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Aptamer-based microfluidic beads array sensor for simultaneous detection of multiple analytes employing multienzyme-linked nanoparticle amplification and quantum dots labels

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ABSTRACT

This study reports the development of an aptamer-mediated microfluidic beads-based sensor for multiple analytes detection and quantification using multienzyme-linked nanoparticle amplification and quantum dots labels. Adenosine and cocaine were selected as the model analytes to validate the assay design based on strand displacement induced by target–aptamer complex. Microbeads functionalized with the aptamers and modified electron rich proteins were arrayed within a microfluidic channel and were connected with the horseradish peroxidases (HRP) and capture DNA probe derivative gold nanoparticles (AuNPs) via hybridization. The conformational transition of aptamer induced by target–aptamer complex contributes to the displacement of functionalized AuNPs and decreases the fluorescence signal of microbeads. In this approach, increased binding events of HRP on each nanosphere and enhanced mass transport capability inherent from microfluidics are integrated for enhancing the detection sensitivity of analytes. Based on the dual signal amplification strategy, the developed aptamer-based microfluidic bead array sensor could discriminate as low as 0.1 pM of adenosine and 0.5 pM cocaine, and showed a 500-fold increase in detection limit of adenosine compared to the off-chip test. The results proved the microfluidic-based method was a rapid and efficient system for aptamer-based targets assays (adenosine (0.1 pM) and cocaine (0.5 pM)), requiring only minimal (microliter) reagent use. This work demonstrated the successful application of aptamer-based microfluidic beads array sensor for detection of important molecules in biomedical fields.

1. Introduction

Development of highly sensitive and selective high-throughput biosensors for simultaneous detection of two or more analytes within a single sample has long been a focus in several important fields such as biomedical diagnostics, environmental monitoring, and industrial process and quality control (Long et al., 2013; Czarnik, 1995). A variety of biomolecules such as DNA, enzymes and antibodies have been utilized to develop highly sensitive and selective biosensors (Hao et al., 2013; Monroe et al., 2013; Iliuk et al., 2011).

Aptamers are short, single-stranded nucleic acids with a three-dimensional shape and can compete with antibodies in assisting a number of analytical methods due to its merits such as convenience in engineering, readily production by chemical synthesis, desirable storage properties, little or no immunogenicity in therapeutic applications, high selectivity to the target molecule and good stability in complex physical and chemical environments (Cho et al., 2009; Liu et al., 2009; Breaker, 2004). Although many aptasensors have presented high applicability in recognition, separation and detection of various analytes such as protein, small molecule, ion and even cells have been developed (Famulok et al., 2007; Lin et al., 2006; Huang et al., 2008; Navani and Li, 2006), one of the key challenges in aptamer-based bioanalytical chemistry is the detection of disease-related molecules in biological fluids with samples volume as small as possible such as laser capture microdissections and the needle biopsy.

For the past several years, increasing efforts have been made to couple aptamers with microfluidics. The introduction of aptamers on a microfluidic chip has been expected to bring several advantages to this field, such as reduced reagent and sample consumption, simplicity, automated processing, faster separation, high throughput, and portability (Dittrich et al., 2006; Vilkner et al., 2004; Tanaka et al., 2007). For example, the aptamers are immobilized in a microfluidic channel to capture rare cells to achieve a rapid assay without any pretreatment (Phillips et al., 2009). An aptamer-based microfluidic device has been developed for simultaneously sorting,

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enriching, and then detecting multiple types of cancer cells from a complex sample (Xu et al., 2009). A microfluidic electrochemical aptamer-based sensor (MECAS) chip is developed by integrating target-specific DNA aptamers for continuous, real-time monitoring of cocaine in undiluted blood serum (Swensen et al., 2009). The flow cytometry is combined with aptamer functionalized magnetic microparticles for the detection of adenosine in serum (Huang and Liu, 2010). A microfluidic-based aptamer sandwich assays are developed for the thrombin determination with higher specificity than direct immunoassays (Tennico et al., 2010). Although many aptamer-based microfluidic technologies have been reported (Xu et al., 2010), few such methods showed characteristics of high-throughput targets measurement.

Recently, the integration of efficient sample handling of microfluidics and high-throughput parallel analysis of microbead based arrays has been demonstrated for multi-target detection, making it an ideal platform for sensitive detection of disease-related biomolecules (Lucas et al., 2007; Gunderson et al., 2005). Due to the distinctive advantages, a series of microfluidic bead-based methods have been developed for bio-analysis, including detection of low-abundant DNA point mutations (Zhang et al., 2008), sensitive DNA detection using microfluidic bead-based rolling circle amplification (Sato et al., 2010), and detection of circulating tumor cell (Zhang et al., 2013).

Moreover, enzyme-functionalized nano-structured materials have become one of the most popular and practical solutions for signal amplification due to the increased enzyme loading toward one reaction event, which subsequently enhanced the ensemble signal for target detection (Tang et al., 2010; Mao et al., 2009). Based on the remarkable signal amplification, the nano-materials functionalized with multi-enzymes have successfully applied for detection of several important biomolecules such as HPV (Zhang et al., 2011), α-fetoprotein (Wu et al., 2009), and prostate specific antigen (Yu et al., 2006).

