Integrative Microfabricated Bioengineering

A soft lithography microfabrication lab has been set up complete with a 12' x 17' softwall class 1000 cleanspace housing spin coater (laurel), UV source(exfo), and microwave plasma system (plasma preen). Currently there are 6 PhD students working in this space and we have published 3 manuscripts to peer reviewed journals, have an additional 5 in preparation, and 16 conference papers on work ongoing in the integrated bioengineering lab. We have the ability to microfabricate polydimethylsiloxane based microfluidic devices and collect and analyze data from the devices. We also have facilities to culture mammalian cell lines within these devices. The devices have been applied to develop a tool to assess tissue within an organ transplantation lab, develop an add-on for common experimental biological tools such as a brain-slice perfusion chamber and multiwell plate, demonstrate the ability to process environmental samples to determine the risk of microbial populations, develop a gradient generator to study chemosensing in yeast, and develop a microfluidic diagnostic device to sort out rare cell populations from patients blood.

1. Students

Kihwan Nam, Fall 2006 - present
Shawn Oppegard, Summer 2007 – present
Hugo Caicedo, Spring 2008 – present
Marie-Elena Brett, Fall 2008 – present
Cari Laurniere, Fall 2008 – present
Elly Sinkala, Fall 2008 - present

2. Published Manuscripts

2.1 Peer Reviewed

Mohammed JS, Wang Y., Halvert TA, Oberholzer J, and Eddington DT. Microfluidic device for multimodal characterization of pancreatic islets. Lab on a Chip 2009; 9 (1): 97-106

Tek P, Chiganos TC, Rousche PJ, Mohammed JS, Eddington D, Fall CP, Ifft P. Rapid Prototyping for Neuroscience and Neural Engineering. Journal of Neuroscience Methods; In Press

Mohammed JS, Caicedo HH, Fall CP, Eddington DT. Microfluidic add-on for standard electrophysiology chambers. Lab on a Chip 2008; 8 (6): 1048-1055.

2.2 In Preparation

S.C. Oppegard, P.A. Anderson, and **D.T. Eddington**, Cnicocytes as a Functional Material in Microdevices, *Journal of Biomimicry and Bioinspiration*

K.Nam and **D.T. Eddington**, Size-Based Separation of Heterogeneous Microparticle Solutions, *Journal of Micromechanics and Microengineering*

S.C. Oppegard, K. Nam, J. Carr, S. Skaalure, and **D.T. Eddington**, Oxygen Regulation in Multiwell Plates Through a Microfluidic Add-On, *Nature Methods*

H.H. Caicedo, C.P. Fall, and **D.T. Eddington** Simulation of a Microfluidic Brain Slice Perfusion Chamber, *Journal of Micromechanics and Microengineering*

M.E. Brett, D. Stone, and **D.T. Eddington** Radially Rotating Phermone Gradients to Study *Cervisae*, *Nature Methods*

2.3 Conference Proceedings

J.S. Mohammed, Y. Wang, T.A. Halvert, J. Oberholzer, and **D.T. Eddington** (2008) "Microfluidic Device for Multiple Functional Assays to Improve Pretransplant Islet Quality Assessment" microTAS, San Diego, CA

H.H. Caicedo, J.S. Mohammed, C.P. Fall, and **D.T. Eddington** (2008) "Localized Brain Slice Chemical Stimulation Using a Microfluidic Device and Off-the-Shelf Perfusion Chamber" microTAS, San Diego, CA

S. Oppegard, K. Nam and **D.T. Eddington** (2008) “Independent Control of Oxygen Concentration for Cell Culture in an Add-on Platform for Multiwell Plates” microTAS, San Diego, CA

J.S. Mohammed, Y. Wang, T.A. Halvert, J. Oberholzer, and **D.T. Eddington** (2008) “Microfluidic Device for Multiple Functional Assays to Improve Pretransplant Islet Quality Assessment” BMES, St. Louis, MO

S. Oppegard, P.A. Anderson, and **D.T. Eddington** (2008) “Jellyfish Nematocysts as Part of an All-In –One Therapeutic Manufacture and Injection Platform” BMES, St. Louis, MO

K.Nam and **D.T. Eddington** (2008) “Size-Based Separation in a Multilayered Microfluidic Device” BMES, St. Louis, MO

H.H. Caicedo, J.S. Mohammed, C.P. Fall, and **D.T. Eddington** (2008) “Microfluidic Substrate Integrated with a Standard Electrophysiology Set-Up” BMES, St. Louis, MO

S. Oppegard, K. Nam and **D.T. Eddington** (2008) “Device for the Control of Oxygen Concentration in Multiwell Cell Culture Plates” BMES, St. Louis, MO

