, the separation between the centroids of the GFP and tdTomato image of a kinetochore cluster provides the average distance separating the two kinetochore proteins. Measurements of the NDC80 complex -

1. the C-termini of Nuf2p and Ndc80p,
2. C-termini of Nuf2p and Spc24p, and
3. N-terminus of Ndc80p and C-terminus of Spc24p yield distances of 1.7+/−7.6 nm, 8+/−11 nm, and 37+/−14 nm.

This is consistent with the EM data showing the 57 nm long NDC80 complex bound to a microtubule with a 40° angle between the axes of the molecule and the microtubule. We will to target C- and N-termini of the largest proteins from each kinetochore protein complex for localization. The distance information for key kinetochore proteins combined with their copy number per attachment site will allow a three dimensional visualization of the protein architecture of around the microtubule plus-end.

3191-Pos Three Dimensional Structure of Sheared Biopolymer Networks using Confocal Microscopy

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Unlike most materials, many biopolymer networks have the interesting property that they strain stiffen. Theoretical models have been proposed to explain the underlying filament dynamics that give rise to this bulk property. To test these models experimentally we visualize in-vitro sheared biopolymer networks using confocal microscopy. Using image processing we accurately locate fiber position and connectivity and thereby 3D structure. We find fiber orientation and distribution of fiber lengths as a function of shear for fibrin, actin bundled with filamin and collagen networks.

3192-Pos The Roles Of Oxldl, Cholesterol And Caveolae In Endothelial Force Generation

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Board B495

Our recent study has shown that exposing aortic endothelial cells to oxLDL results in an increase in force generation as estimated by a gel contraction assay in 3D. We have also shown that a similar effect is induced by cholesterol depletion. These observations are consistent with the earlier studies showing that oxLDL may result in cholesterol depletion from endothelial caveolae. In this study, we show that indeed oxLDL-induced increase of endothelial force generation is strongly inhibited further supporting the hypothesis that this effect is mediated by cholesterol depletion. In contrast, exposing the cells to sphingomyelinase (SMAse), an enzyme that hydrolyses sphingomyelin, the second major lipid component of cholesterolrich membrane domains had no effect indicating that oxLDL-induced hydrolysis of sphingomyelin cannot be responsible for the observed increase in endothelial force generation. Furthermore, we have addressed a question of whether endothelial caveolae are involved in endothelial force generation. To achieve this goal, we compared the degree of force generation in endothelial cells stably transfected either with a caveolin mutant that is phosphorylation deficient (Y14F) or a mutant that is deficient in olygomerization (C156). Earlier studies have shown that expression of either of the two mutants results in a significant decrease in the amount of caveolae. Our observations show, however, that the impacts of the two mutants on the endothelial force generation are significantly different. Specifically, while expression of the phosphorylation-defficient caveolin mutant results in an increase in the force generation, similar to cholesterol depletion, expression of the oligomerization-defficient caveolin mutant has no effect. In addition, we show that depleting the cells of cholesterol further increases the degree of force generation in both mutants similarly to the wild type.

3193-Pos Measuring Forces from Membrane Geometry

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Board B496

Recent advances in cryo-electron tomography have made possible full 3-D reconstructions of subcellular structures. In particular, tomography can be exploited to capture detailed information about complex membrane conformations. We propose that lipid bilayer

membrane shapes, such as the ones observed in cryo-tomography, can be modeled as an elastic material under force. Using results derived from a Helfrich elasticity model, we can determine the forces acting on a membrane directly from the observed shape. We compare these results with in vitro experiments performed on synthetic vesicles, where we apply known forces with optical tweezers.

