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A microfluidic chip for measurement of biomolecules using a microbead-based quantum dot fluorescence assay

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Abstract
This paper describes a custom-designed microfluidic chip for sensitive detection of antibody using quantum dot fluorescence in a microbead-based assay. The microfluidic chip is designed to isolate a single microbead where the binding reaction of antibodies happens on the surface. The microfluidic chip is fabricated on a glass substrate using transparent silicone elastomer, PDMS, for easy access to monitoring and flexible gate operations to capture the single microbead. For antibody detection, a sequence of functionalized assay has been performed in the fabricated chip including: capturing of microbeads; antibody injection into a micro-chamber; quantum dot injection; and fluorescence detection. Various concentrations of antibody have been tested and have shown good agreement with correlated fluorescent intensities.

Keywords: antibody, microbead, quantum dot, microfluidic chip, MEMS

(Some figures in this article are in colour only in the electronic version)

1. Introduction

The analysis using microfluidic chips generally provides low cost, high throughput, fast analysis and high sensitivity [1, 2]. The microfluidic chips can also provide multiple functions for the monitoring of various reactions and consecutive observations. These multi-functional microfluidic systems, which are sometimes referred to as digital microfluidics, have been implemented in many different ways such as droplet-based electrowetting [3], continuous fluidic system with passive components [4] and microbead-based fluidics [5–12]. Especially, the bioanalytical methods using microbeads have been reported by many research groups due to the easiness in modifying the surface of microbeads for specific bindings and manipulating them inside microfluidic channels. Recently, with the advancement of microfluidic technology, a few methods to manipulate a single microbead on a microfluidic chip have been introduced [13–15]. Our group reported a microfluidic chip which can passively manipulate microbeads in a fluid stream and position them in pre-determined target micro-wells [15]. Medoro *et al* used active control to manipulate cells (or microbeads) by using dielectrophoresis [13, 14]. The benefit of active control is that it can provide high adaptability in bead/cell manipulation; however, it requires a complicated layout of control signals in chip implementation. In this paper, we report a microfluidic chip for bio-assay which can allow for the manipulation of microbeads down to single-bead resolution by using simple pneumatic control of a microgate without employing complicated active manipulation.

In this work, we have developed a quantum dot (QD) fluorescence assay by injecting QDs conjugated with antibodies to detect target biomolecules attached to a microbead which is being captured in a micro-chamber. In the optical detection of a bioanalytical assay, QDs have many advantages as a fluorescent material compared with conventional organic dyes. QDs are inorganic fluorophores which can provide much brighter luminescent labels than conventional fluorescent tags, and their emission spectra can be modulated according to the particle size. Therefore, simultaneous excitation of QDs of different sizes can be used to identify various biomolecules in different emission colours due to their broad excitation spectra. Also, long-term photo-stability and narrow emission bandwidth are other benefits of QDs. With these advantages, QDs have been widely used for the detection of biomolecules by attaching QDs to target biomolecules [12, 16–25]. Bakalova *et al* has introduced QD-based western blot technology in recent publications [19, 20]. Sun *et al* utilized QDs modified with anti-human IgG to detect human IgG antibodies on a protein microarray [21]. They reported about 2 μg mL^{-1} detection limit by using a laser conforcal scanner. Goldman *et al* have also reported the detection of protein toxins (staphylococcal enterotoxin B, cholera toxin) by using QD–antibody conjugates [22, 23]. They detected the toxins coated on microtitre plates, and the lowest concentration of toxins that gave detectable signal over background was approximately 15 ng mL^{-1}. Most previous research was focused on the detection of molecules after being fixed on a solid substrate such as well plates or a glass substrate. However, in this paper, we introduce a QD-based bio-assay using microbeads in a custom-designed microfluidic chip, where we can realize multi-functional experiments with the handy manipulation of microbeads which can be easily incorporated in future lab-on-a-chip applications.

An important issue in using microbeads for bioanalytical assay is to find the sensitivity in the assay, which can be explored by monitoring the detection limit of a single microbead. In this work, we investigate the minimum detection level of a single microbead-based assay in the proposed microfluidic chip by exploiting QDs for enhanced fluorescence markers.

