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Microfluidic immunoassay with plug-in liquid crystal for optical detection of antibody

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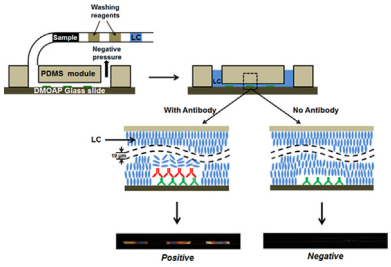
HIGHLIGHTS

- Appearance of liquid crystal (LqC) is dictated by aspect ratio of the microchannel.
- Immunobinding events can be detected by LqC signals inside a microchannel.
- One-step immunoassay is enabled by using tubing cartridges for liquid delivery.
- One-step LqC-based immunoassay is able to detect 1 $\mu g\,mL^{-1}$ anti-IgG in 25 min.
- One-step LqC-based immuoassay shows good specificity and quantitation capability.

GRAPHICAL ABSTRACT



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ABSTRACT

Recent advance in liquid crystal (LqC) based immunoassays enables label-free detection of antibody, but manual preparation of LqC cells and injection of LqC are required. In this work, we developed a new format of LqC-based immunoassay which is hosted in a microfluidic device. In this format, the orientations of LqC are strongly influenced by four channel walls surrounding the LqC. When the aspect ratio (depth/width) of the channel is smaller than 0.38, LqC orients homeotropically inside the microchannel and appears dark. After antigens bind to immobilized antibodies on the channel walls, a shift of the LqC appearance from dark to bright (due to the disruption of LqC orientation) can be visualized directly. To streamline the immunoassay process, a tubing cartridge loaded with a sample solution, washing buffers and a plug of LqC is connected to the microfluidic device. By using pressure-driven flow, the cartridge allows antigen/antibody binding, washing and optical detection to be accomplished in a sequential order. We demonstrate that this microfluidic immunoassay is able to detect anti-rabbit IgG with a naked-eye detection limit down to 1 $\mu g\,mL^{-1}$. This new format of immunoassay provides a simple and robust approach to perform LqC-based label-free immunodetection in microfluidic devices.

1. Introduction

Microfluidic immunoassays possess several advantages such as smaller sample volume, shorter assay time and lower cost compared to conventional immunoassays performed in 96-well

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plates [1,2]. However, because of its miniaturized format, microfluidic immunoassay usually requires special signal detection and enhancement methods such as fluorescence-based detection and enzymatic reaction-based enhancement. Therefore, antibodies/antigens in microfluidic immunoassays are often conjugated with labels such as fluorophores [3,4], enzymes [5,6], nanoparticles [7,8] and redox labels [9], to transduce the immunobinding events into detectable signals. Nevertheless, labeling proteins is tedious and the biofunctionality of antibodies/antigens can be affected upon conjugation with labels [10,11].

Liquid crystal (LqC) has been used as a signal transducer for label-free biodetection [12]. It has been reported that long-range orientation of LqC responds sensitively to surface immunobinding events and changes the optical appearance of LqC, i.e., bright signal upon polar orientation shift [13,14] or change of the brightness upon azimuthal orientation shift [15,16]. These changes enable the development of label-free immunoassays for naked-eye detection. However, because LqC is fluidic in nature, it has to be confined in well-defined space for sensing applications. For example, label-free immunodetection can be conducted either in an optical cell [17] or in a TEM grid [18]. Nevertheless, assembly of these devices requires additional manual steps and is time-consuming. Moreover, rinsing steps are still required after the immunobinding and before filling the cell with LqC [13,14]. Currently, these two issues are the main obstacles to apply LqCs to immunoassays in practice.

Considering the issues mentioned above, we aimed to take advantage of the fluidic property of LqC, and to develop an LqC-based immunoassay in which LqC can be injected directly into a microfluidic channel for detection of antigens/antibodies. So far, some efforts have been devoted to the integration of LqC-based detection with microfluidic devices. For example, Sutarlie and Yang first reported a device with an LqC-doped PDMS film to detect ethanol in microfluidics [19]. Because PDMS is permeable to small organic molecules, ethanol can diffuse into PDMS and interact with LqC to change its color. This device enables real-time monitoring of ethanol produced during microbial fermentation. Nevertheless, this device is only applicable to the detection of organic molecules which can diffuse through PDMS. In another study, Liu et al. developed a microfluidic device with LqC-filled metal grids for the detection of detergent and enzyme on LqC-aqueous interface [20]. Real-time monitoring of different analytes in the microfluidic device was achieved. However, fabrication of micrometer-sized metal grids on glass substrate involves tedious procedures in cleanroom, and syringe pumps were still needed for reagent delivery. Recently, Aliño et al. fabricated a microfluidic device containing printed LqC droplets for detection of protein inside microfluidic channels [21]. These tiny LqC droplets (~pL) embedded in microfluidic channels act as individual protein sensors and generate dark-to-bright response to proteins adsorbed on the LqC-aqueous surface. Nevertheless, a material printer is needed for the generation of fine LqC droplets [22]. Instability of surface immobilized LqC droplets under flowing conditions is another issue.

