A Micro Cell Counter Integrated with Oxygen Micropump

Sang Uk Son*, Yo Han Choi*, and Seung S. Lee*,**

* Department of Mechanical Engineering, Pohang University of Science and Technology, Pohang, Korea
(Tel : +82-54-279-8210; E-mail: chairson@postech.ac.kr)
**Department of Mechanical Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Korea
(Tel : +82-42-869-3046; E-mail: sslee97@kaist ac.kr))

Abstract: This paper describes fabrication of a micro cell counter integrated with an oxygen micropump and Sephadex G-25 beads counting experiment. The device utilized a phototransistor, microwindow, and light source of microscope for beads detection. Microheater and microchannel were used for pumping and guiding of beads to the microwindow. Counting capability of the device was tested with a peristaltic pump and the measured signals (~10 mV) with oscilloscope showed peak shape when beads passed the microwindow. Pumping of beads by the oxygen micropump was carried out by heating paraffin, which enveloped manganese dioxide (catalyst), to trigger the decomposition of hydrogen peroxide into water and oxygen. It lasted for 5 min with 7 μl of wt. 30 % hydrogen peroxide. Beads counting by oxygen micropump showed peaks (2~20 mV) with 30 μl of beads sample and the number of peaks by magnitude was acquired.

Keywords: micro cell counter, integration, oxygen micropump, paraffin, microheater

1. INTRODUCTION

A micro cell counter has been researched as a diagnostic apparatus in medical field or an experimental equipment in biological field for quick results at low cost instead of commercial cell counters like fluorescence activated cell sorter which is desk-top machine-sized, cumbersome, and expensive in operation [1,2]. Cell transport is important function in cell counter and the commercial products adapt pneumatic devices whereas the use of a syringe pump has been general in the micro cell counter [3-5]. Recently, as alternatives, various methods based on microvalves [6-9], electroosmosis [10,11], dielectrophoresis [12], magnet [13,14], or gas [15-17] have been researched to implement on-chip fabrication. The methods can replace the external syringe pump, however still needs external equipments and consumes electric energy during operation. The use of high frequent (hundreds kHz) electric field by dielectrophoresis may also damage on the delicate cell membrane. Therefore new cell transport method meeting the conditions of on-chip fabrication, low energy consumption, and compatibility with cell vitality is required.

In this paper, we implemented integration of an oxygen micropump with the micro cell counter. It utilized hydrogen peroxide which decomposes into water and oxygen when heated or in the presence of light, dust particles, or certain metals [18]. The use of the hydrogen peroxide has been wide and several papers reported the available use of it as pumping source [16,17]. The decomposition was carried out by catalyst which was enveloped in paraffin and separated from the hydrogen peroxide until the paraffin melted by heating. The volume of oxygen reached to 2.8 ml with 7 μl of wt. 30 % hydrogen peroxide, which was enough to pump the 30 μl of cells (beads) suspension. For experiments, Sephadex G-25 beads ranging from 30 to 100 μm in diameter were used for pumping and counting. To generate the signals when the beads passed the detection area, light source of microscope and phototransistor were used. Generated signals were measured with an oscilloscope and analyzed for the distribution of peaks by magnitude.

2. PRINCIPLE AND DESIGN

Fig.1 describes the principle of pumping by oxygen generation and cell detection. In this paper manganese dioxide is used as catalyst and enveloped in paraffin for triggering the decomposition, which is carried out by melting the paraffin with the microheater. After the activation, oxygen generation continues and forms boundary against cells suspension. The cells flow in the microchannel by the inflated oxygen boundary and pass the microwindow through which the light which illuminats the phototransistor. Variation of illumination is converted to electric signal of voltage using load resistor and measured with an oscilloscope.