Our present work is motivated by the promising applications of microfluidic bead-based array in parallel detection of multiple targets with sensitivity enhancement and enzyme-functionalized nanoparticles in signal amplification. Herein, Au nanoparticles functionalized with capture probes and HRP, microbeads functionalized with the aptamer and modified electron rich proteins, and streptavidin-conjugated quantum dot (CdSe/ZnS, 605 nm) are combined for sensitive and simultaneous detection of multiple analytes such as adenosine and cocaine. The combination of aptamer-based microfluidic beads array sensor with enzyme-functionalized nanoparticles for multiple analytes analysis is 3-fold: (1) high detection sensitivity by multienzyme-linked nanoparticle amplification and enhanced mass transport capability inherent from microfluidics, (2) efficient liquid handling capability inherent from microfluidics, (3) and rapid binding kinetics offered by homogeneous microbead assays.

2. Materials and methods

2.1. Materials and reagents

Polydimethylsiloxane (PDMS) prepolymer and curing agent used for the fabrication of microfluidic chip were purchased from Dow Corning Corporation (Saginaw, MI). SuperAvidin™ coated polystyrene microbeads were purchased from Bangs Laboratories and streptavidin-conjugated quantum dot (maximum emission wavelength = 605 nm) was purchased from Life Technologies Corporation (Carlsbad, CA). 3-(4-Hydroxyphenyl) propionic acid *N*-hydroxysuccinimide ester (4-OH-PPA-NHS), tyramine hydrochloride, biotin *N*-hydroxysuccinimide ester (biotin-NHS), casein, horseradish peroxidase, adenosine, cytidine, guanosine, uridine and cocaine hydrochloride were purchased from Sigma-Aldrich Chemical Co. HPLC-purified aptamer and oligonucleotide probes with various modifications were obtained from Sangon Biotech (Shanghai) Co., Ltd (Shanghai, China).

2.2. Microchip fabrication

Microfluidic structures were prepared by molding PDMS against a printed circuit board (PCB) master (Li et al., 2006). In brief, the procedure involved the design of photomasks, the transfer of patterns on the photoresist of the PCB (Kinsten, Chiefskill, Taiwan) by UV irradiation, the removal of the exposed photoresist, the etching of the unprotected copper layer on the PCB, and the casting of PDMS on the PCB master to form negative replicas. The detailed steps of chip fabrication and the loading of functionalized microbeads into the chamber array were described in our previous report (Zhang et al., 2012). Briefly, each bead loading channel containing two disconnected channel segments was aligned with corresponding chamber under a microscope to construct a bead immobilization unit that comprised two gaps with different sizes. While the larger gap allowed beads flowing into the chamber position, the smaller gap prevented the beads from flowing out of the chamber. After the introduction of microbeads, the slab with bead loading channels was removed and the other slab with chambers array bonded with a slab containing a simple micro-channel (i.e. the sampling channel) face-to-face to construct detection device. Since the channel depth of sampling channel was smaller than immobilized beads inside the arrayed chambers, fluidic flow generated during the sample loading would not flush away the beads.

2.3. Synthesis of oligonucleotide probes

The aptamers and corresponding capture probes were designed for the detection of adenosine and cocaine. The designed aptamers for adenosine and cocaine were called Aptamer-A (the DNA sequence was 5′-ACACTGACCTGGGGGAGTATTGCGGA GGAAGGTAAAAAAAAAA-Biotin) and Aptamer-C (the DNA sequence was 5′-ATCTCGGGAGA-CAAGGATAAATCCTTCAATGAAGTGGGTCTCCCAAAAAAAAAA-Biotin) respectively. The corresponding capture probes immobilized on the surface of Au nanoparticles were complementary to above sequences respectively and named Capture A (the DNA sequence was 5′-CCCAGGTCAGTG AAAAAAAAAA-HS) and Capture C (the DNA sequence was 5′-GTCTCCCG AGATAAAAAAAAAA-HS).

2.4. Preparation and functionalization of gold nanoparticles (AuNPs)

AuNPs with narrow size distribution and good dispersion were prepared according to the reported methods (Grabar et al., 1995). All glassware used in this preparation were thoroughly cleansed by aqua regia (three parts HCl, one part HNO_3), rinsed in doubly distilled water, and oven-dried prior to use. In a 250 mL Bunsen beaker, 100 mL of 0.01% $HAuCl_4$ in doubly distilled water was brought to boil with vigorous stirring. To this solution 3 mL of 1% trisodium citrate was added. The solution turned deep blue within 20 s, and the final color changed into wine-red after 60 s. Boiling was pursued for an additional 10 min before the heating source was removed, and the colloid solution was stirred for another 15 min. The resulting AuNP solution was stored in dark bottles at 4 °C and was used to prepare Capture probe–AuNPs–HRP conjugate.

The thiolated capture probes and the HRP were used for conjugation with AuNPs. The 600 μL of the synthesized AuNP (1.2 nM, 15 nm ± 1 nm) solution was concentrated to 150 μL and the pH value was adjusted to 8.0 using 0.1 M K_2CO_3. For the preparation of HRP and capture probe functionalized AuNPs (Capture probe–AuNPs–HRP), 9 μL HRP (5 μg/μL) was added into this solution and placed at room