Tuesday Morning, November 1, 2011

Biofabrication and Novel Devices Focus Topic
Room: 105 - Session BN+NM-TuM

Biofabrication Applications
Moderator: G.F. Payne, University of Maryland, College Park

8:20am **BN+NM-TuM2 Electrically Controlled Biofabrication with Stimuli-Responsive Polysaccharide and Their Visualization in Microfluidic Devices**, ***Y. Cheng***, *X.L. Luo, J. Betz, C.Y. Tsao, H.C. Wu, G.F. Payne, W.E. Bentley, G.W. Rubloff*, University of Maryland, College Park

Stimuli-responsive polysaccharides, such as chitosan and alginate, are useful biomaterials that can be induced to undergo a reversible sol-gel transition to generate biologically-relevant scaffolds. The recent discovery that their gelation can be triggered by imposing an electrical signal opens many avenues for the creation of biologically functional hybrid structures and their localization onto and within microfabricated devices for biofabrication and biosensing applications. Here we report two different mechanisms for creating polysaccharide hydrogels in microfluidics by electrical signal. The cathodic electrodeposition of the cationic chitosan hydrogel was achieved by electrochemically generated OH^- ions at the cathode surface, creating a localized pH gradient at the sol-gel interface. The anodic electrodeposition of calcium alginate hydrogel was achieved by electrical-signal-mediated release of Ca^{2+} ions as a result of electrochemically generated H^+ ions at the anode surface reacting with suspended $CaCO_3$ particles in alginate solution. Localization of the hydrogels in transparent microfluidic devices makes them highly accessible through optical imaging and spectroscopy. The processes of *in situ* gel formation are simple, scalable, spatially controllable, and electroaddressable. Applications in protein immobilization and cell assembly with electroaddressing capability were further demonstrated. With the advantage of spatiotemporal control of gel formation coupled with microfabrication techniques, a variety of novel and useful structures such as multi-layer, multi-address, and even site-programmable arrays of biological components can also be achieved.

8:40am **BN+NM-TuM3 Biofabrication for Interrogating Cell Signaling**, *W.E. Bentley,* ***T. Gordonov***, University of Maryland, College Park **INVITED**

The biological signal transduction process is the means by which external signals are incorporated into information that directly or indirectly alters gene expression and ultimately, phenotype. The hierarchical structure of signal transduction processes is a topic of intense research. Microbial quorum sensing (QS) is responsible for a variety of phenotypes and is rich in diversity and modes of action. As such, quorum sensing represents a "guide" for learning how signals can be translated into altered phenotype.

As microbial communities occupy a confined space over time, concentrations of extracellular signaling molecules accumulate, providing stimulus for unique and varied cellular responses as well as protection from competing microbial communities. Referred to as "quorum sensing" for it's often reported and coincident dependence on high population density, extracellular signaling provides a new basis for control over molecular and cellular processes as well as population behavior, perhaps in a manner more consistent with that of native machinery. Among behaviors guided by QS are the establishment and persistence of bacterial infections.

Our laboratory has uncovered many of the molecular features of the QS autoinducer-2 (AI-2) system using traditional methods that probe bacterial physiology and by exploiting newer princples of biofabrication. That is, we employed electrodeposition methods to assemble complex biological subsystems onto specific sites on microfabricated devices and within microfluidic channels via programmable electrical signals. We have also used genetic engineering techniques to create signal activated fusion tags that covalently link proteins to the device/bio interface. We have designed and synthesized "biological nanofactories" that provide small signal molecule generation at the surface of targeted and captured cells - enabling programmable control of cell function.

Using these methods, we have discovered attributes of the natural switching mechanism that can be exploited for developing next generation antimicrobials. That is, we decomposed elements of the QS "switch" via mutation and a mathematical model of the regulatory elements and coupled this understanding with devices designed to appropriately interrogate these molecular features. Finally, we have developed alkyl analogs of AI-2 that elucidate structural detail and have potential for affecting behavior in natural environments. Correspondingly, these serve as the basis for creating next generation antimicrobials that target the communication between bacteria rather than their survival mechanisms.