3194-Pos Statistical analysis of cell movements during *Drosophila* embryo morphogenesis

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Board B497

Embryo morphogenesis involves a fascinating and complex choreography of cell movements that are highly regulated both in time and space. The recent development of imaging and image processing tools, such as 4D nonlinear microscopy or 3D cell tracking, allows to follow the dynamics of cell movements *in vivo* within a whole embryo and to quantify morphogenetic events among a large population of cells. By using quantitative description inspired by condensed matter physics, we further developed cell movement analysis to investigate the cell kinetic ordering within a large population of cells. An order parameter is defined, which allows to follow the correlations of cell movements and to describe the spatial and temporal propagation of these correlations. This approach allows to identify specific cell collective behaviors leading to tissue and organ morphogenesis and to address questions, such as the spatio-temporal scale and the nature of cell-cell interactions involved in morphogenesis. The final goal of this analysis is to study the emergence of shape during embryo development. We illustrate this approach by analyzing *Drosophila* embryo gastrulation. This early stage of development exhibits the first morphogenetic movements, including the ventral furrow formation, which is both a tractable model of invagination and a beautiful example of cell collective motion.

3195-Pos Is Early Development Affected by Microgravity?

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Board B498

Morphogenesis is under strict genetic control. However organisms acquire forms and shapes through physical mechanisms that may be affected by environmental factors. We focused on microgravity effects on sorting, an essential morphogenetic process that leads to compartmentalization in embryonic development. According to the Differential Adhesion Hypothesis, sorting proceeds in analogy with phase separation in immiscible liquids and is driven by the differential strength of adhesion between populations of motile cells. We followed sorting in mixed 500-micron aggregates of fluorescently labeled Chinese Hamster Ovary (CHO) cells and mouse L-fibroblasts cultured in 1 g and in simulated microgravity using NASA's High Aspect Ratio Vessel (HARV). The spatial variation of the fluorescence density with the evolution of the sorting pattern was analyzed at a depth of 60μm into the aggregates, using confocal microscopy and an in-house produced software. Initially the cells were randomly distributed and the intensity was uniform. With time CHO cells sorted to the interior of the aggregate and the intensity distribution shifted accordingly. We found no detectable difference between the rate of sorting in the presence and absence of gravity. This result is consistent with the successful development of embryos in space. Nevertheless this finding is surprising due to the observed effect of microgravity on tissue mechanical properties such as cohesivity (as measured by tissue surface tensiometry) and viscosity. The variation of these quantities reflects modification at the cellular level. Indeed, under microgravity cellular adhesion and cytoskeletal integrity are strongly altered as we demonstrate by SEM, and comparison with latrunculin treatment. Our findings imply that despite its strong influence on biomechanical properties, microgravity does not derail the normal progression of morphogenetic processes such as sorting.

Supported by NSF-056854

3196-Pos A Digital Heterodyne Laser Interferometer For Studying Cochlear Mechanics

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Board B499

Laser interferometry is the technique of choice for studying the smallest displacements of the hearing organ. During low-level sound stimulation, these displacements may be below 1 nm, which cannot be reliably measured with other presently available techniques in the intact organ of Corti. In a heterodyne interferometer, light is projected against an object, the motion of which is to be studied. Motion of the target along the optical axis causes phase and frequency modulations of the back-reflected light. The membrane of the cochlear cells will reflect less than 0.01% of the incident light back. To recover object motion under these conditions, the reflected light is made to interfere with a reference beam of artificially altered frequency, producing a sinusoidal voltage at the output of the photodetector. In conventional interferometers, this carrier signal is demodulated with analog electronics. In this paper, we describe a digital implementation of the technique, using direct carrier sampling. This results in lower noise and reduces the cost of the system. Within the hearing organ, different structures may move in different directions. It is therefore necessary to precisely measure the angle of incidence of the laser light, and to precisely localize the anatomical structure where the measurement is performed. Therefore, the

interferometer is integrated with a laser scanning confocal microscope that permits us to determine crucial morphometric parameters in each experiment. We provide key construction parameters and a detailed performance characterization. We also show that the system accurately measures the tiny vibrations present in the apical turn of the cochlea during low-level sound stimulation.

