

12-1-2008

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Wang, Hsiang-Yu; Bao, Ning; and Lu, Chang, "A microfluidic cell array with individually addressable culture chambers" (2008). *Birck and NCN Publications*. Paper 333.

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A microfluidic cell array with individually addressable culture chambers

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ARTICLE INFO

Article history:

Received 29 February 2008

Received in revised form 7 May 2008

Accepted 4 June 2008

Available online 12 June 2008

Keywords:

Microfluidics

Cell array

Cell culture

ABSTRACT

Microfluidic arrays of living cells have raised a lot of interests recently due to their potential for high throughput screening of cell-based assays. This report presents a microfluidic cell array with individually addressable chambers controlled by pneumatic valves for cell culture and treatment. There are two modes for the cell array to be operated. In the first mode, different groups of cells are directed into designated chambers for culturing and observation. We demonstrate the delivery and culture of enhanced green fluorescent protein (EGFP) expressing and nonfluorescent Chinese hamster ovary (CHO) cells into specific chambers in the array. In the second mode, the chambers are first seeded with the same cell type and different reagents are delivered to specific chambers for cell treatment. We treat cells in designated chambers with Calcein AM and CellTrace calcein red-orange AM to demonstrate the principle. We envision that this microfluidic cell array technology will pave the way to automated high-throughput screening of biomolecules and drugs based on observing cellular phenotypes and responses.

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1. Introduction

Microfluidics provides a popular tool for studying the response of cells to outside stimuli owing to the reduced sample amount and the high degree of integration. The reduced sample/reagent amount not only decreases the testing cost but also is important for investigating samples from scarce sources such as patient materials. The intense labor required for running multiple trials is another bottleneck for building affordable and high-throughput technique involving testing on cells. Microfluidics provides ideal platform for automated and high-speed operation and analysis on cells of tiny amount (El-Ali et al., 2006). Additionally, microfluidic devices can be inexpensively made since the emergence of soft lithography (Duffy et al., 1998).

Living cell arrays have shown great promise in observing cellular response to external stimuli, functional screening of genes (Bailey et al., 2006; Ziauddin and Sabatini, 2001) and drug discovery (Delehanty et al., 2004). Microfluidics has been used to construct living cell arrays with quantitative and precise treatment of cells (Figallo et al., 2007; Hung et al., 2005a,b; King et al., 2007; Lee et al.,

2006; Liu et al., 2008; Thompson et al., 2004; Yu et al., 2007). These cell culture arrays have been applied to study cell response to concentration gradient (Hung et al., 2005a,b; Lee et al., 2006; Liu et al., 2008), cell culture conditions (Figallo et al., 2007) and dynamic gene expression profile in living cells (King et al., 2007; Thompson et al., 2004). In spite of the rapid progress, very few designs have explored seeding with multiple cell types (King et al., 2007). Furthermore, most of these reports applied the same reagent to entire column or row without the capacity of addressing individual culturing chambers with unique reagents. These problems limit the kinds of tests that can be carried out based on microfluidic cell arrays.

In this study, we report a microfluidic cell array with individually addressable chambers for cell maneuvering and reagent delivery. The device was fabricated using multilayer soft lithography (Thorsen et al., 2002; Unger et al., 2000). Our cell array was able to manipulate cells in suspension in order to seed them in selected culture chambers. We demonstrated two modes of operation for our microfluidic array. First, we delivered two different cell types (CHO cells and EGFP-expressing CHO cells) into designated chambers for culture. Second, all the chambers in the array were seeded with CHO cells and we delivered different fluorogenic dyes (Calcein AM and CellTrace calcein red-orange AM) to cells in designated chambers. We examined the performance of a 6 × 6 microfluidic cell array by observing targeted delivery of different cell types and the specific labeling of cells in different chambers.

