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Electrochemical DNA sensor based on three-dimensional folding paper device for specific and sensitive point-of-care testing

Juanjuan Lu, Shenguang Ge, Lei Ge, Mei Yan, Jinghua Yu*

Key Laboratory of Chemical Sensing & Analysis in Universities of Shandong, School of Chemistry and Chemical Engineering, university of Jinan, Jinan 250022, China

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ABSTRACT

The development of low-cost, accurate and sensitive diagnostic tests is crucial to many clinical, laboratory, and field applications, including forensics and medical diagnostics. Cellulose fiber-based paper is an inexpensive, biodegradable, and renewable resource, the use of which as a diagnostic device confers several advantages compared to traditional substrate. In this work, wax printer was used to produce collapsible microfluidic paper-based analytical devices (μPADs), which were combined with screen-printed electrodes (SPEs) to create simple, low-cost, disposal devices in bulk. Herein, an electrochemical DNA sensor was introduced for the first time into a folding paper based on the AuNPs/graphene modified screen-printed working paper electrode (SPWPE). Thionine (TH) bound to double stranded DNA(dsDNA) as signal tags (TH/D1), together with Complementary ssDNA (S3) immobilized on Nanoporous gold (NPG) forming a S3-TH/D1-NPG bioconjugates. Owing to the chain structure of dsDNA and large specific surface area of NPG, the bioconjugates proved to be an excellent amplification label. Under optimal conditions, the folding μPADs DNA sensor showed high sensitivity, good precision, acceptable stability, reproducibility and excellent performance in human serum assay. In addition, the simple, low-cost, sensitive device can be easily applied for point-of-care testing (POCT), public health and environmental monitoring in remote regions, developing or developed countries.

1. Introduction

DNA biosensors have received significant attention due to their potential applications, including clinical diagnostics, genetics, pathology, forensic study, food/drug industries and environmental monitoring [1–3]. Recent advances in the detection of nucleic acids have allowed the development of different types of DNA biosensors. Although many methods and instruments have been developed, they suffer from several drawbacks such as the need for professional training, high costs and bulkiness, and have not been widely used for point-of-care testing (POCT) applications. Hence, there is an urgent need to develop simple, rapid, inexpensive and accurate assays for DNA detection. Lab-on-a-paper systems that use patterned paper as a substrate, named microfluidic paper-based analytical devices (μPADs), were first reported by Whitesides's group [4,5]. These systems combine the simplicity, portability, disposability, and low-cost of paper-strip tests with the multiplex analysis and complex function of conventional lab-on-a-chip devices for analyte detection. To date, the primary method for the qualitative and quantitative analysis of multiplex analytes on μPADs is colorimetric methods [6–9]. However, the low sensitivity and high detection-limit of colorimetric methods prevent these μPADs from reaching their full potential for competing with the traditional analytical instrumentations. Apart from colorimetric based sensing, electrochemical [10–12] and chemiluminescent [13,14] detection has also recently been used for microfluidic paper-based sensors. Electrochemical method is particularly well suited for this type of technology, owing to its simple instrumentation and operation, fast analysis, high sensitivity and selectivity. This technology holds great promise and has gained increasingly attention and interest over recent years [15–17].

In recent years, great efforts have been made to improve sensitivity for DNA detection. With the rapid development of nanotechnologies, metal or semiconductor nanoparticles with unique physical and electrical properties have been widely used for the amplified detection of DNA sequence [18,19]. Immobilization of ssDNA sequences on the electrode surface has great influence on the performances such as sensitivity, selectivity, accuracy, reproducibility and lifetime. In this work, Au nanoparticles (AuNPs) and graphene assemble platform was used for the modification on electrode surface. Graphene nanosheets (GS), a single layer of carbon atoms with a closely packed honeycomb in a two-dimensional lattice, has recently attracted enormous attention in constructing electrochemical biosensors due to its fast electron transportation, high thermal conductivity, excellent mechanical stiffness and good biocompatibility [20,21]. However, the water solubility of

* Corresponding author. Tel.: +86 531 82767161.
E-mail address: ujn.yujh@gmail.com (J. Yu).

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graphene limits their further application in designing biosensors because graphene is hydrophobic and tends to form agglomerates in water [22]. Primary amine can be introduced into graphene sheets as ionizable functional groups which could form cations or cationic groups. These ionizable groups could not only improve the solubility of graphene, but also facilitate graphene assembly or reaction with other molecules, polymers and biological systems. AuNPs exhibit many advantages such as good biocompatibility, easy synthesis, high conductivity and long stability. In this work, we construct an AuNPs/graphene platform via self assemble rely on the electrostatic interaction or interaction between NH_2 group and Au. This AuNPs/graphene coupling produces a synergic effect in the electroanalytical performance of the resulting electrode material that can be profited for the development of electrochemical DNA sensors, which can improve the electronic transmission rate as well as increase the surface area.

The nanoporous gold (NPG) has attracted considerable attention in recent years due to its unique properties: high surface-to-volume reaction, stability, high in-plane conductivity, and biocompatibility. A simple dealloying strategy, by which silver was dissolved from silver/gold alloys in nitric acid, to make free-standing noble metal membranes with controllable three-dimensional porosity, has been reported [23,24]. There is a considerable amount of published reports about NPG modified electrode, wherein, Zhang's group [25] reported an electrochemical DNA sensor based on NPG modified electrode. However, in this work, NPG was innovatively used for signal amplification label, coupled with complementary ssDNA (S3) and signal dsDNA (D1) decorated with electroactive substance (thionine, abbreviated to TH), forming a S3-TH/D1-NPG signal amplification bioconjugates. The newly fabricated bioconjugates provide a promising platform for the development of high-performance electrochemical DNA sensors.

