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On-chip Temperature Sensing and Control for Cell Immobilization

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***Abstract*— In this study, a temperature sensing and controlling microfluid chip has been developed for cell immobilization using a thermo-sensitive hydrogel (PNIPAAm). The PDMS-based micromagnetic stirrers make microscale fluid mixing to provide the temperature stability in the microchannel. The ITO (Indium Tin Oxide) microheaters and thermosensors, fabricated by micromachining technology, perform *in situ* fluid heating and feedback temperature control. All temperature sensing and controlling devices are integrated on a chip, in which yeast cell immobilization is performed by the gelation of the PNIPAAm solution.**

Keywords- cell immobilization; ITO; PDMS; stirrer; temperature

I. INTRODUCTION

Recent advances in biotechnology rely on the developments of micromanipulation for biochemical experiments. In applied microbiology, individual cell-based diagnosis and pharmaceutical testing are crucial techniques especially in the livestock industry [1]. Despite great interest in analysis, diagnosis and manipulation of single biological cells, most biomanipulation tasks, such as gene and cell injection, are done manually by experienced operators with microscopic information. In order to achieve easier, safer and more reliable cell manipulation, many researches have been studied promising automated on-chip micromanipulation. For example fluid force, electrophoretic force, dielectrophoretic force, optical tweezers and magnet force are often used to manipulate cells in the microchip [2-7]. Flow cytometry is widely used for single-cell separation [8-9] because mechanical micromanipulator is difficult if the sample cells are suspended in a liquid. However the sequentially separation processes, it is impossible to compare multiple objects simultaneously before separation, and positional information is lost by separation. A novel micromanipulation method is recently presented to isolate the target by thermal gelation, in which a 2D projected pattern of the IR laser using the DMD (Digital Micromirror Device: Texas Instruments) is used for local heating of the multiple microheaters on a microchip [10]. However, the local temperature is uncontrolled and unstable due to the microchannel flow. For the present study, in order to achieve temperature sensing and control without externally complicated laser system, temperature sensing and control microdevices are developed on a chip and yeast cells are successfully immobilized.

To observe and operate the cells in a microchip, it is very important to use the transparent materials. ITO is the generally utilized as transparent electrode because it is easy to use and has a very good adhesion to glass. In order to obtain a temperature uniformity in the micro channel, the manipulation of a mixing device is required. Some work has been done on manipulation using magnetic force [11-17] in light of harmless to cells which is easily implemented onto a microchip at low cost. The micro-magnetic microdevices such as valve pump, stirrers, filters and sorter can be controlled by external magnet force which is mounted outside of the microchip and microchips were disposable by making the electromagnet detachable. The present work has proposed that a novel on-chip PDMS-based micromagnetic tools which has merits to use its "softness" to apply to cells and other biological purposes, also it is difficult to be rusted by immersing in the fluid in the microchannels.

In this study, we proposed a temperature sensing and control microchip using ITO and magnetically driven microdevices for cell manipulation. These microdevices are fabricated directly inside the microchannel, so additional chips are not necessary-to be added to the physical package, thus reducing the overall cost and volume.

II. DESIGN

Fig. 1. shows the schematic view of the temperature-controlled microchip which is integrated with a micro stirrer, microheaters and thermosensors.

The micro stirrer rotates by the electromagnets which was mounted one of the branch path of the upstream of Y-shaped micro channel, where a thermo-sensitive hydrogel PNIPAAm and culture fluid are mixed and the coarse temperature adjustment of the fluid has been carried out. Downstream of the micro stirrer, the flow is mixed with the cell flow (cells + DI water) from the other side of branch of Y-shaped micro channel preventing any contaminations. Then the flow is heated by the

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microheaters at the main path of the Y-shaped micro channel. The microheater is mounted along the main path of the micro channel and thermosensors are mounted next to the mocroheater, so that the temperature adjustment can be achieved along the main path of the microflow by the feedback temperature controlling. The cell immobilization can be achieved by using this temperature sensing and control micro chip.

III. FABRICATION

The temperature sensing and control chip is fabricated using micromachining process, and the magnetically driven micro stirrers are made by a mixture of PDMS and magnetite particles. The ITO microheater, thermosensors and micro stirrer are fabricated, respectively. And then all of them are integrated with an in-channel PDMS microcircuit on the same substrate.

