A Microfluidic Microprocessor: Controlling Biomemetic Containers and Cells using Hybrid Integrated Circuit / Microfluidic Chips

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Citable link</td>
<td><a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:4142552">http://nrs.harvard.edu/urn-3:HUL.InstRepos:4142552</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Open Access Policy Articles, as set forth at <a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#OAP">http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#OAP</a></td>
</tr>
</tbody>
</table>
A Microfluidic Microprocessor: Controlling Biomimetic Containers and Cells using Hybrid Integrated Circuit / Microfluidic Chips

David Issadore,\textsuperscript{a,b} Thomas Franke,\textsuperscript{a,c} Keith A. Brown,\textsuperscript{a} and Robert M. Westervelt*\textsuperscript{a,d}

Classification: Physical Sciences, Engineering

\textsuperscript{a} School of Engineering and Applied Science, Harvard Univ., 29 Oxford St, Cambridge, MA.
\textsuperscript{b} David Issadore is now at The Center for Systems Biology, Massachusetts General Hospital / Harvard Medical School, 185 Cambridge St., Boston, MA.
\textsuperscript{c} Microfluidics Group, Univ. of Augsburg, 86159 Augsburg, Germany.
\textsuperscript{d} Dept of Physics, Harvard Univ., 29 Oxford St, Cambridge, MA.
Abstract

We present an integrated platform for performing biological and chemical experiments on a chip based on CMOS (complementary metal–oxide–semiconductor) technology. We have developed a hybrid integrated circuit (IC) / microfluidic chip that can simultaneously control thousands of living cells and pL volumes of fluid, enabling a wide variety of chemical and biological tasks. Taking inspiration from cellular biology, phospholipid bilayer vesicles are used as robust picoliter containers for reagents on the chip. The hybrid chip can be programmed to trap, move, porate, fuse, and deform individual living cells and vesicles using electric fields. The IC spatially patterns electric fields in a microfluidic chamber using 128 x 256 (32,768) 11 x 11 µm$^2$ metal pixels, each of which can be individually driven with a radio frequency (RF) voltage. The chip’s basic functions can be combined in series to perform complex biological and chemical tasks and performed in parallel on the chip’s many pixels for high-throughput operations. The hybrid chip operates in two distinct modes, defined by the frequency of the RF voltage applied to the pixels: Voltages at MHz frequencies are used to trap, move, and deform objects using dielectrophoresis and voltages at frequencies below 1 kHz are used for electroporation and electrofusion. This work represents an important step towards miniaturizing the complex chemical and biological experiments used for diagnostics and research into automated and inexpensive chips.
Introduction

The miniaturization of laboratory functions onto microfluidic (µfluidic) chips is leading a paradigm shift in biotechnology, analogous to the transformation of electronics by the integrated circuit (IC) 40 years ago.\textsuperscript{1-4} The microfabricated pipes, pumps, valves, and mixers of µfluidics enables small volumes of reagents, samples, and individual living cells to be controlled on low-cost, portable chips.\textsuperscript{1-7} However, a µfluidic chip that can be programmed to perform the wide range of chemical and biological tasks required for medical and scientific analysis, akin to a microprocessor in electronics, remains a challenge.\textsuperscript{4,8-16} In this paper ICs are directly combined with µfluidics to control living cells and picoliter (pL) sized vesicles, demonstrating a versatile platform for biological and chemical applications.

Much work has been done in the last decade to develop platforms for programmable µfluidics. Pneumatic control has been used to create reconfigurable µfluidic components such as valves, latches, pumps, and multiplexers.\textsuperscript{11} Recently, µfluidic devices have been developed that replace cumbersome pneumatic lines with electronically activated components that are made of shape memory alloys that can be activated electronically.\textsuperscript{12} Droplet based µfluidic devices have been developed that can programmably trap, move, mix, and separate drops using both electrowetting\textsuperscript{13,14} and dielectrophoresis (DEP)\textsuperscript{10,15} with electronics that are external to the fluidic system. And, hybrid IC/µfluidic chips have been developed that harness the mature technology of ICs to programmably trap and move small drops of water suspended in oil\textsuperscript{9} and living cells suspended in water.\textsuperscript{4,9,10}

