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Short communication

Polypyrrole nanowire modified with Gly-Gly-His tripeptide for electrochemical detection of copper ion

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ABSTRACT

We developed a novel biosensor comprising a transducer of carboxyl end-capped overoxidized polypyrrole nanowire electrode and a probe of tripeptide (Gly-Gly-His) selectively cognitive of Cu^{2+}. The developed sensor was demonstrated to specifically detect Cu^{2+} in the nanomolar range. The diameter of the polypyrrole nanowires, prepared by one step template-free polymerization, was approximately 60–90 nm. For functionalization of the electrode, the carboxyl group was introduced by the addition of pyrrole-α-carboxylic acid which was covalently coupled with the amine group of the tripeptide. The structural features of the peptide functionalized nanowire electrode were confirmed by X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM) spectroscopy. Using cyclic and square wave voltammetry, the nanowire biosensor proved to be highly sensitive to Cu^{2+} in the range of 20–300 nM.

1. Introduction

Nanotechnology is rapidly evolving to open new combination of methods and resolve challenging analytical problems including specificity, stability and sensitivity. Nanomaterials synthesized from conducting polymer (CP) have received considerable attention because of remarkable physical and chemical characteristics originating from their small dimensions and high surface area (Jang, 2006). CP nanomaterials are integrated with various micro-analytical systems and coupled with a variety of chemical/biological species to obtain highly sensitive and specific responses (Yoon and Jang, 2009). Among the CPs, polypyrrole (PPy) is technologically important notably due to its environmental stability and high electronic conductivity especially for fibers having diameter less than 150 nm (Debiemme-Chouvy, 2009; Cai and Martin, 1989). PPy is a very attractive substrate for immobilization of biomaterials, rendering its wide utilization for the design and construction of various biosensors harnessing immobilized enzymes (Laurinavicius et al., 2002), and covalently attached ligands or specific biological species including peptides, antibiotics, DNA and whole cells (Ramanaviciene and Ramanavicius, 2004; Shiu et al., 1994; Lee et al., 2002). Owing to these versatile features, there is an increasing interest in the PPy nanomaterials for various future applications, especially in the use of PPy nanomaterials in analytical sciences (Hou et al., 2008; Li and Lin, 2007).

To minimize possible chemical and/or thermal damages during immobilization of CP nanomaterial transducers onto an electrode substrate for construction of electrochemical sensors, one of the most critical challenges is to ensure high-quality contact between the CP nanomaterials and the electrodes (Yoon and Jang, 2009). Recently, various methods including soft lithography, electrochemical deposition, and ink-jet printing (Xue et al., 2008) have been explored for patterning of conducting polymers in the nanometer regime, but most CPs suffer from insufficient adhesion to the electrode substrate. Those CP nanomaterials have been used to detect analytes predominantly present in the gas phase. To overcome these limitations, several strategies based on chemical binding of nanomaterials onto a substrate surface have been proposed. The PPy nanowire (NW) layer on a silicon substrate using N-(3-trimethoxysilylpropyl)pyrrole resulted in the enhanced adhesion to give rise to ammonia gas detection elements (Dong et al., 2005). Jang and co-workers reported biosensors based on enzyme functionalized poly(pyrrole-3-carboxylic acid) nanotubes which were immobilized onto a microelectrode substrate via covalent linkages (Yoon et al., 2009).

Recently, Debiemme-Chouvy (2009) introduced the preparation of PPy NW without a template. The overoxidized PPy (OPPy) thin layer was formed by one step pyrrole polymerization in the presence of weak-acidic and non-acidic anions, and then PPy NWs were grown on the OPPy thin layer. Generally, PPy can be further overox-

