Feature Article

Microfluidic Devices for the Rapid Analysis of Individual Mammalian Cells

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We are developing techniques to culture and analyze single non-adherent cells on microfluidic devices. The microfluidic modules will be capable of culturing up to 20,000 cells over a period of several days. The cells are arranged in such a way that one of the daughter cells after each division will be released for downstream analysis. The released daughter cells are transported through a channel manifold where they can be subjected to external stimuli in order to examine how they respond. The cells can then be incubated and transported to an area of the chip where they will be lysed. The lysate is then separated and the components of interest detected and quantified.

Introduction

Cells are the fundamental building blocks of life. Almost all diseases result from some type of misregulation at the cellular level. In order to better understand how diseases develop and how to design treatments a complete understanding of cell physiology is necessary. While some of this knowledge can be gleaned from the use of pooled cell lysates, there are many cases in which the interesting results from rare outlier cells would be averaged out in such pooled cell lysates[1]. These cells, however, are the cells that are most interesting as they may indicate an incipient disease. Cancers are a good example of this, as cancers will initially develop from a single cell once it has acquired a sufficient number of mutations to cause it to undergo unregulated cell division. If these cells can be identified early enough for example at a routine annual physical exam, appropriate treatment can be provided to generate the best patient prognosis.

Microfluidic Devices for Single Cell Analysis

While the high throughput analysis of single cells could significantly improve medical diagnosis, the ability to handle single cells in a rapid manner and to look at 10's to 100's of potential biomarkers of potential diseases from each cell is a supreme challenge. Conventional techniques such as flow cytometry are capable of looking at thousands of cells/second; however, the number of markers that can be detected simultaneously is generally < 10. This is primarily due to the spectral bandwidth of the fluorophores used for the detection of the analytes of interest. Fluorescence microscopy has similar spectral bandpass limitations and lower throughput. A new technology is needed that can combine the higher throughput of flow cytometers with the ability to examine a large number of interesting cellular markers. The most promising technology to accomplish this is microfluidics (aka lab-on-a-Chip or micrototal analysis systems (μTAS)). Microfluidic devices consist of a manifold of small channels through which cells and reagents can be moved by applying either pressures or voltages to the termini of the channels. The channels are generally 1 to 100 microns wide and deep. For moving and manipulating non-adherent mammalian cells the channels that we make in our lab are generally 15 to 20 μm deep. The ability to create complex channel manifolds on a single substrate with no dead volume between connecting channels allows one to integrate a variety of chemical handling operations without significant dilution or band spreading. It also allows individual cells (~0.5 pL) to be lysed and injected into a separation channel without significant dilution of their contents. In addition, the large surface to volume ratio allows the application of high electric fields so that

rapid, high efficiency separations of complex samples can be performed. The ability to perform such separations and to integrate these directly with cell handling and lysis are what makes microfluidic devices especially useful for single cell analysis applications[2-4].

We have been working over the past several years to develop methods to transport cells, load cells with reporter molecules, incubate the cells, rapidly lyse cells and then separate out the labeled lysate components on a microfluidic device. This work has been a collaboration among Dr. J. Michael Ramsey (University of north Carolina at Chapel Hill, NC), Dr. Nancy L. Allbritton (University of north Carolina at Chapel Hill, NC), and me and has been supported by the National Institutes of Health.

Our initial cell work[5] was the first to show the potential of microfluidic devices for the rapid analysis of single cell lysates. We were able to hydrodynamically transport cells through a cross intersection and lyse the cells in under 33 ms (one frame of a video rate camera). This lysate was then injected into a separation channel using the same electric field used to lyse the cells. The cells were labeled with two fluorogenic dyes – Oregon green carboxylic acid diacetate and Carboxyfluorescein diacetate. Both of these dyes are neutral and freely partition through the cell membrane. Once inside the cell, esterases cleave the actetoxy groups making the dyes both charged and fluorescent[6]. The Oregon green went through a complex metabolism resulting in 5 reproducible peaks. We were able to separate these peaks from each other and from the hydrolyzed carboxyfluorescein in < 2.2 seconds only 3 mm from the injection intersection.