In this paper, a library of basic functions necessary to perform biological and chemical experiments are demonstrated on a hybrid IC/µfluidic chip. The chip’s functions, listed in Table 1, can be combined in series to perform complex biological and chemical tasks and performed in parallel on the chip’s many pixels for high-throughput operations. Unilamellar lipid vesicles are used as a biologically inspired, robust container for pL volumes of fluid on the chip. These vesicles and living cells can be transported throughout the chip to have their contents mixed with those of other vesicles or delivered to specific cells, creating a programmable microfluidic platform. To accomplish this task, the hybrid chip uses DEP to simultaneously trap and move thousands of individual vesicles and living cells with its large array of microscopic electrodes. The hybrid chip can electroporate a membrane of a vesicle to either mix its contents with that of another vesicle or to deliver its contents to a specific cell. In addition, the hybrid chip can electrofuse any two vesicles together to mix their pL cargo in a single isolated container. The hybrid chip can also electroporate an individual living cell to selectively introduce substances from the solution such as molecular probes, DNA, or drugs. Both living cells and vesicles can be deformed on the hybrid
chip by changing the shape of the DEP trap that holds them. This ability, in combination with the functions listed above, allows the hybrid chip to perform experiments on living cells and vesicles under both controlled chemical and mechanical conditions. Two additional functions, that have been demonstrated in previous papers on similar hybrid chips, are the local deformation of cells and vesicles tagged with magnetic nanoparticles using magnetic fields and the rapid and localized control of temperature using GHz frequency electric fields.

**Results and Discussion**

Taking inspiration from cellular biology, phospholipid bilayer vesicles are used to package pL volumes of liquid on the chip. Lipid vesicles are commonly used in nature by cells to package substances for intercellular and intracellular transport, to store enzymes, and to create isolated chemical reaction chambers. Artificially produced unilamellar vesicles mimic these natural containers and provide robust pL containers that are impermeable and stable for a wide range of environmental conditions such as salinity and pH.

The dielectric properties of cells and vesicles enable the IC / µfluidic chip to carry out the multiple laboratory functions that are listed in Table 1. The hybrid chip operates in two distinct modes, defined by the frequency of the voltage applied to the chip’s pixels. Voltages at frequencies $f > 1$ MHz are used to trap and move objects with DEP. Voltages at frequencies $f < 1$ kHz are used for electroporation and electrofusion to release the contents of vesicles, fuse two vesicles together, or permeabilize the membranes of cells. In this section, a lumped-element model is used to describe the dielectric behaviour of cells and vesicles that enable these functions. Vesicles and cells are modelled as spheres of radius $a$, filled with a solution with a conductivity $\sigma_{\text{int}}$ surrounded by an impermeable thin membrane with a capacitance $C_{\text{mem}}$, suspended in a solution with a conductivity $\sigma_{\text{sol}}$.

Vesicles and cells are trapped and moved on the chip using DEP, the motion of dielectric objects in a non-uniform electric field $\vec{E}$. Any object with a complex permittivity that is different than the surrounding medium can be controlled with DEP. The DEP force on a spherical object is:

$$F_{\text{DEP}}(\omega) = 2\pi \varepsilon_m a^3 CM(\omega) \bar{V} E^2$$

where $\varepsilon_m$ is the real part of the permittivity of the medium, $E$ is the magnitude of the root mean square of the electric field $\bar{E}$, and $CM(\omega)$ is the Clausius-Mossotti factor, a relation between the frequency dependent complex permittivities of the particle $\varepsilon_p$ and the medium $\varepsilon_m$. 

\[\text{Equation 1}\]
When \( CM(\omega) < 0 \) the fluid is more polarizable than the particle, the particle is pushed away from the local maximum of the electric field and this is called negative DEP. When \( CM(\omega) > 0 \) positive DEP occurs and the particle is pulled toward the maximum of the electric field. The Clausius-Mossotti factor \( CM(\omega) \) varies from -0.5 to 1.

