

Cellular BioMEMS

Academic and Research Staff

Professor Joel Voldman

Postdoctoral Associates

Katarina Blagović, Yi-Chin Toh, Wei Mong Tsang

Graduate Students

Hasan Celiker, Salil Desai, Joseph Kovac, Melanie Hoehl, Nick Mittal, Laralynne Przybyla, Somponnat Sampattavanich, Michael Vahey

Undergraduate Students

Sam Sinai

Technical and Support Staff

Chadwick Collins

Laboratory for Cellular BioMEMS – Research Themes

Our group performs research on BioMEMS, applying microfabrication technology to illuminate biological systems, especially at the cellular level. Specifically, we develop technologies that are used to manipulate cells or make measurements from them. Our research builds upon various disciplines: electrical engineering, microfabrication, bioengineering, surface science, fluid mechanics, mass transport, etc. We take a quantitative approach to designing our technology, using both analytical and numerical modeling to gain fundamental understanding of the technologies that we create. We then take our designs through microfabrication to packaging and testing and to biological assay. Our applications have a strong emphasis on stem cell biology and cell sorting.

1. Optical cell sorting for cell cytometry

Sponsors

NDSEG graduate fellowship, NIH NCRR, Singapore-MIT Alliance, Pfizer

Project Staff

Joseph Kovac
Tao Sun

Overview

The goal of this research is to develop new technologies that enable image-based identifying and sorting of mammalian cells. Fluorescence activated cell sorting (FACS) is easily the most widespread sorting technology available for sorting cells, but FACS is unable to resolve sub-cellular or time-varying fluorescence at the single cell level. This limitation prevents researchers from isolating cells based on information such as spatial and/or temporal protein expression and general morphology. This limitation strongly constrains the types of pooled genetic screens that can be performed and greatly increases the amount of effort required to isolate clones of a particular phenotype that requires imaging to recognize. To address this limitation, we have developed an inexpensive, user friendly sorting method that augments a standard automated fluorescence microscope with the ability to sort cells based on images. Because of the low cost, user friendliness, and easy integration of our method into existing microscopes, our approach will

enable fundamentally new biological assays and shorten or eliminate commonplace tedious practices.

Technology Background

This effort has utilized microfabricated /microfluidic approaches to cell sorting, including purely dielectrophoretic (DEP) trap arrays, passive hydrodynamic trap arrays with active DEP-based cell release, and passive microwell arrays with optical cell release to permit sorting of non-adherent cells. As these preceding technologies were best suited to operate with non-adherent cells, we have recently turned our focus to developing a solution for adherent cells. Our approach to sorting adherent cells uses a photolithography-inspired method without the use of microfluidics, illustrated in

Figure 1. We first plate adherent cells into a dish and identify cells of interest using a microscope, noting the locations of desired cells within the dish relative to alignment marks. A computer program interprets the alignment mark and cell locations and generates a mask image, with black features corresponding to locations of desired cells. We then print the mask image to a transparency using a standard inkjet printer. After aligning the transparency mask to the back of the cell culture dish, opaque mask features reside beneath desired cells. We then mix a prepolymer solution consisting of cell culture media, a UV-photoinitiator, and poly(ethylene glycol) diacrylate (PEGDA) monomer. We add the prepolymer to the cell culture dish and shine ultraviolet (UV) light from a standard fluorescence

microscope source through the transparency and into the dish. The prepolymer then crosslinks into a hydrogel in all unmasked locations, encapsulating undesired cells. The desired cells, which are not encapsulated,

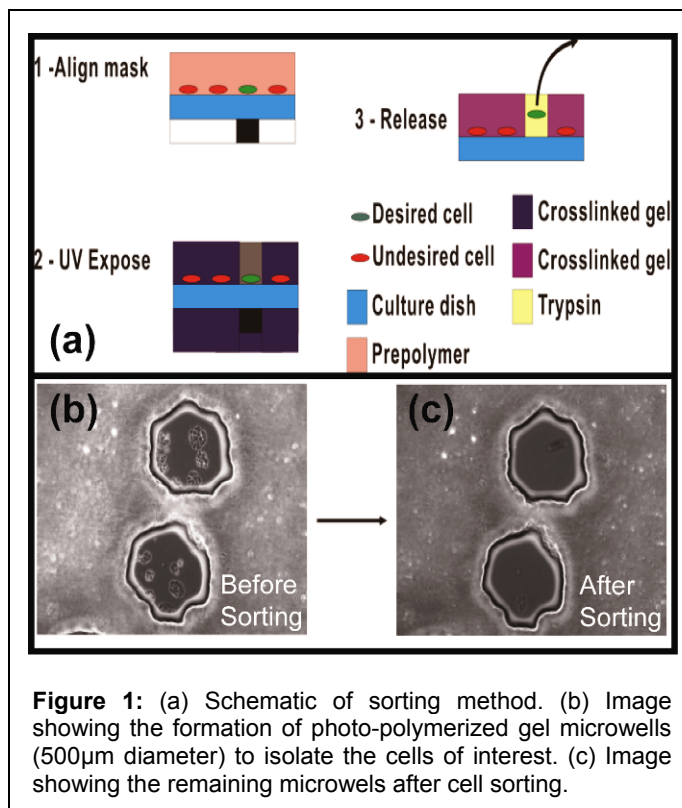


Figure 1: (a) Schematic of sorting method. (b) Image showing the formation of photo-polymerized gel microwells (500µm diameter) to isolate the cells of interest. (c) Image showing the remaining microwells after cell sorting.

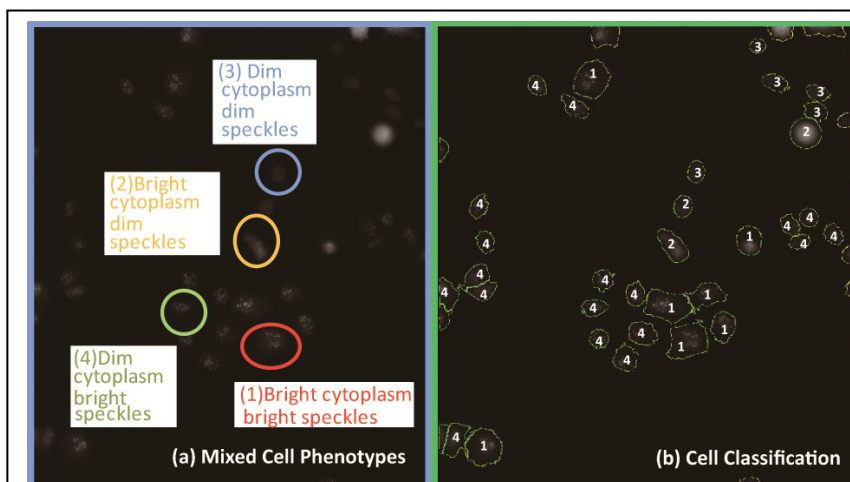


Figure 2: Image showing the automated cell identification and classification using machine learning algorithm-based software: CellProfiler and Cell Analyst. (a) mixed cell phenotypes of four classes (b) results of segmented individual cells and classified cell populations.

can be enzymatically released from the substrate and recovered. The overall technique requires standard equipment found in biology labs and inexpensive reagents (<\$10 per experiment), encouraging widespread adoption.

Current Research

Accurate recognition of the morphological features of cells of variant phenotypes, e.g. localization of intracellular proteins is essential to target the cells of interest as *a priori* to cell sorting. Traditionally manual recognition of cell phenotypes requires the knowledge and experience of experts in cell biology. We are focusing on combining automated fluorescence microscopy and computer-based pattern recognition to identify and classify cells at high throughput. We currently are using the machine learning algorithm-based software CellProfiler and CellAnalyst to quantitatively characterize the morphological features of the imaged cells, covering the cell area, shape, fluorescent intensity and texture. As shown in Figure 2a, four classes were defined according to the fluorescent intensity difference in different cellular compartments. A set of judging rules was generated by iteratively training the classifier based on 171 quantitative cell feature measurements to cluster the cells of similar phenotypes into a particular class, as shown in Figure 2b.

