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Automated liquid operation method for microfluidic heterogeneous immunoassay

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ABSTRACT

In this work, an automated liquid operation method for multistep heterogeneous immunoassay toward point of care testing (POCT) was proposed. A miniaturized peristaltic pump was developed to control the flow direction, flow time and flow rate in the microliter range according to a program. The peristaltic pump has the advantages of simple structure, small size, low cost, and easy to build and use. By coupling the peristaltic pump with an antibody-coated capillary and a reagent-preloaded cartridge, the complicated liquid handling operation for heterogeneous immunoassay, including sample metering and introduction, multistep reagent introduction and rinsing, could be triggered by an action and accomplished automatically in 12 min. The analytical performance of the present immunoassay system was demonstrated in the measurement of human IgG with fluorescence detection. A detection limit of 0.68 μg/mL IgG and a dynamic range of 2–300 μg/mL were obtained.

1. Introduction

Immunoassay is the most important protein measurement method in clinical diagnosis for many diseases such as myocardial infarction [1], AIDS [2] and diabetes mellitus [3]. It is also one of the most important techniques in point of care testing (POCT) for diagnosis of emergent diseases such as acute myocardial infarction and communicable diseases. Currently, the test strip-based immunoassay is the popular method used in POCT, however it can only provide qualitative or semi-quantitative results. Heterogeneous immunoassay techniques such as enzyme-linked immunosorbent assay (ELISA) and fluorescence immunoassay which can provide quantitative results are frequently used in routine laboratories for clinical diagnosis. However, these methods require complicated liquid operation including multistep sample and reagent metering and addition, incubating, and rinsing. These operations still need to be performed by professionals or using complicated and bulky instruments in laboratories. The integration and automation of these complicated liquid operations in a portable system for POCT are still a great challenge.

Microfluidic systems have shown great potentials in immunoassay due to their abilities in achieving system miniaturization, integration and automation. Various microfluidic systems [4–7] based on different strategies have been developed to automate the complicated liquid operation in heterogeneous immunoassay. Kartalov et al. [8] developed a high-throughput immunoassay system using pneumatic actuation pumps. However, the uses of the bulky gas source and multiple control valves make it difficult to be applied in in-situ analysis where portable instruments are usually required. Lai et al. [9] reported a centrifugal-driven disk microchip for ELISA. The flow sequence of the sample and different reagents was controlled by centrifugal and capillary forces. Each step of the ELISA process was carried out automatically by controlling the rotation speed of the disk. In this system, samples were required to be preloaded into the chip in the preparation stage of the chip in laboratory. This may limit its application in practical analysis. Linder et al. [10] proposed a simple cartridge-based liquid handling method for heterogeneous immunoassay. Different reagent plugs were sequentially preloaded in a tube and segmented by air plugs before the analysis. After the sample was loaded into the antibody coated microchannel on the chip and incubated for 7 min, the cartridge was connected with the channel and the reagent plugs were driven through the channel by a vacuum source. The introducing operation for multiple reagents and rinsing solutions was achieved automatically, while the whole analysis still required some human interventions, e.g. sample metering and loading, and cartridge connecting with the chip.

In this work, the automation of multistep liquid handling operation for heterogeneous immunoassay was realized by using a miniaturized peristaltic pump, an antibody-coated capillary and a reagent preloaded cartridge. The whole liquid handling operation for immunoassay including sample metering and introduction, reagent introduction, and rinsing could be automatically achieved with the peristaltic pump programed by a controller. The feasibility and performance of the system was demonstrated in the measurement of human IgG under fluorescence immunoassay mode.

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2. Experimental section

2.1. Chemicals and reagents

All solvents and chemicals used were of reagent grade unless otherwise stated. Deionized water was used throughout. 3-Aminopropyltriethoxysilane (3-APTES), glutaraldehyde solution, human immunoglobulin G (IgG), goat anti-human IgG, and fluorescent goat anti-human IgG were obtained from Sigma-Aldrich (St. Louis, USA). Perfluoro-di-*n*-butylmethylamine (FC-40) used as oil to segment reagent plugs was bought from 3M Co. (St. Paul, USA). The phosphate buffer saline (PBS) solution was purchased from Keyi Biotechnology Co. (Hangzhou, China).