The Clausius-Mossotti factor \( CM(\omega) \) of vesicles and cells can be controlled by varying the internal conductivity \( \sigma_{\text{int}} \) relative to the conductivity of the surrounding medium \( \sigma_{\text{sol}} \). The Clausius-Mossotti factor \( CM(\omega) \) of a vesicle or cell with a radius \( a = 5 \ \mu m \), suspended in a deionized solution \( \sigma_{\text{sol}} = 10^{-3} \ \text{S/m} \), is plotted vs. interior conductivity \( \sigma_{\text{int}} \) in Fig. 1a. If the conductivity inside the vesicle \( \sigma_{\text{int}} > \sigma_{\text{sol}} \) then the CM factor is positive and the object can be trapped and moved with positive DEP. The Clausius-Mossotti factor plateaus at \( CM = 1 \) for \( \sigma_{\text{int}} > 0.1 \ \text{S/m} \). In this paper, the vesicles are filled with NaCl with \( \sigma_{\text{int}} = 0.1 \ \text{S/m} \) and suspended in an iso-osmotic solution with \( \sigma_{\text{sol}} = 10^{-3} \ \text{S/m} \) such that \( CM \approx 1 \) for DEP.

The Clausius-Mossotti factor \( CM(\omega) \) depends on the frequency \( f \) of the applied electric field. Figure 1b shows a plot of \( CM(\omega) \) vs. \( f \) for the vesicle described above. Between 50 MHz and 2 kHz \( CM(\omega) \) is positive and independent of frequency. Positive DEP is used in this frequency range to trap and move vesicles and cells. The high frequency cut-off \( f_d = 50 \ \text{MHz} \) of the DEP regime is set by the dielectric relaxation time \( \tau_d = \varepsilon_p / \sigma_{\text{int}} \) of the solution inside the vesicle. The low frequency cut-off \( f_{\text{mem}} = 2 \ \text{kHz} \) is set by the charging time of the membrane of the vesicle \( \tau_{\text{mem}} \).

Vesicles and cells experience a transmembrane voltage \( V_{\text{TM}} \) in the presence of an applied electric field \( \mathbf{E} \). The transmembrane voltage \( V_{\text{TM}} \) depends on the frequency \( f \) of the applied electric field. Here, we show that this frequency dependence allows vesicles and cells to be unharmed at the frequencies used for DEP and selectively electroporated and electrofused using \( f < 1 \ \text{kHz} \) pulses.

Electroporation is the formation of pores in a cell or vesicle’s membrane by an electric field. Electroporation occurs when a vesicle or cell has an induced transmembrane voltage \( V_{\text{TM}} > 1 \ \text{V} \).\(^{21,22}\) The voltage induced across a membrane \( V_{\text{TM}} \) of a spherical vesicle or cell can be calculated using a lumped-element model:\(^{19,21,22}\)

\[
V_{\text{TM}} = \frac{3Ea}{2\sqrt{1 + (\omega\tau_{\text{mem}})^2}}
\] (3)
The characteristic charging time of the membrane $\tau_{\text{mem}}$ is given by the expression,

$$
\tau_{\text{mem}} = a C_{\text{mem}} \left( \frac{1}{\sigma_{\text{int}}} + \frac{1}{\sigma_{\text{sol}}} \right)
$$

(4)

where $C_{\text{mem}} = 10^{-2} \text{ F/m}^2$ is the specific membrane capacitance.\(^{20}\)

Figure 1b shows the transmembrane voltage $V_{\text{TM}}$ and the Clausius-Mosotti factor $CM(\omega)$ vs. the frequency $f$ of the applied electric field. There is a regime $f < 1/\tau_{\text{mem}}$ where $V_{\text{TM}} \approx 1$ and $CM < 0$. It is in this regime that electric fields can be used to electroporate and electrofuse cells and vesicles. In the regime $f > 1/\tau_{\text{mem}}$ $V_{\text{TM}} \approx 10 \text{ mV}$, which is known to not damage living cells or vesicles,\(^{18,22}\) and $CM \approx 1$. It is this regime that DEP is used to trap, move, and deform objects.

