Sensors and Actuators B 209 (2015) 242–251

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Microfluidic-based multiplex immunoassay system integrated with an array of QD-encoded microbeads

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ARTICLE INFO

Article history:
Received 6 August 2014
Received in revised form
12 November 2014
Accepted 24 November 2014
Available online 30 November 2014

Keywords:
Multiplex immunoassay
QD-encoded microbeads
Microfluidic device
Suspension and the planar microarray

ABSTRACT

Here, we developed a multiplex immunoassay platform within microfluidic devices that combines suspension and the planar microarray format. For the suspension microarray format, QD-embedded polymeric microbeads with an average diameter of 24 μm were prepared using the Shirasu Porous Glass (SPG) membrane emulsification technique. To furnish the microbeads with resolvable spectral codes, QDs with two different colors (450 nm for blue and 520 nm for green) were used and different spectral codes were obtained by changing the ratio of emission intensity of the two different QDs within the microbeads. The surfaces of the QD-encoded microbeads were then functionalized with probe antibodies for immunoassays. The planar microarray format was achieved by an array of microholes fabricated in PDMS. Each microhole was designed to trap a single microbead and eventually generated a microbeads array within the microfluidic system. The feasibility of the microbead array within microfluidic devices for use in a multiplex immunoassay was demonstrated by immunobinding assays between IgG and anti-IgG and/or between IgM and anti-IgM. Furthermore, the resultant microbead-based on-chip assay could be used for a sandwich assay to detect prostate-specific antigen (PSA), a model cancer marker, with a detection limit of 1 ng/mL. The combination of suspension and the planar microarray format enabled the spatial location of individual microbeads within physically separated regions and thus facilitated the simultaneous determination of different targets that interacted with the corresponding microbeads.

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1. Introduction

Multiplex immunoassays are of great importance for various fields, such as medical diagnostics, drug discovery, and other biological studies. Multiplex measurements provide richer information on the biological status of a sample compared to single protein measurements while minimizing the use of sample volume and eliminating the need to run multiple assays [1,2]. To achieve a high-throughput and high-content multiplex immunoassay, antibody-immobilized microarrays have been developed using both chip-based planar microarray and microbead-based suspension (solution) microarray [3–5]. Planar microarrays have been generated by the immobilization of probe antibodies on different microdomains within a plane surface, such as a glass slide [6–8]. Each probe antibody was easily identified by the x and y coordinates on the microarray. Although planar microarrays have been well publicized and have been widely used for applications requiring ultra-high-density analysis, suspension microarrays are becoming popular strategies for high-throughput immunoassays because they have several advantages over planar arrays [9–14]. The high available surface-to-volume ratio of the microbead provides more binding sites for analytes and thus increases the sensitivity of target detection. Moreover, the microbeads enable much faster reactions due to their suspension in a homogeneous solution and the associated advantageous effects on diffusion rates. Suspension microarrays employ self-encoded microbeads as array elements, instead of positionally encoded spots on a planar surface [15]. Among various encoding methods, fluorescence encoding, where both organic and inorganic fluorophores are incorporated into microbeads, has been most widely used due to the large encoding capacity of the microbeads [16–21].

Increasing efforts have been made to incorporate a microbead-based suspension microarray into a microfluidic system, which can be integrated into low-cost devices for biochemical research and point-of-care diagnostic applications [22]. To implement bead-based assays within a microfluidic device, the microbeads should be placed in a specific region. Usually, protein-immobilized microbeads are retained within the microfluidic device using mechanical obstruction, such as a microweir with a small gap between the top of the weir and the channel's roof or microfilter consisting of micropillars with small interspaces [23–26]. Instead of building physical structures to trap the microbeads, an external

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http://dx.doi.org/10.1016/j.snb.2014.11.115
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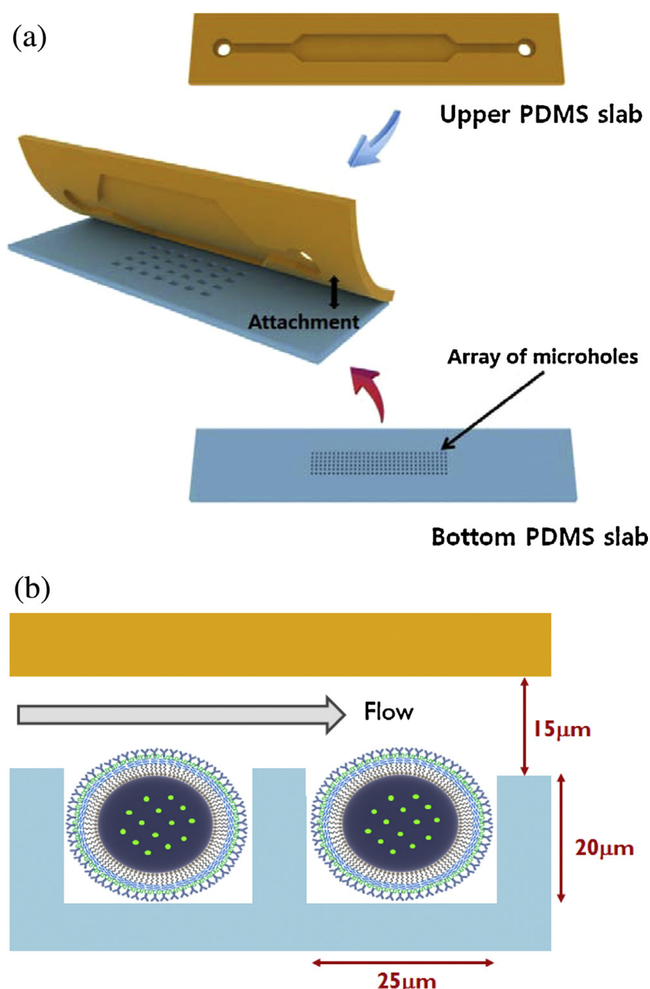

