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An origami electrochemiluminescence immunosensor based on gold/graphene for specific, sensitive point-of-care testing of carcinoembryonic antigen

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ABSTRACT

In this work, an electroluminescence (ECL) immunosensor was introduced into a folding cellulose fiber paper based on gold/graphene modified screen-printed working electrode (SPWE) to develop an ECL microfluidic origami immunodevice. Graphene was used to modify the working electrode for its fast electron transportation, excellent mechanical stiffness and good biocompatibility. A dense gold layer was formed for the first time through the growth of gold nanoparticles on the graphene layer, to further enhance the sensitivity, stability and effective surface area of the SPWE. On this as-prepared SPWE, phenyleneethynylene derivative modified nanotubular mesoporous Pt–Ag alloy nanoparticles were used as signal amplifier for the highly sensitive determination of carcinoembryonic antigen in human serum sample in the linear range from 0.001 to 100 ng mL^{-1} with a low detection limit of 0.3 pg mL^{-1}. The specificity, reproducibility, and stability of this microfluidic origami ECL immunodevice (μ-OECLID) were investigated in this work. This novel μ-OECLID would provide a new platform for low cost, sensitive, specific, multiplex assay and point-of-care diagnosis in public health, environmental monitoring, and the developing world.

1. Introduction

Paper is a porous cellulose fiber web, and the porous nature not only fulfils the primary tasks such as diagnostic tests using body fluids and fluid transport, but also makes it possible to pattern hydrophilic channels separated by hydrophobic walls of photoresist/polymer [1,2], inks [3], wax [4,5], and plasma treatment [6], laser treatment [7] or by cutting method [8]. Recently, paper-based devices have gained more and more attentions, especially after high-speed coating and printing techniques were well established [1,9,10]. Since the first patterned paper was proposed by Whitesides and co-workers [9], many research groups have studied up on the paper-based devices [11,12]. These systems or devices not only combined the simplicity, portability, disposability, and low-cost of paper-strip tests, but also realized the multiplex analysis and complex function of conventional lab-on-a-chip application with small volume of samples [12]. Based on these microfluidic paper analytical devices (μPADs), our group has further made some researches in this area such as 3D electrochemiluminescence (ECL) immunodevice for lab-on-paper and point-of-care testing [13], multiplexed measurement of biomarkers on 3D paper-based ECL immunodevice [14] and electrochemical immunoassay [15]. These devices provide new opportunities in the development of precise and sensitive diagnostic devices.

ECL is a valuable and powerful analytical tool which has attracted more and more attention in the development of analytical methods for μ-PADs [13,14,16–18], due to its inherent features, such as high sensitivity, low background, wide dynamic range, easy controllability and flexibility. Recently, the establishment of ECL on μPADs [19–22] based on the integration of μPADs and commercial screen-printed electrodes, has substantially increased the scope for detections on μPADs. An important requirement in the successful development of ECL immunosensor is their enhanced sensitivity. Graphene (GN) has stimulated intense research interest because of its unique properties, such as high surface area, high electrical conductivity, good chemical stability, and strong mechanical strength. These unique characteristics enable it to hold great promise for application in ECL sensors [23–31].Gold nanoparticles (AuNPs) have attracted much attention in different bio-affinity assay due to their unique physical and chemical properties, such as easily controllable size distribution, long-term stability, superior conductivity, large surface area, and friendly biocompatibility. Given this, a novel immunosensor was developed through the growth of an AuNPs layer on the surfaces of working electrode

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modified by GN. To our best knowledge, there is no report focusing on gold/GN bio-component immobilization for sandwich ECL immunosensor.

In this contribution, taking into consideration the above advantages of AuNPs and GN, a novel ECL-immunosensor based on μPADs was developed. Initially, a GN layer was immobilized on the surface of carbon working electrode, and then gold was assembled on the GN surface through the growth of an AuNPs. The stability of the immunosensor was further improved due to the stability of AuNPs comparing with organic crosslinking agent such as chitosan [14]. The ECL immunosensor could be stored for a longer time. As a novel ECL signal amplifier, phenyleneethynylene derivatives (4,4′-(2,5-dimethoxy-1,4-phenylene) bis(ethyne-2,1-diyl)dibenzoic acid; P-acid) modified nanotubular mesoporous Pt–Ag alloy nanoparticles (P-acid/Pt–AgANPs) [32] were employed as signal amplification label in this work, due to their hollow porous nanostructures [33] having the advantage of highly accessible surface areas, and their rich surface chemistry allowing further vast functionalization of P-acid for ultrasensitive and multilabeling signal amplification. Taking advantages of the AuNPs and GN, and amplification effects of the Pt–AgANPs coupled with P-acid as well as the specificity of immunosensor, our microfluidic origami ECL immunosensor has a low detection limit of 0.3 pg mL^{-1}. In a word, a modified sandwich-type immunosensor was reported with advantages, including high sensitivity, specificity, less reagent consumption, a wider linear range, and lower detection limit. The proposed method was applied to the determination of carcinoembryonic antigen (CEA) in samples with satisfactory results.

