A MAGNETIC-BEAD-BASED IMMUNOASSAY FOR RAPID PURIFICATION AND DETECTION OF INFLUENZA VIRUSES UTILIZING SUCTION-TYPE MICROFLUIDIC SYSTEMS

Kang-Yi Lien[1], Lien-Yu Hung[2], Huan-Yao Lei[3] and Gwo-Bin Lee[1,2*]

[1]Institute of Nanotechnology and Microsystems Engineering, [2]Department of Engineering Science, [3]Department of Microbiology and Immunology, National Cheng Kung University, Tainan, Taiwan

ABSTRACT

The study presents a magnetic-bead-based, suction-type microfluidic system for rapid purification and detection of influenza viral particles from clinical serum samples. Target influenza viral particles in the clinical sample can be first captured by the antibody-conjugated magnetic beads, followed by isolating the viral particles from the bio-samples with the incorporation of a permanent magnet and a suction-type microfluidic control module. Then, another specific secondary developing antibodies labeled with fluorescent dyes were employed to recognize the surface antigen of the virus-bound magnetic complexes. Finally, the fluorescent-labeled magnetic complexes can be then detected and analyzed by utilizing an optical detection module. The entire process can be performed automatically within in 30 minutes. The development of the integrated microfluidic chip may provide a promising platform for fast diagnosis of influenza infection.

KEYWORDS: Magnetic bead, Influenza, Immunoassay, Suction, Microfluidics, MEMS

INTRODUCTION

In recent years, emerging, extremely-contagious, infectious diseases such as severe acute respiratory syndrome (SARS) and avian influenza have attracted considerable concern from public health organizations. Influenza infection is a contagious disease of mainly the upper respiratory tract. The influenza virus can infect patients by viral particles presented in the aerosols generated by sneezes and coughs. It may cause acute respiratory tract infection in human and severe morbidity, especially in elderly and children whose immune responses are weakened or not well established [1]. Therefore, a rapid detection of influenza virus is becoming increasingly significant in face of concerns over an influenza pandemic.

Traditionally, reverse transcription polymerase chain reaction (RT-PCR) assay, a molecular diagnostic method for the detection of influenza infection, provides a highly-sensitive and accurate diagnosis protocol. However, the entire procedure requires a complicated protocol and is relatively costly. Alternatively, enzyme-linked immunosorbent assay (ELISA) is another well-recognized serological diagnostic method for the detection of the influenza infection [2-3]. However, a conventional ELISA is usually performed on 96-well plates, which involves a series of tedious processes, including incubation and washing steps. Not only is it a time-consuming (over 4 hrs) and labor-intensive process, but it also requires well-trained personnel to precisely perform the entire protocol. Therefore, there exists a critical need to develop a platform for the rapid and automatic detection of influenza infection with a higher sensitivity in a short period of time.

To tackle the problems described above, the current study therefore proposes a magnetic-bead-based microfluidic system integrated with sample pre-treatment devices for target viruses purification, concentration and detection. The proposed suction-type, pneumatic-driven microfluidic platform was developed for rapid detection of influenza virus by utilizing fluorescent magnetic-bead-based immunoassay. Two essential modules including a sample purification module consisting of a vortex-type micro-mixer, a sample transportation unit, and an optical detection module are integrated into the miniature microfluidic platform. The entire diagnostic process can be completed within 30 mins with less human intervention.

