

Cellular BioMEMS

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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 screening.

1. Single-cell manipulation platforms for cell cytometry

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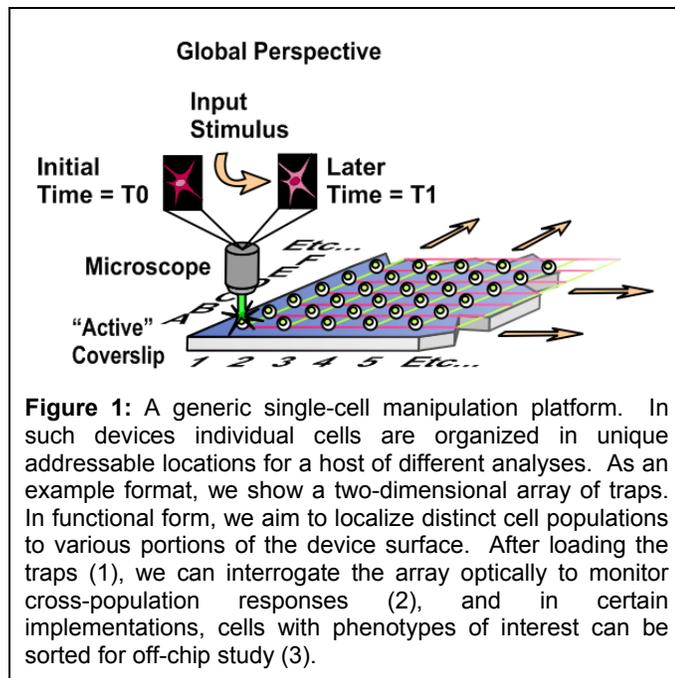
NIH NCRR
NSF Graduate Research Fellowship
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Singapore-MIT Alliance

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Overview

The goal of this research is the development of a series of microfabricated platforms that allow the controlled



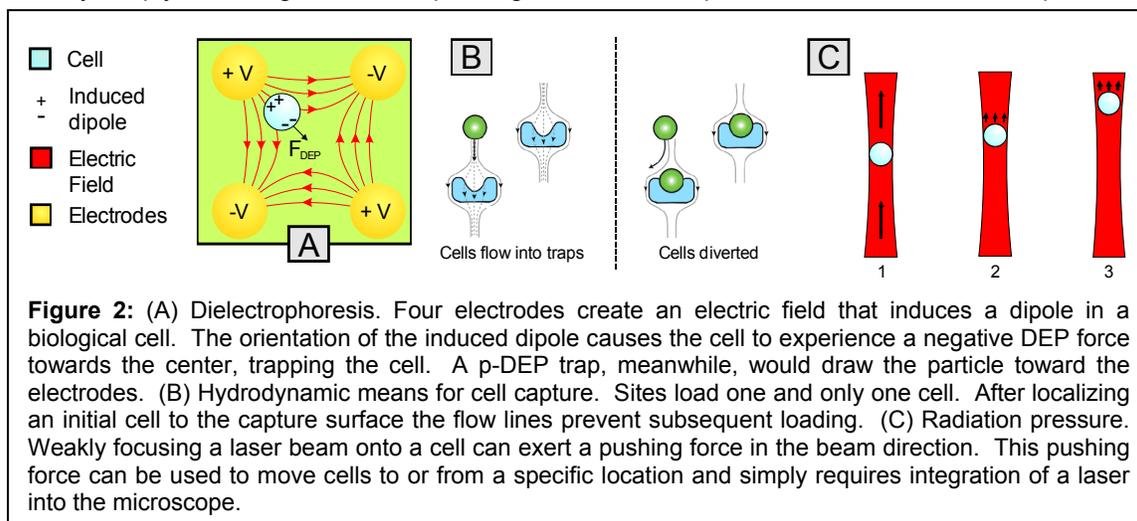
placement and or sorting of multiple single-cell populations (Figure 1). We are currently addressing the challenges of creating useable devices capable of handling and sorting a sufficient number of cells for practical biological assays using a multitude of complementary bioMEMS-based approaches. Our biological goal are to (1) study heterogeneity in cell populations and (2) sort cells based upon imaged dynamic intracellular phenotypes.

In the typical standard techniques used to probe cell-based biological responses, large collections of cells are simultaneously analyzed to develop some understanding of function and system response. As has been recognized in many publications within the last 5-10 years, this bulk perspective often masks the discrete responses of individual cells. Single-cell assays are often able to enable proper understanding of signaling pathway kinetics & dynamics. Our technologies directly address this concern by offering techniques to precisely position and organize large groups of individual cells on a common surveyed surface. For screening applications our work centers on both the observation of cells and the isolation of cells exhibiting a desired phenotype. This functionality offers a unique means to combine the benefits of microscopy with flow-assisted cell sorting (FACS). Our work thus bridges technological gaps between discrete single-cell placement and observation and between observation and isolation.

On some level, these gaps stem from a lack of techniques to physically manipulate cells; microscopy and flow cytometry handle cells differently, leading to their different functionalities. We are thus developing a unique series of tools that can spatially actively organize cell subpopulations and in some implementations release selected cells for extended downstream study. We are realizing these aims by developing massively parallel arrays of switchable cell traps.

Technology Background

The two primary methods for enabling single-cell manipulations in our microsystems-based designs rely upon unique combinations of dielectrophoresis (DEP), hydrodynamic capture geometries, and radiation pressures (Figure 2). *DEP refers to the force on a cell in a non-uniform electric field.* Depending on the properties of cell, media, and applied electric field, DEP forces can either propel cells toward field maxima (positive DEP or p-DEP) or minima (negative DEP or n-DEP) enabling stable holding responses. DEP methods are well-suited for microfabrication-based processes as they incorporate electrode geometries that effectively exert forces on cell-sized objects and scale well for arrayed applications. Hydrodynamic manipulation methods generally involve weir- or well-based geometries sized appropriately for single-cell retention. Such structures mechanically cradle individual cells in specific on-chip locations and passively load by simply "catching" cells from passing fluids in on-chip flow chambers. Radiation pressure, weakly focusing a laser beam onto a cell can exert a pushing force in the beam direction. This pushing force can be used to move cells to or from a specific location and simply requires integration of a laser into the microscope.

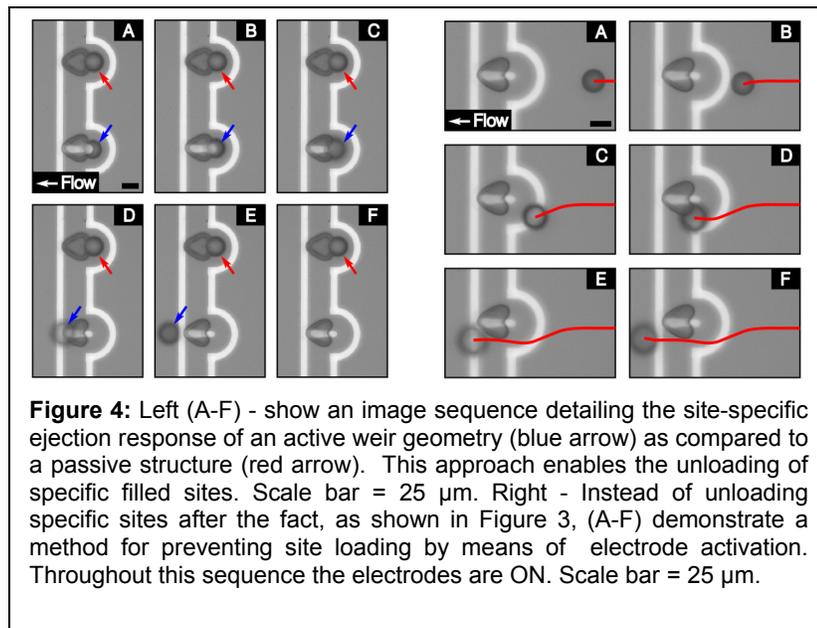
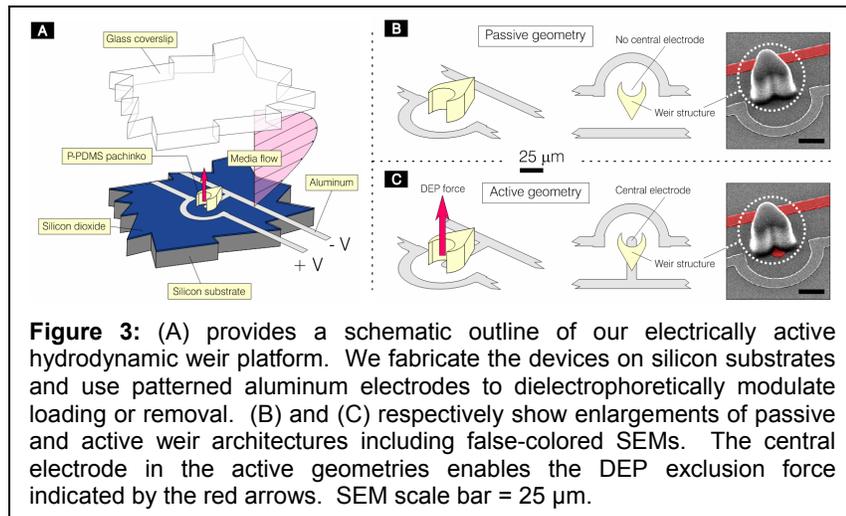


though largely popularized in the biological context by tweezing applications, in more basic forms can serve as a functional means to displace cells. When a laser beam is weakly focused onto a cell, the cell will propagate axially along the propagation direction of the beam. This force arises from momentum transfer from photons to the cell due to light scattering in a fashion analogous to a beach ball being pushed by water from a fire hose. Through unique combinations of the various flexibilities offered by these forcing methodologies our assay platforms provide new functionally active substrates that enable novel investigative potential.