2. Microfluidic perfusion for modulating the stem cell microenvironment

Sponsors

NIBIB

NSF Graduate Research Fellowship

Singapore A*STAR

Project Staff

Katarina Blagović , Laralynne Przybyla, Yi-Chin Toh

Overview

Stem cells are powerful biological models as they may offer insights to fundamental issues in biocomplexity, as well as developmental biology and disease pathogenesis. For these reasons, intense effort is being focused on characterizing and manipulating cellular microenvironment as it plays an important role in determining the biological state of embryonic stem cells (ESCs). However, conventional cell culture methods provide incomplete control over the cellular microenvironment, especially with respect to cell-secreted diffusible factors. Our approach is to use microfabricated culture systems to create a “neutral background” free of cell-secreted factors, and thus enable the study of defined media conditions for self-renewal and differentiation as a supplement to standard techniques. To this end, we have developed microfluidic devices that use perfusion to sweep away diffusible signals (cell-secreted factors), establishing a cleaner experimental system with more control over the soluble microenvironment.

Current Research

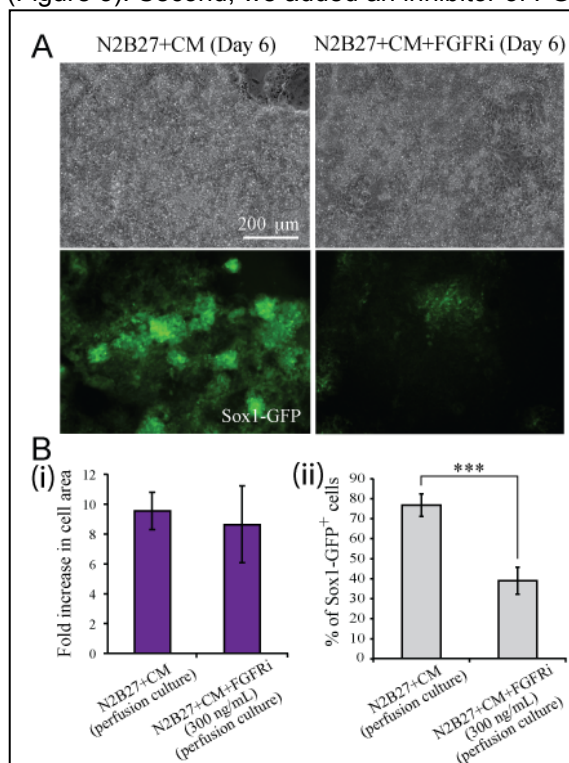
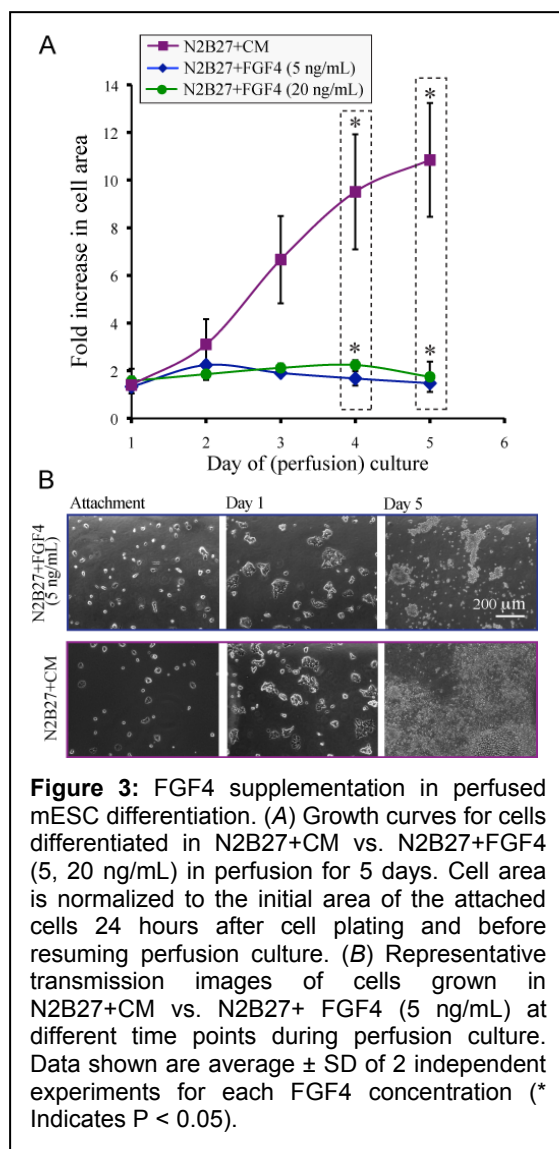
We have been focused recently on three aspects of this technology. First, we are investigating diffusible signals that are important during stem cell differentiation. Second, we are investigating how microfluidic perfusion itself affects stem cell phenotype in self-renewal. Third, we are investigating how shear, even at the low levels used in perfusion, might affect stem cell phenotype.

Microfluidic perfusion for modulating stem cell differentiation

We have previously shown, using our two-layer microfluidic perfusion device, that mouse ESCs (mESCs) failed to proliferate under conditions of minimized autocrine signaling. Briefly, a defined

differentiation medium (N2B27) sufficient to promote growth and differentiation under static monolayer differentiation culture conditions was not sufficient for cells to proliferate under constant flow in perfusion culture when plated at equivalent densities, implying that indispensable cell secreted factors have been swept away. Conversely, medium conditioned from static differentiating cultures restored cell survival and differentiation in perfusion culture as assessed by Sox1-GFP reported cell line used in this study (mESC 46C cell line).

We have further used this neuronal differentiation system to elucidate the role of FGF4 signaling, reported as an obligate autocrine requirement in neuronal differentiation of mESCs. We have probed the contribution of FGF4 in the overall process of acquiring neuronal identity by studying *two extremes*. First, we supplemented *minimal/sufficient* N2B27 medium with different concentrations of FGF4. We found that neither of the doses resulted in the survival recovery of long-term cultures in perfusion, suggesting the presence of other autocrine survival factor(s) implicated in mESC growth under differentiation (Figure 3). Second, we added an inhibitor of FGF signaling to *saturated* medium (N2B27 & cell secreted factors). Inhibition of FGF signaling resulted in significant downregulation of neuronal differentiation, without compromising



growth of mESC cultures undergoing differentiation (Figure 4).

Combining microfluidic perfusion and our “two extremes” approach we have elucidated the role of a specific autocrine signaling in mESC processes. Specifically, we have demonstrated that autocrine FGF4 signaling is implied as a crucial factor in acquiring neuronal identity of mESCs, while alone it cannot restore the growth of mESCs undergoing neuronal specification.

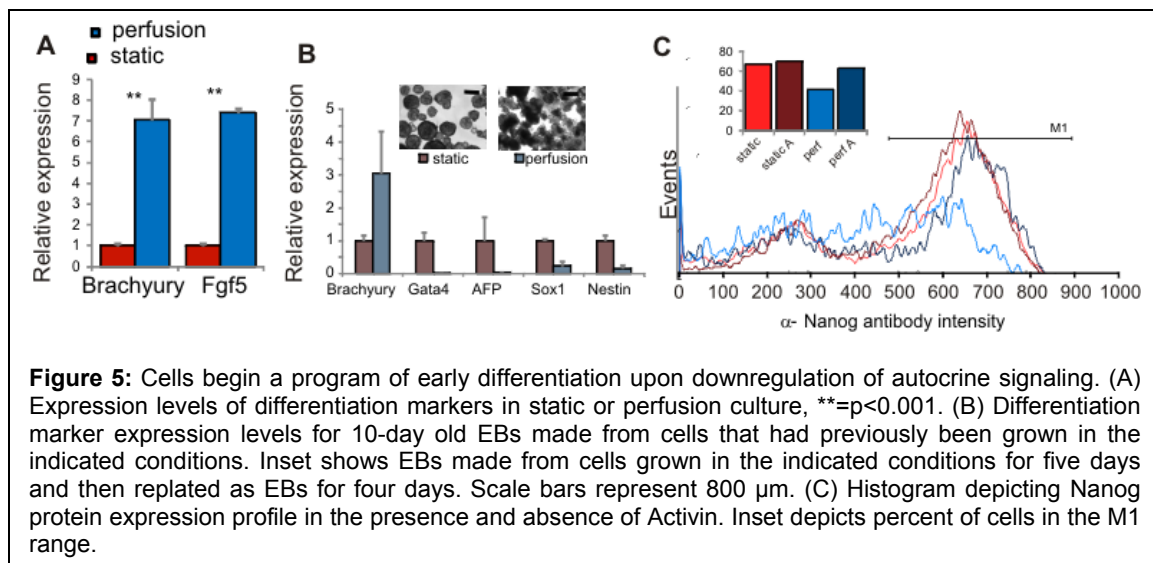
Our results with serum-free cultures set the foundation for using microfluidic perfusion as a powerful tool for investigating the role of autocrine/paracrine signaling in stem cell self-renewal and differentiation.

