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observed. These results suggest that wound healing is predominantly a mechanical process that is modified, but not produced, by cell-cell signaling.

2383-Pos Board B369
Video Force Microscopy (VFM): A New Technique that Allows Cell-Level Driving Forces to Be Determined from Time-Lapse Images
G. Wayne Brodland, Vito Conte, P. Graham Cranston, Shane Hutson, Florian Ulrich, Buzz Baum, Mark Miodownik.
For purposes of video force microscopy (VFM), the forces in the cells of an embryonic epithelium are assumed to consist of active forces, which can be mathematically resolved into equivalent forces along cell boundaries, and passive forces associated with deformation of the cytoplasm and its contained organelles and intermediate filament network, which are represented by an equivalent viscosity, mu. All triple junctions in the time-lapse images are tracked over time and finite element techniques are used to estimate the forces that must act on the passive components of each cell to deform them as observed. A mathematical inverse method is then used to determine the forces that must act along each cell edge in order to produce the net forces needed at each triple junction to drive the observed deformations. The technique has been successfully applied to multi-photon cross-sections of Drosophila embryos undergoing ventral furrow formation. There, it revealed that the ventral furrow is produced by contractions that vary smoothly with time and position in the apical surface of the presumptive mesoderm, by apical-basal contractions in the cells of this tissue and, surprisingly, by spatially more uniform basal contractions in the ectoderm. It was also able to quantify the sometimes subtle force modifications present in mutants that generate abnormal phenotypes. The indicated force alterations are consistent with known genotype-specific structural protein changes. When applied to wound healing in embryonic epithelia, it was able to quantify the forces generated in the purse string that closes the wound and the surrounding cells. It both of these contexts, VFM was able to quantify the forces that drive observed morphogenetic movements and to do so with sub-cellular spatial detail and sub-minute temporal resolution.

2384-Pos Board B370
The Role of the Scar/WAVE Complex in the Mechanics of Cell Migration
Effie Bastounis, Ruedi Meili, Baldomero Alonso-Latorre, Juan-Carlos del Alamo, Richard Firtel, Juan Lasheras.
Cell motility is integral to a wide spectrum of biological phenomena. It requires the spatiotemporal coordination of underlying biochemical processes, resulting in cyclic shape changes associated with mechanical events (*themotility cycle*). A major driving force of cell migration is the dendritic polymerization of actin at the leading edge, regulated through the nucleation activity of the Arp2/3 complex, activated by the Scar/WAVE complex. Our aim is to understand the effect of the different components of the Scar/WAVE complex in the mechanics and in particular the motility cycle of migrating cells.
For this purpose, we acquired time-lapse recordings of cell shape and traction forces of *Dictyostelium* cells migrating on deformable substrates. We compared results for wild-type cells and cells lacking the Scar/WAVE complex proteins PIR121 (Sra-1/CYFIP/GEX-2) (*pirA*-) and SCAR (*scrA*-). We find that mutantcells move slower than wild-type, while maintaining the overall characteristics of the mechanical interaction with the substrate, attaching at front and back and contracting inwards. Although the distribution of applied forces is unchanged, their magnitude is lower than in wild-type for *scrA*- cells and higher for *pirA*- cells. This correlates with the F-actin content of the different cell lines corroborating a role for F-actin in determining the level of the traction stresses. In *pirA*- cells regularity of the motility cycle (quasiperiodic repetition of shape changes and strain energy deposited) seems to be reduced compared to wild-type. This suggests that proper regulation of the Scar/WAVE complex and its role in F-actin turnover is essential for amoeboid motility.

2385-Pos Board B371
Conceptual Models for Synthetic Bipedal Motors
Sara Sadeghi, Nancy R. Forde, Gerhard A. Blab, **Martin J. Zuckermann**, Heiner Linke.
Biomolecular nano-motors have provided the inspiration for the design and construction of artificial nanoscale motors and machines based on several types of molecule including DNA. However, no synthetic nano-motors have yet been constructed from building blocks of protein-based material even though biomotors themselves are proteins. The HFSP motor group (1) are in the process of developing a bottom-up approach to the understanding of bio-motors by designing and constructing synthetic protein motors and numerically simulating their kinetic properties. One such concept is the ''tripedal tumbleweed'' motor (1) and another is the ''Inchworm''. In this context we present the results of numerical simulations for a bipedal motor with two connected peptide legs and a simple model for the ''inchworm'' motor in terms of harmonic bonds. These motors walk on a one-dimensional track of periodically arranged binding sites. The sequence of binding sites on the track of each motor is AB-AB-AB..... and the motors are powered by a temporally periodic sequence of composite washes which modulate the ligand concentrations and the leg angles. The washes cause the motors to undergo directed motion by a hand-over-hand mechanism and an inchworm mechanism, respectively, on a track with asymmetric spacing between the AB and the BA binding sites. We will show simulation results for two-dimensional motor action which includes stepping diagrams, stall forces processivity and first passage times for a range of parameters. These motor has the following properties observed for biological molecular motors: binding, power stroke and diffusional search. Extensions of the model will also be discussed. 1. E. Bromley et al. HFSP Journal Vol. 3 pp.204-212, (2009).