2.2. Microfluidic immunoassay system

The immunoassay system consisted of three parts, including an antibody-coated capillary, a reagent-loaded cartridge and a programmable peristaltic pump (Fig. 1).

2.2.1. Antibody-coated capillary

A fused silica capillary (TSU075375, 75 μm i.d., 363 μm o.d., Polymicro Technologies, Phoenix, USA) was used in the immunoassay. The capillary was pre-activated by rinsing sequentially with 0.1 M NaOH solution for 1 h, water for 30 min, 0.1 M HCl solution for 30 min, and water for 30 min, and then dried with N_2 for 3 h. After the activation, the inner wall of the capillary was silanized with 10% 3-APTES in hexane (v/v) for 12 h at 67 °C. Then, 2.5% glutaraldehyde solution in PBS (w/v) was introduced into the capillary for 60 min. After rinsing with PBS, the capillary was introduced with a goat anti-human IgG solution (500 μg/mL) in PBS, and incubated for 24 h at 4 °C. After that, 1% bovine serum albumin (BSA) solution was used to block the capillary channel surface for avoiding non-special adsorption. Finally, the capillary was washed with PBS and stored at 4 °C.

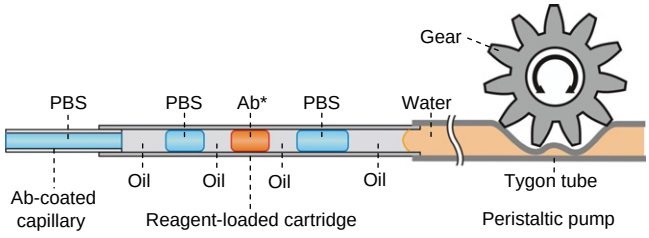

Fig. 1. Schematic diagram of the microfluidic immunoassay system. Ag, antigen; Ab, antibody; Ab*, fluorescently labeled antibody.

2.2.2. Reagent-loaded cartridge

All of the reagents involved in the immunoassay including 1 μL PBS, 1 μL fluorescent-labeled goat anti-human IgG solution (500 μg/mL), and 2 μL PBS, were sequentially loaded in a polytetrafluoroethylene (PTFE) tube (250 μm i.d., 760 μm o.d., Cole-Parmer, Vernon Hills, USA) segmented by 0.2 μL FC-40 oil to form a reagent-loaded cartridge driven by a syringe pump (PHD2000, Harvard, Boston, USA). The FC-40 oil plugs functioned as intervals to prevent the cross-contamination between the adjacent reagent plugs.

2.2.3. Programmable miniaturized peristaltic pump

The appearance and internal structure of the miniaturized peristaltic pump are shown in Fig. 2. A miniaturized reducer motor installed with a plastic gear was used as a rotor and a Tygon tube (0.25 mm i.d., 2.07 mm o.d., IDEX, Chicago, USA) was used as pump tube. The reducer motor was fixed at bakelite pump holder A. The Tygon tube was fixed on another bakelite pump holder B by epoxy. Holder A and holder B were assembled by screws to press the tooth of the gear on the Tygon tube. The pump powered by a 3.6 V Li-ion battery and the size of the whole pump was 5 cm × 3 cm × 3 cm.

The driving principle of the pump is similar to conventional peristaltic pumps, using the tooth of the gear to press the Tygon tube and drive liquids in the tube. The flow rate and flow direction of the pump were controlled by the rotating speed and direction of the motor, respectively. A battery-powered electronic controller based on microcontroller (MSP430F149, Texas Instruments, Dallas, USA) was developed to control the complicated liquid operation in immunoassay. It could control rotating speed, rotating direction and the running time of the motor according to program by applying different supply voltages to the motor. The liquid metering of the pump was realized by controlling the sampling time and flow rate.