2. Materials and methods

2.1. Reagents and apparatus

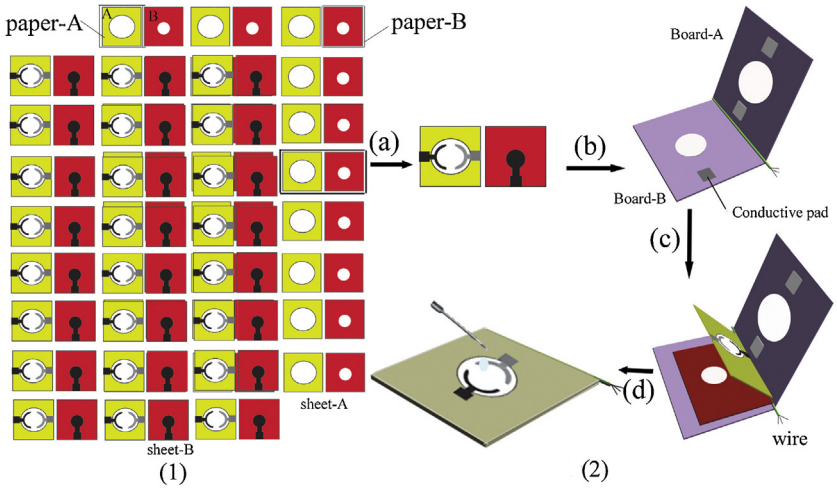
Mouse monoclonal anti-CEA antibodies were purchased from Linc-Bio Science Co. Ltd. (Shanghai, China). All chemicals were of analytical grade and purchased from Sigma-Aldrich or Alfa-Aesar. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ cm, Milli-Q, Millipore) was used in all assays and solutions. Blocking buffer for blocking the residual reactive sites on the antibody immobilized paper was 0.1 M pH 7.4 phosphate buffer solution (PBS) containing 0.5% BSA and 0.5% casein. To minimize unspecific adsorption, 0.05% Tween-20 was spiked into 0.1 M pH 7.4 PBS as washing buffer. The clinical serum samples were from Shandong Tumor Hospital. Carbon ink (ED423 ss) and Ag/AgCl ink (CNC-01) were purchased from Acheson. Whatman chromatography paper no. 1 (200.0 mm × 200.0 mm, pure cellulose paper) was purchased from GE Healthcare Worldwide (Pudong Shanghai, China) and used with further adjustment of size. Tripropylamine (TPA) was purchased from Alfa Aesar.

The scanning electron microscopy (SEM) images of this μ-MEOD were recorded on a JEOL JSM-5510 scanning electron microscope. Transmission electron microscopy (TEM) investigations were performed using JEOL 4000 EX microscope. Electrochemical impedance spectroscopy (EIS) was performed on an IM6x electrochemical working station (Zahner Co., Germany). Wax printer (FUJIXEROX Phaser 8560DN, Japan) was used to print the wax patterns. The fluorescence images were investigated on an inverse fluorescence microscope (ChangFang CFM-500E, China).

2.2. Fabrication of paper-based ECL origami device

The preparation of this paper-based ECL origami device was similarly to our previous work [34] with a modification and detailed procedure described below. Wax was used as the paper hydrophobization and insulation agent in this work to construct hydrophobic barrier on paper. As shown in Scheme 1, this device was fabricated on a piece of pure cellulose paper (60.0 mm × 30.0 mm). The shape of the wax-patterned paper electrochemical cell on ECL device, which contains a paper auxiliary zone (12 mm in diameter) on paper-A and a paper sample zone (6 mm in diameter) on paper-B, was designed using Adobe illustrator CS4. A wax printer was used for wax-printing in bulk (sheet A in Scheme 1(1)). 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 due to the porous structure of paper. After the curing process, the unprinted area (paper auxiliary zone and paper sample zone) still maintained good hydrophilicity, flexibility, and porous structure and will not affect the further screen-printing of electrodes and modifications [4].

Between the paper-A and paper-B, the unprinted line (1 mm in width) was defined as fold line, which could ensure that the paper sample zone on paper-A was properly and exactly aligned to the auxiliary zone on paper-B after folding (Scheme 1) for the difference of flexibility between the printed and unprinted area after baking. The unprinted hydrophilic area (paper auxiliary zone and paper sample zones) constituted the reservoir of the paper



Scheme 1. Schematic representation the fabrication and assay procedure of the paper-based ECL origami device. Paper sheets were firstly patterned in bulk using a wax printer (sheet A). After baking, three electrodes were screen-printed on wax-patterned sheet in bulk, respectively, (sheet B). (a) The prepared sheet was cut to rectangular paper (60.0 mm × 30.0 mm); (b–d) after modification, the rectangular paper was integrated with a device-holder, the device-holder was clamped closely and 40 μL supporting electrolyte was added for electrochemical assay.