A microfluidic chip for highly efficient cell capturing and pairing

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This paper examined the feasibility of a microfluidics chip for cell capturing and pairing with a high efficiency. The chip was fabricated by the polydimethylsiloxane-based soft-lithography technique and contained two suction duct arrays set in parallel on both sides of a main microchannel. Cells were captured and paired by activating two sets of suction ducts one by one with the help of syringe pumps along with switching the cell suspensions inside the main microchannel correspondingly. The effects of suction flow rate and the dimensions of suction channels on the cell capturing and pairing efficiency were characterized. The present chip was capable of creating 1024 pairs of two different cell populations in parallel. The preliminary experimental results showed that the cell capturing efficiency was 100% and the pairing one was 88% with an optimal suction rate of 5 $\mu\text{l}/\text{min}$ in the chip in the 2 μm -sized suction duct chip. The cell viability after capture inside the microfluidic device was $90.0 \pm 5.3\%$. With this cell capturing and pairing chip, interaction between cells in a single pair mode can be studied. The ability to create cell pairs has a number of biological applications for cell fusion, cell-cell interaction studies, and cell toxicity screening. © 2011 American Institute of Physics. [doi:10.1063/1.3623411]

I. INTRODUCTION

Lab on a chip devices allow to manipulate cells precisely at microscale even in a single cell mode, which presents an extremely powerful platform for versatile cell-related studies, such as drug screening,^{1,2} cell communications,³ and fusion.⁴⁻⁶

Hydrodynamic cell trapping is one of the most popular methods to manipulate cell at micro-scale.⁷ Yang *et al.* reported a parallel dam structure to successfully dock cells on chip with a minimal shear stress.⁸ Valero *et al.* used a cross-flow microdevice to trap cells and then analyzed dynamics of programmed cell death.⁹ U-shape (or U-type) hydrodynamic trapping structure has been developed to capture single cell¹⁰ or a small cluster of cells¹¹ in a parallel manner. Single cell enzyme dynamics was analyzed by this technique.¹² Zheng *et al.* fabricated a parylene-C filtering membrane to capture the circulating tumor cells based on their size differences.¹³ Tan *et al.* fabricated a hydrodynamic confinement and employed optically activated microbubble to trap and release single cells.¹⁴ Polydimethylsiloxane (PDMS) microchannel with micro pillars array inside was used to trap single cells and measure their mechanical properties.¹⁵ Microvortices were also proposed to trap particles and showed promising applications in cell capturing.¹⁶ Recently, Chung *et al.* presented a hydrodynamic microwell array for highly efficient single cell capturing, which was able to distribute individual cells into separated microwells.¹⁷

Along with cell trapping, microfluidic cell pairing on a chip has also been widely studied for varied applications.^{3,18,19} Lee *et al.* reported a microfluidic device which allowed trapping

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cells at two opposing side channels set at the bottom of a main microchannel.³ With this chip, an accurate cell pairing was achieved but with very low yield; only 37 pairs were manipulated as reported. Wang *et al.* reported a biotin-streptavidin based cell pairing or conjugating followed by direct electrical fusion through a simple microfluidic channel, where a 25% pairing efficiency was achieved.²⁰ Skelley *et al.*¹⁹ used a microfluidic device to trap and pair thousands of cells. Different cells, including fibroblasts, mouse embryonic stem cells, and myeloma cells, were paired and fused with efficiency higher than 50%, which was five fold larger than that of a commercial electro-fusion instrument. Cao *et al.*²¹ and Tresset *et al.*²² have achieved fusion efficiencies of 40% and 75%, both by electrically activating cell fusion in the microfluidic chips after cell pairing. Recently, micro-orifice structure was reported to assure one-to-one cell pairs and follow-up fusion procedure.^{23,24} A meniscus-based fabrication approach was adopted to create microorifice with a diameter of 2–10 μm on the vertical walls in a microfluidic channel. Only seven cells from each side of the wall were attracted to the microorifice to form a cell pair (95–100%) under a 1 MHz alternating current. Teshima and his colleagues²⁵ developed a meander-shaped 18-unit dynamic microfluidic technology to sequentially pair two different microbeads via a single trapping operation. However, all of the above mentioned microfluidic based cell pairing approaches failed to obtain a high pairing efficiency along with a reasonable pair yield.

In this work, a microfluidic chip with two suction duct arrays set in parallel on both sides of a main microchannel was fabricated and tested for cells capturing and pairing with principle shown in Fig. 1(a). To prove the concept, K562 cells were divided into two subpopulations, one was fluorescently labeled while the other one was not labeled. These two cell populations were paired by sequentially loading cell suspension into the main microchannel and activating suction duct channels imbedded in the walls of the main microchannel arrays one by one. Effects of the flow rate required to create negative pressure in the suction ducts as well as the size of suction ducts required for efficient capturing and pairing of cells were investigated.

