A Microfluidic Array with Micro Cell Sieves for Cell Cytotoxicity Screening

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ABSTRACT

A microfluidic array with micro cell sieves was designed to fabricate multiple cells array for high-throughput cell cytotoxicity screening. Micro channels in both directions (column and row) were individually addressable by using a set of elastomeric monolithic array valves, enabling parallel loading of various cell lines in one direction and introducing of different toxins in the other direction. Several micro cell sieves were designed in each chamber. Cell number and distribution in chambers can be conveniently controlled by the adjustment of cell sieve number, distribution and size. Fluid transport property and cell loading in chamber were simulated and verified experimentally. The capability of the microfluidic array was demonstrated for seeding multiple mammalian cell lines in chambers and observing the cell viabilities after cells exposed to different toxins with a fluorescence microscope.

Keywords: microfluidic, cell array, micro cell sieve, cell cytotoxicity screening

1 INTRODUCTION

There has been tremendous interest in high-throughput cell-based screening platforms [1]. Current methods to perform cell-based drug screening assays are expensive and limited in the number of tests that can be performed [2]. Therefore, developing a technology that can perform such experiments in a cheaper, easier, and higher throughput manner may be beneficial in a number of fields, ranging from drug discovery to tissue engineering.

Recently, microfabrication and microfluidic technologies have attracted a lot of attention in cell-based screening [3]. Unlike high-density microplate systems, microfluidic networks, consisting of micromachined or molded channels with micron dimensions, have the capability to perform experiments in a high-throughput manner in nanoliter or picoliter volumes [4] and to integrate microfluidic components such as valves, pumps and gradient generators can be used to eliminate expensive robotics and labor costs associated with current microplate systems. In recent years, this method has been applied to several cell-based biological studies including mammalian cell patterning in an enclosed array [5], cellular responses to chemical gradients [6,7], investigation of celluar differentiation [8], and observation of dynamic gene expression [9], and potential drug screening [10].

The practical microfluidic cell array for toxins screening will only be possible when substantial progress is made in uniform cell loading and cell distribution in culture chambers, because cells are suspended in medium and very small perturbations to fluid flow will significantly disturb cell positions in culture chamber with nanoliter volume [11]. Inhomogeneous cell loading and distribution can affect cell viability evaluation after toxins exposure, leading to false toxins screening result. There are various reported methods to control cell position, U-shaped weirs to isolate single cell [12-14], PDMS well trapping method [10], Holographic optical trapping [15], dielectrophoretic trapping [16] and so on.

In this report, we developed a microfluidic array platform with lithography molding technology for high-throughput cell cytotoxicity screening. The channels in this platform were individually addressable in both directions (column and row), enabling parallel loading of various cell lines in one direction and introducing of different toxins in the other direction. The channels for cells seeding were orthogonal to channels for toxins exposure, and each region at channel intersection was a circular chamber which was compartmentalized by array valves. Several micro cell sieves were built in each culture chamber to form several low flow velocity regions, and cells can be trapped and immobilized within cell sieves. Cell number and distribution in chambers can be conveniently controlled by the adjustment of cell sieve number, distribution and size.

2 EXPERIMENTAL

2.1 Fabrication of Microfluidic Array

The microfluidic device was fabricated in PDMS (Sylgard 184, Dow Corning) by multilayer soft lithography.

NSTI-Nanotech 2007, www.nsti.org, ISBN 1420061844 Vol. 3, 2007

Two photoresist-based molds were fabricated with the method described in the literature [17] and used to fabricate the multilayer device [18], corresponding to flow and control layers patterned with respective microchannel structures.

In the final assembled devices, the microchannels had the following dimensions: the flow channels were 40 μm (h) × 100 μm (w) with a circular chamber for cell culture (diameter 400μm), and the control channels were 40 μm (h) × 200 μm (w) in the control valving regions, and 30 μm (w) where they cross over flow lines and valve closure is not desired.

