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Microfluidic chip-based silver nanoparticles aptasensor for colorimetric detection of thrombin

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ABSTRACT

In this paper, a colorimetric silver nanoparticles aptasensor (aptamer-AgNPs) was developed for simple and straightforward detection of protein in microfluidic chip. Surface-functionalized microfluidic channels were employed as the capture platform. Then the mixture of target protein and aptamer-AgNPs were injected into the microfluidic channels for colorimetric detection. To demonstrate the performance of this detection platform, thrombin was chosen as a model target protein. Introduction of thrombin could form a sandwich-type complex involving immobilized AgNPs. The amount of aptamer-AgNPs on the complex augmented along with the increase of the thrombin concentration causing different color change that can be analyzed both by naked eyes and a flatbed scanner. This method is featured with low sample consumption, simple processes of microfluidic platform and straightforward colorimetric detection with aptamer-AgNPs. Thrombin at concentrations as low as 20 pM can be detected using this aptasensor without signal amplification. This work demonstrated that it had good selectivity over other proteins and it could be a useful strategy to detect other targets with two affinity binding sites for ligands as well.

1. Introduction

The Microfluidic chip system, also called “lab-on-a-chip”, is a device that integrates a set of fluidic unit operations including fluid transport, fluid mixing, sample separation, detection, etc. on a single chip of only millimeters to a few square centimeters in size [1,2]. Based on the small physical dimensions, microfluidic chip has aroused increasing interests in different specific (bio-) chemical applications because of its distinguishing features such as reduced sample amount and reagent consumption, cost-efficient implementation, high integration and suitability for high throughput analysis [2,3]. In recent years, researchers have developed a variety of affinity separation methods to capture and separate proteins using microfluidic chip systems [4–6]. Among them, quite a lot of methods employ affinity binding between proteins and their specific aptamers [7]. One common method is to immobilize aptamers on microfluidic platform to catch the specific proteins, including aptamer immobilized poly (diallyldimethylammonium chloride) AuNPs multilayers [8], encoded hydrogel microparticles functionalized with aptamer [9], aptamer-functionalized magnetic beads [6,10] and so on [11–13]; other methods involve the combination of pressure or electric force based systems with microfluidic platforms to separate protein-aptamer complex from the free aptamer, such as affinity microchip gel electrophoresis [14,15] and on-chip integrated nanoporous membrane device [16]. The detection limit of these methods is usually at nanomolar level which is lower than that of the common methods mentioned above.

When aptamer-immobilized glass-based microfluidic platform was utilized to capture target protein, a variety of detection methods, such as fluorescent [10,13,17], electrochemical [6,8], and other methods [11,12], have been developed to measure the concentration of the captured proteins. In microfluidic paper-based analytical device, the most common detection technique is colorimetry because colorimetric detection is relatively simple and can be connected with many inexpensive and straightforward reporting systems such as smart phone and flatbed scanner [18]. Colorimetric method is featured with simplicity, rapidness, and low cost without using complicated analytical instruments [19,20]. The mechanism of nanomaterial-based colorimetric detection is generally based on their inherent optical or catalytic properties [21–25]. For example, the surface plasmon resonance properties of metal nanoparticles, AuNPs and AgNPs, depend strongly on the interparticle distance to exhibit easy visualization of color change between individual particles and aggregate ones; red to blue for AuNPs and yellow to brown for AgNPs [26]. Taking advantage of the high molar absorptivity of metal nanoparticles, AuNPs -conjugated immunoassays were developed to detect target proteins

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with low detection limits and high sensitivities [27,28]. AuNPs as nanocatalysts are also capable of catalyzing specific substrates to produce colorimetric signals for sensing of proteins [23,29]. In these studies, AuNPs induced reaction of colored substrates to yield colorless products. Compared with AuNPs, AgNPs-based colorimetric assay has received less attention in terms of its complicated preparation and functionalization processes, and less stability [30]. Nevertheless, the application of AgNPs labels in the analysis of proteins is increasing with the development of synthesis and functionalization of AgNPs [30].

In our previous work, catalytic property of AgNPs has been used for colorimetric sensing detection of proteins [25]. A sandwich-like assay involving catalytically active AgNPs was developed for thrombin separation and colorimetric detection. In that system, AgNPs play the role of catalyst for catalytic reduction of rhodamine B by sodium borohydride, resulting in a color change of the reaction solution which can be visually observed or optically measured by UV–vis spectroscopy. Signal can be amplified with the aid of catalytic reaction system, but additional additives make the experiment more complex. In this report, we tried to detect proteins in microfluidic channels based on the color change of silver nanoparticles themselves without the need of signal amplification, which provides a more straightforward detection manner. We constructed an aptamer-functionalized AgNPs colorimetric biosensing platform in microfluidic chip. The microfluidic chip system is composed by a plurality of parallel single-channels. The binding of thrombin to its specific aptamers were chosen as the model for testing this capture and detection approach. The biotinylated 15-mer thrombin aptamer (Apt 15) was immobilized on the channels to capture target thrombin. The 29-mer thrombin aptamer (Apt 29)-functionalized AgNPs were mixed with thrombin before injecting into the microfluidic channels, which allows thrombin to be captured specifically in a classic sandwich assay format [31,32] and were utilized as the detection bioprobes. The color change causing from the AgNPs captured in the channel was proportional to the concentrations of thrombin. Visualization analysis can be conducted directly according to the color shades degree. Or it can be converted to gray scale value for quantitative analysis through the ScanMaker i900 scanner and the LuxScan3.0 software. This method combined the advantages of the microfluidic system for manipulation of small volumes of sample with the feature of easy and simple detection ability of colorimetric assays to develop a highly sensitive and selective method for thrombin detection, which could be successfully used in protein capture and quantitative analysis.