2. Microchip implementation and experimental procedures

2.1. Operation of microfluidic chip

Figure 1 shows the schematic view and operation of the proposed microfluidic chip for a single microbead bio-assay. The chip is composed of a micro-chamber in which a single microbead can be isolated by gate operation. There are two gates located on either side of a micro-chamber. The gates are operated by pneumatic pressure applied in each gate control channel. These gates are designed to be partially open with a small gap at the initial phase when there is no pressure applied to the gate control channel. The gap is small enough to block the microbead but large enough for a liquid or reagent to flow. When negative pneumatic pressure (or vacuum pressure) is applied to the control channel, the gate is fully opened and microbeads can be introduced.

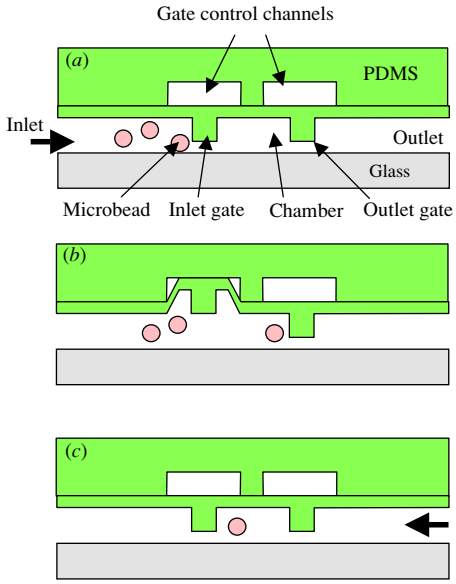

Figure 1. Schematic view of each step for the capturing of a single microbead. (*a*) Bead introduction, (*b*) gate open and (*c*) gate closed/single bead captured.

Initially, the inlet gate is closed (or partially open) to prohibit any microbeads from entering the micro-chamber. In order to capture a single microbead, first, the solution including microbeads is introduced through the inlet (figure 1(*a*)). Next, the inlet gate is opened by applying vacuum (or negative) pressure to the upper gate control channel (figure 1(*b*)). After a microbead enters the micro-chamber, the gate is closed to isolate a single microbead. Finally, the microbeads which remained outside the chamber are washed out by reversing the flow direction from the outlet to inlet (figure 1(*c*)).

2.2. Chip implementation

The proposed microfluidic chip has been implemented using two polydimethylsiloxane (PDMS) layers and a glass substrate which are bonded together. Figure 2 shows the fabrication procedures. For the upper PDMS structure, photo-sensitive epoxy, SU-8 (Microchem Co.), is patterned on a silicon substrate to be utilized as a replica mould for PDMS (figure 2(*a*)). Next, the self-assembled-monolayer (SAM) of tridecafluoro-(1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (United Chemical Technology, Inc.) is coated on both silicon and SU-8 surface for easy peeling off of the PDMS layer. Then, the PDMS prepolymer (Sylgard 184, Dow Corning Co.) is poured onto the mould structure and inherent bubbles are removed in a vacuum chamber (figure 2(*b*)). Finally, PDMS is cured at 80 °C for 1 h and peeled off from the substrate mould (figure 2(*c*)). The lower PDMS layer is fabricated using a similar method. First, SU-8 is coated with a thickness of 5 μm and exposed to ultraviolet (UV) to define the gate structure. Without development, the second coating of SU-8 (thickness of 30 μm) is performed followed by UV exposure to define the channel regions. Then, an unexposed region of SU-8 is developed forming a PDMS mould for channel and gate structures (figure 2(*d*)). Next, thin

