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A versatile quantitation platform based on platinum nanoparticles incorporated volumetric bar-chart chip for highly sensitive assays

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ABSTRACT

Platinum nanoparticles incorporated volumetric bar-chart chip (PtNPs-V-Chip) is able to be used for point-of-care tests by providing quantitative and visualized readout without any assistance from instruments, data processing, or graphic plotting. To improve the sensitivity of PtNPs-V-Chip, hybridization chain reaction was employed in this quantitation platform for highly sensitive assays that can detect as low as 16 pM *Ebola Virus* DNA, 0.01 ng/mL carcinoembryonic antigen (CEA), and the 10 HER2-expressing cancer cells. Based on this amplified strategy, a 100-fold decrease of detection limit was achieved for DNA by improving the number of platinum nanoparticle catalyst for the captured analyte. This quantitation platform can also distinguish single base mismatch of DNA hybridization and observe the concentration threshold of CEA. The new strategy lays the foundation for this quantitation platform to be applied in forensic analysis, biothreat detection, clinical diagnostics and drug screening.

1. Introduction

Over the past decades, biosensors have witnessed significant progress in taking advantage of a large number of technologies and materials (Heath, 2015; Rosi and Mirkin, 2005; Song et al., 2011; Zhou et al., 2015). Among them, microfluidic-based biosensors have attracted a great deal of attention because of its practicality, simplicity and integration (Delaney et al., 2011; Gervais et al., 2011; Heath, 2015; Hou et al., 2011; Song et al., 2014a; Yang et al., 2016). The incorporation of nanomaterials into microfluidic chips shows the potential to revolutionize biosensing technology (Song et al., 2014a). Recently, various nanomaterials with optical, electronic, magnetic and catalytic properties have been introduced into microfluidic chips for development of the new generation of biosensors (Chan et al., 2002; Fan et al., 2008; Gaster et al., 2009; Liong et al., 2013; Stern et al., 2010). Although the new biosensors show significant improvement in simplifying the operation and reducing the reagents and sample volumes, the signal readout still requires expensive and sophisticated instruments, which limit these technologies in a wide range of applications. The emerging of colorimetric biosensing method integrated with microfluidics has been reported to resolve the problems (Chin et al., 2011; Pollock et al., 2012; Song et al., 2014a). Since the results can be directly read out by the naked eye, colorimetric biosensing platforms was used to develop instrument-free biosensors. Many kinds of materials, including gold nanoparticles (Rosi and Mirkin, 2005), carbon nanotubes (Song et al., 2010a), graphene (Song et al., 2010b), Fe_3O_4 nanoparticles (Gao et al., 2007) and other materials (Cai et al., 2015), have been reported to be able to produce color reactions. Although some of materials have been introduced in microfluidic chips, it is still a challenge to give quantitative results without the need of for instruments (Chin et al., 2011; Song et al., 2014a).

Based on Slipchip technology (Du et al., 2009), we developed a volumetric bar-chart chip (V-Chip) to volumetrically measure the production of oxygen gas from hydrogen peroxide and the result displays as ink bar charts, which can be directly read out without any assistance from instruments, data processing, or graphic plotting (Song et al., 2012). In our original design, catalase was employed as the probe to transfer the biorecognition events into visualized and quantitative ink-bar chart results (Song et al., 2013, 2012). Subsequently, platinum nanoparticle (PtNPs) was reported as the perfect substitute for catalase that can react with hydrogen peroxide to produce oxygen gas (Li et al., 2016; Song et al., 2014b). PtNPs outperform catalase with respect to stability at high H_2O_2 concentrations, high temperatures and in long-term reactions, as well as resistance to most catalase inhibitors (Song et al., 2014b). The catalase-mimic property of platinum nanoparticles has been

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applied in V-Chip for detection of lung cancer biomarker CYFRA 21-1 and cell surface biomarkers. However, to detect much broader range of biomarkers, there is a lack of a universal amplification method for improving the sensitivity of PtNPs incorporated V-Chip (PtNPs-V-Chip) system.

Currently, various DNA-amplification technologies have been developed for amplifying the nucleic acids hybridization and antigen-antibody recognition. Although thermal cycling methods, including polymerase chain reaction (PCR) and ligase chain reaction (LCR) (Barany, 1991; Saiki et al., 1988), can amplify the signal in an exponential way, their applications in point-of-care tests (POCT) are limited by requiring long time and complicated thermal cycling settings. Rolling circle amplification (RCA), strand-displacement amplification (SDA) and hybridization chain reaction (HCR) are developed for isothermal amplification (Dirks and Pierce, 2004; Fire and Xu, 1995; Huang et al., 2011; Van Ness et al., 2003; Weizmann et al., 2006; Yin et al., 2008). Among them, HCR does not require an enzyme, which is much more stable than the enzyme amplified reactions and to be suitable for applications in POCT (Dirks and Pierce, 2004; Huang et al., 2011; Yin et al., 2008). In this work, HCR amplification employed in PtNPs-V-Chip as a universal method for highly sensitive detection of DNA, protein and cancer cells. The triggered DNA can be specifically bound to magnetic beads (MB) due to DNA hybridization or antibody-antigen recognition, which will initiate the HCR amplification and result in binding with a large number of repeated biotin-labeled DNA on MB. Thus, a large amount of streptavidin modified PtNPs (PtNP-SA) are able to bind to MB through the recognition between biotin and streptavidin (Weber et al., 1989), which significantly improves the sensitivity of the PtNPs-V-Chip system. Based on the ink-bar chart results, this quantitation platform not only can be used to quantitate the concentrations of DNA and CEA in serum, but also shows the potential to distinguish the biomarker expression on different breast cancer cells.