Current Research

One recent effort has been to newly develop the ability to process a commercially available photopatternable silicone (PPS) from the vantage point of its bioMEMS integration potential. In our hands, this product has proven enabling for manufacturing substrate-affixed micron-scaled patterned geometries that demonstrate low autofluorescence. These properties combined with its ability to function as a cell culture substrate while aligning with pre-processed wafers has expanded many of the polymer-based surface structure capabilities in multi-level bioMEMS designs. Because PPS enables the formation of isolated low autofluorescent structures in devices capable of handling biological cells, efforts to track weakly fluorescent subcellular probes are not hindered.

This use of PPS has enabled the development of devices that combine hydrodynamic weir-structure array site loading with electrode activated n-DEP-based site unloading. In Figure we present a recently developed architecture for cell manipulation that functions even in scaled formats using standard cell culture media on-chip buffers. In this device implementation we approach cell patterning using two primary means. In one mode, the "ejection response," individual sites can be unloaded by expelling loaded cells from the PPS-formed hydrodynamic capture crevices. This operation vertically displaces held cells and allows passing on-chip fluid flow to sweep them downstream and out of the device. The



other manipulation scheme, termed the "rejection response" prevents specific targeted sites from loading altogether. Figure details these two approaches. We have used this new functional platform to perform manipulation assays where interwoven "checkerboard" patterns of green and orange fluorescent beads are organized on-chip (Figure).

Our second major cell manipulation approach targets sorting functionality using a combination of optical and hydrodynamic trapping techniques (Figure). Cells are arrayed by allowing cells to settle into microwell arrays designed to trap, on average, a single cell per microwell. Because loading is passive, fabricated interconnects are not required to be routed to each individual trap site for loading.

Release of a cell from a microwell is achieved by weakly focusing an infrared laser beam onto the cell of interest, exerting radiation pressure on the cell. This pressure establishes an

upward force large enough to overcome the gravitational force on the cell, levitating the cell out of its microwell. Once the cell is levitated, fluid flow can be used to wash the target cell downstream for fractionation. Release therefore requires no interconnects besides a clear optical path to the chip, resulting in a device architecture that is simple to design and fabricate with a traditional SU-8/PDMS replica molding process.

We have fully integrated our optofluidic approach into an upright, automated fluorescence microscope to realize the full scalability of the optofluidic array platform. Additionally, we developed a computer interface to allow rapid inspection of array positions and straightforward removal of target cells. We have successfully fabricated a device containing a 10,000-site microwell array and demonstrated trap and release functionality with both human and murine cell lines. Specifically, we have demonstrated sorting based on whole-cell fluorescence (Figure) and fluorescence localization within cells. Having successfully demonstrated proof of concept assays with this platform which are prohibitively difficult or impossible with existing technologies, we seek to perform an entirely new class of image-based genetic screens in the near future.

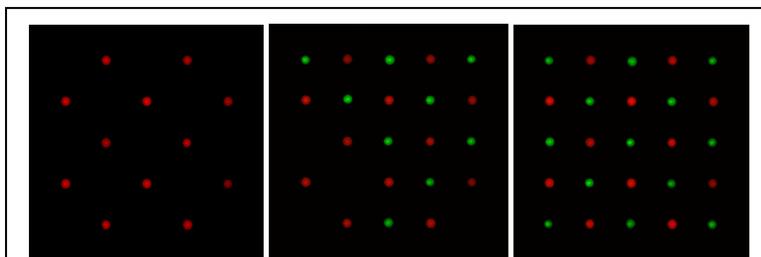


Figure 5: In a sequence of operations we first selectively load (left) the passive weir structures with orange beads using the loading approach demonstrated in Figure 4, where we apply voltages to prevent beads from loading in active sites (showing fluorescent signals alone). We then inject green fluorescent beads (middle) and begin backfilling the remaining sites by turning off all applied voltages. The right image shows a 5×5 "checkerboard" pattern of interwoven alternatively dyed fluorescent beads.

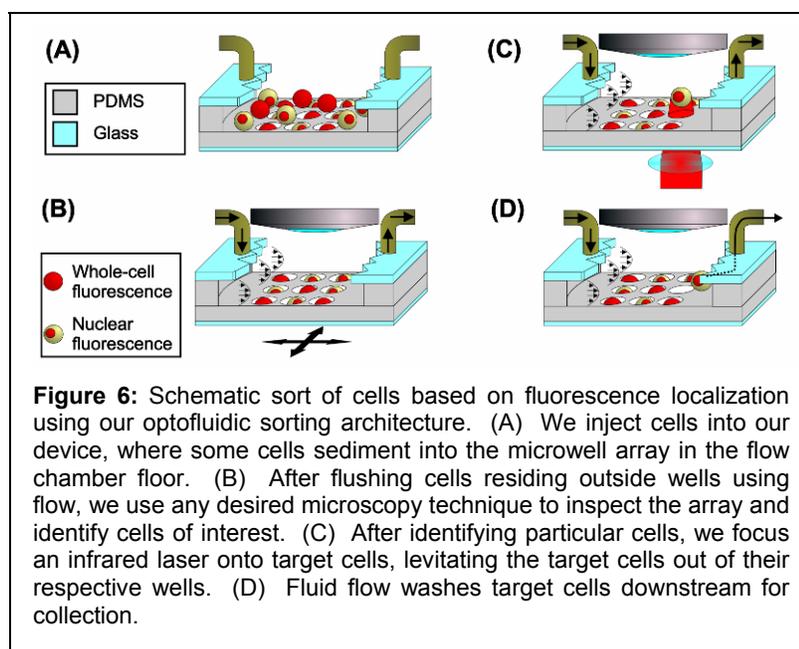
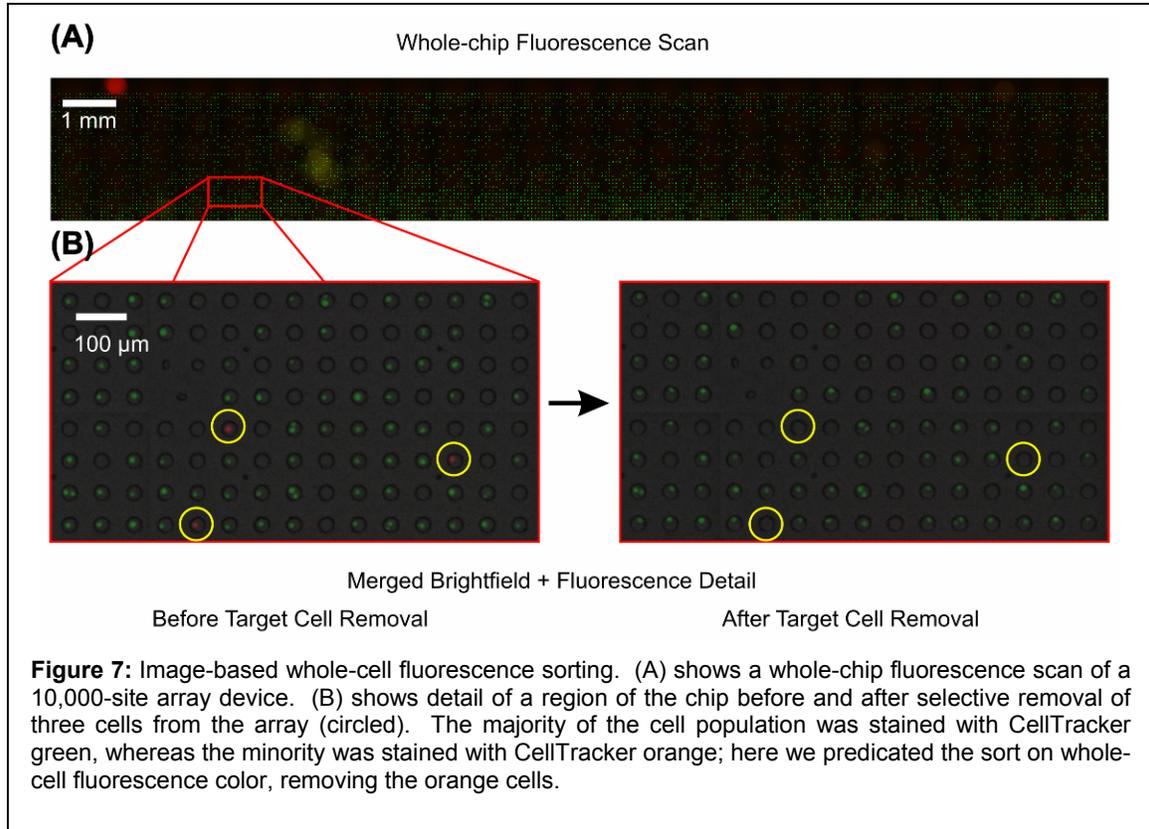


Figure 6: Schematic sort of cells based on fluorescence localization using our optofluidic sorting architecture. (A) We inject cells into our device, where some cells sediment into the microwell array in the flow chamber floor. (B) After flushing cells residing outside wells using flow, we use any desired microscopy technique to inspect the array and identify cells of interest. (C) After identifying particular cells, we focus an infrared laser onto target cells, levitating the target cells out of their respective wells. (D) Fluid flow washes target cells downstream for collection.