The hybrid chip can mix the contents of two vesicles by bringing the two together with DEP and then electrofusing them. Electrofusion is a multi-step process.\(^{8,19,24}\) Two vesicles are brought into tight contact with DEP. Voltage pulses are then used to induce a transmembrane voltage across the contact area of the two vesicles. The transmembrane voltage causes electroporation on the contact-area, and if the pore density is large enough then the vesicles fuse.\(^{24}\) On the chip, voltages at MHz frequency are used to hold the vesicles in contact with DEP while time-multiplexed voltage pulses are used to trigger the fusion. In the duration of the millisecond voltage pulses used for electroporation and electrofusion, when the DEP trap is turned off, cells or vesicles do not diffuse a substantial distance from the trap.

The hybrid IC / µfluidic chip patterns electric fields on micrometer length scales to control cells and vesicles in the µfluidic chamber. The IC’s array of pixels create complex patterns in the electric field, analogous to a display created on a computer screen. Figure 2a shows a quasi-static finite element simulation (Ansoft: Maxwell 11) of the electric field magnitude $E$ a height 5 µm above the chip’s surface. The simulations show that the maximum electric field strength a cell or vesicle will experience on the chip is $E \approx 0.5 \text{ V/µm}$. The dielectrophoretic force $F_{\text{DEP}}$ on cells and vesicles is calculated by combining the finite element simulation from Fig. 2a with the dielectric model described above. Figure 2b shows the force that a vesicle experience vs. its distance $d$ from a DEP trap. The magnitude of the DEP trapping force is $F \approx 1 \text{ nN}$ and the force is localized within two pixel lengths. A line is fit to the force curve in Fig. 2b and the effective spring constant of the trap is found to be $k = 0.5 \text{ nN/µm}$.

The hybrid chip consists of a µfluidic chamber built on top of a custom IC that is fabricated in a commercial foundry. An optical micrograph of the IC is shown in Fig. 3a. The IC consists of 128 x 256 (32,768) 11 x 11 µm$^2$ pixels that can each be individually driven with a 5V peak-to-peak radio
frequency (RF) voltage with frequencies from DC to 11 MHz. The large array of pixels is controlled using static random access memory (SRAM) and logic that is built into the IC. Underneath each pixel is an SRAM element. The state of the SRAM element determines whether the pixel is driven by the external RF voltage source (the pixel turned off) or by the logical inverse of the RF voltage (the pixel turned on). The RF voltage between the pixels creates an electric field above the chip’s surface that is used to control vesicles and living cells. The entire array of pixels can be updated hundreds of times in a second. The IC is designed using Cadence design software and is fabricated with a commercial 0.35 μm process through MOSIS (Metal Oxide Semiconductor Implementation Service, process: TSMC35_P2).

Figure 3 shows the IC / μfluidic chip and the apparatus that is used to connect the hybrid chip to the outside world. The IC is mounted on an 84 pin chip carrier (Global Chip Materials: LCC84). A fluid cell is built directly on top of the IC using a silicone isolator (Invitrogen: p-24744) with a 1.2 mm hole that is cut with a hole punch. A 3 x 3 mm$^2$ glass cover slip is placed on top of the fluid cell to seal it. A photograph of the IC / microfluidic chip mounted on a chip carrier is shown in Fig. 3c. The chip carrier sits on a custom printed circuit board (PCB) that connects the IC to the computer, provides power, and connects the IC to an external RF voltage source, as is shown in Fig. 3d. The PCB sits underneath a fluorescence microscope that is used to image the fluidic environment. The fluorescence microscope (Olympus: BX) is equipped with a CCD camera (Hamamatsu: Orca_ER).

Our IC / μfluidic chip can be programmed to position, electroporate, electrofuse, and deform living cells and vesicles using electric fields. This library of basic functions, listed in Table 1, makes it possible to perform complex biological and chemical tasks on a chip. In this section, we demonstrate these functions.

The IC / μfluidic chip can control the position of thousands of individual living cells and vesicles with DEP using its 128 x 256 array of pixels. In this way, the hybrid chip functions as a programmable microfluidic chip that can trap and move cells and vesicles along individual paths orchestrated by a user at a computer terminal. This programmable chip can be used to mix pL volumes of fluid or to deliver pL volumes of fluid to an individual cell to conduct chemical and biological experiments.

