ABSTRACT
This paper reports a negative-pressure driven microfluidic chip, which is capable of rapidly detecting bladder cancer biomarker, APOA1, with aid of magnetic beads-based Enzyme-linked immunosorbent assays (ELISA). Fluid can be handled through vacuum force and normally-closed valve. Comparing the experimental results between integrated chip and 96 well plate, the former requires less time (4 hr to 30 min), fewer reagent consumption (100 μl to 30 μl). In this study, the beads-based ELISA in chip has higher detection range and shows better performance in both sensitivity and linearity( 99.2%) than conventional ELISA in plate.

KEYWORDS: Microfluidic chip, ELISA, Magnetic beads, Bladder cancer, apolipoprotein A-I (APOA1)

INTRODUCTION
Bladder cancer is one of the most common urinary tract carcinomas. The standard detection methods for bladder cancer, cytology, shows low sensitivity and specificity. Quantitatively detection of candidate biomarkers is another potential method for purposes of making initial diagnosis and monitoring recurrence. Recently, APOA1 has been identified as one potential biomarker in urine for early bladder cancer diagnosis [1]. Besides, ELISA has been widely applied for the clinical detection of biomarkers. However, immunoassays are conventionally requiring large reagent consumption, several hours, and intensive labor easily accompanies with inaccuracy. Alternatively, microfluidic systems have shown great advantages for medical diagnostics [2]. Among several designs, in our perspective, negative pressure-driven device with normally-closed valve [3] seems more promising to be commercialized.

THEORY
Magnetic beads have been frequently used for microfluidic immunoassays because of several reasons: (1) they offer larger surface-to-volume ratio than the traditional 96 well plate to increase the interaction of antigen with antibody. (2) magnetic beads can be easily handled to deliver and purify antibodies through magnetic field [4][5]. Fig.1 shows the principle illustration of the microfluidic chip for beads-based ELISA. Magnetic beads with immobilized antibodies are initially introduced into the chip and then capture antigen specifically. Non-specific antigens are washed away as the immobilized beads are located in the channel through magnet. Second antibodies are followed to interact with antigens and then enzyme react with substrate. Finally the magnetic beads are moved away and the resuspended substrate is for optical density detection.
Figure 1. Schematic illustration of the magnetic beads-based APOA1 ELISA in microfluidic system. (a) 1st Abs coated beads are injected into channel; (b) antigens are captured and non-specific antigens are washed out; (c) 2nd Abs are incubated with the captured antigens; (d) substrates react with enzyme.

Figure 2. (a) The exploded view of microfluidic chip. (b) the photograph of the integrated microfluidic chip. (c) Working principle of normally-closed valve. (d) fluid was blocked by normally-closed valve. (e) valve actuated by negative pressure of vacuum.

EXPERIMENTAL

Fig.2(a) shows the schematic illustration of chip design which consists of three layers including top fluidic channel layer, middle air chamber layer, and bottom substrate layer. Each of three layers is made of poly(dimethylsiloxane) (PDMS) and bonded with O2 plasma treatment. Fig.2(b) shows a photograph of entire microfluidic chip. It enables five reagents to be sequentially introduced into the mixing chamber through negative pressure-driven and normally-closed valves control. Fig.2(c) shows that fluids flow through valves when the negative air pressure is given and then thin PDMS layer of valve is actuated. Fig.2(d) and 2(e) shows the opened valve and closed valve through vacuum control. Besides, a four chambers type micromixer is designed to enhance mixing efficiency during incubation.

RESULTS AND DISCUSSION

Fig. 3 shows the concentration distributions of fluid for a period of mixing time. After 5 second, the mixing efficiency increases from 15 to 90% at the driving frequency of 4 Hz. In order to define the detection range with 20 μl (1x10⁹ beads/ml) beads employed, concentration of APOA1 ranging from 0 to 10000 ng/ml are conducted. As the results, the detection ranges was defined from 0 to 1000 ng/ml when 1x10⁹ beads/ml are applied. Comparing the detection ranges of ELISA in chip with 96 well plate, the detection ranges of beads-based ELISA (0-1000 ng/ml) are higher than conventional ELISA (0.2-20 ng/ml). Therefore, it shows benefit to omit the sample dilution process when high APOA1 concentration in urine sample are detected. The detection limit of APOA1 is calculated to be 15 ng/ml in chip, which is close to the detection limit of APOA1 in traditional plate. Comparing the experimental results between developed chip and traditional 96 well plate, the former has better performance in both sensitivity and linearity (99.2%). The reason would be the higher surface-to-volume ratio and the higher reaction probability among the molecule. In this study, the entire consuming time from antigen capture to substrate development is about 30 min, which is 8 times less than traditional plate ELISA.
Figure 3. Four chamber type micromixer was driven by 5 Hz. The mixing effect at different times (a) 0, (b) 0.5, (c) 0.7, (d) 1.0, (e) 1.8, (f) 5 sec are shown as the frames. (g) The profile of the concentration distribution before and after mixing (5 sec). D+: the normalized location (d/D) measured across the center of the mixing chamber (x–x’ line). C+: normalized concentration.

Figure 4. Comparison of the detection result of APOA1 ELISA in microfluidic chip and the traditional 96 well plate. The microfluidic chip has better performance in both sensitivity and linearity.

CONCLUSION
In this study, we propose an entire vacuum force driven microfluidic chip with normally-closed valve, which is capable of quantifying APOA1 biomarker for early detection of bladder cancer. Comparing the experimental results between developed chip and traditional 96 well plate, the former has better performance in both sensitivity and linearity. In conclusion, the development of this integrated microfluidic chip can provide a rapid and robotic platform for medical research and clinical diagnosis.

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REFERENCES

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