**2414-Pos Board B400**
**Reconstitution of Actomyosin Bundles**
**Todd Thoresen**, Margaret Gardel.
Many vital processes among diverse cell types are dependent on proper actomyosin contraction. Despite muscle and non-muscle cells containing many different proteins believed to form the requisite structure necessary for contraction, the minimal requirements for effecting contractile motion and sustaining a tensile load are unknown. While myosin motors are known to be responsible for force generation, it is generally thought that long range force transmission requires accessory proteins to cross-link F-actin. We show that a minimal set of proteins, F-actin and smooth muscle myosin thick filaments, spontaneously assemble into contractile bundles that undergo dramatic reductions in contour length and generate contractile forces on the bundle end points. These contractile bundles are responsive to changes in boundary conditions, whereby dynamic remodeling seeks to minimize the distance and build tension between end points. The degree of contraction is sensitive to myosin/actin stoichiometries; at sufficiently low myosin concentration, contraction fails to commence. The rate of contractile is proportional to bundle length and, thus, these in vitro bundles show similar telescoping phenomenon observed in striated muscle. Contraction and further remodeling is abrogated at a critical tension, consistent with force-induced stalling of myosin motors within a bundle. Thus, we have found a two protein component system comprised of F-actin and thick filaments of smooth muscle myosin is sufficient to reconstitute a dynamic material that rapidly remodels through contraction when tension is low and forms a stable structure under high tension. This serves as a model to understand adaptive force sensing and transmission in the actomyosin cytoskeleton.

**2415-Pos Board B401**
**Self-Organization in Reconstituted Actomyosin Bundles**
**Matthew R. Stachowiak**, Patrick M. McCall, Todd Thoresen, Ben Stratton, Margaret L. Gardel, Ben O'Shaughnessy.
Actomyosin bundles such as muscle myofibrils, stress fibers and cytokinetic rings are used by cells to exert force, contribute to the cell's structural integrity and accomplish morphological change. In muscle and some types of stress fibers actin, myosin and other components are organized into sarcomeric repeat units but in many other cases the structure is far more disordered. The mechanisms whereby such disordered architectures produce tension are not established. Here we used mathematical modeling to quantitatively understand the behavior of reconstituted in vitro actomyosin bundles consisting solely of F-actin and myosin thick filaments. In the presence of ADP, actin and myosin formed stable (>1 hour), non-tensile bundles ~5-50 microns long anchored between polystyrene beads. The myosin is initially uniformly distributed along the bundles. Upon addition of ATP, the bundles contracted and became taut while the myosin reorganized into discrete clusters. We developed a mathematical model to account for this behavior. The random actin filament locations and polarities leads to a random net actin polarity at different locations along the bundle. We found the self-organization of myosins into clusters is driven by the tendency of myosin to migrate to zeros of the polarity profile. In agreement with experiment myosin clusters develop over ~10 s by myosin translation. We calculate the distribution of myosin cluster separations and predict the mean cluster separation increases with actin filament length. Thus, a minimal bundle of actin and myosin alone has the inherent capacity to self-organize into a heterogeneous structure exhibiting morphological similarity to tension-producing cellular actomyosin structures such as stress fibers.

**2416-Pos Board B402**
**Actin Disassembly is Mechanically Regulated in Spontaneously Fracturing Stress Fibers**
**Matthew R. Stachowiak**, Mark A. Smith, Elizabeth Blankman, Laura Luettjohann, Hayri Balcioglu, Ben O'Shaughnessy.
Stress fibers are contractile bundles of actin, myosin and other components which mechanically interact with the extracellular matrix and maintain the cell's structural integrity. As part of the cytoskeleton they must be able to rapidly remodel in response to dynamic stimuli, but detailed mechanisms are not established. Here we use mathematical modeling and quantitative image analysis of cells expressing tagged stress fiber components to study stress fiber remodeling. We observed occasional acute elongation of stress fibers followed either by repair (82% of the time) or spontaneous fracturing followed by rapid recoil over ~25 s (18%). Fractured stress fibers shortened by ~80% total regardless of their initial length, suggesting significant attachments to other cytoskeletal elements were absent. We discovered that fiber shortening is accompanied by substantial actin disassembly with a ~30 s delay. The disassembly processes shed ~40% of the actin initially present. Thus, the fiber actin density increases during recoil, peaks, and then decays to twice its initial value. To quantitatively explain this behavior we extended an earlier mathematical model of stress fibers to spontaneous fracture and recoil [Stachowiak and O'Shaughnessy, New J. Phys., v10, p025002 (2008)]. The model predicts that, following breakage, fiber shortening due to myosin contractile force increases actin density which in turn augments actin-actin compressive elastic stresses. These stresses promote actin depolymerization, thus allowing the fiber to remodel its actin. Model predictions agree quantitatively with experimental data. The measured 30 s delay is the time for actin density during contraction to reach the threshold to trigger depolymerization-promoting stresses. These results demonstrate that remodeling of cytoskeletal structures can be mechanically regulated by coupling between shape, elastic stress and component turnover rates.

