2094-Pos
Microscale Colocalization of CD3 and CD28 is Required for Activation of Human CD4+ T Cells
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It is increasingly recognized that intracellular cell signaling is dependent on the spatial organization of signaling molecules. We previously introduced a platform for investigating spatially-dependent signaling, in the context of the immune synapse, a small (~70 square micrometer) region of contact between T cells and Antigen Presenting Cells. Multiple rounds of microcontact printing are combined to produce glass surfaces with independently defined, microscale patterns of antibodies to CD3 (part of the TCR complex) and CD28 (a major costimulatory signal), surrounded by ICAM-1; antibodies locally engage and activate their respective ligands, organizing signaling complexes in cells on these substrates. We demonstrated that Il-2 secretion by naïve mouse CD4+ T cells is sensitive to the position of CD28 signaling within the region of cell-substrate contact, analogous to the immune synapse. Mouse T cell activation is less sensitive to the organization of CD3 both within the cell-substrate interface and with respect to the location of CD28. In sharp contrast, we demonstrate here that Il-2 secretion by human CD4+ T cells requires colocalization of CD3 and CD28 signaling. All patterns we examined in which antibodies to CD3 and CD28 are separated by micrometer-scale distances were ineffective in inducing Il-2 secretion. Immunohistochemcial staining using phospho-specific antibodies after 15 and 60 minutes following cell-substrate binding revealed that colocalized patterns are more effective than segregated counterparts in maintaining Lck phosphorylation at Y394, a site associated with full activation of this kinase. No differences in Zap70, PI3K, or PKC-theta were observed as a function of pattern geometry. Together, these results identify a dramatic difference between mouse and human T cell physiology, and suggest that Lck may be responsible for spatial integration of CD3 and CD28 signaling.

2095-Pos
Monte Carlo Study of B-Cell Receptor Clustering by Antigen Cross-Linking
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B cell signaling is triggered by the recognition of antigens by the surface proteins of the cell known as B-cell receptors (BCRs). It is known from experiments that, in the presence of soluble antigens BCRs assemble into small micro clusters and then structure into a macro cluster. However the underlying mechanisms of the antigen interaction with the BCRs and their cluster formation remain unclear. In our recent effort we have investigated, using Monte Carlo simulations, the mechanism of BCR clustering which would arise due to the intrinsic attractions among them. Such mutual attraction between two adjacent BCR molecules could arise, among other possibilities, due to cross-linking by bivalent soluble antigens. Recently, we have developed and studied a Monte Carlo model of B cell receptor clustering caused by binding and cross-linking of soluble antigens. The results of our study demonstrate the formation of small micro-clusters of BCR molecules (typically of size 2-10 molecules). But antigen cross-linking only is not adequate enough for the formation of large macro-clusters. A simple model of biased diffusion where BCR molecules experience a biased directed motion towards the largest cluster is then applied, which results in a single macro cluster of receptor molecules. The types of receptor clusters formed are analyzed using various network-based metrics such as the average distance between any pairs of receptors and number of adjacent pairs. The effect of BCR and antigen concentrations on the receptor clustering, the stability of the formed clusters over the time, and size of BCR-antigen cross-linked chains are all analyzed using suitable network-based metrics.

2096-Pos
Amplification & Analysis of the Synechococcus Os-B' Crispr Region from Single Cells
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The Octopus Hot Spring at Yellowstone National Park, which can reach near-boiling temperatures at its surface, is home to a microbial mat that has been found to be a good model for microbial diversity [90]. Among the many species of prokaryotes found in the hot spring mat are thermophilic strains of *Synechococcus*, which can be found in the photic zone at locations that correspond to a wide range of temperatures [2]. Fluorescence measurements of vertical mat slices have revealed a large degree of heterogeneity in the *Synechococcus* populations [3].
Conventional methods of genetic analysis require axenic cultures of bacteria, but less than 1% of bacteria species can be cultured in such a fashion [4]. To examine the genetics related to the aforementioned heterogeneity at the single-cell level, we have utilized and adapted a microfluidic chip for multiple displacement amplification (MDA) of DNA, which can amplify the DNA of a single cell for off-chip PCR and subsequent analysis [5,6]. Specifically, we are interested in examining the variation of the clustered regularly interspaced short palindromic repeats (CRISPR) region of the *Synechococcus* species found in the mat.
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2097-Pos
Cavitation Bubble Based Measurement of Red Blood Cell Elasticity
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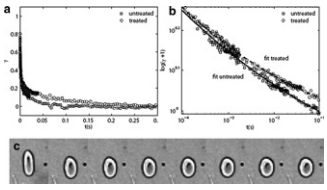
We present a novel technique to measure the red blood cell's (RBC) elasticity by exposing RBCs to an impulsive and transient flow. The flow is created from a rapidly expanding laser-induced cavitation bubble. The expanding bubble leads to a stretching deformation of cells close by. The fast flow lasts for approximately 20 microseconds and quickly ceases afterwards. Thereafter the RBCs relax back to their original shape, however on a much slower timescale. This relaxation is studied with high-speed photography and analyzed with digital image processing. In particular we determine the relaxation of the RBC's major and minor axis and find excellent agreement with a power law over several decades.
We compare findings on the elasticity of RBCs treated with neuraminidase and confirm the results from the common but much more laborious aspiration method. The figure above depicts the measured strain in linear and logarithmic scaling of treated and untreated RBCs, and a typical relaxation process of a single cell as a function of time.