Fig. 1. Schematic illustration of the microfluidic device for on-chip multiplex immunoassay using an array of microbeads. (a) Microfluidic devices consisting of two different PDMS slabs. (b) Side view of microchannels integrated with antibody-immobilized and QD-encoded microbeads within microholes.

magnetic field can be applied to a position in the microfluidic system when the paramagnetic beads are utilized [27–30]. However, several challenges exist in current microfluidic systems that have attempted to implement suspension microarray-based assays. In most cases, many microbeads were trapped within the same region, contacting each other, which may result in not only the deactivation of surface-immobilized probe proteins but also inhibition of the interaction between the probe and target molecules. It is also very difficult to monitor the same single microbead continuously because many microbeads are placed in the same area and they can change their location. Furthermore, because the number of microbeads incorporated within the microfluidic device cannot be precisely controlled, it is very difficult to obtain consistent results between devices.

In this study, as an effort to overcome the drawbacks associated with current suspension microarray systems within microfluidic devices, we designed microfluidic systems that combine the planar microarray format with the suspension microarray format. For the suspension microarray, QD-encoded polymeric microbeads were prepared using the SPG membrane emulsification technique. Instead of immobilizing probe antibodies onto the microbeads directly, we used the layer-by-layer (LbL) deposited multilayer as a spacer between the protein and microbead because this soft layer has been reported to minimize the dehydration and denaturation of immobilized proteins due to its "cushioning effect" as well as large hydration volume [31–33]. For the planar microarray format, an array of microholes was fabricated in the PDMS-based microfluidic system, where each microhole was designed to trap a single microbead and eventually generated the microbead array within a microfluidic system. Thus, each microbead was separated from other microbeads and easily identified via the *x* and *y* coordinates as well as by color, thereby facilitating observation of the multiplex immunoassay. After establishing the microbead array in the microfluidic system, the feasibility of the developed on-chip assay was successfully confirmed using immunobinding assays between IgG/anti-IgG and between IgM/anti-IgM as well as the sandwich immunoassay to detect prostate-specific antigen (PSA).

2. Materials and methods

2.1. Materials

Polystyrene (PS, MW 192,000), sodium lauryl sulfate (SLS), poly(allylamine hydrochloride) (PAH, MW 50,000), poly(acrylic acid)(PAA, MW 50,000), (1-ethyl-3(3-dimethylaminoprophyl)carbodiimide (EDC), N-hydroxysuccinimide (NHS), and phosphate buffered solution (PBS, 0.1 M, pH 7.4) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). A solution of 10 mg/mL CdSe/ZnS quantum dots (QDs) in chloroform (NSQDs-OS, emission wavelength 450 nm and 520 nm) was obtained from NanoSquare Inc. (Seoul, Korea). Mouse-IgG (IgG), mouse-IgM (IgM), Cy5-rabbit anti-mouse IgG (Cy5-anti-IgG) and Cy5-goat anti-mouse IgM (Cy5-anti-IgM) were purchased from ZYMED Laboratories (San Francisco, CA, USA). Prostate-specific antigen (PSA) and anti-PSA antibody (anti-PSA) were obtained from Pierce (Rockford, IL, USA). The Dow Corning Sylgard 184 poly(dimethylsiloxane) (PDMS) elastomer was purchased from Dow Corning (Midland, MI, USA). Photomasks for photolithography were prepared using AUTO CAD and were printed onto transparencies using a standard laser jet printer (LaserWriter 16/600 PS, Apple Inc., Cupertino, CA, USA).

2.2. Preparation of QD-embedded PS microbeads

QD-embedded PS microbeads were prepared using the Shirasu Porous Glass (SPG) membrane (SPG Technology Co, Miyazaki, Japan) with a pore size 15 μm, as shown in Fig. S1. PS and QDs were dissolved in methylene chloride at 10 w/v% and 1 mg/mL concentration, respectively, and the resultant solution was stored in the tank as the dispersed phase. SLS was dissolved in water at 1% (w/v) in the continuous phase. By applying nitrogen pressure, the PS solution containing QDs passed through the SPG membrane pores and was dispersed in the continuous phase to form the monodispersed emulsion. QD-embedded PS microbeads were finally obtained after the evaporation of methylene chloride in the emulsion. Scanning electron microscopy (SEM) was performed using a JEOL T330A at 15 kV (JEOL, Ltd., Tokyo, Japan) to observe the morphology of the microbeads.

2.3. Immobilization of antibodies on the microbeads

The surfaces of the QD-embedded PS microbeads were first modified with PAH/PAA layers using the layer-by-layer (LbL) deposition method, and subsequently immobilized with antibodies (Fig. S2). Briefly, negatively charged microbeads were incubated in 1.0 mg/mL PAH-0.4 M NaCl solution (pH 7.4) for 15 min. The excess PAH was removed by three washings with water using a centrifugation (at 1400 g)/washing protocol. The PAH-layered PS particles were then resuspended in 1.0 mg/mL PAA-0.4 M NaCl solution for 15 min, followed by three washings.

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