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2. Materials and methods

2.1. Device fabrication

The microfluidic cell array with individually addressable chambers was fabricated using multilayer soft lithography technique (Unger et al., 2000). The microscale patterns were first created using computer-aided design software (FreeHand MX, Macromedia, San Francisco, CA) and then printed out on high-resolution (5080 dpi) transparencies. The master for channels in the control layer was fabricated using a negative photoresist SU8-2025 (MicroChem Corp., Newton, MA) while AZ 9260 (Clariant Co., Charlotte, NC) was used to construct the master for the fluidic layer. The fluidic layer master (AZ 9260 photoresist on silicon wafer) was heated at 125 °C for 10 min after development to produce round cross-section for the channels. The channel depth (translated from the photoresist thickness) was 25 μm for the control channels and 18 μm for the fluidic channels. Polydimethylsiloxane (PDMS, GE RTV-615 with a ratio of A:B = 10:1.2, GE Silicones, Wilton, CO) was poured onto the control layer master to cast a 5 mm thick control layer. The fluidic layer was fabricated by spinning the same polymer mixture onto the fluidic layer master at 1500 rpm for 35 s (the acceleration was 330 rpm/s). Both layers were baked at 80 °C for 30 min and the control layer was peeled off from the master. After oxidation of both PDMS surfaces using a high-voltage discharger (Tesla Coil, Kimble/Kontes, Vineland, NJ) in air, the control layer was then brought into contact with the fluidic layer and the two-layered chip was baked at 80 °C for another 30 min. The two-layered chip was peeled off from the wafer and the holes for connection were punched using a blunt-ended needle before the fluidic layer surface was sealed to a pre-cleaned glass slide by oxidizing both surfaces using the high-voltage discharger. The composite was then baked at 80 °C for 10 min to ensure high-quality bonding among different interfaces. The connection holes had a slightly smaller diameter than that of tubing to provide airtight connection and to ensure aseptic environment after sterilization. Glass slides were pre-cleaned in a basic solution (H₂O:NH₄OH (27%):H₂O₂ (30%) = 5:1:1, volumetric ratio) at 75 °C for 1 h and then rinsed with DI water and blown dry.

2.2. Pre-treatment of the microfluidic cell array before cell seeding

The channels in the fluidic layer were sterilized by flushing 70% alcohol and then rinsing with Millipore ultra pure water and phosphate-buffered saline (PBS). The solutions were filtered through 0.2-μm filter to prevent clogging. The channels were then filled with 250 μg/ml fibronectin (Sigma, St. Louis) in PBS and incubated at 37 °C for 1 h. The devices can be stored at 4 °C for days if not used immediately. The fluidic layer channels were rinsed and degassed by flushing PBS through before experiments.

2.3. Cell culture

Chinese Hamster Ovary cell (CHO-K1) was chosen as the model cell line in this research. Cells were incubated at 37 °C, under 5% CO₂ in the Dulbecco's-modified Eagle's medium (DMEM, Mediatech Inc., VA) supplemented with 10% (v/v) fetal bovine serum (FBS, Sigma, MO), penicillin (100 U/ml, Sigma, MO), and streptomycin (100 μg/ml, Sigma, MO). To maintain cells in the exponential growth phase (~1 × 10⁶ cells/ml), they were diluted at a ratio of 1:5–1:8 every 2 days. The cells expressing EGFP were produced by transfecting CHO-K1 cells with pEGFP-C1 plasmid using a lipofection reagent (Polyfect, Qiagen, Valencia, CA) based on the manufacturer's protocol. The pEGFP-C1 plasmid (Clontech, Mountain View, CA) used for transfection was propagated in the DH5α *E. coli* culture and then extracted and purified using a commercial kit