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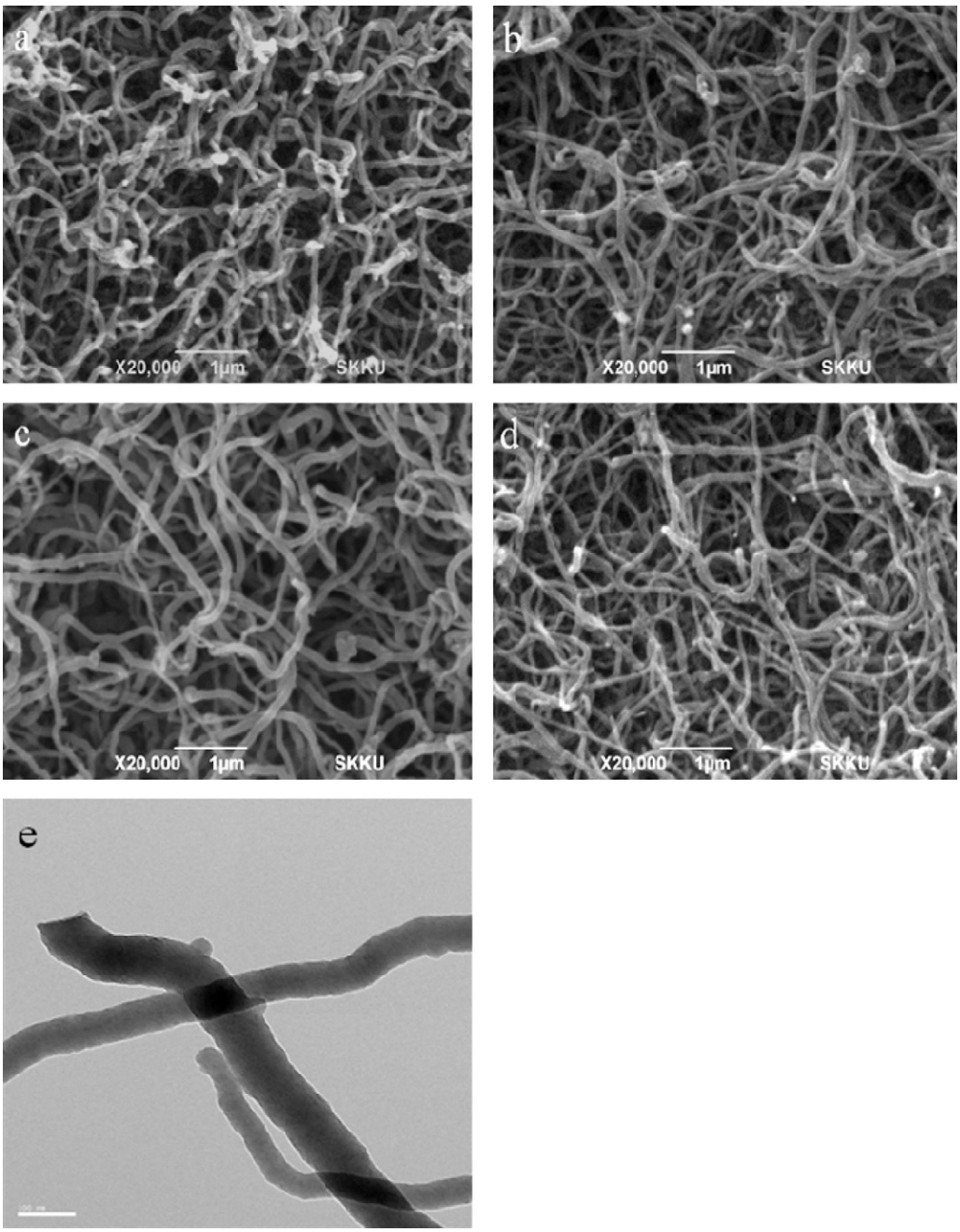

Fig. 1. SEM images of (a) PPy NW, (b) PPy-COOH NW, (c) OPPy-COOH NW, and (d) OPPy-GGH NW. (e) TEM image of OPPy-GGH NW.

idized at a higher positive potential, expelling the doping ions and thereby losing its conductivity. The irreversibly OPPy has excellent cation exchanging and molecular sieving properties (Majidi et al., 2007; Witkowski and Brajtertoth, 1992), thereby imparting superior selectivity and sensitivity toward the analytes (Li and Lin, 2007).