Modulation of diffusible signaling affects properties of mouse embryonic stem cells

Many factors contribute to the decision made by an embryonic stem cell (ESC) to either remain as a stem cell or to differentiate into a specific cell type. These opposing options form the basis for the definition of an embryonic stem cell as a cell that can either maintain itself by self-renewal, or can differentiate into any cell type in the body, a property known as pluripotency. A major factor in this decision involves the external cues received by the cell, which are often molecules secreted by surrounding cells in the microenvironment. However, the ability to specifically control these external factors remains difficult.

We use a microfluidic system in which cells can be cultured under continuous media perfusion. In these conditions, cell-secreted diffusible molecules can be removed by flow, establishing culture conditions in which signaling pathways are not obscured by autocrine signals. This more neutral background allows determination of the minimal complement of extracellular signals required to achieve and maintain a given cell state, and allows us to uncover more specific roles of added or inhibited molecules.

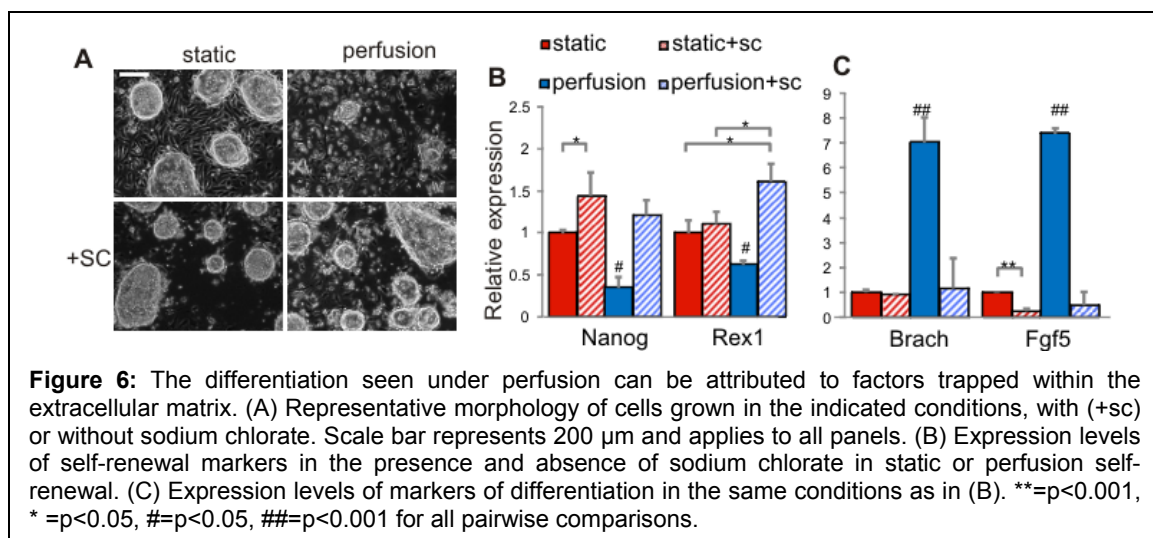
Using this microfluidic device to grow mouse ESCs, we determined that upon removal of autocrine signals under perfusion, cells begin a program of differentiation, tending towards an epiblast-like cell state of early directed differentiation. This state is characterized by transcriptional and functional changes, including an increase in Brachyury and Fgf5 marker expression (Figure 5A) and an alteration of downstream differentiation markers that are expressed upon downstream embryoid body (EB) differentiation (Figure 5B). In addition, an important feature of the early differentiation epiblast stem cell state is a dependence on exogenous Activin supplementation to maintain expression of Nanog, which allows for continued self-renewal. Under perfusion, cells produce lower levels of Nanog protein, but these levels can be upregulated to the levels seen in



static cultures by addition of exogenous Activin (Figure 5C). These results are important because together, they indicate that normal mESC cultures rely on secreted autocrine factors to maintain their self-renewal and pluripotency characteristics.

To explain the spontaneous differentiation that occurs upon a downregulation of diffusible autocrine factors, we turned towards the extracellular matrix (ECM), which acts as a sink or reservoir for many secreted proteins. To remove the effects of proteins trapped within the ECM, we use the sulfation inhibitor sodium chlorate, which blocks the ability of heparan sulfate proteoglycans to act as protein tethers or reservoirs within the ECM. As has been previously described, addition of sodium chlorate under static conditions increases culture homogeneity and increases expression of the self-renewal marker Nanog while decreasing expression of the differentiation marker Fgf5 (Figure 6). Similarly, addition of sodium chlorate under perfusion allows for growth of morphologically normal ESC colonies (Figure 6A). In addition, expression of the self-renewal markers Nanog and Rex1 are increased to static levels, and expression of the early differentiation markers Brachyury and Fgf5 are decreased. (Figure 6B,C).

Together, these data indicate that an ECM component acts in normal cultures to promote differentiation, but that effect is masked in the presence of diffusible autocrine signals. We show that the presence of self-renewal media is not sufficient to maintain ESCs as such, as had been previously thought. These results demonstrate the utility of perfusion culture to assess behavior in the absence of diffusible autocrine signals, and to uncover more specific roles of exogenously added factors on cell state.



Microfluidic perfusion for shear stress studies

Stem cells are important for regenerative medicine and as *in vitro* models for drug testing and developmental studies. The stem cell microenvironment plays a vital role in determining stem cell fate; thus we need to understand and control these microenvironmental cues to fully exploit the potential of stem cells. While there has been substantial research on how soluble factors and extracellular matrices (ECM) affect stem cells, our understanding on stem cell responses to fluid shear stress is limited. Prior work has shown that by applying fluid shear stress corresponding to that in the developing heart, differentiation of stem cells into the cardiovascular lineage is significantly augmented. Although stem cell exposure to shear stress *in vivo* is predominantly limited to developing cardiovascular tissues, *in vitro* culture can subject bulk population of stem cells to shear stress, which can alter their phenotype. This is particularly true during the expansion of pluripotent stem cells for downstream applications in regenerative medicine, where bioreactors are typically used to culture the cells. Unintended alteration of stem cell phenotypes induced by fluid shear stress in bioreactors may jeopardize the application of the cultured stem

cells for downstream applications. We aim to quantitatively investigate the effect of fluid shear stress on stem cells using a microfluidic perfusion system with multiple shear stress magnitudes.

To this end, we have used a multiplex microfluidic array (Figure 7), which overcomes the limitations of macro-perfusion systems in shear application throughput and precision, to initiate a comprehensive, quantitative study of shear effects on self-renewing mouse embryonic stem cells (mESCs), where shear stresses varying by more than 1000 \times (0.016–16 dynes/cm²) are applied simultaneously. When compared to static controls in the presence or absence of a saturated soluble environment (i.e., mESC-conditioned medium) we ascertained that flow-induced shear stress specifically upregulates the epiblast marker, Fgf5 (Figure 8). Epiblast-state transition in mESCs involves heparan sulfate proteoglycans (HSPGs), which have also been shown to transduce shear stress in endothelial cells. By disrupting (with sulfation inhibitors and heparinase) and partially reconstituting (with heparin) HSPGs function, we show that mESCs also mechanically sense shear stress via HSPGs to modulate Fgf5 expression. This study demonstrates that self-renewing mESCs possess molecular machinery to sense shear stress and provides quantitative shear application benchmarks for future scalable stem cell culture systems.