It is imperative that our cell manipulation techniques do not adversely affect the cell and that we completely understand the physiological ramifications of manipulation. Clearly, each manipulation technique will have a different level of physiological impact which dictates the guidelines for usage and the appropriate controls necessary. This is especially important for studying complex and subtle phenotypes which could easily be masked by the simple act of manipulation. To this end, we have demonstrated the design and implementation of a microfabricated platform for assaying the physiological impact of dielectrophoretic trapping. While dielectrophoresis (DEP) has been widely used to manipulate mammalian cells, little is understood about the effects of electric fields and Joule heating on gene expression. We have developed a microfabricated platform through which we subject fluorescence-based reporter cell lines to electric fields and subsequently assay their gene expression. We have used this platform with NIH 3T3 cells containing a GFP-based heat shock reporter. After trapping cells using negative DEP and allowing them to adhere to the substrate, we activate the stimulating electric field. We then use quantitative fluorescence microscopy to assay the physiological state of the cells. In one experiment, we assayed the effect of changing voltage (and thus the induced Joule heating) on stress response. Cells stimulated at 10 MHz and 20 Vp-p for 30 min showed $2.4X \pm 0.2X$ ($n = 4$) increase in fluorescence as compared to the control, while cells stimulated at 5 MHz and 5 Vp-p for 30 min showed $1.3X \pm 0.1X$ ($n = 4$) increase in fluorescence. Positive controls performed on chip using a known stressor showed a $9.5X \pm 0.4X$ ($n = 4$) fluorescence increase. These results are in agreement with finite element models predicting greater Joule heating at elevated stimulating voltages. We are using this platform to map out optimal operating regimes, in terms of both the frequency and intensity of the applied field, and the types of controls necessary for using DEP in the future.

To enable more high-throughput studies of electromagnetic field effects on cells we are building a platform for culturing cells between parallel plate electrodes and then stimulating them via a computer-controlled interface. We have consequently focused live-cell-based assays using (1) heat-shock-factor 1 (HSF1) linked GFP reporters for studying heat stress, (2) dichlorodihydro-

fluorescein diacetate (DCFDA) based dyes for studying oxidative stress granule and (3) stress granules for studying transcriptional/translational silencing. These live-cell assays coupled with quantitative fluorescence techniques serve as powerful tools for analyzing the physiological states of thousands of cells with single-cell resolution.

Finally, we are continuing to build vesicle-based sensors for measuring physiologically relevant phenomena within a DEP trap. We have successfully electroformed giant unilamellar vesicles (GUVs) with temperature-sensitive (Laurdan) and voltage-sensitive (di-8-ANEPPS) dyes bound to their membranes as “probes” for temperature change and induced transmembrane potential. We are continuing to develop this *in situ* metrology technique to build a detailed map of temperature and induced transmembrane potentials for a wide range of trapping frequencies, voltages and durations.

2. Microsystems for modulating the stem cell microenvironment

Sponsors

NIH NCRR

NSF Graduate Research Fellowship

Project Staff

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Overview

Stem cells are powerful biological models for many different processes, ranging from fundamental issues in biocomplexity to developmental and disease processes. For these reasons, intense effort is being focused on characterizing and manipulating stem cells in culture, identifying culture conditions that can propagate stem cells, and controlling their phenotype in culture. However, despite the impressive progress made on these fronts, the techniques and technology used are predominantly based in traditional culture techniques that are labor-intensive and qualitative. *Our long-term objective is to streamline the ability to manipulate stem cells in vitro while automating the extraction of quantitative in situ phenotypic data.*

Our approach is to develop a *microfabricated culture system* that can precisely, accurately, and automatically deliver nanoliter to microliter volumes of

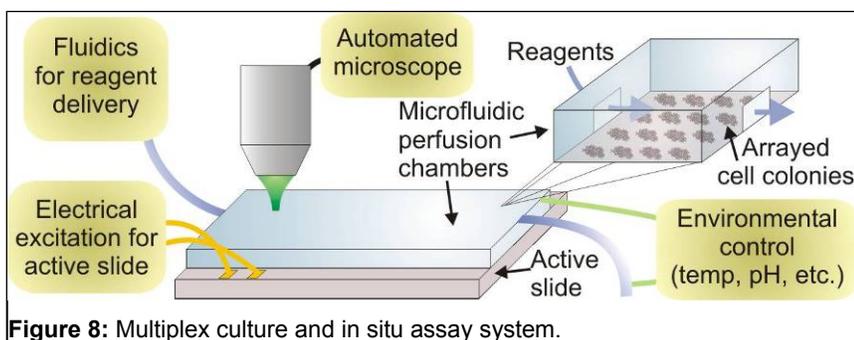


Figure 8: Multiplex culture and in situ assay system.

reagents to a multiplexed array of millimeter-sized perfusion chambers containing cells, and couple this to *automated light microscopy to extract images* of cells in the chambers (Figure). We will keep the system sufficiently low cost to be accessible to a single-investigator laboratory and disseminate its plans and protocols in an open-source format to ensure wide distribution. *Our initial goals are to develop the technology base for such a system.*

Technology Background

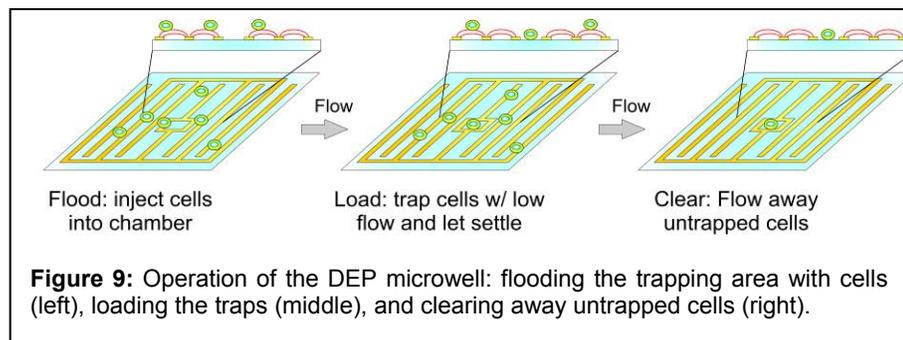
Our system uses two main microtechnologies to achieve our aims. First, we use microfluidics to precisely deliver combinatorial reagents to a large array of chambers, and second, we use cell patterning techniques to position cells in those chambers.

Controlling liquid flows at the microscale is well-developed technology. At the small size scales (<1 mm) and typical flow rates ($\sim\mu\text{l}/\text{min}$) encountered in these systems, the fluid flow is laminar and well controlled. Mixing occurs only by diffusion, which means that one can force two liquids to mix quickly ($\sim\text{sec}$) or stay separate by changing geometries and flow rates. Using accepted technologies, one can make arrays of microfluidic chambers containing cells, and “hard-wire” different concentrations and combinations of input reagents to each of these wells, allowing one to run many experiments in parallel.

The other technology that we are using is cell patterning. We have developed a number of cell patterning techniques suitable for patterning sensitive embryonic stem cells. First is dielectrophoresis, as described in Project 1. The second is bioflipchip technology, which is a physical cell transferring approach. Finally, we have used stencil patterning to pattern colonies of stem cells. All three techniques have the property that they can pattern cells without patterning the extracellular matrix, an important property when trying to pattern dividing, motile cells.