In our previous research, a fabrication of Cu^{2+} biosensor based on Gly-Gly-His (GGH) tripeptide attached onto a poly(3-thiopheneactic acid) film modified gold electrode has been reported (Lin et al., 2009a,b). Herein, we report a novel electrochemical biosensor harnessing the tripeptide covalently attached to overoxidized PPy NW on a gold electrode for enhanced determination of Cu^{2+} in the lower concentration regimes. The carboxyl group of the polymer was modified to accommodate tripeptide molecules for Cu^{2+} capture. The tripeptide functionalized PPy nanomaterials was anchored onto a gold electrode to form a biosensor without any chemical linker. In the present study, the tripeptide modified PPy nanowire biosensor proved its capability to determine the concentration of Cu^{2+} in the range of 20–300 nM, which has rarely been reported by the conventional biosensors scaffolded with film type electrodes (Heitzmann et al., 2007; Shiu et al., 1994).

2. Experimental

2.1. Preparation of OPPy-COOH electrodes

The gold electrodes (diameter: 2 mm) were polished and cleaned according to the literature (Priano and Battaglini, 2005). The PPy NW was electropolymerized on a gold electrode by chronoamperometry at 0.8 V in 0.15 M pyrrole (Py) solution con-

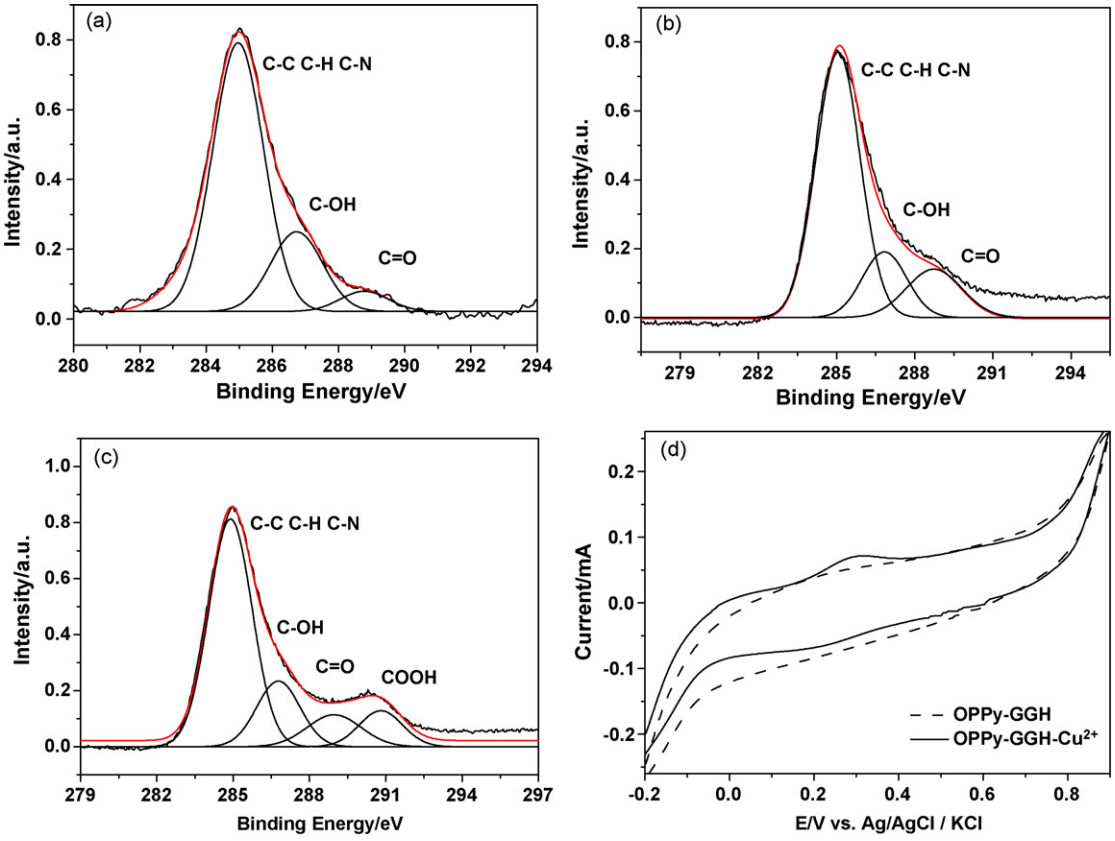

Fig. 2. XPS spectra (C_{1s}) for (a) PPy NW, (b) OPPy NW and (c) OPPy-COOH NW electrodes. (d) CV of OPPy-GGH NW electrode (dash line) and OPPy-GGH NW electrode with Cu^{2+} captured (solid line).

