Bacteria & Motile Cells: Mechanics, Motility, & Signal Transduction

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Fluid Transport in the Extracellular Matrix:Kinetic Changes Induced by Metabolic Inhibitors

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Water and solutes are transported to and from cells across the extracellular matrix by osmo-mechanical forces, the fiber-mesh, glycosaminoglycans, and myofibroblasts in as yet unclear ways. Here, we analyze fluid transfer in/out of the dermal matrix as a function of pressure and time and explore the effects of glucose-metabolism inhibitors. Osmotic-stress techniques were adapted to measure transfer-kinetics in full-thickness dermal cultures at six different polyethylene glycol concentrations adjusted by membrane osmometry (range =3-211 mmHg). Influx/outflux at each pressure was followed over time by precision weighing. Progression curves were modeled using:

$Volume\ Transfer = V_{max}/[1+(time/T_{1/2})^d]$,

where V_{max} is total volume transferred; $T_{1/2}$ is the time at which volume is half-maximal; and d is proportional to the rate at $T_{1/2}$. Rates and Vmax were found to be proportional to pressures and varied with temperature, as did the pH of cultures, suggesting that flow regulation is energy-dependent. Na Azyde (0.4mg-2.5 mg/ml) increased outflow, while Iodoacetamide (1.8-4.5 mg/ml) decreased it relative to that in explants without inhibitors; at 208 mmHg, Vmax values were 0.438 $\pm$ 0.0058 (Na Azyde); 0.284 $\pm$ 0.036 (Iodoacetamide); 0.359 $\pm$ 0.029 (control) below the initial value. Inflow increased with inhibitors; at 3 mm Hg, Vmax values were 0.392 $\pm$ 0.032, 0.463 $\pm$ 0.093, and 0.265 $\pm$ 0.012 above the initial volume, respectively. With the caveat that the inhibitors' effects on transfer could be unrelated to changes in the myofibroblasts' glucose metabolism, these differences suggest that energy for flow regulation is derived primarily from anaerobic metabolism during outflow but from aerobic and anaerobic metabolism during inflow. Globally, these observations are consistent with a model where myofibroblasts respond to pressure and oxygenation changes by adjusting fiber tension to control glycosaminoglycans hydration.

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Untangling Hyaluronan-Protein Networks and Function

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Hyaluronan (HA), a polysaccharide present in the extracellular matrix (ECM) and attached to the plasma membrane of many cell types, is suspected to mediate a surprising number of biological functions. Researchers have puzzled over how such a relatively simple polysaccharide influences so many physiological processes. It is suspected that the wide array of hyaluronan binding proteins helps to modify the structure and activity of HA. These hyaluronan binding proteins are located in hyaluronan-rich tissues throughout the body: neurocan in the brain, versican in the skin, aggrecan in cartilage, TSG-6 in inflammatory processes. However, very little is understood about how these proteins interact with hyaluronan and lead to a restructuring of the local matrix. We employ video-based Particle Tracking Microrheology (PTMR) and Fluorescence Recovery After Photobleaching (FRAP) to characterize the mechanical and structural properties of hyaluronan-protein solutions. We have started by measuring the dependency of the viscoelastic shear moduli on frequency (1-1000 kHz) for highly-monodisperse HA solutions at four different molecular weights (160kDA, 500kDa, 1000kDa, 2500kDa). The HA concentration was varied to explore the scaling laws of the mechanical properties as predicted by polymer theory. FRAP is applied as a complimentary method to determine the mesh size and transport properties of the networks. These control studies of monodisperse HA prepare us for a detailed PTMR and FRAP study of hyaluronan-protein networks, particularly those formed with hyaluronan binding protein (HABP), the G1-domain of versican, and the link domain of TSG-6.

