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Multiplex electrochemical origami immunodevice based on cuboid silver-paper electrode and metal ions tagged nanoporous silver–chitosan

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ABSTRACT

A 3D microfluidic electrochemical origami immunodevice (denoted as μ-ECOI in this work) for sensitive detection of tumor markers was designed. High sensitivity was achieved by using novel cuboid silver modified paper working electrode (CS-PWE) as sensor platform and different metal ions-coated nanoporous silver–chitosan (NSC) as labels. The CS-PWE was fabricated through a seed-mediated growth approach and served as a promising platform for antibodies attachment. The metal ions could be detected directly through square wave voltammetry without metal preconcentration, and each biorecognition event produced a distinct voltammetric peak, whose position and size reflected the identity and amount of the corresponding antigen. The large number of metal ions loading on the NSC greatly amplified the detection signals, and the good biocompatibility of CS-PWE retained good stability for the sandwich-type immunoassay. Using cancer antigen 125 (CA125) and carcinoma antigen 199 (CA199) as model analytes, the simultaneous multiplex immunoassay showed linear ranges of over 4 orders of magnitude with the detection limits down to 0.02 and 0.04 mU mL^{-1}, respectively. Moreover, this strategy accurately detected the concentrations of CA125 and CA199 in human serum samples. The detection limits of CA125 and CA199 were 0.08 and 0.10 mU mL^{-1}, respectively. This facile biosensing μ-ECOI exhibited high sensitivity and specificity with excellent stability, reproducibility, and accuracy, indicating its wide range of potential applications in point-of-care testing.

1. Introduction

Microfluidic devices have received significant attention for the applications in diagnostics, primarily in resource-limited settings, due to the low requirements of volumes of reagents and the easy to transport and storage (Chin et al., 2007; Gubala et al., 2012). Paper is an attractive substrate for microfluidic devices; it is abundant, low-cost, disposable or biodegradable, easy to use, and it is easy to be modified chemically (Bruzewicz et al., 2008). Since Whitesides' group presented the first microfluidic paper-based analytical devices (μ-PADs) (Martinez et al., 2007), μ-PADs have emerged as ideal platforms for point-of-care testing (POCT). Importantly, μ-PADs are easily fabricated in bulk using wax-printing, which is among the cheapest and the most easily implemented means of mass production available, and it has been found effective in the production of μ-PADs at minute cost (Carrilho et al., 2009). Inspired by this simple technique, many groups have paid great efforts to the development of μ-PADs (Tao et al., 2011; Chen et al., 2012; Liu and Crooks, 2012; Ge et al., 2013).

Immunoassay based on the antibody–antigen interaction is one of the most important analytical techniques in the quantitative detection of cancer biomarkers due to the highly specific molecular recognition of immunoreaction. Electrochemical (EC) immunoassay has attracted considerable interest for its intrinsic advantages such as good portability, low cost, and high sensitivity (Chikkaveeraiah et al., 2012; Kimmel et al., 2012). Therefore, different EC immunosensors, particularly amperometric immunosensors, have been developed and extensively applied to the determination of cancer biomarkers (Feng et al., 2012; Akter et al., 2012). Meanwhile, owing to the limited specificity of tumor markers to a particular disease, multiplex immunoassay (MIA) methods for simultaneous detection of a panel of cancer biomarkers in a single run with improved diagnostic accuracy have attracted considerable attention (Tang et al., 2011). Moreover, the

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MIA can enhance detection throughput, shorten analytical time, and decrease detection cost and sampling volume.

Great efforts have been made toward bio-component immobilization and signal amplification for sandwich-type EC immunoassay in order to significantly enhance the sensitivity for cancer biomarker detection. Cuboid silver (CS) is chosen as a model biocompatible nanomaterial due to its large surface area, strong absorption ability, and superior conductivity, which maintains the bioactivity and stability of immobilized biomolecules (Hu et al., 2012). In this work, an interconnected CS layer was grown on the surfaces of cellulose fibers from silver nanoparticle (AgNP) seeds in the paper sample zone of paper working electrode (PWE) to fabricate novel CS-PWE on a 3D electrochemical origami device. Due to the high ratio of surface area to weight (9.5 m^2/g) (Pelton, 2009) and porous structure of paper as well as the high conductivity of CS, the active surface area and the sensitivity of this CS-PWE was much higher than that of a bare one (unmodified PWE). On the other hand, nanomaterial-supported multiple labels were widely used in EC-based bioassay. Nanoporous silver (Xu et al., 2010) has been chosen as a signal amplifier due to its high surface-to-volume reaction, high in-plane conductivity, and convenient labeling to biomolecules. Chitosan, a natural biocompatible polysaccharide, could provide abundant amino and hydroxyl functional groups, which was largely used as a chelating agent for the absorption of metal ions (Liu et al., 2012). Herein, we have successfully fabricated nanoporous silver–chitosan (NSC) hybrids as nanocarriers for loading different metal ions such as Ag^+ and Cu^{2+}. The fabrication of versatile labels was used for simultaneous EC detection of cancer markers. Taking into consideration the above advantages, it would be an effective way to integrate CS-PWE with metal ions functionalized NSC hybrids as magnified elements for constructing sensitive EC biosensor with a particular analytical sensing design.