Figure 4a shows the hybrid chip can independently trap and move individual vesicles along controlled paths using DEP. In Fig. 4a (i) three vesicles are held in individual traps that are arranged in a triangle. In Fig. 4a (ii) the upper right vesicle is moved upwards by deactivating the pixels that are currently trapping it and activating a set of pixels nearby. The vesicle moves at an average speed of 70 μm/sec or 6 pixels/sec. In Fig. 4a (iii) the leftmost vesicle is moved to the right, through the other two vesicles. The pixels trapping the leftmost vesicle are deactivated, and the pixels to the right of the vesicle are activated to move...
it to its new position. The membranes of the vesicles are stained with rhodamine to be easily viewed with a fluorescence microscope.

Figure 4b shows the hybrid chip can trap and position hundreds of vesicles simultaneously, using the chip’s large array of pixels. The chip simultaneously uses its entire array of 32,768 pixels, allowing thousands of individual objects to be independently controlled. The ‘H’ pattern shown in Fig. 4b was made by attracting vesicles from a suspension onto activated pixels on the chip.

Figure 4c demonstrates the hybrid chip trap and move both vesicles and living cells suspended in the same solution. The simultaneous control of vesicles and living cells on the chip allows pL volumes of fluid to be delivered to specific living cells. Figure 4c (i) shows a vesicle and a budding yeast cell trapped on the chip. The fluorescence image of the rhodamine filled vesicle is colored red and is superimposed onto the brightfield image of the yeast cells on the chip’s surface. In Fig. 4c (ii) the vesicle is moved upwards while the cell is trapped in place. In Fig. 4c (iii) the yeast cell is moved to the left while the vesicle is held in place. In Fig. 4c (iv) the yeast cell is moved downwards as the vesicle is simultaneously moved to the left.

In this demonstration, baker’s yeast cells are cultured overnight in YPD broth (BD Inc.) at 37°C, and then resuspended with the vesicles in an iso-osmotic glucose solution.

The IC / µfluidic chip can selectively electroporate any individual vesicle on its 128 x 256 array of pixels, allowing the contents of these micrometer-sized containers to be released into the surrounding solution on demand. By combining this function with the hybrid chip’s ability to position cells and vesicles, the chip can deliver pL volumes of fluid to other vesicles or to cells enabling a host of biological and chemical experiments. The sequence of images in Fig. 5a shows the hybrid chip hold an individual vesicle in place using DEP and controllably release its contents into the solution via electroporation. The vesicle is filled with 50 mM NaCl and is suspended in a concentrated, self-quenched fluorescein solution. When the vesicle is electroporated, fluorescein diffuses into the vesicles and causes the vesicle to fluoresce. The vesicle is electroporated using a pulse train that consists of a 1 ms voltage pulse that repeats every 5 ms for 0.5 seconds, time multiplexed with DEP. The sequence of images in Fig. 5a (i-iii) show the vesicle begin to fluoresce as it is electroporated. When the pulse train is turned off, the vesicle’s membrane heals and the contents of the vesicle cease mixing with the solution. Figure 5a (iv) shows that the vesicle continues to fluoresce after it is held in a DEP trap for 2 minutes. When the pulse train is again turned on, the mixing of the contents of the vesicle with the solution resumes. The sequence of images in Fig. 5a (iv-vi) show the vesicle ceasing to fluoresce as the concentration of fluorescein in the vesicle begins to match that of the self-quenched solution.
The IC / μfluidic chip can selectively electrofuse any two individual vesicles together, allowing the contents of these micrometer sized containers to be mixed. By combining this function with the hybrid chip’s ability to control the position of vesicles, the chip can be programmed to mix isolated pL volumes of fluid to perform combinatorial chemical and biological experiments or for preparing and diluting reagents on the chip. Figure 5b shows that the hybrid chip can controllably fusing two vesicles together using electrofusion. In Fig. 5b (i) two vesicles are brought into contact using DEP. In Fig. 5b (ii) a pulse train that contains a 1 ms voltage pulse that repeats every 5 ms for 0.5 sec electrofuses the vesicles, causing the two to fuse into one. In Fig. 5b (iii) the DEP is turned off and the fused vesicle relaxes into a sphere. The voltage pulses used to electrofuse the vesicle also create pores between the vesicle and the solution; however, the voltage pulses do not lead to an appreciable mixing of the contents of the vesicle with the solution. In the experiment shown in Fig. 5a, it takes ~4 sec for appreciable mixing of the contents of a similar vesicle using the same pulse sequence.