The advantages of this novel technique are its simple implementation and the study of many cells simultaneously.

2098-Pos
Mechanical Properties of Desmin in Skinned Fibers from Normal and *desmin-Null* Mice
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Biomechanical properties of desmin, an intermediate filament protein, and its links through costameres to the contractile apparatus and the subsarcolemal cytoskeleton in single mammalian myofibers of *Extensor digitorum longus* single myofibers isolated from wild (**WT**) and desmin-null (**des-/-**) mice were measured. Suction pressures (**P**) applied through a pipette to the sarcolemma generated a bleb, the height of which increased with increasing **P**. At large **P**s, the connections between the sarcolemma and myofibrils broke; eventually the sarcolemma itself burst. We determined the values of **P** at which these changes occurred, and used these to determine the tensions and stiffness of the system and its individual elements. Tensions of the whole system and the maximal tension sustained by the costameres were (1.6-fold) lower in **des-/-** muscles than in **WT**. Separation and bursting **P**s, as well as the stiffness of the whole system and the isolated sarcolemma were ~1.4-fold lower in **des-/-** than in **WT**. The viscoelastic parameters of the entire system and costameres were also reduced in desmin-null myofibers. Our results indicate that the absence of desmin reduces muscle stiffness, increases sarcolemmal elasticity, and compromises the mechanical stability of costameres and their connections

to nearby myofibrils as desmin act as scaffold around the Z disk. We develop an elastic model of the sarcolemma and its links through costameres to the contractile apparatus based on our results.

2099-Pos

Spatial Correlation of Speckle Fluctuations Reveals Thickness and Features of the Ocular Surface Tear Film

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Here we present Fluctuation Analysis of Spatial Image Correlation (FASIC), a non-invasive method for evaluating the complex dynamics of the tear film surface by spatial correlation analysis. Tear film stability and its interaction with the corneal surface play an important role in maintaining ocular surface integrity and quality of vision. Dry Eye Syndrome (DES) refers to abnormalities of tear film secretion and/or stability diagnosed by conventional methods such as the Schirmer test and tear break-up time (TBUT). Several different physical methods have been developed to measure non-invasively the structure and function of the tear film including high-speed videokeratography and dynamic wavefront aberrometry. Interferometry and optical coherence tomography are amongst new proposed methods to measure tear film thickness that have remained in research phase.

With FASIC, a series of images are obtained using a laser illumination and a cMOS camera. The spatial correlation is calculated for every frame. A sinusoidal background due to interference of the tear film appears in this spatial correlation together with other features. We have developed a mathematical model to obtain the thickness of the tear film from this sinusoidal background. The model includes the macroscopic dynamics of small lipid droplets in the tear film. Consistent data with live animal model and human clinical study has been obtained. The authors gratefully thank the support from NIH grant numbers: PHS-5P41-RR003155 and P50-GM076516.

2100-Pos

A Bluetooth Device for Wireless Communication of in vivo Data from Freely Moving Research Animals

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Collecting neurophysiological data through electrodes can impact behavior when the animal is connected to wires and less able to move. In Parkinson's disease there is a clear link between reduction in dopamine availability and Parkinson's symptoms, which include tremor, slowness of movement and postural alterations. To better study the link between dopamine release in the basal ganglia and motor behavior, we are developing the implementation of a Bluetooth wireless technology for the measurement of neurotransmitter release. Data of dopamine release can be collected by means of fast scan cyclic voltammetry in which voltage ramps between 450 mV and +1000 mV are applied at a rate of ~300 V/s to a carbon fiber electrode (CFE) implanted in the striatum. The oxidation and reduction currents can be converted to cyclic voltammograms to identify the dopamine signal. The voltage ramp signals are wirelessly delivered to a remote unit connected to the implanted CFE and the resulting currents are amplified and sampled at 44.1 kHz at the remote unit. Using stereo headset protocol to transmit the data back to the computer, a recording bandwidth of ~1.3 kHz has been achieved. As usual, the voltammetric current collected before dopamine release is subtracted from the voltammetric signal collected after dopamine release within the computer to extract the net oxidation and reduction currents due to dopamine release alone and to generate the cyclic voltammogram. We anticipate that this technology will be useful for the study of the mechanisms of Parkinson's disease and possibly other electrophysiological recordings from freely moving research animals.