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Functional Analysis Of Intravital Two-photon Cell Motility Data

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Intravital two-photon microscopy is a powerful tool which allows to observe cell migration and interaction in living tissue. Recently this method was applied to lymphocytes in lymph nodes. The motility of lymphocytes has important implications on the functionality of immune responses. Different cell types entering and leaving lymphoid tissue have to home to their respective compartments and to find suitable interaction partners. Both processes crucially depend on chemotaxis which is mediated by corresponding chemokines and membrane bound receptors. While this knowledge was based on corresponding block- or knock-out-experiments, the aim of the recent two-photon experiments was to clarify the migration mode of lymphocytes in vivo under realistic conditions. Surprisingly two-photon experiments of lymphocyte motility in lymph nodes do not exhibit any sign of chemotactic activity. Three groups have found that the cells basically move in a random walk with directional persistence of 2 minutes (Schwickert etal Nature 2007 446:83; Allen etal Science 2007 315:528; Hauser etal Immunity 2007 26:655). These results induced a controversial discussion about functional implications that could not be resolved on the basis of the measured data alone. We show that a mathematical analysis of cell motility can clarify the interpretation of two-photon motility data for this specific case and in general. Data about tissue morphology and functionality are used together with a simulation of lymphocyte motility to understand the details of chemotaxis in lymphoid compartments. It is found that the chemotactic sensitivity of lymphocytes is tightly regulated and the simulations predict a frequent up and down regulation of chemokine receptors on the plasma membrane. It is shown that under these conditions both the motility data and lymphocyte functionality can be reconciled.

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Sarcomere Mechanics in the Stress Fiber

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Actin filaments inside endothelial cells assemble into tensed bundles called stress fibers. Stress fibers are observed in vivo in a variety of tissues including the aorta, the heart, the spleen, the eye, and hair follicles. Sarcomeres are the force generating units of stress fibers and are responsible for generating intracellular tension. Little is known about the mechanical behavior of individual sarcomeres in living cells. Using femtosecond laser ablation to sever individual stress fibers in living capillary endothelial cells, we are able to measure the mechanics of sarcomeres in living cells. Our results indicate that the length of a sarcomere after severing decreases in two phases- an initial elastic response, followed by slower contraction at constant speed. The latter phase, interpreted as active myosin-mediated contraction, ceases abruptly after a minimum sarcomere length is achieved, suggesting a rigid resistance that prevents further contraction. We model this with an equivalent mechanical circuit, allowing us to estimate the speed of myosin motor walking in sarcomeres for the first time. We find that this speed ranges from 0.02 to 0.1 microns/s, which compares well with in vitro measurements. Our analysis suggests a novel mechanical model of a sarcomere that includes an active force generating component in parallel with an infinite barrier and in series with a stiff elastic spring.

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Transport Theory For HIV Migration Through In Vivo Distributions Of Microbicide Epithelial Coating Layers

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Topical microbicide products are being developed for preventing HIV transmission. These include vaginally applied gels that deliver anti-HIV molecules. Gels may also provide partial barriers that slow virion diffusion from semen to vulnerable tissue, increasing the time during which anti-HIV molecules can act. Previously, our group developed a mathematical model for HIV transport and neutralization for a uniform layer of microbicide gel. Hindrance of HIV diffusion was found to have significant potential to impact efficacy. In vivo, however, gels do not deploy to form complete, uniform layers - not all tissue is coated, and thickness is not constant. Here, we further developed our model to assess salient parameters that determine a gel's ability to hinder HIV diffusion in vivo. We applied this model to experimental data for coating distributions of two vaginal gels in women. Time required for a threshold number of virions to reach the tissue surface was used as a metric to compare different hypothetical and experimental scenarios. We found that time-to-threshold increased with increasing gel layer thickness and with decreasing diffusion coefficient. For gel layers with average thickness $>$ 100um, fractional area coated rather than gel layer thickness was the primary determinant of time-to-threshold. For gel layers $<$ 100um, time-to-threshold was brief, regardless of fractional area coated. Application of the model to experimental data showed little difference in time-to-threshold between the gels tested. However, the protocol