휴대용 POC 시스템을 위한 원터치형 면역 센싱 랩온어칩

論 文
56-8-12

One-Touch Type Immunosenging Lab-on-a-chip for Portable Point-of-care System

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Abstract - This paper presents a simple and reliable one-touch type multi-immunosensing lab-on-a-chip (LOC) detecting antibodies as multi-disease markers using electrochemical method suitable for a portable point-of-care system (POCS). The multi-stacked LOC consists of a PDMS space layer for liquids loading, a PDMS valve layer with 50 ìm in height for the membrane, a PDMS channel layer for the fluid paths, and a glass layer for multi electrodes. For the disposable immunoassay which needs sequential flow control of sample and buffer liquids according to the designed strategies, reliable and easy-controlled on-chip operation mechanisms without any electric power are necessary. The driving forces of sequential liquids transfer are the capillary attraction force and the pneumatic pressure generated by air bladder push. These passive fluid transport mechanisms are suitable for single-use LOC module. Prior to the application of detection of the antibody as a disease marker, the model experiments were performed with anti-DNP antibody and anti-biotin antibody as target analytes. The flow test results demonstrate that we can control the fluid flow easily by using the capillary stop valve and the PDMS check valves. By the model tests, we confirmed that the proposed LOC is easily applicable to the bioanalytic immunosensors using bioelectrocatalysis.

Key Words : Lab-on-a-chip, Capillary Attraction Force, Check Valve, Immunosensor, Point of Care

1. Introduction

Lab-on-a-chip (LOC) devices that perform the complex diagnostic procedures, such as pre-treatment, sample separation, signal amplification, and signal detection, have been realized for biochemical analysis [1]. However, the difference between academic world and real industry world puts LOC technologies unproven state [2]. The main focus of LOC systems in the academic researches is how to get the novelty or high sensitivity in the device. Sometimes they use the complex handling systems based on piezoelectric, electrokinetic, optic operating systems according to their strategies [3]. But the real industries require simplicity and reliablility for the easy control. Also the cost of the LOC devices which includes both the material and manufacturing process must be kept low [3].

For the application to the point-of-care system, the fixed instrument and the disposable part must be portable and cheap. To satisfy these viewpoints, on-chip operated mechanisms without external electric power are suitable for the real market.

The typical LOCs consist of a number of microfludic components according to their operating method. Various researchers have explored active control devices such as active micropumps and microvalves [4]. However, active microfluidic control is not acceptable in portable diagnostic systems due to high cost, difficulty in integration, complexity in fabrication and the need for an external power supply. Passive microfluidic control is viable because the fabrication is simple and the integration with other components is easy. Among the passive components, micro check valves are used as flow control components due to easy integration.

In this paper, the multi-stacked PDMS LOC integrated with the PDMS check valves are presented for the application to the disposable platform. The sequential flow of sample and buffer liquids according to strategies is controlled by on-chip operation mechanisms without any external electric power. The driving forces of liquid transfer are capillary attraction force and pneumatic pressure applied by one-touching air bladder for on-chip operation.

We applied the designed LOC to the diagnostic immunosensors integrated with biospecific electrodes. The

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接受日字 : 2007年 6月 7日
最終完了 : 2007年 7月 11日

immunosensing mechanism in this LOC is electrochemical signaling from an antigen-antibody interaction with bioelectrocatalyzed enzymatic amplification [7]. Prior to the application of detection of the antibody as a disease marker, the model experiments were performed with anti-dinitrophenyl (DNP) antibody and anti-biotin antibody as target analytes.

2. Design

For the disposable and handheld usage, materials and fluid transport methods of the device are important issues in biochip design. The key design point of this LOC is that operating power source is supplied in a chip, called "on-chip operation" without any external electric power. Figure 1 shows the schematic diagram of the handheld-immunosening system and LOC. Since immunosensing LOCs are to be disposable, cheap and reliable, the complication associated with the flow control of liquid samples needs to be removed. We use one-touch type of pneumatic pressure as an operating power source which is adaptable for handheld or portable diagnostic systems. The materials we used are PDMS which has large compliance and simple molding fabrication and easy integration.

