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Conference Paper**Author(s):**

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Publication date:

2017

Permanent link:

<https://doi.org/10.3929/ethz-b-000192239>

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Originally published in:

<https://doi.org/10.1109/TRANSDUCERS.2017.7994414>

A MICROFLUIDIC 16×6-CELL-TRAP ARRAY FOR COMPARATIVE CELL CULTURING

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ABSTRACT

This work presents a microfluidic chip with a 96-cell-trap array (16-row × 6-column) for immobilization and comparative culturing of single yeast cells. In this microfluidic system, budding yeast (*S. cerevisiae*) cells can be delivered to each column of the cell-trap array by means of a laminar focused flow and be reliably captured at the traps in each column through hydrodynamic forces. Immobilized cells can then be comparatively cultured while being exposed to different media. The developed system allows for performing experiments and control experiments in parallel or for executing comparative experiments with on-site monitoring of the dynamic cellular development in the microfluidic 96-cell-trap array.

KEYWORDS

Single-cell analysis, cell trapping, comparative cell-culturing, laminar flow

INTRODUCTION

Traditionally, studies on cells are always conducted on groups of cells. Under different external stimuli, cells can exhibit different behaviors during their growth and proliferation. Results concluded from these studies are based on the assumption that cells used in experiments are uniform, and that the behavior of a cell group represents the behavior of single cells. However, single cells are often known to be heterogeneous [1, 2]. Cellular heterogeneity has been widely proven to play an important role in the development of cells, organs and organisms. Therefore, innovative techniques for single-cell analysis are required as suitable tools to study cellular heterogeneity: single cells have to be isolated and captured at specific positions so that they can be cultured continuously and analyzed on-site to investigate the dynamics and cellular differences.

Methods for single-cell immobilization have been developed in the past decades. One method included to first dilute the cell samples to a low concentration of about one single cell in hundred microliter of medium. Then, the diluted cell samples in suspension were loaded to a cell-culturing platform, such as a 96-well plate or a specific microwell array [3]. However, this method featured low efficiency and reproducibility for single-cell immobilization. Microfluidic technologies offer significant advantages for single-cell studies, such as small sample volumes and precisely controllable medium conditions for cell culturing, and high sensitivity and accuracy in the analysis of cellular dynamics. Thus, microfluidic systems have been widely used for single-cell

studies [4].

In order to culture single cells in microfluidic chips, cells have to be first isolated and captured at multiple positions. Researchers have developed several methods for cell trapping in microfluidic chips [5, 6]. For example, one method used cup-shaped microfluidic structures to immobilize single cells upon flowing a cell suspension through the chip [7]. This simple mechanism for single-cell trapping does not require external equipment for precise control of sample perfusion. However, the cell-trapping process was stochastic, and could not be precisely controlled during the experiments. To achieve a high throughput, cell traps were patterned at a high density in the microfluidic chip.

Compared to conventional cell-culturing methods, microfluidic systems can provide suitable and dedicated environmental conditions for cell growth and proliferation. Cell-culturing media delivered to the cells can be precisely controlled with respect to reagent concentration, dosage and stimulation duration. Cellular dynamics in proliferation can be observed or measured in real-time. Previously reported microfluidic systems for single-cell immobilization usually featured cell-trapping structures in one channel, which only enabled one cell-culturing condition at a given time [8, 9]. To perform control experiments, multiple chips had to be used sequentially or in parallel, so that the complexity of the experiments was increased. Moreover, there may be subtle differences among microfluidic chips even if they have been designed, fabricated and handled according to the same protocol. Such differences may entail additional variability in comparing cell-culturing results of different devices.

To address the aforementioned questions, we designed and fabricated a microfluidic chip with 96 cell traps. The 96 traps were patterned in an array format (16-row × 6-column). Each trap was constructed as a narrow slit between two fluidic channels, a main flow channel and a side flow channel. Cells can be captured and retained at the ends of these narrow slits based on the hydraulic pressure drop across the slits. The pressure drop across the cell traps in each column can be controlled by adjusting the flow rates of the main flow and side flow channels, so that an adequate pressure drop for cell immobilization can be generated. In the microfluidic chip, 6 parallel columns were designed for comparative cell culturing, and each column featured 16 cell traps. In order to deliver cells to each column, the inflow of the cell suspension was focused by means of laminar sheath flow. After cell immobilization in all 6 columns, comparative cell culturing was performed by exposing the immobilized cells in the various columns to different cell-culturing media.

