Sensors and Actuators B 269 (2018) 143–150

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Multiple optical trapping assisted bead-array based fluorescence assay of free and total prostate-specific antigen in serum

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ARTICLE INFO

Article history:
Received 12 January 2018
Received in revised form 25 April 2018
Accepted 28 April 2018
Available online 30 April 2018

Keywords:
Multiple optical tweezers
Prostate-specific antigen
Fluorescence imaging
Bead-array based assay
Prostate cancer

ABSTRACT

Although suspension bead-based assay technology has been widely used owing to its advantages of high-throughput and microvolume detection, its sensitivity is greatly limited because it detects the fluorescence signal emitted by microbeads for a short time in the flowing fluid. In this work, we present the approach for prostate-specific antigen (PSA) detection of both free PSA (fPSA) and total PSA (tPSA) based on bead-array based fluorescence imaging by combining multiple optical trapping and bead-based bioassays. The polystyrene beads were employed to enrich the targets using the classic sandwich immuno-binding and tagged with fluorescent quantum dots (QDs), and the QDs-tagged beads in suspension were trapped array-by-array using multiple optical tweezers constructed with a diffraction optical element and excited with a 405 nm fiber laser for wide-field fluorescence imaging. The distinctive size information from the image of the trapped beads enabled the sorting of different targets. Moreover, the limits of detection for fPSA and tPSA are 3.8 pg/mL and 2.5 pg/mL respectively with good specificity. More importantly, this strategy was successfully used to detect fPSA and tPSA simultaneously in real serum samples. The high sensitivity, good selectivity, and tiny sample volume make this strategy a promising method for life sciences and clinical applications.

1. Introduction

Bead-based fluorescence assays are well established for biosensing applications such as DNA detection [1–3], SNP genotyping [4,5], and immunoassays [6–9]. The bead-based technology relies on the high surface-to-volume ratio of the microbeads, resulting in faster binding kinetics and a large number of binding sites. Importantly, the bead-based assays achieve remarkable signal-to-noise levels and high sensitivity and are stable to the variations in sample volume and the number of beads under "ambient conditions" that proposed by Ekins [10] by reducing the amount of capture beads [11]. The micron-sized beads are floating in the liquid environment and the most common detecting method is the suspension array technology (SAT) [12] relies on flow cytometry to excite and collect the fluorescence signal from the individual bead in a short time. While for fast and stable fluorescence imaging detection the beads should be captured to form the bead array, therefore the assistant manipulation such as electric, magnetic or acoustic manipulation is required for the construction of an array [13–15]. However, the manual actuation by magnetic-tipped micromanipulator is slow with a reported time of five minutes to place a single bead [16], and the acoustic manipulation by the ultrasonic standing-wave always results in an irregular array that is away from the focus of the objective which brings difficulties in precise fluorescence imaging detection [15].

Optical tweezers which was first developed by Ashkin et al. [17–19] has been a technology for optically trapping microparticles that can compensate for the shortcomings of the above methods. This technique employs a highly focused laser beam to trap dielectric particles or living cells. It is a highly useful tool in the bead-based assay for its advantages of non-contact and non-invasion manipulation. However, initial setups were only capable of trapping single particles and it is not suitable for bead array based fluorescence assay [20]. Optical trapping array can be created by either time-sharing optical tweezers using galvano-mirrors [21,22], acousto-optical deflectors [23], or by modifying wavefront of the beam using spatial light modulator [24,25]. A simple and effective means to quickly create the arrays of trapped particles in small volume in arbitrary patterns is using diffractive optical elements (DOE) to split a single laser beam into a bunch of equal power laser

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https://doi.org/10.1016/j.snb.2018.04.169
0925-4005/

directly [26], and with the high utilization of laser energy, the bead array can be stably captured with lower power lasers. As beads can be easily encoded physically, it is easy to identify a different kind of beads just by the bead size through imaging [27].

Prostate-specific antigen (PSA) is a glycoprotein produced by the prostate to liquefy the seminal fluid [28]. In the past twenty years, serum PSA has been the most frequently used tumor marker. The level of PSA in serum is used for screening, staging, and prognosis of prostate cancer [29,30]. Serum PSA circulates in two different forms, uncomplexed PSA (free PSA (fPSA)) and complexed PSA [31]. Complexed PSA is predominantly bound to a-1-antichymotrypsin (PSA-ACT), while a smaller percentage bound to a-2-macroglobulin (PSA-A2M). PSA-ACT is immunoreactive but enzymatically inactive, while PSA-A2M maintains weak protease activity despite having no exposed immunoreactive epitopes. PSA-A2M is thus unmeasured by current assays. fPSA exists in an enzymatically inactive, unbound state [32,33]. In the actual clinical diagnosis, detecting of fPSA has been chosen as an assistant to total PSA (tPSA) detecting to increase the specificity and prevent the false-positive prostate cancer (PCa) diagnosis of benign prostatic hypertrophy (BPH) as patients with prostate cancer tend to have a lower fPSA/tPSA ratio than patients with BPH [34–36].

In this work, we utilize the multiple optical trapping constructed by a DOE for bead-array based fluorescence assay for simultaneous detection of fPSA and tPSA in serum. Therefore, a simple and efficient approach is applied to form multi-optical tweezers to build stable bead array based immunoassay to detect both fPSA and tPSA simultaneously to improve the accuracy and reliability of prostate cancer diagnosis, and this proof-of-principle study shows that the as-developed setup and detection approach has great potential in the biological and clinical analysis.