I. Papautsky, C. Maltbie, **D.T. Eddington**, A.S. Bhagat, H.H. Caicedo, (2008) “Introducing Microfluidics in a Problem Based Learning Course”, ASEE, Pittsburgh, PA

Oppegard S., Anderson P.A., and **Eddington D.T.**, (2008) "Cnidocytes as a functional material in microfabricated systems ", Institute of Biological Engineering, Raleigh, NC

Eddington D.T., (2008) “High Throughput Hypoxia”, Lab Automation, Palm Springs, CA

Nam K., **Eddington D.T.**, (2007) "Independent control of gas concentrations in a multiwell-format", Student Research Forum, University of Illinois at Chicago

Nam K., **Eddington D.T.**, (2007) "High Throughput Hypoxia", Catalyzing Collaboration between Industry and Academia in the Life Sciences, Baxter Healthcare, Round Lake, IL

Caicedo H.H., Mohammed J.S., Fall C.P.**, Eddington D.T.**, (2007) "Spatiotemporal brain slice stimulation using a microfluidic network and standard perfusion chamber",10th Annual Illinois Louis Stokes Alliance for Minority Participation Student Research Symposium in Science, Technology, Engineering, and Mathematics, Glenview. Illinois

Caicedo H.H., Mohammed J.S., Fall C.P.**, Eddington DT.**, (2007) "Spatiotemporal brain slice stimulation using a microfluidic network and standard perfusion chamber",BMES, Los Angeles

Eddington, D.T., Higgins, J., Bhatia, S.N, Mahadeven, L., (2007) “Collective hydrodynamics and kinetics of sickle cell vaso-occlusion and rescue in a microfluidic device”, BMES, Los Angeles

3. Major Equipment Purchased

Laurell Spin Coater, $4690
Microwave Plasma Chamber, $4423
UV Light Source, $4800
Softwall Cleanroom, $5925

4. Summary of Research Work

Microfabrication enables many exciting experimental possibilities for medicine and biology that are not attainable through traditional methods. However, in order for microfabricated devices to have an impact they must not only provide a robust solution to a current unmet need, but also be simple enough to seamlessly integrate into standard protocols. Broad

dissemination of bioMEMS has been stymied by the common aim of replacing established and well accepted protocols with equally or more complex devices, methods, or materials. The marriage of a complex, difficult to fabricate bioMEMS device with a highly variable biological system is rarely successful. Instead, the design philosophy of the integrative bioengineering lab aims to leverage a beneficial microscale phenomena (e.g. fast diffusion at the microscale) within a bioMEMS device and adapt to established methods (e.g. multiwell plate cell culture) and demonstrate a new paradigm for the field **(adapt instead of replace)**. In order for the field of bioMEMS to mature beyond novel proof-of-concept demonstrations, researchers must focus on developing systems leveraging these phenomena AND integrating into standard labs, which have largely been ignored. Towards this aim, I will outline three ongoing projects from my lab that span many disciplines and dimensions, target various funding agencies, and are in various stages of publication below.

I. Microfabricated Device for Detection of Circulating Tumor Cells

Metastasis of prostate cancer poses a great challenge as diagnostics/therapies often fail. Metastasis is known to be caused by circulating tumor cells (CTCs) in blood, thus clinically significant detection of CTCs is critical for disease diagnosis and prognosis. However, CTCs in blood are extremely rare, hindering highly efficient and sensitive detection of CTCs. The first step in metastasis involves transient, adhesive interactions between the endothelial cells and the invasive CTCs, known as cell rolling. In the second step, the cells firmly attach to the endothelial cells, followed by diapedesis. By mimicking this naturally occurring process, we hypothesize that CTCs will be selectively captured on a microfluidic chip based on iterative rolling and stationary multivalent binding. The separation of CTCs from whole blood will be achieved by two sequential biomimetic steps: 1) E-selectin-induced cell rolling of CTCs and 2) polyamidoamine (PAMAM) dendrimer mediated strong multivalent binding between CTCs and micropatterned anti-EpCAM antibody. Additionally, engineered microfluidic channels will induce rotation of flow that increases the cell interaction with the biofunctional surfaces. The device will be utilized to answer important questions such as how CTCs correlate with clinical response and whether CTCs can be used to diagnose metastatic prostate cancer. A Schematic of the device is shown in Figure 1.

