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Facile fabrication of polyaniline nanotubes/gold hybrid nanostructures as substrate materials for biosensors

Shiyong Bao [b], Mingliang Du [a,b,*], Ming Zhang [a,b], Han Zhu [b], Pan Wang [b], Tingting Yang [b], Meiling Zou [b]

[a] Key Laboratory of Advanced Textile Materials and Manufacturing Technology, Zhejiang Sci-Tech University, Ministry of Education, Hangzhou 310018, PR China
[b] Department of Materials Engineering, College of Materials and Textiles, Zhejiang Sci-Tech University, Hangzhou 310018, PR China

HIGHLIGHTS

- H_2O_2 detection biosensor using PANI nanotubes/Au nanostructures was fabricated.
- *In situ* reduction and electrospinning technique were utilized.
- The biosensors exhibit fast response, broad linear range and low detection limit.

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ABSTRACT

Polyaniline (PANI) nanotubes/Au hybrid nanostructures with well-dispersed and tunable densities of Au nanoparticles (AuNPs) were fabricated through a novel, simple and green approach using electrospun polyacrylonitrile (PAN) nanofibers as sacrificial temples. Potential applications of the as-prepared PANI nanotubes/Au hybrid nanostructures as biosensor substrate materials were demonstrated through experimental studies. Transmission electron microscopy (TEM), field emission scanning electron microscopy (FE-SEM), Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and X-ray photoelectron spectrometer (XPS) were employed to study the morphology and crystal structure of the novel nanostructures. Hollow nanotubular structures were shown to readily facilitate ion diffusion and improve the electronic response performance of the PANI nanotubes/Au hybrid nanostructures. The HRP–PANI nanotubes/Au hybrid nanostructures embedded with horseradish peroxidase (HRP) by immobilization methods were used as biosensor substrate materials for H_2O_2 detection. The HRP–PANI nanotubes/Au hybrid nanostructure biosensors were highly sensitive with a detection limit of 0.25 μM and signal-to-noise ratio (S/N) of 3.

1. Introduction

Following the recent development of more advanced fabrication technologies, the electrodes can now be produced at the nanoscale [1]. Compared to conventional electrodes, nanoelectrodes possess several beneficial features, such as higher spatial resolution, and low voltammetric time scales and background noise [2]. Polyaniline (PANI), a common conducting polymer, was considered one of the most promising nanomaterials due to unique properties, including superior electronic properties, low cost, excellent environmental stability, and high reactivity through oxidation and protonation reactions [3–5]. Recently, various morphologies of PANI was used as the base material in the fabrication of nanoelectrodes for target detection. For example, Viktor Mazeiko et al. have identified GO_X/Au-NPs/PANI nanocomposite and found that this novel nanostructure increased the amperometric signal more significantly than GO_X/PANI [6]. Zhang' group has designed a PANI/Au nanocomposite modified nanoelectrode which displays high electrocatalytic activity toward dopamine over a wide linear range (200–0.3 μM) and with a detection limit of 0.1 μM [1]. Weng et al. prepared gold decorated SiO_2@polyaniline core–shell microspheres as sensors for ascorbic acid [7]. Xu et al. demonstrated that graphene/polyaniline/AuNPs could be fabricated into modified nanoelectrodes in order to observe direct electron transfer of glucose oxidase and glucose. These electrodes had a detection limit of 0.6 μM and S/N of 3 [8]. Despite improvements in sensitivity, the electrochemical performance of the PANI was unsatisfactory

* Corresponding author at: Key Laboratory of Advanced Textile Materials and Manufacturing Technology, Zhejiang Sci-Tech University, Ministry of Education, Hangzhou 310018, PR China.
E-mail address: du@zstu.edu.cn (M. Du).

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under high mass loading due to the decreased accessible surface area available for participating in the electron transfer process [9]. Thus, the development of novel nanostructured PANI with high specific surface area and high ion diffusion is of importance in the field of electrochemical signaling. Chen et al. fabricated PANI nanotubes through a one-step *in situ* chemical polymerization process utilizing MnO_2 nanotubes as sacrificial templates. The resulting PANI nanotube pseudocapacitors exhibited significantly improved electrochemical performance compared with that of PANI nanofiber pseudocapacitors in both acidic aqueous and ionic liquid electrolytes [10]. Miao et al. used the PAA nanofibers as templates to generate PANI nanotubes as base materials for high performance supercapacitors, which demonstrated superior electrochemical performance during charge–discharge processes [11]. Meanwhile, AuNPs are of particular interest in analytical and bioanalytical applications, because of their unique physical and chemical properties. Jena et al. developed a AuNPs-based nanostructured electrode that displayed high sensitivity and selective electrochemical detection toward sub-ppb levels of chromium (VI). The detection limit and S/N were 0.1 ppb and 9, respectively [12]. Willner et al. fabricated a versatile biosensor using AuNPs which showed high sensitivity for detection of Thrombin [13,14]. Hydrogen peroxide is a vital strong oxidant, and it is known to take part in several fields such as medical disinfectant and chemical analysis. However, some studies have demonstrated that the excess H_2O_2 in the environment could be harmful to human health. From the recent literature, enzyme modified electrodes for detection of H_2O_2 have been frequently applied due to their advantages such as low operating expense and short analytical time.