3197-Pos Shear-Stress Induced Mechanical and Structural Changes in the Vicinity of Endothelial Cell Mechanosensors

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Board B500

Hemodynamic forces have been shown to play a significant role in atherosclerotic plaque localization; regions of arteries exposed to relatively lower fluid shear stresses (less than 4 dyn/cm^2) are prone to these plaque formations, while regions exposed to higher stresses (greater than 15 dyn/cm^2) are atheroprotected. Endothelial cells, whose dysfunction leads to atherogenesis, are constantly exposed to forces from the blood, and have mechanisms of sensing differences in fluid shear stress. In this study, we examined structural and mechanical changes in the vicinity of integrin, Caveolae, and PECAM mechanosensors as a response to fluid shear stress conditions that mimic in vivo athero-protected and athero-prone regions. We employed an optical tweezer-based cytorheometer - confocal microscopy dual setup to simultaneously probe the storage (G') and loss (G'') moduli and visualize structural changes in the vicinity of the three mechanosensors as a response to different fluid shear stress conditions.

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3198-Pos Studies of Mitochondrial Dynamics in G1 Arrested S. cerevisiae by Fourier Imaging Correlation Spectroscopy

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Board B501

The intracellular environment can be described as a complex, heterogeneous fluid. The size of various intracellular species span many length scales, so that cytoskeletal motor proteins are sometimes necessary to transport cargo faster than simple diffusion would allow. Mitochondria in mammalian cells are known to associate with elements of the cytoskeletal machinery, and the stability of microtubules and microfilaments affects the dynamics of the organelle. Here we implement Fourier Imaging Correlation Spectroscopy (FICS) to study mitochondrial motility in G1 arrested *S. cerevisiae*. Our method permits observation of mitochondrial dynamics on four discrete length-scales from 0.6 μm to 1.19 μm, providing detailed information about the dependence of the organelle's motion on critical aspects of the cytoskeleton. Pharmaceutical assays with Nocodazole and Latrunculin A, responsible for destabilizing microtubules and microfilaments respectively, indicate that the actin cytoskeleton is essential for the motility of mitochondria while the microtubule cytoskeleton has no effect on the dynamics of mitochondria in G1 arrested cells. Mitochondrial motility in actin filament depleted yeast cells also exhibit simplified dynamics, which is consistent with observations made of mitochondria in mammalian cells treated with Nocodazole and Latrunculin A. A strain with the mutation Act1- V159N exhibits mitochondrial dynamics faster than wild-type on lengthscales upwards of 0.79 μm. This information supports a model that mitochondrial dynamics are driven by actin polymerization since in vitro studies of Act1-V159N also reveal this protein to have a higher rate of polymerization than wild-type actin. When Arc18p, which is a subunit of the Arp2/3 complex, is deleted from the yeast genome, mitochondrial dynamics are slowed across length-scales larger than 0.79 μm. This is consistent with the actin-polymerization model of mitochondrial motility in yeast.

3199-Pos Distance-Dependent Relaxation of Adherent Cells

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Board B502

Cells in their native environment are constantly exposed to local mechanical stresses and strains. The global cellular response to these stimuli depends on the propagation of mechanical perturbations through the cell. Here we directly quantify intracellular mechanical coupling of adherent cells by locally perturbing the apical cell surface with an AFM cantilever and observing the cellâ™s response away from the perturbation in three dimensions by video microscopy of defocused fluorescent beads. We find that both the deformation and time-scale of the cellular response at a given point depend on the distance to the point of the original perturbation. These results are consistent with a role for cytoplasmic flow in mechanical coupling within cells.

3200-Pos Non-Contact, Low Pressure Interrogation of Live Cells Using Scanning Ion Conductance Microscopy (SICM)

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Board B503

SICM uses a scanned nano pipette to obtain high resolution, non-contact images of soft surfaces such as live cells. A potential is applied between an electrode in the pipette and an electrode in the surrounding physiological-like bath. The resulting flow of electrons is used as a distance feedback control mechanism maintaining a constant pipette-sample separation.

Using hydrostatic pressure, a known and controllable force can be generated at the pipette tip, typically 50 nm - 2 μm in radius, while measuring the sample's dimensional response. The pressure is small, 0–100 kPa, and is effectively coupled to the surface underneath without pipette contact. We have automated and calibrated this new pressure application method. Using both neurons and osteoblasts, we demonstrate this capability for live cell measurement in multiple ways. A cell's Young's modulus can be quantitatively determined and variations in cell stiffness between different sections of a cell mapped and compared to surrounding cells. In addition, the effect of the cytoskeleton on cell stiffness is studied by using the cytoskeletal disruption agents Cytochalasin D and Latrunculin B. Changes to the rate of pressure application or periodic pressure excitation of a cell can be used to determine a cell's viscoelastic characteristics. This work demonstrates just some of the possibilities for this new investigative method.

