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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.

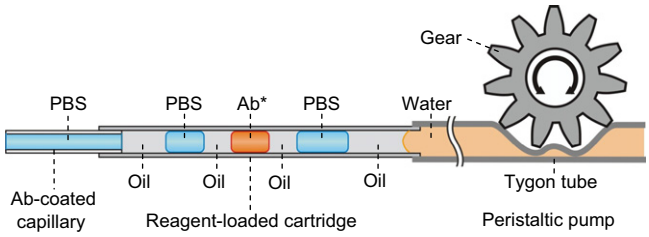

Fig. 1. Schematic diagram of the microfluidic immunoassay system. Ag, antigen; Ab, antibody; Ab*, fluorescently labeled antibody.

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.

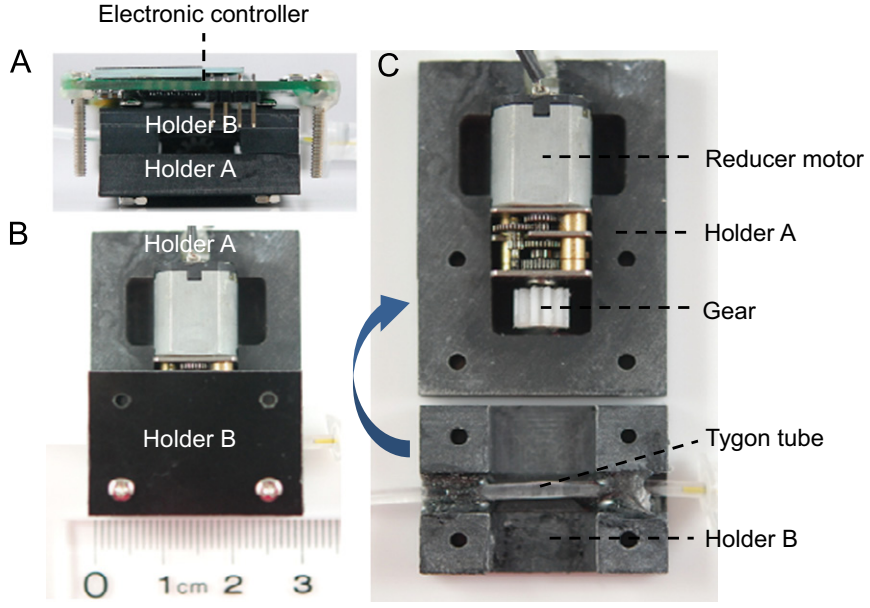

Fig. 2. The side view (A), top view (B), and internal structure (C) of the programmable peristaltic pump.