2386-Pos Board B372
Mechanical Control of Bacterial Cell Shape
Hongyuan Jiang, Fangwei Si, William Margolin, Sean X. Sun.
The most visible phenotype of an organism is its geometrical shape, yet a mechanistic connection between genotype and the organismal shape is elusive. For bacterial cells, several genes have been implicated in cell shape determination, including the cytoskeletal filaments bundles such as MreB, which controls whether the cell takes on a spherical or a rod-like shape. Here we describe the role of cell wall growth, mechanics and cytoskeletal filaments in determining the bacterial cell shape. We show that a growing rod-like cell by itself is mechanically unstable. But this growth-induced instability can be suppressed by cytoskeleton filaments such as MreB. We propose that MreB exerts an additional force on the cell wall. We use simulation and experiments to demonstrate the negative control of cell shape by MreB. In the process, we demonstrate that our model explains a range of MreB function, including: (a) Depletion of MreB leads to a reversible transformation from a short rod to a sphere. (b) Over-expression of MreB results in the filamentation of bacterial cells. (c) The depolymerization of MreB helix around the septum is a prerequisite for cell division. (d) Partial disassembly and non-uniform distribution of MreB can lead to the bulging of a filamentous cell.

2387-Pos Board B373
Cells CRAWL from Soft to Stiff Surfaces as Myosin-II Polarizes the Cytoskeleton
Matthew D. Raab, Dennis E. Discher.
Scarring in the heart after a myocardial infarction, or scarring in the skin after wounding - lead to rigidification of tissue through extensive collagen crosslinking and can also lead to homing of adherent mesenchymal stem cells (MSCs). 'Durotaxis' describes the tendency for a cell to crawl from a soft, collagen-coated gel to an adjacent stiff matrix, but clear evidence for accumulation of any cell type has been lacking as has insight into molecular mechanisms. We cultured human MSCs on matrices with scar-like gradients in elastic modulus (stiffness) that are on order of 10 Pa/micron and document a bias in migration toward the stiff matrix with proliferation-independent accumulation taking just a couple of days. As found with other cell types, MSCs on stiff substrates show myosin-IIB is polarized toward the rear while the centrosome and microtubule (MT)-network are polarized toward the front, but such polarization is surprisingly absent from cells on soft substrates. With myosin-II inhibition, we find cells on stiff matrix crawl faster whereas cells on soft matrix are initially impeded but then transition to motile cells as their centrosomes and MT-networks polarize as they would on stiff matrix. While myosin-II is required for contractility but not migration, MTs are required for any migration - including durotaxis - but contractility on gels remain intact after destabilization of MTs. The model gel results thus show that the progressive polarization of myosin-II on stiff substrates is particularly key to durotaxis. The broader relevance of this conclusion is tested with decellularized heart tissue that permits an examination of MSC adhesion, contraction, and migration. Decellularized heart tissue was stiffened with chemical crosslinking to increase elastic modulus measured with atomic force microscopy. We see that this increased stiffness alters cell migration behavior.

2388-Pos Board B374
How Deeply Cells Feel: From Soft Matrices of Controlled Thickness to Nuclear Readouts
Amnon Buxboim, Edward C. Eckels, Dennis E. Discher.
Tissue cells constantly probe their surroundings. They lack eyes to see and ears to hear but sense their microenvironment by adhering and physically deforming, which allows cells to feel into the depths of a matrix. To address how deeply cells feel we cultured mesenchymal stem cells, as prototypical but particularly sensitive adhesive cells, on collagen-coated gels-made microfilms of controlled elasticity (E) and thickness (h). After just 36 hrs in culture, cell spread area was distinctively smaller on thick and soft compared to either thin or stiff films, correlating well with nuclei morphology. Transition from small-to-large spread area was obtained at <5 microns gel thickness, which defines a tactile length scale for mechanosensitivity. Matrix-dependent cytoskeletal organization exhibits

thickness-dependent nematic and smectic-like assembly, and nuclear component Lamin-A (LMNA), which is widely implicated in cell differentiation, was also found to be different for thin films relative to thick gels of the same E. Transcriptional profiles of many nuclear genes, including LMNA and histones, also showed a dependence on h. The changes suggest mechanical links to regulation of gene expression by matrix physics.