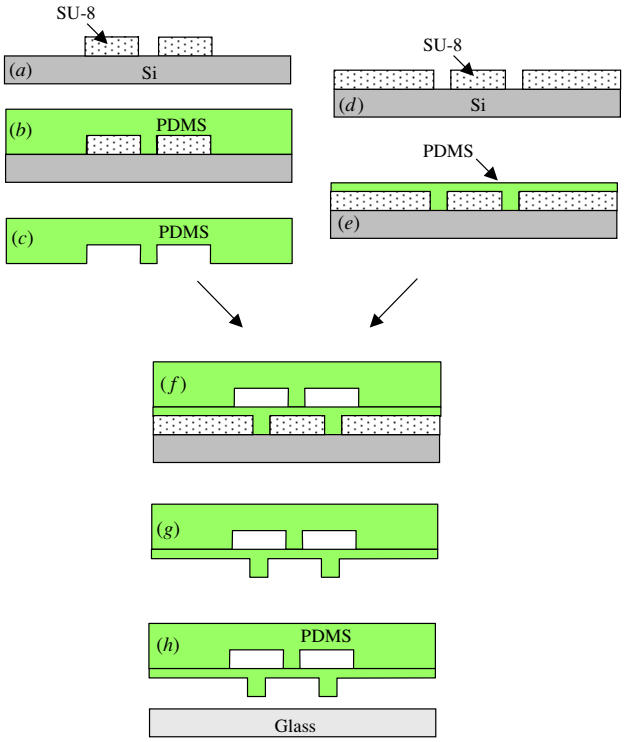

Figure 2. Fabrication processes. (*a*) SU-8 mould patterning for the top PDMS plate, (*b*) PDMS pouring and curing, (*c*) PDMS peeled off from the mould, (*d*) SU-8 mould patterning for the bottom PDMS membrane, (*e*) PDMS spin coating and curing, (*f*) bonding the top and bottom PDMS plates, (*g*) peeling off of the bonded PDMS layer from the substrate and (*h*) bonding with the glass substrate.

PDMS prepolymer is coated on the fabricated SU-8 mould by spin coating (thickness of 60 μm), followed by curing in an oven (figure 2(*e*)). The fabricated upper and lower PDMS layers are bonded together after surface modification by using oxygen plasma (figure 2(*f*)) and peeled off from the silicon substrate (figure 2(*g*)). The access holes for sample inlet, outlet and vacuum control are formed by manual punching. Finally, the completed PDMS structure is bonded with the glass substrate after surface treatment by oxygen plasma (figure 2(*h*)).

Figure 3 shows the fabricated microfluidic chip and a magnified view of a micro-chamber. The size of a micro-chamber is 50 μm $\times$ 50 μm square and the height is 30 μm. The micro-chamber is located between two gate valves. The initial gap of the gate valve is 5 μm in order to block the microbeads (10 μm) while maintaining liquid flow. Platinum electrodes (bright areas in magnified view) were formed on the bottom surface of the microchannel and micro-chamber for future applications. The width and height of inlet and outlet micro-channels are 50 μm and 30 μm, respectively.

2.3. Assay using single microbead on microfluidic chip

The fabricated microfluidic chip was cleaned with ethanol, sterilized in a commercial autoclave machine and fully dried out in an oven overnight. The surface of the microchannel was coated with 0.5% bovine serum albumin (BSA, in PBS)

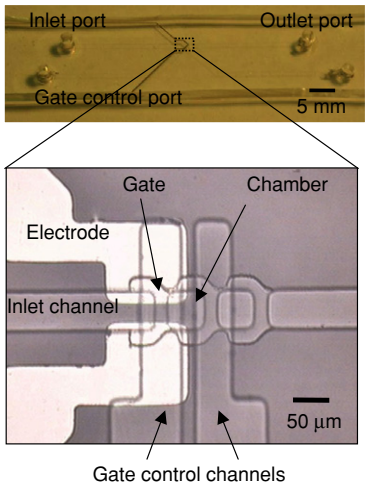

Figure 3. Photograph of the fabricated microfluidic chip and a magnified view of a micro-chamber.

for 2 h to prevent non-specific binding of proteins. Before the introduction of microbeads, first, the channel was filled with a phosphate buffer solution (PBS) to prevent air trapping in the microchannel. Then the solution containing microbeads was deposited in the inlet port using a pipette. To avoid the entrance of many microbeads into a microfluidic chip at a time, a diluted solution (about 10^4 mL^{-1}) was used in the experiment. Then, the introduced microbeads were loaded into the inlet gate by suction operation at the outlet port using a micro-syringe. When a microbead arrived at the inlet gate, the gate was opened to allow the microbead to pass through and then closed immediately while keeping the outlet gate closed. This results in the capture and isolation of a single microbead inside the micro-chamber. The gate manipulation was manually performed by a syringe connected to the gate control channel through a silicone tube. After trapping a single microbead, the rest of the microbeads loaded in the microchannel were washed out by reverse flow of PBS from the outlet to the inlet. During the period of reverse flow of PBS, the two gate valves are closed (or partially open) so that the captured microbead remains inside the micro-chamber.

