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Paper-based microfluidic devices for electrochemical immunofiltration analysis of human chorionic gonadotropin

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ABSTRACT

An electrochemical immunofiltration analysis was introduced into microfluidic paper-based analytical devices (μPADs) for the first time, which was based on photolithography and screen-printing technology. The hydrophilic test zones of the aldehyde-functionalized screen-printed electrodes (SPEs) were biofunctionalized with capture antibodies (Ab_1). A sensitive immune detection method was developed by using primary signal antibody functionalized gold nanoparticles (GNPs/Ab_2) and alkaline phosphatase conjugated secondary antibody (ALP-IgG). Differential pulse voltammetry (DPV) was performed to detect the electrochemical response. The microfluidic paper-based electrochemical immunosensor (μ-PEI) was optimized and characterized for the detection of human chorionic gonadotropin (HCG), a model analyte, in a linear range from 1.0 mIU mL^{-1} to 100.0 IU mL^{-1} with a detection limit of 0.36 mIU mL^{-1}. Additionally, the proposed μ-PEI was used to test HCG in real human serum and obtained satisfactory results. The disposable, efficient, sensitive and low-cost μ-PEI has exhibited great potential for the development of point-of-care testing (POCT) devices that can be applicated in healthcare monitoring.

1. Introduction

Human chorionic gonadotropin (HCG), a well-known and important biomarker presented in the blood and urine of pregnant women, can protect against complications during prenatal care (Roushani and Valipour, 2016; Xia et al., 2016a). Recently, elevated levels of HCG were found in many cancerous tumors, including choriocarcinoma, germ cell tumors, hydatidiform mole, prostate cancer, and islet cell tumor (Roushani et al., 2016; Zhao et al., 2016a). The normal HCG range for men is between 0 and 5 mIU mL^{-1}, a positive result in males can be a test for testicular cancer. Therefore, exact calculation of the concentration of HCG in urine or serum is useful in the monitoring germ cell and trophoblastic tumors. Many sensitive and selective biosensors for quantitative detection of HCG in human blood and urine were studied in recent years, such as fluorescent analysis (Xia et al., 2016a), Surface enhanced Raman spectroscopy (SERS) (Wen et al., 2016), and electrochemical immunoassay (Teixeira et al., 2014; Roushani and Valipour, 2015; Roushani et al., 2016). However, these methods are usually expensive, time-consuming and lab-dependent. Therefore, developing a simple, cheap, sensitive, high reliable and fully disposable point-of-care testing (POCT) devices for quantitative assay that can operate at home are highly demanded recently.

Immunoassays based on the specific antibody-antigen recognition is widely applied in various fields including drug detection, toxicological analysis, bioanalysis, clinical diagnosis, food industry and environmental protection due to the excellent properties such as high selectivity, sensitivity and rapid detection without extensive pretreatment (Chen et al., 2016; Feng et al., 2016; Miranda et al., 2013). The conventional immunoassay is carried out on microplate, which involves a series of mixing, incubation, and washing steps need to be operated by professional personnel in well-equipped laboratory. Thus lead to the expensive, sample- and time-consuming diagnostic service in hospital. Direct-to-consumer (DTC) diagnostic tests and POCT have emerged as two of the most prominent forms of “personalized” healthcare due to portable, simple to operate, and fast response (Chen et al., 2016). The desire to produce disposable and low-cost POCT devices has prompted the development of microfluidic systems in recent years (Eletxigerra et al., 2015; Lei et al., 2015). Microfluidic devices based on several different substrates like silicon, glass, or polymer are developed extensively for the application in POCT. Furthermore, there are a large number of simple and excellent microfluidic analysis systems have been reported recently (Armbrecht et al., 2015; Chen et al., 2015; Lin et al., 2016; Ma et al., 2015; Tomazelli Coltro et al., 2014). However, few commercialized POCT products appeared in the market due to the

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inaccessible to untrained personnel.

