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

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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.

Biofabrication and Novel Devices Focus Topic
Room: 105 - Session BN-TuA

Biofabrication Methods and Devices

Moderator: L. Gamble, University of Washington

2:00pm **BN-TuA1 Microengineered Hydrogels for Stem Cell Bioengineering and Tissue Regeneration**, ***A. Khademhosseini***, Brigham and Women's Hospital, Harvard Medical School, MIT, and Harvard University **INVITED**

Micro- and nanoscale technologies are emerging as powerful tools for controlling the interaction between cells and their surroundings for biological studies, tissue engineering, and cell-based screening. In addition, hydrogel biomaterials have been increasingly used in various tissue engineering applications since they provide cells with a hydrated 3D microenvironment that mimics the native extracellular matrix. In our lab we have developed various approaches to merge microscale techniques with hydrogel biomaterials for directing stem cell differentiation and generating complex 3D tissues. In this talk, I will outline our work in controlling the cell-microenvironment interactions by using patterned hydrogels to direct the differentiation of stem cells. In addition, I will describe the fabrication and the use of microscale hydrogels for tissue engineering by using a 'bottom-up' and a 'top-down' approach. Top-down approaches for fabricating complex engineered tissues involve the use of miniaturization techniques to control cell-cell interactions or to recreate biomimetic microvascular networks within mesoscale hydrogels. Our group has also pioneered bottom-up approaches to generate tissues by the assembly of shape-controlled cell-laden microgels (i.e. tissue building blocks), that resemble functional tissue units. In this approach, microgels were fabricated and seeded with different cell types and induced to self assemble to generate 3D tissue structures with controlled microarchitecture and cell-cell interactions.

2:40pm **BN-TuA3 Nanoscale Architectures for Probing Cell Mechanics**, ***S. Wind***, *M. Schvartzman, M. Palma, M. Biggs, T. Fazio, R. Piqueras Jover, M. Sheetz*, Columbia University

The physical properties of a cell's environment are important factors in determining cell behavior and ultimately, phenotype. Two key factors that have been associated with major changes in cell morphology and behavior are (1) spatial organization of extracellular matrix (ECM) molecules and (2) rigidity. In order to understanding how cells sense these factors at the nanoscale and how these factors affect cell function, we have developed new nanofabricated surfaces in which these physical characteristics of the ECM are simulated.

The first type of surface combines nanoimprint lithography with selective biofunctionalization to precisely control the placement and geometric arrangement of integrin binding sites. The binding sites consist of sub-10 nm metallic nanodots functionalized with ECM binding ligands, designed so that each site can accommodate only a single integrin molecule. Cell spreading and motility assays were performed using 3T3 fibroblasts on arrays in which binding site spacing, density and number were independently varied. Cell spreading efficiency was markedly enhanced for clusters comprising at least 4 liganded sites spaced $\leq$ 60 nm apart, with little or no dependence on global density. This points to the existence of a minimal matrix adhesion unit defined in space and stoichiometry.

A second type of surface consists of elastomeric substrates with locally variable rigidity. We have found that exposure of poly(dimethylsiloxane) (PDMS) to an electron beam alters the rigidity of the elastomer, with the modulus of the exposed regions increasing with the applied electron dose. In addition to planar surfaces, pillared substrates can be patterned with no measurable change to the pillar dimensions. Immortalized mesenchymal stem cells plated on soft PDMS surfaces patterned in this manner displayed a distinct preference for the more rigid, exposed regions, forming focal adhesion nearly exclusively there. Furthermore, focal adhesion formation diminished significantly as the size of the exposed features was reduced below 1 μm, indicating that there is a length scale for cellular rigidity sensing, with the critical length in the range of a few hundred nanometers.

By adapting the tools of nanomanufacturing to cellular systems, we are able to define important parameters that can control aspects of cell function and behavior and will help identify conditions under which these functions may be altered. Potential applications range from therapeutic treatments that block metastasis to the development of new adoptive immunotherapies, as well as the development of new guidelines for the design of tissue scaffolds that can optimize healing without scarring.

3:00pm **BN-TuA4 Production of Functionalized 3D Micro Environment for Cell Culture**, ***J. Nowak***, *D. Mehn, P. Colpo, M. Zurn, T. Martin, F.J. Rossi*, European Commission, JRC Institute for Health and Consumer Protection, Italy

One of the main challenges for the robust *in-vitro* studies is to obtain adaptable 3D culture systems that may mimic the tissue environment. Unfortunately the universal condition used in 2D cell culture techniques may hinder the full functionality of cells and generate misleading results.