Figure 4 shows the experimental protocol for antibody detection using a microbead loaded in the microfluidic chip. In this experiment, we used human IgG antibody as an example to demonstrate the feasibility of QD fluorescent detection using a single microbead. We used polystyrene microbeads (diameter 10 μm) coated with streptavidin (ProActive® streptavidin coated microspheres, Bangs Laboratories, Inc.). For a specific binding of human IgG, the surface of the microbead was coated with biotinylated protein-A. All of the liquids including PBS, antibody solution and QD solution were introduced into the microfluidic chip through a silicone tube connected to the inlet port. In order to maintain a steady flow at a very low flow rate, all the media and reagents were supplied at a constant pressure by applying the same height difference of liquid levels between the inlet and outlet ports. The reservoir containing the injection liquid was placed about 40 cm higher than the outlet, which creates about 4 kPa of constant pressure making a continuous flow rate of about 2 μL min^{-1} in our microfluidic chip.

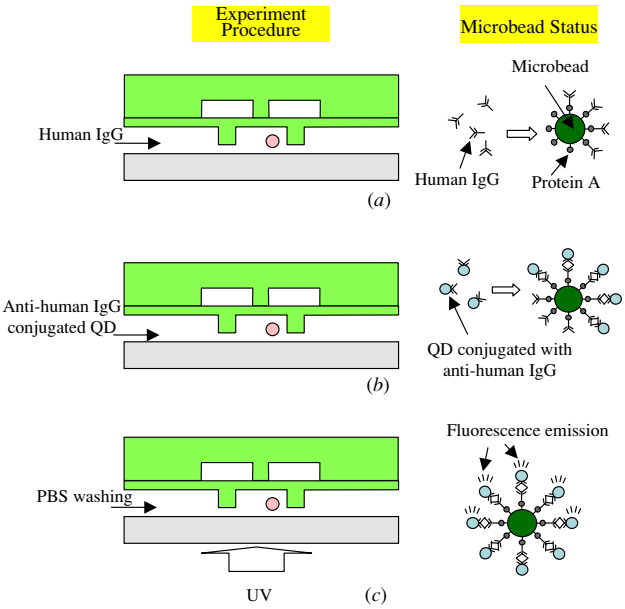

Figure 4. The experimental procedures. (*a*) Human IgG injection after bead capturing, (*b*) introduction of anti-human IgG conjugated with QD after washing and (*c*) washing with PBS and fluorescence detection.

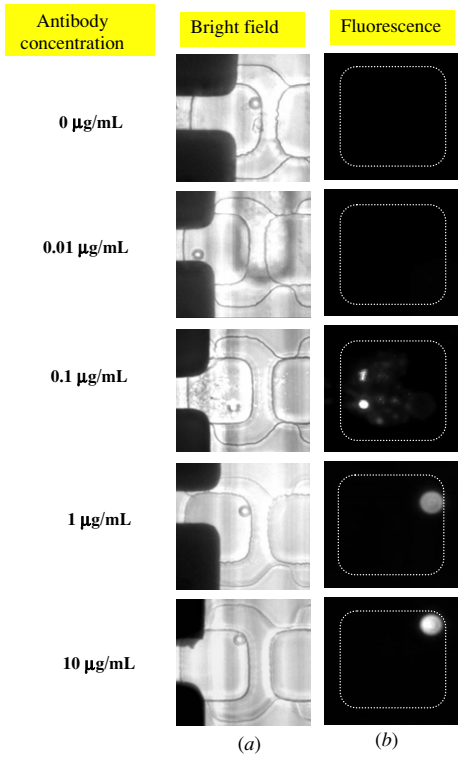

Figure 5. Experimental results. (*a*) Bright field (not fluorescence) images of each micro-chamber with the captured single microbead and (*b*) fluorescence images for various concentrations of antibodies in a corresponding micro-chamber.

After isolating a single microbead inside a micro-chamber, the human IgG antibody was introduced through the inlet (figure 4(*a*)). In order to study the detection limit of a single-bead assay, we conducted our experiment with five different microfluidic chips. Five different concentrations of human IgG were injected into each chamber for 30 min. The injected concentrations were 0 μg mL^{-1}, 0.01 μg mL^{-1}, 0.1 μg mL^{-1}, 1 μg mL^{-1} and 10 μg mL^{-1}, respectively. The injected antibody was attached to the surface of the microbead which was coated with biotinylated protein-A. After this, the micro-chamber was washed with PBS for 30 min. Then, 5 nM of QDs, which were conjugated with anti-human IgG, was injected into each micro-chamber for 30 min (figure 4(*b*)). In these experiments, CdSe/ZnS QDs conjugated with anti-human IgG were used ($\lambda_{emission} = \sim$605 nm, Qdot Corp.) for the fluorescence detection of human IgG. Finally, the channels and micro-chambers are completely washed with the PBS solution (figure 4(*c*)).

