Balance of Isotropic and Directed Forces Determines Cell Shape

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Balance of Isotropic and Directed Forces Determines Cell Shape
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The shape of a cell membrane is largely defined by the underlying actin cytoskeleton and membrane mechanics. The actin cytoskeleton asserts contractile forces on the membrane that can be divided in isotropic and directed forces. We present a theory which is an extension of the Young-Laplace equation. It models cell edges as parts of one uniform ellipse, which changes from cell to cell. The ellipse parameters are characterized by the ratio of isotropic to directed contractility of the cell.
We demonstrate the capabilities of this model using fibroblasts seeded on an elastic micro-pillar array. In this way adhesion forces exerted by the cell at single adhesion sites are measured. We show that isotropic and directed forces balance the line tension in cortical actin. Furthermore, for cells with homogeneous contractile forces and a single orientation of stress-fibers any part of the cell edge follows a universal ellipse, enabling us to calculate the magnitude of isotropic and directed contractility in a single cell.
We show that in 3T3 fibroblasts the directed contractility is about three times as strong as the isotropic contractility. If myosin motors are inhibited, however, directed contractility decreases, effectively disabling forces generated by stress-fibers, and the elliptical cell cortex turns into a circular shape predicted for an isotropic contractile cytoskeleton.
Our analysis shows that a simple two-parameter model explains polarity, shape of the cell cortex and cellular forces as experimentally observed. Potentially this model can be used to predict stresses and forces on the extracellular matrix and tissue.

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A Computational Model of Cell-Generated Traction Forces and Fibronectin Assembly
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The extracellular matrix (ECM) is an assembly of proteins that surround cells and serves as the cell substrate *in vivo*. A primary component of newly synthesized ECM is fibronectin (FN), critical for embryonic development and wound healing. Despite years of research, the mechanism of FN assembly is still not understood. We hypothesize that FN assembly occurs through the revelation of buried FN-FN binding sites within *any* of the 15 Type III FN domains, and that these binding sites are exposed by stretching of FN-III beta strands, allowing attachment of additional FN molecules via a beta strand addition mechanism.
To investigate this hypothesis, we developed a biophysical computational model of cell-FN-substrate biomechanical-chemical interactions. In the model, FN-III domains are represented by Hookean springs with distinct stiffnesses; thus, each FN dimer is represented by 30 springs in series. Integrin binding/unbinding is represented by a stochastic first-order reversible chemical reaction with force-dependent off-rate. Model results are validated using *in vitro* tools: FN fibril growth and traction forces applied over time are measured using microfabricated pillar arrays in a human fibroblast cell line. Beta-strand exposure is probed using Thioflavin T, a fluorescent probe that binds to free beta strands. Results indicate that the computational model recapitulates three unique features that are observed in experimental measurements of FN fibrils: 1) FN fibrils are highly elastic, but have a maximal 4-fold elongation of their resting length; 2) FN fibril length and force are not strongly correlated; and 3) FN fibrils have discrete stable lengths, suggesting local minima of force balance within growing fibrils. These results will be discussed and compared with simulations in which FN fibril assembly is mediated by either a single cryptic binding site or a subset of cryptic binding sites.

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Deletion of Calponin 2 in Macrophages is Anti-Inflammatory and Attenuates the Development of Atherosclerosis
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Arterial atherosclerosis is an inflammation disease. Macrophages play a central role in the pathogenesis and progression of atherosclerosis. Substrate adhesion influences macrophage differentiation into pro- or anti-inflammatory phenotypes. Calponin is an actin-filament-associated protein that inhibits myosin-ATPase and traction force, and stabilizes actin cytoskeleton and enhances cell adhesion. Encoded by the Cnn2 gene, calponin isoform 2 is expressed in macrophages. The development of atherosclerosis lesions in apolipoprotein E knockout (ApoE$^{-/-}$) mice was effectively attenuated when accompanied by myeloid cell-specific Cnn2 gene knockout. Studies of peritoneal macrophages of Cnn2$^{-/-}$ and WT mice demonstrated that the deletion of calponin 2 increases cell motility and phagocytosis, whereas weakens cell adhesion. Cnn2$^{-/-}$ macrophages produced lower levels of pro-inflammatory cytokines than that in wild type macrophages. The up-regulation of pro-inflammatory cytokines in foam cells produced by loading acetylated-low-density lipoprotein in culture was also attenuated in Cnn2$^{-/-}$ cells. Macrophages growing on low stiffness substrate exhibit decreased calponin 2 and weakened adhesion compared to macrophages on high stiffness substrate, indicating a mechanism by which cell adhesion and mechanosignaling regulate macrophages function. Deletion of calponin 2 removes an inhibition of myosin-motors and increases the dynamics of actin cytoskeleton, which form a foundation for faster migration, enhanced phagocytosis and reduced pro-inflammatory cytokine production, corresponding to the attenuated inflammatory lesion and the development of atherosclerosis. These findings suggest a novel therapeutic approach to the treatment of coronary heart disease and other inflammatory diseases.

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Provisional Matrix Citrullination Contributes to Enhanced Fibroblast Migration
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Activated fibroblasts—characterized by various altered behaviors including invasiveness, altered protein secretion profiles, and resistance to apoptosis—contribute to the development and exacerbation of numerous chronic human inflammatory diseases including cancer, rheumatoid arthritis, and interstitial lung disease. Citrullination, a post-translational protein modification known to occur extensively in inflammatory environments, alters the provisional extracellular matrix (ECM) and key arginine-containing cell-binding sites therein. We hypothesize that citrullination alters fibroblast interactions with provisional ECM such that adhesion is reduced and related downstream functions such as motility and contraction are also altered. Results have thus far indicated that provisional matrix citrullination does contribute, solely or in part, to several characteristics of activated fibroblasts. Adhesion assays, whereby human foreskin fibroblasts (HFFs) were allowed to attach to modified or normal provisional ECM and subsequently challenged with shear forces, suggest that citrullination significantly decreases fibroblast attachment and spreading. Blocking experiments have shown that these effects appear to be mediated, in part, by both RGD and αvβ3 integrin interactions. Despite this decreased adhesion, in vitro random migration and wound healing assays have paradoxically demonstrated the ability of citrullinated provisional ECM to significantly enhance fibroblast migration rates, indicating that adhesion differences alone do not fully explain differences in citrullination-mediated cell motility. This research will ultimately provide insight for understanding a fundamental pathway linking citrullination of provisional ECM proteins with fibroblast activation. It therefore constitutes an important step in the development of novel treatments to both prevent and ameliorate a panoply of human diseases, many of which currently have inadequate or nonexistent therapeutic solutions.

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Active Dynamic Mechanics of Blood Clot Contraction
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Despite the biological and clinical importance of blood clot contraction, it is a poorly understood mechanical process driven by forces that are generated by platelets and propagated by fibrin, resulting in the compression and deformation of red blood cells (RBCs) and the overall volume shrinkage of the clot. Optical tracking and dynamic rheometry were used to examine unconstrained (free to reduce volume) and constrained (fixed volume) clot contraction over time, which allowed for the kinetics of clot contraction and generation of contractile force to be tracked. The presence of RBCs enhanced the generation of platelet contractile forces by 60% but lessened the rate and extent of clot contraction by 30% when compared to samples without RBCs. Structurally, blood clots have distinct areas showing fibrin and RBCs in association with platelets and areas where they spatially removed from platelets. We developed a three-element active poroviscoelastic model to improve the understanding of how composition and distribution of the clot, particularly the presence of RBCs, affects the process of clot contraction. Our model revealed that clot response to