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Multivalent aptasensor array and silver aggregated amplification for multiplex detection in microfluidic devices

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ABSTRACT

Herein, we developed a rapid and sensitive aptamers-based sandwich assay in microfluidic devices based on multivalent aptasensor array (MAA) chip and silver aggregated amplification (SAA) strategy for the detection of two biomarkers. Firstly, aptamers-modified silver nanoparticles were dotted in array to form MAA chip. Then PDMS was used to form a microfluidic device. After that, target proteins and two kinds of aptamer-modified silver nanoparticles (Tag-A and Tag-B) were rapidly injected into the microfluidic device. The aptamer on MAA chip recognized target, and the target also bound with Tag-A and Tag-B which could aggregate with each other to amplify fluorescence signal. Based on MAA chip and SAA strategy in microfluidic device, a linear response to PDGF-BB (r = 0.999) was obtained in the concentration range from 16 pg mL^{-1} to 250 ng mL^{-1}, and the detection limit was 1.4 pg mL^{-1}. In addition, a linear response to PDGF-BB (r = 0.992) was obtained in the concentration range from 16 pg mL^{-1} to 250 ng mL^{-1} in 10% blood serum with detection limit of 7.8 pg mL^{-1}. Ultimately, this assay was used to simultaneously detect PDGF-BB and VEGF-165, and the results showed good specificity and sensitivity. This assay can also be expanded to sensitive and high-throughput detection of other protein biomarkers by coupling of various aptamers with nanoparticles.

1. Introduction

Microfluidic system, also called micro-total-analysis-system (μTAS) or lab-on-chip (LOC), has received a growing number of attention in various fields. Compared to conventional techniques, microfluidic system has the advantages of rapid, high-throughput, miniaturization, automation, and parallel processing. Owning to these merits, the microfluidic systems have been applied widely in chemical and biological sensors [1], drug discovery [8], aptamer screening [23], medical diagnostic [39] and point of care testing [4]. Protein microarray chip with high-throughput capacity provides a promising way for multiplex detection in parallel [16,2]. In our previous work, we designed a structure switching aptamer-based silver microarray nanosensor for sensitive and selective detection of proteins [38]. We also reported a simple, ultrasensitive, and cost-effective electrochemical aptamer microarray sensor for specific determination of multiplied proteins using SPE array chip as sensing platforms [33]. Recently, Shivani Sathish developed a simple technology to create micro- and nanoarrays of biomolecules within microfluidic devices [31]. Antoine-Emmanuel Saliba developed a liposome-microarray-based assay (LiMA) that was capable of measuring protein recruitment into membranes in a quantitative and high-throughput manner [30]. María Díaz-González presented an automated electrical readout system incorporating microfluidic channels for low-cost glass-slide microarrays [7]. Although those methods provide simplicity, automation, high-throughput potentials for achieving multiplex detection in microarray based microfluidic devices, the solutions to enhance the sensitivity and reduce the detection limit are still limited. We aim to develop a totally new platform based on microarray in microfluidic devices to fulfill simple, sensitive, automated and multiplex detection.

Platelet-derived growth factor-BB (PDGF-BB) [29] and vascular endothelial growth factor-165 (VEGF-165) [27] have been reported to be important biomarkers which play a vital role in diagnosis and prognosis of many diseases. Until now, a variety of analytical platforms for protein biomarkers detection have been reported, such as enzyme-linked immunosorbent assay (ELISA) [31,46], electrochemical assay [32,43], optical methods [21,47], surface plasmon resonance (SPR) [28,3] and other method [9]. In addition, a new class of nucleic acid affinity elements called aptamers has been developed for high specificity and selectivity of protein assay [12,15]. In general, Aptamers are short single-stranded nucleic acids selected in vitro for binding certain molecules due to their high affinity and specificity, and they have been playing important roles in biosensing, medical diagnosis and diseases treatment [42,45]. Especially, when combined with nanomaterials,

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aptamers bring great improvement of performance in molecular recognition [25,5]. Nanomaterials are used to increase the binding ability of aptamers [22,35] as well as amplify signal [14], especially amplifying fluorescence signal [44]. Our group have developed a series of methods based on aptamers and silver nanoparticles for sensitive detection of proteins [19–21,37,6]. However, it was the first time that we integrated aptasensor array, silver amplified signal and microfluidic devices into a new platform.