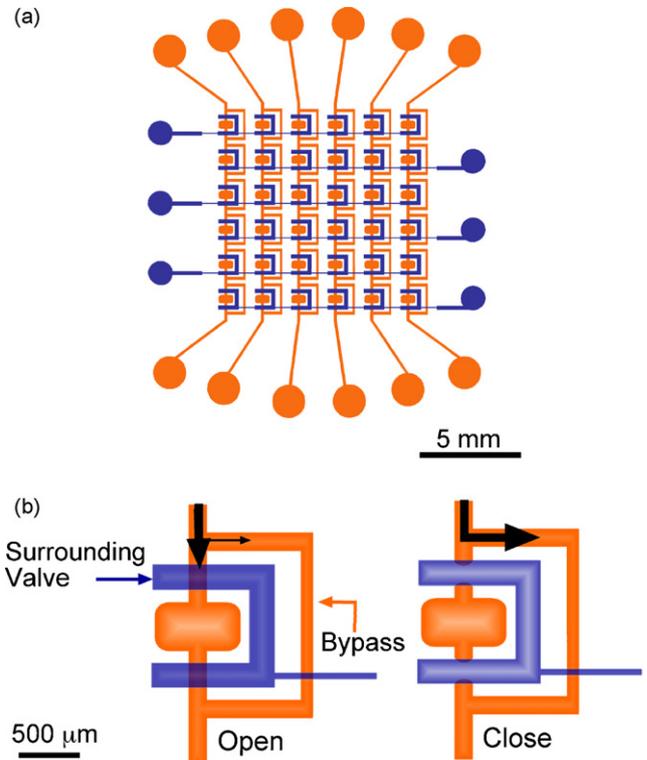


Fig. 1. (a) The schematic of the multilayer microfluidic cell array. The fluidic channels and chambers (in the fluidic layer) are shown in orange while the surrounding valves (in the control layer) are shown in blue. (b) The operation of a surrounding valve and the microfluidic culture chamber under its control. The channel depth is 18 μm in the fluidic layer and 25 μm in the control layer. The sample flows mainly through the chamber when the valve is open due to the higher flow resistance in the bypass channel. The sample flows through the bypass channel when the valve is closed. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

(HiSpeed, Qiagen, Valencia, CA). Cell concentration was adjusted to 2×10^6 cells/ml in the DMEM medium for all experiments in this report.

2.4. Cell culture in the microfluidic cell array

Cells were delivered into the array using a syringe pump (at an infusion rate of 1.5 μl/min) from all six inlets simultaneously. As we detail below, the microfluidic surrounding valves (in Fig. 1b) in the control layer determined the destination of transported cells. The control layer channels were filled with water and connected to a gas source of 30 psi through a number of solenoid valves (ASCO Scientific, Florham Park, NJ). The solenoid valves were able to close/open the microfluidic surrounding valves by connecting/disconnecting them to the pressure source. The solenoid valves were automated by a LabVIEW (National Instruments Co., Austin, TX) program. Seeding of different cell types (CHO cells and EGFP-expressing CHO cells) was achieved by changing cell suspension in the syringes after seeding the first cell type in selected chambers by actuating the surrounding valves. Before loading a different cell type, the connecting channels were flushed using culture medium with relatively high flow rate while all the valves were closed to remove the residual cells inside to prevent cross-contamination between different cell samples. The cells were allowed to settle down for 30 min before incubation. After the seeding, 200 μl pipette tips filled with fresh medium were inserted into the inlets to the fluidic layer to provide continuous gravity-driven perfusion into the chambers. The entire setup including the chip and the pipette tips was then trans-

ferred to a 37 °C, 5% CO₂ incubator and incubated for 48 h before the observation of EGFP expression.

2.5. Controlled delivery of different dyes into the cell array

In this set of tests, one cell type (CHO cells) was delivered into the array using the same approach described above in Section 2.4. After incubation of 24 h, the medium-filled pipette tips were removed and replaced with tubings, which were connected to syringes containing either 3 µg/ml Calcein AM or 1 µg/ml CellTrace calcein red-orange AM (producing green and red fluorescence, respectively, after being metabolized by cells). The surrounding valves direct the dyes into specific chambers. The dyes flowed into chambers at a flow rate of 1 µl/min for 3 min for labeling the cells when the surrounding valves were open.

