Microbead based micro total analysis system for Hepatitis C detection

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Abstract - This paper describes a micro total analysis system (μTAS) for detecting and digesting the target protein which includes a bead based temperature controllable microchip and computer based controllers for temperature and valve actuation. We firstly combined the temperature control function with a bead based microchip and realized the on-chip sequential reactions using two kinds of beads. The PEG-grafted bead, on which RNA aptamer was immobilized, was used for capturing and releasing the target protein. The target protein can be chosen by the type of RNA aptamer. In this paper, we used the RNA aptamer of HCV replicase. The trypsin coated bead was used for digesting the released protein prior to the matrix assisted laser desorption ionization time of flight mass spectrometer (MALDI TOF MS). Heat is applied for release of the captured protein binding on the bead, thermal denaturation and trypsin digestion. PDMS microchannel and PDMS micro pneumatic valves were also combined for the small volume liquid handling. The entire procedures for the detection and the digestion of the target protein were successfully carried out on a microchip without any other chemical treatment or off-chip handling using 20 μl protein mixture within 20 min. We could acquire six matched peaks (7 % sequence coverage) of HCV replicase.

1. Introduction

Immunoassay is one of the most important and widely used analytical methods in clinical diagnoses and biochemical studies because of its extremely high selectivity and sensitivity. There have been some papers about microchip-based immunoassays, but most of them were miniaturization of capillary electrophoresis-based immunoassay. These systems are based on separation of the free form and the complex of the antigen and the antibody by microchip-based capillary electrophoresis, so that accuracy and sensitivity of the assay are generally dependent on the quality of the separation, which is easily affected by sample composition. Moreover, if an analyte is a protein or a macromolecule, the antigen-antibody complex often precipitates, or several kinds of complexes are formed, which affects the analysis. These reasons mean these methods are not suitable for every analyte contained in biological samples. As an example of a small immunoassay format, a dipstick or thin-layer chromatography based immunoassay system has been developed. However, the method is not suitable for most medical and biochemical applications because of its problems concerning its ability to quantify and its determination limit. On the other hand, enzyme-linked immunosorbent assay (ELISA) or other immunosorbent assay systems, in which antigen and antibodies are fixed on a solid surface, are applicable for many more analytes with high sensitivity and are used practically in many fields, including clinical samples. Therefore, integration of the immunosorbent assay system into a microchip with a much shorter reaction time and a high sensitivity has been welcomed. There have been several papers about the application and merits of bead packed microchip based immunoassay. However, the entire previous bead packed microchips only used one kind of bead and the enzyme reaction was conducted at the room temperature [1, 2, 3]. Thus, their applications are restricted to simple processes, such as enrichment of the protein, trypsin digestion of the protein or capturing the target protein among the several process of the heterogeneous immunoassay.

In this paper, we propose a new structure composed with silicon, glass and PDMS, which can pack two kinds of beads and control temperature of the reaction chambers independently to expand the functionality of bead based microchip. With the PDMS pneumatic microvalves more accurate and compact fluid control system was also realized. From the following subsections, we will describe design and fabrication of the proposed microchip, the whole control system and their interfaces.

2. Design

Figure 1 shows the structural schematic drawing of the bead-based temperature controllable microchip. The microchip consists of PDMS micro fluidic device, glass cap, silicon structure and Pt electrodes. The PDMS micro fluidic device includes four micro pneumatic valves and microchannel. The pneumatic valves are normally open and have a push-down structure. The control channel is located on the flow channel so that pressure is applied in the upper channel to deflect the membrane downwards. The glass cap has four holes for inlet and outlet of each reaction chamber. In the silicon structure, there are two reaction chambers (3.13 μl). Each reaction chamber has a column of posts (60×60×200 μm³) for bead packing. Since the trypsin immobilized on the beads is highly sensitive to the high temperature, two reaction chambers are thermally isolated by removing unnecessary silicon parts to reduce the thermal interferences derived from the high thermal conductance of the silicon. Two reaction chambers are physically separated each other and are to be connected only by the PDMS microchannel. Pt electrodes on the bottom of the reaction chambers are used for the micro heaters and temperature sensor. The total dimension of the glass/silicon microchip is 10×37×0.75 mm³. The cross sectional dimension of PDMS microchannel is 200×60 μm². The height of the PDMS pneumatic valve is 20 μm and the dimension is 500×500 μm². Since the thickness of microchip is very thin (0.75 mm) compared to top side area (10×37 mm²), there are two supporting bar to reinforce the strength of middle region.