3201-Pos Motion Analysis of Actin Flaments and Microtubules In Neuronal Growth Cones

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Board B504

Neuronal growth cones are highly motile structures at the tip of neurites, exploring the environment in search for its final destination. Growth cones are composed by lamellipodia and filopodia. In this study, we have analysed the kinetics of filopodial motion of live PC12, DRG and hippocampal neurons. DIC images of moving growth cones were obtained with a confocal microscope and a stack of four images at different focal depths (0, 1, 2 and 3 μm above the glass where the neurons are plated) were acquired every 5–10 seconds. Using an operator assisted program, we recovered the three dimensional motion of several tens of entire filopodia emerging from growth cones. Filopodia moved quickly lifting their tips up to 3 μm from the bottom of the glass. The velocity of their tips could reach values up to 1–2 μm sec-1. Three different types of exploratory motions were identified: rigid growth or retraction, lateral motion and bending. In some growth cones, retraction and growth of neighboring filopodia was significantly correlated. During their exploration, filopodia visited a large portion of the free space around them. We also analysed interactions between neighboring growth cones and we quantified the dynamics and properties of the formation of physical contacts between them. Often when a stable contact was formed one of the growth cone retracted, maintaining the established connection. To investigate the role of microtubules and actin filaments in filopodia motion, we fluorescently labeled these cytoskeletal components in live neurons. PC12 cells and hippocampal neurons were electroporated with plasmids encoding for ds-red actin and EB1-GFP and a 3D reconstruction of moving actin filaments and microtubules was obtained.

3202-Pos Role of Cigarette Smoke in the Transendothelial Invasion of Ovarian Carcinoma Cells

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Board B505

Although the literature is equivocal concerning the role of cigarette smoking and the risk of developing ovarian cancer, little is known about the role of cigarette smoke in transendothelial invasion of ovarian cancer cells. We investigated this issue by examining the effects of cigarette smoke condensate (CSC) on barrier function and directional migration of human umbilical vein endothelial cells (HUVECs), and on the invasive activities of ovarian carcinoma cells through HUVEC monolayers as well. Central to this work was the use of electric cell-substrate impedance sensing (ECIS), a cell-based biosensor that monitors motility and other morphology changes of cells adherent on small gold electrodes. Upon addition of different concentrations of CSC, the wound healing rate of the HUVEC layer decreases as CSC concentration increases from 0.01 to 0.10 mg/ml, whereas the transcellular resistance increases within the first 10 hours of CSC exposure, indicating an increase in cell adhesion molecule (CAM) expression. Following the addition of ovarian cancer cells to HUVEC layers pre-exposed to different CSC concentrations for 20 hours, dose-dependent changes of the resistance curves were observed. An initial decrease due to the retraction of HUVEC layer was followed by a progressive increase due to the penetration of ovarian cancer cells. Parallel with the ECIS measurement, transendothelial penetration of ovarian cancer cells was verified by confocal fluorescence microscopy using CellTracker dyes. Our results suggest that CSC is detrimental to normal endothelial cell function in maintaining vascular integrity. In addition, the chemicals present in CSC may facilitate transendothelial invasion of ovarian cancer cells.

3203-Pos Spontaneous cell movement and galvanotactic response in Dictyostelium cells

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Board B506

Cells can show not only spontaneous movement but also tactic responses to environmental signals. Since the former can be regarded as the basis to realize the latter, it is important to investigate spontaneous cell movement and its fluctuations quantitatively and identify its control mechanism. For that purpose, we took a series of spontaneous movement of Dictyostelium cells under development and characterized these trajectories through statistical analysis. We successfully applied a Langevin model with non-linear decay rate, memory, and additive and multiplicative noises of its velocity dynamics to the experimental data. Based on the results, next we analyzed tactic response in view of the modulation of spontaneous movement. We took a series of galvanotactic movement of Dictyostelium cells under various field strengths and analyzed these trajectories by applying the proposed Langevin model. We estimated the degree of produced bias in the movement and characterized various dynamical properties. Finally, we would discuss about the relating molecular system and the consistent relationships between spontaneous movement and tactic response.

