generation and network formation. In contrast, exposing the cells to Bacterial SMase C to hydrolyze of sphingomyelin didn't affect neither endothelial biomechanics not morphogenesis indicating that OxLDL-induced stimulation of SMAse cannot be responsible for these effects.

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A Zipper Network Model of Extracellular Matrix Failure Reveals a New Role for Proteoglycans

Bela Suki[1], Michael C. Ritter[1], Arnab Majumdar[1], Rajiv Jesudason[1], Dimitrije Stamenovic[1], Jo Ann Buczek-Thomas[2], Philip J. Stone[2], Matthew A. Nugent[2].
[1]Boston University, Boston, MA, USA, [2]Boston University School of Medicine, Boston, MA, USA.

Mechanical failure of soft tissues is characteristic of life threatening diseases, including emphysema and vessel wall aneurysms. Failure occurs when mechanical forces are sufficient to rupture the enzymatically weakened extracellular matrix (ECM). Elastin is an important structural protein of the ECM, and is known to stretch beyond 200% strain before failing. However, ECM constructs and native vessel walls composed primarily of elastin and proteoglycans (PGs) have been found to fail at much lower strains. In this study, we hypothesized that PGs significantly contribute to tissue failure. To test this, we developed a novel Zipper Network Model (ZNM), in which springs representing elastin are organized into long wavy fibers in a zipper-like formation and placed within a network of springs mimicking PGs. Elastin and PG springs possessed distinct mechanical and failure properties. The elastin does not percolate while the PGs can serve as bridges between elastin fibers as well as hinder folding of the fibers via bond-bending. During stretching, elastin fibers first become straight, then start stretching the PG bridges. Simulations using the ZNM showed that the failure of PGs alone reduces the global failure strain of the ECM well below that of elastin and hence digestion of elastin does not influence the failure strain. Network analysis also suggested that elastin determines the peak and failure stress while PGs transmit the load and define the failure strain of the network. Predictions of the ZNM were experimentally confirmed by measuring the failure properties of engineered ECM constructs before and after digestion with trypsin that cleaves the core protein of PGs without affecting elastin. This study reveals a novel role for PGs in the failure mechanics of engineered and native ECM with implications for the design of engineered tissues.

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Biophysical Regulation of Endoderm By 3-Dimensional Fibronectin Matrix

Adam J. Engler[1,2], Jean Schwarzbauer[2].
[1]University of California, San Diego, La Jolla, CA, USA, [2]Princeton University, Princeton, NJ, USA.

Fibronectin (FN), a major extracellular matrix (ECM) component that assembles into a 3-dimensional (3D) network, plays a significant role in the development and maintenance of most tissues. In the embryonic stem (ES) cell niche, ECM composition, elasticity, and architecture likely contribute to the decision between self-renewal and differentiation. ES cells differentiating as multicellular embryoid bodies (EBs) exhibit a 10-fold drop in expression of Nanog, a self-renewal marker, concurrent with a 3-fold upregulation in FN production as well as the onset of differentiation markers Fgf 5 (ectoderm), brachyury (mesoderm), and GATA4 (endoderm). However, FN and GATA4 appear to be temporally and spatially correlated within the EB while FN and Nanog are inversely correlated with each other. To probe any specific FN-GATA4 interaction and its biophysical regulation, FN-coated surfaces and 3-dimensional, soft fibrillar FN matrices were used as substrates for ES cells grown in monolayer culture. ES cells on FN-coated surfaces displayed a well spread morphology but did not significantly increase their FN production or GATA4 expression. In contrast, ES cells grown on fibrillar matrices were less spread, displayed a 4-fold upregulation of FN production similar to that of EBs, and expressed GATA4 via immunofluorescent detection. However, when crosslinked to increase 3D FN matrix elasticity from 350 Pa to 4500 Pa, FN expression dropped 2-fold and GATA4 staining was significantly reduced. Though the specific molecular mechanisms require elucidation, these findings suggest important temporal, spatial, and mechanical roles for FN matrix in regulation of ES cell development.

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Dynamic Behavior Of Heterogeneous Cell Populations Growing Under Mass Transport Limitations

Kyriacos Zygourakis[1], Gang Cheng[2], Pauline Markenscoff[3].
[1]Rice University, Houston, TX, USA, [2]Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA, [3]University of Houston, Houston, TX, USA.