The microheater is placed on a glass plate due to its transparence for the illumination to the phototransistor. Space for the manganese dioxide enveloped in the paraffin and the hydrogen peroxide is designed to be 2 × 3.5 × 0.5 mm^3. The microwindow is made with silicon nitride membrane for the illumination, and is designed to be 100 × 100 μm^2 considering the bead size. The microchannel and reservoirs for beads suspension are fabricated with PDMS (Polydimethylsiloxane) due to its transparence and the height is designed to be 100 μm also considering the bead size. A meander is placed between hydrogen peroxide and beads suspension reservoir to make the oxygen form boundary easier, and a branch is added to the meander for injection of the beads (Fig. 3). The microchannel is designed to be trapezoidal and the same dimension of 100 μm width near the microwindow to make the beads pass inside the microwindow.

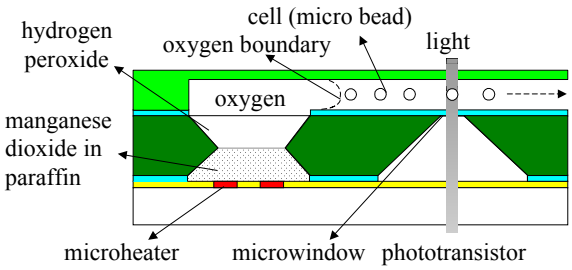

Fig. 1 Schematic view

3. FABRICATION

Fig. 2 shows the fabrication process for the microwindow (A), microheater (B), and microchannel (C). The fabrication started with a 2 × 2 cm^2 (100) n-type silicon wafer. A 1.5 μm thickness of low stress silicon nitride layer was deposited on

the silicon wafer by LPCVD and patterned using RIE for a reservoir and a 100 × 100 μm^2 membrane. The silicon layer was etched in wt. 25 % TMAH at 90 ℃. A 150/3000 Å thickness of Cr/Au layer was deposited on a 2.2 × 2.2 cm^2 glass by thermal evaporation and patterned for the microheater which was measured 240 Ω. For electric insulation a 0.8 μm thickness of SOG (Spin-On-Glass) layer was spin-coated and cured. To open the pads the SOG layer was patterned and etched using RIE. For a mold having the reservoirs and microchannel, SU-8 50 negative photoresist was spin-coated with height of 100 μm and baked at 65/95 ℃ for 10/30 min. The SU-8 layer was patterned through UV expose of 500 mJ/cm^2, bake at 65/95 ℃ for 3/10 min, and development in PGMEA for 10 min. The fabricated SU-8 mold was coated with trimethyl silane (DC(R) 9-5170, Dow Corning) in a vacuum chamber over night to make the peeling off process with PDMS easy. A 1 mm thickness of PDMS layer was poured on the SU-8 mold after mixing monomer with agent by wt. ratio 10:1 and removing the bubbles from the mixed PDMS in the vacuum chamber. After curing at 65 ℃ for 5 hours in a convection oven the PDMS layer was peeled off and cut with a razor.