2.1 Structure

The designed multi-stacked LOC consists of a PDMS space layer for liquids loading, a PDMS valve layer for the membrane, a PDMS channel layer for the fluid paths, and a glass layer for multi electrodes as shown in Figure 2. The micro check valves in LOC are designed with membrane-type vertical structures using PDMS. To make the check valve part and liquid inlet parts, the space layer and the membrane layer are stacked.

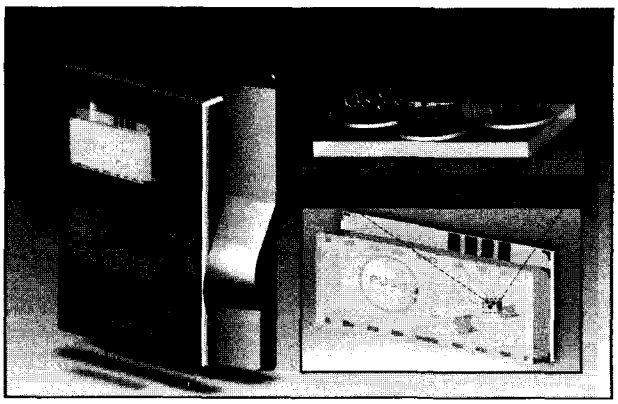
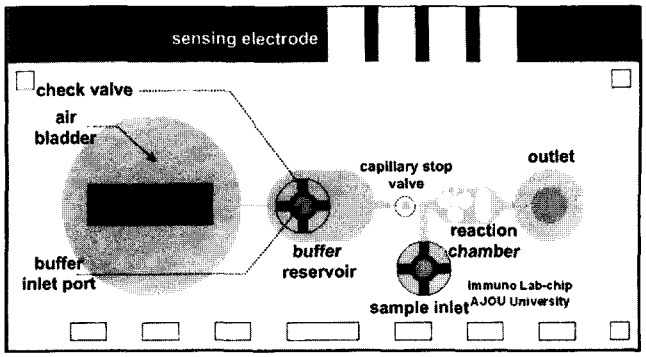
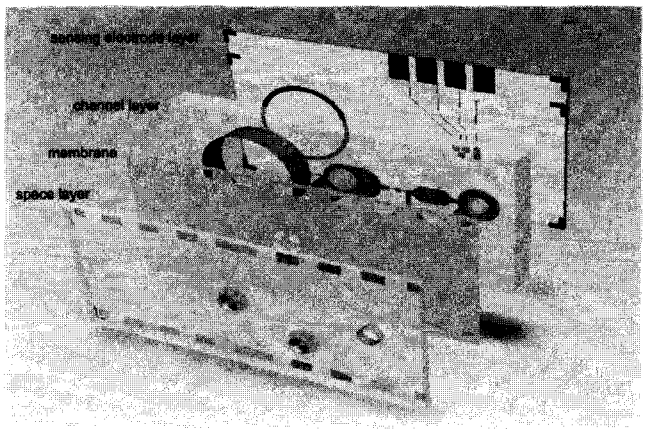


Fig. 1. Schematic diagram of the portable immunosensing system and LOC



(a)



(b)

Fig. 2. Schematic illustration of the LOC. (a) top view of LOC, (b) deal drawing

The PDMS space layer has two inlet ports for fluid loading, which are 800 μm in diameter and 1 mm in height. The PDMS valve membrane with 70 μm in height has the superior flexibility so that the membrane is deflected by small amount of driving force. We use the compressed pneumatic pressureas membrane driving force by pushing the air bladder. The channel layer has the passive microfluidic components including the two liquid channels, the sudden expanded zone between the two channels, the reaction chamber and the outlet.

2.2 Operation principle

In this LOC, the operation comprises three steps. The first step is to load the buffer solution. The second is to immobilize the antibodies in the sample liquid on the multi-electrodes and the last is to wash with the buffer solution. Sample and buffer liquids are controlled by two kinds of passive valving mechanisms which are capillary stop valve and check valves.