We have demonstrated that our logarithmic microfluidic array is an effective platform to quantitatively investigate shear stress effects on stem cells over varying magnitudes. This improvement over current perfusion devices used for shear stress studies, where only one shear

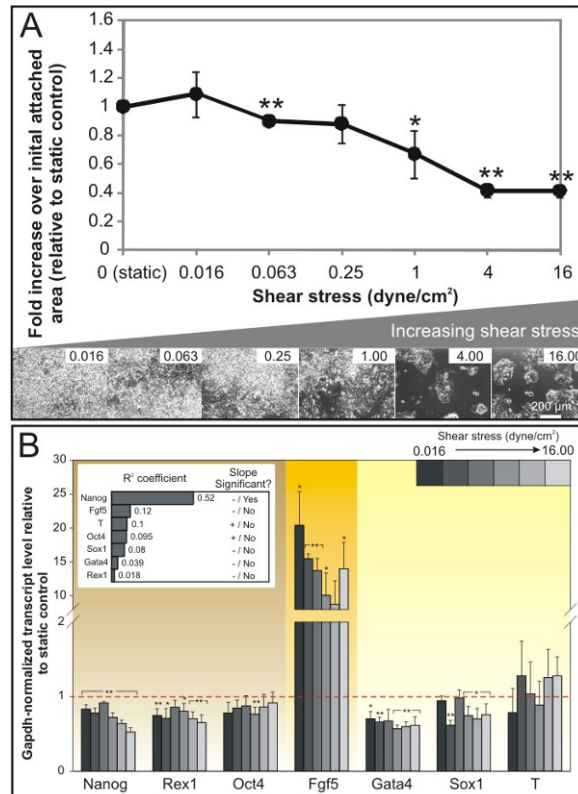
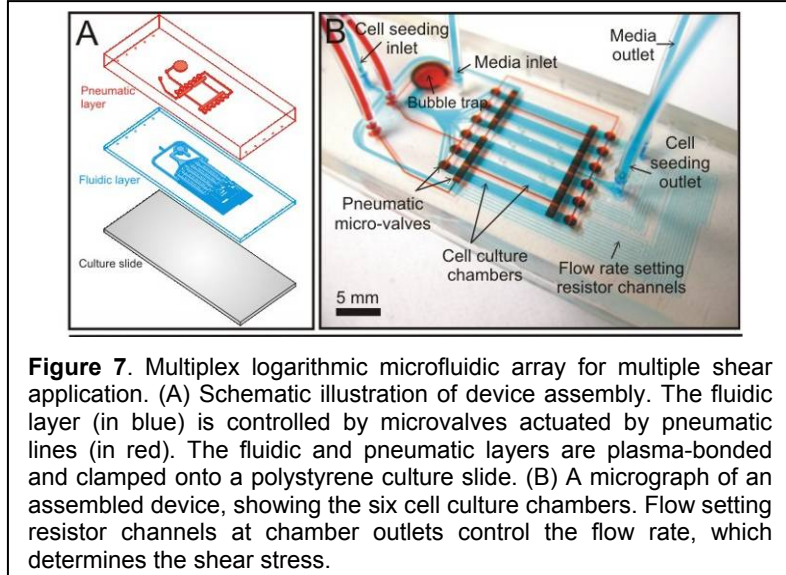


Figure 8. Perfusion culture of mESCs at varying shear stresses. (A) Proliferation of mESCs after 72 hours. Top: fold increase in cell area over initial cell attachment area relative to static culture. Bottom: phase images of mESCs at different shear stresses (indicated at the top right hand corner). (B) Gene expression of mESCs after 72 hours perfusion. Inset shows the R^2 and whether the regression slope is significantly different from zero. Data are average \pm s.e.m of 3 independent experiments. Asterisks indicate statistically significant difference between perfusion and static cultures, * $p < 0.1$, ** $p < 0.05$.

magnitude is applied at a time, will greatly facilitate our understanding of how fluid shear stress modulate stem cell fate.

3. Stem cell interactions and growth

Sponsors

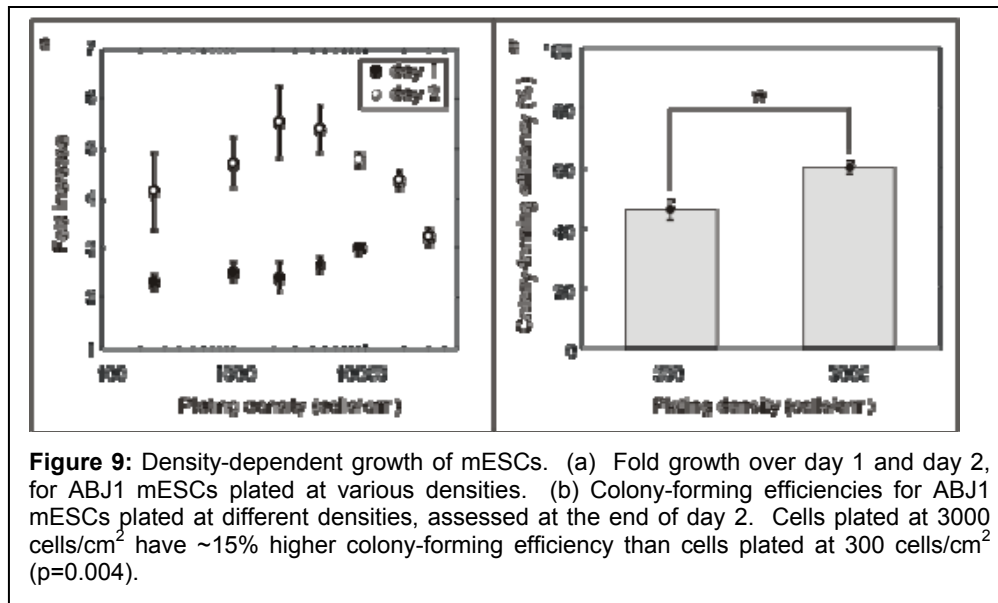
NIH NCRR

Project Staff

Nikhil Mittal

Embryonic stem cells (ESCs) have the unique potential of being able to produce any cell type in the adult animal. While ESCs can differentiate to produce any type of cell, they can also divide to produce two daughter stem cells via self-renewal. Establishing proper culture techniques that permit self-renewal is a crucial step for maintaining stem cell cultures, as well as for expanding stem cell populations – a step that would likely precede any stem cell therapy. When expanding ESCs for therapeutic use, it will likely be important to do this via self-renewal, since the expansion potential of these cells decreases with increasing differentiation. For example, blood stem cells, which are currently the best characterized adult stem cells, can only be expanded ~20-fold *in vitro*. To study the requirements for cell growth, we have been investigating the effects of cell-cell interactions on the growth of mouse ESCs (mESCs). Cell-cell interactions consist of diffusible signaling and cell-cell contact (juxtacrine signaling), and are important in numerous biological processes such as tumor growth, stem cell differentiation, and stem cell self-renewal.

By carefully investigating the density-dependence of mESC growth and determining whether components of the media enhance or diminish growth (Figure 9), we demonstrate the existence of one or more survival-enhancing autocrine factor(s) in mouse ESC cultures. Proteomic analysis of proteins secreted by mouse ESCs revealed a number of candidate autocrine molecules, which we have been following up on using a variety of assays. These findings identify in turn novel survival-enhancing autocrine factors in mouse ESC cultures.



4. Regularly-spaced cell patterns for the examination of diffusive intercellular communication in autocrine systems

Sponsors

NIH NIBIB

Project Staff

Somponnat Sampattavanich

Overview

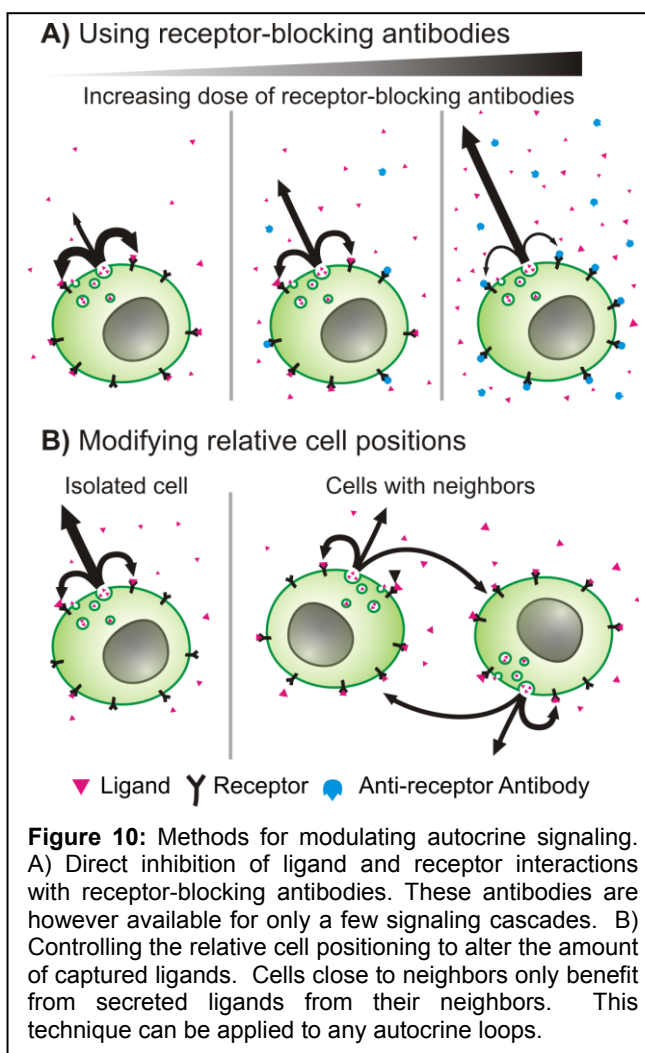
Autocrine signaling is a major cause of tumorigenesis and is involved in positive feedback control of many physiological processes. While commonly referred to as the underlying cause of inoculum cell-density effects, variation of cell responses with altering cell plating densities can also be caused by other signaling cues. For biological systems that are affected by multiple signaling cues, the contribution of autocrine signaling on a specific phenotype can be challenging to determine. Because of its close-loop nature, direct perturbation of autocrine loops without interfering with other signaling cues requires loop-specific inhibitors and such antagonists have limited availability (Figure 10A). Our project goal is to develop a novel method that can be used to accurately evaluate the impact of autocrine signaling on specific phenotypes without requiring specific inhibitors.