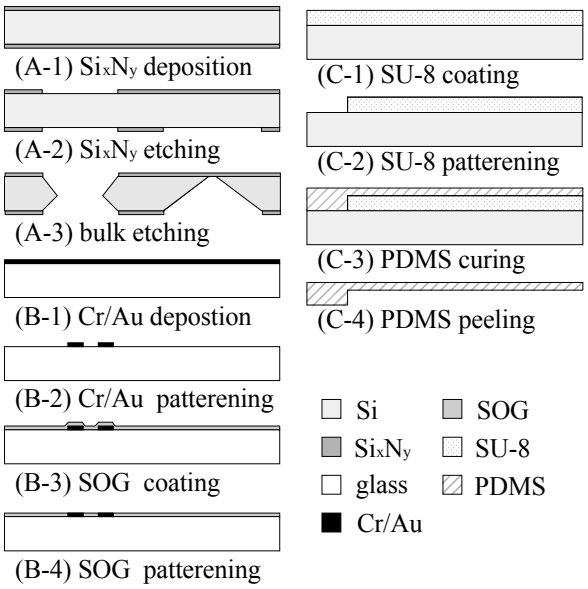

Fig. 2 Fabrication process

Fig. 3 shows the assembled device after three steps: (i) adhering the silicon wafer (A-3) to the glass (B-4) by using a spin-coated SU-8 2 layer at 5000 rpm on (A-3) as glue and curing them at 95 ℃ for 1 min, (ii) filling the reservoir by putting the debris of the paraffin (32,720-4, Sigma-Aldrich) on the powder of manganese dioxide (M3138, Sigma-Aldrich) placed on the microheater and melting the paraffin using a hot plate, and (iii) adhering the PDMS layer (C-4), which was oxidized for 1 min in a plasma cleaner (PDC-32G, Harrick), to (A-3) by aligning them using methanol as lubricant and curing them in the convection oven at 70 ℃ over night. In the assembling, the paraffin filling process required careful attention because the paraffin was easy to overflow the reservoir during melting because of its volume expansion. In the event of overflowing the silicon wafer cannot be used in successive process because the paraffin on the silicon wafer was hardly removed. The PDMS layer adhering process was also important in that leakage of the cell suspension or the hydrogen peroxide was frequent during experiment. To solve the leakage problem, adhering process should be done as soon as possible (at least within 1 hr) after peeling off and the enough curing time (more 5 hr) with high temperature (above 70 ℃) are adapted.

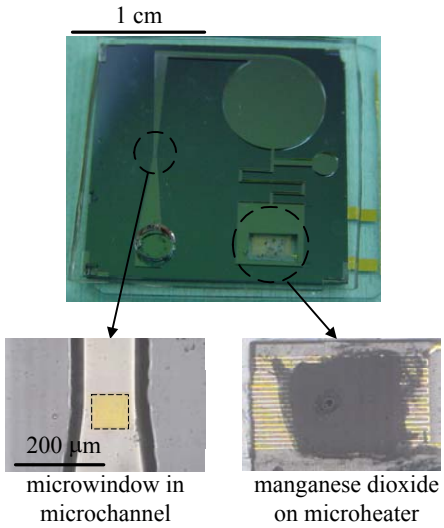

Fig. 3 Fabricated device

4. EXPERIMENTS

4.1 Bead counting test with peristaltic pump

Fabricated device was connected to a peristaltic pump and 200 μl of water with 10 μl of dyed beads (powder) with black oil ink were injected into reservoir to test the counting capability. For illumination light source of microscope was used and the illuminated area was focused on the microwindow by setting magnification to 195. Fig. 4 shows the measured signals when the beads passed the microwindow at velocity of 13.9 mm/s using phototransistor, load resistor (1 kΩ), and reverse voltage (3V) in circuit of Fig. 5. The peaks in Fig. 4 were up to 10 mV according to beads size and dyeing degree. To match bead size and peak magnitude, threshold value of voltage to bead size should be established, which is ongoing research. In the experiment microchannel clogging by beads occurred and the reasons seemed to be from high density of beads and microhannel height caused by uneven spin coating. The problems were solved by dilution and enough soft bake time.

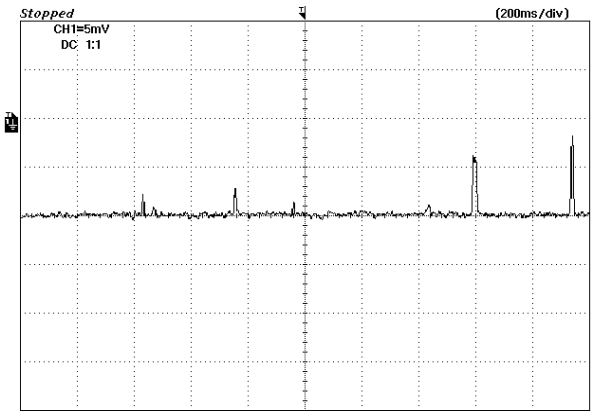

Fig. 4 Measured signal when beads pass the microwindow at velocity of 13.9 mm/s with a peristaltic pump. Reverse voltage to phototransistor was 3V and load resistor was 1 kΩ.

