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A multianalyte electrochemical immunosensor based on patterned carbon nanotubes modified substrates for detection of pesticides

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ABSTRACT

A novel multianalyte electrochemical immunosensor based on the assembly of patterned SWNTs on glassy carbon (GC) substrates was developed for simultaneous detection of endosulfan and paraoxon. Based on aryldiazonium salt chemistry, forest of SWNTs can be patterned on GC substrates by C–C bonding using micro contact printing (MCP), which provides an interface showing efficient electron transfer between biomolecules and electrodes. Then redox molecules FDMA and PQQ can be attached to the SWNTs, respectively followed by the attachment of specific epitopes and antibodies. The modified sensing surfaces were characterized by XPS, SEM, AFM and electrochemistry. Based on the current change of specific redox probes, the fabricated immunosensor array can be used for simultaneous detection of endosulfan and paraoxon by a displacement assay. In phosphate buffer solution (50 mM, pH 7.0), there is a linear relationship between electrochemical signal of FDMA and the concentration of endosulfan over the range of 0.05–100 ppb with a detection limit of 0.05 ppb; the linear range between electrochemical signal of PQQ and the concentration of paraoxon is 2–2500 ppb with a detection limit of 2 ppb. The immunosensor array demonstrates high repeatability, reproducibility, stability and selectivity for the detection of endosulfan and paraoxon.

1. Introduction

Immunochemical techniques such as immunoassay based on the antibody-hapten reaction have lately gained great attention for the analysis of agrochemicals because of their specificity, cost-effectiveness, and high sample throughput (Gabaldon et al., 2003). Sensors that can be used for detecting many species simultaneously are of particular importance for routine analysis in the field. The ability to perform multiple analyses on a single sensing surface has a number of advantages (Rowe et al., 1999) over performing multiple parallel analyses on different substrates, such as a single set of positive and negative controls can be used for all the assays, use of a single substrate provides more effective (and valid) comparisons of the experimental data and controls, and performing multiple assays simultaneously decreases the assay times compared with sequential analyses et al. Studies on optical biosensors capable of simultaneous detection of multiple analytes are very active (Anderson et al., 2000; Blawas et al., 1998; Ekins and Chu, 1993; Venkatanarayanan et al., 2012). However, demonstration of the ability to use a single sensor substrate for simultaneous, multi-analyte detection based on electrochemical biosensors is limited (Plowman et al., 1999; Rowe et al., 1999; Taitt et al., 2002). Maquieira's group has successfully developed a multianalyte immunosensor for on-line determination of organic compounds (Gonzalez-Martinez et al., 2001). However, the reproducibility is low, and the effect of saturation could reduce the number of analytes that can be monitored.

Single walled carbon nanotubes (SWNTs) have attracted increasing attention since their discovery two decades ago (Iijima, 1991), due to their unique structural, mechanical and electric properties. Especially SWNTs have good biocompatibility characteristics, which is beneficial to enhance electron transfer and to maintain the biological activity of enzyme (Gooding et al., 2003). With the deepening of research on properties of SWNTs, more and more people use SWNTs in surface modification for biosensors. The most applied method to anchor SWNTs is based on the formation of amide bonds from the reaction between the amines located on the modified electrode and the carboxylate groups at the ends of sidewall defects of nanotubes (Garrett et al., 2010). Carbon nanotubes can also be fabricated to substrates based on H-bonding interactions between the carboxylate groups of on nanotubes and the –OH groups at the ITO surface (Diakowski et al., 2010). Pinson and coworkers firstly reported the covalent attachment of nanotubes to substrates by C–C bonds using diazonium salt reactions (Joyeux et al., 2009), and they claimed that nanotubes were tethered to the surface in an approximately horizontal orientation with respect to the surface. Recent studies by Ferri's group show vertically aligned SWNTs are covalently modified on surfaces through C–C bondings

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(De Fuentes et al., 2011). Although the precise orientation of SWNTs tethered on substrates by C–C bonds using diazonium salt reactions is argumentative, it is widely agreed that the formed SWNTs interface is very stable which is in consistency with the known stability of tether layers grafted from diazonium salts (Liu et al., 2007). Meanwhile, the assembly of SWNTs on the tethered aryldiazonium salt layer has greatly increased the electronic coupling between the electrode and outside biological environment. Our previous studies have demonstrated that immunosensors based on SWNTs modified substrates have the ability for rapid, sensitive and quantitative analysis of antibodies and small molecules (Liu et al., 2011a, 2013, 2012b). Venkatanarayanan et al. (2012) reported an electrochemiluminescence sensor array based on vertically aligned SWNT , which shows a wide linear dynamic range, a remarkably low detection limit of 1.1 pM for IgG and is promise for future development of multiplexed assays. An additional feature of this kind of immunosensor becomes more and more important: the immunosensor should be able to discriminate between multiple analytes in a single pot of samples. Fabrication of the sensing interface with different redox reporters might be able to make this additional feature possible. Small redox molecules (Harper et al., 2007; Liu et al., 2008; Pheeney and Barton, 2012) ferrocene derivatives (Beheshti et al., 2012; Diakowski et al., 2010), and redox enzymes (Guo et al., 2013; Jin et al., 2012) are popular redox reporters. However, to our knowledge no report shows multiple redox reporters are simultaneously fabricated on the sensing interfaces for multianalyte immunosensors.