Fabrication of firm and flexible micro-structures from organic polymers offers benefits for making smart 3D environments capable of driving cell behavior and surpassing the limitations of the 2D systems. These 3D bio-scaffolds can be employed to study various aspects of cell biology. Furthermore upon functionalization with the extra-cellular matrix proteins or signaling molecules they can be used as platforms for governing stem cell differentiation into the specialized cell types.

Here we present the straightforward approach to generate 3D bio-scaffolds that can facilitate cell growth under controlled geometrical and chemical conditions.

The technique involves UV cross-linking of the polymeric precursors to create the micro-well structures. The geometrical features of the structures are obtained by introducing a physical mask in contact with a liquid precursor, therefore restricting the region of the polymerization. We used PDMS mold as a physical mask to direct the polymerization of the PEG-DA and epoxy based polymers. However the technique can be used with various UV-sensitive polymeric materials.

The chemical and geometrical properties of the structures were characterized by XPS and microscopic techniques.

The features of the scaffolds lead to the development of a geometrically defined neuronal network when applied as platforms in a primary-neuron culture. Cell morphology and expression of the neuronal markers were characterized by fluorescent microscopy.

Biofabrication and Novel Devices Focus Topic
Room: East Exhibit Hall - Session BN-TuP

Biofabrication and Novel Devices Poster Session

BN-TuP1 Photoluminescence Characterization of Polythiophene Films Incorporated with Highly-Functional Molecules Such as Metallophthalocyanines, ***H. Kobe***, *K. Onaka, H. Kato, S. Takemura, T. Hiramatsu, K. Shimada, K. Matsui*, Kanto Gakuin University, Japan

Conducting polymer polythiophene (PT) films incorporated with highly-functional molecules such as phthalocyanines with different center metals were synthesized and characterized by x-ray photoelectron spectroscopy (XPS) measurements, photolumimescence measurements (PL) and time correlated single photon counting (TCSPC) measurements in order to obtain fundamental photoluminescence properties of various PT-phthalocyanine complexes prepared by different solvents. The electrochemical polymerization was performed in acetonitrile containing thiophene monomer and $(ET)_4NBF_4$ as a supporting electrolyte and the polymerization on an indium tin oxide (ITO) was conducted by applying positive voltage to the anode. The dopant molecules were iron phtalocyanine (FePc), copper phtalocyanine (CuPc) magnesium phtalocyanine (MgPc), lithium phtalocyanine (Li_2Pc) and cobalt phtalocyanine (CoPc). Those molecules were doped in the polymer film by the diffusion method. The solvents used in the doping process were acetonitrile and toluene. At first, it was confirmed by XPS measurements that the metallophthalocyanines were introduced in the PT films. In the photoluminescence measurement, emission peaks were different in intensity and wavelength according to the additional dopant molecules. Those peaks were influenced by Soret and Q bands. The solvent used in the doping process also influenced the emission characteristics. In the case of CuPc using acetonitrile as a solvent, emission peaks which originated from Soret and Q bands were observed in the photoluminescence emission spectrum. On the other hand, emission peaks only due to Q band were observed in the cases of FePc, MgPc, Li_2Pc and CoPc. Using toluene as a solvent drastically changed the emission characteristics. In the case of FePc, Li_2Pc and CoPc, the emission peaks only due to Soret band were observed. As for CuPc, an emission peak due to Soret band was dominated. In the TCSPC measurements, it was confirmed that the number of life time components fitted to the decay curve ranged from 3 to 5 with several nanoseconds to several hundreds of nanoseconds. The life time and the number of components depended on the center metals of the doped phthalocyanine and the solvent used in the doping process. The present work clarified that the photoluminescence emission peak position, intensity and life time were varied by solvents and center metals of phthalocyanines.

BN-TuP3 Towards F_1-ATPsynthase Based Hybrid Nanobiodevice Fabrication, ***J.K. Settle***, *M.L. Richter, C.L. Berrie*, University of Kansas

Incorporation of biomolecules into nanoscale devices, termed nanobiodevices, requires control over biomolecule placement within the device. Nonspecific adsorption of the tiny molecular motor, F_1-ATPsynthase (ATPase), results in a variety of protein orientations on the surface. To improve the functionality of the immobilized protein, only one orientation (gamma unit upright) is desired. Therefore, controlling orientation is also imperative in increasing the number of functional molecules. Several techniques have been utilized to study and control this adsorption process. Atomic force microscopy was used to graft a dithiol into a resist matrix monolayer, exposing a thiol group. Through maleimide chemistry, a maleimide-nitriloacetic acid (NTA) group can be attached to the terminal end of the dithiol pattern. NTA will coordinate with nickel ions, which then coordinates with the histidine tag on the ATPase, thus controlling orientation. This process has also been studied via surface plasmon resonance. By coordinating these efforts with nanoelectrode construction, a functional nanobiodevice may be engineered.

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