In this work, we developed a sensitive 3D microfluidic EC origami immunodevice (μ-ECOI) for simultaneous detection of cancer antigen 125 (CA125) and carcinoma antigen 199 (CA199) using novel CS-PWE as sensor platform and different metal ions functionalized NSC as labels. This novel CS-PWE was fabricated on this 3D electrochemical origami device through a seed-mediated growth approach. The prepared CS-PWE was biocompatible and conductive, creating an ideal sensor platform to improve electron transfer and absorb abundant antibodies. Due to the outstanding absorption capability, a large amount of metal ions such as Ag^+ and Cu^{2+} were incorporated into the NSC hybrids to form NSC–metal ion labels. CA125 antibodies and CA199 antibodies were conjugated with NSC–Ag^+ and NSC–Cu^{2+} to fabricate anti-CA125/NSC–Ag^+ and anti-CA199/NSC–Cu^{2+} probes, respectively. The metal ions in the labels could be detected directly without acid dissolution and preconcentration, which would greatly simplify the detection steps and reduce the detection time. Ultimately, with the aids of a simple home-made device-holder, two tumor markers in real human serum samples were simultaneously detected based on dual-signal amplification technique. This proposed strategy improved the sensitivity and made contributions for simple, high-throughput, rapid and portable multiplex electrochemical immunoassay on μ-PADs.

2. Experimental section

2.1. Materials and reagents

Antigens (CA125 and CA199), mouse monoclonal capture antibodies (Ab_1) and signal antibodies (Ab_2) of two antigens were purchased from Linc-Bio Science Co. Ltd. (Shanghai, China). The clinical serum samples were obtained from Shandong Tumor Hospital. Chitosan, poly-(allylamine hydrochloride) (PAH), bovine serum albumin (BSA), and ascorbic acid (AA) were obtained from Sigma-Aldrich Chemical Co. (USA). Glutaraldehyde (GA), $AgNO_3$ (as the precursor for the formation of AgNP seeds), $NaBH_4$, and trisodium citrate was obtained from Shanghai Reagent Company (Shanghai, China). 10 mM $AgNO_3$ solution and 5.0 mM AA solution were prepared daily for silver-growth enhancement. Phosphate buffered solution (PBS) (pH 7.4, 10.0 mM) was prepared with NaH_2PO_4 and Na_2HPO_4. The washing buffer was PBS (10.0 mM) containing 0.05% (w/v) Tween-20. PBS (10.0 mM) containing 0.5% (w/v) BSA and 0.5% (w/v) casein was used as blocking solution. HAc/NaAc solutions with different pH values were prepared by mixing the stock solutions of HAc and NaAc. Ultrapure water obtained from a Millipore water purification system (>18.2 MΩ, Milli-Q, Millipore) was used in all assays and solutions. All other reagents were of analytical grade and used as received. The Ag/Al alloy (23:77 wt%, 50 μm thick) foils were obtained from Monarch. Carbon ink (ED423ss) and Ag/AgCl ink (CNC-01) were purchased from Acheson. Whatman chromatography paper #1 (58.0×68.0 cm^2) (pure cellulose paper) was obtained from GE Healthcare Worldwide (Pudong, Shanghai, China) and used with further adjustment of size (A4 size).

2.2. Apparatus

All electrochemical immunoassay measurements were performed on a CHI 760D workstation (Chenhua, Shanghai, China). Electrochemical impedance spectroscopy (EIS) was performed on a CHI 604D electrochemical workstation (Shanghai CH instruments, China). X-ray photoelectron spectra (XPS) were measured using an ESCALAB 250 spectrometer (Thermo Fisher Scientific) with monochromatized Al-Kα X-ray radiation (1486.6 eV) in ultrahigh vacuum ($<10^{-7}$ Pa). All samples were powdered and pressed into thin plates before the measurement. In every case a neutralizer was used to eliminate the charge effect which occurs for non-conducting samples and the binding energies were calibrated by taking C1s peak (284.6 eV) of adventitious carbon as reference. Broad survey scan with the scan range from 0 to 1300 eV was run to identify all elements present in the samples, and the detailed scans (20 eV wide) were recorded to establish precise elemental peak locations. The data were analyzed using Avantage software and the peaks were deconvoluted after background subtraction, using a mixed Gaussian–Lorentzian function. Fractional atomic concentrations of the elements were calculated using empirically derived atomic sensitivity factors (Wagner et al., 1981). UV–vis absorption spectra were recorded on a UV-3101 spectrophotometer (Shimadzu, Japan). Transmission electron microscopy (TEM) investigations were performed using JEOL 4000 EX microscope (operating voltage 100 KV, applied current 62 μA). Scanning electron microscopy (SEM) images and energy-dispersed spectrum (EDS) experiment were recorded on a QUANTA FEG 250 thermal field emission SEM (operating voltage 10 KV, applied current 250 μA, working distance 6.6 mm, FEI Co., USA).

2.3. Preparation of this 3D electrochemical origami device

This 3D electrochemical origami device was fabricated on pure cellulose paper within 10 min, and a detailed procedure was described in the Supporting information. As shown in Scheme 1, on each origami device (30.0×15.0 mm^2), there were two circular paper zones (one auxiliary zone (8.0 mm in diameter) and one sample zone (6.0 mm in diameter)) for screen-printing of electrodes. The electrode array consisted of a screen-printed Ag/AgCl reference electrode and carbon counter electrode on the paper auxiliary zone and a screen-printed carbon working electrode on the paper sample zone, respectively. One angle of the square