2.6. Phase contrast and fluorescence microscopy

The microfluidic cell array was mounted on an inverted fluorescence microscope (IX-71, Olympus, Melville, NY) equipped with 1.25×, 2× and 10× dry objectives for observation. The epifluorescence excitation was provided by a 100-W mercury lamp, together with bright field illumination. The excitation and emission from cells loaded with calcein AM or cells expressing EGFP were filtered by a fluorescence filter cube (Exciter HQ480/40, emitter HQ535/50, and beamsplitter Q505lp, Chroma technology, Rockingham, VT)

while another filter cube (Exciter HQ540/25, emitter HQ605/55, and beamsplitter Q505lp, Chroma technology, Rockingham, VT) was used to observe cells loaded with CellTrace calcein red-orange AM. The images of cells and the cell array were taken with a CCD camera (ORCA-285, Hamamatsu, Bridgewater, NJ).

3. Results and discussion

3.1. Device design

The microfluidic cell array with individually addressable chambers was composed of two layers of microchannels: the fluidic layer containing channels and chambers for transporting and culturing cells and the control layer containing inverse C-shaped surrounding valves for flow control (Fig. 1). In the fluidic layer, the microfluidic fluidic channels were connecting culture chambers as shown in Fig. 1. The relatively large size of the culture chambers created a fairly slow cell velocity and facilitated cell trapping in the chamber. Each culture chamber was connected to a bypass channel to allow liquid/cells to detour around the chamber while the surrounding valve was closed. A surrounding valve controlled the access of an underlying culture chamber by solution and cells. The surrounding valves in the same row were connected by narrow channels. Due to the correlation between the channel width and the actuation pressure (Unger et al., 2000), these narrow control channels would not close the bypass channels at the given pressure (~30 psi). When