In this work, to develop a fully functional LqC-based microfluidic immunoassay, we first investigated how LqC behaves in a PDMS–glass hybrid microchannel. We point out that this system is substantially different from a traditional optical cell, because LqC in a microchannel is in contact with four channel walls with different length scales (rather than two surfaces as in the case of an optical cell). Apparently, the orientations of LqC are determined by not only the surface functionality but also the geometric dimensions of the channel walls. Understanding the orientational behavior of LqC in a microchannel is important, because it allows us to design microchannels with optimized dimensions and control the orientational response of LqC to immunobinding events. Secondly, we investigated the feasibility of the integration of LqC-based detection with the microfluidic immunoassay to detect immunobinding events on the microchannel surface. Since LqC is immiscible with water, we hypothesize that LqC can push out any residue aqueous solutions from the microchannels after the immunobinding and washing steps. Detection of antigens/antibodies can be accomplished by simply observing the optical appearance of LqC inside the microchannel. To further streamline the immunoassay procedure, we introduced tubing cartridges loaded with sample solution and other reagents [23]. This approach enables all steps (immunobinding, rinsing and detection) to be conducted in a sequential manner with minimal manual procedures.

2. Experimental

2.1. Reagents and materials

All glass slides were purchased from Marienfeld (Germany). Polydimethylsiloxane (PDMS) prepolymer (Sylgard 184) was purchased from Dow Corning (USA). *N*,*N*-dimethyl-*N*-octadecyl-3-aminopropyltrimethoxysilyl chloride (DMOAP), trichloro (1*H*,1*H*,2*H*,2*H*-perfluorooctyl) silane (passivation silane), rabbit IgG (IgG), anti-rabbit IgG (anti-IgG) and bovine serum albumin (BSA) were purchased from Sigma–Aldrich (Singapore). Liquid crystal 4-cyano-4′-pentylbiphenyl (5CB) was purchased from Merck (Japan). PBS buffer (10×, pH 7.4) was purchased from 1st BASE (Singapore). Mylar transparency mask was purchased from Infinite Graphics (Singapore). Fluorinated ethylene propylene (FEP) tubing (i.d/o.d = 0.8 mm/1.6 mm) was purchased from Cole Parmer (USA). Negative photoresists, SU8 2010 and SU8 2050, were purchased from Microchem (USA). Silicon wafers were purchased from LaTech (Singapore).

2.2. Fabrication of PDMS microchannels

Silicon masters for PDMS molding were fabricated by using standard photolithography with soft masks and negative photoresists (SU8 2010 and SU8 2050) [24]. The height of the microchannel feature on the silicon wafer was controlled by applying different speeds during spin-coating of photoresist and verified by using a P-16+ surface profiler (KLA-Tencor, USA). The silicon master was then treated with passivation silane to passivate its surface. PDMS microchannels were fabricated using standard soft lithography [25]. In brief, prepolymer and curing agent (in 10:1 w/w ratio) were mixed and degassed for 30 min to remove air bubbles. The mixture was then poured onto the silicon master and cured at 65 °C for 2 h. Finally, the cured PDMS microchannels were peeled off from the master, cut into appropriate size and punched with holes to form inlets and outlets.

2.3. Detection of surface-immobilized IgG in microchannel

A piece of glass slide was modified with DMOAP based on a protocol reported previously [26]. To activate the surface, the DMOAP-coated slide was placed 3 cm below a UV pencil lamp (254 nm, from Spectroline, USA) and exposed to UV light for 40 s. The UV/ozone oxidation of the alkyl chain on DMOAP leads to the formation of aldehyde group [27]. Two microliters of immobilization buffer (50 mM $NaHCO_3$, 100 mM $MgCl_2$, 10 mM $NaBH_3CN$, pH 10.0) containing different concentration of IgG was manually spotted onto the UV-treated glass slide. The slide was then incubated for 1 h in a home-made humidified chamber to allow IgG to be covalently immobilized through reductive amination [28]. Next, the slide was rinsed thoroughly with PBS buffer and DI water to remove unbound IgG, and dried with nitrogen gas. Subsequently, PDMS microchannels were conformally attached to the IgG-