AUTOMATED MICROFLUIDIC IMMUNOASSAY (AMI) SYSTEM UTILIZING A POLYMER CHIP EQUIPPED WITH A BLOOD FILTER AND REAGENT STORAGE CHAMBERS
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ABSTRACT
We report an automated microfluidic immunoassay system, which consists of a highly-integrated polymer microfluidic chip, a world-to-chip interface, and a control system. We also demonstrate quantitative detection of prostate-specific antigen in the range of 1–10ng/ml using UV absorbance detection. Microfluidic components for immunoassay, such as a blood filter and reagent-storage chambers, are integrated on a planer polymer chip. The capture antibody is coated on a gold surface before introducing a sample. The microfluidic actuations for the immunoassay are accomplished sequentially and automatically without external intervention. A novel interface structure enables on-chip storage of reagents and easy handling of the chip.

KEYWORDS: Immunoassay, Automation, Interfacing, Absorbance.

INTRODUCTION
Recently, enormous efforts have been focused on the development of lab-on-a-chip(LOC)s for the detection of specific biomolecules [1]. The ultimate purpose is enabling fast, cheap, highly-sensitive, quantitative, and reproducible detection. However, a chip that satisfies all of these requirements has not been realized [1-2]. Also, the integration of microfluidic components is one of the main issues of LOC currently under development. We present a novel AMI system pursuing the concept of ‘sample to result’ diagnostics. The requirements are largely satisfied by realizing the automated and reproducible fluid manipulations, the disposable polymer chip, the integration of microfluidic components, and the simple world-to-chip interface.

Figure 1. Automated microfluidic immunoassay(AMI) system. (a) Overall view, (b) Chip-to-world interfacing jig.
EXPERIMENTAL

Figure 1 shows an overall view of the present AMI system and a close view of the interfacing jig. After dropping a sample into the sample inlet of the chip and installing the chip onto the interfacing jig, automated sequential processes for immunoassay are designed to be accomplished by a syringe pump (Cavro™ XCalibur) operated by a control software. The chip-to-jig fluidic connection is completed by pressing down the needle array to pierce through sealing tape on the chip. After the completion of the immunoassay processes, the intensity of the target protein, prostate-specific antigen (PSA) in this case, is detected by an optical absorbance method.

Figure 2. (a) Polymer microfluidic chip, (b) Nickel template for injection molding.

Figure 3. Schematic of optical detection.

Figure 4. Numerical simulations on filling to detection chamber.

The present polymer microfluidic chip (cycloolefin copolymer, COC; 29*39*6 mm³), which includes all the necessary components for a sandwich enzyme-linked immunosorbent assay (ELISA), is shown in Figure 2(a). The chip contains a capture antibody-coated surface, a blood filter, a micromixer, the capillary-stop valves, the reagent-storage chambers, a reaction chamber, a waste chamber, and a detection chamber. The hybrid injection molding technique was utilized to fabricate both the micro-patterns and the macro-structures in a single polymer chip. A nickel mold having the micro-patterns was fabricated by electroplating nickel on an etched silicon wafer, was placed at one surface of the injection molding cavity. The macrostructures were formed by inserting structures into the molding cavity through a wire-cut nickel surface (Figure 2(b)). The polymer top substrate produced by injection molding was thermally bonded with the polymer bottom substrate having gold surfaces formed by e-beam evaporation.
After thermal bonding of the chip, a capture antibody (mouse anti-PSA monoclonal antibody) was introduced through the inlet of capture antibody to coat it on the gold surface. The antibody-coated surface was treated with casein solution. The reagent storage chambers were filled with a washing buffer (PBS) and a substrate solution (tetramethylbenzidine, TMB). Capillary-stop valves were located in the micro-channel connected with the storage chamber to prevent overflowing of reagents. All the open holes were sealed by adhesive tape to inhibit evaporation. The blood filter was formed by stacking a few sheets of membrane and a sealing rubber ring.

RESULTS AND DISCUSSION

About 7ul of plasma was separated from 30ul of whole blood with high filtering efficiency (>99.9%). The leakage of blood particles was mostly reduced by the sealing rubber ring. The filtered plasma containing PSA was mixed with the detection antibody (HRP-conjugated anti-PSA antibody) via a micromixer in the chip, and pumped into the reaction chamber. After incubation and washing steps, TMB was supplied into the reaction chamber for enzyme reaction. After a fixed reaction time, the reacted TMB solution was pumped into the detection chamber, which was designed to have a long optical path. The degree of absorbance dependent on the concentration of PSA was measured by absorbance-based optical detection method (Figure 3). Figure 4 demonstrates numerical simulations on the bubble-free filling to the detection chamber. Figure 5 displays results of the PSA detection, which indicates detection resolution of 1ng/ml is possible in range of 1~10ng/ml.

CONCLUSIONS

The presented AMI system using a polymer chip, which was developed under the concept of ‘sample to result’ diagnostics, enables a simple, quantitative and sensitive microfluidic immunoassensing. It could be used for the universal clinical diagnostics for various target proteins with simple modifications of the immunoassay protocols.

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REFERENCES