Herein, we developed a rapid and sensitive aptamers-based sandwich assay in microfluidic devices based on multivalent aptasensor array (MAA) chip and silver aggregated amplification (SAA) strategy for the detection of two biomarkers at the same time. Based on MAA chip and SAA strategy in microfluidic device, the detection limit for PDGF-BB was 1.4 pg mL^{-1} in buffers and 7.8 pg mL^{-1} in 10% serum. Additionally, this assay was used to simultaneously detect PDGF-BB and VEGF-165 with good specificity and sensitivity.

2. Materials and methods

2.1. Materials and reagents

SG-2506 borosilicate glass was purchased from Changsha Shaoguang Chrome Blank Co., Ltd. Normal human serum was purchased from Zhong Ke Chen Yu (Beijing) Trading Co., Ltd. Sylgard 184 elastomer base and curing agent for polydimethylsiloxane (PDMS) were purchased from Dow Corning (Midland, MI). The dechroming liquid was a mixture of 200 g L^{-1} ceric ammonium nitrate and 3.5% (v/v) glacial acetic acid. The etching solution contained 18.6 g L^{-1} NH_4F, 4.64% (v/v) HNO_3 and 5% HF. silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$). Polyvinylpyrrolidone (PVP), sodium L-ascorbate (L-SA), sodium citrate (SC) were ordered from Sigma-Aldrich, Co. LLC. Tween-20 was obtained from Nanjing Bookman Biotechnology Co. Ltd. Phosphate buffered saline (PBS) (Shanghai Sangon Biotechnology Co. Ltd.) was used for preparation of the following solutions: 1 × PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, PH 7.4), 1 × PBSM (1 × PBS + 1 mM $MgCl_2$), BSAM (1 × PBSM + 1 mg mL^{-1} BSA), Blocking solution (1 × PBS + 10 mg mL^{-1} BSA). All the reagents were of analytical grade. All aqueous solutions were prepared using ultrapure water (≥ 18.20 MΩ) from a Millipore system. The oligonucleotides (Table 1 in supporting information) used in this work were synthesized and purified by Shanghai Sangon Biotechnology Co.

2.2. Apparatus

A microfluidic pump (Model LSP04-1A, Longer pump Corp., Baoding, China) was applied for liquid manipulation. Plasma Cleaner (Model PDC-MG, Chengdu Ming Heng Science & Technology Co., Ltd., China) was applied to bond glass slide with PDMS piece. The aptamer-silver nanoparticles array was made by SmartArray 48 arrayer (Beijing CapitalBio Co. Ltd., China) and scanned by Luxscan-10K/A microarray Scanner (488 nm laser source for TMRNA, Beijing CapitalBio Co. Ltd., China). The images were collected and analyzed by LuxScan 3.0 software (CapitalBio Ltd., China). UV–Vis spectrum was performed on a Synergy Hybrid Reader (BioTek, USA). MIKRO 220 R refrigerated centrifuge from Hettich (Andreas Hettich GmbH & Co. KG, 78532 Tuttlingen, GERMANY) was used for centrifugation. Scanning electron microscopy (SEM) (S-4800, Japan) and Transmission electron microscope (TEM) (JEM-200CX, Japan) were used for collecting SEM and TEM images.

