

**1612-Pos Board B456****Multivariable Parameter Optimization Of Microfluidic Post Arrays For DNA Fractionation**

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Length-based fractionation of long DNA is a fundamental process in genomic analysis. Traditional methods, such as pulsed-field gel electrophoresis or capillary electrophoresis, are slow and not easily coupled to downstream analytical processes. Microfluidic devices containing arrays of micron-scale posts have previously been described for length-based fractionation of kilobase length DNA. [1] Here, we present experimental and computational studies defining and optimizing a broad array of parameters in order to maximize mass throughput and isolation of DNA > 150 kb. Parameters explored include post field geometry, electric field intensity, and field oscillation timing. Experimental studies were performed by analyzing the mobility of single YOYO-1 intercalated DNA molecules in PDMS post arrays. Computational studies simulated kilobase length DNA as a worm-like chain model and investigated the interaction of such molecules with post arrays of various geometries. Optimization of our system results in the ability to isolate 165 kb DNA from 125 kb DNA and process 5 ng of DNA within 30 minutes of operation. This research was supported by the Department of Homeland Security, Science and Technology Directorate. [1] Huang et al., Nature Biotechnology, 2002, 20, 1048.

**1613-Pos Board B457****Nanopore Sequencing with MspA**

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Nanopores may provide the basis for a high-speed and inexpensive de novo DNA sequencing technique that could revolutionize medical and biological science. In this technique, single-stranded DNA is electrophoretically translocated through a pore with inner dimensions similar to that of DNA. The co-passing ion current is recorded to obtain sequence information. Since its inception, nanopore sequencing has had promising results with only one bacterial pore  $\alpha$ -Hemolysin and various solid-state pores. The geometry of another bacterial pore, MspA, found in the outer membrane of *Mycobacteria Smegmatis*, appears to be ideally suited for nanopore sequencing. We used site-directed mutagenesis on MspA to produce mutants that allow DNA translocation. These mutants can resolve small chains of the nucleotides A, C, and T when a duplex region of hairpin DNA arrests translocation. Additionally, DNA interaction with the mutant MspA is significantly and predictably altered with further mutations to the MspA structure. Our results introduce MspA as a promising and engineerable framework for nanopore sequencing technology.

**1614-Pos Board B458****Mechanotransduction in Single Cardiac Myocyte Studied Using Laser Tweezers and FRET**

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Deletion or mutation of a variety of proteins localized at cell-matrix and cell-cell junctions, such as vinculin and its splice-variant metavinculin, can lead to dilated cardiomyopathy in mice and humans, leading some to hypothesize that these molecules are involved in mechanotransmission or mechanotransduction in the heart.

To investigate cardiac mechanotransduction mechanisms in single cells, we have combined laser tweezers with a fluorescence resonance energy transfer (FRET) biosensor to apply localized forces and probe localized signaling events in isolated mouse ventricular myocytes.

Isolated murine ventricular cardiac myocytes were transfected with a focal adhesion kinase (FAK) FRET reporter to monitor integrin-mediated activation events. An integrin ligand-coated microsphere was adhered to the cell surface. Then laser tweezers were used to apply localized piconewton forces parallel or normal to the image plane either cyclically or statically. To ensure a constant force application on the microsphere, 10 $\mu$ M of blebbistatin was added to the imaging media. In conjunction with force application, a pulsed Ti:sapphire infrared laser was used for two-photon excitation of the FRET reporter.

Phase contrast and fluorescent images were captured simultaneously, allowing quantification of applied forces and FRET ratio changes. Results indicate that piconewton level forces can be applied to the microsphere and FRET ratio

changes validate that integrin-mediated events are being activated by the locally applied forces. This data suggests that the use of laser tweezers combined with FRET provides a means to study integrin-mediated events in cardiac mechanotransduction. Future studies include varying the type of integrin ligand-coated microsphere and FRET biosensors, as well as studying genetically manipulated murine lines in isolated adult cardiac myocytes.