2. Material and methods

2.1. Materials

SG-2506 borosilicate glass was purchased from Changsha Shaoguang Chrome Blank 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. Human serum albumin (HSA), immunoglobulin A (IgA), immunoglobulin G (IgG), lysozyme (Lys) and bovine serum albumin (BSA) were purchased from Biosharp, Japan. Human α-thrombin (thrombin), silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), polyvinylpyrrolidone (PVP), sodium L-ascorbate (L-SA), sodium citrate (SC) and methoxypolyethylene glycol thiol (mPEG-SH, average MW 5 kD) were ordered from Sigma-Aldrich, Co. LLC., USA. Streptavidin (SA) was obtained from Beijing Biosynthesis biotechnology Co., LTD. 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 $Na_2HPO_4 \cdot 12H_2O$, 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 used in this work were synthesized and purified by Shanghai Sangon Biotechnology Co., Ltd. The thrombin-binding aptamers were derived with biotin or thiol at the 5′ terminus and their sequences were as follows:

Apt 15: 5′-biotin-AAA AAA AAA AAA AAA GGT TGG TGT GGT TGG-3′,

Apt 29: 5′-SH-AAA AAA AAA AAA AAA AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-3′.

2.2. Apparatus

A microfluidic pump (Model LSP04-1A, Longer pump Corp., Baoding, China) was applied for liquid manipulation. DT-02 cold plasma generator from Suzhou OPS Plasma Technology Co., Ltd (China) was applied to bond pre-cleaned glass with PDMS piece. The devices were scanned by ScanMaker i900 (MicroTek, Shanghai, China). The images were analyzed through LuxScan3.0 software (Beijing Capital Bio Co. Ltd., China). UV–vis spectrum was performed on a Synergy Hybrid Reader (BioTek, USA). Transmission electron microscope (TEM) (JEM-1011, Japan) was used for collecting TEM images. A CT15RT versatile refrigerated centrifuge (Shanghai Tianmei Scientific Instrument Co., Shanghai, China) was used for centrifugation.

2.3. Preparation of nanoparticles

Silver nanoparticles were synthesized according to the previous reports [33–35] with some modifications. Briefly, $AgNO_3$ (10 mL, 2 mM) was added into $NaBH_4$ solution dropwise (20 mL, 3 mM) with 2 mL PVP (5%) in an ice bath. When the reaction was completed, a yellow solution AgNPs (0) was obtained. After that, a mixture of 10 mL SC (50 mM), 10 mL of L-SA (10 mM), 10 mL of PVP (6%) and 10 mL of AgNPs (0) was reacted for 5 min. Subsequently, 10 mL of 10 mM $AgNO_3$ was added to the mixture and continuously stirred for another 60 min to get an orange AgNPs solution. 5 mL of AgNPs was further mixed with 5 mL of PVP (6%), 20 mL of 10 mM L-SA, 5 mL of 50 mM SC and 20 mL of 10 mM AgNPs to make large silver nanoparticles (AgNPs (2)). AgNPs (0), AgNPs and AgNPs (2) were all stored at 4 °C for further use.

2.4. Preparation of aptamer-functionalized AgNPs (Apt 29-AgNPs)

The AgNPs functionalized with Apt 29 (Apt 29-AgNPs) were prepared based on a previously described protocol [22]. 1 mL of AgNPs was mixed with Apt 29 (10 μM, 50 μL) and mPEG-SH (10 μM, 50 μL) first. Then, 64 μL of 2 M NaCl and 5 μL of 0.5% Tween-20 were added and reacted for 4 h at 37 °C. After standing overnight, excess reagents and unmodified Apt 29 were removed by centrifugation (15 min, 15,000 rpm for three times). The resulting precipitate of Apt 29-AgNPs was washed with 1 × PBS and re-dispersed in 1 mL of 1 × PBSM. The produced Apt 29-AgNPs was used for the subsequent reaction with thrombin.

2.5. Fabrication of microfluidic devices

The microfluidic device was designed to fit in the size of a microscope slide, consisting of seven parallel channels as shown in Scheme 1. The microfluidic channel designed for protein capture