II. METHODS

A. Materials and equipments

Sylgard 184 silicone elastomer, including base and curing agent, was purchased from Dow Corning Corp., USA. RZJ-304 photoresist used in the lithography was from Ruihong Comp.,

FIG. 1. Schematic illustration of the microfluidics cell capturing and pairing chip. (a) Working principle of the present chip. (b) Chip layout. (c) Cross-sectional view of two captured and paired cells.

Shanghai. PRMI 1640 was obtained from GIBCO™ Invitrogen Corp., USA. Fetal bovine serum (FBS) was obtained from MDgenics Inc. USA. Celltracker Orange (CMTMR) was purchased from GIBCO Invitrogen Corp., USA. Tryptan blue was purchased from Beijing Jingke Comp., China. The other chemicals were of analytical grade. The human erythroleukemia cell line K562 used in the experiment was purchased from Institute of Basic Medical Sciences, Chinese Academy of Medical Science, China. The syringe pump (TS-2A/L0107-2A) used in this work was purchased from Baoding Longer Precision Pump Co. Ltd., China. An inverted microscope (Olympus IX71, Tokyo, Japan) equipped with a CCD camera served as the imaging platform.

B. Microfabrication

Schematic illustration of the present microfluidic cell capturing and pairing chip was shown in Fig. 1(b) and (c). The chip contained three basic functional units: one main microchannel and two suction duct networks (suction network 1 and suction network 2). Considering the cell line used in the work was human leukemia K562 with a diameter of 16–17 μm , the present main microchannel was 35 μm wide and 40 μm deep, which were larger enough for the second cells freely flowing through the microchannel without displacing the captured first cells. In the suction networks, the suction duct connected to the main microchannel was 100 μm long and set with a 40- μm pitch in parallel on each side of the main microchannel. To study the effect of the orifice size on the capturing and pairing efficiency, the suction duct was designed to have a square-shaped cross-section with the edge length of 2 μm and 8 μm .

Microfabrication process was schematically illustrated in Fig. 2. Fabrication of the PDMS mold started with a 4 in. silicon wafer. First, 5000 Å SiO_2 was formed by a thermal oxidation, Fig. 2(a). Then, the first lithography step was conducted to define the main microchannel, as indicated in Fig. 2(b), followed by selectively removing the exposed SiO_2 by RIE (reacting ion etching). The second lithography defined the suction ducts and then a first short-term DRIE (deep reactive ion etching) was conducted with the patterned photoresist as the etching mask to

FIG. 2. Schematic illustration of the microfabrication process of the present chip.

form the initial depth, exactly the height of the suction duct, as shown in Figs. 2(c) and 2(d). After stripping the photoresist, the second DRIE was applied directly to form the two-depth silicon structure with the pre-defined SiO_2 as the etching mask, Fig. 2(e). The final silicon mold was obtained after removing SiO_2 in a BHF (buffered fluorhydric acid) bath, shown in Fig. 2(f). Fig. 2(g) illustrated the PDMS molding process. PDMS pre-polymer was prepared by mixing the base and the curing agent with a weight ratio of 10:1. The mixture was degassed for 20 min in a vacuum desiccator. Then, the pre-polymer was poured onto the silicon mold and cured in an oven at 70°C for 60 min. After released from the mold, the PDMS were punched through to function as inlet/out, and then bonded with a flat PDMS sheet by an O_2 plasma treatment of 10 s, as indicated in Fig. 2(h).

C. Cell culture and staining

The K562 cell line was maintained in PRMI 1640 supplemented with 10% FBS. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 . Before experiments, cells were harvested and divided into two experimental groups. One was kept at the original status and another one was stained by CMTMR at a final concentration of $10\ \mu\text{M}$. After incubated at 37°C in black for 30 min, centrifuged and washed in PBS 3 times, and then re-suspended in PBS at a density of 1×10^6 cells/ml, the cell suspension was ready for the microfluidic capturing and pairing experiment. Viability of the captured and paired cells was evaluated by trypan blue staining, the living cells and dead cells were counted under a light microscopy.

D. Cell capturing and pairing

Experimental setup was shown in Fig. 3. The microfluidic chip was connected to a syringe pump system, which included two independently controlled pumps for main microchannel and two set of suction networks, respectively. The chip was first rinsed with ethanol and distilled water sequentially. About $30\ \mu\text{l}$ of K562 cell suspension (the fluoresced group) prepared as aforementioned was dropped into the reservoir, Fig. 1(b), and then sucked into the main microchannel by a syringe connecting with pump from opening 2 (Fig. 1(b)). The syringe pump connected with the suction duct network 1 through opening 3 (Fig. 1(b)) was then started to trap

FIG. 3. Experimental setup for the present microfluidic chip. Inset figure shows the PDMS chip with a penny for comparison.

cells in the main microchannel. After capturing the first cells, PBS was used to wash away all the free cells inside the main microchannel by adding PBS into reservoir and sucking out from opening 2. Then, the second cells (the non-fluoresced group) were introduced into the main microchannel in the same manner and captured by starting the syringe of the suction duct network 2 (opening1 in Fig. 1(b)). The rest cells were washed away after pairing. The whole cell operation process was observed under microscope, and the final cell capturing and pairing yields were calculated based on the images.

