MICROFLUIDIC CHIP FOR ACTIVE AND AUTONOMOUS SINGLE-CELL ISOLATION BY USING DIELECTROPHORESIS AND IMPEDANCE MEASUREMENT

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ABSTRACT

This paper presents the design, fabrication, and characterization of a microfluidic chip to manipulate and sense a cell and a microbead by DEP and impedance detection method. Combining deflective dielectrophoretic barriers with controlled pressure-driven liquid flows allows the accurate control of a cell/microbead in suspensions. An MCF7 cell and a polystyrene microbead were successfully isolated in the trapping chamber at the proper DEP conditions The impedance change caused by the blockage of the electrical conducting path between sensing electrodes with the trapping of an MCF7 cell and a polystyrene microbead was measured.

KEYWORDS: Dielectrophoresis, single-cell, impedance spectroscopy, microfluidics

INTRODUCTION

Many researches have been reported for manipulation of cells in single-cell level in order to apply to chemical and biological analysis [1]. Recently the active control of single-cell using dielectrophoresis (DEP) has been developed for single-cell isolation into a pocket structure [2]. However, the needs for eye monitoring using optical microscope and manual control of electrical signal hinder the automation of microfluidic system. In this work, we propose a new scheme for active manipulation of single cell using DEP with electrical monitoring of cell isolation into specific chambers for automatic microfluidic assay system.

THEORY

Figure 1 shows the schematic view and operation principles of the proposed microfluidic chip. The chip is composed of trapping chambers connected to the main channel, DEP electrodes, and sensing electrodes. First, cells are introduced into inlet and move through main channel. When a cell arrives at 1st Actuation electrodes, it is forced into trapping chamber by negative DEP (a → b → c → d → e). Then, trapping of the cell can be electrically monitored by measuring the impedance change at sensing electrodes after the positioning of the cell between two sensing electrodes. Next, the DEP on 1st actuation electrodes is turned off when impedance change was monitored at the 1st sensing electrodes. Then the next cell passes the 1st trapping chamber (a → b → f) and captured into the following trapping chambers by the same method.

The strength of DEP force depends on the permittivity of the medium and the particles, the size of the particles, and the frequency of the electric field.[3] Because the planar actuation electrodes are deposited on bottom surface of microchannel, the particle will experience both of lateral deflection and vertical lifting force by the combination of negative DEP and hydrodynamic force. When a particle is pushed to the side of microchannel and arrives at the ending region of actuation electrodes, it is pushed into trapping chamber by negative DEP force.Then, the trapping of a cell is monitored by measuring the impedance between the sensing electrodes. When a cell is placed between electrodes, the cell membrane and the intercellular medium create additional impedance, which is much larger than the impedance of the culture medium or Phosphate buffered saline (PBS). Thus, the cell trapping can be electrically monitored by measuring the impedance between the sensing electrodes.