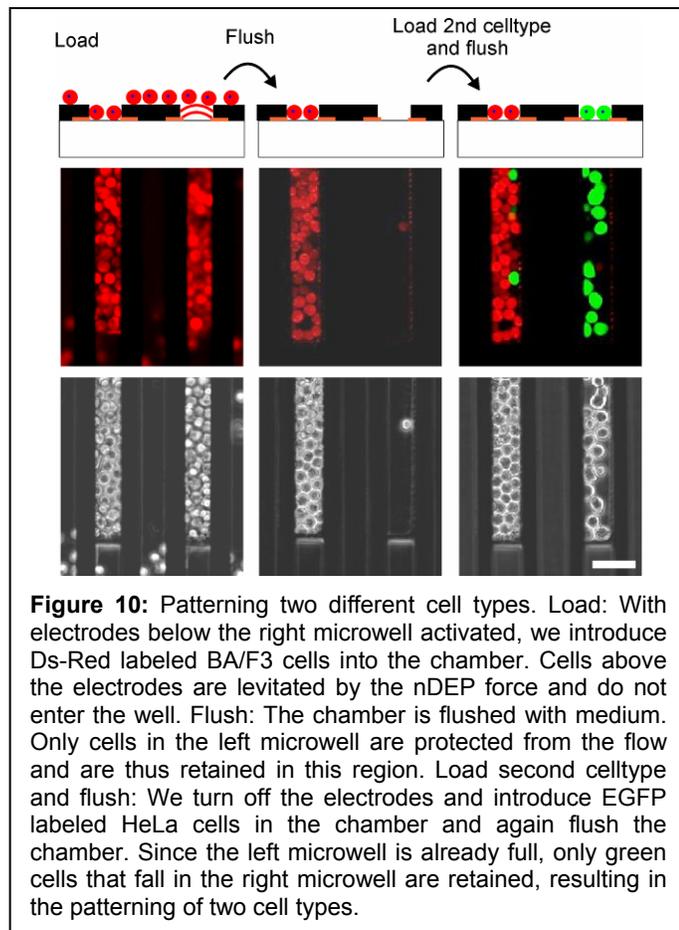
Current Research

Our current research is focused on two areas: cell placement and microfluidic cell-culture systems.



Cell placement via DEP traps

We are currently focusing on the development of a dielectrophoretic trap that can be used to array single cells in flow chambers. Prior single-cell DEP traps are strong but are either not appropriate for patterning cells, would not allow unobstructed cell division, are difficult to package or fabricate, or cannot be used with normal cell culture media. The challenge is fabricating a strong trap that allows single-cell patterning, is planar, and uses nDEP. To meet this challenge, we have designed a novel method of trapping called a dielectrophoretic microwell. The DEP microwell allows cells in the trap to settle while cells outside the traps are levitated and flowed away (Figure). We have used our previously validated modeling software to identify operating conditions that minimize cell heating and induced transmembrane voltage and maximize trap strength.



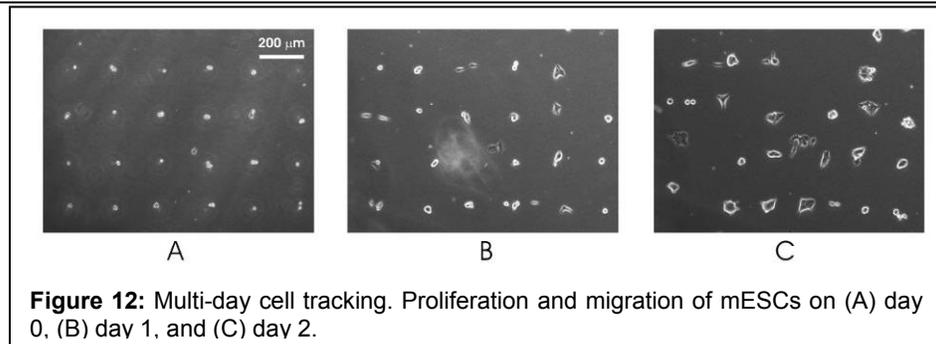
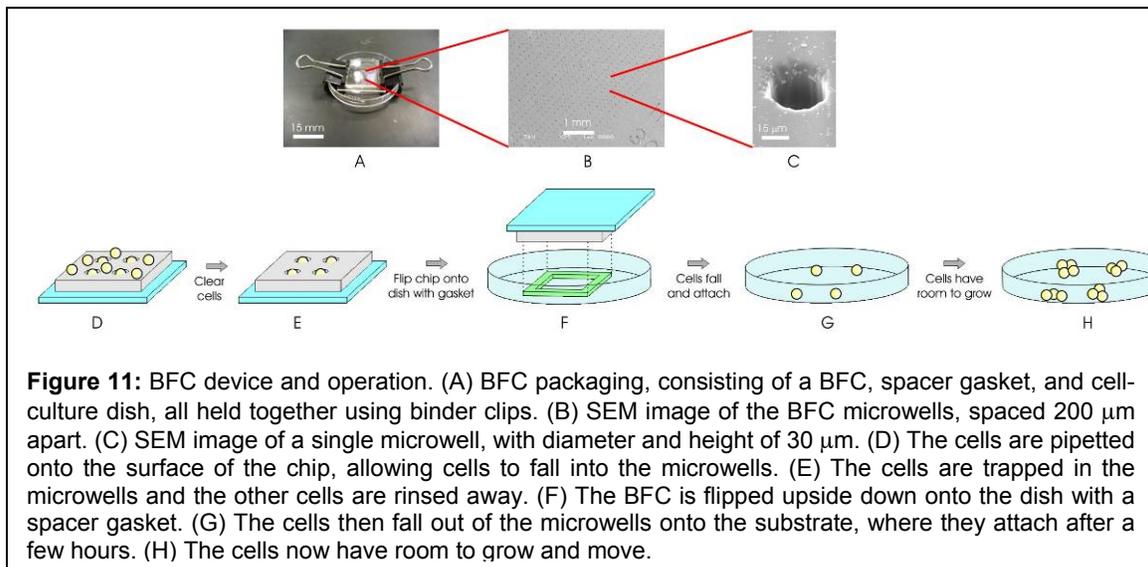
Patterning multiple heterogeneous cells using active microwells

Cellular physiology, *in vivo*, is regulated by a multitude of factors which include cell-cell as well as cell-matrix interactions. The ability to recreate complex cellular microenvironments *in vitro* enables the study of such interactions and the construction of tissues *ex vivo* for implantation and organ regeneration.

The use dielectrophoretic forces to position cells offers the ability to pattern multiple celltypes since electrical trapping sites can be easily switched between on and off states. Additionally, we have recently demonstrated that nDEP offers high resolution (up to single cells) and is bio-compatible. Building on our previous work, we have developed a method to pattern multiple heterogeneous cells using active microwells. Our microwells have electrodes at the bottom that are used to provide a repulsive nDEP force which enables cells to be ejected from the microwells (Figure). By addressing these electrodes individually, we are able to selectively clear particular wells and then refill them with a different celltype.

Bio-flipchip cell patterning

We have also recently developed a physical method for patterning single cells without patterning matrix. Term bio-flipchip cell patterning, this approach uses a microfabricated polymer chip, containing thousands of microwells, that enables cell patterning with single-cell resolution anywhere on a substrate and onto any substrate (Figure). We have used this approach to pattern embryonic stem cells and watch them proliferate and move (Figure), pattern them on other cells (e.g., feeder cells), onto pre-patterned matrix, three-dimensional matrix, and finally to study how different numbers of starting cells affect colony forming efficiency.



Colony patterning using stencils

We have also been investigating the role of colony-colony interactions via diffusive signaling factors. We have used stencil cell patterning to localize ESC colonies with varying inter-colony spacing on a substrate (Figure A-B). This patterning technique allowed ESC colonies to move and expand naturally. We monitored the behavior of ESC colonies for different intensity of colony-colony interactions by processing time-series images of ESC colonies to quantify their self-renewal, growth and motility (Figure C). The developed platform can examine ESC colonies at multiple space and time points.

