plate-plate rheometer by applying a cyclic sinusoidal strain of a given amplitude, and analyze the non-linear stress-strain relationship. With each cycle, the maximum stress and the linear modulus of the material decrease, and the transition from a linear to a strain-stiffening response occurs at higher strains. If the strain amplitude is increased and a new set of cycles is taken, this 'working' of the material repeats for the now higher strain amplitude. However, for each strain step, the first oscillation of the set of cycles is qualitatively different from the following ones. First, when compared to the stress-strain response of a gel that has not been previously worked at smaller strains, the two match closely. Secondly, upon unloading, this first oscillation shows a significantly increased dissipation.
Upon addition of covalent crosslinks by incubating the collagen gels with 2% glutaraldehyde solution after polymerisation, the stress-strain relationship becomes independent of the loading history. We hypothesize that the microscopic mechanism responsible for the history dependence is due to intra-fibrillar slip of adjacent collagen monomers. We present evidence from direct observation using confocal microscopy to image collagen gels under shear that supports this hypothesis.

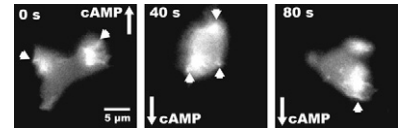
2881-Pos
Direct Detection of Tension Recovery After Local Stretching of Cell Surface
Takahiro W. Nakayama[1], Shin-ichi Machida[1], Ichiro Harada[1], Hiroshi Sekiguchi[2], Rehana Afrin[1], Atsushi Ikai[1].
[1]Tokyo Institute of Technology, Yokohama, Japan, [2]University of Tokyo, Kashiwa, Japan.
Cellular response to the externally applied force has a vital effect on cell proliferation, propagation and finally on its ultimate fate. Details of the molecular basis of the mechanical response are , however, little known. Here we measured mechanical and structural responses of a cell under locally applied force on an atomic force microscope (AFM) equipped with a fluorescence microscope for live cell imaging. When a colloidal AFM probe was first pressed on the cell surface and then pulled up, the tensile force sensed by the cantilever was recovered after initial relaxation. This recovery of the tensional activity was inhibited when cells were treated with cytochalasin D, the inhibitor of actin polymerization, or blebbistatin, the inhibitor of ATPase activity in myosin II, suggesting that the tension-recovering activity was driven by actin-myosin contractility. Our method allows us to investigate the dynamic processes of the mechanical maintenance of subcellular structures in a single cell.

2882-Pos
Probing the Micrheology of Mesenchymal Stem Cell Migration to Tumors
Daniel McGrail, **Michelle Dawson**.
Georgia Institute of Technology, Atlanta, GA, USA.
Mesenchymal stem cells (MSCs) are excellent candidates for the development of cell-based gene delivery systems; however, extended cell culture, required for therapeutic development, alters MSC morphology, reducing MSC migration upon reinfusion. Spontaneous migration of MSCs to tumors is mediated by tumor secretion of proangiogenic chemokines. Multiple particle tracking microrheology was used to investigate the effect of tumor-secreted molecules on MSC viscoelasticity, which was correlated with MSC migration and morphology. Within 24 hours after MSC treatment with tumor-conditioned media (TCM), MSCs were elongated, with more than 5-fold difference in the length of lamellipodia. Within 24 hours, the migration of MSCs, measured using a Boyden chamber assay, toward TCM was increased 10-fold over control media. The mean squared displacements (MSDs) of 100-nm carboxylated polystyrene particles, injected into the cytoplasm of human MSCs using the Biolistic Particle Injection System, were determined with 33 ms temporal and 5 nm spatial resolution using multiple particle tracking. The frequency dependent elastic and viscous moduli were calculated from the complex shear moduli, which were determined from the Fourier transform of the time-dependent MSDs, using the frequency-dependent Stokes-Einstein equation. Pretreatment of MSCs with TCM resulted in rapid changes in cytoplasmic viscoelasticity with a 9.8-fold increase in the average elastic moduli, which increased from 35 to 344 dyn/cm2, and a 3.5-fold decrease in the average viscous moduli, which was reduced from 99 to 28 dyn/cm2, within 1 hour ($n = 6$-8 cells per group). We hypothesize that tumor-secreted molecules increase MSC mobility by altering cytoskeletal organization. Changes in MSC viscosity may be in part to reduced actin cross-linking during cytoskeletal reorganization. Increased MSC rigidity may be due to MSC elongation, which leads to the formation of polymer entanglements as the ratio of cell length to width is greatly increased.