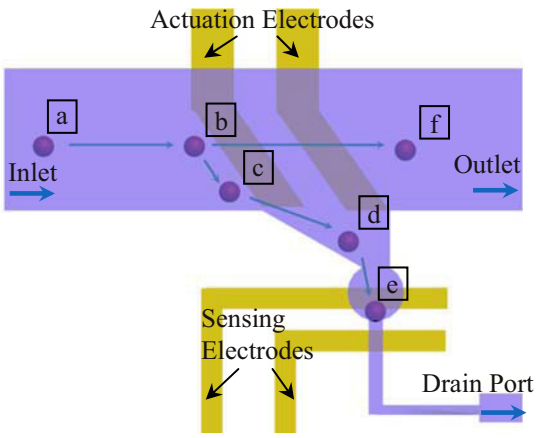

Figure 1. Schematic diagram of proposed microfluidic chip

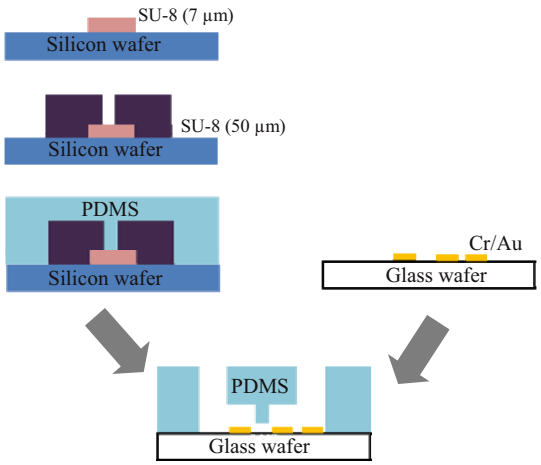

Figure 2. Fabrication process of proposed device

EXPERIMENTAL

Figure 2 shows the fabrication procedures, which is simple PDMS replica molding method. To form two different channel thicknesses, we used the double exposure and single development technique [4]. For the glass substrate, a Cr/Au (200 Å/2000 Å) layer was deposited and patterned by conventional wet etching process with AZ1512 as a masking layer to form electrodes. Then, a PDMS prepolymer (Sylgard 184, Dow Corning Co.) was poured onto the mold structure and inherent bubbles were removed in a vacuum chamber. Finally, the PDMS was cured at 80°C for 1 h and peeled off from the substrate mold. The access holes for the sample inlet, outlet, and drain port were formed by manual punching. Finally, the completed PDMS structure was bonded with the glass substrate after surface treatment by using oxygen plasma treatment.

Figure 3 shows the fabricated microfluidic chip and a magnified view of a microchamber. The width and height of the main channel were 50 μm and 50 μm, respectively. The drain channel was located at the bottom of the trapping chamber, which had dimensions of 10 μm width and 7 μm height. The actuation electrodes were placed at the entrance of the trapping chamber to guide the cell into the chamber. For sensing electrodes, one electrode was placed in the trapping chamber and the other in the drain channel to maximize the impedance change when a cell was positioned between these electrodes.

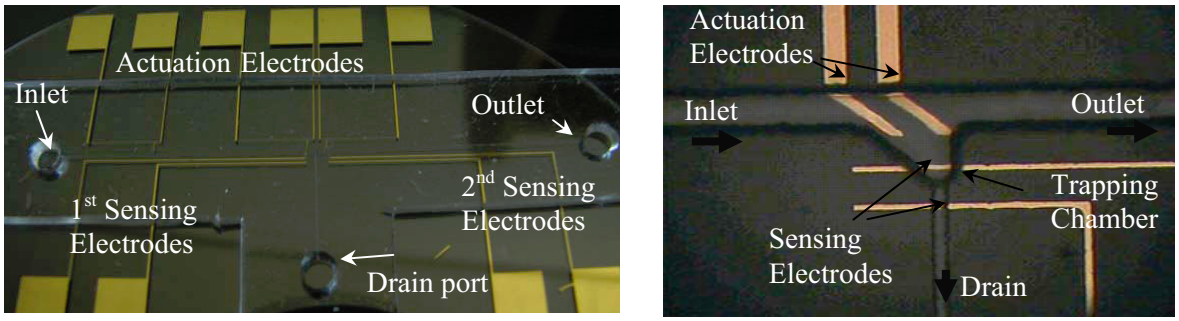

Figure 3. Fabricated microfluidic chip and magnified view.

RESULTS AND DISCUSSION

Figure 4 shows the sequence of single cell capturing experiment into trapping chamber by using MCF7 cells in medium. The flow velocity was 500 μm/s and the voltage applied on Actuation electrodes was 10 V_{pp} at 10 MHz. We were able to clearly monitor that a cell was carried toward the trapping chamber and exactly positioned between the sensing electrodes when the negative DEP force prevailed over drag force.

Figure 4. Sequence of capturing single cell (MCF7).

Figure 5 shows the measurement result of MCF7 and microbead experiments. The impedance was measured at 1 V_{pp} with the LCR meter. For cell experiment, the average of initial impedance was 11 MΩ, that was increased to 12 MΩ (~ 10% change) after a cell was positioned between the sensing electrodes, because the cell blocked the electrical conducting path between the sensing electrodes. For the microbead experiment, the impedance was increased by 0.4 MΩ (5% change) after trapping. The impedance change after microbead trapping was small compared to the cell experiment. For the cell experiment, the drain channel had been tightly blocked by the cell. However, the sealing was not perfect for the hard polystyrene microbead experiment, resulting in an electrical conducting path through the media around the microbead.