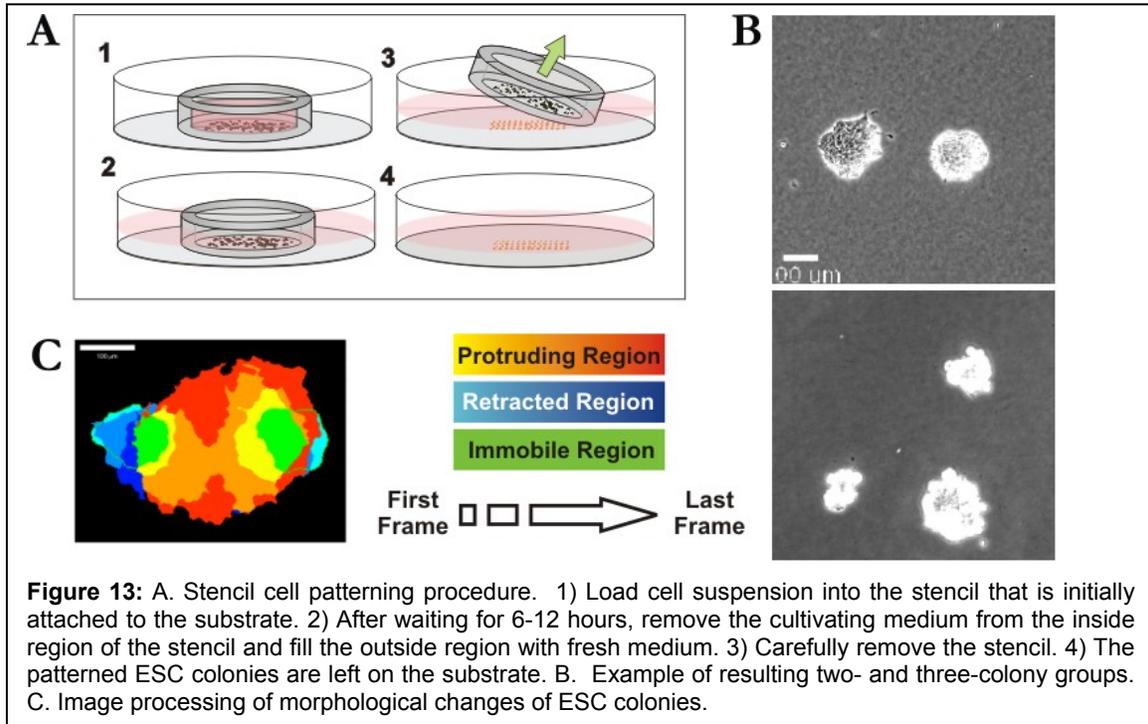


Figure 13: A. Stencil cell patterning procedure. 1) Load cell suspension into the stencil that is initially attached to the substrate. 2) After waiting for 6-12 hours, remove the cultivating medium from the inside region of the stencil and fill the outside region with fresh medium. 3) Carefully remove the stencil. 4) The patterned ESC colonies are left on the substrate. B. Example of resulting two- and three-colony groups. C. Image processing of morphological changes of ESC colonies.

Microfluidic cell culture and assay

We are also developing microfluidic systems for culturing adherent cells over a range of flowrates and reagent concentrations. Such systems enable greater control over the cell culture microenvironment by controlling media composition via continuous perfusion. Specifically we have designed and fabricated a device for performing adherent cell culture over a logarithmic range of flowrates. Logarithmic ranges are commonly used in experimental biology to explore a large parameter space but to-date have been lacking in microscale cell culture. The device, shown in Figure, sets the flowrates through each culture chamber using syringe-driven flow and a network of fluidic resistances. Each cell culture chamber has one input channel and two output channels. When cells are loaded, one set of output channels

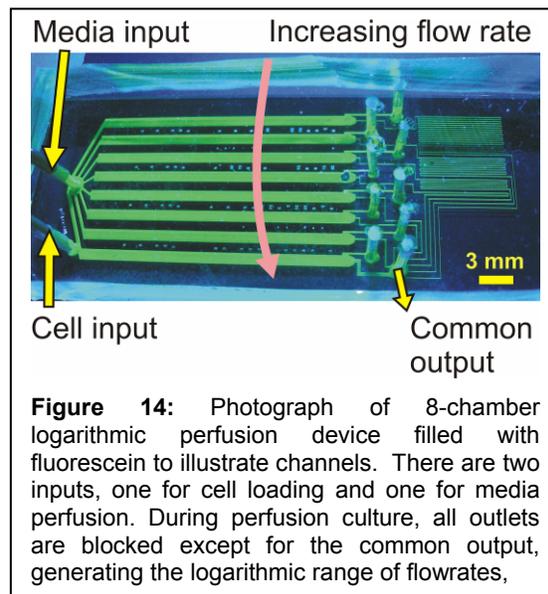
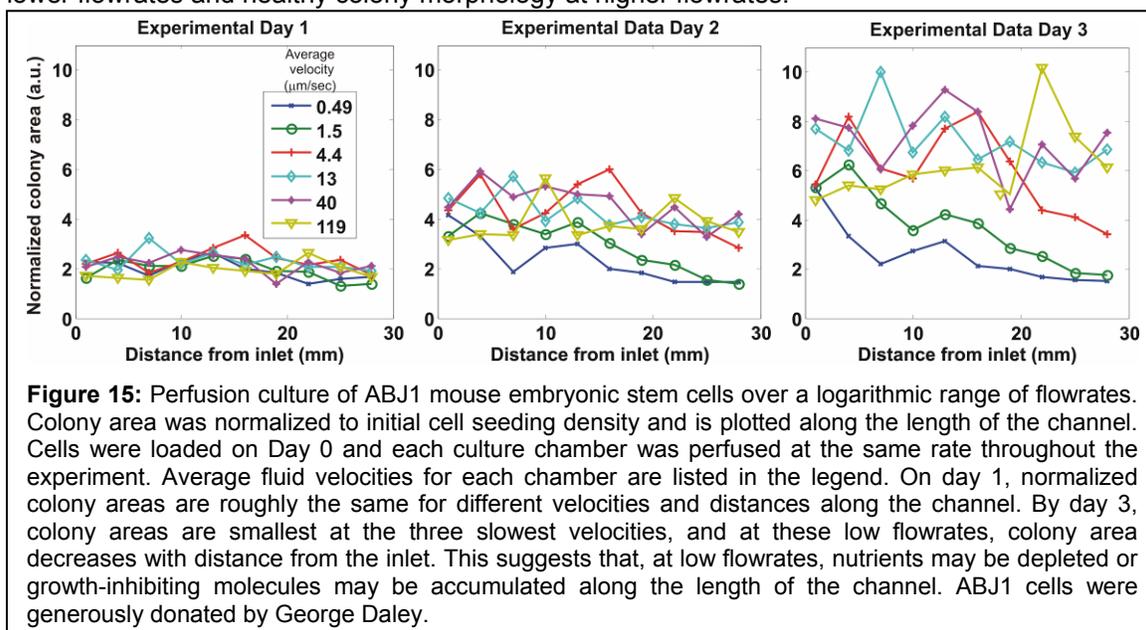


Figure 14: Photograph of 8-chamber logarithmic perfusion device filled with fluorescein to illustrate channels. There are two inputs, one for cell loading and one for media perfusion. During perfusion culture, all outlets are blocked except for the common output, generating the logarithmic range of flowrates,

creates the same flowrate through all four chambers. After loading and cell attachment, the other set of output channels creates a logarithmic range of flowrates across the device. The 30-mm-long channels enable characterization of media depletion along the length of the channel. The design is easy to fabricate, scalable, and consists of a single-layer network of channels in PDMS bonded to a glass substrate to which cells adhere.

To better understand the design requirements for a cell culture and *in situ* assay system, we are focusing on mouse embryonic stem cells as an example biological system. Stem cells are particularly sensitive to their microenvironment and are slow to culture using traditional techniques. We have successfully cultured embryonic stem cells in continuous, logarithmically scaled perfusion for >4 days using a device with the same design as in Figure 13. Results are shown in Figure 14. Perfusion rates varied >2000 \times across the array, with poor proliferation at lower flowrates and healthy colony morphology at higher flowrates.



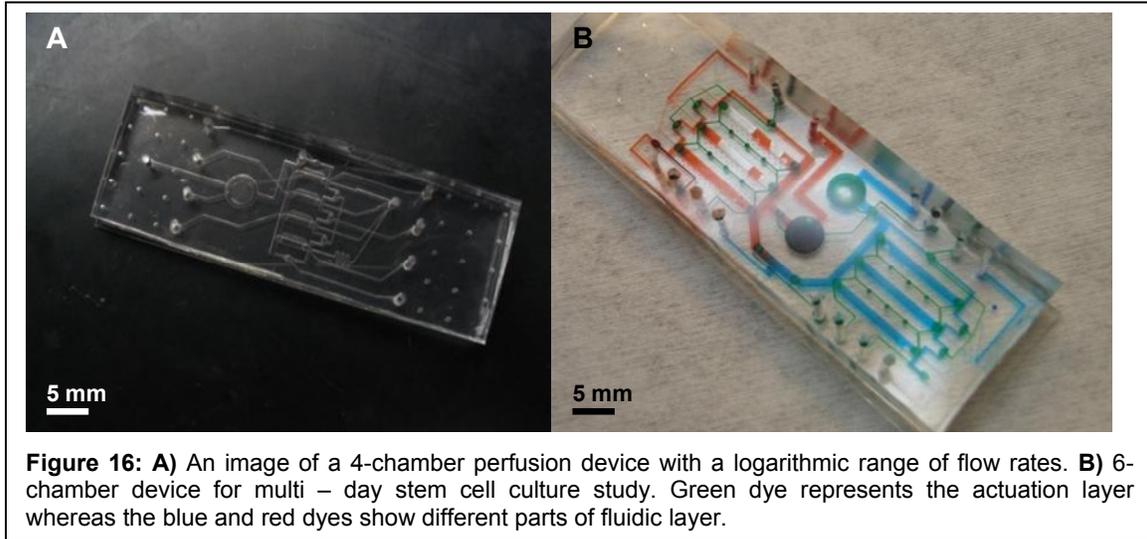
Valved microfluidics for cell culture

We have designed and fabricated a valved version of the microfluidic perfusion device previously developed in our lab for culturing adherent cells across a logarithmic range of flow rates. The incorporated valve architecture is similar to one developed by Irmia and Toner (*Lab Chip*, 2006). The two layered device is easy to fabricate by pouring PDMS into plastic molds. The fluidic and manifold layer are bonded together and clamped to a microscope slide to which cells adhere.