2389-Pos Board B375
Stem Cells Behaviour on Nano-Films of Collagen Fibrils
Irena Lambrova Ivanovska, Dennis E. Discher.
Commitment of stem cells to different lineages is regulated by many cues in their local microenvironment, including mechanical aspects of the extracellular matrix [Engler et al, Cell 2006]. We study the behavior of human mensechymal stem cells (hMSC) cultured on molecularly thin highly ordered collagen films that are transglutaminase-crosslinked or not. Cells pull on the collagen films, and their ability to deform the collagen fibrils is greatly influenced by the films' rigidity. Nanotopography and mechanics of the films are evaluated by Atomic Force Microscopy techniques (AFM) with the AFM stylus used to deform the fibrils, mimicking cellular processes of collagen remodeling. Crosslinked films require higher forces for similar plastic deformations of native collagen films. Cells cultured on different films initially respond by altering their morphology, cytoskeleton organization and nuclear shape. Mechanically anisotropic native collagen films promote strong polarization and orientation along the highly aligned fibrils whereas cells on crosslinked films flatten, spread and resembles cells undergoing osteogenesis. At least two major osteogenic markers are upregulated on such films, in contrast to cells cultured on the native collagen films. How cell and nuclear elasticity are altered in differentiating cells was also addressed in relation to expression of nuclear lamina proteins. The results suggest that the mechanics of collagen matrix - not just composition - is a major cue to nuclear state and stem cell differentiation.

2390-Pos Board B376
Leukocyte Transmigration is Mediated by Endothelial Cell Contractile Forces and Substrate Stiffness
Kimberly M. Stroka, Helim Aranda-Espinoza.
Leukocyte transmigration through the vascular endothelium is a crucial step in the normal immune response. However, in the cardiovascular disease (CVD) of atherosclerosis, an excess of leukocytes adhere to and transmigrate through the endothelium, and progression of this disease is associated with arterial stiffening and variance in mechanical force transduction. In this study we investigated the mechanics of leukocyte transmigration in an *in vitro* model of the vascular endothelium. We modeled healthy versus diseased blood vessels through manipulation of substrate stiffness using polyacrylamide gels, coated with extracellular matrix protein and plated with human umbilical vein endothelial cell (HUVEC) monolayers. The HUVEC monolayers were activated with tumor necrosis factor-alpha to mimic inflammatory conditions. We observed that leukocyte transmigration through HUVEC monolayers increases with stiffness below the endothelium, and we hypothesized that substrate stiffness changes the biophysical properties of the endothelium to produce this effect. Using an array of biophysical techniques, we first evaluated the adhesion protein expression, stiffness, morphology, cytoskeletal arrangement, and cell-substrate adhesion of HUVEC monolayers as a function of substrate stiffness; however, none of these properties could account for the transmigration behavior. We also explored the role of endothelial cell-cell adhesion and myosin light chain kinase (MLCK)-dependent endothelial cell contraction. We observed that (1) decreasing cell-cell adhesion increases transmigration on soft substrates and (2) inhibition of MLCK and endothelial cell contraction normalizes the effects of substrate stiffness by reducing leukocyte transmigration on stiff substrates without affecting transmigration on soft substrates. These results provide strong evidence that neutrophil transmigration is regulated by MLCK-mediated generation of gaps at cell borders through endothelial cell contractile forces which depend on substrate stiffness.

2391-Pos Board B377
The Origin and Limits of Natural Variation in Cell Mechanical Behavior
John M. Maloney, Krystyn Van Vliet.
Investigations of natural variation in cell mechanics within a population are essential for understanding the stochastic nature of cell deformation under applied load. Historically, a wide range of methods to measure cell stiffness have revealed a weak power law that governs the relationship between complex elastic modulus and excitation frequency; similarly, measurements of creep compliance find a corresponding power-law dependence on load application time. In both experimental regimes, researchers have reported a Gaussian distribution of power-law exponent along with a log-normal distribution of cell stiffness/compliance. However, the underlying analytical relationship between these two distributions has not yet been fully explored. Do these mechanical distributions stem from inherent variations within a cell population, from stochastic mechanisms of single-cell deformation, or both? Here, we develop this relationship to generate new predictions regarding the evolution of mechanical parameters during testing in the frequency and time domains. These predictions are in agreement with literature reports that were originally presented as empirical findings lacking theoretical explanation, as well as with our own studies of creep compliance in stem cell populations. Our work thus serves to link two fundamental findings in the cell rheology literature, produce nontrivial predictions supported by experiment, and motivate further study into constitutive laws describing the mechanical variation of living matter.

