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A microchannel immunoassay chip with ferrofluid actuation to enhance the biochemical reaction

Yaw-Jen Chang*, Chih-Yu Hu, Chu-Hsuan Lin

Department of Mechanical Engineering, Chung Yuan Christian University, 32023, Taiwan, ROC

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ABSTRACT

This paper presents a novel microchannel immunoassay chip comprised of a nitrocellulose slide and a microchannel film. The microchannel film has a circular channel and four reaction zones and is fabricated using PDMS. An analytical reagent, such as protein, can be immobilized at the reaction zones on a nitrocellulose slide, and the sample to be analyzed is manipulated by ferrofluid to circulate through the reaction zones for immunological tests. The ferrofluid forming plugs in the circular channel was actuated by an external NdFeB permanent magnet affixed to a stepper motor. The purpose of circulative fluid actuation is to produce enough agitation to increase the collision opportunities of molecules and, hence, the reaction rate. After immunoassay, the PDMS microchannel film can be peeled off for fluorescence scanning. In this study, four different concentrations of biotin were immobilized at each zone, respectively. Streptavidin–Cy5 conjugate was driven by ferrofluid. The experimental results showed that adequate fluid actuation was contributive to enhance the immunoassay reactions. The incubation time can be reduced, providing high throughput experiments. Moreover, slow fluid actuation cannot produce enough agitation to increase the reaction rate, while rapid fluid actuation has a negative effect on immunoassay, as the immobilized biotins may be removed from the chip surface.

1. Introduction

Immunoassay is a widely used biochemical test for measuring analytes normally presented at very low concentrations. It is based on the high affinity recognition capabilities of antibodies with their antigens. In recent years, immunoassay can be performed on microarray chips due to the maturity of bio-MEMS technologies. The analyte-specific reagents (ASRs), such as antigens, are spotted on the microarray surface. Each microspot works as a capture molecule, and can be incubated with a specific type of analyte from a complex mixture. Various classes of capture scenarios, e.g. sandwich assay, antigen–antibody interaction, and protein–protein interaction, have been developed [1]. In the sandwich immunoassay, for example, after the incubation and washing steps, the solution containing the analytes to be detected is added to cover the entire microarray chip, and with the help of a second tagged molecule, an experimental readout based on fluorescence represents the relative amounts of the captured analytes. A microarray allows high throughput study of protein abundance, and has become an indispensable tool in the development of proteomics [2–4]. However, immunoassay is a labor-intensive and time-consuming procedure even though it is conducted using microarray chips.

In BioMEMS applications, microfluidic platforms can provide transportation for reagents and samples in the microchannels for the purposes of dilution, particle separation, mixing, and incubation. Thus, microfluidic devices are the primary components of most lab-on-a-chip (LOC) devices, as they offer the possibility of a smaller consumption volume of reagents, shorter reaction time, and parallel operation in a single chip. Recently, many research achievements reported that the merging of microfluidics and microarray technologies can enhance reaction efficiency [5–8]. A variety of applications have been proposed for DNA hybridization [9–11], pathogenic nucleic acid detection [12,13], and validation of oligonucleotide [14]. Consequently, the microfluidic-based immunoassay chips have also evoked great research interest [15–18].

Fluid actuation is the core technique for microfluidic devices. Some existing fluid actuation methods include electrical, pneumatic, thermal actuation, etc. However, they generally involve complicated and expensive fabrication processes, and some are restricted to certain limited samples. The use of ferrofluid is an innovative actuation method for fluid handling. Ferrofluid is a colloidal suspension of nanosize magnetic particles in an organic or aqueous medium [19]. Due to the properties and behavior in magnetic fields, ferrofluids have been used in many applications, such as dynamic sealing in rotating shafts, heat dissipation for

* Corresponding author. Fax: +886 32654399.
E-mail address: justin@cycu.edu.tw (Y.-J. Chang).

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loudspeakers, and contrast enhancement for magnetic resonance imaging [20]. In recent years, ample implementations to MEMS devices were reported as actuation mediums in micropumps [21–28], microvalves [29,30], and micromixers [31]. The microchannels of these ferrofluidic applications were commonly fabricated using PMMA or silicon wafer and manufacturing is time-consuming and costly. Moreover, there are few researches on its implementation for biological applications.

In this paper, a novel microchip design, with PDMS microchannel film adhered to a nitrocellulose slide, is presented. The analytical reagent, such as protein, can be immobilized at four reaction zones on a nitrocellulose slide, and the sample to be analyzed is manipulated by ferrofluid to circulate through the reaction zones for immunological tests, which differs from most applications by unidirectional transportation of the sample from a location to pass through the microarray. After immunoassay, the PDMS microchannel film can be peeled off for fluorescence scanning. The purpose of circulative fluid actuation is to produce enough agitation to increase the collision opportunities of molecules and, hence, the reaction rate. The influence of a flowing reagent upon the immunoassay was systematically investigated in this study.