Fig. 2. shows the process flow of the temperature sensing and control circuit, which is started with a 150-μm-thickness ITO-sputtered glass plate. The ITO-sputtered glass plate is patterned by photolithography (a1), and then the heaters and thermosensors are defined by both wet and dry etching methods (b1). On the other hand, the micro channel is patterned on a Si wafer by photolithography using a thick film resist SU-8 (a2). And then PDMS is molded by this wafer (b2) and bonded to the patterned ITO-sputtered glass plate using plasma bonding technique (c).

In wet etching process of ITO, $FeCl_3$ is used as the etchant. With a water bath heating, the etching rate is about 6 Å/min. In dry etching process, a homemade reactive ion etching (RIE) equipment using Xe gas is employed. The etching conditions are controlled with a gas pressure of 0.2 Pa, a stage-temperature at 20 °C, and the RF power of 90 W. The etching rate of ITO is approximately 350 Å/sec, and the selectivity of photoresist to ITO is 5. Wet etching of ITO is widely used in industry because of its low cost, excellent selectivity, simplicity and large yield. However, the isotropic etching may cause undercutting problem that is not suitable for small dimension pattern. Dry etching can perform an excellent anisotropic etching, while for a thick film, a high selectivity mask or a thick photoresist should be employed. For different applications, the suitable etching method can be selected.

Fig. 3. shows two methods to produce micro stir bars which are made of a mixture of 50 wt% PDMS and 50 wt% magnetite.

The first method use SU-8 photoresist in order to make pattern on the Si-wafer, the wafer is used as the mold for soft lithography by PDMS. The surface of PDMS mold is activated by plasma process [18] in order to remove stir bars easily. The second method use KMPR photoresist. This magnetite-mixed PDMS is put directly to the photoresist-patterned Si-wafer, and then KMPR photoresist is dissolved by the stripper therefore the stir bars come out from the Si-wafer and collected by a large stir bar by magnetic forces. By using these techniques many kinds of magnetic micro-stir bars can be produced. Finally the surface of the micro stirrers are processed by platinum sputtering or Tefron coating by C_4F_8 gas in order to remove any stiction between PDMS.

The fabricated temperature-controlled microchip is shown in Fig. 4. The width of the microheater and thermosensor are 30 μm and 15 μm, respectively. And both of them have a thickness of 350 nm. Two kinds of representative stir bars (plus, 2-wings) have also fabricated with a diameter of 1500 μm and placed in a 2000 μm-diameter chamber.

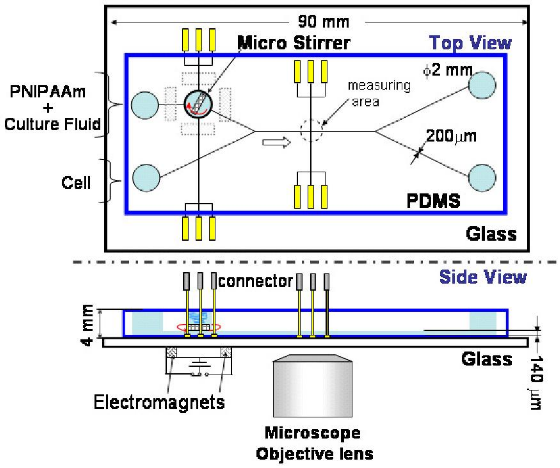


Fig. 1. A schematic view of temperature sensing and control microchip.

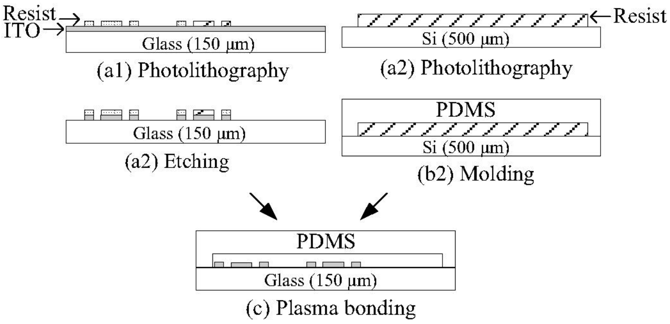


Fig. 2. Process flow of the temperature sensing and control microfluid chip.