9:20am **BN+NM-TuM5 Surface Modified Magnetic Microparticles for Bioreactor Applications**, ***A. Khaing***, *E. Milkani, A. Maziarz, C. Lambert, W. McGimpsey*, Worcester Polytechnic Institute

A magnetically-stabilized, continuous-flow bioreactor was designed and applied for the controlled growth of rat aortic smooth muscle cells (RASMC) in a pre-determined shape in a three-dimensional environment. The cells were immobilized on magnetic agarose beads (MABs) and grown into a tube-shaped tissue. By adjusting the experimental parameters, the size of the MABs were controlled. The surfaces of the MABs were biochemically modified and RASMC cell growth on the modified MABs was tested. Initial RASMC tissue rings with MABs grew in the magnetic field inside the continuous flow of culture medium in the first few days. The RASMC tissue tube was formed in a week, and allowed to mature up to about a month before removing from the bioreactor to characterize it. Histological staining of RASMC tissue tube showed that RASMC were circumferentially aligned perpendicular to the direction of the flow of culture medium. The majority of the cells in the RASMC tissue tube grown out of the MABs stabilized in the magnetic field in the continuous flow were healthy and highly proliferating. The system has applications in the fields of tissue regeneration, pharmaceutical production, stem cell amplification and biofuel production.

9:40am **BN+NM-TuM6 Bacterial Communication in Controlled 3D Microenvironments**, ***X.L. Luo***, *H.C. Wu, C.Y. Tsao, Y. Cheng, J. Betz, G.W. Rubloff, W.E. Bentley*, University of Maryland

Antibiotic resistance is a growing and widely recognized public health issue. Today, more than 70% of bacteria are resistant to at least one of the most commonly used antibiotics. Bacteria evolve with increasing antibiotic resistance due to the selective pressure that administration of conventional antibiotics creates on cell viability, wherein those bacteria that survive antibiotics become dominant. The emergence of "super" bacteria that carry multiple resistant genes calls for the development of novel antimicrobial strategies that place *less* selective pressure on the target bacteria. Rather than killing bacteria with antibiotics, interruption of bacterial communication networks - or quorum sensing (QS) - might delay the population-scale behaviors of target bacteria in gene regulation and buy time for the host immune system to fight back. Microfluidic environments provide a controlled and attractive opportunity to study bacterial QS and to explore these strategies.

Here we report *in vitro* signaling between localized, spatially distinct cell populations in controlled 3D fluidic microenvironments. First, a freestanding chitosan membrane was fabricated by using pH gradients generated at the flow interface of two converging flows. Next, alginate membranes were fabricated by cross-linking alginate sequentially on both sides of the chitosan membrane using diffusion of calcium ions through the semi-permeable chitosan membrane. Finally, cell assembly was achieved by suspending cells in the alginate solution to embed the target cells into the alginate scaffolds, realized as a micro-sandwich structure of cells in alginate on both sides of the chitosan membrane. Signal molecules transmitted *in situ* from one cell population were transported either by diffusion to (1) surrounding cells and (2) nearby segregated cell population, or by convection to (3) cell populations that are relatively far away in a separated microchannel. Induced quorum sensing responses, the production of fluorescence proteins functionally linked to QS genes, were observed for all three configurations. Importantly, these membrane-based 3D scaffolds offer convenient top-down visualization and easy access to both sides of the scaffolds. These approaches provide a versatile and powerful platform to understand and modulate collective and interruptive cellular responses in bacterial quorum sensing.

10:40am **BN+NM-TuM9 "Body-On-A-Chip": Combining Microfabrication, Cell Cultures, and Mathematical Models**, ***M.L. Shuler***, Cornell University **INVITED**

We seek to understand the response of the human body to various pharmaceuticals. Our platform technology is an in vitro system that combines microfabrication and cell cultures and is guided by a computer model of the body. We called this in vitro system a micro cell culture analog (microCCA) or a "Body-on-a-Chip". A microCCA device contains mammalian cells cultured in interconnected micro-chambers to represent key body organs linked through the circulatory system and is a physical representation of a physiologically based pharmacokinetic model. [1, 2] MicroCCAs can reveal toxic effects that result from interactions between

organs as well as provide realistic, inexpensive, accurate, rapid throughput toxicological studies that do not require animals. The advantages of operating on a microscale include the ability to mimic physiological relationships more accurately as the natural length scale is order of 10 to 100 microns.