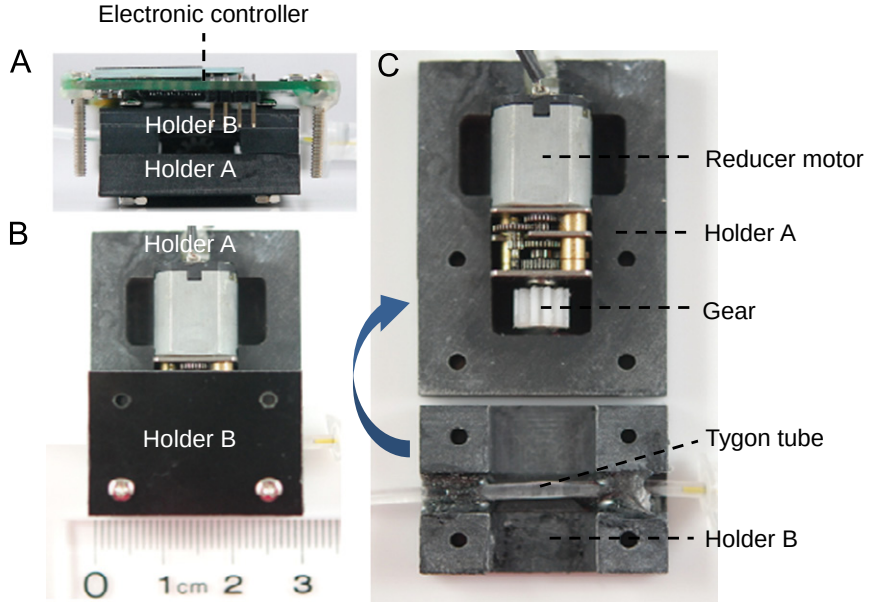

Fig. 2. The side view (A), top view (B), and internal structure (C) of the programmable peristaltic pump.

2.3. Measurement of human IgG

The antibody-coated capillary, reagent-loaded cartridge and the programmable peristaltic pump were connected sequentially. After inserting the capillary inlet into the human IgG sample solution, the analysis procedure was started by pressing the "Analysis" key of the pump controller. In the sampling stage, the pump motor rotated anticlockwise and the IgG sample was aspirated into the capillary at a flow rate of 2.0 μL/min for 0.5 min. Then, the flow direction of the pump was reversed and the flow rate was set at 0.5 μL/min. The sample and preloaded reagents in the cartridge were sequentially pushed out through the capillary. The whole immunoassay operation including sample metering and loading, multiple immune reactions and rinsing were performed automatically (Fig. 3). After the above operation, the capillary was imaged by an inverted fluorescence microscope (Nikon Co., Kanagawa, Japan) equipped with a CCD camera (Andor Technology, Belfast, Northern Ireland). The capillary fluorescence intensities were quantified using image analysis software ImageJ (NIH, USA).

3. Results and discussion

3.1. Programmable miniaturized peristaltic pump

In the present work, the miniaturized peristaltic pump was adopted to automate the complicated and multistep liquid operation of solid-phase immunoassay for in-situ analysis. It could work in "pull" and "push" mode under command of a programmable electronic pump controller. The sample metering and introducing operations which often are carried out manually using pipettes in previous immunoassay systems [9,11,12], were achieved by setting the pump in "pull" mode and controlling the sample aspirating time of the pump. The subsequent immunoassay operations were simply performed by using the peristaltic pump under "push" mode to deliver the reagents preloaded in the cartridge through the capillary at a definite flow rate. The whole operation for multistep immune reactions could be automatically achieved with the peristaltic pump without the need of human intervention and the use of expensive and nonportable syringe pump.

The performance of the programmable peristaltic pump was tested. The effect of motor supply voltage in the range of 0.6–3.0 V on flow rate of the pump was investigated and the results are shown in Fig. 4A. For flow rate measurement, a water plug was first delivered into the capillary by the pump. By observing the movement of the water plug with the microscope, the time of the water plug passing through a definite length of the capillary channel was measured. The flow rate was calculated using the data of the transit capillary length and the transit time. The flow rate increased with the supply voltage from 0.4 μL/min to 2.5 μL/min. The error bars indicate the standard deviation of the flow rates in 11 repetitive experiments at each supply voltage. Thus the pump flow rate could be easily adjusted by regulating the supply voltage. Furthermore, we also tested the stability of the flow rate at definite supply voltage. The supply voltage was fixed at 1.0 V and the flow rate was measured every 2 min in 72 min (Fig. 4B). The average flow rate was 0.5 μL/min. The relative standard deviation (RSD) of the flow rates was less than 1.2% ($n=35$). The stable pump flow