Figure 6 shows the results of impedance measurements at various frequency ranges before and after trapping of the cell and the microbead, respectively. The impedance measurement over wide frequency range

revealed that the impedance decreased as the frequency increased because of the double layer capacitance on each electrode.

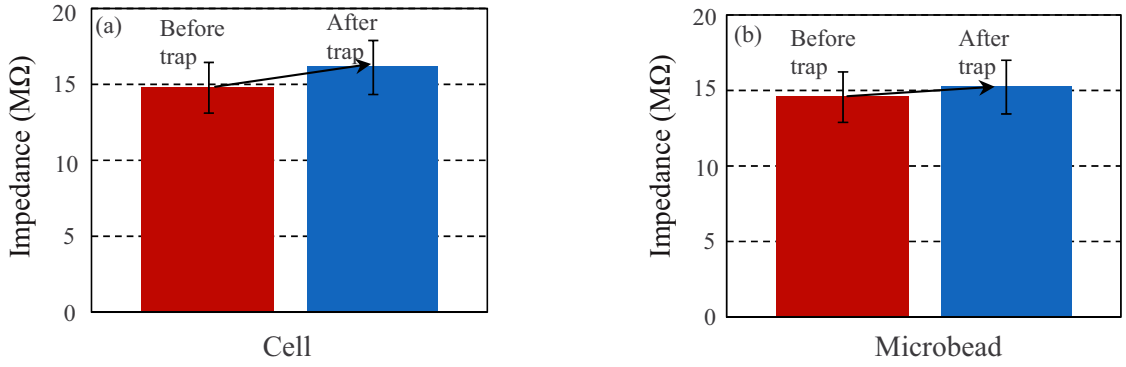

Figure 5. (a) Average impedance values before and after trapping of cell. (b) microbead.

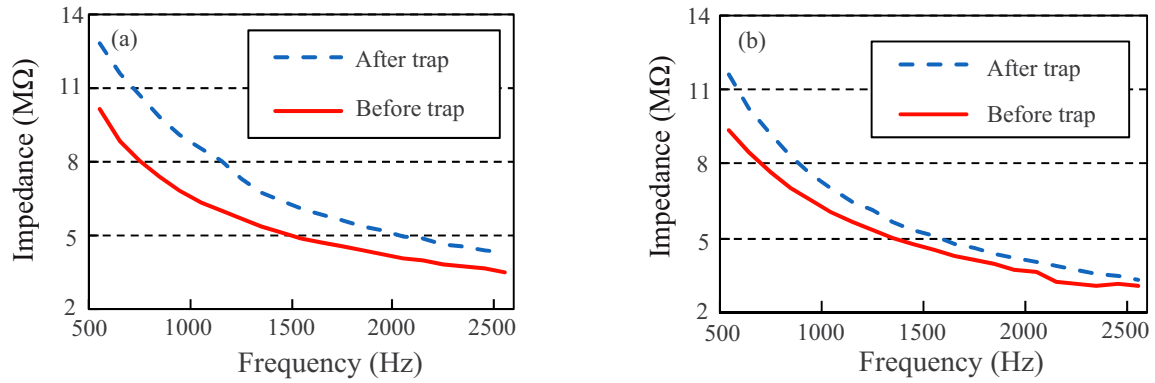

Figure 6. (a) Impedance measured at various frequency ranges before trapping, after trapping of cell, and (b) impedance measurement results of polystyrene microbead experiments.

CONCLUSION

In this experiment, a microfluidic chip to isolate a single cell or a microbead was developed. The cell and microbead were electrically manipulated by DEP actuation applied on parallel planar electrodes in a fluidic channel. The drain channel design and sensing electrodes placed in the trapping chamber allowed electrical monitoring of single-cell isolation into desired locations. The impedance change was clearly monitored during the trapping and releasing of the cell and microbead.

ACKNOWLEDGEMENTS

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