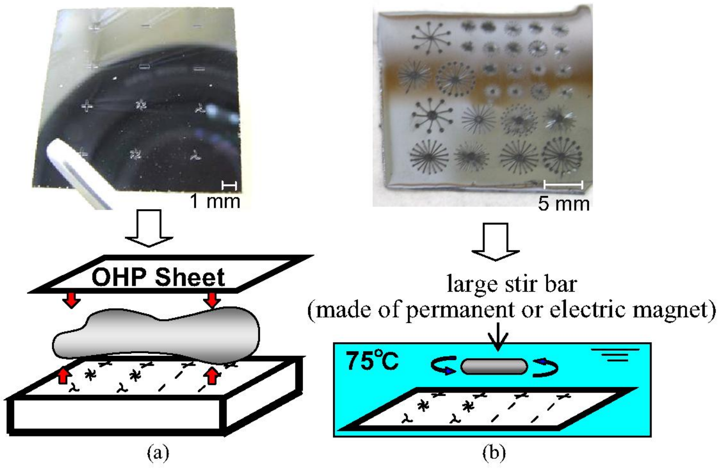


Fig. 3. Processes to produce series of micro magnetic-stir bars using Si wafer patterned with (a) SU-8 and (b) KMPR photoresists.

IV. CHARACTERIZATION

In the temperature sensing and control chip, the characterization of micro thermosensor, ITO heater, and micro magnetic stirrer are investigated.

A. Micro thermosensor

The principle of the thermosensor use the temperature dependent property of the electrical resistance. A calibration data, the temperature dependence of the electrical resistance of ITO thermosensor, is determined as shown in Fig. 5., in which the width of the sensor is 15 μm. The resistance was measured by using the four probe method and the temperature was controlled with a temperature controlled bath. The four probe method consists of two current carrying probes and two voltage measuring probes, and it is preferable to measure the resistance of the thermosensor because of the advantage of showing no regard for the contact and spreading resistances.

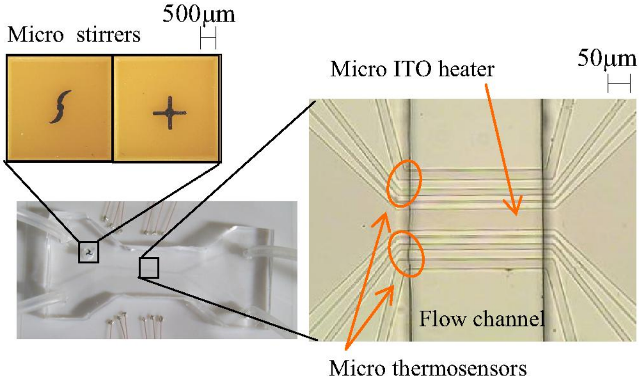


Fig. 4. Fabricated temperature-controlled microchip with the microheater, thermosensors, and micro stirrers.

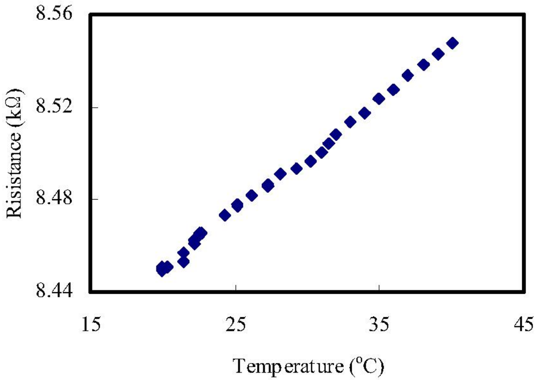


Fig. 5. Temperature dependence of the electrical resistance of ITO thermometer in air.

B. ITO heater

The ITO heater has been made on a glass plate, which is a transparent, conducting material and therefore can form a resistive heater distributed over the surface of the substrate while still allowing light to pass through.

Passing an electric current through the ITO electrode generates heat due to the larger resistance. The temperature increases with the applied voltage enhancing to the ITO heater. The gelation situation in the flow channel under various voltages is shown in Fig. 6. By enhancing the applied voltage, the temperature increase, and eventually produce the various areas of gelation.

C. Micro Magnetic Stirrer

The micro-stirrer is actuated by placing the micro stir bars on a rotating couple of disk-shaped ferrite magnet (130-550mT each). The rotation speeds are measured using a laser tachometer (DT-2234BL). The fluid flow through the micro-channel is set-up using controlled micro-syringe pump (Kd-Scienctific model 230) which generated a pressure flow with a flow rate set to 5μLmin^{-1}.

Fig. 7. shows the rotating speed of the stir bars and motor on which the disk shaped ferrite magnets are attached. Two kinds of representative stir bars have been examined. It was observed that stir bar rotated following the rotation of magnet for most of the cases. Also, it was confirmed that the micro stirrer can rotate at least 5 hours without any stiction between PDMSs. It is expected to keep steady mixing condition for long hours enough to culture cells.

