2377-Pos Board B363
Species-Specific PKC Activation of IK_S Channels
Angelica M. Martinez-Perez, Jin O-Uchi, Coeli M. Lopes.
Calcium activated potassium channels (IKs) is a slow activating potassium channel that is one of the main channels controlling cardiac repolarization. The IKs channel is formed by a pore subunit, KCNQ1 and an auxiliary subunit, KCNE1. These can be modulated by two different secondary messenger kinase proteins, PKA and PKC. Previous reports have suggested that activation of PKC decreases the IKs current in mice, while in humans, PKC activation increases the IKs current. However the detailed mechanism underlying this species-different regulation of IKs by PKC is not known. Here, we expressed the channel in Xenopus oocytes by injecting them with RNA encoding for the rat and human KCNQ1 and KCNE1 subunits. In addition, the muscarinic receptor (M1), a Gq-protein coupled receptor which downstream signaling activates PKC, was also expressed. We used two-electrode voltage clamp techniques to measure channel current. We measured the effect of PKC on both channel ability to conduct current and voltage to activate the channel by measuring the maximal channel conductance (Gmax) and the voltage that activates half of the maximal current (V1/2), respectively. Our results shows that upon short acetylcholine stimulation, human IKs channels show an increase in Gmax and a shift in V1/2 whereas rat channels exhibit a shift in V1/2 without Gmax changes. Finally, prolonged acetylcholine stimulation abolished Gmax increase on human channels whereas decreases it on rat channels. Thus, understanding of the PKC regulation of human IKs current as compared with other species, may allow the development of specific drugs to control cardiac rhythm pathologies.

Cell and Bacterial Mechanics & Motility II

2378-Pos Board B364
Mechano-Stimulation of Fibroblasts by Adjusting Viscous Drag of Mobile Cell Linkers in Biomembrane-Mimicking Substrates
Daniel E. Minner, Yu-Hung Lin, Andreas Schoenborn, Wolfgang Goldmann, Ben Fabry, **Christoph A. Naumann**.
Recent advancements in the design of polymeric substrates of tunable rigidity have shown that cells probe the viscoelasticity of their environment through an adaptive process of focal contact assembly/disassembly that critically affects cell adhesion and morphology. However, the specific mechanisms of this process of mechano-sensitivity have not yet been fully uncovered, in part due to the limitations of existing engineered cell substrates, which are characterized by immobilized cell linkers. To overcome this limitation, we here present a biomembrane-mimicking cell substrate based on polymer-tethered lipid mulibilayers, in which laterally mobile cell linkers enable the free assembly and disassembly of focal adhesions. In this experimental platform, the mechanostimulation of plated cells is accomplished by altering the viscous drag of cell linkers through the number of lipid bilayers in the solid-supported multibilayer stack. Results from microscopy experiments are discussed, which illustrate that the number of bilayers in the multi-bilayer stack has a profound impact on various cellular properties of plated 3T3 fibroblasts, including adhesion, morphology, shape fluctuations, migration, and cytoskeletal organization. Furthermore, this biomembrane-mimicking substrate is integrated into a force traction microscopy assay, which confirms that the presence of the fluid multibilayer system leads to a notable reduction in cellular traction forces. Our experiments illustrate that the described biomembrane-mimicking cell substrate is particularly well suited to monitor plated cells under conditions of weak force transduction conditions between cells and underlying substrate.

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Molecular-Detailed Modeling of Red Blood Cells in Stokes Flow
Zhangli Peng, Qiang Zhu.
To relate the fluid dynamics loading on red blood cells (RBCs) with the detailed mechanical loads inside their molecular architectures, we carried out a multiphysics simulation by coupling a three-level multiscale approach of RBC membranes with a boundary element method (BEM) for the extracellular and intracellular Stokes flows. Our multiscale approach of the membrane includes three models: in the whole cell level (Level III), a finite element method (FEM) is employed to model the lipid bilayer and the cytoskeleton as two distinct layers of continuum shells. The skeleton-bilayer interaction is depicted as a sliding-only contact. The lipid bilayer possesses a large area stiffness and negligibly small shear stiffness, and the mechanical properties of the cytoskeleton are obtained from a molecular-detailed model (Level II) of the junctional complex. The spectrin, a major protein of the cytoskeleton, is simulated using a constitutive model (Level I), including its folding/unfolding reactions. To incorporate fluid effects, a BEM model is coupled with the FEM model of the membrane through a staggered coupling algorithm. Using this method, we simulated the tumbling, tank-treading and swinging behaviors of RBCs in shear flow and RBC dynamics in capillary flow. The predicted cell shapes, motion frequencies, membrane stress and their dependencies on the fluid viscosities are in excellent agreements with existing studies. Furthermore, we predicted the protein density variation of the cytoskeleton and the interaction force between the lipid bilayer and the cytoskeleton in the molecular level. The results showed that sufficiently large fluid dynamics loading may lead to structural failure and remodeling of the cytoskeleton and its connections with the lipid bilayer. Finally, we found that the lipid bilayer and the cytoskeleton may undergo a 'double' tank-treading with slightly different frequencies in shear flow with high shear rates.

