

MICROFLUIDIC SINGLE-CELL CULTURE CHIP FOR INDIVIDUAL TRAPPING, CULTIVATION, AND SELECTIVE RELEASE OF YEAST CELLS

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ABSTRACT

A microfluidic cell-culture chip is presented that enables cultivation and release of selected individual cells. Single cells are trapped in a microfluidic channel using mild suction at defined sites, where they are cultivated under controlled environmental conditions. Cells of interest can be selectively released for further downstream analysis by applying a negative dielectrophoretic force via electrodes located at the trapping sites. The combination of pneumatic and dielectrophoretic methods enables highly versatile single-cell manipulation in an array-based format. Yeast cells have been used to demonstrate and characterize different functions of this platform, which is intended to serve applications in Systems Biology.

KEYWORDS: Microfluidics, Cell Culture, Single-Cell Analysis, Yeast

INTRODUCTION

Single-cell analysis is important in order to obtain more precise information and to reveal the properties of individual cells and cell-to-cell differences. Meanwhile, with the rapid development of MEMS and microfabrication technologies, the concept called 'Lab-on-a-Chip' (LOC) or 'Micro Total Analysis Systems' (μ TAS) based on microfluidics has been increasingly attracting the interest of researchers in biological, chemical, and medical diagnostic applications. As a result of the micro-dimensional features of the microfluidic devices, these LOC or μ TAS systems are appropriate to the micro-environmental mimesis, manipulation, reaction, separation and detection of single cells.

In order to perform single-cell analysis on cell-based microfluidic chips, researchers developed single-cell culture systems featuring simultaneous cell isolation, cultivation and selection capabilities. One of the most popular methods is the microwell array that features a high-throughput cell-trapping platform [1]. This system, however, can only be used for cultivating isolated cells, without offering the possibility to sort or select cells of interest for further analysis. Another proposed single-cell culture system involves dielectrophoresis (DEP) [2]. A 'ring-dot' electrode geometry in a row/column array format has been used on a microfluidic cell-sorting chip to capture and retain the individual cells above the dot by pDEP, and release the targeted single cell by simply switching off the stimulus applied at the corresponding row/column electrodes [3]. The pDEP, however, appears to damage the trapped cells, which evidently affects cell proliferation.

Another strategy employs the hydrodynamic force (negative pressure) to capture single cells at the entrance of narrow channels [4]. Although this chip has been used for electroporation with low-throughput trapping and is unable to selectively release the single cells, it offers the potential to integrate more trapping sites and to achieve stable immobilization of various single cell types of different size. We developed a simplified fabrication processes and integrated the electrical components on the chip to realize the selective cell-releasing function. The experimental results based on yeast cells demonstrate that our microfluidic single-cell culture chip enables trapping of single cells, which are subsequently cultivated and can be selectively released.

THEORY

The microfluidic cell culture device and the principle for individual trapping, cultivation and selective release of single cells are schematically illustrated in Figure 1. The microfluidic chip consists of a cell culturing channel and two sucking channels that are located beside it. Several orifices that connect the cell culturing and sucking channels are placed along the flow, which results in an array format of cell-immobilization sites located at these orifices in the cell culturing channel, as shown in the three-dimensional close-up of Figure 1a. The cells are loaded by introducing the cell suspension at the inlet of the culturing channel using a conventional syringe pump. To generate a sufficiently high sucking force in order to achieve reliable cell immobilization, a controlled negative pressure is applied through these orifices in the cell culturing channel wall via the sucking channels. After immobilization of single cells, cells are cultivated under constant perfusion.

Since this microfluidic device is designed to generate a local nDEP force that repels the respective cell from the immobilization site in order to release a cell of interest, each orifice is further equipped with a microelectrode (shown in red color in Figure 1), which is situated under the orifices. In the center of the cell culture channel, there is a long common electrode. Therefore, an AC voltage is applied between the microelectrode at the corresponding orifice and the long common electrode. Due to the specific geometry of the design, a non-homogeneous electric field is generated, with a strong electric field located around the microelectrode under the trapping orifice. The non-homogeneous electric field produces the corresponding DEP force on the cells through polarization (Figure 1b).

The DEP is a phenomenon, in which a force is exerted on a dielectric polarizable particle when it is subjected to a non-uniform electric field; cells are typical dielectric particles [5]. The DEP can produce a positive force (pDEP) that propels the particle towards the region of strong electric field or a negative force (nDEP) that repels the particle from the regions of

strong electric field. The force direction depends on the frequency of the applied AC voltage and the conductivity and permittivity of the particle and the surrounding medium. When the cells are less polarized than the medium, the DEP force repels the cell from the region of strong electric field at the trapping site (Figure 1b). After release, the selected cell is flowing along the cell culturing channel towards the outlet for further analysis.