Modification of carbon surfaces with aryldiazonium salts to give covalent attached layers has attracted wide attention since it is firstly reported it 1992 (Delamar et al., 1992). Recent studies, such as *in situ* aryldiazonium salt fabrication (Baranton and Belanger, 2005), microcontact printing (MCP) using spontaneous reduction of aryldiazonium salts (Garrett et al., 2007), and forming mixed layers of aryldiazonium salts with something close to molecule level control of sensing interfaces (Liu et al., 2010), demonstrate that applications of aryldiazonium salt chemistry on sensing interface have been progressively attractive. As a simple and relatively fast patterning method, MCP using adhering a poly(dimethylsiloxane) (PDMS) stamps and aryldizonium salt inks becomes an attractive route to pattern and covalently attach reactive tether layers. A broad range of substrates can be patterned by adhering a PDMS mold to the surface to form microchannels (Downard et al., 2006; Lehr et al., 2010). However, little work is reported on using patterned surfaces for multianalyte immunosensors for detection of pesticides.

In this contribution, as illustrated in Scheme 1, we report a novel multianalyte electrochemical immunosensor based on the assembly of SWNTs formed by MCP on glassy carbon (GC) substrates. The flow chamber modules can be made on GC substrates by placing a 4-channel PDMS patterning template in contact with the surface, applying pressure to create a fluid-tight and airtight seal. Two redox probes ferrocenedimethylamine (FDMA) and pyrroloquinoline quinone (PQQ) were loaded to different channels, respectively. Binding of the antibody to the surface-bound epitope immerses the redox molecules in a protein medium. A consequence of this change in environment is the attenuation of electron transfer to the redox molecules due to the inaccessibility of a counterion. Association or dissociation of the antibody with the sensing interface causes a modulation of the electrochemistry from the redox molecules. Based on different electrochemistry signals from two redox reporters FDMA and PQQ, this fabricated immunosensor array can be used to simultaneously detect two analytes in one sample. Hence the approach we established here is a prototype research that is promising for designing portable device for on-site detection of pesticides in environmental monitoring.

2. Experimental section

2.1. Reagents and materials

Sodium nitrite, potassium ferricyanide, hydrochloric acid, 4-phenylenediamine, aniline, 2-[4-(2-Hydroxyethyl)-1-piperazine]ethanesulfonic acid (HEPES), 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and pyrroloquinoline quinone (PQQ) were purchased from Sigma-Aldrich (Shenshi Huagong, Wuhan). SWNTs prepared by the HiPco process were purchased from Carbon Nanotechnologies Incorporated. Cut SWNTs were prepared as reported previously (Liu et al., 1998). The aryldiazonium cations for 2-(2-(2-(4-aminophenoxy)-ethoxy) -ethoxy)-ethanol (PEG) was custom synthesized (Liu and Gooding, 2006). Ferrocenedimethylamine (FDMA) was synthesized by the literature method (Ossola et al., 2003). Endosulfan and paraoxon were purchased from Fluka (Sanyi Chemicals, Wuhan). The endosulfan hapten, anti-endosulfan IgG, paraxon hapten, and anti-paraoxon IgG were prepared as reported previously (Heldman et al., 1985; Liu et al., 2013, 2012b). All other reagents were used as received. Aqueous solutions were prepared using Mill-Q water ($>$ 18 MΩ cm). Phosphate buffered saline (PBS) solutions were 0.15 M NaCl and 0.1 M phosphate buffer, pH 7.3. Phosphate buffer solution for electrochemistry was prepared using 0.1 M buffer with added 0.05 M KCl (pH 7.0).

2.2. Apparatus

All electrochemical experiments were conducted using the GaossUnion EC510 potentiostat (GaossUnion, China). All experiments utilized a 3 mm disk GC working electrode, a Pt secondary electrode and a Ag/AgCl (3.0 M NaCl) reference electrode. X-ray photoelectron spectra (XPS) were collected from GC plates on a VG multilab 2000 spectrometer with a monochromated Al Kα source (1486.6 eV), hemispherical analyzer and multichannel detector. SEM was carried out using a Hitachi S-900 SEM (Berkshire, England). Atomic force microscopy (AFM) images were taken on GC plates using a Digital Instruments Dimension 3100 scanning probe microscope.

2.3. Preparation of aryldiazonium cation ink and microcontact printing

Aryldiazonium salt modified SWNTs were prepared according to the literature (de Fuentes et al., 2011). Specifically, cut SWNTs (1 mL, 1 mg/mL) was added to the mixture of HCl (1 mL, 0.5 M), *p*-nitroaniline (10 mM), and $NaNO_2$ (10 mg) which was left to react overnight. The functionalized SWNTs suspension was centrifuged, and the residue was washed with water and acetonitrile. Then the residue (*p*-nitrobenzene modified SWNTs) was treated in acetonitrile solution containing 5 mM PEG diazonium salts for 6 h to attach PEG molecules to the sidewall of SWNTs. The PEG functionalized SWNTs suspension was centrifuged, and the residue was washed with acetonitrile. Finally *p*-nitrobenzene/PEG-modified SWNTs were suspended in a basic solution (pH 10) and treated with $NaBH_4$ for 1 h to reduce $-NO_2$ to $-NH_2$. Subsequently the mixture was centrifuged and rinsed with water. The *p*-aminophenyl/PEG-modified SWNTs were suspended in HCl (0.5 M) containing $NaNO_2$ (10 mg) to get the aryldiazonium cation ink.

PDMS stamps were immersed in the aryldiazonium cation ink for 10 min, dried to tackiness in a stream of N_2 gas, and placed on the substrate for 25 min to obtain the GC-Ph-SWNTs modified interface. All printed samples were rinsed with acetonitrile solution followed by Milli-Q water and dried in a stream of N_2 prior to analysis or further treatment. Control samples were prepared in