MATERIALS AND METHODS

Design of the Microfluidic System

The microfluidic chip consists of two poly(dimethylsiloxane) (PDMS) layers and a glass substrate (Figure 1). The fluidic channel network in both PDMS layers is 25 μm high. The chip has four inlets (Inlets 1-4) and two outlets (Outlets 1 and 2) for fluidic interfacing. Three inlets (flow rates: Q1, Q2, and Q3) were designed to perfuse cell suspension (Inlet 2) and different cell-culturing media (Inlets 1 and 3) through the main flow channel (Figure 2a). The channel network for the side flow was patterned in both, top and bottom PDMS layers that were connected through punched via holes (Figure 2b). Medium was infused into the side flow channel through Inlet 4 (flow rate: Q4).

Figure.1: 3D schematics of the microfluidic cell-trap array. The top PDMS layer (dark gray) includes a fluidic channel network used to deliver medium into the side flow channel through Inlet 4. The bottom PDMS layer (light gray) includes 6 parallel columns for cell trapping and culturing, and each column has 16 cell traps, thereby forming a 16 $\times$ 6-cell-trap array. The top and bottom PDMS layers are connected through punched via holes in the bottom layer. The cell suspension and different cell-culturing media are delivered into the chip through Inlets 1-3.

There are 6 parallel columns for cell culturing in the bottom PDMS layer (Figure 2). In each column, the main flow and side flow channels are interconnected with 16 narrow slits, serving as cell traps, thereby forming a 96-cell-trap array of 16 rows by 6 columns. Both, main flow and side flow channels are 300 μm wide. In order to capture single yeast cells, the width of slits is about 4 μm , which is smaller than the diameter of budding yeast cells. When the overall flow rate of the main flow (Q1+Q2+Q3) is larger than the flow rate of the side flow (Q4), a hydraulic pressure drop is generated across the slit. Thus, cells in the main-flow channel can be dragged towards the narrow slits and be captured and retained at the ends of slits [10, 11].

Figure.2: Fluidic channel network in the bottom PDMS layer. Light blue region represents the main flow channel, and the orange region represents the side flow channel. (a) 6 parallel columns in the bottom PDMS layer are designed for cell immobilization and culturing. Each column has 16 cell traps, located between the main flow and side flow channels. The close-up view shows one cell trap, which is 4 μm wide. (b) Cross-sections of a cell trap (A-A') and a via-hole connection (B-B') between the top and bottom PDMS layers.

Chip Fabrication

The microfluidic chip was fabricated by using a soft-lithography technique. SU-8 3025 photoresists (MicroChem Co., USA) were used to fabricate the masters for PDMS replication. The thickness of both SU-8 masters for the top and bottom PDMS fluidic channels was about 25 μm . After casting the PDMS replicas from SU-8 masters, the top PDMS layer was punched with two holes for Inlet 4 and Outlet 2 at defined positions, and the bottom PDMS layer was punched with twelve via holes for the fluidic connections of the side flow channel network. Then, the top PDMS layer was bonded to the backside of the bottom PDMS layer with a precise alignment of the side flow channel network and via holes. Afterwards, four holes for Inlets 1-3 and Outlet 1 were punched into the bonded PDMS layers, which were finally bonded to a glass slide to seal the fluidic network in the bottom PDMS layer. During the bonding process, an oxygen plasma cleaner (PDC-002-HP, Harrick Plasma, USA) was used to activate the surfaces of PDMS and glass, so that irreversible sealing was achieved.

Cell Culturing

In this work, a budding yeast cell strain was used for the biological validation of the comparative cell culturing with the microfluidic chip. The *S. cerevisiae* strain was engineered with an estrogen-dependent transcriptional activator that triggers the expression of a yellow fluorescent protein (YFP) upon the addition of inducer, β -estradiol, to the cell-culturing medium. Yeast cell suspension was grown in an YPD medium made of 1% w/v Yeast Extract (YE), 2% w/v Peptone and 2% w/v glucose at 30°C. Before infusing cells into the microfluidic chip, YPD medium with 10 μ M β -estradiol, cell suspension, and YPD medium without β -estradiol were loaded in three syringes, which were connected to Inlets 1-3, respectively. Another syringe was loaded with YPD medium without β -estradiol, and was then connected to Inlet 4 as the side flow. Flow rates of the four inflows were precisely controlled by using a syringe pump (neMESYS, Cetoni GmbH, Germany) with four independent dosing units.