Technology Background

Our approach for modulating the impact of autocrine signaling is to precisely define the spatial arrangement of cells on the tissue culture substrate. Because the amount of ligand captured by each cell varies with the amount of diffusive interaction between cells, we have utilized stencil cell patterning technology to modulate the amount of intercellular interactions and therefore cellular phenotypes in autocrine systems (Figure 10B). Our platform maintains cells as regularly-spaced arrays of circular cell patches with varying patch sizes and spacing. To assist the investigation of autocrine signaling, a mathematical simulation was developed to predict the levels of ligand/receptor interactions for different patterning configurations. Analysis of cellular phenotypes is then measured at the single-cell level using high-throughput microscopy.

Current Research

Using the TGF- α /EGFR autocrine loop in A431 epidermoid carcinoma cells as our model, we first showed that A431 growth is influenced by multiple signaling cues when the cells are cultivated in conventional randomly-



plated culture. We then used the developed cell-patterning platform to evaluate the direct impact of autocrine signaling on A431 cell growth. Specifically, our platform can modulate diffusive interactions between the patterned cell patches while keeping other signaling cues identical. Our mathematical model identified two important spatial regimes of intercellular

communications, 1) the 'isolated' mode where each cell cluster only obtains ligands from self, and 2) the 'communicative' regime where all cells can diffusively interact to one another (Figure 11). We are currently testing this prediction experimentally by quantifying cell growth and the amount of captured ligands for the different patterning designs.

Ultimately, we want to apply the developed platform to compare autocrine contribution on growth and metastatic properties of different cancer cell types. Our platform is also useful for the examination of autocrine signaling roles in maintaining homeostasis of different physiological processes.

5. Microfluidic pairing and fusion for studying stem cell fusion and reprogramming

Sponsors

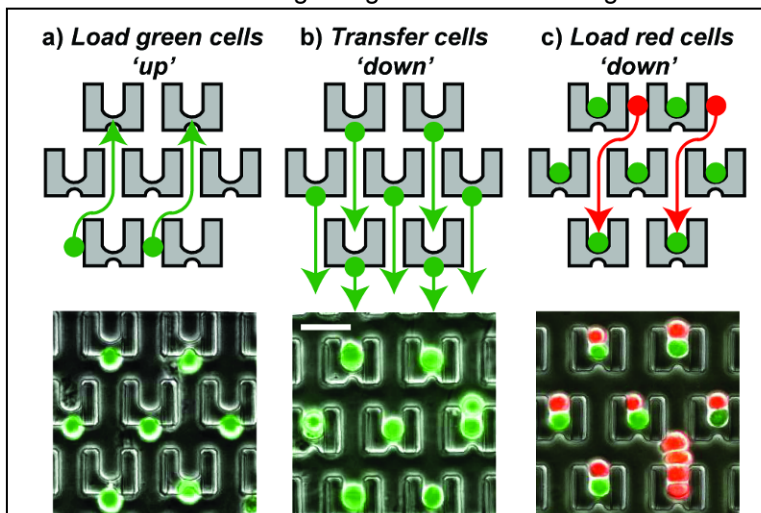
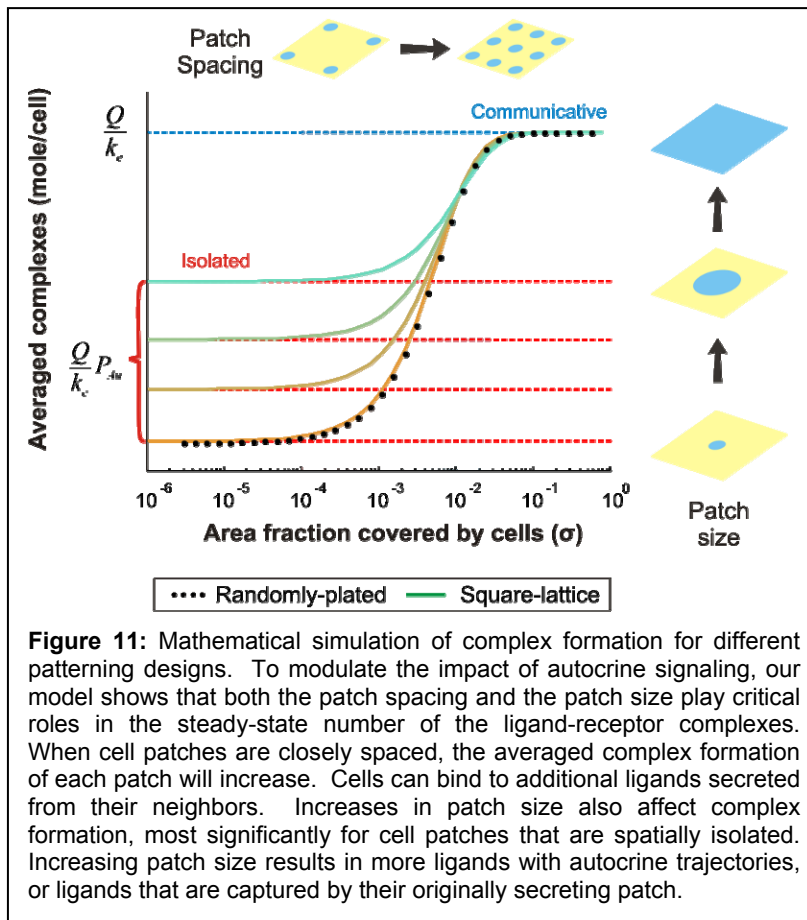
NIBIB, Singapore-MIT Alliance

Project Staff

Melanie Hoehl

Overview

Cell fusion is an appealing method by which to study reprogramming of somatic cells to an embryonic stem-cell-like state as the delivery



of cells is easily visualized. However, conventional methods to fuse cells *en masse* do not control the pairing between cell populations, resulting in heterogeneous output populations that must be further purified. We have developed a microfluidic device for high yield cell-pairing and fusion measurements using stem cells. We are developing similar methods for the statistical, kinetic study of immune cell interactions.

Technology Background:

Cell fusion has been used for many different purposes, including generation of hybridomas and reprogramming of somatic cells. The fusion step is the key event in initiation of these procedures. Standard fusion techniques, however, provide poor and random cell contact, leading to low yields. While different approaches can be used successfully for reprogramming, cell lines generated are not yet suitable for potential therapeutic applications in humans and many questions remain about the process of nuclear reprogramming. These questions could be answered with a more efficient cell pairing and fusion method. Skelley et al have therefore developed a microfluidic device to trap and properly pair thousands of cells (Figure 12). The device consists of thousands of microscale cell traps in a millimeter-sized area. The traps consist of larger frontside and smaller backside capture cups made from a transparent biocompatible polymer. The key to pairing cells efficiently is to load them sequentially in a 3-step loading protocol enabling capture and pairing of two different cell types. The geometry of the capture comb precisely positions the two cells, and flow through the capture area keeps the cells in tight contact in preparation for fusion. With this approach we have obtained pairing efficiencies of ~70%.