2. Experimental

2.1. Materials and reagents

Carboxyl-modified polystyrene microbeads (diameter 3.0 ± 0.1 and 5.0 ± 0.1 μm, 5% w/v, PA = 3 Å^2, PA means the "parking area" of a single carboxyl) were obtained from Huge Biotechnol. Co. Ltd. (China). Streptavidin-modified 605 nm CdSe/ZnS QDs (quantum yield 85%) was supplied by Wuhan Jiayuan Quantum Dots Co., Ltd (China). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl, 99%), N-hydroxysulfosuccinimide (Sulfo-NHS, 98%), 2-(N-Morpholino) ethanesulfonic acid, (MES, 99%), Tween 20 and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Co. (USA). DyLight488 Goat anti-mouse IgG (A23310-2) was purchased from Abbkine Scientific Co., Ltd. (USA). Anti-PSA biotin-conjugated antibodies (5A6) were purchased from Abcam (ab182031, Cambridge, MA). Prostate-specific antigen (PSA), carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), mouse monoclonal anti-fPSA (Ab1) and mouse monoclonal anti-tPSA (Ab2) were purchased from Zhengzhou Biocell Biotechol. Co., Ltd. (China).

2.2. Serum samples

Serum samples were supplied by Hubei Cancer Hospital (China). They were centrifuged and frozen at −80 °C and were thawed on the day of analysis. All the patients' blood was obtained with the approval of medical ethics committee.

2.3. Preparation of the capture beads

Take the fPSA capture beads as an example to explain the preparation process. 10 μL (2×10^7) of the 3 μm stock bead suspension was pelleted by centrifugation at 10,000 rpm for 2 min. The beads were resuspended in 25 μL water by vortex and sonication. After the microbeads were pelleted again, the supernatant was removed, and the washed beads were resuspended in 70 μL of MES (75 mM, pH 6.0). 10 μL of N-hydroxysulfosuccinimide (Sulfo-NHS, 50 mg/mL) and 10 μL of EDC (50 mg/mL) were added to the bead suspension and gently mixed. Thereafter the suspension was incubated for 20 min at room temperature with vortex at 10 min intervals. The activated beads were then washed twice with 200 μL of PBS buffer (0.1 M, pH 6.2) and resuspended in 200 μL of PBS (pH 6.2). 12 μg of capture antibody Ab1 was diluted in 100 μL PBS (pH 6.2) and then added to the activated beads. The suspension was stirred for 3 h with the vortex at 30 min intervals. The antibody-conjugated beads were washed with the wash buffer (0.1 M PBS, 0.05% Tween 20) twice, and then resuspended in 100 μL of 1% BSA for 1 h to block the extra binding site on the bead surface. Finally, the capture beads were washed and stored at 100 μL of PBS buffer (0.1 M, pH 7.4) for further use. For the tPSA capture beads we use an equal volume of 5 μm stock beads and 10 μg Ab2 is added to the reaction system and the other steps are the same.

2.4. Quantification of the capture antibody on the beads

First, capture beads conjugated with various amounts of mouse monoclonal Ab1 and Ab2 were prepared by reacting various volume (0, 2, 4, 6, 8, 10, 12 μL) with 10 μL of each bead. Afterwards, each group of capture beads was incubated with 2 μL of 1 mg/mL DyLight488-labeled goat anti-mouse IgG for 1 h at 37 °C with gentle shaking to acquire the immunobeads tagged with DyLight488 and then the corresponding fluorescence intensities of the DyLight488-labeled beads were measured by flow cytometry, which the excitation laser wavelength was selected as 488 nm and the collecting channel was set at 530/40 nm.

2.5. Bead-based immunoassay of the PSA and QDs tagging

A 1.5 mL Eppendorf tube was pre-wetted with washing buffer (1 × PBS/0.5% BSA), then 200 μL PSA standard solution of different concentration was added and diluted with the working buffer (1 × PBS/5% human whole serum) to each tube. The capture beads were mixed immediately before use, an appropriate amount of beads and 1 μL of biotinylated antibody (anti-PSA, 5A6) were added to each tube. After incubation at 37 °C with shaking for 3 h, these assays were washed thrice with the washing buffer by centrifugation to remove the unbound antibody. Finally, 0.5 μL SA-QDs solution were added to the washed beads that resuspended in 200 μL working buffer to label the biotinylated antibody on the beads, after 30 min gentle shaking at 37 °C these assays were washed thrice to remove the unbound QDs. Finally, the QDs labeled beads that resuspended in 10 μL of detecting buffer (1 × PBS, pH 7.2) were transferred to the micro-volume sample chamber for detection.

2.6. The setup of the multiple optical tweezers

The schematic diagram in Fig. 1 shows the implementation of diffractively generated optical trapping arrays. This design is used to create a 3 × 3 array of multiple optical trapping. A 980 nm continuous-wave (CW) fiber laser (VLSS-980-B-900, Connet Fiber Optics, China) was used as the trapping laser. After being collimated by the collimator (F260APC-980, Thorlabs), the 980 nm laser beam was expanded to 7.5 mm diameter by the beam expander, and then the beam passes through the diffractive 3 × 3 square array generator (MS-585-K-Y-X, HOLO/OR) that placed at the eyepoint of a 4*f* lens system (telescope). The laser beam transports into the back illumination port of an inverted microscope (IX70, Olympus) and is reflected into the objective using a shortpass dichroic mirror DM1