taining 0.1 M $LiClO_4$ and 0.1 M Na_2CO_3. The obtained polymer electrode was rinsed with distilled water and then kept into 10% $HClO_4$ solution for 12 h to remove the carbonate ions. Subsequently, the PPy NW electrode was soaked into a 0.1 M pyrrole-α-carboxylic acid (Py-COOH) acetonitrile (ACN) solution for 3 h and dried. Py-COOH was physically adsorbed onto the surface of the PPy. Py-COOH was chemically polymerized in the presence of iron chloride (0.5 M) to form the PPy-COOH NW electrode. The normal PPy film was electropolymerized by chronoamperometry at 0.8 V in 0.15 M pyrrole solution containing 0.1 M $LiClO_4$ electrolyte. The PPy-COOH film was also prepared in the same manner.

The overoxidized PPy-COOH (OPPy-COOH) polymers were prepared by cyclic voltammetry (CV) between 0 and +0.9 V in 0.15 M NaOH. Then the OPPy-COOH electrodes were immersed in 0.1 M HCl solution for 1 h before modification with the tripeptide.

2.2. Modification of OPPy-COOH electrode with GGH tripeptide

The modification of OPPy-COOH electrode with tripeptide (GGH) was performed as reported elsewhere (Chow et al., 2005; Lin et al., 2009a). The carboxyl terminal groups of the OPPy-COOH were activated in a solution of 20 mM EDC/20 mM NHS in 100 mM MES (pH 6.8) for 3 h. After a thorough rinse with 25 mM MES buffer, the polymer electrode was reacted overnight with GGH (3 mg/mL) in MES buffer to form the peptide conjugated OPPy-COOH (OPPy-GGH) electrode (Scheme 1S). The modified electrode was rinsed thoroughly with MES buffer and stored at room temperature in 50 mM ammonium acetate buffer (pH 7.0) prior to use.

2.3. Metal ion sensing

The OPPy-GGH electrode was immersed in 20 mL of 50 mM aqueous ammonium acetate buffer solution (pH 7.0) at various concentrations of copper ions for 10 min and washed with copper-free ammonium acetate buffer. The bound metal ions on the OPPy-GGH electrode were reduced at −1.2 V for 120 s, and the electrochemical response was measured by square wave voltammetry (SWV) using a frequency of 50 Hz, an amplitude of 20 mA, and a step voltage of 5 mV. After each SWV measurement, the electrode was rinsed with ammonium acetate buffer, placed in 0.1 M EDTA solution for regeneration, rinsed with acetate buffer, reduced and measured by SWV in a fresh ammonium acetate buffer to ensure complete stripping of all metal ions.

All electrochemical analysis was performed in a conventional three-electrode cell comprising the modified electrode as a working electrode, a platinum plate as a counter electrode, and an Ag/AgCl in saturated KCl solution as a reference electrode.

3. Results and discussion

3.1. Surface morphology of PPy NW electrodes

The structures of the prepared PPy and OPPy electrodes were investigated by SEM-analysis. The structure of PPy layers produced by electrochemical technique depends on the preparation conditions such as the nature of the used counter ions, and medium. When $LiClO_4$ was used as an electrolyte in aqueous medium, the PPy film (Fig. 1Sa) with a cauliflower structure was obtained. On the other hand, in the presence of Na_2CO_3 and $LiClO_4$, the interconnected, branched network-like nano-fibrous PPy NW was obtained