The device is compatible with both chemical and electrical fusion, and, in agreement with the literature, we have obtained higher performance with electrofusion. When we compared fusion performance in our device to commercial approaches, we obtained significant improvements in overall performance for both PEG-mediated fusion and electrofusion. Specifically, we have measured fusion efficiencies of ~80% in our device using

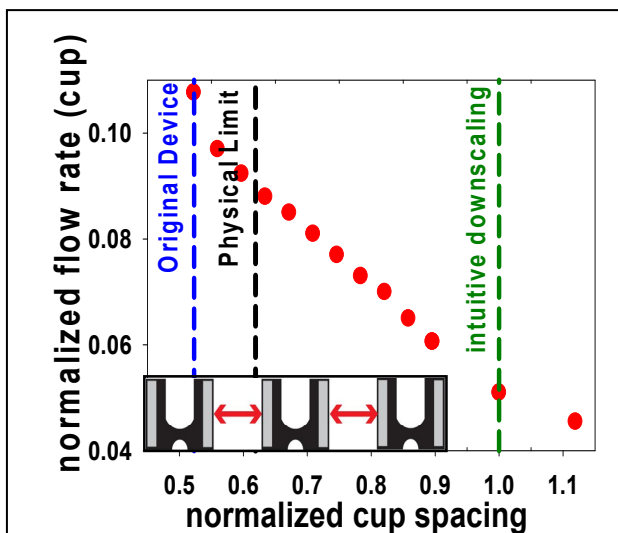


Figure 14: To redesign the cell-pairing device for immune cells we have established a finite element fluids model. The plot shows the predicted effect of reducing cup spacing (normalized by the downscaled immune cell device) on the relative flow rate through the capture cups. The blue line represents the original cell fusion device. The green line represents simple downscaling of the original device, yielding low flow and, presumably, pairing efficiency. The physical limit (black line) indicates the smallest cup spacing possible that still permits cells to pass between two cups. The model shows that reducing the horizontal cup spacing can increase flow through the cups and ultimately pairing efficiencies for immune cells.

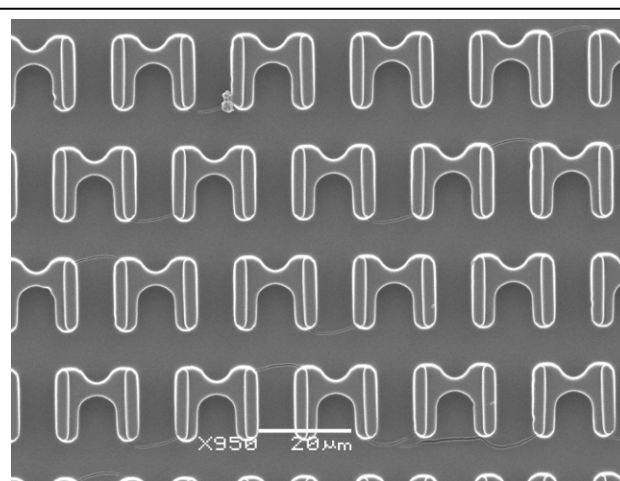


Figure 13: SEM image of the immune cell pairing PDMS device. It was designed and fabricated according to the results from the finite element fluid model. The scale bar is 20 μm .

electrofusion, about 5× greater than that obtained in commercial systems.

Having established the basis for high yield cell-pairing measurements using stem cells, we are also developing a similar device for the statistical, kinetic study of immune cell populations. Immune responses are largely mediated by cell-cell interactions. In particular, natural killer cells and cytotoxic T cells form conjugates with pathogenic and cancer cells in order to fight disease. Errors in these and other immune cell-cell interactions can lead to fatal immune diseases. The study of these intricate cell-cell interactions at the molecular scale is crucial for understanding the dynamics and specificity of the immune response. Conventional techniques, such as bulk measurements or immobilization of cell pairs on a dish, preclude gathering of sufficient data on single cell pairs for meaningful statistics of cell-cell interactions. We propose to overcome the limitations of traditional methods by both controlling the pairing and visualizing thousands of individual immune cell pairs simultaneously. Primary mouse lymphocytes (~6 μm diameter), which are a common model system in immunology are significantly smaller than stem cells (~18 μm diameter) —hence making a device redesign necessary. We have employed a numerical, finite element fluid dynamic model as a guide to optimize pairing efficiencies by altering geometric properties (Figure 14, Figure 13). Our immune cell pairing device is furthermore compatible with standard staining methods, such as antibody staining and ratiometric calcium flux measurements.

6. Iso-Dielectric Separation for Continuous-Flow Cell Screening

Sponsors

NIBIB

Singapore-MIT Alliance

National Science Foundation

Project Staff

Michael Vahey, Hasan Celiker

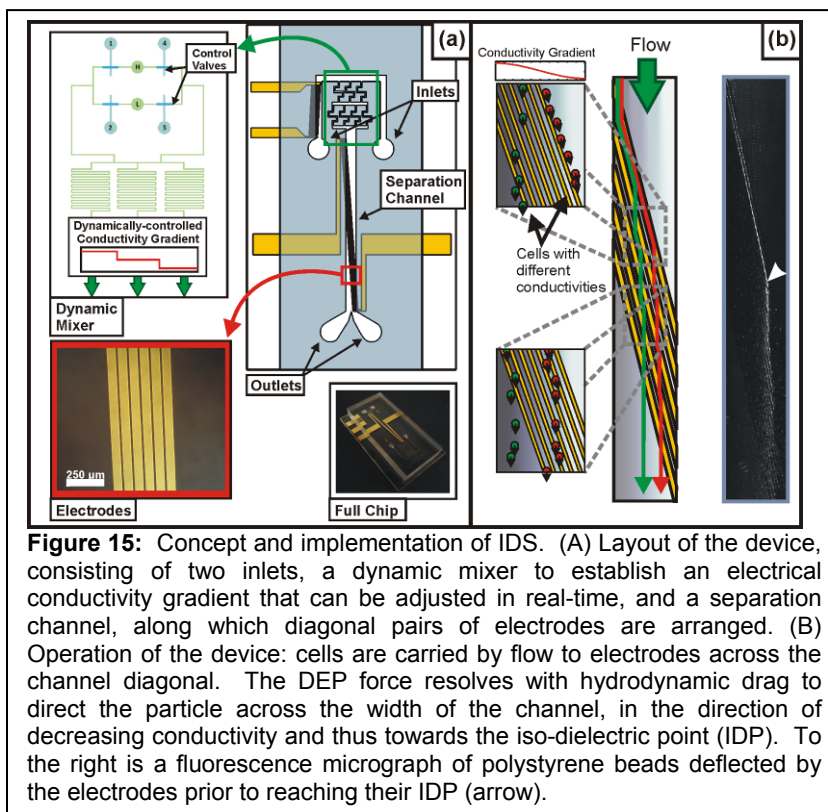
Overview

Genetic or phenotypic screens require the ability to select a small fraction of targeted cells from a large, heterogeneous background. One of the greatest challenges in applied biology is to perform these screens in a way that possesses both high throughput and high purity. Our approach to this problem is to develop a new equilibrium method, called iso-dielectric separation (IDS), for sorting cells based upon electrically distinguishable phenotypes. Equilibrium methods sort cells according to their intrinsic properties, and thus do not require that any labels be developed and applied to the targeted cells. Furthermore, they have the potential to be both preparative and analytic, meaning that they are able to provide both separation as well as quantitative information about the population of cells. The IDS device developed in our lab exhibits all of these characteristics, and offers the additional advantage of operating under continuous-flow. This enables high throughput, label-free, analytic and preparative separations capable of resolving multiple sub-populations of cells from heterogeneous backgrounds in a microfluidic format.

Figure 15 presents an overview of the device concept and operation. A particle or cell in a spatially non-uniform electric field experiences a force proportional to its polarizability. Because the polarizability of a particle is a function of the electrical conductivity of that particle as well as that of the surrounding medium, a cell placed in a conductivity gradient spanning an appropriate range will be characterized by the point along this gradient where its polarizability vanishes. This corresponds to the matching of cell and medium electrical properties at a particular location, which we refer to as the iso-dielectric point (IDP). Using dielectrophoresis, it is possible to direct particles to their IDPs, enabling the separation of electrically distinguishable particles. Importantly, although the polarization of a cell generally depends on factors other than the electrical conductivity and permittivity (e.g. size), by selecting cells based upon their IDPs, we are

able to suppress sensitivity to these additional factors. Thus IDS offers the potential for conductivity-specific separations, even in the presence of large variability in size.