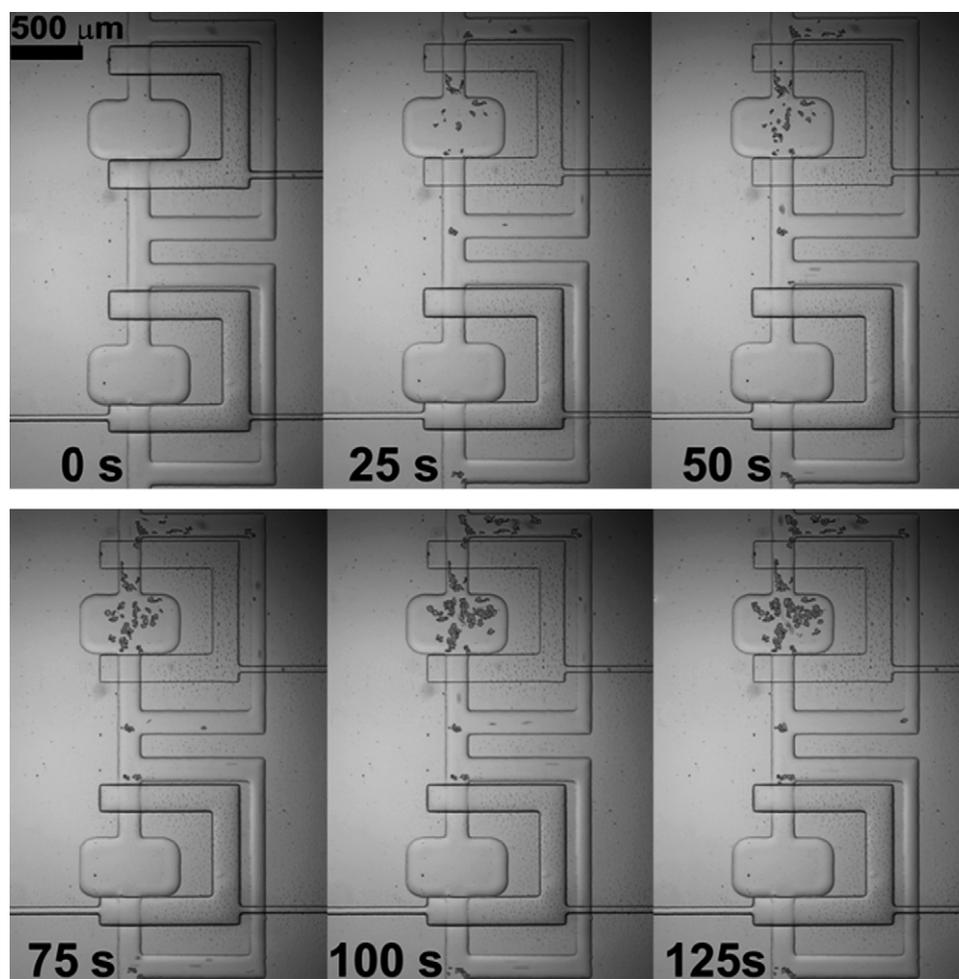


Fig. 2. The controlled delivery of CHO cells into a designated chamber. The CHO cell suspension (2×10^6 cells/ml) had a flow rate of 1.5 µl/min. The surrounding valve controlling the upper chamber was opened at 0 s while the surrounding valve for the lower chamber remained closed during the process.

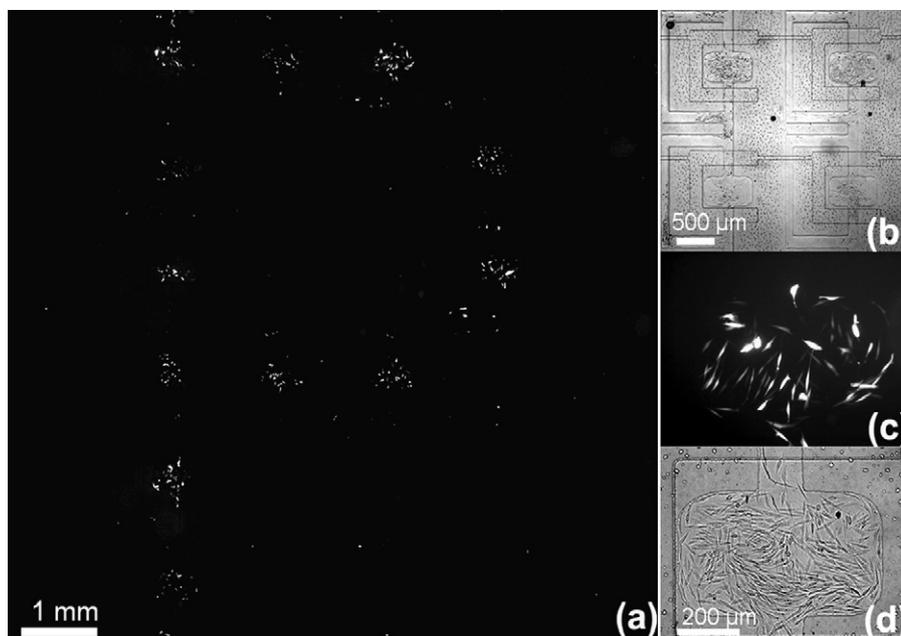


Fig. 3. Culturing of different cell types (CHO cells and GFP-expressing CHO cells) in selected chambers. (a) The fluorescent image of the entire array. (b) The phase contrast image of four adjacent chambers in the array. (c and d) The fluorescent and phase contrast images of one chamber in the array which contained GFP-expressing CHO cells. These images were taken after 48 h cell culturing in the array.

the pressure was applied in a particular row of control channels, the surrounding valves completely closed the chambers in the row so that the sample (either cells or reagents) did not go into the chambers but flowed through the bypass channel to the next chambers in the same column instead. In this way, we controlled the delivery of cells or reagents to designated chambers. Switching samples in the syringes connected to the inlets allowed us to deliver different samples into specific chambers in the same column (the previous sample in the connecting channels needed to be flushed out before loading a new sample). We used an array of 6×6 to demonstrate the principle. The array can be expanded in the same format by adding more rows and columns (such expansion will be likely to increase flow resistance and time for loading the samples). The layout and geometry of the chambers and channels can also be varied to accommodate in case the array is read by commercial array or plate readers.