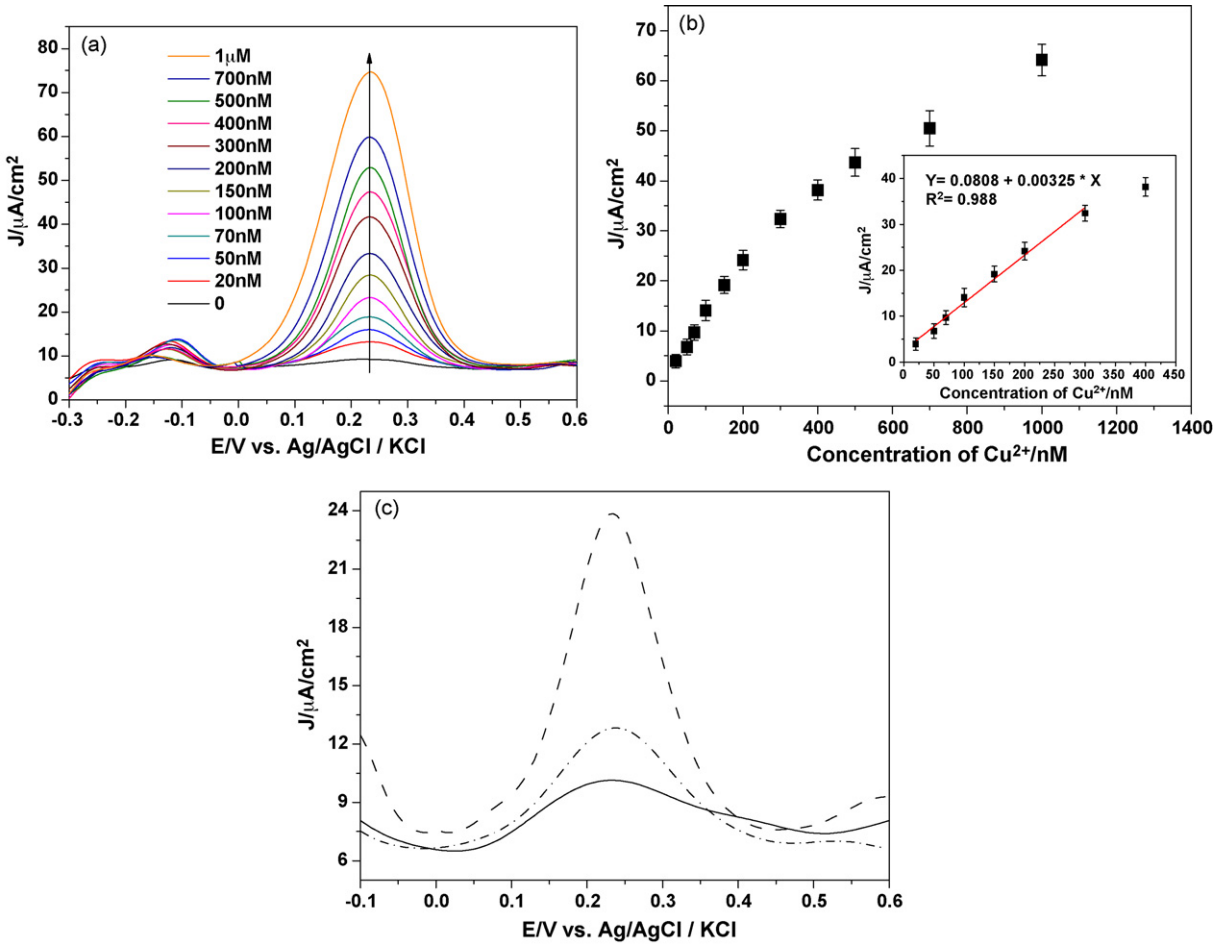

Fig. 3. (a) SWV curves for dissolution of Cu^{2+} captured by OPPy-GGH NW electrode. (b) SWV peak current for OPPy-GGH NW electrode. (c) SWV curves for dissolution of Cu^{2+} captured by OPPy-GGH electrodes: NW 100 nM (dash line), film 100 nM (solid line) and film 300 nM (dash–solid line).

(Fig. 1a) (Özcan et al., 2008). The carboxylation of PPy NW to give rise to PPy-COOH NW electrode (Fig. 1b) rarely affected the morphological features of the electrode since neither the overall network-like structure nor the apparent diameter of the NW changed. As shown in Fig. 1c, overoxidation of PPy-COOH exerted little effect on the NW structure, and the morphology of OPPy-COOH NW remained almost similar to that of PPy-COOH NW. On the other hand, it is interesting to note that OPPy-COOH film (Fig. 1Sb), following the oxidation and carboxylation, comprised cauliflower shaped agglomerates of increased diameter when compared to those of the PPy film. It is worth mentioning that the overall structure of the OPPy-COOH is mostly governed by the PPy layer, and that there should be only slight changes on the carboxyl end-capped surface (Shamsipur et al., 2008). With conjugation of the tripeptide (GGH) to the surface of OPPy-COOH NW electrode, the surface (Fig. 1d) shows little departure from the original one presumably due to the small size of the peptide molecules. Fig. 1e shows the TEM image of OPPy-GGH NW, and its diameter is in the range of 60–90 nm.