Tissue growth in biomimetic scaffolds is strongly influenced by the dynamics and heterogeneity of cell populations. A significant source of heterogeneity is nutrient (or growth factor) depletion. Cells slow down, stop dividing or die when the concentrations of key nutrients or growth factors drop below critical levels in the scaffold interior. As a result, we still cannot grow *in vitro* tissue samples thicker than a few millimeters for metabolically active cells.

To provide theoretical guidance for the *in vitro* cultivation of bioartificial tissues, we have developed a multi-scale model that can describe how the complex interplay among key intracellular processes, cell population dynamics and nutrient depletion regulates the growth of tissues in 3D scaffolds. We use a discrete, stochastic algorithm to describe the population dynamics of migrating, interacting and proliferating cells. Diffusion and consumption of a limiting nutrient is modeled by a partial differential equation subject to boundary conditions appropriate for common bioreactors. This PDE is solved numerically and the computed concentration profiles are used to modulate cell proliferation rates and migration speeds. The hybrid discrete-continuous model was parallelized and solved on a distributed-memory multicomputer to study how mass transport limitations affect tissue regeneration rates under conditions encountered in typical bioreactors.

Simulation results show that the severity of mass transport limitations can be estimated by the magnitude of two dimensionless groups. Critical system parameters like cell population heterogeneity, the initial spatial distribution of seed cells, the distribution of cell migration speeds, and the hydrodynamic environment are shown to affect not only the overall rate, but also the pattern of tissue growth. More specifically, the interplay of cell population heterogeneity and cell death due to nutrient depletion can lead to dynamic self-assembly of cells and the formation of stratified structures.

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Velocity-dependence of Cargo Loading onto Molecular Shuttles Demonstrates the Glue-like Character of Biotin/Streptavidin

Ashutosh Agarwal, Henry Hess.
University of Florida, Gainesville, FL, USA.

Molecular shuttles based on biomolecular motors and their associated filaments are being developed to function as conveyor belts in the molecular factories of the future. An essential design element in these active nanoscale transport systems is cargo loading onto the shuttles. We demonstrate that molecular shuttle velocity has to be optimized to facilitate cargo attachment of nanospheres via biotin-streptavidin linkages. The biotin-streptavidin bond gains its ultimate strength on a timescale of milliseconds due to existence of metastable binding states. As a consequence of the glue-like character of this widely used intermolecular bond, the velocity of molecular shuttles has to be optimized to permit efficient attachment of cargo via biotin-streptavidin linkages.

In our experiments, kinesin motor proteins adsorbed to a casein precoated surface were used to propel biotinylated microtubules which were coated with streptavidin at saturating dosages. The microtubule gliding velocity was varied between 50 nm/s and 450 nm/s by changing the kinesin substrate ATP concentration. Finally, biotinylated fluorescein-labeled nanospheres were added in concentrations ranging from 25 pM to 100 pM. Nanospheres attached to the surface and were loaded onto microtubules only as a result of collisions between gliding microtubules and nanospheres. Nanosphere attachment showed an unexpected optimum at an intermediate shuttle velocity.

The attachment and detachment processes were modeled by combining rigorous mechanical engineering analysis with detailed physico-chemical models. This contribution will present both, the experimental details of our velocity dependent loading experiments and the theoretical model which explains the optimum on the basis of the complex binding energy landscape of the biotin streptavidin linkages.

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Correlation Between Antibody Affinity and Activity: Understanding the Molecular Basis for a picomolar to femtomolar Increase in Affinity

Eilyn R. Lacy, Juan C. Almagro, Gabriela Canziani, Debbie Gardner, Michael Brigham-Burke.
Centocor R&D, Radnor, PA, USA.

A chimeric antibody was human adapted and then affinity matured. Biological activity studies revealed that the affinity matured antibody is 10-fold more potent that the chimeric antibody. To determine the correlation between affinity and activity of these antibodies, the binding profile to their antigen was analyzed by Biacore and Kinexa. The studies showed the two mAbs have different thermodynamic profiles. These differences, particularly the equilibrium dissociation constant, K_D, revealed a positive correlation with potency (biological activity). The data showed that the affinity of the chimeric antibody is picomolar, whereas the affinity of the human adapted antibody is femtomolar. Molecular modeling studies showed that several of the mutations introduced in the CDRs during the affinity maturation process were hydrophobic replacements