2.3. Preparation of the aptamer-silver nanoparticle

Two different sizes of AgNPs (AgNPs-1 and AgNPs-2) were synthesized according to our previously reported method with some modification [18,20]. The AgNPs-1 functionalized with oligonucleotides of Apt-PD-TAMRA and hybrid-A to form detection probe (Tag-PD-A) according to our previous protocol [17]. And oligonucleotides of Apt-PD-TAMRA and hybrid-B (Tag-PD-B) were used to form detection probe (Tag-PD-B). 1 mL of AgNPs-1 was mixed with Apt-PD-TAMRA (50 μL, 10 μM) and hybrid-A (50 μL ,10 μM)/ hybrid-B (10 μM). Then, 5 μL of Tween-20 and 64 μL of NaCl (2 M) were added and reacted for 4 h at 37 °C with gentle shaking. After standing overnight, excess reagents and unmodified Apt-PD-TAMRA, hybrid-A and hybrid-B were removed by centrifugation (10 min, 15,000 rpm for three times). Based on the same method, Tag-VE-A and Tag-VE-B were prepared for detection of VEGF-165.

The AgNPs-2 was functionalized with Apt-PD and 5′SH-oligo(d)A_{12}-NH_2 to form capture probe (PD-AgNP). 1 mL of AgNPs-2 was mixed with Apt-PD (50 μL, 10 μM) and 5′SH-oligo(d)A_{12}-NH_2 (50 μL ,10 μM). Then, 5 μL Tween-20 and 64 μL of NaCl (2 M) were added and reacted for 4 h at 37°Cwith gentle shaking. After standing overnight, excess reagents and unmodified Apt-PD, 5′SH-oligo(d)A_{12}-NH_2 were removed by centrifugation (10 min, 15,000 rpm for three times). In addition, PD &VE-AgNP were prepared for multiplexed capture of PDGF-BB, VEGF-165 detection.

2.4. Fabrication of MAA chip in microfluidic devices

The Apt-AgNP array was designed and manufactured by robotic printing. Glass slide was cleaned with ethanol and deionized water and dried in a drying oven. Each slide had 4 identical array which consisted a total of 36 (4 × 9) spots. For multiplex protein assay, each array consists of three kinds of capture probes. Glass substrate and PDMS layer were plasma treated under the optimal parameters (705 w, 1 min) to obtain a hydrophilic surface. After plasma treatment, the slides were immediately spotted.

PDMS architecture was designed and fabricated by standard soft lithography techniques. Briefly, the laser printing film with customized patterns was transferred to the borosilicate glass, and UV exposure was immediately used for 35 s, followed by rinsing for 1 min with NaOH (0.5% (w/v)) 5 min with dechroming liquid. For etching, the glass with micropatterns was fist etched by solution at 37 °C for 30 min and then rhomboidal chamber was protected by tape during the etching process. The resulting glass molds were then washed with ultrapure water and dried at 80 °C. A 10:1 mixture of the PDMS pre-polymer and the curing agent was degassed in a vacuum chamber and then poured onto the mold and cured at 80 °C for at least 1.5 h. After curing, the PDMS replica was removed from the mold to produce 5 mm thick PDMS layer. The PDMS layer was cut, punched, and plasma treated. After the plasma treatment, bonding between the PDMS layer and the glass slide were immediately performed.

2.5. Analytical procedure

The Apt-AgNP array were immobilized at 37 °C for 1.5 h. Then, 10 mg mL^{-1} BSA was used to block the nonspecific binding sites of Apt-AgNP array for 1 h followed by 3 min of washing step using 1xPBST at a flow rate of 25 μL min^{-1}. After that, The MAA chips were ready to use or stored at 4 °C. The sample of proteins, Tag-A and Tag-B were injected into three inlets of the microfluidic device with a flow rate of 10 μL min^{-1} for 20 min followed by 3 min of washing step using 1xPBST at a flow rate of 20 μL min^{-1}. Finally, A Luxscan-10K/A Microarray Scanner (λex = 532 nm, λem = 570, PMT = 560) was used to scan the slide and collect data. An MAA chip containing capture probe of PD-AgNP, VE-AgNP and PD&VE-AgNP was used for multiplex proteins detection.

3. Results and discussion

3.1. The principle of MAA chip and SAA strategy in microfluidic devices

In this work, a new platform based on multivalent aptasensor array and silver aggregated amplification strategy in microfluidic devices was