2. Experimental methods

The miniaturized immunoassay system consists of a microchannel immunoassay chip and a control device. The microchannel immunoassay chip was produced by coating a layer of nitrocellulose (NC) membrane on the glass substrate on which a PDMS microchannel film attached. The nitrocellulose membrane was used to immobilize analytical reagent, such as protein, for immunological tests. Inside the circular microchannel, ferrofluid was injected for driving the sample to be analyzed. By controlling the speed of stepper motor, the sample flowed through the reaction zones for enhancing the biochemical reaction. In this study, the principle of biotin–streptavidin binding specificity was applied to examine the performance of this microchannel immunoassay chip. The chip fabrication and the experimental procedure are elaborated as follows.

2.1. Coating of nitrocellulose membrane

Immobilization of proteins on nitrocellulose membrane is one of the most widely used techniques for protein analysis. Nitrocellulose membrane works as a universal blotting surface. With the porous fiber structure and electrostatic force, the membrane can immobilize nonspecific biospecies via noncovalent surface interactions. Hence, the feature was utilized in this study by coating nitrocellulose on the chip as the biologic recognition layer.

Nitrocellulose paper was purchased from Whatman International Ltd. (Maidstone, Kent, UK). Due to the solid phase, nitrocellulose paper cannot be directly coated onto the surface of a chip. It must be dissolved by alcohol or ketone to form an aqueous solution before coating. Moreover, nitrocellulose paper does not have physical or chemical adsorbability with most common substrates such as glasses. 3-Glycidoxypropyltrimethoxysilane (GPTS) is a medium to assist in coating the nitrocellulose solution onto the surface of glass slide.

The fabrication process of nitrocellulose slide was complex. The production steps of NC membrane are as follows:

(1) Firstly, with mixing ratio of 25:1, acetone and GPTS (purity: 98%, manufactured by Dow Corning) were added in a test tube and sealed with parafilm to avoid evaporation. Parafilm (produced by Pechiney Plastic Packaging Company, USA) is a ductile and cohesive plastic paraffin film with a paper backing for sealing vessels. The solution was shaken using high-speed rotator for 6 h until both materials were completely mixed.
(2) The nitrocellulose paper was torn into pieces and added into the solution with shaking again for 6 h until the paper was fully dissolved. Then, the nitrocellulose solution was ready for further bio-experiments. In this study, 3% nitrocellulose solution was prepared.
(3) The NC solution of 1 mL was applied to the surface of 1 in. × 3 in. glass by the spin coater with high-speed velocity.
(4) Glass substrates were placed in an oven at 60 °C for 1 h to dry the surface moisture for obtaining stable NC membranes.
(5) The NC slides were preserved in the drier more than a week before usage.

2.2. Fabrication of PDMS microchannel film

PDMS microchannel film has one circular channel and four reaction zones, as illustrated in Fig. 1(a). The width and depth of circular channel are 2 mm and 500 μm, respectively, with inner radius of 7.5 mm. Thus, the volume of the circular channel is 53.4 μL. The dimension of each reaction zone is 14 mm × 7 mm. Certainly, the design of PDMS microchannel film can be changed according to the demand of user.

A bakelite mold, as shown in Fig. 1(b), having opposite pattern of microchannel design was manufactured by machining. This bakelite was adhered to a glass and surrounded with a PMMA frame so that the microchannel film can be fabricated by casting PDMS in the bakelite mold. Prior to the casting process, a layer of silicon oil was applied on the mold so that the PDMS film can be peeled off from the mold easier. PDMS (SYLGARD®184) purchased from Dow Corning (Midland, MI, USA) is a silicone elastomer, with the supply of base and curing agent as a two-pair kit, comprising of liquid components. The two components must be thoroughly mixed using a volume ratio of 8:1. After pouring PDMS mixture, the mold is placed in the vacuum chamber of oven for de-airing such that there are no bubbles in PDMS mixture. Finally, the PDMS mixture is cured at 50 °C in an oven for 4 h. Once cured, the PDMS microchannel film is carefully peeled off from the bakelite mold. The bakelite mold is reusable to fabricate more PDMS microchannel films. Fig. 1(c) shows the fabrication procedure.

In order to enhance the resistance of PDMS microchannel film to acid and alkali, surface modification is necessary by O_2 plasma under vacuum of 10^{-1} Torr and plasma intensity of 29.6 W for 3 min.

PDMS microchannel film attaches on the substrate by physical absorption so that it can be peeled off easily. The leakage in bonding of PDMS microchannel film and glass substrate is not allowed. If the microchannel film cannot adhere to the substrate tightly, the fluid (reagent) may outflow. It might significantly influence the result of analysis. Basically, PDMS possesses excellent physical absorption property to adhere to the surface of glass substrate. However, the absorption property is reduced due to the coating of NC membrane, resulting in leakage. Therefore, clamps were used to tightly clip both together with an additional PMMA template on the top of PDMS film for obtaining uniform pressure distribution, as shown in Fig. 1(d).

2.3. Actuation by ferrofluid

Ferrofluids are colloidal suspensions of nanosize magnetic particles in an organic or aqueous medium. The magnetic particles are coated with a surfactant layer to prevent agglomeration due to the short-range van der Waals force between individual particles. These suspensions are stable and preserve their properties at extreme temperatures and over a long period of time. In this paper, the nanosize magnetic particle is Fe_4O_3 and the carrier liquid of ferrofluid is the mixture of silicone oil and hexane, which is immiscible with the water. The ferrofluid was purchased from Tan Bead (Taiwan).