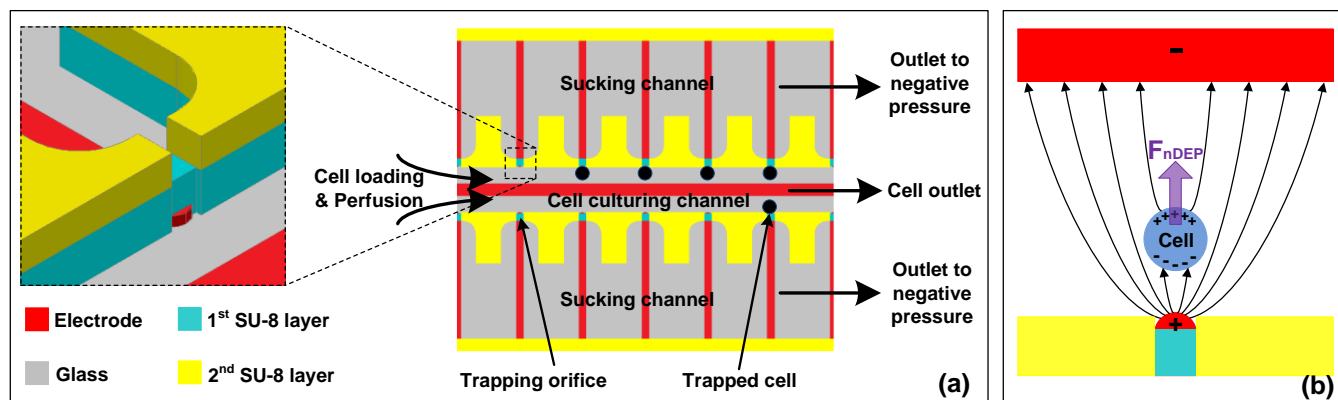


Figure 1: Schematic top view of the microfluidic cell culture chip without PDMS cover for better visibility (a) and nDEP force moving the cell at the trapping orifice towards the region of lower electric field (b)

EXPERIMENTAL

The microfluidic chip is fabricated using a two-layer SU-8 process on a glass substrate, as shown in Figure 2. First, Pt electrodes are patterned on a 4-inch Pyrex wafer using a common lift-off metallization process (Figure 2a). Afterwards, the first layer of SU-8 (Figure 2b) with a thickness of 25 μm is spin-coated on the Pt-patterned wafer, soft-baked on a hotplate and exposed to UV light in order to define the main fluidic channels. After the post-exposure bake (PEB), a second layer of SU-8 (Figure 2c) with a thickness of 5 μm is directly spin-coated onto the first, undeveloped SU-8, again followed by a soft-bake and an exposure under precise alignment with respect to the electrode structures by means of a mask aligner. Then, both SU-8 layers are developed to realize both main fluidic channels (30 μm depth in total) and cell-immobilization orifices (5 μm depth). The diced wafer is then irreversibly bonded to an unstructured PDMS cover, which seals the microfluidic channels (Figure 2e). For a tight seal, the SU-8 surface of each chip must be modified with 3-aminopropyltriethoxysilane (APTES) by vapor phase silanization (Figure 2d) [6].