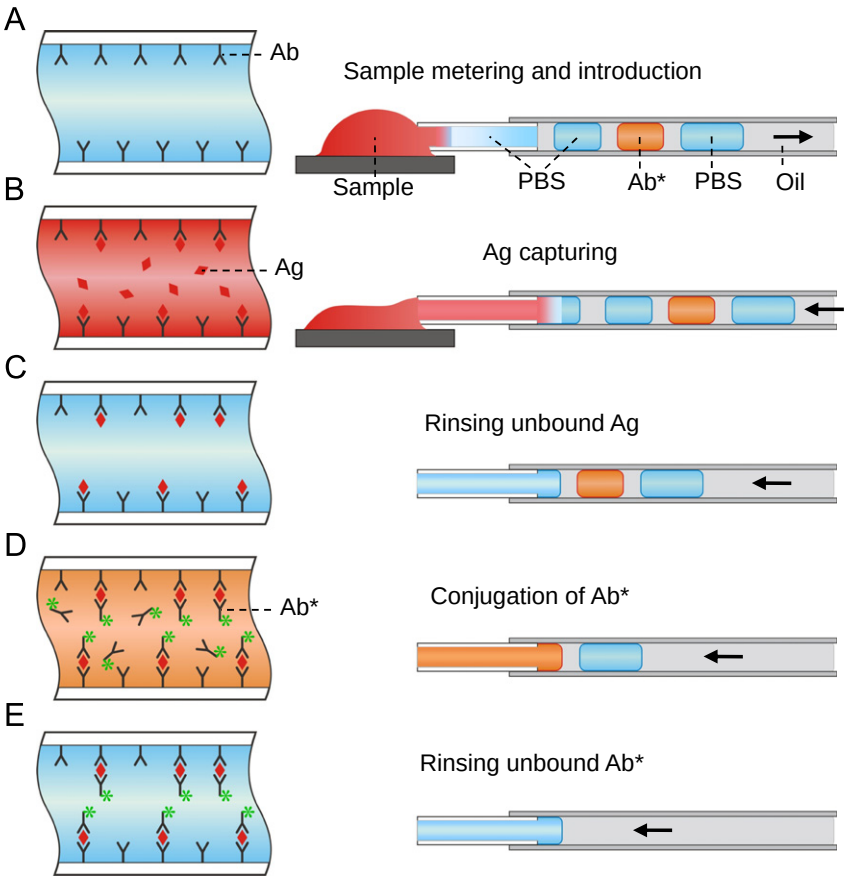

Fig. 3. Schematics of the automated immunoassay process. (A) Aspirating and metering of sample; (B) IgG in the sample was captured by the coated antibody on capillary wall; (C) rinsing unbound antigen with PBS solution; (D) conjugation of fluorescently labeled secondary antibody with the bound antigen; (E) rinsing unbound fluorescently labeled secondary antibody with PBS solution. Ag, antigen; Ab, antibody; Ab*, fluorescently labeled antibody.