Inspired by prior research, the current study involves the fabrication of PANI nanotubes through the dissolution of PAN by *N*,*N*-dimethyl acetamide (DMAc). In order to enhance the recognition sensitivity of the biosensor toward H_2O_2, AuNPs were introduced to the surface of PANI nanotubes through reducting reactions and cheating effects [15–17]. This preparation of PAN nanofibers through electrospinning is more flexible and controllable, when compared with previously reported processes [18]. In addition, the electrospun fibers obtained using the new process possessing high surface area to volume ratio and high porosity, which may allow such fibers play a significant role in the fabrication of various hierarchical nanostructures for sensors, catalysts, and energy storage applications [19–23]. Combined with the advantages of the novel structure and the electrospinning progress, the HRP–PANI nanotubes/Au hybrid nanostructures for H_2O_2 detection exhibit significant advantages such as fast response, high sensitivity and low background noise. However, these immobilization methods for introducing HRP to PANI nanotubes/Au hybrid nanostructures allow leakage of enzyme, resulting in a low stability of the electrode. On the whole, these PANI nanotubes/Au hybrid nanostructures are expected to be superior modified material of glassy carbon electrode (GCE) for target detection.

2. Experimental

2.1. Materials

Perchloric acid ($HClO_4$), aniline, hydrogen peroxide (H_2O_2), hydroquinone (HQ), phosphate buffer (PB), horseradish peroxidase (HRP), polyacrylonitrile (PAN), *N*,*N*-dimethylacetamide (DMAc), dimethyl formamide (DMF), and thioglycolic acid (TA) were acquired from Aladdin Chemistry Co., Ltd. Chloroauric acid ($HAuCl_4{\cdot}4H_2O$, 99.9%) and ammonium persulfate (APS) were commercially obtained from Shanghai Civi Chemical Technology Co., Ltd. All reagents were used without further purification. Deionized water (DIW, 18.2 MΩ) was used for solution preparation.

2.2. Preparation of electrospun PAN nanofibers

The precursor of the PAN nanofibers was prepared by dissolving the PAN powder in DMF under strong stirring. This method produced a PAN solution of 12 wt.%. The PAN solution was electrospun at speed of 0.6 mL/h with a distance of 20 cm between the tip of the needle and collection plate. The voltage applied was 15–18 kV.

2.3. Preparation of the hollow PANI nanotubes

PAN/PANI nanofibers were produced via *in situ* polymerization of aniline in the presence of PAN nanofibers. A typical preparation process began by adding 30 mg of electrospun PAN nanofibers into a solution containing 0.01 mol aniline, followed by the dropwise addition of 4 mL $HClO_4$ under gentle stirring. Then, 1 mL ethanol was added to prevent the solution from freezing at low-temperature and a 30 mL solution contain 0.04 mol APS was injected into the flask to initiate the polymerization. The above mixture was shaken at 250 rpm and maintained under a nitrogen atmosphere in an ice-water bath at 4 °C. After approximately 7 h, the PAN/PANI nanofibers were separated from the solution by filtration with deionized water and then submerged in a DMAc solution in order to remove the PAN section. The resulting solution was washed repeatedly with deionized water and ethanol using suction filtration and then vacuum dried at 50 °C for 12 h to acquire hollow PANI nanotubes.

2.4. Preparation of PANI nanotubes/Au hybrid nanostructures

PANI nanotubes/Au hybrid nanostructures were synthesized as follows. The as-prepared hollow PANI nanotubes were redispersed in the 0.1 mol $NH_3{\cdot}H_2O$ to eliminate the doping effect and then doped into 20 mL of 1.0 M TA solution. The reaction mixture was then centrifuged and redispersed in deionized water, and kept under stirring for 1 h. A 3 mL aliquot of 10 mM $HAuCl_4$ was added into the mixture of TA doped PANI nanotubes and maintained under gentle stirring for 24 h. The solution was then washed repeatedly with deionized water and ethanol using suction filtration and vacuum dried at 50 °C for 12 h to acquire PANI nanotubes/Au hybrid nanostructures.

2.5. Biosensing experiments

The electrocatalytic activity of the PANI nanotubes/Au hybrid nanostructures for the detection of H_2O_2 was evaluated using cyclic voltammetry (CV) and amperometry. In our work, the HRP/(PANI nanotubes/Au)/GCE was used as the working electrode. The GC electrode was polished carefully with 1.0, 0.3 and 0.05 μm alumina powder and washed with DIW and ethanol before the surface coating. Water stable PANI nanotubes/Au hybrid nanostructures were incubated with 1 mg/mL HRP solution (1 mL) at 4 °C overnight in a humidity chamber. Appropriate amount of HRP/PANI nanotubes/Au hybrid nanostructures were mixed with nafion (0.2%), respectively, and then were coated on the surface of the GCE. After that, the electrodes were allowed to dry under nitrogen for 1 h. A Pt foil and an Ag/AgCl electrode were used as the counter and reference electrodes, respectively. The CV measurements were performed in 0.1 M PBS (pH = 6.8) at a corresponding scan rate and voltage. Amperometric measurements were conducted in a gently stirred solution at an applied potential of −0.25 V.

2.6. Characterizations

Fourier transform infrared (FTIR) spectra of the synthesized PAN nanofibers, PAN/PANI nanotubes, and PANI nanotubes/Au