2. Experimental

2.1. Materials and chemicals

Materials for device fabrication are included in the Supporting Information. Tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane was obtained from Pfaltz and Bauer, Inc. (Waterbury, CT). FC-70 (a mixture of perfluoro-tri-*n*-butylamine and perfluoro-di-*n*-butylmethylamine) was purchased from Hampton Research (Aliso Viejo, CA). PBS buffer (0.1 M, pH 7.4) was obtained from Lonza, Inc. (Allendale, NJ). Bovine serum albumin (BSA), (3-glycidoxypropyl) trimethoxysilane (3-GPS), hydrogen peroxide solution (35% wt in H_2O), p-phenylene diisothiocyanate (PDITC), dimethylformamide (DMF), 1-butanol, 3,3',5,5'- tetramethylbenzidine (TMB) and streptavidin were obtained from Sigma-Aldrich, Inc. (St. Louis, MO). Magnetic beads (MBs, Dynabeads M-270 epoxy, 2.8 μM) were obtained from Invitrogen (Carlsbad CA). Amicon Ultra-15 Centrifugal Filter Units (NMWL, 10 kDa and 100 kDa) were purchased from Millipore (Billerica, MA). Red ink was purchased from Fisher Scientific and diluted 2 × before use. The carcino-embryonic antigen (CEA) and the antibodies for CEA and HER2 were purchased from Abcam (Cambridge, MA). All DNA oligomers were synthesized by Integrated DNA Technologies, Inc (Coralville, IA) and used as received:

Name	Sequences of oligonucleotide
EV1	5′-GGA GTA AAT GTT GGA GAA CAG TAT CAA CAA-3′
EV1′	5′-GGA ATA AAT GTT GGA GAA CAG TAT CAA CAA-3′ (one-base mismatch)
EV1″	5′-GGA ATA AAC GTT GGA GAA CAG TAT CAA CAA-3′ (two-base mismatch)
EV2	3′-NH_2-A12-CCT CAT TTA CAA CCT-5′
EV3	5′-biotin-C6-A12-TTG TTG ATA CTG TTC-3′
EV3i	5′-GACCCTAAGCATACATCGTCCTTCATTTTTTGTTGATACTG TTC-3′
Ai	5′-GACCCTAAGCATACATCGTCCTTCATAAAAAAAAAA AA-NH_2 − 3′
H1	5′-ATGAAGGACGATGTATGCTTAGGGTCGACTTCCATAGACC CTAAGCATACAT-TEG-biotin-3′
H2	5′-biotin-TEG-GACCCTAAGCATACATCGTCCTTCA-TATGTATGCTTAGGGTCTATGGAAGTC-3′

Ebola virus specific target DNA is designated as EV1

2.2. Devices fabrication

The devices were prepared as our reported methods (Song et al., 2013; Song et al., 2012). The standard photolithography and wet etching processes were used to fabricate the 3-plex V-Chip (3V-Chip) device. By controlling the etching temperature at 35 °C, 45-min etching could produce the structures with a depth of approximately 50 μm. Access holes were prepared with a diamond drill of 0.031-in. diameter. After thoroughly clean using piranha solution and oxygen plasma treatment, the glass slides were given a hydrophobic coating by silanizing with tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane (Song et al., 2012). Finally, FC-70 was completely spread between the glass slides to assist the device assembly. The oil layer served as a lubricant and prevent air pockets during operation.

2.3. Preparation of PtNP-streptavidin complexes (PtNP-SA)

PtNPs with an average diameter of 6 nm were prepared as the previously reported methods (Huang et al., 2005). The size distribution of PtNPs was characterized by using transmission electron microscope (TEM). Zeta potential measurement and the hydrodynamic diameter analysis of 15 ug/mL PtNPs or PtNP-SA in PBS were all performed on a Malvern instrument NanoZS Zetasizer using a universal dip cell at 25 °C. To prepare streptavidin conjugated PtNPs, 25 μL streptavidin (1 mg/mL) was mixed with 500 μL PtNPs (1 mg/mL) in PBS buffer at pH 6.5 and the mixture was kept at 4 °C for overnight. Next, BSA was added to block the surface of PtNPs with the final concentration of 0.5% and the unconjugated streptavidin was removed by dialysis. Finally, the streptavidin conjugated PtNPs were suspended in 500 μL PBS solution (10 mM, pH 7.2, 0.5% BSA).

2.4. Preparation of MB-DNA or MB-antibody complexes

Conjugation of capture DNA (EV2), CEA or HER2 capture antibody onto MB was done according to a standard protocol (Song et al., 2012). 9 mg of epoxy magnetic beads (2.8 μm) were suspended in 500 μL PBS buffer and then mixed with 10 μL EV2 (1 mM) or 10 μL polyclonal antibodies (1 mg/mL). To speed up the reaction, 100 μL of 7 M ammonium sulfate solution was added to mixture. The solutions were incubated on a shaker overnight and unbound DNA or antibodies were removed by magnetic separation. Then, the coated magnetic beads were washed four times with 2% BSA in PBS solution. Finally, the coated magnetic beads were re-suspended in 500 μL PBS buffer.

2.5. Preparation of DNA-antibody complexes

For preparation of DNA-antibody complexes, 30 μL 0.1 M sodium borate buffer (pH 9.2) was firstly added to 60 μL of 1 mM