Cellulose paper, a thin, flexible, cheap, disposable, biodegradable and permeable porous material composed of a homopolymer of (1,4)-β-glucopyranose linked through acetal bonds (Ahmed et al., 2016). The excellent chemical compatibilities make it utilize extensively in analytical and clinical chemistry. μPADs, a combination of low-cost paper test strips and conventional lab-on-chip devices, is attractive for the application in a new class of POCT device in recent years (Fosdick et al., 2014; Liu et al., 2014, 2016; Rattanarat et al., 2014; Tian et al., 2016; Xia et al., 2016b). Paper-based microfluidics are fabricated by patterning sheets of paper into hydrophilic channels surrounded by hydrophobic barriers based on various technologies such as photolithography (Dungchai et al., 2009; Nie et al., 2010), wax printing (Ueland et al., 2016), polydimethylsiloxane (PDMS) printing (Zhao et al., 2016b), and plasma treatment (Xia et al., 2016b). The dimension and height of the hydrophilic channels were defined by the hydrophobic barriers and the thickness of paper, respectively. Therefore, aqueous solution can be transported passively by the capillary action of paper fibers. Disposable detection electrodes could be produced by screen-printing with conductive carbon and Ag/AgCl ink on the surface of paper. Meanwhile, biological analyses such as glucose, uric acid, and lactate detection were demonstrated on paper fluidics based on this development (Labroo and Cui, 2014; Malon et al., 2014; Noiphung et al., 2013; Teengam et al., 2017; Yang et al., 2014).

It is well-known that sandwich immunoassay is the most widely used analytical method due to the high specificity and sensitivity. The stability of antibodies anchoring on paper surface is of great importance to the formation of sandwich immunocomplex. However, the backbone of pure cellulose paper offers only hydroxyl groups, which is impossible to direct covalent immobilization of biomolecules. Although there are many chemical methods for the modification of cellulose paper to covalently immobilize biomolecular (Ahmed et al., 2016; Kong and Hu, 2012), most of them refer to hazardous solvents, require complex modification steps, and are suitable only for specific kinds of biomolecules (Ge et al., 2012; Rosa et al., 2014). Thus, there is an urgent need to develop a simple and stable paper modification strategy to allow the conjugation of a great many of molecules that enables biosensing to be applied to the paper-based materials. Periodate oxidation, a highly specific reaction to directly convert 1,2-dihydroxyl (glycol) groups of paper to a pair of aldehyde groups, has been used as a novel and simple fabrication strategy for the covalent immobilization of antibodies on μPADs (Ahmed et al., 2016; Badu-Tawiah et al., 2015; Wang et al., 2012; Zhu et al., 2014b). Researches about the covalent conjugation of antibodies on paper-based device found that the antibodies still possess high binding stability for the development of low-cost POCT (Chen et al., 2016).

Recently, many nanomaterials such as GNPs, carbon nanotubes, and graphene, were used as carriers for loading a lot of signal tags such as enzymes, antibodies, oligonucleotide and dyes to prepare semi-quantitative and quantitative paper-based electrochemical immunosensor (Burrs et al., 2016; Li et al., 2014a, 2014b, 2015; Ma et al., 2015; Zhu et al., 2014a). The excellent signal amplification function and large surface areas of these nanomaterials can generate various strategies for sensitive detection of proteins or nucleic acids. Immunoassay based on GNPs including immunochromatographic and immunofiltration analysis are benefit to the development of remarkable industrial and engineering applications with biotechnological systems (Zhou et al., 2012; Zhu et al., 2014a).