III. RESULTS

A. Silicon mold and PDMS chip

SEM photos of the silicon mold and the PDMS replica were shown in Fig. 4. The silicon mold was fabricated by the two-step DRIE etching described previously. Geometrical size of the suction duct was defined by the etching mask along with the first etching depth. PDMS replica showed a very good replication from the silicon mold, which guaranteed the cell capturing and pairing performance as designed.

FIG. 4. SEM photos of the silicon mold (a) and the PDMS replica (b). Inset figures are connections between the suction duct and the main microchannel with high magnifications and 45° tilted. All scale bars are 100 μm .

B. Cell capturing

The negative pressure in the suction channels depended on the fluid flow rate through the network of suction duct fluidic channels. Cells in the $8\text{ }\mu\text{m}$ -sized suction duct chip were physically destroyed when the flow rate was higher than $2\text{ }\mu\text{l/min}$ (around -23.7 kPa calculated at the suction port, gauge pressure), especially in the case of $8\text{ }\mu\text{l/min}$ ($\sim -94.8\text{ kPa}$) suction as shown in Fig. 5(a). If the flow rate was lower than $2\text{ }\mu\text{l/min}$ but higher than $1\text{ }\mu\text{l/min}$ ($\sim 11.9\text{ kPa}$), cells

FIG. 5. Photos of the cells trapping at the suction flow rates of $8\text{ }\mu\text{l/min}$ (a), $2\text{ }\mu\text{l/min}$ (b), $0.8\text{--}1\text{ }\mu\text{l/min}$ (c). All scale bars are $40\text{ }\mu\text{m}$.

easily went through the suction ducts and entered the two sets of suction duct networks (Fig. 5(b)). When the flow rate was decreased down to 0.8–1 $\mu\text{l}/\text{min}$, cells were successfully captured after about 5 min of applying negative pressure through the suction ducts and kept alive during the whole experiment, as exhibited in Fig. 5(c). In the chip with 2 μm -sized suction ducts, cells were captured much more easily than that with 8 μm -sized ones, even at a higher suction flow rate of 5 $\mu\text{l}/\text{min}$ (~ 15.2 kPa), as shown in Fig. 6. Moreover, the negative pressure applied through the smaller suction ducts could keep the captured cells from being washed away. However, cell damage risk rose when the suction duct was too small, especially at a high suction flow rate. The 2 μm -sized suction ducts with the suction flow rate of 5 $\mu\text{l}/\text{min}$ resulted in the optimal cell capture efficiency of 100% and viability of $90.0 \pm 5.3\%$.

C. Cell pairing

After determining device parameters leading to optimal cell capture, experiments were performed to demonstrate assembly of cell pairs in the microfluidic device. Fluorescently labeled cells were introduced into the main microchannel and trapped by applying flow rate of 5 $\mu\text{l}/\text{min}$ to the network of suction duct fluidic channels (Figs. 6(a) and 6(b)). Uncaptured cells were washed away with PBS. In the second step, unlabeled cells were introduced into the main microchannel and were captured at the opposite wall of the main microchannel by activating

FIG. 6. Photos of the cells pairing in the chip with 2 μm -sized suction duct and suction flow rate of 5 $\mu\text{l}/\text{min}$. Phase contrast (a) and fluorescently labeled cells (b) merged picture (c). All scale bars are 40 μm .

fluidics in the other network of suction duct channels (Fig. 6(c)). The whole pairing process took 15 min to complete. As shown in Fig. 6(c), fluorescent cells and non-fluorescent cells were successfully paired. Our microdevice allowed to achieve cell pairing efficiency $88 \pm 12\%$ (calculated based on three independent experiments) with 1024 cell pairs formed.

IV. CONCLUSION

This paper described a microfluidic chip for efficient capture and pairing of cells. The experimental results indicated that both the flow rate and the size of the suction ducts were important for cell manipulation in the microfluidic device. A 100% cell capturing and $88 \pm 12\%$ cell pairing efficiency was achieved with an optimal suction flow rate of 5 $\mu\text{l}/\text{min}$ using 2 μm -suction ducts. The viability of cells captured in the devices was $90.0 \pm 5.3\%$. The chip will find immediate application in cell-cell fusion, where it is important to achieve high-yield of heterocaryon–fusion cell without generating extra homocaryon.

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