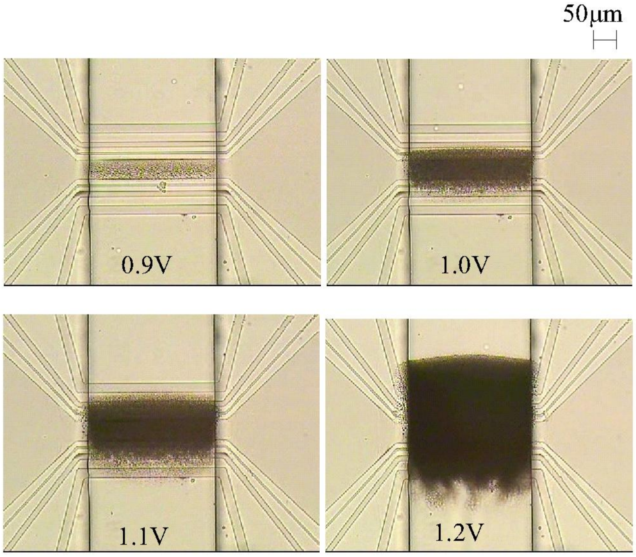
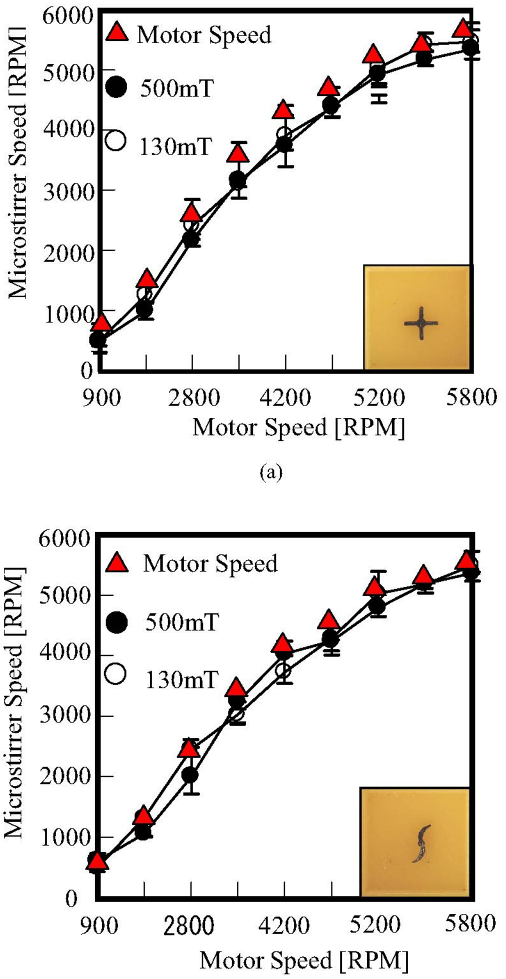


Fig. 6. Fabricated temperature-controlled microchip with the microheater, thermosensors, and micro stirrers.



(a)

(b)

Fig. 7. Rotating speed of micro stir bars (a) plus and (b) 2-wings shapes, and motor on which a couple of ferrite magnet are attached. Open circle indicates the rotation speed of stir bar with lower magnet force (150mT) and black circle indicate higher magnet force (500mT). The red rectangular indicate the rotating speed of motors.

V. CELL IMMOBILIZATION

The cell immobilization experiment was carried out with the temperature sensing and control chip. Fig. 8. exhibits that a yeast cell is immobilized when PNIPAAm solution heated by the ITO microheater.

Firstly, PNIPAAm 5% solution and yeast cells were injected into the microfluid channel. Then, PNIPAAm solution gelated while it heated over 32 °C [19] by the ITO microheater with a direct electric current. At the same time, the temperature can be detected by measuring the resistance change of the thermosensor. A temperature control can be obtained by using a feedback temperature controlling.

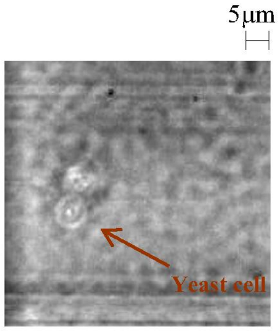


Fig. 8. Immobilization of a yeast cell with gelation of PNIPAAm 5% solution in the microchannel.

VI. CONCLUSION

On-chip micro-mixing and temperature sensing and controlling microdevices were developed for the present study in order to achieve temperature sensing and controlling without mounting externally complicated system such as lasers. By using this machine, yeast cells are successfully immobilized efficiently with temperature control. The proposed method can be applied to various biological and chemical applications.

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