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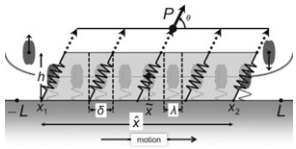
The Non-Equilibrium Thermodynamics and Kinetics Governing Focal Adhesion and Cytoskeletal Dynamics: Application to Cancer Cell Motility
Joseph E. Olberding, Aslan Dizaji, Michael Thouless, Ellen M. Arruda, **Krishna Garikipati**.
We consider the chemo-mechanical growth dynamics of actin stress fibers (SFs) and focal adhesions (FAs). Free energy changes drive the binding and unbinding of proteins to SFs and FAs, thereby controlling their dynamic modes of growth, treadmilling and disassembly via four mechanisms (Ref. 1) : (a) work done during addition of protein molecules, (b) the chemical free energy change associated with the addition of protein molecules, (c) the elastic free energy change of deforming SFs, FAs, cell wall and substrates (d) the work done by molecular conformational changes. We treat these dynamics as nonlinear kinetic processes driven by out-of-equilibrium thermodynamic driving forces. The above four mechanisms admit the full range of experimentally-observed behavior. The model has been coupled with cellular structure to explain the observed modes of motility in highly metastatic breast cancer cells of the MDA-MB-231 cell line, as well as in genetically-modified versions of this line. The graphc shows a grey FA with elastic elements and binding/unbinding proteins.



Reference
[1] Olberding JE, Thouless MD, Arruda EM, Garikipati K (2010) The Non-Equilibrium Thermodynamics and Kinetics of Focal Adhesion Dynamics. PLoSONE 5(8): e12043. doi:10.1371/journal.pone.0012043

2381-Pos Board B367
Biophysical and Biochemical Studies of Bacterial Predation
Megan E. Nunez, Eileen M. Spain, Megan A. Ferguson.
The ubiquitous Gram-negative bacterium Bdellovibrio bacteriovorus preys upon a wide variety of other Gram-negative bacteria, even those living in the relatively protected environment of a biofilm. Bdellovibrio swims through the environment propelled by a long polar flagellum. After collision with and recognition of a prey cell, it burrows into the prey periplasm where it digests the cellular contents and multiplies into progeny cells. Though the basic parameters are known, any questions remain about this organism and its life cycle. How does the predator find its prey, and once found, how does it recognize it? How does the predator penetrate the prey cell? What molecules and chemical signals are involved in communication between cells during Bdellovibrio's life cycle? Our collaboration uses a wide variety of biophysical and biochemical techniques, including atomic force microscopy, Langmuir Blodgettry, nuclear magnetic resonance spectroscopy, and microfluidics to attempt to answer some of these questions, and to demonstrate that despite its diminutive size Bdellovibrio is a complex and fascinating organism.

2382-Pos Board B368
Crawling Cells Can Close Wounds Without Purse Strings or Signaling
Charles W. Wolgemuth, Pilhwa Lee.
When a gash or gouge is made in a confluent layer of epithelial cells, the cells move to fill in the ''wound''. In some cases, such as in wounded embryonic chick wing buds, the movement of the cells is driven by cortical actin contraction (i.e., a purse string mechanism). In adult tissue, though, cells apparently crawl to close wounds. At the single cell level, this crawling is driven by the dynamics of the cell's actin cytoskeleton, which is regulated by a complex biochemical network, and cell signaling has been proposed to play a significant role in directing cells to move into the denuded area. However, wounds made in monolayers of Madin-Darby canine kidney (MDCK) cells still close even when a row of cells is deactivated at the periphery of the wound, and recent experiments show complex, highly-correlated cellular motions that extend tens of cell lengths away from the boundary. These experiments suggest a dominant role for mechanics in wound healing. Here we present a biophysical description of the collective migration of epithelial cells during wound healing based on the basic motility of single cells and cell-cell interactions. This model quantitatively captures the dynamics of wound closure and reproduces the complex cellular flows that are