The capillary stop valve is a sudden expansion port which stops the buffer solution from flowing into the reaction chamber before the sample liquid filling by the capillary attraction force. The principle of valving operation is based on the pressure barrier that cross

section of capillaries change abruptly in neck and expansion regions [5]. The capillary stop valve is used as the buffer liquid reservoir valve by stopping flow of the buffer liquid at the sudden expanded zone. The external pressure are applied to overcome the pressure barrier and to reestablish a flow. The external pressure to open the valve in this device is pneumatic pressure by pushing air bladder with hand.

The main purpose of the check valve in this device is to prevent the unwanted reverse flow toward the inlet ports when pneumatic pressure is applied. Figure 3 shows the operation of the PDMS check valve. The designed PDMS check valves stop the buffer solution and the sample liquid from flowing backward when we push the air bladder to wash the reaction chamber with the buffer solution. In initial state, the sample and buffer liquid flow in the channel naturally according to their passages. On the valve-closed state when pneumatic pressure is applied, the valve membrane is attached to the inlet holes and blocks back flow of the liquids into the inlet ports perfectly. On the valve-released state, the valve membranes are released and the air from inlet fills the empty buffer liquid reservoir. The air in the reservoir restricts back-flow of the preceded liquid in the reaction chamber. Therefore, unidirectional flow can be achieved during entire valving procedure.

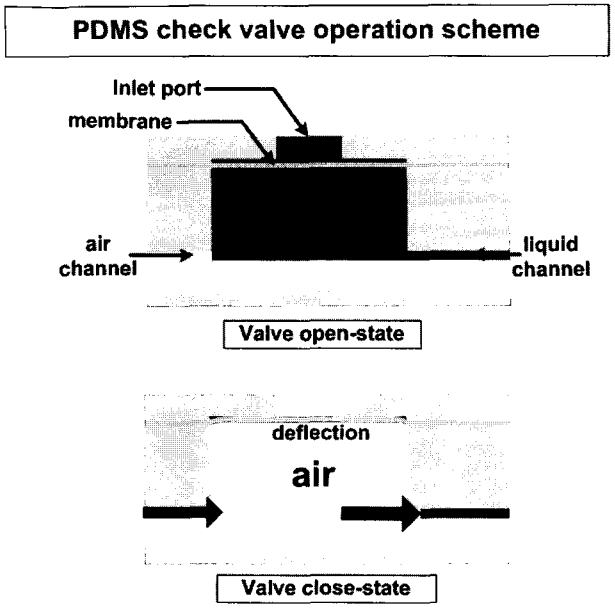


Fig. 3. Schematic illustration of the operation of the PDMS check valve (cross-section view).

3. Fabrication

The fabrication procedure of LOC includes replica molding and peeling, aligning, and stacking. The molding master was fabricated with the SU-8 photoresist (SU-8 2100 for channel layer and membrane molding, SU-8 2050 for the space layer molding) on a silicon wafer using transparency film as photo masks.

The fabrication process is shown in Figure 4. To fabricate the thin and flexible valve membrane, we used the sandwich molding process [6]. The transparent film (PVC film) was lowered onto the SU-8 master for the easy separation from the cast PDMS. To prevent breaking master during clamping, the dummy PDMS was stacked onto the film. The sandwiched dummy PDMS-PVC film-master was taped between two glass plates tightly instead of using clampers. Then liquid PDMS prepolymer was sucked into the empty space of 70 μm in height between master and PVC film by capillary attraction. To remove any air bubbles in the sheet, we degassed for 2 hour in vacuum and cured at 65°C for 3 hours. We peeled off each layer from the SU-8 master carefully and punched out the sample inlet, buffer solution inlet, air vent, and interconnection holes. And then, the peeled layers were bonded using O2 plasma treatment.