WORKING PRINCIPLE

A suction-type microfluidic system integrated with a sample transportation unit and micro-valves is designed and fabricated for rapid diagnosis of influenza infection by using monoclonal antibody (mAb)-bound magnetic beads in a sandwich-based immunoassay. To utilize the high affinity between the antibodies and the antigens [4], the target viruses in the clinical serum sample can be captured by the mAb-bound magnetic beads with a high selectivity. Figure 1(a) shows a schematic illustration of a sandwich-like magnetic-bead-based immunoassay for rapid detection of influenza virus with the incorporation of fluorescent dye-labeled developing mAb. Figure 1(b) illustrates the experimental assay of the proposed magnetic-bead-based microfluidic system. By utilizing the specific mouse α-influenza nucleoprotein (NP) mAbs conjugated magnetic beads, the target influenza viral particles in the clinical sample can be recognized and then adhered onto the surface of the magnetic beads during the incubation process. After that, a washing process is performed with the incorporation of the permanent magnet and the microfluidic control module to wash out all the other biological substances in the clinical samples. Next, another developing mouse anti-α-influenza NP mAb labeled with R-phycoerythrin (RPE) is applied to incubate with the virus-conjugated magnetic complexes in the second incubation process, follow by repeating the washing process to rinse the un-bound developing mAbs away. Finally, the magnetic-bead-based fluorescent immunoassay can be completed by analyzing the fluorescent signals with the incorporation of the optical detection module.

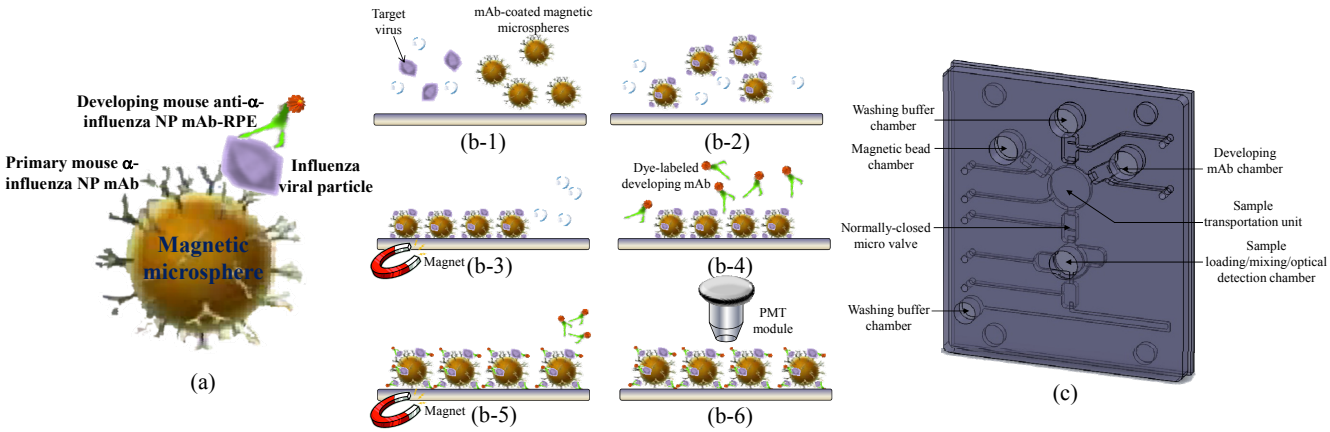

Figure 1: (a) Schematic illustration of a sandwich-like magnetic-bead-based immunoassay for rapid detection of influenza virus. (b) Schematic illustration of the protocol for sample purification and optical detection of viral particles by using the mAb-bound magnetic beads in the microfluidic system. (c) The schematic illustration of the proposed magnetic-bead-based microfluidic system.

CHIP DESIGN

The schematic design and of the proposed magnetic-bead-based microfluidic system are shown in Figure 1(c). The microfluidic system integrated several functional modules including a central sample transportation unit, a magnetic bead loading/mixing/optical detection chamber with a suction-based vortex-type micro-mixer, a clinical chamber, a washing buffer chamber, a developing mAb chamber, five normally-closed micro-valves, micro-channels and a waste collection chamber. The dimensions of the microfluidic system are measured to be 33 mm × 38 mm.