Off-chip control of the valves, which are pressurized by a nitrogen source gated by miniature solenoid check valves (The Lee Co,) is carried out using an analog-to-digital logic board (National Instruments PCI-DIO-32HS) driven by the Java operating software [19].

2.2 Microfluidic Simulations

Optimal layout of the cells sieves in each microchamber was achieved by initially simulating the cell loading process. Computational fluid dynamics (CFD) simulations were carried out using commercial CFD tool (STAR-CD 3.15a, CD-adpaco), which is based on finite volume method (FVM). Momentum equations and continuity equation were solved using SIMPLE (Semi-Implicit Method for Pressure Linked Equation) algorithm.

Within the simulation, the transient motion of every cell was calculated by using one-way coupled Lagrangian approach for the pre-computed Newtonian flow fields. This includes spatial interpolation of the flow velocity at the cell's position. Each cell was assumed to be a solid sphere with a hydraulic diameter of 10μm, and the forces considered in the equation of motion for the cells were the Stokes drag, the pressure gradient, diffusive and buoyant forces, and a spring force to model the elastic collision between cells. The cell trajectory code was validated against the experiment results of the annular expansion channel [20,21]. A fourth-order Rosenbrock method based on an adaptive time-stepping technique was utilized as the integration method, as it is more reliable for stringent parameters than the Runge–Kutta method [22].

2.3 Cell Culture

BALB/3T3 murine embryonic fibroblast and HeLa cell were purchased from Advanced Type Culture Collection (ATCC), and cell culture reagents were purchased from Gibco Invitrogen Corp. Bovine endothelial cell was a generous gift from Professor Forbes Dewey's group. Cells were manipulated under sterile tissue culture hoods and maintained in a 5% CO2 humidified incubator at 37 °C. BALB/3T3 was maintained in dulbecco's modified eagle medium (DMEM) supplemented with 10% calf serum. HeLa was maintained in eagle's mimimum essential medium (MEM) supplemented with 10% fetal bovine serum. Endothelial cell was maintained in DMEM supplemented with 10% fetal bovine serum. Once the cells were confluent, they were trypsinized (0.25% in EDTA, Sigma) and passaged at a 1:5 subculture ratio.

2.4 Cell Seeding

The microfluidic devices were autoclave sterilized, rinsed with 1x phosphate-buffered saline (PBS), and degassed by driving trapped air through the walls of the gas-permeable device. Fluidic networks were pre-coated with 20 mg/mL gelatin (Sigma) for 1 hour to promote cells attachment. Excess gelatin was removed by rinsing with PBS. To channel the cells into the circular cell culture chambers, a vertical array valve was actuated, and a suspension of cells (about 10^6 cells/mL) was injected into the device through the inlets and allowed to settle under static conditions in incubator for ~5 h. After primary incubation, the devices were aseptically connected to peristaltic pumps to perfuse channels with fresh medium at 0.5 μL/min to replenish critical metabolites and remove potentially toxic waste.

3 RESULTS AND DISCUSSION

The microfluidic array platform was designed to fabricate multiplex live cell arrays for cell cytotoxicity screening （Figure 1）. The microfluidic array allowed flow discrimination in two dimensions. The microchannels in this microfluidic array for cells seeding were orthogonal to the microchannels for toxin exposure, and each region at channel intersection was a circular chamber which was compartmentalized by two array valves, horizontal array valve and vertical array valve. When vertical array valve was actuated, the microchannels in row direction are individually addressable, various cell lines can be delivered simultaneously into different channels, respectively. Compartmentalization of cells into chambers was achieved by the actuation of the horizontal array valve. When the horizontal array valve was actuated and vertical array valve was open, the microchannels in column direction was individually addressable, and different toxins can be loaded into different channels to expose to each cell line seeded in the microfluidic array.