We have used a microCCA to test potential combination therapies (Tegafur and uracil) for colon cancer. [3] Tegafur is a prodrug for 5-FU and uracil an inhibitor of DPD, an enzyme which deactivates 5-FU. Simple microwell plates cannot probe this system, but the microCCA predicts the types of responses observed experimentally. A "pumpless" system that would be easy to utilize has been demonstrated with Tegafur also.[4] We have coupled these body modules with a micro model of the GI tract to examine the response to oral exposure of drugs, chemicals, or nanoparticles. [5]

Overall, we believe that in vitro, microfabricated devices with cell cultures provide a viable alternative to animal models to predict toxicity and efficacy in response to pharmaceuticals.

References

1. Sin, A., K.C. Chin, M.F. Jamil, Y. Kostov, G. Rao, and M.L. Shuler. The Design and Fabrication of Three-Chamber Microscale Cell Culture Analog Devices with Integrated Dissolved Oxygen Sensors. *Biotechnol. Prog.* (2004), *20*:338-345.

2. Khamsi, R. Meet the Stripped Down Rat. *Nature* (2005), *435*(5 May):12-13.

3. Sung, J.H. and M.L. Shuler. A Micro Cell Culture Analog (microCCA) with 3-D Hydrogel Culture of Multiple Cell Lines to Assess Metabolism-Dependent Cytotoxicity of Anti-Cancer Drugs.

Lab Chip (2009), *9*:1385-1394.

4. Sung, J.H., C. Kam, and M.L. Shuler. A microfluidic device for pharmacokinetic-pharmacodynamic (PK-PD) model on a chip. Lab Chip (2010) 10: 446-455.

5. Mahler, G.J., M.B. Esch, R.P. Glahn, and M.L. Shuler. Characterization of a gastrointestinal tract microscale cell culture analog used to predict drug toxicity. *Biotechnol. Bioeng. (2009) 104*:193-205.

11:20am **BN+NM-TuM11 Simultaneous Bacterial Transformation and Localization within a Microfluidic Device**, ***J. Betz**, Y. Cheng, C.Y. Tsao, G.F. Payne, W.E. Bentley, G.W. Rubloff*, University of Maryland

Transformation, the process by which a bacterium takes up and incorporates extracellular DNA, is one of the primary enabling technologies in the biotechnology field. This allows a researcher to program bacteria, equipping them with a complement of genes to accomplish a task, such as producing a molecule of interest or acting as a sensor. We describe the simultaneous transformation and localization of *Escherichia coli* bacteria in response to an electric signal within a microfluidic device. We demonstrate that these transformed bacteria can act as fluorescent sensors of isopropyl β-D-1-thiogalactopyranoside (IPTG), a chemical stimulus, or low dissolved oxygen levels, an environmental stimulus.

This method focuses on bacterial transformation with the added benefit of simultaneous entrapment within an alginate hydrogel at a desired electrode address. This offers the ability to create microfluidic cell-based sensors in a single, simple step. To transform and deposit bacteria, the device was filled with a mixture of electrocompetent cells, 200ng plasmid, 0.5% alginate, and 0.125% $CaCO_3$ and subjected to a 30V/cm DC electric field for 3 minutes on ice. The cells were allowed to recover at 37ºC for an hour, cultured for 16 hours, and induced with a chemical signal, IPTG, for 4 hours. This resulted in increased expression of DsRed, a red fluorescent protein.

Dissolved oxygen is an important parameter for many cell culture experiments. To create a dissolved oxygen sensor, *E. coli* were transformed with a plasmid that causes production of green fluorescent protein (GFP) in response to decreased dissolved oxygen concentration in the surrounding medium. Following the above transformation and culturing method, the cells were induced with media that had been deoxygenated in a vacuum chamber, resulting in an increase in GFP expression.

This method is versatile in terms of creating microfluidic cell-based sensors. We envision many exciting applications of this work, including the development of dynamically reconfigurable microfluidic biosensors and high-throughput screening methods for plasmid libraries generated by protein engineering and directed evolution experiments.

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