3.2. Controlled delivery of cells into chosen chambers

The process of cell delivery into designated chambers was shown in Fig. 2. These images presented the time course of exclusive cell delivery into the upper culture chamber. Both the upper and lower chambers in one column were closed at time 0 by the surrounding valves. The surrounding valve controlling the upper chamber was opened after time 0 to allow cells to enter while the lower chamber remained closed. We pre-coated all the chambers and channels with fibronectin. The retention of cells in the chamber was low when the surfaces were not coated with fibronectin. The fibronectin coating also provided favorable surface for cells to adhere and proliferate during the culture phase. During the first 100 s, the number of cells retained in the upper chamber increased gradually. The cell number remained relatively stable after 100 s. The chamber was able to retain up to hundreds of cells and the number of cells held within a chamber could be decreased by allowing a shorter period of opening the surrounding valve.

3.3. Culturing different cell populations in designated chambers in the array

Cell culture inside microfluidic devices was explored in our previous work (Wang et al., 2008). In this work, we demonstrated that different cell populations (either different cell types or cells treated differently) could be delivered to designated chambers for culture and observation. As a proof-of-concept, CHO cells and EGFP-expressing CHO cells were delivered into different chambers in the array while controlling their destinations using the surrounding valves. As shown in Fig. 3a, we were able to form a fluorescent “P” pattern in the 6×6 array by placing the EGFP-expressing CHO cells in specific chambers. The close-up phase contrast and fluorescent images of the culture chambers in the array confirmed that healthy cell culture was maintained after 48 h of incubation (Fig. 3b–d). This mode of operation offers flexibility for assays that require cells to be treated in devices or locations other than the culture chambers. Cells can be treated in the suspension and in other locations of the chip and then transported and sorted into designated chambers in the array. For example, such mode will be compatible with flow through electroporation we demonstrated previously (Wang and Lu, 2006a,b) and cells can be delivered with different genes before they are transported into different chambers for observation of phenotypes.

3.4. Controlled delivery of different reagents to designated chambers in the array

Our array platform also allows the delivery of different reagents into designated cell chambers for treating cells and observing cellular responses. CHO cells were seeded in all chambers of the array and cultured for 24 h before the test. Fig. 4 shows that cells in the chamber became increasingly fluorescent after the surrounding valve was open and the cells started to expose to calcein AM (nonfluorescent calcein AM is converted to a green-fluorescent calcein after hydrolyzed by intracellular esterases in live cells). We