Based on previous work of our group in μPADs [26,27], a high-performance electrochemical DNA sensor was established on a novel folding paper-based 3D device. The use of collapsible paper-based device successfully replaced the stacking process onto another paper by just simply folding the device, which was operated before the electrochemical assays were conducted. The device was based on AuNPs/graphene modified SPWPE, which construct an effective DNA immobilization matrix and made the immobilized capture ssDNA (S1) possessed high stability and bioactivity. The capture DNA was immobilized on AuNPs through the interaction between SH group and Au. In addition, a sandwich type DNA biosensor was constructed by using S3-TH/D1-NPG bioconjugates as amplification label. The experimental results showed that the 3D-μPAD DNA sensor not only exhibited excellent analytical performance, but also showed potential for large-scale integration through combination with the emerging class of paper electronic devices to further develop high-throughput POCT devices.

2. Experimental

2.1. Chemicals

6-Mercapto-l-hexanol (MCH), glutaraldehyde, thionine was obtained from Sigma Chemical Co. (St. Louis, MO, USA). The following buffer solutions were employed in this study: DNA immobilization buffer: 10 mmol L^{-1} Tris–HCl and 0.1 mol L^{-1} NaCl (pH 7.4), hybridization buffer: 10 mmol L^{-1} phosphate buffered saline (PBS, pH 7.4) with 0.25 mmol L^{-1} NaCl. Washing buffer: 10 mmol L^{-1} phosphate buffer (PBS), and 0.1 mmol L^{-1} NaCl (pH 7.4). 10 mmol L^{-1} Tris–HCl solution (pH 7.4) was used as the supporting electrolyte. Other chemicals employed were analytical grade. Ultrapure water obtained from a Millipore water purification system (g18 MΩ, Milli-Q, Millipore) was used in all assays and solutions. The 25-μm-thick Au/Ag alloy (42:58, wt%) foils were purchased from Changshu Noble Metal Company. Carbon ink (ED423ss) and silver/silver choride (Ag/AgCl) ink (CNC-01) was purchased from Acheson. Whatman chromatography paper #1 (200.0 mm × 200.0 mm) (pure cellulose paper) was obtained from GE Healthcare Worldwide (Pudong, Shanghai, China) and used with further adjustment of size.

All of the synthetic oligonucleotides were purchased from Shanghai Linc-Bio Science Co. Ltd. (Shanghai, China).

Their base sequences are as follows:

Capture probe ssDNA sequence (S1): 5′-TGG AAA ATC TCT AGC AGT CGT-$(CH_2)_6$-SH-3′
Target ssDNA sequence (S2): 5′-ACT GCT AGA GAT TTT CCA CAC TGA CTA AAA GGG TCT GAG GGA-3′
Complementary ssDNA sequence (S3): 5′-NH_2-$(CH_2)_6$-ATG TCC CTC AGA CCC TTT-3′
Signal dsDNA sequence (D1): 5′-SH-$(CH_2)_6$-GGC GCG CGG CCC GGC CCG-3′
3′-SH-$(CH_2)_6$-CCG CGC GCC GGG CCG GGC-5′
Two-base mismatched ssDNA sequences: 5′-ACT GCT AGA GAT TTT CCA CAC TGA CTA AAA GCG TCT GTG GGA-3′
Non-complementary ssDNA sequences: 5′-ACT GCT AGA GAT TTT CCA CAC TGA CTA CTT CAA CAG TGC CCC-3′

2.2. Apparatus

Electrochemical measurements were carried out with a CHI 660D electrochemistry workstation (Shanghai CH Instruments Co., China). Electrochemical impedance spectroscopy (EIS) was performed on a CHI 604D Electrochemical Workstation (Shanghai CH Instruments Inc., China). Scanning electron microscopy (SEM) images were recorded using a JEOL-JSM-6300 scanning electron microscope. Transmission electron microscopy (TEM) investigations were performed using JEOL 4000 EX microscope. Atomic force microscope (AFM) images were achieved by scanning probe microscopy (SPM, Vecco, USA).

2.3. Design and fabrication of 3D-μPAD

The 3D paper-based device was fabricated on a piece of rectangular pure cellulose paper (60.0 mm × 30.0 mm), and the fabrication process (Scheme 1) consists of wax-printing, baking the wax-patterned sheet, screen-printing electrodes, followed by cutting, and the whole fabrication process took less than 10 min. The detailed fabrication procedures were as follows: firstly, the configuration for wax-printing (wax-printing is a rapid, efficient, and inexpensive method that has been used in most applications of microfluidic paper-based analytical devices [28–30].) on rectangular paper sheet (12.0 cm × 15.0 cm) was designed using Adobe illustrator CS4, and a wax printer was used for wax-printing in bulk (sheet A in Scheme 1). On each wax-patterned paper, there were two circular paper working zones (named circle-A: 6.0 mm in diameter and circle-B: 8.0 mm in diameter) for screen-printing electrodes. The wax-patterned paper sheet was baked in an oven at 130 °C for 150 s to melt the printed wax so that it penetrated through the paper to form the hydrophobic and insulating patterns. Then, the wax-patterned sheet was used as substrates for screen-printing electrodes (sheet B in Scheme 1). In circle-A, carbon ink was used for screen-printing working electrode (4.0 mm in diameter); in circle-B, carbon ink and Ag/AgCl ink were used for screen-printing half-ring like counter electrode and reference electrode, respectively. Then, the conductive wires and contact pads were screen-printed in the defined area. After that, the prepared paper sheet was cut to rectangular paper (60.0 mm × 30.0 mm) (folding sheet C in Scheme 1). Finally, by folding the rectangular