3204-Pos Behavior of signaling molecules in electrotaxis of *Dictyostelium* cells

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Board B507

The importance of electric signal has been gradually realized in variety physiological phenomena such as embryogenesis, neurogenesis and wound healing. Typical response triggered by electric signal is directional cell migration toward cathode or anode, called electrotaxis. However, the underlying mechanism of electrotaxis remains unknown. Previously, we have reported that phosphatidylinositol-3-OH kinase (PI3K) and guanylyl cyclases (GC) dependent signaling pathways are involved in electrotaxis in Dictyostelium cells. Here, we investigated the intracellular dynamics of these signaling molecules during electrotaxis of Dictyostelium cells. By using green fluorescent protein (eGFP), we observed PI3K and PIP3-binding protein, PH-Akt/PKB for PI3K-dependent pathway. PI3K-eGFP and PH-Akt/ PKB-eGFP were localized at the leading edge pseudopod facing to cathode in electrotaxis. Such localization of PI3K-eGFP and PH-Akt/PKB-eGFP at the regions facing to cathode was inhibited by the treatment of latrunculin A, a F-actin-depolymerizing reagent. Next, we examined the soluble guanylyl cyclase (sGC) and cGMP-binding protein C (GbpC) for GC-dependent pathway. Since GbpC has been known as a major binding target for intracellular cGMP, sGC and GbpC are the upstream and downstream molecules of cGMP, respectively. Both sGC-eGFP and GbpC-eGFP exhibited the similar behavior as observed in the case of PI3K-eGFP for both latrunculin A-treated and -untreated cells. These results suggest that PI3- kinase and PH-Akt/PKB do not involved in the directional sensing of electric field directly and that PI3-kinase and GC dependent signaling pathways may enhance electrotactic efficiency through the stabilization of polarized shape by controlling actin and myosin-dependent signaling pathways.

3205-Pos Traveling Waves In Inositol Phospholipids Signaling Pathways For Spontaneous Cell Migration And Tactic Responses

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Board B508

Spontaneous cell migration is the basis to realize the tactic response to environmental cues. In Dictyostelium cells, the spontaneous migration and shape changes are not random, but driven in an oscillatory manner. How spontaneous cell migration is generated remains to be unsolved. To investigate the mechanisms of spontaneous cell migration, we have focused on intracellular dynamics of both phosphoinositide-3 kinase (PI3K) and phosphatase tensin homolog deleted on chromosome 10 (PTEN) molecules. PI3K and PTEN are localized exclusively on membrane, where catalyze the production and degradation of PIP3 respectively. Such complementary localization of PI3K and PTEN is a molecular basis of PIP3 localization at the PI3K-dominant side, which leads to pseudopod formation. Thus, the PI3K-PTEN system can be regarded as a compass for cell migration. Here we report spontaneous generation of traveling waves in the PI3K-PTEN system on membrane of Dictyostelium cells. Fluorescent-labeled pleckstrin homology domain of Akt/PKB, a reporter for PIP3, and PTEN were observed simultaneously in the cells, which revealed self-organizing oscillatory changes on membrane without external signals. This means that spontaneous pseudopod formation was driven by oscillatory activities of PI3K-PTEN system. External signals may bias the direction of cell via modulation of PI3K-PTEN system for tactic responses. We will discuss the possible mechanisms for the traveling waves in the inositol phospholipids signaling pathway based on reaction-diffusion dynamics of PI3K and PTEN.