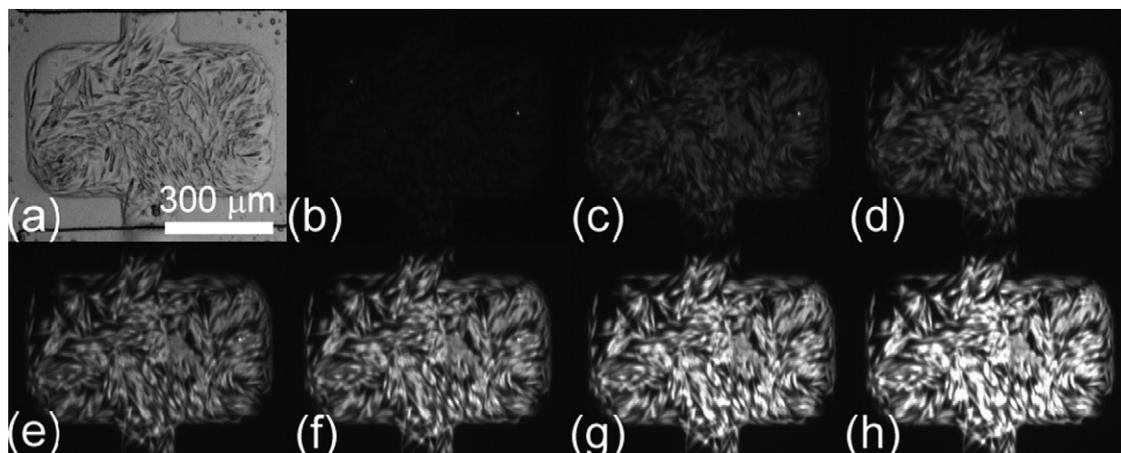


Fig. 4. The controlled delivery of calcein AM at a concentration of $3 \mu\text{g/ml}$ and a flow rate of $1 \mu\text{l/min}$ to CHO cells in the chosen chamber. (a) The phase contrast image of the cells in the chamber. (b–h) Time lapse fluorescent images of the cells in the same chamber. The interval between the images was 1 min. The surrounding valve was opened at the time of taking image (b).

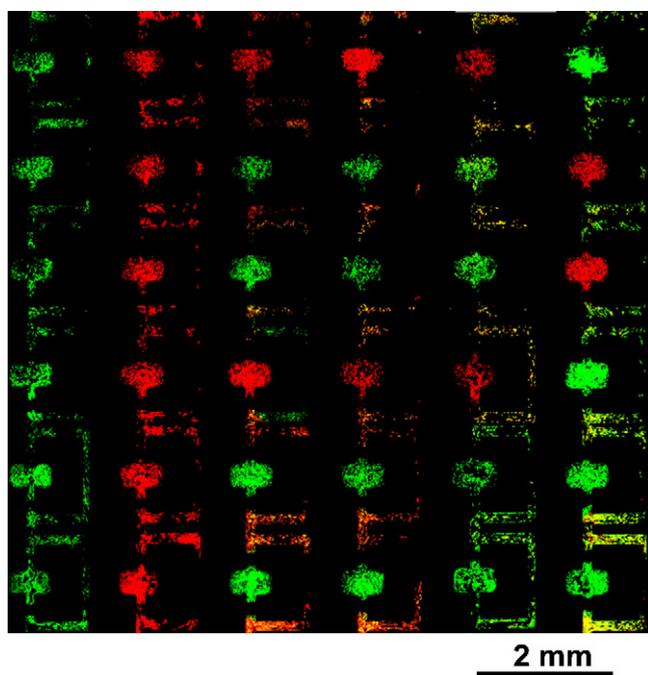


Fig. 5. The delivery of $3 \mu\text{g/ml}$ Calcein AM (turning cells green) and $1 \mu\text{g/ml}$ CellTrace calcein red-orange AM (turning cells red) to designated chambers. CHO cells were cultured in the array for 24 h before the delivery. The reagents were flowed into the array with an infusion rate of $1 \mu\text{l/min}$. This image was obtained 15 min after the delivery of the reagents. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

also demonstrated selective labeling of cells in different chambers. Two different dyes (calcein AM and CellTrace calcein red-orange AM) were delivered into designated chambers by manipulating the surrounding valves. The chambers in the array were labeled either green or red to generate a red “P” with green background as shown in Fig. 5. The surrounding valves enabled the isolation of specific chambers from the rest during the application of the reagents and prevented crosstalk between chambers. However, the residual cells in the bypass channels were labeled by different reagents while they flowed through. The interference from these residual cells in the bypass channels with array reading is a solvable problem. In principle, the number of residual cells in the bypass channels can

be reduced by decreasing the channel dimensions. Furthermore, the signal from the residual cells in the bypass channels can be discarded by taking the localization information of the chambers into account during image analysis or scanning.

4. Conclusions

We demonstrated a microfluidic cell array with the capacity for cell/reagent manipulation and cell culture. The culture chambers in the array were individually addressable with the aid of pneumatic valves on the chip. The array was automated by a computer for cell and sample manipulation. The cellular responses or phenotypes were observed based on fluorescence-based assays. Our microfluidic cell array offers a platform that is ideal for high-throughput cell-based screening assays.

Acknowledgement

This research was supported in part by NSF through grant CBET-0747105.

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