RESULT AND DISCUSSION

In order to capture cells in all 96 traps, cell suspension must be delivered to the 6 parallel columns in sequence. By using the laminar focused flow, the cell suspension (Q2) can be focused through two sheath flows of cell culturing media (Q1 and Q3). Thus the cell suspension can be sequentially guided to each column by varying the flow-rate ratio of Q1:Q2:Q3. During the laminar-flow perfusion and cell trapping process, the cell suspension (Q2) was set to a constant flow rate of 1.5 μ L/min. The total main flow (Q1+Q2+Q3) was set to 18 μ L/min, and the side flow (Q4) was set to a constant flow rate of 10 μ L/min. As shown in Figure 3a, cell suspension from Inlet 2 was delivered to column 1 when Q1:Q2:Q3 was set to 8:1:1. After 10 to 15 seconds of perfusion, cells were immobilized at all traps in column 1. Then, the flow-rate ratio Q1:Q2:Q3 was set to 6.5:1:2.5, and the cell suspension was guided to column 2 for cell trapping (Figure 3b). Q1:Q2:Q3 was set to 5:1:4, 3.5:1:1.5, 2:1:7 and 0.5:1:8.5, so that cells were sequentially delivered to columns 3 to 6. Therefore, based on the laminar flow focusing by means of two sheath flows (Q1 and Q3), cell suspension Q2 could be focused and guided to each column in sequence, and cells could be immobilized at 96 traps in the cell-trap array.

After the cells were immobilized in the microfluidic chip, the comparative cell-culturing was performed. The inflows of cell suspension and media were set as follows: the cell suspension from Inlet 2 was switched off (Q2=0 μ L/min); the cell-culturing medium with β -estradiol from Inlet 1 (Q1), and the medium without β -estradiol from Inlet 3 (Q3) were set to the same flow rate of 9 μ L/min; the side flow from Inlet 4 (Q4) was set to 10 μ L/min to maintain the same hydraulic pressure drop across the traps. With this regime, cells immobilized at the traps of columns 1-3 were exposed to the medium without inducer, while the cells in columns 4-6 were exposed to the medium with β -estradiol from Inlet 1 (Figure 3c).

Figure 3: Schematics of the liquid perfusion of cell suspension and cell-culturing media during cell trapping and comparative culturing. (a) Cell suspension was guided to the first column of the cell traps by setting the flow-rate ratio Q1:Q2:Q3 to 8:1:1. (b) Cell suspension was guided to the second column by setting Q1:Q2:Q3 to 6.5:1:2.5. (c) After cell immobilization, the inflow of cell suspension Q2 was set to 0, while media flows (Q1 and Q3) were set to the same value to perform comparative cell-culturing. Cells captured in the left three columns were exposed to medium 2, while cells in the right three columns were exposed to medium 1.

Time-lapse fluorescence images of cells, immobilized in different columns, were taken every 15 min with an inverted microscope. Experimental results are shown in Figure 4. In the beginning of the comparative cell culturing, yeast cells were successfully captured at the traps along columns 2 and 5. After continuous perfusion of cell-culturing media for 6 hours, cells at column 2 did not develop any fluorescence, as they were exposed to the medium without inducer. In contrast, cells captured in

column 5 shown clearly detectable fluorescence, since they were cultured under a perfusion of medium with β -estradiol, which triggered the expression of YFP in the employed cell strain. The result demonstrates that on-site comparative cell-culturing or control experiments can be achieved by using laminar flow perfusion.

Figure 4: Comparative culturing of immobilized budding yeast cells in the microfluidic array. Cells captured in columns 1-3 and in columns 4-6 were exposed to cell-culturing media without and with inducer (β -estradiol), respectively. Part of columns 2 and 5 are exemplarily shown here. After culturing for 6 hours, YFP was induced in the cells in column 5, while cells in column 2 did not develop any fluorescence.

CONCLUSION

A microfluidic 16×6 -cell-trap array has been presented in this work. The microfluidic system features 16 cell traps in each column and 6 parallel columns in the array. By using laminar focusing and two sheath flows, cell suspension has been successfully guided to the 6 columns in sequence, and cells have been reliably immobilized for further culturing. In the biological experiments, we have employed an engineered yeast strain, which can express YFP upon exposure to an inducer, β -estradiol. During the cell culturing, yeast cells have been exposed to different media (namely medium with and without β -estradiol) through laminar-flow perfusion. The experimental result has demonstrated that this microfluidic 16×6 -cell-trap array enables comparative culturing of immobilized cells.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Fabian Rudolf at ETH Zurich, Switzerland, for providing the yeast cell strain. This work was supported by the National Natural Science Foundation of China (No. 61404027), the National Key Basic Research Program of China (No. 2015CB352100), and the Fundamental Research Funds for the Central Universities.

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