2101-Pos

Modeling the Relative Effects of Biofouling, Fibrous Encapsulation and Microvessel Density on Implanted Glucose Sensor Performance

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The formation of a foreign body capsule around implanted sensors is purported as a key contributor to sensor failure. A number of different processes during the wound healing sequence not only decrease the vascular density proximal to sensor but also provide diffusive and bioactive barriers to the transport of analytes from the few remaining vessels that are near the implanted sensor. While a number of surface treatments have mediated this response, the relative contributions of the different stages of wound healing to the attenuation of sensor response have yet to be elucidated. A 1D partial differential equation model was constructed to examine glucose transport through the interstitium and assess the effects that different results of the inflammatory and wound healing processes will have on glucose transport to the sensor surface. By incorporating the effects of biofouling, macrophage adhesion, and fibrous encapsulation, we have been able to recreate subcutaneous glucose traces with attenuated signals and delayed responses that mimic those seen in previous experiments. Such a tool will allow us to probe the characteristic traits of the foreign body capsule (avascularity, dense fibrous matrix, inflammatory cell presence, etc.) to gain a better understanding of what aspects of the wound healing process contribute most to sensor failure. With a more thorough knowledge of the relative contributions of the wound healing process to the decrease of sensor effectiveness, researchers can more rationally address issues of biocompatibility in the design of subcutaneous sensors.

2102-Pos

Jet-Fluid Effects on the Stented-Flow Structure in the Cavity of Cerebral Aneurysm

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The endovascular treatment of cerebral aneurysms using coils and stents, which are metal mesh cylinders, provides a promising alternative to open surgery. Although various analyses on the property of stented flow have been presented [1,2], the flow reduction mechanisms are not completely understood.

Our numerical simulation indicates that the jet flow through stent struts can reduce near the aneurysm mouth but increases the flow speed far from the mouth (Fig. 1). In this work, based on this observation, we reveal the effect of the phenomenon that the pulsed jet flow drives the fluid with different velocity on the flow structure in the aneurysm cavity. As a result, we found a possibility that the shape of aneurysm may induce the self-oscillation of jet flow. We expect that our findings introduce new strategies in stent development and improve the endovascular treatment of cerebral aneurysms.

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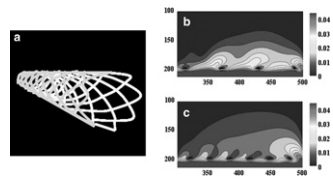

(a) Stent image. (b,c) Velocity distribution of stented flow.

2103-Pos

Development of Non-Viral Gene Delivery Carriers for Ischemic Heart Disease (IHD)

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Ischemic heart disease (IHD) or coronary artery disease (CAD) is a leading cause of death in the United States resulting in a major financial burden to the health care system and is projected to be one of the main contributors to disability by 2020. The poor prognosis of IHD is directly related to a build-up of atherosclerotic plaque that produces narrowing of the coronary artery lumen. The rupture of the artery and/or narrowing of the artery lumen results in myocardial ischemia, which can lead to myocardial infarction or death of the heart muscle tissue. Current treatments include bypass surgery, angioplasty, stent implantation, and pharmacotherapy but unfortunately many patients with IHD remain refractory to pharmacological treatments and are unsuitable candidates for surgical interventions. Also, restenosis of the vessel lumen due to neointimal hyperplasia is a recurrent problem. Gene therapy is a promising alternative to traditional treatment strategies since the delivery of angiogenic cytokines can stimulate neovascularisation in a process known as therapeutic angiogenesis. To this end, we have designed, synthesized, and characterized novel biodegradable polymeric carrier systems for the delivery of therapeutic angiogenic plasmids. The polymers were found to have a MW of ~3.2 kDa. A gel retardation assay showed condensation of DNA at N/P ratios higher than 20/1. The particle sizes of the polymer/DNA complexes were 100-231 nm with surface charges of 0.8-20 mV. Preliminary data with the reporter gene luciferase showed that the complexes produced significantly higher transfection efficiencies and lower cytotoxicities in several cell lines as compared to