There are some difficulties to fabricate microfluidic live cell array for toxin screening. Uniform cell loading in a whole microfluidic array and uniform cell distribution in each cell chamber are main challenges in a large array format. In microfluidic array, the volume of each cell chamber is typical in nanoliter, even in picoliter. The volume of each cell chamber in the microfluidic array shown in Figure 1 is only 5 nL. It is hard to control the cell number and distribution in each cell chamber, because very small perturbations to fluid flow will significantly disturb cell positions in cell chambers. Inhomogeneous cell loading and cell distribution in microfluidic array will affect cell viability evaluation after toxins exposure, leading to false

toxins screening result. In this regard, we designed several micro cell sieves in each cell chamber to control cell number and distribution (Figure 1).

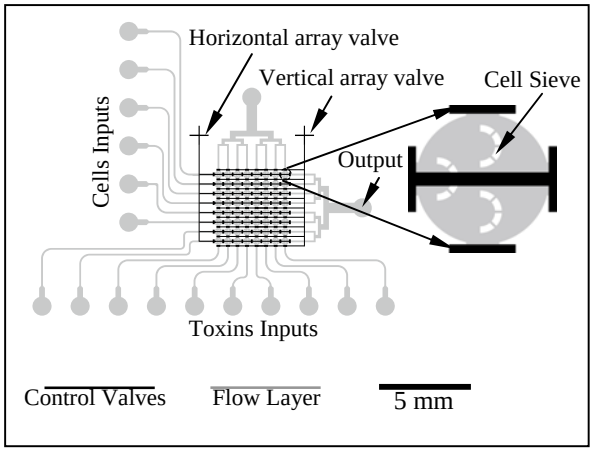

Figure 1: Schematic of the microfluidic array for parallel cell cytotoxicity screening.

Each cell chamber in the microfluidic array shown in Figure 1 is 400 μm in diameter and contains three cell sieves. Each sieve is semicircular, 100 μm in diameter, and 20 μm in width, with three apertures (8 μm). The fluid velocity was simulated as described in Experimental Methods for one 3D cell chamber containing three cell sieves. The velocity profile through the single cell chamber is shown in Figure 2. The fluid velocities in the regions in the cell sieves are reduced as is expected, and three low fluid velocity regions are formed in the cell chamber. 8 μm apertures on the cell sieve allow a fraction of fluid streamlines carrying cells to enter into the sieve.

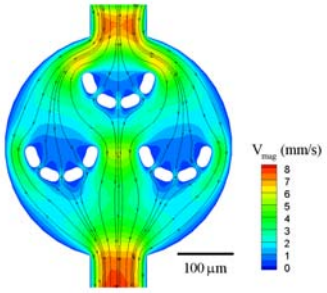

Figure 2: Theoretical flow velocity profile through single chamber with three cell sieves.

Cell loading in cell chamber was simulated as described in Experimental Methods to show the whole process of cells trapping in cell sieves. A series of images of the cell loading simulation were shown in Figure 3. In Figure 3A, cells were delivered into the chamber from the inlet. In Fig. 3B, cells moved further and passed the first cell sieve, and the flow velocity of few cells was reduced and cells moved into the first cell sieve and were trapped in the sieve. In Fig. 3C, most of cells moved out this chamber and into the next chamber, and some cells were trapped in these three cell sieves.

Cell loading uniformity was determined by individual loading of eight rows of the microfluidic array at 1 μL/min for 2 min. Uniform cell loading in the microfluidic array was obtained with the help of cell sieves, the average cell number per chamber was 19.3 ± 1.6, and deviation was 8%. Uniform cell loading and distribution in this microfluidic array significantly increases the reliability to perform toxins screening experiments compared with a similar array without cell sieves in chambers. Cells distribution in the microfluidic array without cell sieves was random, some chambers contained tens of cells, some chambers only several cells, even some chambers empty (deviation over than 100%).