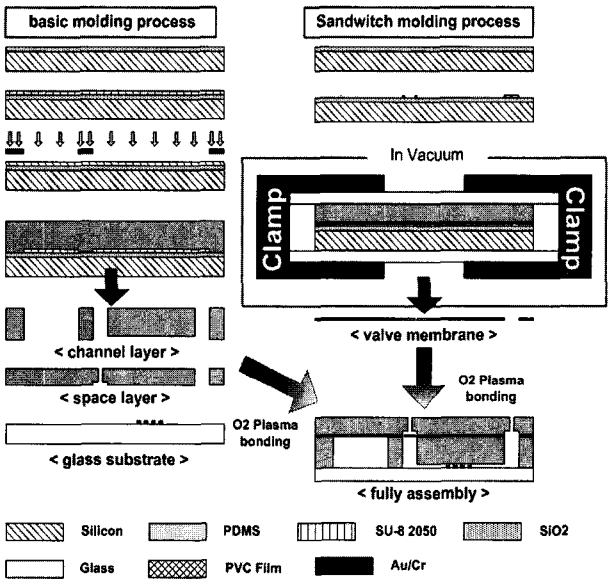


Fig. 4 Fabrication process of the PDMS LOC with check valve.

In order to carry out the immunosensing gold electrodes on the glass substrate, Ti/Au (500 Å / 2500 Å) were deposited onto the pyrex glass using sputter system and patterned using the general photolithographic technology. antigens (DNP, dinitrophenyl and biotin) were immobilized on the immunosensing electrode as a possible reactant for the target molecule. Amine-reactive SAM was formed by dipping the surface into a 5 mM DTSP solution in DMSO for 2 hours. After washing with DMSO and ethanol, the electrodes were transferred to the 0.5 % (w/w) poly(amidoamine) G4 dendrimer solution in ethanol for 30

minutes. Finally antigens were functionalized on the dendrimer-modified surface. The Figure 5 shows images of (a) the assembled LOC, (b) SEM image of released capillary stop valve, (c) top view of channel layer, (d) SEM image of the patterned PDMS check valve membrane and (e) photography of the assembled PDMS check valve membrane and space layer.

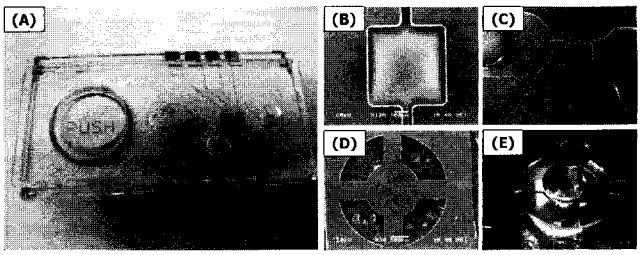


Fig. 5. Photography of the fabricated LOC. (a) the assembled LOC, (b) SEM image of the sudden expanded zone, (c)the channel layer, (d) the patterned PDMS valve membrane, (e) the assembled the PDMS valve membrane and the space layer.

4. Result

4.1 Flow characterization

The characteristics of this device can be mainly described by its fluidic test. Figure 6 shows the captured video clips showing the sequential liquid transfer. As depicted in Figure 6 (a), the abrupt change of the channel expansion angle made a pressure barrier and the buffer liquid driven by the capillary attraction force was stopped at the desirable place. The sudden expanded zone trapping the bubble drive the sample liquid into the reaction chamber by capillary attraction force (b). Figure 5(c) shows the successful result of check valve actuation by pneumatic pressure from pushing air bladder. After pushing buffer liquid into the reaction chamber, the valve membrane moved back to its original place and the empty reservoir was filled with the air entered from inlet. Therefore, we drove the sample and buffer to the desired place using the passive valves according to the liquid transfer steps. The results demonstrate that the multi-liquid transfer can be controlled well by using the capillary stop valve and the PDMS check valves.