(Figure 1) Schematic drawing of the bead based temperature controllable microchip
3. Fabrication

Figure 2 shows the fabrication process of the glass/silicon chip. The starting wafer was a double-sided polished silicon wafer (100) with a diameter of 100 mm and a thickness of about 250 μm. First, SiN₄ is deposited using low-pressure chemical vapor deposition (LPCVD) to a thickness of 1 μm. The SiN₄ layer on one side of the silicon wafer was etched away using reactive ion dry etcher (RIE). The microchannel and reaction chambers were patterned using photolithography then the silicon was etched to the depth of 200 μm by deep reactive ion etcher (DRIE). A glass wafer with a diameter of 100 mm and a thickness of 500 μm was cleaned with H₂SO₄ and laminated with a dry film photosist (BF410: Tokyo Ohka Kogyo Co., Kanagawa, Japan). The BF410 photosist was patterned by photolithography to define four holes for inlet and outlet of the reaction chambers. The laminated glass wafer was sandblasted. After alignment, the etched silicon wafer was anodically bonded with the sandblasted glass wafer. After anodic bonding, Pt heater and the sensor were fabricated using the lift-off process on the back side of the silicon wafer. A 200 Å Ti and a 1000 Å Pt were deposited using low energy magnetron sputtering. The Ti was deposited as an adhesion layer. Finally, the thermal isolation shape of the silicon part was defined by photolithography using AZ 4330 photosist (Clariant Co., North Carolina, USA) on the opposite side of the structured silicon wafer, and any unnecessary silicon was completely removed using DRIE.

![Figure 2] Fabrication process of the glass/silicon chip

A PDMS microvalve and a PDMS microchannel were fabricated using the soft lithography. On a single side polished silicon wafer, 60 μm mold for microchannel and 20 μm mold for microvalves were fabricated using SU-8 photosist (Microchem Co., Chestnut Street Newton, USA). The syugal 184 silicone elastomer base and curing agent ( Dow Corning, Midland, MI) were used for the fabrication of PDMS micro fluidic device. The base elastomer and the curing agent were mixed in specific ratios (30:1 and 10:1). The 30:1 PDMS was spun on the microchannel mold at 1500 rpm for 10 s and cured for 18 min at 80 °C. The 10:1 PDMS was poured on the grommatic valve mold to the thickness of 2 mm and cured for 18 min at 80 °C. Subsequently, the cured thick PDMS layer for microvalves was removed from its mold and holes were punched completely through to serve as inlet port for gas injection. Holes were punched by hand using a commercialized hole-punching machine (Technical Innovations, Brazoria, TX). The thick PDMS layer for microvalves was then aligned and bonded to the thin PDMS layer for microchannel. And the bonded PDMS layers were cured again for 12 h at 80 °C for permanent bond. When bonding was complete, the two-layer PDMS device was removed from the mold of microchannel and the holes for inlet and outlet of the microchannel were punched completely through to access the microchannels in the thin layer. The fabricated glass/silicon microchip and the PDMS micro fluidic device were permanently bonded each other to be a bead based temperature controllable microchip. The glass surfaces of the microchip and PDMS were treated in oxygen plasma for 15 s under the following conditions prior to bonding: oxygen gas flow of 100 sccm, pressure of 300 mTorr, and electric power of 75 W. After bonding, the completed bead based temperature controllable microchip was cured for 2 h at 80 °C. The cured microchip was mounted and wired on a PCB board for the external connection. The packaged microchip was connected to the computer through the interface board containing several amplifying circuits, wheat stone bridge circuit, noise filter and power supply. The controllers for temperature and valve actuation and process monitoring unit were realized using a data acquisition board (DAQ PCI-MIO-16E-1) and the LabVIEW software package.

4. Detection of the Hepatitis C

Two kinds of beads were used for capturing and digesting the target protein. The first reaction chamber was packed with the PEG-grafted bead on which RNA aptamer of HCV replicase was immobilized. This bead can be prepared by coating of organosilane, grafting of PEG on PS bead and immobilizing of RNA aptamer. The second reaction chamber was packed with the PEG-grafted bead on which trypsin was immobilized covalently. Heat was applied to release, denaturation and digestion of the captured protein. First of all, 20 μl of 68 g/μL HCV replicase was injected into the first reaction chamber and then incubated for 5 min at 25°C. After incubation, the first reaction chamber was washed with buffer intensively to remove non-specifically adsorbed protein. The HCV replicase was released from beads through protein denaturation by heating the first reaction chamber at 95 °C for 1 min. 20 μL HCV replicase was transferred to the second reaction chamber packed with trypsin-immobilized PS beads and incubated for 10 min at 37 °C to digest HCV replicase into peptides. 20 μL solution was eluted from microchip finally and analyzed with MALDI-TOF. As a result, we could acquire six matched peaks (7% sequence coverage) of HCV replicase within about 20 min using a fabricated microchip and a control system (figure 3).

![Figure 3] MALDI-TOF MS spectrum result of the captured hepatitis C

5. Conclusion

In this work, a bead based iRTAS for detection of the target protein and sample preparation of the detected protein prior to the MALDI TOF MS has been successfully implemented. The micro fluidic technology, the temperature control technology and the bead based immunosensor technology were successfully merged on a microchip so that more sophisticated reactions could be carried out on our bead based temperature controllable microchip. The hepatitis C could be easily detected from the protein mixture within 20 min using the implemented iRTAS.

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[References]