On a device, shown in FigureA, we have successfully grown 3T3 fibroblasts and ABJ1 mESCs. Cells are cultured in 4 chambers with varying flow rates. Individually addressable valves facilitate cell loading and seal the culture chambers during the cell adhesion stage. While the culture chambers are sealed by valves, the bus design allows easy flushing of the rest of the device, thereby restricting cell to the culture chambers. Bubbles are an important issue in microfluidic systems causing experiments to fail, and moreover are cytotoxic to cells as they may rupture the cell membranes and create a poor cellular environment in general. This device design addresses this problem by introducing an active bubble trap in the input channel.

In order to run different biological experiments on a same chip, we have designed and fabricated the device as shown in FigureB. This design enables running multi-day perfusion experiments with varying input parameters, i.e. perfusing cell culture chambers with different medias or loading different cell types. The device has two sets of 3 chambers each with the same flow rate across all the chambers. Two distinct media inputs with active bubble traps facilitate cell culture under

different media conditions. This design provides us with a platform for a multi – parameter study of the effects of diffusible signaling via flow.



3. Microfluidic cell traps for studying stem cell fusion and reprogramming

Sponsors

NIH

Postdoctoral Associates

Alison Skelley

Overview

Stem cells have the ability to differentiate into mature tissues and organs. Cloning experiments, such as Dolly the sheep, have demonstrated that adult cells can be reprogrammed back to an embryonic-like state, and that these cells can give rise to further organs and animals. Such advances show that the process of reprogramming can potentially yield donor-specific tissues, generated from differentiated adult cells, preventing immunological rejection. Several reprogramming methods have been demonstrated in the literature, such as fusion of a somatic cell with an embryonic stem cell, or transfer of the nucleus of the somatic cell into an embryonic cell. Cell fusion promises to be a more scalable approach than nuclear transfer, and millions of cells in theory can be fused in parallel using commercially available chemical or electrical stimuli. One significant limitation, however, is that the cells are not properly paired prior to fusion, resulting in a high background of incorrectly fused or non-fused cells and necessitating days of culturing and colony picking to isolate the desired fusions. As a result, little is known about the cellular mechanisms behind reprogramming.

Our goal is to develop a microfluidic device that can properly pair cells prior to fusion, generating larger numbers of successfully fused cells.

Technology Background

Our devices are fabricated using standard SU8 photolithography. PDMS is cured on these SU8 molds to create the channel geometries and cell capture features. The PDMS is plasma-bonded to a glass slide to seal the channel and capture array.

Our system relies upon the principle of hydrodynamic trapping. The top PDMS surface of the device has features, or “capture combs,” that extend vertically into the flow-through channel. The combs are fabricated with cell-sized pockets, and are held off the bottom surface by small pillars on either side of the capture pocket. Cell suspensions are loaded into one end of the channel and drawn past the capture combs. Single cells are trapped by each comb and held stationary against the fluid flow. While trapped, the cells experience little-to-no shear, and can be cultured in the device by perfusing with media.

We have developed novel capture geometries that allow sequential capture and pairing of two different cells types. The capture combs have been re-designed to have capture pockets on both sides of the comb. The combs are in a symmetric staggered configuration. During loading (Figure), the first cell suspension is loaded (UP) so that single cells are captured in the smaller cup on the backside of the capture comb. This cup is sized to fit only single cells. Next, the flow is reversed, and the cells are all transferred into the larger capture cup. This transfer step takes advantage of the laminar flow in the microfluidic channel, and all of the cells are transferred and re-capture simultaneously within ~ 0.5 s. Finally, the second cell suspension is loaded (DOWN) and the second cell type is captured in the larger comb that is sized to fit only 2 cells.

Current Research

Optimizing the two-cell capture

We are currently optimizing the capture geometry to obtain 1000s of properly paired cells in a 2mm x 2mm array. Using red and green 3T3 cells we have demonstrated >50 - 70% pairing over the entire device (Figure). We are also investigating different channel geometries to provide easy removal of the cells from the device, and geometries with 4 capture arrays to explore different fusion conditions within a single experiment.

Cell Fusion

A distinct advantage of our microfluidic device is that once the cells are immobilized fusion can be initiated using different techniques. Chemical fusion is initiated by flowing polyethylene glycol

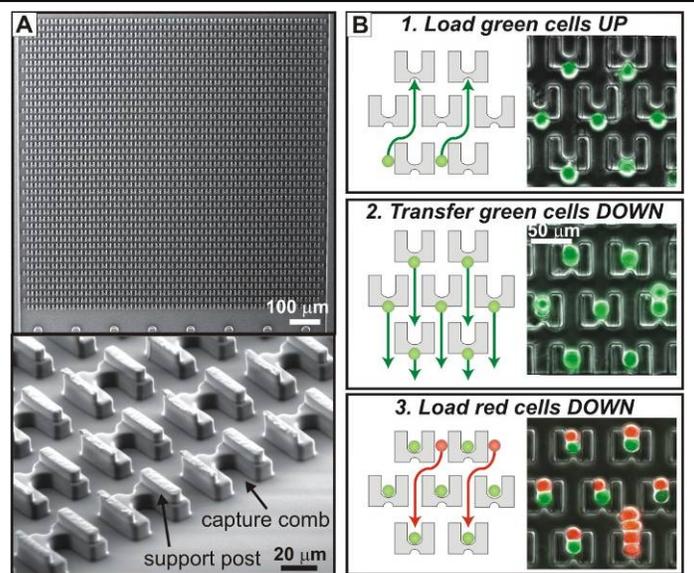


Figure 17: (A) SEM images of the silicone cell fusion microdevice, showing an overview (top) of a 2-mm \times 2-mm capture area, and (bottom) a close-up of the capture comb and support posts. (B) Schematic and red/green/transmitted-light overlay photos of the two-cell loading process.

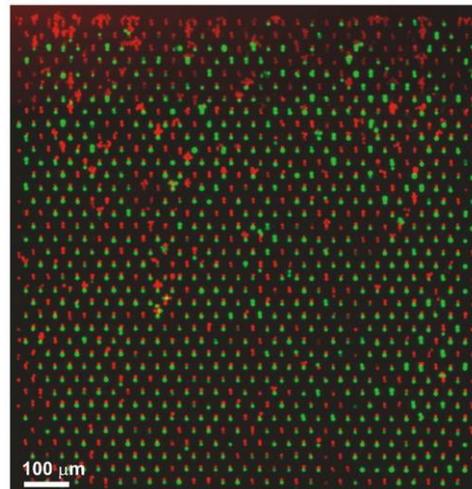


Figure 18: Red/green overlay images of the capture array loaded with fluorescently labeled 3T3 cells. The red/green pairing efficiency is $\sim 50\%$ over the entire device and $\sim 70\%$ in the bottom half.

(PEG) past the cells, and then washing out the PEG with cell culture media. In conventional systems, the cells have to be pelleted to obtain cell contact, but no specific pairing is obtained. As a result, the fusion yields of A+B are ~ 1-5%. Using our device loaded with red and green 3T3 cells we have demonstrated fusion yields of ~ 15% with a single dose. In addition, because the cells remain immobilized and paired, we can do multiple doses of PEG to increase the fusion efficiency, and have demonstrated up to 35% successfully fused cells with 4 doses of PEG.

We have also demonstrated fusion using an electric field as the fusion pulse. The device is prepared by bonding the PDMS to a glass slide with chrome electrodes that lie on either side of the capture array. Once the cells are immobilized, an electric pulse is generated using a commercially-available power supply.

Finally, using both of the methods above, we have been able to track the progression of fusion by monitoring the exchange of fluorescent dye. Red and green fluorescent images taken every 2.5 minutes during and after the initiation of fusion demonstrated that the exchange of dye takes place over a 15 minute period.

We are currently optimizing the capture and fusion conditions for mouse embryonic stem cells and mouse embryonic fibroblasts to study the reprogramming process.