The IC / microfluidic chip can selectively electroporate an individual living cell on the chip, to introduce substances such as molecular probes, DNA, or drugs from the solution. Figure 5c demonstrates that the hybrid chip can controllably electroporate a living cell. In Fig. 5c a yeast cell is shown suspended in a glucose solution with 0.4% trypan blue viability stain. When trypan blue enters the cell, via electroporation, it stains the cell blue. The hybrid chip electroporates the cell using a pulse train consisting of a 1 ms voltage pulse that repeats every 5 ms, time multiplexed with DEP. After 40 sec of the pulse train the cell is observed to turn dark blue, as is shown in Fig. 5c (ii).

The IC / microfluidic chip can selectively apply mechanical stress to individual cells and vesicles on the micrometer scale with its 128 x 256 array of pixels using DEP. This ability, in combination with the functions listed above, allows the hybrid chip to perform experiments on living cells under controlled chemical and mechanical conditions. Figure 6 shows that the hybrid chip can controllably deform a vesicle into a variety of different shapes by changing the geometry of the DEP trap that is holding it. In Fig. 6 (a) the vesicle is held in a simple trap of 4 pixels and the vesicle’s cross-section is circular. In Fig. 6 (b) the trap is spread into two displaced bars, and the vesicle is pulled into an oblong shape. More complicated pixel patterns lead to shapes such as diamonds Fig. 6 (c), hexagons Fig. 6 (d), and squares Fig. 6 (e). The ability to deform vesicles using DEP traps is an important proof-of-concept for performing rheological experiments on vesicles and cells.24,25

This paper describes the development of a versatile platform for performing biology and chemistry experiments on a chip, using the sophisticated IC technology of the commercial electronics industry. Integrated circuits, ubiquitous in modern electronics, enable vastly complex devices to be manufactured inexpensively. For instance, cell phones, which contain more than 100 million intricately connected...
transistors, the product of decades of research and development, are manufactured at a cost low enough to be affordable in rural areas that do not yet have the infrastructure for running water. The potential for applying the capabilities of ICs to chemical and biological problems is great. Handheld devices, with the size and cost of cell phones, will perform complex tasks that are currently carried out in medical and biological laboratories. The hybrid chip, demonstrated in this paper, can be programmed to trap, move, electroporate, electrofuse, and deform individual living cells and vesicles using electric fields. These functions provide the basic building blocks for using ICs to perform biological and chemical experiments.

While the hybrid chip demonstrated in this paper can fit into a package the size of a cellular phone, in its current configuration the chip requires a laboratory microscope, light source, and computer to function. Integration of sensors onto these hybrid chips, such as NMR sensors, electric field sensors for measuring eletrogenic cells, and integrated optics for imaging and fluorescence measurements using semiconductor technology will further expand the reach of this powerful approach. In addition, the hybrid chip can be connected to complex fluidic networks, that transport chemical and biological samples onto and off of the chip. In this way, the hybrid chip can function as a module in larger microfluidic networks, performing functions that are not possible using only passive microfluidic devices.

Hybrid IC / microfluidic chips, fully realized, will have a far reaching impact on how science and medicine are performed. These chips can be programmed to carry out complex, multi-step experiments and be reconfigured in real-time to perform a wide variety of experiments. Tasks such as the detection and counting of cells, sorting of cells, genetic analysis, protein detection, and combinatorial chemistry could be performed on these low-cost, automated devices. These lab-on-a-chip systems can be used for applications as wide ranging as point-of-care disease detection or the detection of toxic substances or parasites in drinking water. By making use of sophisticated IC technology, these hybrid chips have the potential to bring complex biological and chemical tests from laboratories out into the clinic and the field.