3206-Pos Stem cell fate directed by Matrix Elasticity and Ligands

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Mesenchymal stem cells (MSCs) from adult bone marrow have recently been found responsive to matrix elasticity in their differentiation. Collagen-I coated hydrogels induce MSCs to express neurogenic, myogenic, and osteogenic markers depending on the Young's modulus E (~1 - 34 kPa) that is used to approximate the elasticity of native tissue. While collagen is the most abundant protein in mammals, hyaluronic acid (HA) is the major non-protein factor in the marrow and is a widely distributed load-bearing matrix polysaccharide that promotes proliferation and migration during embryonic development and other processes. We show that MSCs dynamically express an HA-receptor, and we use the tunable elasticity of novel HA hydrogels to understand the morphology, motility, and fate choices of MSCs as they depend on matrix elasticity and adhesive ligands. Marrow-derived hematopoietic stem cells (HSCs) are also studied, and the results amplify the influence of matrix elasticity in stem cell fate choices.

3207-Pos Stalled Contraction of a Biological Spring, *Vorticella Convallaria*, in a Microchannel

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The stalk of *Vorticella convallaria*, a sessile peritrich, generates speeds and forces hundreds to thousands of times greater than those of other cellular machines based on molecular motors or polymerizing fibers. When stimulated, the stalk of *Vorticella* contracts over a few hundreds micrometers in a few milliseconds using Ca^{2+} not ATP. The contractile organelle inside the stalk is the spasmoneme, and its major components are EF-hand Ca^{2+}-binding proteins including spasmin and centrin. Therefore, spasmoneme is biochemically related to motile processes relying on centrin in other eukaryotes. This study describes biomechanical characteristics of spasmoneme contraction based on stalling contraction, and a key measure is the maximum force that it can develop. We found that Poiseuille flow of highly viscous PVP solution in a microchannel exerts significant viscous drag force on live *Vorticella* attaching to the bottom of the channel. As PVP concentration increased to 4% (flow rate = 15.15 ml/min), cells contracted to shorter distances taking a longer time to complete contraction, the maximum contraction speed decreased being approximately proportional to viscosity^{-1}, and time lag between body contraction and stalk contraction increased. From the external drag force, the estimated maximum contraction force of live *Vorticella* is 100 ~ 400 nN, which is comparable to the 180 nN force obtained from micropipette experiments. The stall force places physical bounds on any possible models of spasmoneme contraction. The microchannel format is an optimized setup to stall the contraction of *Vorticella*, and it enables further investigation into the relationship between Ca^{2+} and contraction force development of *Vorticella*.

3208-Pos Elasticity, Mechanosensing And Traction By Bone Cells Under Varying Morphologies

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It is unclear whether morphology difference, dictated by the geometry of attachment is critical for cell rheology and mechanosensing. Thus, we studied the rheology and mechanosensitivity of bone cells under different morphologies using atomic force microscopy and our two-particle assay for optical tweezers. Primary osteoblasts were isolated from avian bone using a sequential collagenase treatment. MLO-Y4 cells were used to model osteocytes and MC3T3-E1 cells for osteoblasts. All cells were incubated at room temperature in CO_2-independent medium without serum immediately before measurements. Elasticity was determined using a Hertzian contact model for both flat and round morphologies. We found that the elastic modulus of MLO-Y4 osteocytes when flat and adherent ($>$ 1kPa) largely differed when round but partially adherent ($<$ 1kPa). The elasticities of round suspended MLO-Y4 osteocytes, MC3T3-E1 osteoblasts, and primary osteoblasts were similarly $<$ 1kPa. The mechanosensitivity of round suspended MLO-Y4 osteocytes was investigated by monitoring nitric oxide (NO) release, an essential signaling molecule in bone. These cells were stimulated by oscillatory undulations of the integrin-bound spheres upto 30pN force. Interestingly, the NO released increased in response to 5pN force stimulation, in contrast with flat cells, which required 20nNforce stimulation while releasing lesser NO. Furthermore, force traction fluctuations by round suspended cells increased amplitude positively correlating to increased optical trap stiffness. Our results suggest that a round cellular morphology supports a less stiff cytoskeleton configuration compared with flat cellular morphology. This implies that osteocytes take advantage of their ellipsoid morphology *in vivo* to sense small strains benefiting bone health. Our assay provides novel opportunities for *in vitro* studies under a controlled suspended morphology versus commonly studied adherent morphologies.