Notably, all the fluidic samples in the micro-device can be manipulated and transported by the incorporation of the suction-type sample transport unit capable of liquid transportation in an automatic manner with the incorporation of normally-closed micro-valves. In principle, transportation of samples can be achieved when the normally-closed PDMS membranes are deflected upwards sequentially by the negative pressure in the air chambers, which is generated by an external vacuum pump, such that the fluidic sample can be drawn into the fluidic reservoirs underneath the central PDMS membrane. Next, the normally-closed micro-valve on the left side is released while the normally-closed micro-valve on the right side is then switched on, such that the floating-block structure in the left micro-channel can be switched off to push the sample forward. Afterwards, all the PDMS membranes of the suction-type microfluidic pumps/valves are released to push the fluidic sample from the fluidic reservoirs into the reaction chamber. In addition, the suction-based vortex-type micro-mixer consists of 2 microfluidic side-channels so that the biological samples can be rapidly mixed during the incubation in the miniature platform. Briefly, the vortex-type micro-mixer is comprised of a thick PDMS structure and a thin pneumatically-driven PDMS membrane to generate a vortex flow within the mixing chamber. The thick PDMS structure contains two air chambers with connecting air channels and the bottom thin-film PDMS membrane is comprised of a mixing chamber with two normally-closed fluidic side-channels in each layer. The normally-closed PDMS membrane of the microfluidic side-channels would be then uplifted sequentially and the sample would flow into the microfluidic side-channels by the low air pressure in the air chamber, generated by the suction from the vacuum pump. This is followed by the generation of a vortex flow field within the mixing chamber when the PDMS membrane is released. The vortex-type micro-mixer is connected to an electromagnetic valve (EMV) that is driven by a digital controller with the incorporation of a vacuum pump. As a result, a rapid mixing effect can be generated for the incubation of the virus sample with the magnetic beads.

RESULTS AND DISCUSSION

The vortex-type micro-mixer and the suction-type micro-pump consisting of a sample transportation unit and micro-valves are used in the proposed microfluidic platform for bio-sample incubation and transportation, respectively. The vortex-type micro-mixer is designed to generate the vortex flow field within the mixing chamber for rapid mixing of magnetic beads and the bio-samples. A mixing efficiency index is used to quantify the mixing performance of the proposed vortex-type micro-mixer. The mixing efficiency is evaluated by measuring the concentration distribution along a cross section of the mixing chamber. 1 μL of blue ink and 50 μL of DI water are loaded into the mixing chamber to measure the efficiency of the vortex-type micro-mixer. The vortex-type micro-mixer is actuated at 4.0 Hz with an applied negative pressure of -80 KPa. The experimental data reveals that the mixing efficiency of the vortex-type micro-mixer as high as 96% can be realized after mixing for 1 sec. Consequently, the efficient mixing within the mixing chamber can be produced for the incubation process of the bio-samples.

