

# Thursday Afternoon, November 12, 2009

## BioMEMS Focus Topic

Room: A8 - Session BM+MN+MS+TF+BI-ThA

## Advances in Microfluidics for BioMEMS

Moderator: G.W. Rubloff, University of Maryland

2:00pm **BM+MN+MS+TF+BI-ThA1** **Advances towards Programmable Matter**, *D. Erickson*, Cornell University **INVITED**

A dichotomy exists between the bottom-up self-assembly paradigm used to create regular structures at the nanoscale, and top-down approaches used to fabricate arbitrary structures serially at larger scales. The former of these enables rapid, highly parallel assembly but lacks critically important features of the latter such as the ability to arbitrarily direct the assembly location and perform error correction. We and our collaborators have recently proposed an alternative approach which combines these two based on dynamically programmable self-assembling materials, or *programmable matter*. The uniqueness of our approach is that it uses dynamically-switchable affinities between assembling components facilitating the assembly of irregular structures. In this talk I present an overview of our approach and detail some of the analytical and experimental advances towards a programmable matter system we have recently made. These include: the development of a multi-chamber microfluidic chip for improved far-field assembly, the demonstration of near-field inter-tile affinity switching using a thermorheological assembly fluid and the ability to enhance assembly in three dimensions using unique fluid-structure interactions.

2:40pm **BM+MN+MS+TF+BI-ThA3** **A Multilayered Microfluidic System with Buried Channels and Cell Compartmentalization for Engineering Heterogeneous Neural Networks**, *C. James, A. Greene, A. Schiess, G. Bachand*, Sandia National Laboratories, *M. Romero-Ortega*, University of Texas at Arlington

Current technology for engineering *in vitro* neural networks utilizes cell guidance cues that yield only temporary networks (< 1 month) as the cells rapidly diverge from their designed guidance cues during development of the culture. In addition, these engineered networks are typically comprised of homogeneous populations of neurons, thus the lack of multiple neuron types produces oversimplified networks that do not adequately represent *in vivo* networks. In addition, effective control over synaptic connections between different populations of neurons has not been demonstrated. Here, we describe a novel hybrid technology of multi-layered microfluidics with compartmentalized chambers containing multiple neuron types for engineering robust and complex neural networks with high resolution organization of synaptic connections. The device contains a first level of microfluidic channels etched 1-2 microns into the base glass substrate. These channels are fabricated with a novel process using a silicon nitride mask for hydrofluoric acid undercut etching to create buried microfluidic channels for robust containment and guidance of neurons. After the etching process, photoresist liftoff is performed to selectively adsorb poly-L-lysine (PLL) within the buried channels for improved neuron attachment and outgrowth at pre-defined locations. Polarity control of neurons is provided through a continuous set of guidance cues to promote axon development, while interrupted sets of guidance cues promote dendrite development. Current results show that axons and dendrites are positioned at predefined locations with a >65% accuracy. A second level of microfluidic channels and large (~mm) cell chambers are fabricated in polydimethylsiloxane (PDMS) from two-level SU-8 master molds. The base glass substrate and the PDMS substrate are aligned and bonded to create interconnects between channels in both substrates. These interconnects provide interaction regions for the development of synapses between growing neurites from cells in different chambers. We are currently applying this technology to engineer corticostriatal networks, an important region of the brain responsible for integrating multiple informational inputs crucial to complex decision-making in higher mammals. Specifically, we are using patch-clamp electrophysiology to track the development of synaptic memory in the form of long-term depression and potentiation (LTD/LTP) in these engineered networks.

3:00pm **BM+MN+MS+TF+BI-ThA4** **Vesicle Production on a Microfluidic Platform using pH Sensitive Block Copolymers**, *L.E. Brown*, The University of Sheffield, UK, *S.L. McArthur*, Swinburne University of Technology, Australia, *G. Battaglia, P.C. Wright*, The University of Sheffield, UK

The development of pH sensitive, biocompatible block copolymer vesicles has enabled the intracellular delivery of water soluble drugs and proteins.

Improving the encapsulation efficiency of the vesicles is now a critical parameter. Transferring the production method to a microfluidic device creates the potential to vary the encapsulation conditions and improve this efficiency. In this work, a flow focussing microfluidic device is used. The self assembly of PMPC-b-PDPA block copolymer vesicles is induced within the device by changing the pH of the flows within the microchannels. The use of pH shift eliminates the need for organic solvents currently required for glass capillary production methods. This enables the biocompatibility of the block copolymers to be maintained, an essential factor for their application as molecular delivery vehicles.