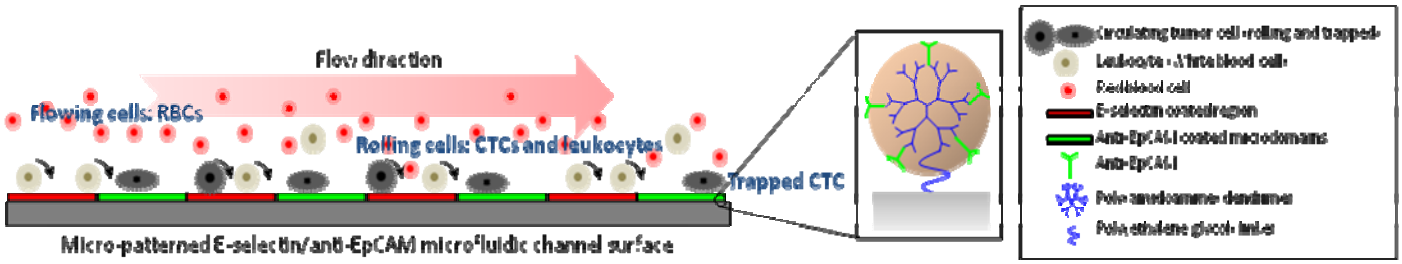

Figure 1. A schematic diagram of CTC capturing on a micropatterned surface using iterative cell rolling (E-selectin: red) and multivalent stationary adhesion (anti-EpCAM: green). The inset diagram represents immobilized anti-EpCAM through flexible polymer nanolinkers (dendrimers and PEG: blue) by which the multivalent effect can be achieved through locally concentrated anti-EpCAM. The flow direction appears to be linear for simple illustration but actual flow in the chip will be rotated by grooves in the channel ceiling.

II. Microfluidic Device for Rapid Islet Assessment

Diabetes Mellitus (DM) is a group of metabolic disorders in which the body does not secrete enough insulin to regulate blood glucose levels properly, resulting in elevated levels of blood glucose. In the year 2000, the Edmonton group reported a series of 7 patients reaching insulin-independence after islet transplantation from multiple donors using steroid-free, sirolimus based immunosuppression. With the new protocol, islet transplantation is progressively becoming a promising treatment for Type I DM with benefits of minimal surgery, less mortality and morbidity. Currently, prior to transplantation, the islets are put through a rigorous quality control to assess their purity, morphology, sterility, pyrogenicity, viability, and potency (static glucose-stimulation studies). However, the standard assays for evaluating islet quality prior to transplantation such as static glucose incubation and viability assay provide us with little information about β-cell function and does not address defects in β-cell morphology, metabolism, and signaling levels. Therefore, it is essential to develop a standardized, accurate, real-time assessment of β-cell function based on β-cell physiology that can be used as a gold standard for assessing the functionality of islet preparations post isolation prior to transplantation. In this project we developed a microfluidic device for multiplexing several well-accepted islet functional assays to better predict transplantation outcome. The assays include the temporally resolved analysis of the kinetics of insulin secretion, mitochondria potential, and $[Ca^{2+}]_i$ will provide in depth understanding of the functionality of the islets. Microfluidics

offers a practical solution to this unmet clinical need to rapidly asses islet function. Parallel microchannels enable multiple experiments to be performed simultaneously and imaging of cells within the microchannels, which is currently not possible with current commercially available dynamic perfusion platforms as shown in Figure 2. Additionally, microfluidic delivery of stimulatory solutions reduces the mechanical perturbation that will reduce stress to the islets. To successfully deploy a device at multiple transplantation centers, our device is simple, robust, user-friendly, and provide quick, reproducible results that are predictive of transplant outcome; as a device failure during a characterization process may lead to discarding potentially lifesaving donor tissue.

III. Microfabricated Insert for High-Throughput Hypoxic Experimentation

The objective of this project is to optimize and disseminate a platform to independently control the local gas concentrations within each well of a multiwell plate (currently not possible) as a simple yet robust technique to explore this vital yet largely ignored metabolic variable in cell biology. The majority of cell biology is conducted in incubators set to 21% O_2 and 5% CO_2, yet gradients of oxygen are found throughout all biological systems and are not replicated under these conditions. This platform will break down experimental barriers to impose these oxygen gradients in standard cell culture materials. The platform is comprised of a microfabricated modular add-on for the ubiquitous multiwell plate found throughout biomedical research labs and requires no special equipment or training to operate. Just as the transwell assay allows in vitro assays of phenomena previously limited to in vivo animal models such as migration and paracrine signaling pathways, this platform creates a new in vitro high-throughput tool to probe a previously difficult to control variable and thus enhance experimental efficiency. The platform consists of a polydimethylsiloxane (PDMS) insert that nests into a standard multiwell plate as shown in Figure 3. The insert contains a series of pillars matching the number and

Figure 3. Image of a 6-well, 24-well, and a 96-well insert.