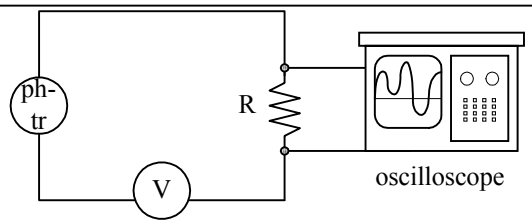

Fig. 5 A diagram showing the circuit to measure the signal with a phototransistor (ph-tr), a voltage source (V), and a load resistance (R).

4.2 Oxygen generation by catalytic reaction and beads counting

For oxygen generation, 7 μl of wt. 30 % hydrogen peroxide was injected using a syringe into the rectangular reservoir in the device (Fig. 3) carefully not to be contacted with skin. Fig. 6 shows oxygen generation and microfluid flow in the microchannel. The volume of oxygen was about 2.8 ml which was sufficient to pump 30 μl of the beads suspension. Two methods of heating were tried. One was heating the hydrogen peroxide (Fig. 7a) with power of 960 mW and the other was melting the paraffin (m.p. 54-56 ℃) for the catalytic reaction (Fig. 7b). The heating method was easy to switch the oxygen generation, however, had a drawback of continuous energy consumption whereas the catalytic reaction method needed heating once at initial stage to allow the contact between the hydrogen peroxide and the manganese dioxide. The pressure by the catalytic reaction was dependent on wt. % of the hydrogen peroxide, quantity of the catalyst, geometry of the microchannel, and the remained amount of the suspension in the reservoir. The microfluid flowed at velocity of 150 μm/s in the meander after melting the paraffin and the velocity varied according to the pressure.

Fig. 7 shows experiment with Sephadex G-25 beads (Amersham Pharmacia) ranging from 30 to 100 μm in diameter which were dyed with black oil ink. The beads were injected using a syringe into the inlet and filled the circular reservoir in the device (Fig. 3). The pre-filled hydrogen peroxide in the rectangular reservoir hindered inflow of the beads whereas relatively lower pressure in the circular reservoir which was connected to the outlet allowed the inflow. The oxygen from the rectangular reservoir passed the meander and formed boundary to the beads suspension. The boundary inflated as the oxygen generation continued and pumped the beads (Fig. 7a,b). The beads flowed by pumping in the microchannel and pass the microwindow through which the light illuminated the phototransistor (Fig. 7c,d).

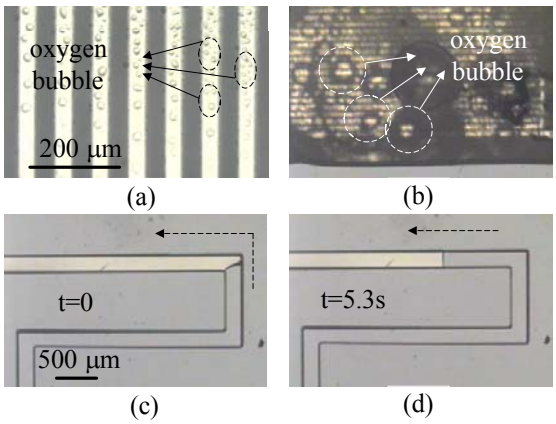

Fig. 6 Photomicrographs showing oxygen generation: (a) heating the hydrogen peroxide, (b) melting the paraffin which brings the catalytic action of manganese dioxide, and (c),(d) microfluid flowing in the microchannel at velocity of 150 μm/s.

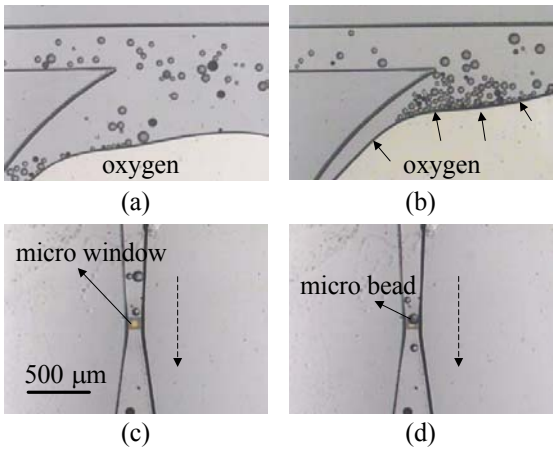

Fig. 7 Photomicrographs showing inflation of oxygen boundary (a,b) and the beads passing the microwindow (c,d).