The flow focussing microfluidic device was produced through standard soft lithography techniques. A three-channel flow system is used with the copolymer in solution at pH6 in the central channel and aqueous buffered solutions flowing in the channels either side. The laminar flow conditions within the microfluidic device result in a pH gradient at the interfaces where the three channels meet and where the block copolymers self-assemble into vesicles. These vesicle formation processes have been imaged using confocal microscopy via FRET with a block copolymer containing both rhodamine and fluorescein isothiocyanate groups. Dynamic light scattering and TEM were used to confirm vesicle formation.

With 50nm to 250nm vesicles continuously being produced within the device it was then possible to investigate whether higher encapsulation efficiencies can be achieved using the microfluidic device. The protein myoglobin was introduced through the central channel along with the copolymer. Spectrophotometric analysis indicated the overall the efficiency of the encapsulation process within the device is not a significant improvement on the standard bulk methods currently used, involving sonication of the vesicle solution containing the molecule to be encapsulated. Despite this, the continuous nature of microfluidic devices, as well as the lack of organic solvents being used in the production process indicates that the development of these devices offers a viable alternative production method for polymer vesicles that may enable the increases in encapsulation efficiency to be achieved. Work is ongoing to achieve this using the same pH shift mechanism within a glass capillary microfluidic device.

3:40pm **BM+MN+MS+TF+BI-ThA6** **Integration of a Microfluidic Flow Cell Array with SPR Microscopy for In Situ Microarray Formation and Biomolecule Interaction Analysis**, *J. Liu, M. Eddings*, University of Utah, *A. Miles*, Wasatch Microfluidics, *B. Gale, J. Shumaker-Parry*, University of Utah

Analysis of biomolecule interactions based on surface plasmon resonance (SPR) microscopy provides a label-free approach to monitoring arrays of biomolecule interactions in real time. Typically the microarray sensing surface for these measurements is prepared *ex situ* and a single or few channel flow cell is used for the biomolecule interaction studies. The multiplexing nature then is derived from the microarray and the number of samples that can be run simultaneously is rather limited, diminishing the potential application for assays requiring a high-throughput approach due to a large number of samples. One example of this is the need to monitor for anti-drug antibodies from a large pool of patient samples during clinical trials of biotherapeutics. We demonstrate the capability of a multi-channel microfluidic flow cell array (MFCA) to expand the throughput capability when integrated with SPR microscopy. In addition, the MFCA provides an *in situ* approach to array fabrication that allows full characterization of the biomolecule immobilization process. We use the MFCA for delivery of sample solutions with continuous flow in 48 channels in parallel for rapid microarray creation and binding analysis while using SPR microscopy for real-time monitoring of these processes. Label-free measurement of antibody-antibody interactions demonstrates the capabilities of the integrated MFCA-SPR microscopy system and establishes the first steps of the development of a high-throughput, label-free immunogenicity assay. We demonstrate a limit of detection (LOD) of ~ 80 ng/ml for the particular antibody pair we studied. This LOD is ~6 times lower than the industry recommended immunogenicity assay detection limit. The high-throughput nature of the integrated system allows a large number of replicate experiments, including control experiments, to be performed simultaneously on the same sensor surface in a short time. The integrated system also will be applicable for more general high-throughput protein-array based analysis.

4:20pm **BM+MN+MS+TF+BI-ThA8** **Nanochannel Stretching of Nucleic Acids: Towards Epigenetic Analysis**, *D.E. Streng, S.-F. Lim, A. Karpusenka, J. Pan, J.A. Hook, R. Riehn*, NC State University

Nanochannels with a diameter of about 100nm<sup>2</sup> are a novel method for stretching DNA for genomic investigations. Such devices are implemented through standard nanolithography in fused silica. The elongation of DNA

results from an interplay of steric and entropic effects. Previous applications of nanochannel stretching included sizing, restriction mapping, and observation of transcription factor binding.

We show here that nanochannels can also be used to map the site-specific epigenetic state of DNA. In particular, we show here that the concept by nanoconfinement can be extended to chromatin, or DNA complexed to histones, and that the stretching is within the range expected from the de Gennes theory. We also demonstrate that the location-resolved cytidine methylation state of DNA can be mapped by specific fluorescent labeling. We will discuss the basic operation of these technique, and the application to artificial substrates with predefined epigenetic marks.