2392-Pos Board B378
Stiffness and Load-Dependent Spreading of Cell-Surface Adhesions are Emergent Properties of a Molecular Model
Sam Walcott, Sean X. Sun.
Cells plated on a 2-D substrate form adhesions with that surface. These adhesions, consisting of aggregates of various proteins, including integrins, paxillin, vinculin, talin and others, are thought to be important in mechanosensation, the process by which the cell senses and adapts to mechanical properties of the surface, such as stiffness. At a molecular level, integrins in the adhesion bind to the extracellular matrix, while the various other proteins are involved in, among other things, binding to actin filaments that, in turn, apply a load to the adhesion. Several of these proteins (e.g. talin) undergo load-dependent state transitions that are thought to be important in both the load-dependent and the surface stiffness-dependent stability of the adhesions. Based on these molecular-level observations, we consider a grossly simplified version of an adhesion, made up of "molecules" that can bind to the surface in a strain-dependent manner and can undergo a load-dependent state transition. Remarkably, we find that, in Monte-Carlo simulations of these molecules, molecular aggregates are formed in a load- and stiffness-dependent fashion that closely mimics that seen in experiment. Furthermore, we find that these adhesions exhibit three phases of growth: 1) nucleation, where small, transient molecular aggregates form; 2) maturation, where adhesions grow quadratically in time; and 3) decay, where a short steady-state is followed by adhesion disassembly. These three phases of adhesion growth are also experimentally observed. These various properties of the Monte-Carlo simulation may be simply understood by analytic calculations. We therefore conclude that many experimental observations of stiffness- and load-dependent adhesion growth are emergent properties of molecular-mechanical systems with strain-dependent surface binding and a load-dependent state transition.

2393-Pos Board B379
Membrane Feedback Controls Signaling Regulation of Cell Spreading
Padmini Rangamani, Marc-Antoine Fardin, Yuguang Xiong, Azi Lipshtat, Olivier Rossier, Michael Sheetz, Ravi Iyengar.
Cell motility and spreading are regulated by signaling from the integrin receptors. Interaction between the integrin receptors and substrates such as fibronectin triggers the activation of downstream signaling pathways, resulting in the activation of actin regulating proteins such as Arp2/3, gelsolin and profilin. We have developed an integrated model of cell spreading regulated by integrin signaling network to understand the role of signaling during fast isotropic spreading, when fibroblasts spread on fibronectin coated slides. The three dimensional stochastic spreading model was developed using differential geometry techniques and is coupled with a deterministic model for integrin signaling regulation. We find that cell spreading is a robust process and depends on signaling only for the initiation of spreading but not for maintaining the spreading dynamics. Our model further predicts that signaling dynamics in the absence of Cdc42 and WASP reduce the spreading rate to a small extent but do not affect the shape evolution of the spreading cell. These predictions were verified by experiments conducted with dominant negative Cdc42 cells and wiskostatin effects on cell spreading. Computational analyses predicted that the spreading shape evolution is controlled by the physical properties of the plasma membrane such as membrane surface load and membrane bending ridigity. Simulations from our model identified that changing these properties affects the spreading dynamics, in particular the shape evolution. In contrast, changing information flow through the cell signaling network has little effect. Overall isotropic fast spreading of fibroblasts on fibronectin-coated surfaces depends strongly on the biophysical properties of the plasma membrane and is robust to changes in the signaling dynamics.

2394-Pos Board B380
Differentiation of Hematopoietic Stem Cell Modulated by Actomyosin Forces
Jae-Won Shin, Kyle R. Spinler, Joe Swift, Dennis E. Discher.
Specificity of fate decisions during stem cell differentiation appears determined in part by biophysical processes that include cellular contractility and matrix elasticity, and we had previously demonstrated that human mesenchymal stem cells (MSCs) specify lineage based on these cues [Engler et al., Cell 2006]. Here, we show the importance of actomyosin force as a central node