3.2. X-ray photoelectron spectroscopy

The addition of Py-COOH to the surface of PPy NW was confirmed by XPS analysis. The C_{1s} spectrum of PPy NW (Fig. 2a) has been fitted into neutral carbons at 285.0 eV and oxidized carbon species at 286.8 and 288.8 eV, which correspond to –C–OH and –C=O, respectively (Ge and Qi, 1994; Malitesta et al., 1995). The C_{1s} spectrum of PPy NW is in a good agreement with the spectrum reported by Debiemme-Chouvy (2007). In the case that weakly acidic anions (monohydrogenophosphate, acetate or carbonate) are present in the electrolyte, the electropolymerization of pyrrole led to the overoxidized insulator film. The extent of overoxidation process was monitored by CV current profiles which show a continuous current decay per each cycle, followed by a plateau reached with the completion of oxidation (Özcan et al., 2008). After overoxidization, the C_{1s} spectrum of OPPy NW shows the similar peak fitting to that of PPy NW, while the relative intensity of the peak at 288.8 eV corresponding to the overoxidized PPy (–C=O) increases (Fig. 2b). The XP survey spectrum for the formation of the OPPy-COOH NW shows a peak centered at 290.5 eV in the C_{1s} high resolution spectrum (Fig. 2c), accounting for successful incorporation of the carboxyl group from Py-COOH to the surface of PPy NW.

3.3. Electrochemical performance of OPPy-GGH electrode

Fig. 2d shows the cyclic voltammograms recorded for the OPPy-GGH NW electrode (Fig. 2d, dash line) and the modified electrode with Cu^{2+} captured in 100 nM copper solution (Fig. 2d, solid line) for 10 min. The electrochemical response for the bare OPPy-COOH NW shows no obvious electrochemical response between −0.2 and +0.9 V for the Cu^{2+}-captured electrode, however, a few apparent small redox peaks appeared at +0.3 and +0.2 V suggesting that the oxidation process of the polymer itself does not interfere with Cu^0 oxidation. These peaks are clearly attributed to the redox reaction of Cu^0/Cu^{2+} (Lin et al., 2009a,b; Chow et al., 2005), confirming the attachment of GGH tripeptide to the OPPy-COOH electrode.

GGH tripeptide used in this study has been known for its selective affinity to Cu^{2+} despite the presence of other divalent metal ions (Lin et al., 2009b; Chow et al., 2005). In addition, its small size is ideal for locating the peptide probe in close proximity to the transducer, thereby allowing the OPPy-GGH NW sensor to detect Cu^{2+} in a selective and reproducible manner in the nanomolar range.

We investigated the SWV response of the modified PPy electrodes with and without overoxidation between −0.2 and +0.9 V. The PPy was overoxidized to avoid its large background current that may hinder an accurate determination of a SWV peak current arising from oxidation of Cu^0 in the polymer matrix. Fig. 3a shows the SWV peaks originating from OPPy-GGH NW electrode. Following the Cu^{2+} capture step, the modified electrodes were transferred into a metal ion-free buffer (50 mM ammonium acetate, 50 mM NaCl, pH 7.0) for detection of Cu^{2+} where the captured metal ions on the electrode surface were reduced before recording the stripping current using SWV (Heitzmann et al., 2007; Lin et al., 2009a; Yang et al., 2003; Bi and Yang, 2007). When the tripeptide was modified on the surface of the PPy-COOH NW electrode without the prior overoxidization process, the electrochemical performance of the resulting PPy-GGH NW electrode (Fig. 2S) was remarkably different from that of the OPPy-GGH NW electrode as evidenced by emergence of a large background current peak at −0.1 V presumably due to oxidation of the PPy-GGH. Each peak around 200 mV accounted for oxidation of Cu adsorbed at various concentrations, but determination of the SWV peak current for each Cu oxidation peak from PPy-GGH electrode is not as evident as from the OPPy-GGH electrode. In contrast, a small oxidation peak hence a negligible background current was observed for OPPy-COOH NW electrode at −0.1 V. Furthermore, each Cu oxidation peak observed at 230 mV for the OPPy-COOH NW electrode was distinctive from the background in a concentration dependent manner, rendering a clear determination of the peak current for each SWV curve. Overoxidation is an entirely irreversible process leading to dedoping of the PPy NW (i.e. ClO_4^- in this study) hence the decreased conductivity of the polymer. This, however, was found to be conducive to significantly reducing the background current by minimizing electrochemical change of PPy NW itself, thereby rendering an improved discerning of electrochemical changes caused by Cu^0/Cu^{2+} oxidation from the background electrochemical responses.