2883-Pos
Toward Magnetic Control of Cell Polarity
Mathieu Coppey[1], Fred Etoc[1], Domenik Lisse[2], Yohanns Bellaiche[3], Jacob Piehler[2], Maxime Dahan[1].
[1]Ecole normale superieure, paris, France, [2]Institute of Biophysics, Osnabrück, Germany, [3]Curie Institute, paris, France.
Cell polarity is involved in many aspects of cell and developmental biology. It is of fundamental importance for processes as diverse as cell motility, division, or differentiation. Cell polarization manifests itself through complex signaling and transport mechanisms by which molecules are asymmetrically localized within the cell. Whereas usual genetic and biochemical approaches are adapted to identify the elements in a transduction pathway which are necessary for the emergence of a cell polarity, they are not sufficient to know if the sole localized activity of a given effector is sufficient for the cell to acquire a polarity or to determine the kinetics of polarity formation. To address these important issues, we present a novel approach based on functionalized magnetic nanoparticles which are used to induce a localized signaling event of polarization. By doing so, we are able to monitor the cellular dynamic response to a local perturbation while preserving the complexity of the interaction feedbacks needed for the emergence of a global polarity. In our experiments, fluorescent magnetic nanoparticles (100-500 nm in size) are coated with purified constitutively active Cdc42 proteins, a key regulator of cell polarity. Once injected in the cytoplasm of live cells, these nanoparticles are manipulated using a customized magnetic setup able to exert forces on the order of 1-100 pN. We monitor at the single cell level the dynamics of nanoparticles and analyze the role of diverse factors (cytoskeleton, ER, substrate rigidity) on their intracellular mobility. Finally, we measure in different cell lines (Hela, 3T3) the effect of the local signalization on downstream effectors such as actin dynamics.

Bacteria & Motile Cells: Signal Transduction

2884-Pos
Generating Alternating Bidirectional Gradient Fields for Dynamic Measurements of Chemotactic Response
Boern Meier[1,2], Alejandro Zielinski[1], Christoph Weber[1,2], Delphine Arcizet[1,2], Simon Youssef[1,2], Thomas Franosch[1,2], Joachim O. Rädler[1,2], Doris Heinrich[1,2].
[1]Ludwig-Maximilians-Universität, München, Germany, [2]Center for NanoScience, München, Germany.
Prerequisite to a quantitative analysis of biochemical signalling networks is a well defined stimulus in space and time and a defined marker of biochemical response at the single cell level. We combine time-lapse fluorescence microscopy with a microfluidic chamber, which allows applying a defined temporal sequence of a spatially homogeneous chemical gradient over an ensemble of cells. The distribution of a chemoattractant in the flow chamber is characterized and the performance of the device found in good agreement with finite element calculation. To elucidate the dynamics of cellular response we investigate the velocity distribution of the amoeba Dictyostelium discoideum as a response to alternating cAMP gradients in opposing directions. We find pronounced directional migration at low switching frequencies, while at switching frequencies above 0.01 Hz stochastic cellular motility exhibiting seemingly non-responsive cells is observed. We demonstrate that the dynamics of intracellular polarization as displayed by the distribution of Lim-GFP is delayed with respect to the external change in gradient. We expect the microfluidic set-up to be useful for comparison of experimental data and computational systems modelling of cellular responses.



2885-Pos
The Response of Single E. Coli Cells to Changes in External Osmolarity
Teuta Pilizota, Yi Deng, Joshua W. Shaevitz.
Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, USA.
The extreme concentrations of chemicals in a bacterium's cytoplasm generate a large osmotic pressure that inflates the cell. Using a number of interconnected systems, bacteria actively regulate their turgor pressure to resist changes in their local environment. In response to osmotic shock and changes in internal ion concentration, the osmosensory transporters ProP/U, BetT/U, TrkAH and KdpFABC transport external chemicals such as proline, choline and potassium into the cell, whereas the mechano-sensitive channels MscS and MscL export solutes from the cell in response to increased membrane strain. Although each has been shown to play a role in the regulation of turgor, details of how the different systems are coordinated by a cell is poorly understood. Previous measurements of osmoregulation in bacteria have been unable to directly probe the adaptation of turgor pressure, focusing instead on the activity of various transporters, or the change in cellular survival rates. Here we move beyond these limited measurements using AFM and fluorescence imaging to monitor turgor pressure and cell volume adaptation on a single cell level with a time