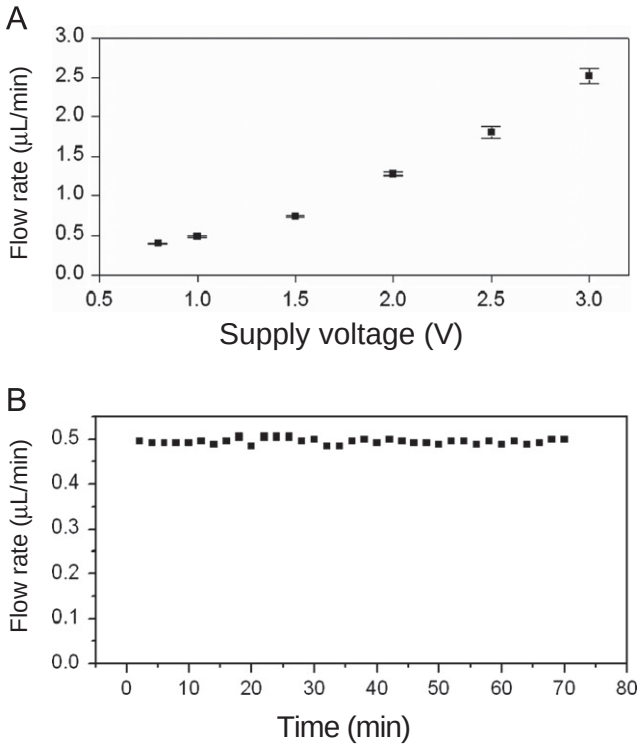

Fig. 4. (A) Effect of supply voltage on pump flow rate. (B) Stability of pump flow rate in 72 min.

rate ensured the sufficient precision for liquid metering and reaction efficiency in the immunoassay. The whole cost of the programmable miniaturized pump is less than $15.

3.2. Optimization of the system

In a microfluidic immunoassay system, the sample and reagents introducing flow rate and volume play significant parts in immune reaction efficiency and system detection sensitivity [13]. We used the reaction of the fluorescently labeled antibody with the bound antigen on the capillary wall to investigate the effects of sample introducing volume and flow rate on the immune reaction. Before the experiment, a 200 μg/mL human IgG solution was introduced into the antibody-coated capillary for 20 min to allow the antigen to be bound with the immobilized antibody. Then a 100 μg/mL fluorescently labeled antibody solution was continuously introduced into the capillary at different flow rates of 0.25, 0.5, 1.0 and 1.5 μL/min. The capillary was imaged by the inverted fluorescence microscope at definite times corresponding to introducing volumes of 0.5, 1.0, 1.5 and 2.0 μL. The results are shown in Fig. 5. The fluorescence intensity of the capillary indicated the reaction efficiency of fluorescently labeled antibody and IgG. With a fixed flow rate, the capillary fluorescence intensity increased with the increase of the sample introducing volume, and this increasing trend slowed down after the volume exceeded 1.0 μL. For the cases with fixed sample volume and different flow rates, the capillary fluorescence intensity increased with the decrease of the flow rate. The result shows that the faster flow rate will reduce the binding efficiency of the antigen and antibody. This is in agreement with those reported previously [14,15]. At lower flow rates, more free antibody were captured by the immobilized antigen due to the longer residence time of the antibody solution in the capillary channel. However, the complete capture of antibody usually needs a long time of several tens of minutes, which is not suitable for rapid POCT application. With the sample volume of 1.0 μL, the fluorescence intensity at

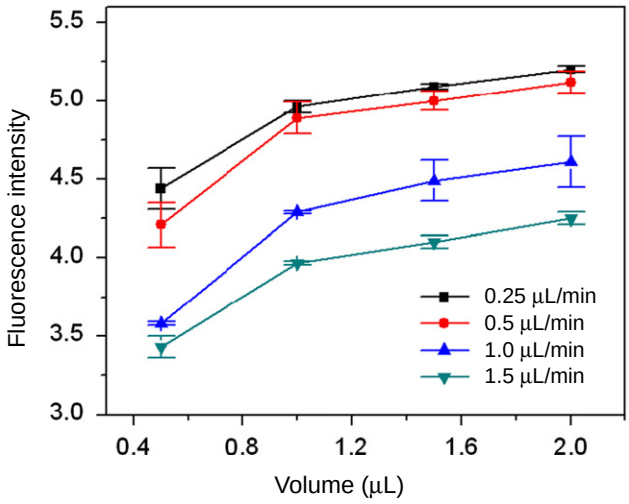

Fig. 5. Effects of the sample introducing flow rate and volume on the capillary fluorescence intensity.

0.5 μL/min is almost equal to that at 0.25 μL/min, but the reaction time takes only half of that at 0.25 μL/min. Thus, sample volume of 1.0 μL and flow rate of 0.5 μL/min were employed in the present system as a compromise between the detection sensitivity and the analysis time.