While successful, it was difficult to get reproducible injections on the glass chips. Our group at Kansas State, therefore, decided to move to a chip design that would allow us greater control over setting the cell transport speed through the chip and cell positioning at the intersection. In order to implement this design we had to
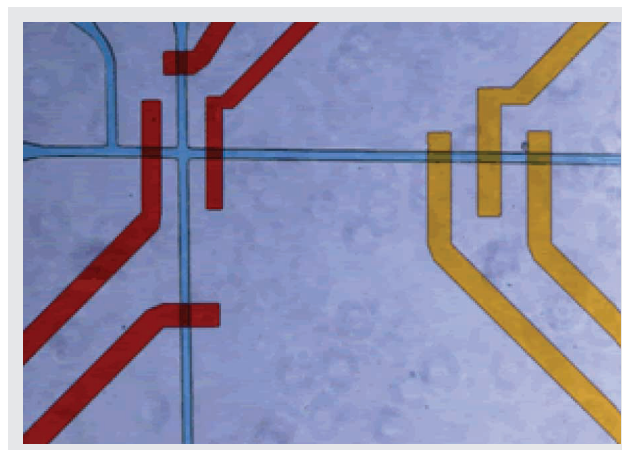

Figure 1   Optical micrograph of cell handling microfluidic device.

move to PDMS and use the soft layer multi-lithography techniques originally developed by Steve Quake's lab at Stanford[7, 8]. Figure 1 shows one iteration of this design where we have implemented both a peristaltic pump and series of valves around an intersection to control fluid flow and cell transport. This device has two channel layers separated by a thin PDMS membrane. One layer contains the fluid filled channels used to transport cells and perform separations. The second layer contains a series of control channels that contain only air. When air at approx. one atm. is applied to the control channels the membrane between the control and fluid channels expands into the fluid channel and closes it. The 3 valves in yellow are closed sequentially to produce a peristaltic pump. The 4 red valves are used to trap a cell in the fluid channel intersection so that it can be lysed through the application of an electric potential along the vertical blue fluidic channel.

Figure 2 (first frame) shows a slightly modified incarnation of the device in which a T-lymphocyte cell has been trapped. The cells are trapped by actuating the valves situated above the horizontal fluid channel. Cell detection is performed automatically by detecting the cell fluorescence. A laser is focused just to the left of the
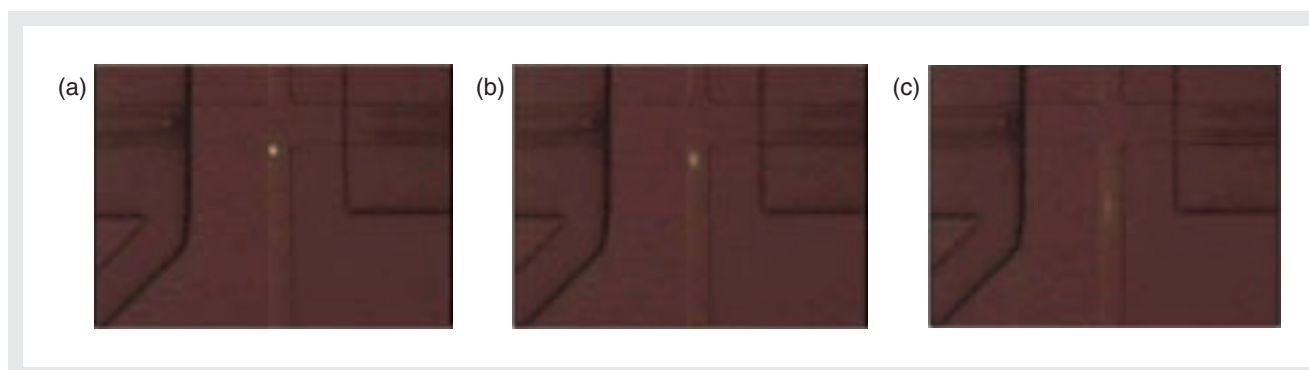