The experimental results on this microbead-based assay have been monitored using a fluorescence microscope (IX71, Olympus Co.) with a specialized filter set (XF304-2, Omega Optics). The wavelength of excitation light was 470 nm and the emission wavelength from QDs was about 605 nm. The fluorescence images from the microbead were captured using a CCD camera and the average brightness was extracted using graphic software.

3. Results and discussion

Figure 5 shows the experimental results presenting both bright field and fluorescent images of the micro-chamber with a single microbead. The images were captured after the injection of human IgG followed by a consequent introduction of QDs conjugated with anti-human IgG. The bright field images show the successful isolation of a single microbead in each micro-chamber (figure 5(*a*)). In these experiments, we could control the number of microbeads loaded into the microfluidic chip by diluting the solution. Typically, a very small number of microbeads (less than three microbeads in most cases) were loaded into the microfluidic chip and reached the inlet gate. Because the outlet gate was always closed, no microbead has been lost during the isolation of a single microbead. Sometimes, more than one microbead was captured inside a micro-chamber. In this case, we had to repeat the isolation experiment after removing all microbeads from a micro-chamber by opening both gate valves. Although we used purified distilled water for our experiment, we have observed contaminations. Sometimes unknown substances flow into the microfluidic chip and obstruct the fluid stream as shown in the case of 0.1 μg mL^{-1} in this figure.

Figure 5(*b*) shows initial experimental results of fluorescent images for various concentrations of antibody. From the fluorescent image of the antibody concentration of 0 μg mL^{-1}, we could verify that non-specific binding of anti-human IgG (conjugated with QDs) to the bare microbead is negligible. The fluorescence signal can be detected when antibody concentration becomes larger than 0.1 μg mL^{-1} and the intensity increases at higher concentrations. The normalized average intensities are plotted in figure 6 as a function of antibody concentration. Deviation from a linear relationship at the concentration of 0.1 μg mL^{-1} may come from the reduced binding of antibody due to the reduced flow rate of reagent in the contaminated microchannel. The detection limit of our work is much lower than that of the

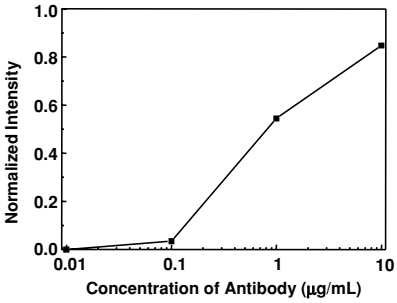

Figure 6. Measured average fluorescence intensity for various antibody concentrations.

previous report which used the same biomolecules analysed in this work. The detection limit in our experiments, 0.1 μg mL^{-1}, is by about one order of magnitude lower than that reported in [21], where the human IgG antibodies were detected down to 2 μg mL^{-1} on a glass plate. In our experiment, the utilization of commercialized QDs—while the QDs synthesized in their own laboratory were used in [21]—may have also contributed to the increased sensitivity. In the microfluidic chip-based assay, binding efficiency can be improved because the reagents are continuously transferred to the surface of microbeads, although this requires more experiments and analyses to be confirmed quantitatively.

For the establishment of the proposed detection scheme, we need to further optimize the chip design and experimental conditions including injection time of each reagent such as antibody and QDs, chamber volume and microbead size in future research. Once we know the detection limit of a single-bead assay, we can enhance the sensitivity by increasing the number of microbeads captured in a micro-chamber. We will leave this to a future study to examine the relationship between sensitivity and number of microbeads used in a micro-chamber.

4. Conclusion

In this paper we have demonstrated specific antibody detection by using a microbead-based assay with QDs on a custom-designed PDMS microfluidic chip that allows single microbead manipulation. Various concentrations of human IgG antibodies have been introduced to bind onto a single microbead captured and isolated inside a designated micro-chamber in a small volume of 75 pL. Fluorescence detection has been monitored by using a CCD camera after the second binding with QDs conjugated with anti-human IgG. In this experiment, a human IgG antibody concentration below 0.1 μg mL^{-1} has been successfully detected. The proposed microfluidic chip and measurement scheme can be extended to be applicable to sensitive detection of any types of biomolecules such as DNAs, proteins and cell secretions in future work.

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