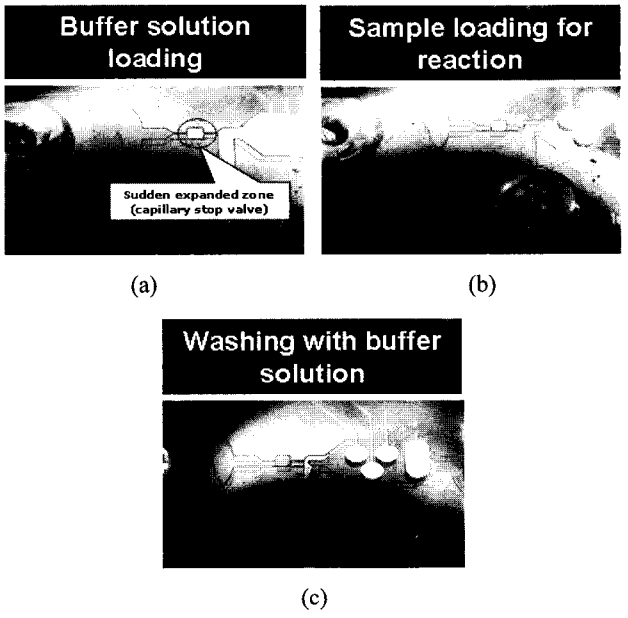


Fig. 6 The captured video clips showing the sequential liquid transfer. (a) The flow of fluid 1 driven by the capillary attraction force stopped at the sudden expanded zone (β = 90) as indicated inside the circle. (b)The sudden expanded zone trapping the bubble forced the fluid 2 being driven into the reaction chamber. (c) Washing step: pushing the air bladder, the buffer liquid was driven to the reaction chamber by effective action of check valve membrane.

4.2 Electrochemical immunoassay

Prior to the application of detection of the antibody as a disease marker, the model experiments were performed with anti-DNP antibody and anti-biotin antibody as target analytes. The antibodies conjugated with glucose oxidase (GOX) were used as a signaling molecule [7]. The buffer solution we used are PBS in presence of 0.1mM ferrocene-methanol and 10mM glucose. And the sample are the antibodies (anti-DNP or anti-biotin) tagging the activated enzyme (GOx).

Electrochemical measurements were carried out with an electrochemical analyzer (CH Instruments). To confirm the occurrence of the GOx bioelectrocatalysis at the sensing electrodes, voltametric measurements were conducted with buffer solution containing different concentrations of glucose substrate. The electrochemical signal was amplified due to the enzyme reaction of GOX and glucose. Figure 7 and Figure 8 show the cyclic voltamograms of the current variation depending on the concentration of anti-DNP antibody and anti-biotin antibody, respectively. The cyclic voltammetric trace represents the results of the electrochemical signal generation and amplification from the fabricated immunosensing LOC. From the results, possibility of electrochemical analysis using GOx was confirmed. When the concentration of the anti-DNP antibody in the sample is high, the amount of tagged GOx attached on the electrodes increases and results the high amplification of the electrochemical signal.

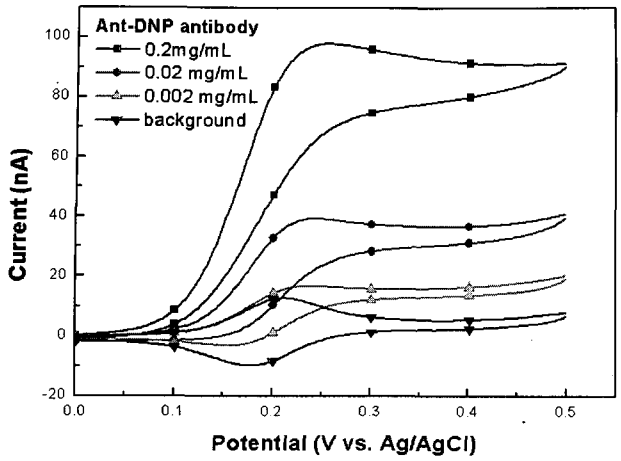


Fig. 7. Cyclic voltammetric traces for the DNP/anti-DNP affinity as a function of target protein concentration.