**Materials and Methods**

The vesicles are prepared using liposome electroformation. This method yields robust vesicles with sufficient dielectric contrast to be controlled by the hybrid chip. Briefly, a small amount of lipid in chlorophorm (ca. 20 µL of a 5mg/ml solution) is deposited between two indium-tin-oxide (ITO) coated glass slides. After evaporation of the organic solvent for at least 6 hours the slides are assembled in parallel with a 2 mm teflon spacer to form the preparation cell. The preparation cell is then incubated for one hour in a saturated water vapor at room temperature for prehydration. Subsequently, the electroformation cell is filled with 200 mOsm aqueous solution (50 mM Sodium Chloride and sucrose) and an electric field with a frequency $f = 500$ Hz and an amplitude $E = 1$ V/mm is applied to the ITO-slides. After approximately 8 hours lipid vesicles of diameters
larger than 10 µm are harvested. The external solvent is removed by repeatedly washing with an iso-osmotic aqueous glucose solution and gentle centrifugation at low rotation frequencies.

Acknowledgements

We thank Donhee Ham for his generous help on the design of the integrated circuit and Jim MacArthur for his work on the supporting electronics. This work was supported in part by the Harvard-MIT Center for Cancer Nanotechnology Excellence, in part by the Department of Defense through the National Defense Science and Engineering Graduate Fellowship Program, in part by Bayerische Forschungsstiftung, and in part by the German Excellence Initiative.

References


Figure Legends

Table 1. List of Functions Performed on the Hybrid IC / Microfluidic Chip

Fig. 1 (a) The Clausius-Mossotti factor $CM$ versus the interior conductivity $\sigma_{int}$ calculated for a vesicle with radius $a = 5 \ \mu m$ suspended in a solution with conductivity $\sigma_{sol} = 10^{-3} \ \text{S/m}$. (b) A plot of the $CM$ (blue) and the transmembrane voltage $V_{TM}$ (red) vs. frequency $f$ for a vesicle with interior conductivity $\sigma_{int} = 0.1 \ \text{S/m}$.

Fig. 2 (a) Finite element simulation of the electric field strength $E$ a distance 5 $\mu m$ above the chip’s surface. The green sphere represents a vesicle. (b) A plot of the force that a vesicle experiences vs. distance $d$ from the DEP trap. The blue pixels are held at 5 V and the white pixels are grounded.

Fig. 3. (a) Optical micrograph of the integrated circuit (IC). (b) A magnified view of the IC, showing its pixels. (c) Photograph of the 84 pin chip carrier holding the IC, on top of the IC is the fluid cell. (d) A photograph of the experimental setup of the hybrid IC / microfluidic system.

Fig. 4 (a) Time sequence of vesicles positioned with dielectrophoresis (DEP). The green line shows the direction that the vesicles are moving. The blue squares show pixels that are turned on. Each frame is separated by ~1 sec. (b) Hundreds of vesicles simultaneously positioned to draw an ‘H’ using DEP. (c) Time sequence of vesicles and cells simultaneously controlled on the chip. The fluorescence image of the vesicles (red) is superimposed onto the brightfield image of the cells. Each frame is separated by ~1 sec.

Fig. 5 (a) Time sequence of the electroporation of a vesicle held in place with dielectrophoresis (DEP). The electroporation causes the vesicle to mix its contents with that of the self-quenched fluorescein solution, causing the vesicle to fluoresce. In the first three frames, each separated by 4 seconds, the vesicle is electroporated. The vesicle is then held in a DEP trap for 1 minute 52 seconds. In the next three frames, each separated by 4 seconds, the vesicle is electroporated until the contents of the vesicle fully mixes with the solution and the fluorescein is self-quenched. (b) Two vesicles are brought into contact with DEP and electrofused using voltage pulses. Each frame is separated by ~1 sec (c) A cell is held in place with DEP and electroplated in a solution containing trypan blue stain. The left and right frame show the cell before electroporation and after 40 sec. of electroporation, respectively.

Fig. 6 A single vesicle is deformed into a variety of shapes by changing the shape of its DEP trap. The schematics on the left show which pixels are turned on. Blue and white pixels indicate pixels that are turned on
or off, respectively. The center graphics show plots of the simulated electric field strength $E$ a distance $5 \, \mu m$ above the chip’s surface. On the right are micrographs of the deformed vesicle.