The most recent implementation of the device uses a valve scheme that enables real-time control of the conductivity gradient, along with the ability to rapidly switch samples for sorting and characterization (Figure 1a). The ability to dynamically adjust the range of conductivities within which cells are resolved effectively increases the dynamic range of the separation. This feature, together with rapid sample loading, considerably decreases the time needed to characterize cells, making the systematic characterization of both pooled and unpooled cell libraries feasible.



Current Research

Electrogenomic Profiling

We are using this platform to perform a genome-wide analysis of electrical properties in the budding yeast *Saccharomyces cerevisiae*. Although methods to sort cells according to electrical differences have been explored for several decades, this process has largely been *ad hoc* due to a lack of information regarding the biological basis for altered electrical properties. A better understanding of how an organism's genotype is manifested by its electrical phenotype could enable the systematic development of electrical separations and lead to a new class of phenotypic screens. Towards this end, we are using IDS to obtain the first genome-wide mapping of an organism's genes to its electrical phenotype, focusing on the budding yeast *Saccharomyces cerevisiae*, an important model of eukaryotic biology.

We have screened the haploid yeast deletion library (a collection of ~5000 yeast strains each lacking a single gene not essential for growth and identifiable by a molecular barcode) using IDS, fractionating cells into different outlets according to their effective conductivities. By collecting cells from these outlets, extracting and amplifying the DNA barcodes encoding the genetic identity of each cell and then sequencing these barcodes, we are able to quantify the abundance of each strain across the four outlets. Normalizing the strain distributions in each outlet to that from the original unsorted pool reveals genes whose deletions increases or decreases the effective conductivity of a cell under the conditions of the screen (i.e. conductivity range and electric field frequency).

Figure 16a shows the comprehensive deletion strain enrichment across each of the four outlets, spanning a range in conductivities from 0.075 to 0.015 S/m. Here, the strains have been arranged in ascending order according to their enrichment at higher medium conductivities.

Selecting strains associated with substantial enrichment across the four outlets has identified ~50 genes whose deletion results in a “high conductivity” phenotype (i.e. distribution to the left of the wildtype strain in Figure 16b) and ~15 gene deletions conferring a “low conductivity” phenotype (i.e. distribution to the right of the wildtype strain in Figure 16b). Pathway analysis on the “high conductivity” and “low conductivity” sets of genes identified in this screen suggests several biological processes and genetic pathways closely associated with distinct electrical phenotype. These results provide the first connection between genotype and a cell’s electrical properties, suggesting the feasibility of developing more systematic biological assays based on electrical differences between cells.

7. Cyborg moth flexible multi-electrode arrays

Sponsors

DARPA

Project Staff

Spencer Murray

Wei Mong Tsang

Overview

Significant interest exists in creating insect-based Micro-Air-Vehicles (i-MAVs) that would combine advantageous features of insects—small size,

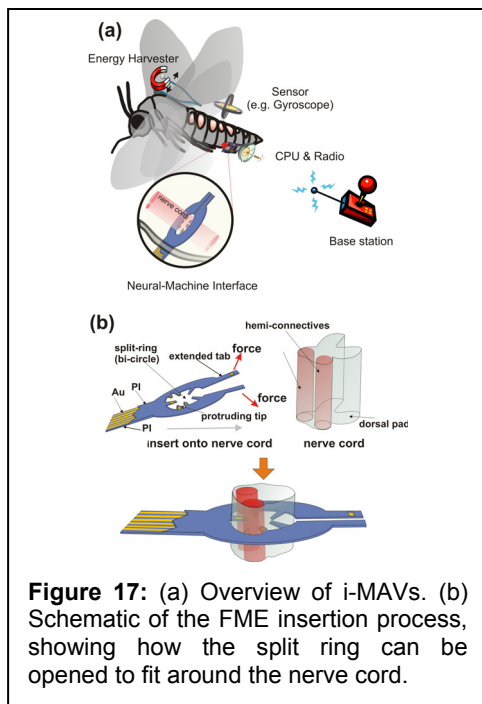


Figure 17: (a) Overview of i-MAVs. (b) Schematic of the FME insertion process, showing how the split ring can be opened to fit around the nerve cord.

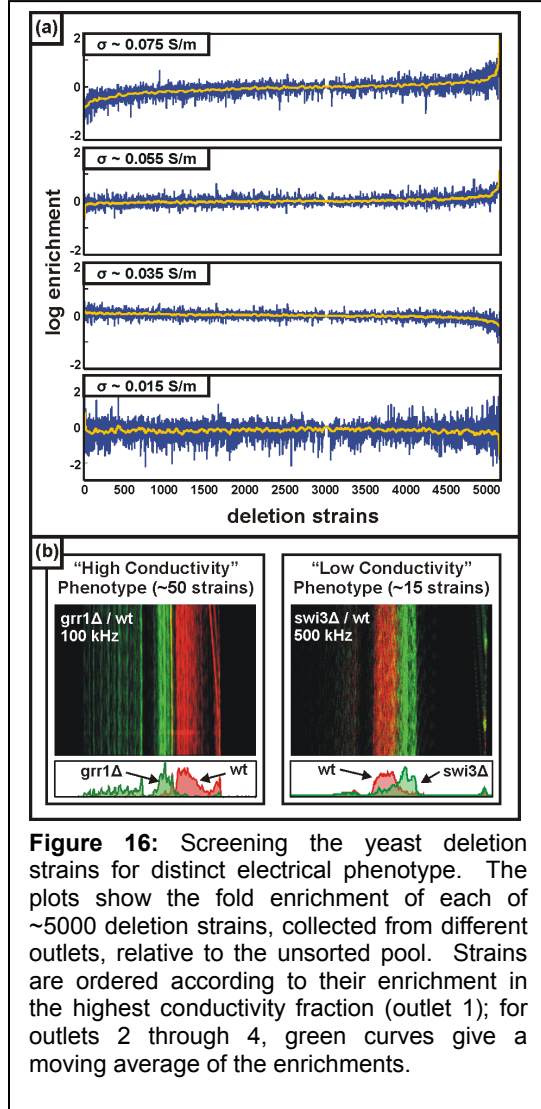


Figure 16: Screening the yeast deletion strains for distinct electrical phenotype. The plots show the fold enrichment of each of ~5000 deletion strains, collected from different outlets, relative to the unsorted pool. Strains are ordered according to their enrichment in the highest conductivity fraction (outlet 1); for outlets 2 through 4, green curves give a moving average of the enrichments.

relatively large payload

capacity, navigation ability—with the benefits of MEMS and electronics—sensing, actuation and information processing. The i-MAV includes four types of components—the animal, interfaces, energy sources, and communication links—and requires overcoming four main challenges—the small size, mass, and energy budget of the animal, along with robust interfacing of the components to the animal (Figure 17).

Our goal is to develop a flexible electrode array that interface with the moth *Manduca sexta*. These flexible multisite electrodes (FMEs) must be suitable for implantation into moth pupae, and directly interface with the central nervous system (CNS) of the moth for flight control. This effort is a part of a larger joint project between the Massachusetts Institute of Technology, the University of Arizona and the University of Washington to develop the tools and

technologies capable of guiding the flight of the self-powered moth.

Technology Background

In this work, we have developed FMEs for insect flight control. The FMEs are made of two layers of polyimide with gold sandwiched in-between in a split-ring geometry using standard MEMS processing. The FMEs have a novel split-ring design that incorporates the anatomical bi-cylinder structure of the nerve cord of the Moth and allows for an efficient surgical process for implantation (Figure 17). Additionally, we have integrated carbon nanotube (CNT)-Au nanocomposites into the FMEs to enhance the charge injection capability of the electrode.