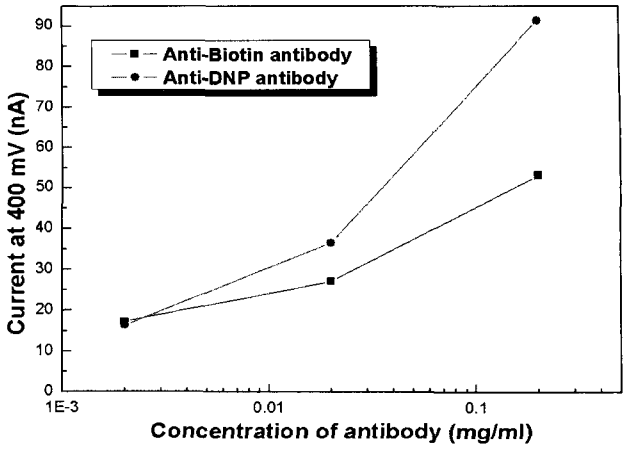


Fig. 9. The modulated plot of immunosensing sensitivity at 400 mV

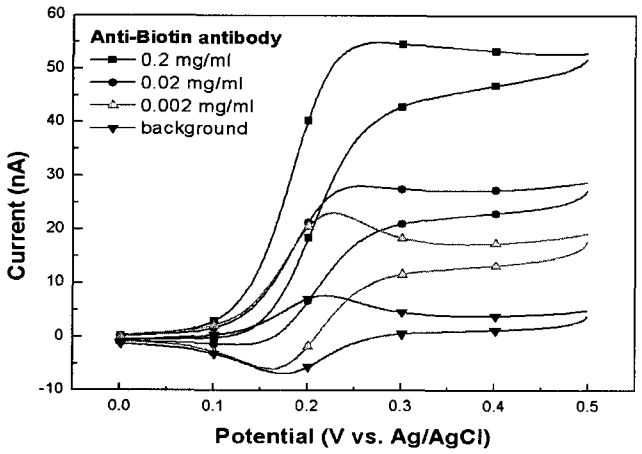


Fig. 8. Cyclic voltammetric traces for the biotin/anti-biotin affinity as a function of target protein concentration.

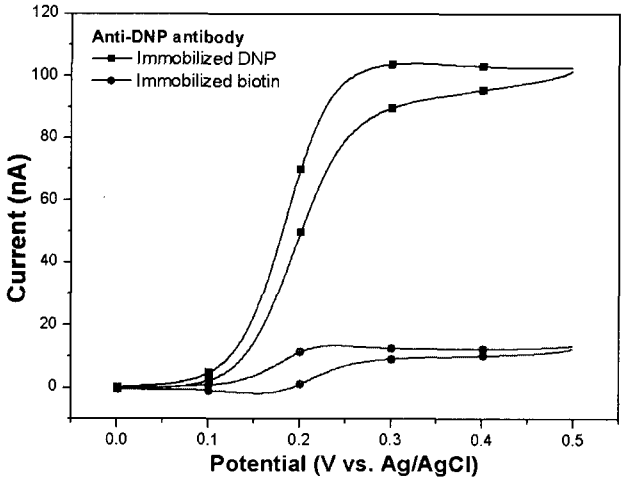


Fig. 10. Cyclic voltammogram of multi-immunosening test on the different patterned immuno-electrode

Figure 9 shows the measured sensitivity of the target analyte at + 400 mV according to the decease of the concentration of antibodies from the data of Figure 7 and Figure 8. With the sample having 0.002 mg/ml to 0.2 mg/ml of anti-DNP antibody, the amplified signal current is registered 16.4 nA to 91.58 nA at 400 mV. And the sample having 0.002 ug/ml to 0.2 ug/ml of biotin antibody, the amplified signal current is registered 17.3 nA to 53.33 nA at 400 mV. This result shows that different amplified signal current are registered according to different pattern of sensing electrode and provides possibility of multi-immunosensor LOC which have different antigen on the electrode surface.

Figure 10 shows the cyclic traces of multi-immunosensing test of LOC which have two immunosensing electrodes, one is DNP antigen and the other is Biotin antigen. The sample mixed with anti-DNP antibody and Biotin antibody is immobilized on the specific affinity sensing electrodes.

5. Conclusion

We have developed a one-touch type PDMS LOC with the check valve and capillary stop valve components. The sequential flow of the liquids is controlled by on-chip operation mechanisms without any external electric power. These passive fluid transport mechanisms are suitable for single-use immunosensing LOC module. The flow test results demonstrate that we can control the fluid flow easily by using the capillary stop valve and the PDMS check valves. By the model tests, we confirmed that the proposed LOC is easily applicable to the bioanalytic immunosensors using bioelectrocatalysis. Our future works are the integration of the fabricated LOC with immunosensor and the development of portable monitoring system proper to the disposable LOC.

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