invasion of hosts and disease initiation and progression. In order to monitor the pathogen invasion process, most often one mixes a solution containing pathogens into a culture of host cells, and then hopes the host cell being monitored is invaded by a pathogen. These chance encounters have been monitored using fluorescence microscopy, allowing for example fluorescent effector proteins to be monitored as they are injected into a host cell (Enninga, Mounier et al. 2005; Schlumberger, Muller et al. 2005). Such ''mix and hope'' strategies are simple, direct, and necessary for initial studies. However, they do not provide a path to obtain large sample sizes, control of timing of pathogen invasion, or a way to determine how many bacteria are required to defeat host defenses. We present recent results from using optoelectronic tweezers (OET) (Chiou, et al. 2005) as a tool for manipulating single pathogenic bacteria, opening a promising route for controlled initiation of HP interactions. Optoelectronic trapping uses laser-excited carriers inside a thin film of amorphous or crystalline Si to create a non-uniform electric field. These carriers, coupled with an electric field modulated in the MHz range produces strong field gradients. The object being trapped is polarized by the electric field, and gradients in the electric field create a potential well which traps the object. This trapping is obtained at extremely low optical intensities ($<1 W/cm^{\wedge 2}$) and does not require functionalization of the pathogen in order to facilitate delivery to the host, opening up novel possibilities for massively parallel studies of HP interactions.

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A Suitably Compliant Microenvironment Commits Mesenchymal Stem Cells to Differentiate into Muscle Like Cells Which Restore Muscular Defects in Dystrophic Models

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Human mesenchymal stem cells (hMSCs) express markers of different lineages when grown on matrices of an elasticity that mimics various tissues of mesenchymal origin. Of particular interest to our group is the possibility of using this approach to drive hMSCs into skeletal muscle lineage for repair of damaged skeletal muscle. In order to identify and determine the necessary *in vitro* culture conditions that will lead to optimal commitment of cells to the muscle lineage *in vivo*, we have been developing biophysical techniques to allow stem cell precommitment to a specific lineage prior to implantation in a diseased tissue by controlling matrix stiffness under *in vitro* culture conditions. This technology has led to a highly reproducible approach to optimizing stem cell fate for human transplantation. The physical nature of a cell's microenvironment - including elasticity of the surrounding tissue - appears to exert a significant influence on cell morphology, cytoskeleton and gene expression. Numerous gel systems - particularly polyacrylamide gels - have tunable elasticity that can be adjusted over several orders of magnitude from extremely soft to stiff, mimicking the elasticity of a wide range of tissues by controlling the extent of polymer cross-linking. Here, we use cross-linked polyacrylamide hydrogels that mimic the true *in-vivo* muscle-like elasticity (10-15 kPa) to induce hMSCs to differentiate into myoblasts expressing key early markers of muscle differentiation program (Pax7, myoD). They also fuse to form myotube like structures expressing late skeletal muscle markers like troponin I in vitro. We have recently used these committed cells on our *in vivo* animal models of muscular dystrophy and successfully demonstrated that they have a very high potential for integrating into skeletal muscle and rescuing the muscular defects.

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Single-Molecule DNA Biosensors for Quantitative Transcription Factor Detection

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We have developed two single-molecule fluorescence biosensors that can quantitatively detect single or multiple transcription factors (TFs) with high sensitivity and specificity. The first sensor is based on TF-based coincidence of two DNA fragments, each containing half of a particular TF binding sequence. In the absence of the particular transcription factor, the DNA fragments diffuse independently. The coincidence is detectable using 2-colour alternating laser excitation (ALEX) spectroscopy using either solution or surface-based approaches.

We have detected a single transcription factor (lactose repressor - lacR) at a concentration of 100pM without the need for amplification steps. Further we can detect TFs in a quantitative manner using a simple kinetic model, without the need for a calibration curve. Using an inducible plasmid for catabolite activator protein (CAP), we show quantitative detection of changes in gene expression in bacterial cell lysates over time.

We extend this first sensor to simultaneously detect two transcription factors (lacR and CAP) in the same solution. Two assays were designed to implement two basic Boolean logic operators (AND and OR). We demonstrate correct functioning of these operators using solution and surface-based approaches with nM TF concentrations. These operators can be cascaded to form arbitrarily complex intelligent sensing assays for true multiplexed detection of several TFs in one experiment.

The second sensor is based on the common phenomenon of TF-induced DNA bending. The design is uni-molecular with a donor-acceptor pair in close proximity and a binding site for the TF of interest. On binding of the TF (CAP), the DNA is bent, increasing the donor-acceptor distance. This can be detected via a change in FRET (Förster Resonance Energy Transfer) in a quantitative manner. Current investigations are focused on using this sensor for TF detection in cell lysates and in-vivo.

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A Microfluidic Device to Maintain Islet-Associated Endothelial Cells During Long-Term Tissue Culture

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Endothelial cells (EC) are integral to the characteristically dense vasculature of pancreatic islets. This vasculature enables accurate blood-glucose sensing and rapid secretion of insulin into the blood stream. It also provides pro-survival hemodynamic signals to EC. How EC and beta cells interact to affect glucose stimulated insulin response is an actively debated topic. However, long-term studies in the *ex vivo* tissue are limited by the loss of EC over a period of days in traditional culture. We postulate that the EC die in part from an absence of the shear and media exchange provided by hemodynamic fluid flow. To test the role of hemodynamic forces on EC, we created a microfluidic device capable of supplying a range of fluid flow to *ex vivo* islets. Our protocol controls temperature, pH and bubble formation using two hot plates and a syringe pump for long-term desk top experimentation. Using this microfluidic device with immunofluorescence microscopy, we examined the morphological response of islet-EC to a variety of flow rates for 24 and 48 hours. Our results show more than twice the average percent area and connectivity of EC in islets treated in the device as compared to no-flow controls stored in traditional cell culture. Using this device with varying media viscosity, we determined that the differences in morphology are due to media exchange and not shear-activated survival. As well, we are currently evaluating the effect of fluid flow on beta cell survival by ensuring normal glucose stimulated calcium and insulin response. Overall, our data indicates that flow in a microfluidic device provides a reliable *co-culture* environment enabling the long-term study of cell biology in the pancreatic islet.

3175-Plat

Engineering Lipid Bilayer Platforms for High Throughput Cell-Free Electrophysiology

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Ion channels are crucial physiologically, involved in nearly all biological processes. Their many roles make them important drug targets as well as targets to avoid, as for drug safety screening (e.g. the hERG channel). Patch clamp provides the highest quality measurements of ion channel function and is used by the pharmaceutical industry to test drug interactions with ion channels, but has poor cost and throughput performance. As an alternative ion channel measurement platform, artificial lipid bilayers are well established to provide a highly controllable environment capable of measurement at the single molecule level, although they also suffer from their own technological shortcomings. Previously, we developed an artificial lipid bilayer platform which is capable of shipping and indefinite storage. Here we present the continued technological development of this system which has resulted in faster bilayer formation times and the ability to package, store, and ship ion channels with the bilayer chip. We have also begun to integrate array chip designs with automated and high throughput solution manipulation and ion channel measurement hardware, advancing this platform closer to operator-free involvement and low cost high throughput cell-free electrophysiology.

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Towards Dark Quencher Based Real Time DNA Sequencing

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Third-generation DNA sequencing technologies are expected to transform biomedical research and health care. Although powerful single-molecule DNA