**2417-Pos Board B403**
**Investigating the Mechanism of Pulsed Actin-Myosin Contraction**
**Adam C. Martin**, Eric F. Wieschaus.
During embryonic development, masses of cells undergo dramatic rearrangements to organize into separate layers that will give rise to different parts of the body. This incredibly dynamic process is called gastrulation and is driven by cell shape changes that collectively deform the tissue. Apical constriction is a common cell shape change that converts a columnar shaped cell into a wedge or cone shaped cell. The resulting change in cell geometry facilitates the bending or folding of an epithelial sheet. Previously, we imaged the dynamics of apical constriction during the embryonic development of the fruit fly, *Drosophila melanogaster*. We found that apical constriction is driven by a ratchet-like contraction of the actin-myosin meshwork across the apical surface of these cells. This involves pulses of myosin driven contraction that are repeated cyclically to constrict the cell. Here, we examine the mechanism of pulsed contractions. It was possible that contraction pulses result from mechanical signaling (i.e. stretching) between neighboring cells. However, using RNAi of adherens junction proteins to disrupt cell-cell adhesion, we find that contraction pulses can occur even when cells are not mechanically coupled. This suggests that contraction pulses in the embryo result from the dynamic properties of actin-myosin networks. We are currently examining the importance of actin and myosin turnover during pulsed contraction in the embryo.

**2418-Pos Board B404**
**Structure and Dynamics of Epithelial Cell Cortical Actomyosin Networks**
**Philipp Diesinger**, Anoop Cherian, Christoph Klingner, Roland Wedlich-Soldner, Mark Bathe.
Cortical actomyosin networks consist of filamentous actin (F-actin) bundles crosslinked into highly dynamic networks that are linked physically to the extracellular matrix via integrins. These networks play an active role in tissue morphogenesis during development as well as metastasis in cancer, by enabling rapid remodelling of cell shape via F-actin polymerization and interaction with myosin-II motor proteins. Here we investigate a newly discovered isotropic actomyosin network on the dorsal surface of epithelial MDCK cells and characterize its structure and dynamics using computational analysis of timelapse fluorescence microscopy data provided by Lifeact, a highly specific marker for F-actin in living cells. At low cell density, F-actin bundles depolymerise and polymerize continuously, splitting and merging into large bundle clusters. Spatial Temporal Image Correlation Spectroscopy and Optical Flow are used to characterize the oscillatory dynamics of the bundled networks, and reveal characteristic length- and time-scales associated with their motions.

**2419-Pos Board B405**
**Non-Muscle Myosin IIA Facilitates Vesicle Trafficking for MG53-Mediated Cell Membrane Repair**
**Pei-Hui Lin**, Chuanxi Cai, Noah Weisleder, Hua Zhu, Hiroshi Takeshima, Jianjie Ma.
Repair of disruptions to the plasma membrane is an essential mechanism for maintenance of cellular homeostasis and integrity, a process that involves coordinated movement of intracellular vesicles to membrane injury sites for patch formation. We have previously identified MG53 as an essential component of the cell membrane repair machinery. In order for MG53 and intracellular vesicles to translocate to membrane injury sites, motor proteins must be involved. Here we show that non-muscle myosin type IIA (NM-IIA) interact with MG53 to regulate vesicle trafficking in both muscle and non-muscle cells. In cells that are deficient for NM-IIA expression, MG53 cannot translocate to acute injury sites, whereas rescue of NM-IIA expression in these cells can restore MG53-mediated membrane repair. Compromised cell membrane repair is observed in cells with RNAi-mediated knockdown of NM-IIA expression, or pharmacological interventions of NM-IIA motor function. Thus, our dada reveal NM-IIA as a key cytoskeletal motor that facilitates vesicle trafficking for MG53-mediated cell membrane repair.

**2420-Pos Board B406**
**Myosin Cortical Assembly and Spindle Oscillations During Hela Cell Division**
**Ming-Tzo Wei**, Dimitrios Vavylonis, H. Daniel Ou-Yang.
The cortical actomyosin layer below the membrane of dividing cells and the microtubules of the mitotic spindle are coupled through internal feedback