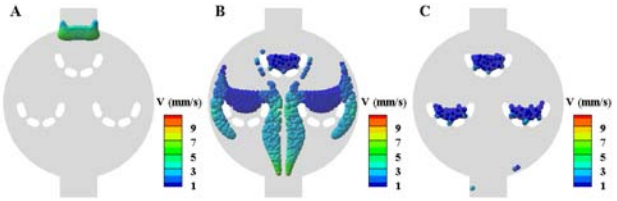

Figure 3: Cell loading simulation in one chamber.

We designed several cell chambers with different cell sieve numbers and distribution (Figure 4), cell loading in these chambers were investigated. In Figure 4A, there are nine cell sieves in the cell chamber, each sieve contains one aperture, and about fifteen cells were trapped in this chamber. The chamber shown in Figure 4B contains three cell sieves and each sieve contains three apertures, and about twenty cells were trapped in this chamber. In Figure C, the chamber also contains nine cell sieves, each sieve contains two apertures, and about forty cells were trapped in this chamber. Cell number and distribution in chambers can be conveniently controlled by the cell sieve number, distribution and size. According to different requirements, more chambers with different cell sieves can be designed to control cell number and distribution.

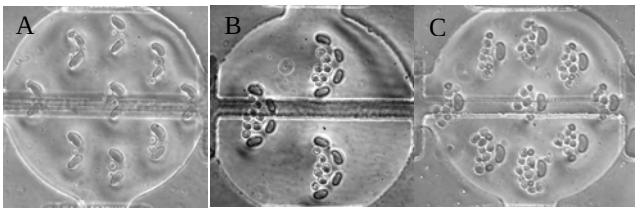

Figure 4: Cell loading in chambers with different cell sieve numbers and distribution.

As a proof-of-concept experiment, we seeded endothelial cell, 3T3, and HeLa cell in the microfluidic array, and used ethanol and methanol as toxins. These three cell lines were seeded in different channels. Microfluidic

NSTI-Nanotech 2007, www.nsti.org, ISBN 1420061844 Vol. 3, 2007

toxin exposure experiments were performed by delivering 75% ethanol 75% methanol in PBS into selected horizontal channels at 0.5 μL/min after vertical array valve actuated, and the left channels were perfused with PBS. After 30 min, channels were rinsed with PBS, and cells were stained with the LIVE/DEAD viability stain. Cell viabilities in the channels loaded with ethanol and methanol were 0%, while cell viabilities in the channels perfused with PBS were 100% (Figure 5).

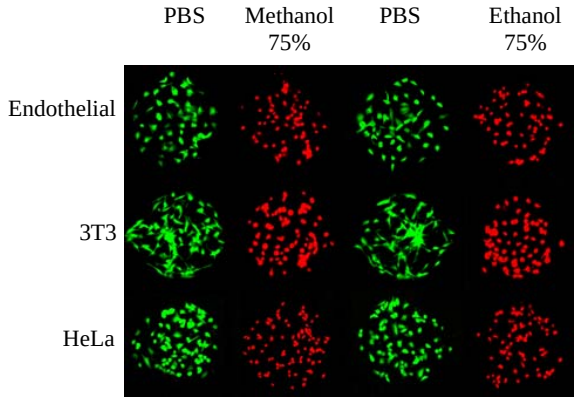

Figure 5: Endothelial, 3T3 and HeLa cell array exposed to PBS, methanol and ethanol.

4 CONCLUSIONS

A PDMS microfluidic array with micro cell sieves was developed to fabricate multiple mammalian cell lines array for cell cytotoxicity screening. Uniform cell loading and distribution in microfluidic array was obtained with the help of cell sieves. High-throughput microfluidic device can be fabricated with the method demonstrated in this paper, a microfluidic array with M channels for cell seeding and N channels for toxins screening can be used to perform M×N unique tests simultaneously. By using standard soft lithography, microfluidic device containing thousands of nanoliter-scale cell chambers in a 1 cm^2 can be envisioned, and thousands of tests can be performed in the same time, which is much greater than the densities achieved using existing multi-well plate technologies.

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