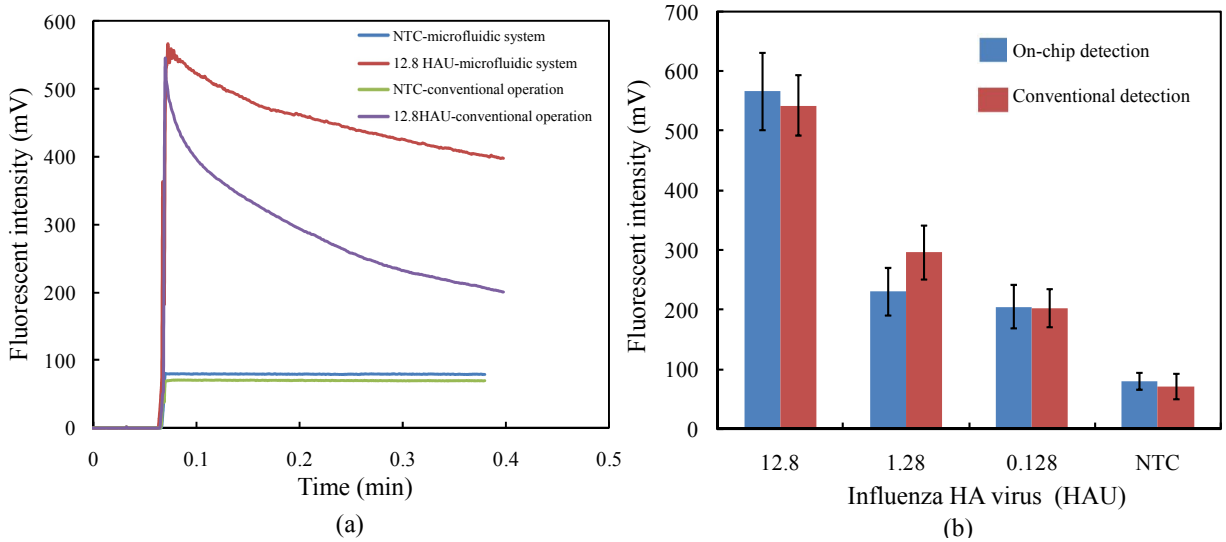

Figure 2: (a) Optical signals for the detection of influenza virus HA in the microfluidic system. (b) Sensitivity test of the proposed microfluidic system. 0.128 HAU of influenza viruses can be detected in the microfluidic system within 30 mins

To verify the performance of the developed magnetic-bead-based microfluidic system, a sandwich-like fluorescent immunoassay (FIA) is then performed according to the protocol mentioned previously. Figure 2(a) shows the experimental results for the detection of influenza virus A by the mAb-conjugated magnetic beads. The selectivity of the developed system is investigated by incubating the clinical serum samples with influenza infection (positive cases) and serum samples with dengue infection (negative cases) with the mAb-bound magnetic beads, which can only selectively detect the influenza viral particles. Therefore, the results indicate that only the serum samples with influenza viral particles can only be specifically captured by the mAb-bound magnetic beads. In addition, the sensitivity of the proposed microfluidic system is also explored. The optical intensities of each sample with different concentration are shown in Fig. 2(b). From the experimental results, it can be found that the detectable fluorescent intensity is measured to be 204.8 mV in the sample with a viral concentration of 0.128 HAU. Note that the fluorescent intensity of the negative control case is around 80.0 mV. From the comparable results, 0.128 HAU of influenza viruses in the clinical sample can be detected by the proposed microfluidic system within 30 mins, which is much faster than the manual operation of magnetic-bead-based FIA (more than 3 hrs). Consequently, the proposed microfluidic system integrated with FIA and a sample pretreatment module can be used as a potential platform for rapid diagnosis.

CONCLUSION

A new magnetic-bead-based, suction-type microfluidic system has been demonstrated for rapid, automatic immunological diagnosis of influenza infection by utilizing mAb-bound magnetic beads. A suction-type microfluidic control module and an optical detection module were integrated into a single chip to carry out bio-sample incubation, purification and optical analysis automatically. Experimental results indicated that the microfluidic system can reduce the total detection time to 30 mins. In addition, the results also showed that influenza virus with a concentration of 0.128 HAU can be detected successfully by this miniature system. Therefore, this integrated system may provide a powerful platform for rapid diagnosis of influenza infection.

ACKNOWLEDGEMENTS

The authors would like to thank the National Science Council in Taiwan for financial support (NSC98-2120-M-006-001).

REFERENCES

[1] N. J. Cox, T. L. Brammer and H. L. Regnery, Influenza: Global surveillance for epidemic and pandemic variants, European Journal of Epidemiology, 10, pp. 467-470, (1994).

[2] G. F. de Boer, W. Back and A. D. M. E. Osterhaus, An ELISA for detection of antibodies against influenza A nucleoprotein in humans and various animal species, Archives of Virology, 115, pp. 47-61, (1990).

[3] B. W. Lee, R. F. Bey, M. J. Baarsch and R. R. Simonson, ELISA method for detection of influenza A infection in swine, Journal of Veterinary Diagnostic Investigation, 5, pp. 510-515, (1993).

[4] J. Wrammert, K. Smith, J. Miller, T. Langley, K. Kokko, C. Larsen, N. -Y. Zheng, I. Mays, L. Garman, C. Helms, J. James, G. M. Air, J. D. Capra, R. Ahmed and P. C. Wilson, Rapid cloning of high-affinity human monoclonal antibodies against influenza virus, Nature, 453, pp. 667-671, (2008).

CONTACT INFORMATION

*Dr. Gwo-Bin Lee, Tel: +886-6-2757575 ext. 63347; gwobin@mail.ncku.edu.tw