4. Iso-Dielectric Separation: A New Technology for Continuous-Flow Cell Screening

Sponsors

MIT Buschbaum Fund
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Merck-CSBi Fellowship

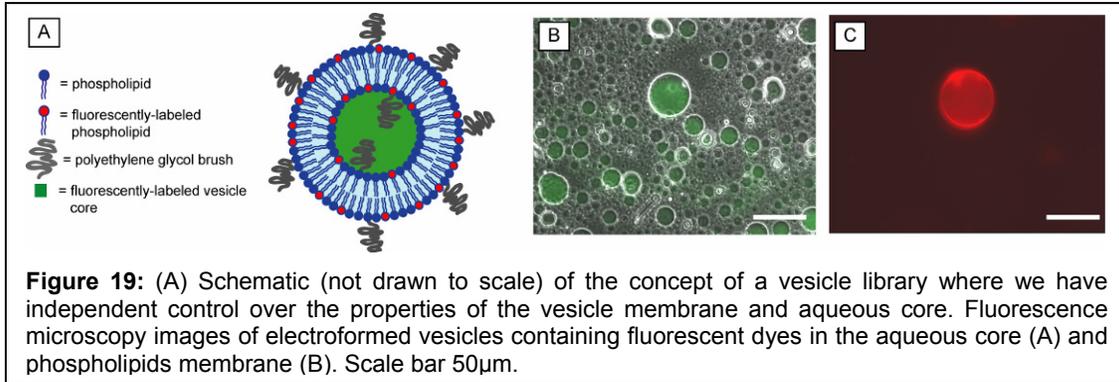
Project Staff

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Overview

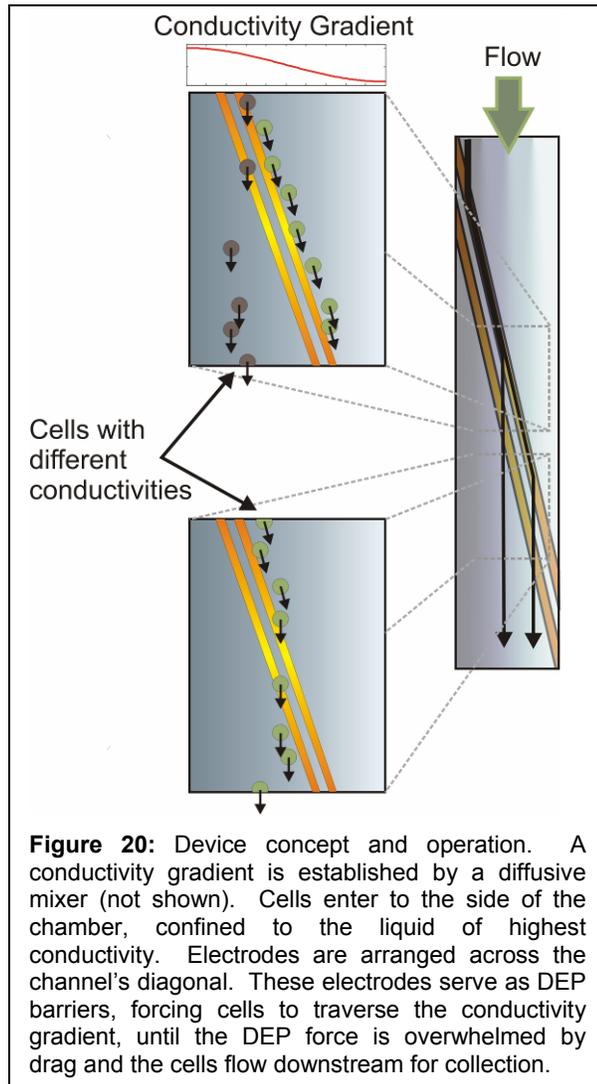
Genetic or phenotypic screens require the ability to select a small fraction of targeted cells from a large, heterogeneous background. One of the largest challenges in applied biology is to perform these screens in a way that possesses both high throughput and high purity. Our approach to this problem 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.

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 and shape), 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.

One challenge in characterizing IDs and other techniques that depend on dielectrophoresis is that commercially available test particles are limited in their range of electrical properties. Polystyrene microspheres commercially available in several sizes and surface functionalizations (e.g. carboxyl groups) are the most widely used test particles for characterizing DEP systems. While functionalized microspheres (FMs) can be further engineered with phospholipids in an effort to mimic biological membranes, they serve as poor models for live cells. We have used phospholipid vesicle electroformation techniques to develop a new class of metrology tools with specifically engineered electrical properties to enable identifiable dielectrophoretic responses in microfabricated systems. These cell-sized phospholipid vesicles (commonly called giant unilamellar vesicles or GUVs) enable the creation of large libraries of test particles for DEP. They offer exquisite control of both their inner aqueous core and outer membrane properties (Figure 22). By encapsulating solutions of different electrolyte strength inside the vesicle we can control its electrical polarizability. This allows for a large range in conductivity (varying over ~100X of physiologically relevant conductivities) which cannot be achieved easily with commercially available FMs. Furthermore, we can encode information about the properties of the vesicle in its fluorescence signature. This facilitates tracking and visualization of vesicles in microsystems and provides valuable internal consistency checks when building libraries with distinct properties. Additionally, vesicle size can be tuned by post-filtration and lipid film patterning, enabling preferential selection of vesicles with sizes that are appropriate to the DEP system under test.



Current Research

Isodielectric separation

The current implementation of IDS uses a diffusive mixer to generate a smooth, linear conductivity gradient from two input solutions. Cells enter the device with the higher conductivity solution, where they remain through the mixer into the main separation channel. Here, electrodes arranged across the diagonal of the channel provide a DEP barrier which deflects particles in the direction of decreasing conductivity (Figure). If the range of the conductivity gradient is chosen appropriately, this will continue until the cells are sufficiently close to their IDPs that the drag force overwhelms DEP, and they flow unobstructed to the outlets for collection. In this way, we are able to map an electrically distinguishable phenotype to a position along the width of a microfluidic channel.

We have modeled the relevant physics – electrostatics coupled with heat, mass, and momentum transfer – so as to converge on a design which will allow for separations of a broad range of particles subject to the constraints of diffusion, force balance, and electrohydrodynamics. Because of these constraints, IDS is an inherently microscale technology. We then fabricated the devices by patterning gold electrodes on a Pyrex wafer and bonding the molded PDMS channel to the top.

To date, we have tested the device using polystyrene beads, vesicles, yeast, and *E. coli*. Specifically, we have demonstrated separation of polystyrene beads based on surface conductance (Figure) and yeast, according to viability (Figure). Present and on-going work focuses on the development of alternate architectures for IDS, as well as pursuing actual genetic screens using this technology.

Vesicle Libraries

To demonstrate the capabilities of vesicle-based metrology, we construct vesicles encapsulating different conductivity solutions and with

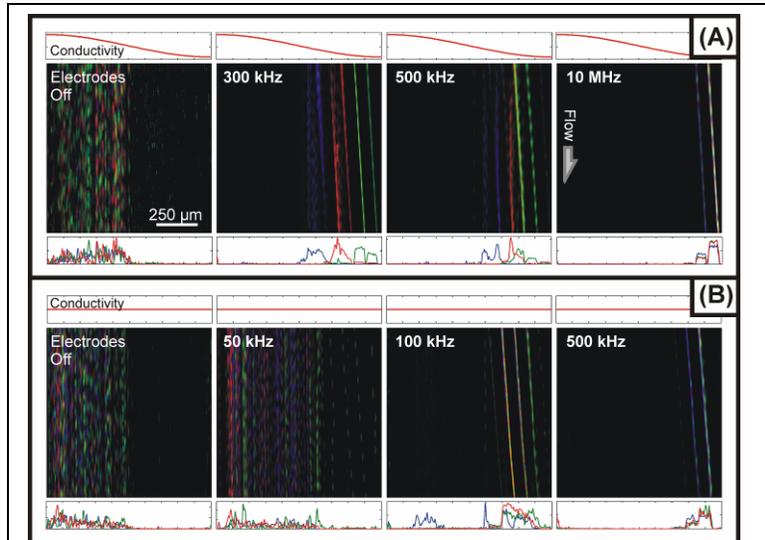


Figure 21: (A) Simultaneous separation of three types of polystyrene beads. At lower frequencies, the DEP force is determined by the surface conductance of the beads, as opposed to higher frequencies, where the electrical permittivity of bulk polystyrene becomes dominant. (B) In the absence of a conductivity gradient, no separation is observed.