Current Research

Images of the fabricated FMEs are showed in Figure 18. They can be connected to the stimulator by either FFC\FPC connectors or metal wires with conductive epoxy. The FMEs show excellent flexibility (Figure 18a), allowing us to open the ring of the FME during insertion around the nerve cord (Figure 17b). The two extended tabs at the tip of the FNP act as "handles" to manipulate the implant and lock the FME in place after the insertion. As opposed to the initial FME design (WH6, Figure 18b) that assumed a cylindrical nerve cord structure, the new FME designs (BC6 & BC8, Figure 18c-d) contain either 5 or 7 stimulation sites at the bottom of the split-ring in a bi-circle geometry. The bi-circle geometry allows multi-site stimulation that is anatomically matched to the insect nerve cord. Moreover, they have an additional stimulation site on the extended tab to act as a reference electrode (Figure 18a). In addition, the holding-tips at the top of the split-ring insert into the dorsal pad of the nerve cord to physically support the probe and prevent it from moving. The holding tips do not contain any stimulation sites in these new designs as there are no neuronal processes in the dorsal pad. We have been able to insert the electrode into both pupae and adult moth. We are able to stimulate multi-directional abdominal motions in both pupae and adult moths as shown in Figure 19. The direction of the abdominal movements depends on the

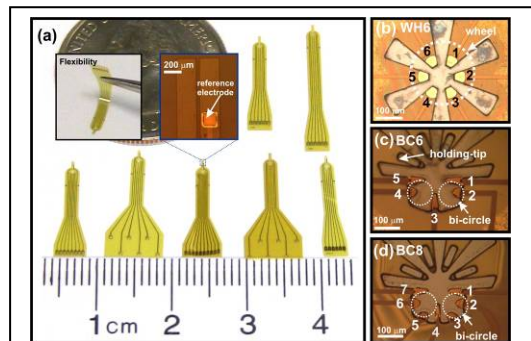


Figure 18: (a) Image showing various FME designs. (b)-(d) Close-up images of the split-ring structure of these FMEs.

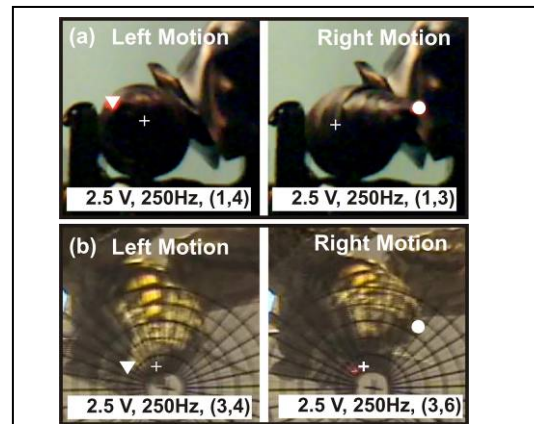


Figure 19: (a) Images showing the multi-directional, graded abdominal movements of a pupa following the CA-FME stimulation. (The location of the abdomen apex of the pupa before stimulation is marked by '+'). (b) Analogous stimulation of an adult.

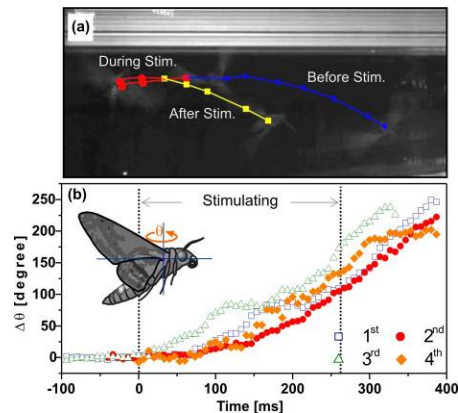


Figure 20: (a) Side view image of a freely flying moth that has been stimulated to perform right turns following the elicited abdominal motions. (b) The changes of the yaw angle (θ) of the moth with 4 successive stimulations.

particular pair of stimulation sites excited. Moreover, we are developing a process for electroplating Au-CNT nanocomposite coatings on FMEs to reduce the stimulation voltage and enhance the charge injection capacity at the interface between the electrode and nerve tissue of the moth. The CNT coated FMEs are able to elicit abdominal motion of the moths with a stimulation voltage significantly less (1.0 V vs. 2.0 V, $p < 0.001$, $n=10$ moths) than that of uncoated FMEs.

Finally, we have integrated the FMEs into a wireless system and in the flight control experiment, we are able to force a freely flying animal to perform turning motions (Figure 20a) using the abdominal ruddering with these elicited abdomen motions. These turning motions are well repeatable and the changes in the yaw angle of the moth with 4 successive stimulations are shown in Figure 20b.

Publications

Journal Articles, Published

W. M. Tsang, A. L. Stone, Z. Aldworth, J. G. Hildebrand, T. Daniel, A. I. Akinwande, and J. Voldman, "Flexible Split-Ring Electrode for Insect Flight Biasing using Multisite Neural Stimulation," *IEEE Transactions on Biomedical Engineering*, 2010, in press.

Y.-C. Toh, K. Blagovic, and J. Voldman, "Advancing stem cell research with microtechnologies: opportunities and challenges," *Integrative Biology*, vol. 2, pp. 305-25, 2010.

N. Tandon, A. Marsano, R. Maidhof, K. Numata, C. Montouri-Sorrentino, C. Cannizzaro, J. Voldman, and G. Vunjak-Novakovic, "Surface-Patterned Electrode Bioreactor for Electrical Stimulation," *Lab on a Chip*, vol. 10, pp. 692-700, 2010.

D. C. Daly, P. P. Mercier, M. Bhardwaj, A. L. Stone, Z. Aldworth, T. L. Daniel, J. Voldman, J. G. Hildebrand, and A. P. Chandrakasan, "A Pulsed UWB Receiver SoC for Insect Motion Control," *IEEE Journal of Solid-State Circuits*, vol. 45, pp. 153-66, 2010.

H.-H. Cui, J. Voldman, and K.-M. Lim, "Separation of particles by pulsed dielectrophoresis," *Lab on a Chip*, vol. 9, pp. 2306-12, 2009.

S. P. Desai, M. D. Vahey, and J. Voldman, "Vesicle libraries - tools for dielectrophoresis metrology," *Langmuir*, vol. 25, pp. 3867-75, 2009.

S. P. Desai, D. M. Freeman, and J. Voldman, "Plastic masters - rigid templates for soft lithography," *Lab on a Chip*, vol. 9, pp. 1631-7, Jun 7 2009.

M. D. Vahey and J. Voldman, "Characterization of diverse cell and particle types using iso-dielectric separation," *Analytical Chemistry*, vol. 81, pp. 2446-55, 2009.

Books/Chapters in Books

J. R. Kovac, B. M. Taff, and J. Voldman, "Enabling technologies for image-based cell sorting," in *Microdevices in Biology and Medicine*, Y. Nahmias and S. Bhatia, Eds.: Artech House, 2009, pp. 129-48.

Meeting Papers, Published

W. M. Tsang, A. Stone, Z. Aldworth, D. Otten, T. Akinwande, T. Daniel, J. G. Hildebrand, R. Levine, and J. Voldman, "Remote control of a cyborg moth using carbon nanotube-enhanced flexible neuroprosthetic probe," in *IEEE MEMS*, Hong Kong, China, 2010.

Y.-C. Toh and J. Voldman, "In vitro construction of complex stem cell microenvironments by stencil-and-flip cell patterning (SAF-CP) " in *Micro Total Analysis Systems*, Jeju, Korea, 2009, pp. 1327-9.

S. P. Desai, D. M. Freeman, and J. Voldman, "Plastic masters - rigid templates for soft lithography," in *Micro Total Analysis Systems*, Jeju, Korea, 2009.

K. Blagovic, S. P. Desai, and J. Voldman, "Micro-patterned polystyrene substrates for highly integrated microfluidic cell culture " in *Micro Total Analysis Systems 2009*, Jeju, Korea, 2009, pp. 144-6.

D. C. Daly, P. P. Mercier, M. Bhardwaj, A. L. Stone, J. Voldman, R. Levine, J. G. Hildebrand, and A. P. Chandrakasan, "A Pulsed UWB Receiver SoC for Insect Motion Control," in *ISSCC*, San Francisco, 2009.

Theses

S. P. Desai, *Building Integrated Cell-based Microsystems: Fabrication Methodologies, Metrology Tools, and Impact on Cellular Physiology*, Ph.D. thesis, Department of Electrical Engineering and Computer Science, 2009.

M. D. Vahey, "A Microfluidic Platform for the Genome-Wide Analysis of Electrical Phenotype: Physical Theories and Biological Applications," in *Electrical Engineering and Computer Science* Cambridge, MA: Massachusetts Institute of Technology, 2010.

N. Mittal, "Cell-Cell and Cell-Medium Interactions in the Growth of Mouse Embryonic Stem Cells," in *Physics* Cambridge, MA: Massachusetts Institute of Technology, 2010.

J. R. Kovac, "Inexpensive Technologies Enabling Widespread Utilization of Image-predicated Cell Sorting," in *Electrical Engineering and Computer Science* Cambridge, MA: Massachusetts Institute of Technology, 2010.