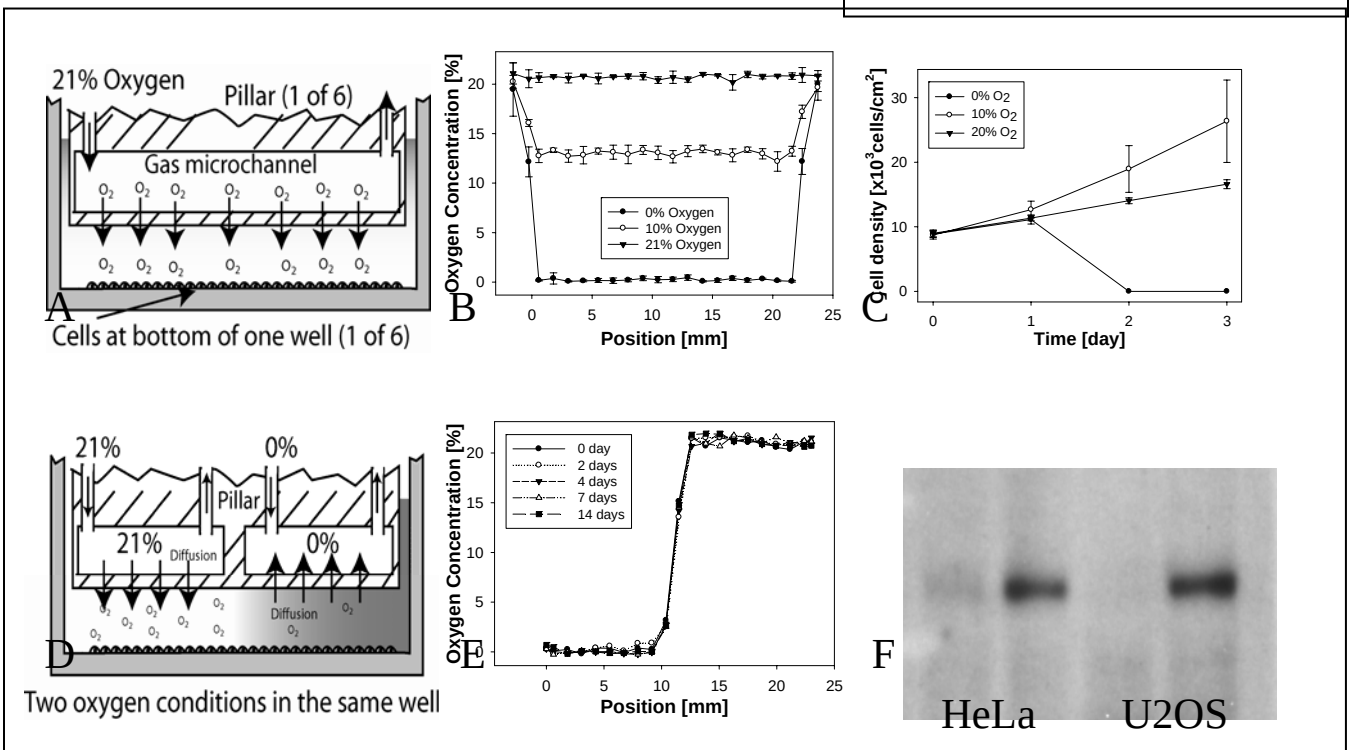

Figure 4. *(A-C) Single oxygen concentration per well.* A) Schematic crossection of a single oxygen concentration imposed on a well of a 6-well plate. B) Oxygen quantification of three oxygenation conditions imposed sequentially in a device shown in A with error bars of standard deviation. C) Cell growth under the various oxygen tensions quantified in B (21%, 10%, and 0%). *(D-F) Step oxygen concentration profile in a single well.* D) Schematic crossection of the step condition. E) Oxygen quantification over 14 days of the device depicted in D. F) Western blot of HIF-1α upregulated by hypoxia delivered through our device for HeLa cells which is comparable to using $CoCl_2$ in U2OS cells.

spacing of wells of the plate it is designed to nest into. Oxygen is injected through microchannels embedded at the base of each pillar and will be separated from the fluidic contents of the culture well by a thin gas permeable PDMS membrane. The oxygen microchannels connect to gas cylinders which provide the pressure to deliver the gas throughout the insert. The insert acts as a sink or source of oxygen depending on the concentration of the oxygen in the microchannels and be immersed in the cell media of the well. The gas is delivered to the cell monolayer through diffusion of oxygen across a thin PDMS membrane separating the fluidic contents of the multiwell plate from the oxygenation microchannels embedded into each pillar. The oxygenation routing and pillar microchannels are fabricated through standard soft lithographic techniques and the pillar array is fabricated through casting PDMS into a machined Delran mold. We are very exciting about this project and have received enthusiastic offers to validate the technology in several varied applications including docking to a microelectrode array (Dr. Banach, Department of Surgery, UIC) in addition to studying hematopoietic stem cells (Dr. Losordo, Feinberg School of Medicine, Northwestern University).