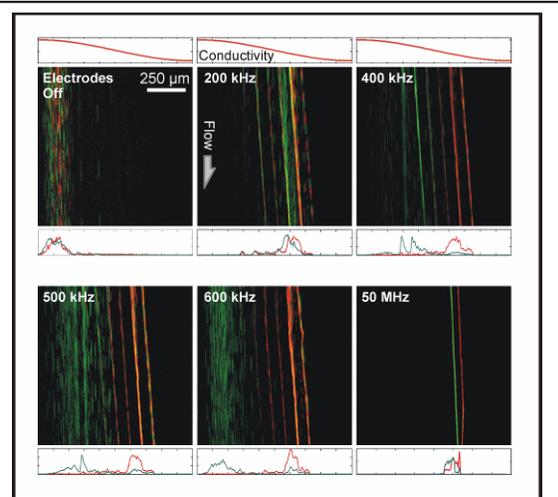
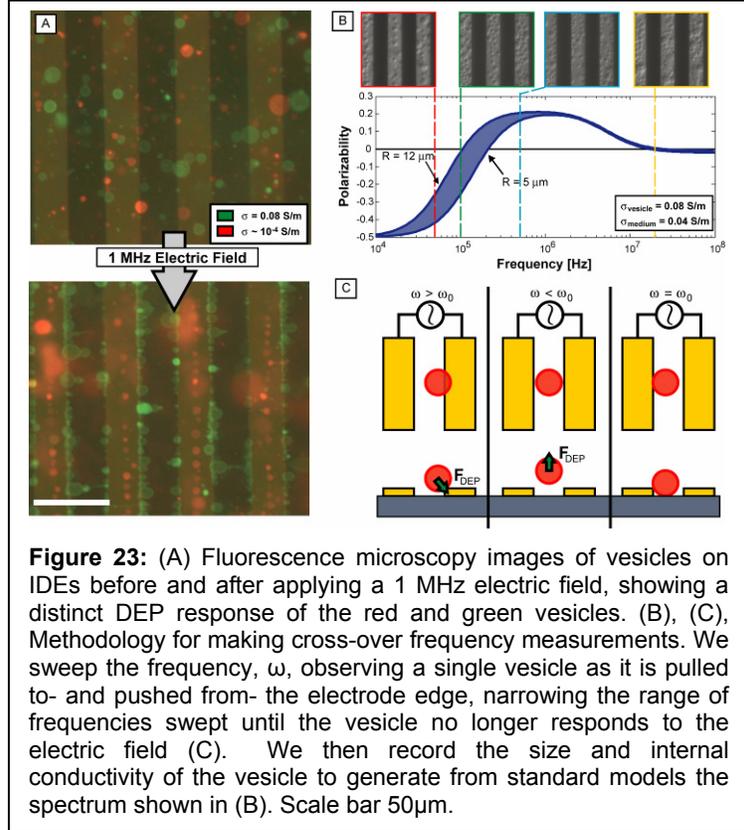


Figure 22: Live/dead separation of yeast using IDS. Blue depicts viable cells, while green depicts heat-treated cells. At sufficiently low (~200 kHz) or high (~50 MHz) frequencies, the cells are electrically very similar, while at intermediate frequencies, live cells exhibit higher conductivities, a consequence of their uncompromised membrane.

different membrane properties. Figure shows our technique for characterizing the electrical properties of the vesicles, applied to vesicles labeled with red and green fluorescent dyes, corresponding to different internal conductivities. Application of a 1 MHz waveform results in the more polarizable green vesicles undergoing positive DEP and the less polarizable red vesicles undergoing negative DEP. Similar characterization of electroformed vesicles with functionalized phospholipids containing PEG brushes attached to their hydrophilic head group suggest that we are also able to control the effective membrane thickness and thus the low frequency electrical properties of the vesicles. We believe these proof-of-principle studies are the first to demonstrate that the electrical properties of vesicles can be specifically engineered to allow their dielectrophoretic manipulation. These vesicles form the first steps toward the development of vesicle libraries as metrology tools for any DEP-based microsystem.



5. Cyborg moth flexible multi-electrode arrays

Sponsors
DARPA

Project Staff
Wei Mong Tsang

Overview

The object of this project is establishing an interface between the nervous system of the moth and appropriate computational and MEMS systems (i.e. flexible electrode for nervous stimulation) to control the flight of moths in real time. 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 giant hawkmoth *Manduca sexta*.

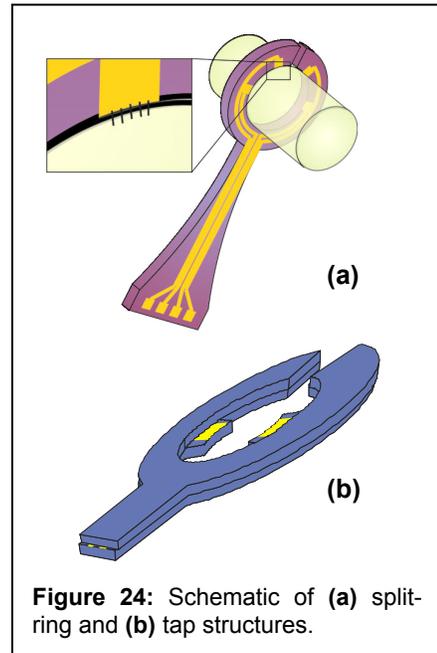
Historically, research using machine/neural interfaces has focused on the use of either rigid silicon-based probe electrodes or extremely flexible polymer electrode systems that penetrate neural tissues. Penetrating electrodes can potentially damage critical neurons. In live, freely behaving insects, rigidity is problematic because nearly all neuromuscular systems undergo large

shape changes during movement. Moreover, existing electrode structures are often sized for use in mammals, whose neural systems are significantly larger than those of insects. Finally, for implantable systems that are incorporated in developing animals, the large-scale morphogenetic rearrangements that take place suggest that rigid electrodes might not be appropriate for interfacing in such systems.

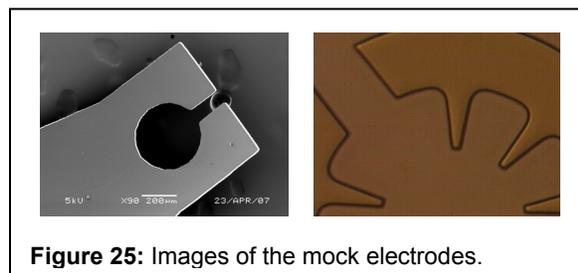
Accordingly, we will formulate a new electrode technology that uses compliant polymer-based MEMS that will surround nerves with multi-site electrodes. The new electrode will be stiff enough to be easily inserted into the animal but flexible enough not to cause injury during or after implantation. Additionally, it will have the capability of multi-channel stimulation. Finally, carbon nanofibers (CNFs) will be integrated into the electrode to enhance electrical contact with the nerve, and integrated organic electronics will provide on-site amplification and mux/demux capabilities.

Current Research

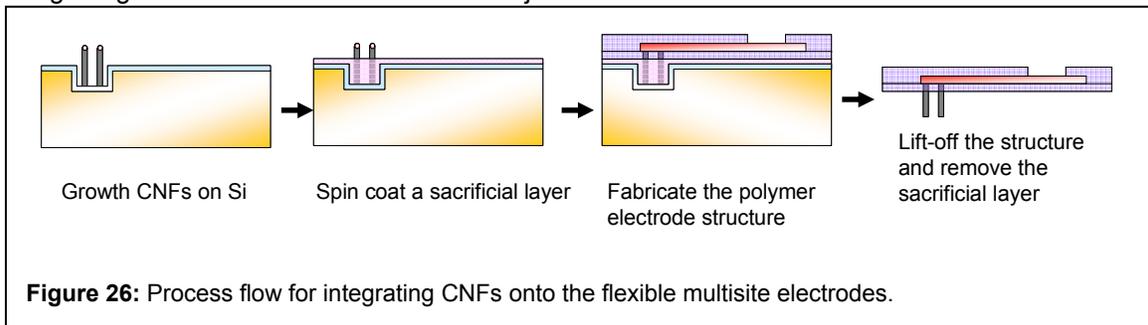
Our initial designs consist of a split-ring structure (FigureA) for the electrode. It allows for easy insertion around a nerve bundle while still making conformal contact. It is stiff in the radial direction, ensuring intimate and conformal contact with the nerve bundles, yet flexible in the axial direction to allow for easy insertion around the nerve bundle with minimal injury. Furthermore, with an aim of improving the electrical contact (increasing the contact area) between the nervous and the electrode, we also propose another structure where tabs containing the electrodes will protrude into the split ring (FigureB). Upon insertion around a connective, the tabs will fold out-of-plane and create electrical contact with the connective sheath thus effectively increase the contact area as compared with the split-ring structure.



We fabricated mock electrodes (a single-layered polymer structure without electrical element) of various sizes and structures for moth implantation experiments to optimize the physical shape of the electrode. These mock electrodes are fabricated using either SU-8 or polyimide because of their excellent biocompatibility and mature fabrication process. Representative images of the mock electrodes of split-ring and tap structures are shown in Figure.



Finally, we have also started working on integrating CNFs into the device. The major



challenge will be to grow CNFs at low temperatures in order to be compatible with polyimide. We are currently considering two approaches to this challenge. One is to convert our existing CNFs growth system to lower temperatures. The second is to develop a revised process flow that allows us to grow the high temperature CNFs on Si substrate and then perform the low temperature polymer fabrication thereafter. The schematic of the proposed process flow is shown in Figure.

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