3209-Pos Continuum-scale modeling of Stress Fibers requires accounting for condensation forces

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Stress fibers are bundles of aligned actin filaments forming between points of cytoskeletal attachment to substrate, at which the contractile actin-myosin stress can be resisted. An important characteristic of a stress fiber is that it acts like a separated phase, forming a distinct channel that delivers focused tension between two distant points. Recent models of stress fiber formation in contractile cells capture a number of experimentally-observed features, but not the focused nature of stress transmission. The stresses at constraints typically diffuse out. This was attributed to the elliptic nature of the continuum equations solved, due to the implicit underlying assumption of continuous strain gradients. In this paper we suggest that the inability to capture the discrete nature of stress-fiber force transmission arises from the mechanistic assumptions in modeling actin-myosin interaction. We argue that, in cytoskeletal actin networks, the direction of contractile force is more likely to be determined by the direction of myosin filaments than actin filaments. Consequently, an additional force acts perpendicular to actin filaments in tension, which has the effect of condensing the filaments into a phase separation, and results in a continuous line of un-dissipated force in our simulations.

3210-Pos Bidirectional Motility Of Membrane Tubes Pulled By Non-processive Motors

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Dynamic interactions between the cell's cytoskeletal components and the lipid membranes that compartmentalize the cell interior are critical for intracellular trafficking. A trademark of these cytoskeletal-membrane interactions is the presence of continuously changing membrane tube networks. Membrane tubes are formed when a localized force is exerted on a membrane by e.g. clusters of cytoskeletal motors. We investigate how the tube pulling process depends on the fundamental properties of the motor collections involved. Previously, it has been shown that tubes can be pulled by processive motors that dynamically associate at the tip of a membrane tube [Koster et. al *PNAS* 2003, Leduc et. al *PNAS* 2004]. Here, we use a minimal *in vitro* model system to show that membrane tubes are also formed by cooperating non-processive motors and that these tubes display rich dynamics as compared to those pulled by processive motors. We report the emergence of a distinct switching behavior: the tubes pulled by non-processive Ncd alternate between forward and backward movement with variable speeds, ranging from +120nm/s to -220nm/s. This bidirectional switching is entirely absent in membrane tubes extracted by processive Kinesin motors which proceed at constant positive speeds up to 400nm/s. We present a model assuming only a simple motor clustering mechanism along the length of the entire tube and the presence of a length-dependent tube tension. We show the switching phase to be an attractor of the dynamics of this model, suggesting that the switching observed experimentally is a robust characteristic of nonprocessive motors. Our findings present non-processive motors as an alternative source for *in vivo* bidirectional tube dynamics, often credited to the presence of a mixture of plus and minus ended motors.

3211-Pos Native Increase in Intercellular Contact between Lateral Epidermis and Amnioserosa in *Drosophila* Embryonic Dorsal Closure Requires Microtubules

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During the development of organisms, cells relate to one another via intercellular contacts maintained by cellular junctions. In the *Drosophila* embryo, a specific intercellular contact extends while the embryo proceeds through a program of events that lead to dorsal closure. Dorsal closure is an model morphogenetic process that allows for study of *in vivo* biomechanical forces as well as the necessary molecular components of morphogenesis. The two main tissues involved in dorsal closure include the amnioserosa and the lateral epidermis. A sheet of cuboidal to columnar epithelial cells makes up the amnioserosa and is surrounded by lateral epidermis. Before closure begins, the amnioserosa and lateral epidermis connect via vertical (dorso-ventral) cell boundaries comparable to the intercellular contacts within each individual tissue. However, as the embryo prepares to undergo closure, extended intercellular contacts develop between the juxtaposed row of amnioserosa cells and its overlying lateral epidermis. Closure occurs when the amnioserosa cells contract and contribute to dorsal-ward movement of the two opposing sheets of lateral epidermis. Without stable connection between the amnioserosa and the lateral epidermis, closure fails. This study shows that micro-injection of colchicine to block microtubule polymerization abrogates the native increase of intercellular contact between amnioserosa and lateral epidermis. As an extension of this observation, the intercellular contacts between amnioserosa and lateral epidermis were challenged with laser microsurgery of interior amnioserosa cells. This incision produced a recoil that resulted in a transient reduction in amnioserosa/lateral epidermis contact. Despite this reduction, apical junctions between the tissues remained intact. Together, these results develop a model of the morphogenetic movement of dorsal closure whereby the necessary contact between amnioserosa and lateral epidermis is most resilient at apical junctions and sensitive to microtubule loss.

