SUCTION-TYPE MICROFLUIDIC IMMUNOSENSING SYSTEMS FOR RAPID DETECTION OF DENGUE FEVER

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ABSTRACT

This study reports an integrated microfluidic system for rapid immunosensing of antibodies associated with dengue fever by utilizing enzyme-linked immunoabsorbent assay (ELISA) poly(dimethylsiloxane) (PDMS)-based chip. In this study, we successfully developed a rapid immunosensing chip with pre-functionalized surfaces to avoid protein adsorption. The functionalized PDMS surface was found to be highly effective in reducing the adsorption time in microchannels. The functionalized surfaces can be stable at least for 1 month when stored at 4°C. In addition, a suction-type microfluidic transportation module was used to form a single-fluid-slug transportation and to mix the reagents for rapid detection of dengue virus. The entire process can be finished within 30 min. The sensitivity was found to be superior to the conventional methods.

KEYWORDS: microfluidics, ELISA, surface modification, dengue fever

INTRODUCTION

Microfluidic technologies have made a substantial impact on the development of in-vitro diagnostic (IVD) devices by providing a rapid and cost-effective platform for the implementation of immunoassays [1, 2] and molecular diagnosis assays [3]. In particular, the immunology techniques are known to be reasonably sensitive and selective with a simple and cheap operation. However, their applications may be hindered by high sample/reagent consumption, expensive equipment and high labor cost. For many such applications, microfluidics may be used to develop a platform for fast diagnosis. In this study, a microfluidic system capable of performing the entire immunological protocol on a single chip was reported. A compact instrument for detection of dengue fever was developed to demonstrate that a promising platform for fast and sensitive detection of dengue fever infection is feasible.

SURFACE MODIFICATION

PDMS is one of the most popular polymeric materials employed for the fabrication of microfluidic devices owing to a number of advantages including simple fabrication, biocompatibility, and permeability to gases. In spite of these advantages, the native hydrophobicity and biofouling tendency of PDMS has been one of its biggest limitations for biomaterial applications. For example, nonspecific protein adsorption on a material is recognized as the first incident leading to subsequent events. Consequently, there are tremendous needs for methods to quickly and easily modify the surface properties of PDMS. In order to perform affinity analysis on a PDMS surface, hydrophilic modification and protein immobilization are two basic requirements. A highly hydrophilic environment not only can increase the affinity interaction between the bio-species but also reduces nonspecific binding that would be caused by hydrophobic interaction. In this study, a layer-by-layer approach was demonstrated in creating a long-term bioaffinity surface on a PDMS substrate.

Figure 1. Schematic illustration of the surface modification process.

The method using poly(ethyleneimine) (PEI) and poly(acrylic acid) (PAA) with subsequent cross-linking is an efficient method for obtaining long-term or permanent stability on a PDMS surface. Besides, PEI and PAA are commonly used polyelectrolytes which are biocompatible and can be easily functionalized. Figure 1 shows a simplified...
fabrication process to modify the PDMS surface. After oxygen-plasma treatment, poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer (Pluronic P123) was coated on the hydrophilic surface firstly. The P123-coated substrates were then coated with PAA solution for 15 min. The PDMS substrates were exposed to PEI solution for 15 min. Next, the multilayers were stabilized by adding a glutaraldehyde solution to form amide bonds between the polyelectrolyte layers for 5 min. For detection of dengue virus, protein A solution (10% weight ratio) was added to the substrates for 8 hr to allow affinity binding with the exposed antibody. After protein A binding, the surface was activated with the capture antibody for 12 hr. The blocking buffer (5% Tween 20 in the PBS buffer with 3% BSA) was finally added to the PDMS for 2 hr to reduce nonspecific binding. In this study we repeat 2 times to form the (P123)₂/(PAA)₂/(PEI)₂ polyelectrolyte multilayer.

CHIP DESIGN

After surface modification, the entire ELISA process can be performed automatically on a microfluidic chip, as shown in Fig. 2. This study presents a new suction-type, pneumatically-driven microfluidic device for reagent delivery and incubating (mixing). The major components, including three detection chambers namely tested chamber, negative control chamber, and internal control chamber. Nine normally-closed micro-valves and a sample transport/mixing unit, are also integrated in this device, as shown in Fig. 2. The microfluidic system employed one vacuum pump to drive and control the sample fluids. We also used the vacuum pump to suck out the waste reagent across the waste chamber. Figure 3 shows a cross-sectional view of one detection chamber. Briefly, the system was made of one glass substrate and two PDMS layers. It is composed of one membrane-type transportation/mixing unit, and three detection chambers that namely tested chamber, negative control chamber, and internal control chamber. Figure 4 shows the working principle of the chip for the sample transportation. Note that the air chambers connected with electromagnetic valves can be driven by a digital controller with the corporation of a vacuum pump. The PDMS membrane would be deformed and the sample would flow into the detection chambers when the air in the air chamber was sucked out with the aid of the vacuum pump. When the PDMS membrane was released and sucked, the membrane also can be used as a micro-mixer. Therefore, no extra micro component was required to mix the sample. Therefore, a rapid transportation and gentle mixing effect would be generated in detection chambers.

Figure 2. A schematic diagram of the microfluidic chip.

A: dengue virus  
B: HRP-labeled antibodies  
C: wash buffer  
D: TMB substrate (NeA blue)  
E,F,G: Stop solution  
H: rabbit anti-mouse IgG antibody  
I: waste chamber

Figure 3. The cross-sectional view of the detection chambers in the microfluidic chip.
RESULTS AND DISCUSSION

Figure 5(a) shows a photograph of the microfluidic chip. We designed micro-columns inside the reaction chambers for adding the surface area to capture the virus. SEM images of the micro devices are shown in Figs. 5(b) & (c). The diagnosis was performed by employing a conventional sandwich-type immuno-assay for dengue virus (DV). The PDMS surface is first conjugated with the capture antibodies. The DV is bound to the antibody-conjugated PDMS surface by the affinity between the antibody and the antigen. Then, the DV is recognized by the specific developing antibodies labeled with horse reddish peroxidase (HRP). Next, the tetramethylbenzidin (TMB) substrate as NeA-blue is added; the substrate generates a blue color when catalyzed by the HRP. Finally, the stopping buffer is added to stop the substrate reaction. The resulting yellow color is then read at 450 nm using an ELISA reader.

The virus samples with concentrations ranging from 104 to 101 PFU/ml were tested. Figure 6 shows the OD value of the ELISA reader for samples. It indicates that the detection limit of the integrated microfluidic system is 101 PFU/ml, which is superior to the conventional method (103 PFU/ml). Note that the detection time is decreased from 4.5 hr to 30 min by using the microfluidic device. Therefore, this integrated system provides a powerful platform for rapid diagnosis of dengue fever infection.

CONCLUSION

This study has demonstrated an integrated microfluidic chip equipped with a multi-functional micro-component to transport, meter and mix samples. The surface modification can ensure a high specificity. A detection limit of 101 PFU/ml was experimentally found, which is superior to the conventional ELISA (103 PFU/ml). The fully automated microfluidic chip is able to perform the entire process within 30 min, which is also much faster than the 96-well microtiter plates (4 hr).

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REFERENCES


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