The illumination from the light to the phototransistor varied when the micro beads passed the microwindow, which resulted in variation of the current in the circuit, and the signals were measured with an oscilloscope. As the beads entered in the microwindow the height of the signal increased and reached maximum when the all area of the micro bead was in the microwindow, therefore the signal took peak shape. Fig. 8 shows experimental result with intensified light by the magnification 390 of microscope and reverse voltage of 9V in the circuit aimed for higher magnitude. The height of peaks seemed to be related the dimension and dyeing degree of the beads provided that they passed the microwindow one by one. In the experiment, the dyeing degree of the beads was not uniform therefore the peaks did not provide the numbers of the beads by size. The cases of several beads passing together were not observed through screen due to high speed. The spaces of peaks were not uniform because of non homogeneous beads density and non constant oxygen generation rate. The heights of peaks were up to 20 mV and distribution by magnitude is shown in Fig.9.

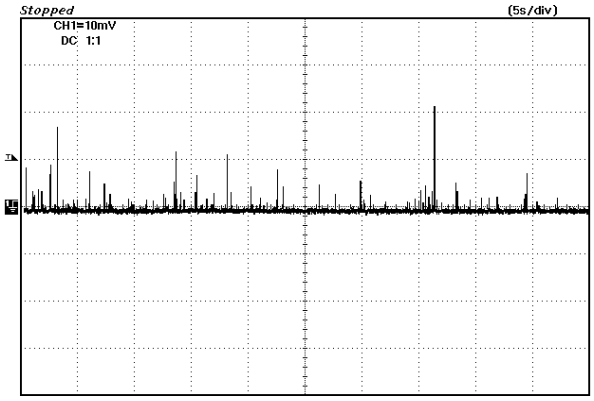

Fig. 8 Signals when 30 μl of beads sample passed the microwindow by oxygen micropump during 50s. Reverse voltage to phototransistor was 9V and load resistor was 1 kΩ with intensified light from microscope (x390).

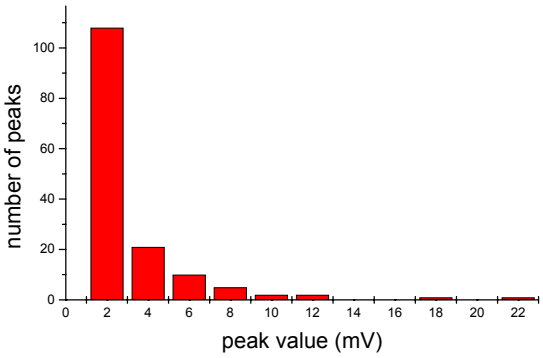

Fig. 9 Peaks distribution by magnitude.

5. CONCLUSIONS AND FURTHER STUDIES

Integration of an oxygen micropump with a micro cell counter was implemented. The micro oxygen pump generated oxygen with hydrogen peroxide by catalytic reaction and pumped beads suspension, which lasted for 5 min with 7 μl of the hydrogen peroxide. In the catalytic reaction manganese dioxide was used as catalyst and paraffin was used to envelop the manganese dioxide to enable the trigger when the paraffin melted. When Sephadex G-25 beads ranging from 30 to 100 μm in diameter passed the detector consisting of the light source of microscope, the microwindow, and the photodiode, peaks of up to 20 mV height were measured and the distribution by magnitude was acquired. The velocity of beads varied by wt. % of the hydrogen peroxide, quantity of the catalyst, geometry of the microchannel, and the remained amount of the micro bead suspension in the reservoir. The integrated oxygen micro pump had advantages of limited energy consumption and bio-compatible byproducts (water and oxygen) during operation.

Establishing threshold of signal to bead size will be studied.

ACKNOWLEDGMENTS

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