In this study, electrochemical immunofiltration analysis was firstly introduced into μPADs to display a novel fabrication strategy for stable, rapid and portable sandwich immunoassay. Periodate oxidation was used as a simple modification strategy to activate the hydrophilic test zones of the SPEs for the covalent immobilization of antibodies. A sensitive immune detection method was developed by using ALP-IgG labeled immunogold as the signal probe. Ultimately, a new μ-PEI was optimized and characterized for detection of HCG in human serum samples. The proposed paper-based immunofiltration analysis not only shows acceptable specificity and sensitivity for the application in POCT, but also exhibits advantages including low-cost, simple operation, time- and sample-saving without professional conditions. This work could also make a great contribution to further expand the application of analytical method on μPADs.

2. Experimental section

2.1. Chemicals and materials

HCG (10000IU), rabbit polyclonal anti-HCG antibody (2.0 mg mL^{-1}) and mouse monoclonal anti-HCG antibody (2.0 mg mL^{-1}) were bought from Shuang Liu Zheng Long biochemical laboratory (Chengdu, China). $HAuCl_4{\cdot}4H_2O$, sodium citrate, HCl (37%), tris (hydroxymethyl) aminomethane (Tris), bovine serum albumin (BSA), para-nitrophenyl phosphate (p-NPP), ferrocenecarboxylic acid (FCA), phosphate buffer saline (PBS, 0.02 M, pH 7.4) and alkaline phosphatase conjugated secondary antibody (ALP-IgG) were obtained from Sigma-Aldrich. Follicle-stimulating hormone (FSH), luteotropic hormone (LH), thyroid stimulating hormone (TSH) and immunoglobulin G (IgG) were provided by XiBao biological technology Co., Ltd (Shanghai). Whatman No. 1 chromatography paper was purchased from GE Healthcare Worldwide. SU-8 2007 negative photoresist was bought from MicroChem Corp. (Newton, MA). Carbon ink (ED423ss) and Ag/AgCl ink (ED7019) were bought from Acheson. Human serum was provided by the People's Hospital of Guilin. Potassium periodate, 2,4-dinitrophenylhydrazine (2,4-DNP), glycerol were purchased from Sinopharm Chemical Reagent Co., Ltd. All chemical reagents used in this study were of analytical grade. All solutions used in this study were prepared with deionized water purified by a MilliQ Water Purification System (Milli-Pore, Bedford, MA, USA). Electrochemical substrate for DPV detection was 3.0 mM p-NPP in 0.01 M Tris-HCl buffers (pH 9.5). The original HCG solution was diluted to serial concentrations by PBSA (1×PBS, 1% BSA) for further experiments. The purchased ALP-IgG were diluted to 10 folds with PBS prior to use.

2.2. Apparatus

UV–vis spectra were obtained on a UH-5300 spectrophotometer (Hitachi, Japan). The morphologies and structures of paper-based SPEs were characterized using field emission scanning electron microscope (FESEM, JSM-7610F, Japan). The synthesized GNPs/Ab_2 was characterized by transmission electron microscope (TEM, JEM-2100, Japan). All electrochemical measurements were carried out on a CHI660E electrochemical analyzer (CH Instruments Co., Shanghai, China). Automatic screen-printing machine, screen-printing boards and scraper were bought from Jinjian Screen-printing Company (Hangzhou, China). The connection between paper-based SPEs and the CHI660 workstation was a simple sensor connector.

2.3. Fabrication of the paper-based SPEs

The fabrications of paper-based microfluidic electrochemical immunosensor are illustrated in Scheme 1. Sheets of Whatman No. 1 filter paper were soaked in a KIO_4 (0.03 M) solution at 65 °C for 2 h to functionalize aldehyde groups (Chen et al., 2016). Afterwards, each of the disposed sheets was washed twice for one minute with fresh deionized water. The prepared aldehyde-functionalized papers were treated with some absorbent paper and dried in a desiccator. Photolithography was applied to prepare the μPADs (Martinez et al., 2007). The photomask used to fabricate the μPADs was designed with Corel Draw 9 and produced by Weiyu co. (Guilin, China). Briefly, an adequate of photoresist was poured on the aldehyde-functionalized paper and spread over it using a spin-coater (500 rpm, 15 s; 6500 rpm,