4:40pm **BM+MN+MS+TF+BI-ThA9 Microfluidic Models of Endothelial Cell Sprouting in Response to Biomechanical and Biochemical Microenvironments**, *A.M. Shamloo, S.C. Heilshorn*, Stanford University

A novel microfluidic device was designed in order to generate stable, quantifiable concentration gradients of biomolecules in a cell culture chamber for 2-D and 3-D studies of shear-sensitive cell types such as endothelial cells. Endothelial cells form the inner lining of blood vessels and initiate a critical step in angiogenesis (the sprouting of new blood vessels) during wound healing and cancerous tumor growth. Therefore, a deeper understanding of the critical biomechanical and biochemical factors regulating endothelial cell sprouting can lead to improved clinical therapies for a multitude of diseases. Concentration distribution of soluble growth factors inside the microfluidic cell culture chamber was determined by simulation and experiment, and the stability of the gradient was verified over multiple hours. This device allows independent tuning of the matrix rigidity, the growth factor absolute concentration, and the growth factor concentration gradient steepness within a single experimental platform. Sprout formation of dermal microvascular endothelial cells was studied within collagen gels of varying density (0.3 - 2.7 mg/mL, corresponding to shear moduli of 8 - 800 Pa) that contained stable gradients of soluble vascular endothelial growth factor (VEGF). These experiments revealed that endothelial sprouting into multi-cellular, capillary-like structures is optimized at an intermediate collagen matrix density ( $G' \sim 100$  Pa). At lower matrix densities, cells were more likely to lose their coordinated motion and migrate as individual cells through the matrix; while at higher matrix densities, the cells formed broad cell clusters that rarely elongated into a sprout. Sprout thickness directly correlated with matrix rigidity, with thicker and less frequent sprouts present in gels with the highest shear moduli. Intriguingly, our 3D experiments also found that endothelial sprouts alter their sensitivity to VEGF depending on the matrix density, suggesting a complex interplay between biochemical and biomechanical factors. As matrix stiffness increases, steeper VEGF gradients and higher VEGF absolute concentrations are required to induce directional sprouting. In more compliant gels, endothelial sprouts that originally misaligned were able to turn and properly reorient parallel to the VEGF gradient; however, this turning phenomenon was only rarely observed in stiffer gels. These results demonstrate that matrix stiffness is an effective factor in stabilization and orientation of endothelial cells during sprouting and suggests new anti-angiogenic strategies for potential cancer treatment as well as pro-angiogenic strategies for regenerative medicine scaffolds.

5:00pm **BM+MN+MS+TF+BI-ThA10 Plasma Polymerisation of PDMS for Microfluidic Applications**, *S. Forster, A.G. Pereira-Medrano, G. Battaglia, P.C. Wright*, University of Sheffield, UK, *S.L. McArthur*, Swinburne University of Technology, Australia

Polydimethylsiloxane (PDMS) has become the most popular material choice for a wide range of microfluidic bioengineering applications, including proteomics, protein separations and drug discovery and development. The reasons its popularity lie mainly in its highly advantageous fabrication requirements when compared to traditional materials such as glass and silicon. However, PDMS has some fundamental drawbacks, namely a lack of functionality present at the surface, high protein fouling and an inability to retain stable surface modification due to its motile hydrophobic monomer. These factors can lead to the loss of specificity and sensitivity in many bioassays. Due to this reason much work has been completed looking into surface modification of PDMS for such applications. Here an alternative method of stable surface modification of PDMS for many microfluidic applications through enhanced curing conditions and plasma polymerisation is shown. Stable and functional surface coatings have been achieved on bulk PDMS and within microfluidic channels. Bulk surfaces were characterised using a combination of XPS and ToF-SIMS, while coated micro-channels were tested using confocal microscopy and various assays. This methodology has been used in many applications and one area where it has proven extremely useful is in microfluidic proteomics where surface properties are of paramount importance due to the inherently small volumes and quantities associated with biological samples. Firstly, plasma polymer coated PDMS micro-

channels were utilised for on-chip IEF protein separations (i.e. separating proteins bases on charge) and showed reduced electroosmotic flow (EOF) and protein adsorption within the device. Secondly, a  $\mu$ IMER (micro-immobilised enzyme reactor) was produced using plasma polymer coated PDMS devices. The  $\mu$ IMER was then used in 'shotgun' protein digestion applications in conjunction with Mass Spectrometry where it was shown to have numerous advantages over untreated PDMS devices, as well as comparing favorably to published work on other  $\mu$ IMER systems. The device was used to digest single and multiple protein samples as well as complex membrane protein samples. Finally, successful covalent bonding of plasma polymer coated devices has led to the completion of polymer vesicle immobilisation within a microfluidic channel. Initial work looking at the immobilisation of polymer vesicles with an encapsulated digestive enzyme has shown to increase proteomic digestion efficiency. This success opens up the possibility of translating this technique into many potential microfluidic applications through the extensive versatility of encapsulation within polymer vesicles.

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