3212-Pos Biophysical Studies Of The Effects Of 2.1 Tesla Static Magnetic Field On The Neural Cell Growth

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In this study, we investigated the effects of 2.1T static magnetic filed on the mixed culture of neurons and astrocytes obtained from rat's spinal cord, focusing on their viability and morphology. The study of the effect of these fields at cellular level are of high importance due to high incidence of exposure of human tissues to high static magnetic fields applied in magnetic resonance scanning. Our results showed that the exposure of a 2.1T magnetic field for 24 and 48 hours had no effects on the viability of either dorsal root ganglion neurons or astrocytes, checked by Ethidium Bromide/Acridine Orange staining. However, the exposure of the mentioned field for 72 hours caused neural cell death ($p<0.001$), while, astrocytes remained alive. There were neither changes in neural/astroglial cell surface area nor cellular migration detected following a two-hour exposure to the field. Analysis of the neurite growth pattern showed that when neurons were exposed to the field for four hours, the normalized parallel components of the neurites with respect to the field direction were statistically greater than the corresponding perpendicular ones ($p<0.01$). This fact indicates that the neuritis could be aligned in parallel to the applied magnetic field direction. However, this effect was not observed when cells were exposed for only two hours. There was no statistically significant change in neurite number identified after two hours exposure to 2.1T static magnetic field. The results can be further elaborated at molecular level when considered with respect to the polymerization and arrangement of microtubules in neural cells exposed to magnetic field.

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Keywords

Biophysics, neural culture, magnetic field, astrocyte

3213-Pos Switching microtubule molecular motors during melanosome transport in Xenopus Laevis melanophores

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Xenopus laevis melanophores are one of the cellular systems commonly used to study the function of molecular motors in vivo. An unexpected but common observation in trajectories of single organelles moving along microtubules is that the organelle frequently revert its direction. This observation was explained by the switching of the motor responsible for the transport but the mechanism by which motors with a given polarity turns on and motors with opposed polarity turns off is not known.

In this work, we explore the mechanism of in vivo coordination of microtubule motors by using a fast and precise tracking method. We analyze the interval in the trajectories where reversals of directions are observed and postulate a model to explain the reversals in living cells.

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Cell Mechanics & Motility - III

3214-Pos Analysis of Ciliary Coordination: The Making of Waves

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Coordinated ciliary beating is essential for mucociliary transport. Discrete coordination patterns vary both with organism and cell type. In all coordination patterns, there exist orthogonal directions of synchrony and metachrony that are uniquely defined with respect to power stroke direction. Coordination parameters quantified previously include metachronal wave direction, wavelength and velocity. These parameters assume cilia are tightly coupled. But how does one quantify coordination parameters of cilia whose beat cycles are only partially coupled in space or time? This is often the case in primary ciliary dyskinesis (PCD). Here, we describe a new approach to analyze ciliary coordination using high speed DIC images of primary explant cultures from respiratory epithelium.

Digital movies (500 fps) of beating cilia were acquired using a Redlake Motion Meter camera in conjunction with a Zeiss Axioskop microscope equipped with high extinction DIC optics, including a 63X (NA 1.4) oil immersion objective. Movies were assembled into 3D stacks with Oncore Image software, the z direction representing time. Image stacks then were preprocessed and ciliary centroids were determined. For each individual cilium within a group, beat direction was precisely determined and the phase of the beat cycle was determined as a function of time. These data then were analyzed with respect to coordination for specific ciliary groups along specific directions. Coordination, in this sense, represents constant phase relationships within a ciliary group.

Using this approach, we defined the variance of beat frequency (phase) with time for single cilia. In addition, we determined how distance and positional angle between cilia, as well as the angle between their power strokes, affect coordinated beating. Finally, we defined directions of synchrony and metachrony, as well as meta-