Figure 2   Cell trapping and lysis followed by the immediate injection of the lysate into the separation channel.
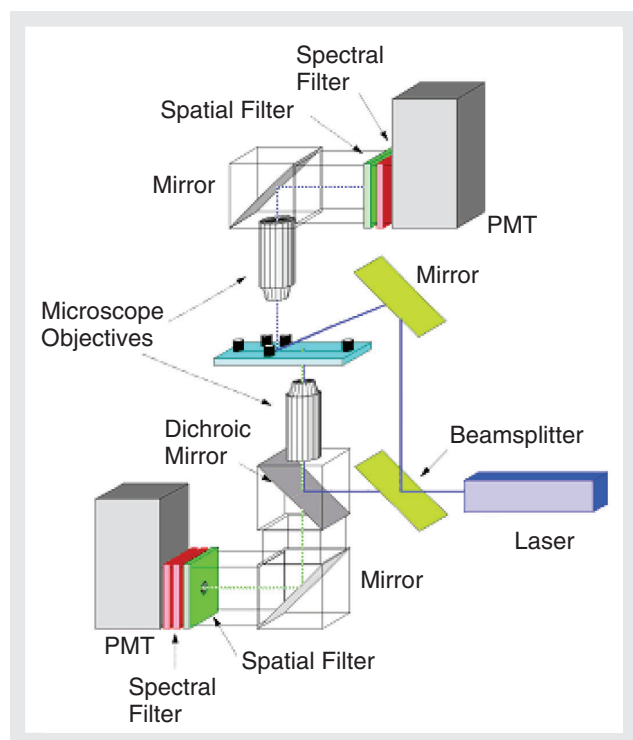
Figure 3   Schematic of dual detection system.
One laser beam and detector are used to detect the intact cell just prior to lysis while the second beam and detector are used to detect the lysate.

Figure 4   Calcein and Oregon Green released from 10 Jurkat cells. Five cells were loaded with Calcein and 5 cells were loaded with Oregon Green.

vertical fluidic channel. As the cell passes through the laser it fluoresces and is detected by a PMT attached to a microscope that is also focused on the channel intersection (Figure 3). This causes the valve to actuate. It also initiates the cell lysis and lysate separation sequence as shown in Figure 2.

Once the cell has been lysed, the labeled components of the lysate are electrokinetically injected into the separation channel. About 5 mm downstream, a second laser is focused into the channel. As the analytes pass through this beam they are excited and emit light that is collected by a microscope objective and detected by a PMT. Figure 4 shows some separations of Calcein AM and Oregon Green 488 dyes in < 2 sec.