For the analytical purpose, SWV is used due to its lower detection limit afforded by square wave techniques. As shown in Fig. 3a, SWV for the OPPy-GGH NW electrode reveals a distinctive peak at 230 mV associated with oxidization of Cu^0/Cu^{2+} (Heitzmann et al., 2007; Lin et al., 2009a). The peak current from SWV is proportional to the amount of copper ions bound to the OPPy-GGH NW electrode surface. The calibration curve (Fig. 3b inset) shows a linear increase in current density with the Cu^{2+} concentration from 20 to 300 nM ($R^2 = 0.988$), illustrating a high sensitivity of the OPPy-GGH NW electrode to Cu^{2+}.

The reproducibility of the biosensor was found to be excellent as evidenced by a relative standard deviation of 3.9% from six separate measurements. After each measurement, the regeneration of electrodes was performed by soaking the used electrode in 100 mM EDTA solution. The regenerated OPPy-GGH electrode retained 98% of its initial sensitivity, indicating that the regenerated electrode can be used for repeated Cu^{2+} detection in a reproducible manner. Each regenerated electrode was confirmed to be used for determination of copper ions without significant loss of sensitivity for 2 months.

The selectivity of the OPPy-GGH NW electrode was investigated in the presence of common metal ion interferences, such as Ni^{2+} and Cr^{3+}. The OPPy-GGH NW electrode has a preferential high affinity toward Cu^{2+} and is nearly insensitive to Cr^{3+} and Ni^{2+} (Fig. 3S), which is in good agreement with our previous results (Lin et al., 2009a).

Moreover, the OPPy-GGH NW electrode exhibits a three-dimensional structure with a large specific surface area and a large number of micropores dispersed in the networked NW (Özcan et al., 2008), thereby providing more sites for ligand functionalization and Cu^{2+} capture. A large number tripeptide molecules immobilized into the NW resulted in the enhanced sensitivity of the OPPy-GGH NW electrode. This is best illustrated in Fig. 3c. The electrochemical response of OPPy-GGH NW electrode for 100 nM Cu^{2+} (Fig. 3c dash line) is significantly higher than that of the OPPy-GGH film electrode (Fig. 3c solid line) for the same Cu^{2+} concentration. The superiority of the NW scaffolded biosensor, in the context of more efficient capturing of Cu^{2+} through the networked polymer–peptide complex, is further highlighted when its electrochemical response for 100 nM Cu^{2+} is compared with that of the film scaffolded biosensor for 300 nM Cu^{2+} (Fig. 3c dash–solid line). Taken together, the demonstrated peptide functionalized OPPy-COOH NW is expected to provide an excellent platform for the construction of highly sensitive, accurate and reproducible biosensors capable of detecting various target compounds whose detection is otherwise difficult.

4. Conclusions

A novel electrochemical nano-biosensor based on carboxy end-capped overoxidized PPy NW electrode modified with the use of Cu^{2+} cognitive GGH tripeptide as a functional probe was constructed. The OPPy-GGH NWs were anchored to gold surface without any chemical linker in order to facilitate efficient electrical contact, and the peptide molecules were covalently bound to OPPy-COOH NW electrode without any prior surface treatment. This simple strategy is highly conducive to solving one of the major challenges in electrochemical sensor design (i.e. immobilization of nanomaterials onto an electrode substrate for high-quality contact), providing a new route to fabrication of electrochemical sensors based on polymer nanomaterials. The performance of the OPPy-GGH NW electrodes, demonstrated for Cu^{2+} detection by square wave voltammetry in comparison with the conventional film type equivalent, proved that the developed sensor is highly sensitive to detect Cu^{2+} in the range of 20–300 nM reproducibly. The OPPy-GGH NW electrodes are also very stable and reusable with regeneration through a simple wash in EDTA solution.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.06.030.

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