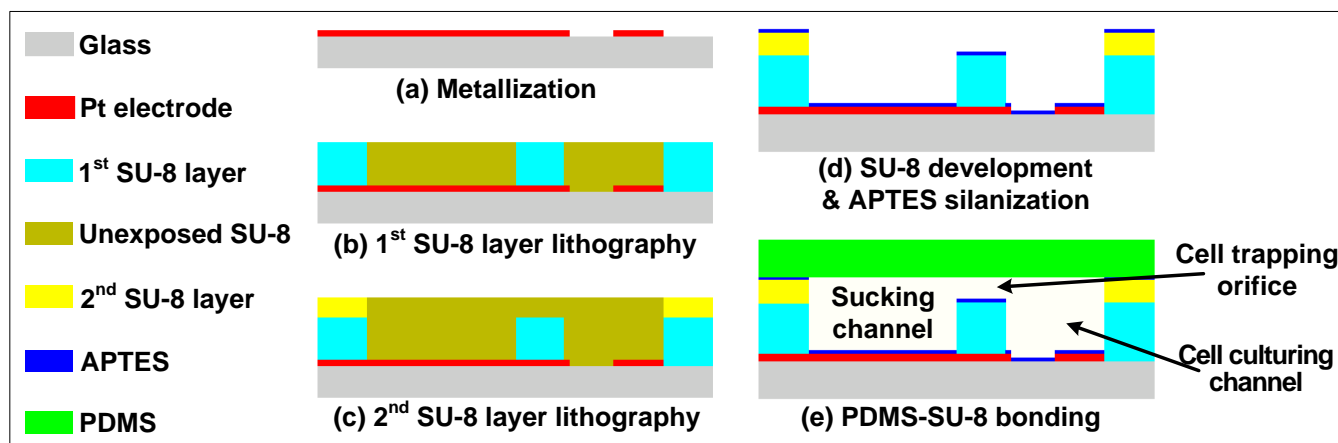


Figure 2: Fabrication process

We performed the biological experiments with yeast cells to illustrate the functionality of the microfluidic single-cell culture chip. First, the bonded microfluidic chip is placed on an aluminum holder, and the chip is screwed tightly onto the holder by a PMMA cover-flat with holes, through which tubings connect the inlets and outlets of the chip to the corresponding fluidic control units. The cell suspensions or media are loaded in syringes and are then injected into the cell culture channel with a controllable continuous-flow by the dedicated syringe. Then, single yeast cells are individually trapped by a sufficient negative pressure in the sucking channels. The negative pressure is optimized manually by a pressure controller, since it highly depends on the flow rate of the cell suspension delivery. To implement the release of selected cells

by the nDEP force, the electrode pads are electrically connected to a printed circuit board (PCB) with switches that enable the activation of the AC voltage from the signal generator. During the experiment, continuous imaging of the cell trapping, proliferation and selective release is performed by using a CCD camera.

RESULTS AND DISCUSSION

In Figure 3, the flow rate of the cell suspension was $0.1 \mu\text{l}/\text{min}$ and the optimized sucking pressure was -20 mbar . As a result of the fluidic condition, both of the immobilization sites captured single yeast cells, and the cell trapped on the left site was a budding yeast cell, as shown in Figure 3a. Afterwards, we stimulated the electrode under the right immobilization site by applying a $20 V_{pp}$, 5 MHz AC voltage (the long common electrode was grounded and is not shown for the reason of space), which generated a nDEP force to repel the cell from the original position, pushing it then into the fluid flow (Figure 3b). Subsequently, the left electrode was stimulated with the same signal as the right one, which released the budding yeast cell from its immobilization site (Figure 3c). This demonstrates the overall process of trapping and releasing single yeast cells and proves the functionality of this microfluidic single-cell culture chip.

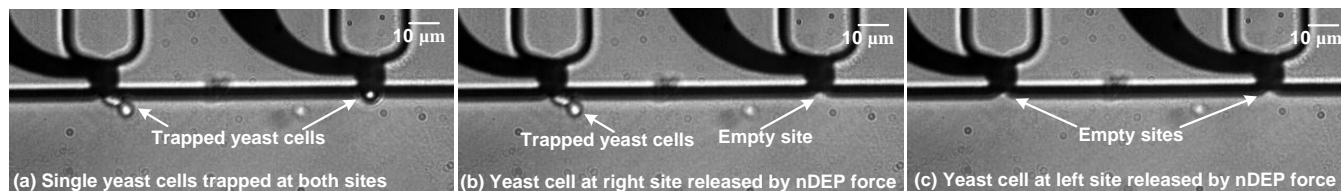


Figure 3: Selective release of trapped single yeast cells (budding yeast cell at left site) by nDEP force

CONCLUSION

We presented a microfluidic cell-culture chip that integrates the functions of trapping, culturing and selective release of single cells. The chip, based on SU-8 microfluidic structures, was fabricated on a glass substrate with electrodes and was bonded to a PDMS cover for sealing. PDMS is transparent for optical imaging without fluorescent staining and SU-8, serving as microfluidic channels, helps to precisely align the pre-patterned structures. Yeast cells were used in the experiments as cellular sample. Cells were isolated and captured in a microfluidic channel through mild suction at the narrow orifices, then cultivated under controlled perfusion with cell culture medium. Immobilized cells were selectively released by applying nDEP force via microelectrodes at the trapping orifices. The experimental results with yeast cells successfully demonstrate the functionality of our microfluidic single-cell culture chip, which constitutes a promising approach to single-cell cell manipulation and analysis.

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