Cell Culturing on Microfluidic Devices

In our original paper[5] we also noticed that the separation patterns of the Oregon green hydrolysates that we saw from some of the cells were substantially different from other cells. One potential reason for this could have been that the cell line that we were using (Jurkat cells) is immortalized so the cells being examined were in different stages of the cell cycle. As we are interested in eventually examining cells mostly in the G0/G1 phase of
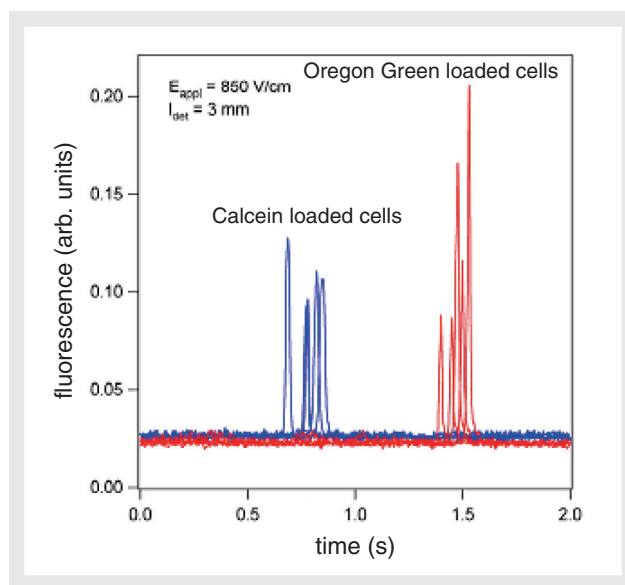
the cell cycle as most cells in the human body are after reaching adulthood, we need a source of synchronized cells in order to appropriately validate our device. In addition, as one also begins to think of using such devices to potentially screen for potential pharmaceuticals against a variety of conditions one needs a source of well-characterized cells all in the same phase of the cell cycle. Therefore, we have begun to incorporate a cell culturing system on our upstream of the lysis intersection. The cell culturing functional element can be seen in Figure 5. It consists of 8 parallel channels. In each channel there are 2500 cell wells that are 15 μm in diameter and that hold on average one cell. The wells are only 15 μm deep. The idea of using this type of design is that as a cell divides one of the daughter cells will be pushed out of the well. It will then be entrained in the fluid flow above the well and transported rapidly to the lysis intersection. Assuming a 24 hour doubling time a filled array and non-synchronized cell division, the cell array would release a new cell approximately every 4.3 seconds for analysis. We have thus far been able to culture cells on these devices for a period of 48 hours with a cell viability of ~ 90% and for 10 days with a viability of 40%.
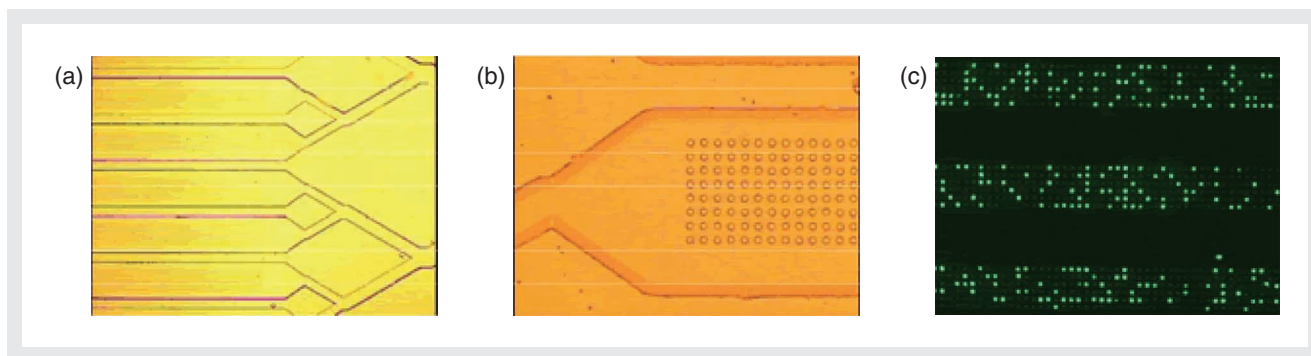
Figure 5   Single cell array in a microfluidic device.
(a) image of 5 of 8 cell well containing channels.
(b) expanded view of one channel.
(c) fluorescent image of wells filled with T lymphocytes labeled with Calcein (Jurkat cell line).

Conclusion

We have been developing several cell handling components on microfluidic devices that can be used to analyze the contents of individual non-adherent mammalian cells in a rapid manner. The small channels in these devices allow for the precise transport of cells throughout the chip and the injection of cell lysates without significant dilution. With the additional integration of cell culturing with the cell lysis, we should be able to better control the basic physiological state of the cell and, therefore, improve the robustness and information content of our results. In the near future we plan on adding the capability of loading the cells with other fluorescent reporters of enzyme activity and agonists and antagonists to enzymes of interest such as kinases.

References

[1] Martin, R. S.; Root, P. D.; Spence, D. M. *Analyst (Cambridge, United Kingdom)* **2006**, *131*, 1197-1206.

[2] Price, A. K.; Culbertson, C. T. *Analytical Chemistry (Washington, DC, United States)* **2007**, *79*, 2614-2621.

[3] Roman, G. T.; Chen, Y.; Viberg, P.; Culbertson, A. H.; Culbertson, C. T. *Analytical and Bioanalytical Chemistry* **2007**, *387*, 9-12.

[4] Sims, C. E.; Allbritton, N. L. *Lab on a Chip* **2007**, *7*, 423-440.

[5] McClain, M. A.; Culbertson, C. T.; Jacobson, S. C.; Allbritton, N. L.; Sims, C. E.; Ramsey, J. M. *Anal. Chem.* **2003**, *75*, 5646-5655.

[6] Haugland, R. P. *The Handbook: A guide to Fluorescent Probes and Labeling Technologies*, 10th ed.; Invitrogen: USA, 2005.

[7] Fu, A. Y.; Chou, H.-P.; Spence, C.; Arnold, F. H.; Quake, S. R. *Anal. Chem.* **2002**, *74*, 2451-2457.

[8] Unger, M. A.; Chou, H.-P.; Thorsen, T.; Scherer, A.; Quake, S. R. *Science* **2000**, *288*, 113-116.

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