3.3. Analytical performance

The analytical performance of the system was demonstrated in human IgG measurement. The relationship between the capillary fluorescence intensity and the concentration of human IgG solution is shown in Fig. 6. In the concentration range of 0–300 μg/mL, the fluorescence intensity signals increased with the human IgG concentration, and a linear relationship ($I=0.19C+1.73$, $r^2=0.9869$) was obtained in relatively low concentration range of 0–10 μg/mL. The error bars indicate the standard deviation of the fluorescent signals for three repetitive measurements at each concentration. The limit of detection for human IgG based on three times the standard deviation of the blank values was 0.68 μg/mL. The precision of the system was 2.4% RSD ($n=5$) for 100 μg/mL human IgG solution. The sample and fluorescent reagent consumption for each measurement were both 1 μL. The time for liquid operation and immune reaction was reduced to 12 min.

4. Conclusion

In summary, an automated liquid handling method for heterogeneous immunoassay was developed using the programmable peristaltic pump and the reagent cartridge to fully automate the labor-intensive and time-consuming multistep liquid handling operation. Compared with the previously reported microfluidic immunoassay systems, the present system provides a simple and rapid method for liquid handling in multistep immunoassay and is particularly suitable for POCT. The disposable antibody-coated capillary and reagent-loaded cartridge can be fabricated easily without the need of microfabrication techniques, and their costs are quite low, only $0.5 for one measurement.

In the present work, an inverted fluorescence microscope was used to detect the fluorescence intensity of the capillary. The bulky microscope is not suitable for POCT application. A miniaturized laser induced fluorescence detector is being developed in the authors' group which could be used to achieve the whole immunoassay operation from sampling to results display on-site.

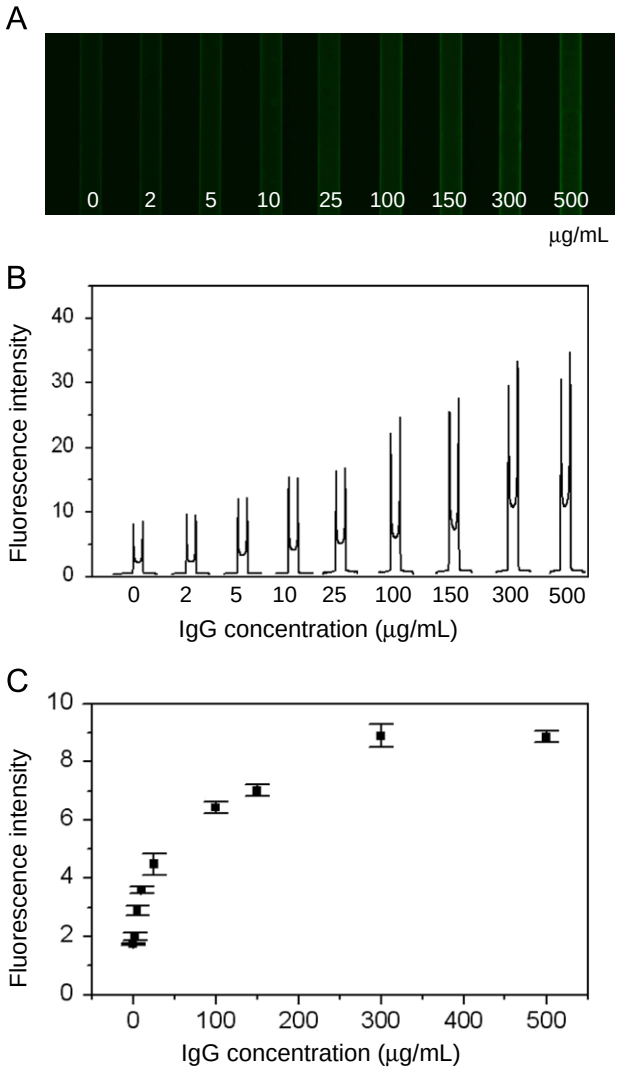

Fig. 6. (A) Fluorescence images of the capillaries with different human IgG concentrations. (B) Quantified fluorescence intensity distribution of the capillaries in (A). (C) Calibration curve of human IgG sample in the range of 0–500 μg/mL.

In addition, the programmable miniaturized peristaltic pump has the advantages of simple structure, small size, low cost, and easy to build and use. It can also be used in other microfluidic systems as an automated liquid handling device.

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