**1615-Pos Board B459****Engineering Polymeric Drug Delivery Systems For Cancer Therapeutics Using Multi-scale Modeling**

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Polymers are of particular interest as drug delivery vehicles due to their ability of targeting drugs to tumors while simultaneously decreasing drug exposure to normal tissues. The classical method of designing polymer-drug conjugates invokes trial-and-error testing of chemical substances on animals and subsequently matching apparent effects to treatments. While effective, this procedure can be time-consuming and expensive. In our study, we use an *ab initio* approach to elucidate certain physicochemical properties of polymer-drug therapeutics that cannot as readily be determined by traditional experimental methods: bottom-up atomistic-to-mesoscale computational modeling.

Our polymeric DDS is poly-L-glutamyl-glutamine (PGG) covalently bound to Paclitaxel, a widely used anticancer therapeutic. Physicochemical properties of polymer-drug conjugates that have been shown to potentially affect the delivery and targeting of drugs to tumors are particle size and shape. The size and shape of polymer-drug conjugates have been shown to affect their abilities of adhering to tumor endothelium, being endocytosed by tumor cells, and diffusing through fenestrations of leaky tumor vasculature. We have developed coarse-grained models of PGG Paclitaxel in effort to achieve a variety of sizes and shapes by varying the Paclitaxel % weight loading (18%, 24%, 37% of total wt) and distribution (even, random, clusters, middle, side, ends) on PGG. Parameterization of PGG and PGG Paclitaxel was accomplished using the MARTINI force field, and simulations were run in GROMACS in explicit water solvent in 310 K for  $\sim 1\mu$ s. The aggregation of PGG Paclitaxel molecules into different sizes and shapes were then observed.

We plan to demonstrate multi-scale modeling as a novel tool that allows us to successfully engineer a polymer-drug cancer therapeutic. With this model we expect to suggest optimal physicochemical properties of PGG Paclitaxel for future synthesis and testing.

**1616-Pos Board B460****Impact Of Oxysterols On Endothelial Elastic Properties, Contractility And Morphogenesis**

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Our previous studies, have shown that exposing bovine aortic endothelial cells (BAECs) to oxLDL resulted in an increase in cell stiffness, force generation, and endothelial network formation. The mechanisms responsible for these effects, however, were completely not clear. Since all three effects could be simulated by cholesterol depletion, we suggested that oxLDL may be mediated changes in the membrane cholesterol. However, the total amount of the cholesterol remains the same after exposing oxLDL to BAECs. To resolve this discrepancy, we tested whether oxLDL-induced effects can be reversed by supplying a surplus of cholesterol. To achieve this goal, we used the MBCD complexed with cholesterol, a known cholesterol donor. In all the experiments, cells were exposed first to oxLDL for one hour and then subsequently to MBCD-cholesterol for an additional 1 hour. Our observations show that after treating with MBCD -cholesterol, cell elastic modulus, force generation and network formation were back to the normal level as compared to control cells. It suggests that cholesterol plays an important role in oxLDL induced cell mechanics. Furthermore, we show that the impact of oxLDL on endothelial biomechanics and morphogenesis can be simulated by specific oxysterols, known components of oxLDL. Here, we show that specific oxysterols have distinct effects on endothelial biomechanical properties. Specifically, we tested four different oxysterol species that are present in oxLDL: 7B- hydroxycholesterol, 7-ketocholesterol, 25-hydroxycholesterol and 27-hydroxycholesterol to study their mechanic properties. The results show that 7k and 27HC are potent factors to increase cell elastic modulus. However, only 27HC raises the force

generation and network formation. In contrast, exposing the cells to Bacterial SMase C to hydrolyze of sphingomyelin didn't affect neither endothelial biomechanics nor morphogenesis indicating that OxLDL-induced stimulation of SMase cannot be responsible for these effects.

#### 1617-Pos Board B461

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

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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.

#### 1618-Pos Board B462

##### Biophysical Regulation of Endoderm By 3-Dimensional Fibronectin Matrix

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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.

#### 1619-Pos Board B463

##### Dynamic Behavior Of Heterogeneous Cell Populations Growing Under Mass Transport Limitations

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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 multicompiler 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.

#### 1620-Pos Board B464

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

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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.

#### 1621-Pos Board B465

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

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A chimeric antibody was human adapted and then affinity matured. Biological activity studies revealed that the affinity matured antibody is 10-fold more potent than 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