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



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HIGHLIGHTS

• H₂O₂ 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.

A R T I C L E I N F O

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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 H₂O₂ detection. The HRP–PANI and signal-to-noise ratio (S/N) of 3.

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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 $(200-0.3 \,\mu\text{M})$ and with a detection limit of $0.1 \,\mu\text{M}$ [1]. Weng et al. prepared gold decorated SiO₂@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

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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 MnO₂ 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 H₂O₂ 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 H₂O₂, 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 H₂O₂ 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 (HClO₄), aniline, hydrogen peroxide (H₂O₂), 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 (HAuCl₄·4H₂O, 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 HClO₄ 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 · 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 HAuCl₄ 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 H₂O₂ 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 μ m 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



Fig. 1. Schematic illustration of the achievement of PANI nanotubes/Au hybrid nanostructures.



Fig. 2. FE-SEM of (a) the PAN nanofibers, (b) PAN/PANI nanofibers, (c, d) hollow PANI nanotubes and (e, f) PANI nanotubes/Au hybrid nanostructures; (inset in (f)) EDS of PANI nanotubes/Au hybrid nanostructures.

hybrid nanostructures were recorded on a Nicolet 5700 FTIR spectrometer in transmittance mode at a resolution of 4 cm⁻¹ and 32 scans. X-ray diffraction (XRD) patterns of the hollow PANI nanotubes and PANI nanotubes/Au hybrid nanostructures were characterized with a SIEMENS Diffraktometer D5000 X-ray diffractometer using Cu Ka radiation source at 35 kV and a scan rate of $0.02^{\circ} \ 2\theta \ s^{-1}$ in the 2θ range of 5–80°. Field emission scanning electron microscopy (FE-SEM) images of the synthesized PAN/PANI nanofibers, hollow PANI nanotubes, and PANI nanotubes/Au hybrid nanostructures were obtained using a ISM-6700F field-emission scanning electron microscope (JEOL, Japan). Transmission electron microscopy (TEM) was performed on a JEOL JEM-2100 instrument operating at 200 kV. X-ray photoelectron spectra of PANI nanotubes and PANI nanotubes/Au hybrid nanostructures were recorded using an X-ray photoelectron spectrometer (XPS) (Kratos Axis Ultra DLD) with an aluminum (mono) Ka source (1486.6 eV). The aluminum Ka source was operated at 15 kV and 10 mA. Cvclic voltammograms were recorded using a computer controlled CHI660E electrochemical workstation (CHI, Shanghai Chenhua, Shanghai).

3. Results and discussion

As shown in Fig. 1, the PAN/PANI nanofibers were chemically synthesized using *in situ* chemical polymerization in the presence of aniline as the monomer, APS as oxidant, and $HClO_4$ as dopant.

Typically, the aniline monomer would attach to the PAN nanofibers due to the high surface area to volume ratio and high porosity of electrospun nanofiber. After modification of TA, a large number of –SH groups were loaded onto the surface of the PANI nanotubes. AuNPs were gradually reduced from HAuCl₄ with the assistance of PANI and uniformly bound the nanotubes between –SH and AuNPs. This process is illustrated in the following figure.

The surface morphology of pure PAN nanofibers, PAN/PANI nanofibers, hollow PANI nanotubes, and PANI nanotubes/Au hybrid nanostructures were explored through SEM. The SEM images in Fig. 2 show the evolution in morphology during the preparation of PANI nanotubes/Au hybrid nanostructures. As shown in Fig. 2a, the diameter of as-prepared PAN nanofibers is uniform with an average size of about 400 nm. As illustrated in Fig. 2b. the PAN/PANI nanofibers were formed by coating the PANI onto the nanofibers using an *in situ* polymerization process. After a thin laver of PANI coating was applied, the surface of nanofibers became rough and the overall diameter increased in comparison to pure PAN nanofibers (Fig. 2a). As shown in Fig. 2c and d, the PAN nanofibers were removed by DMAc solution to obtain PANI nanotubes. The hollow nanotubular structures are shown in greater detail in Fig. 3c and d. As illustrated in Fig. 2e and f, the morphology of the PANI nanotubes/Au hybrid nanostructures is significantly different with regards to surface roughness and diameter compared to the as-prepared hollow PANI nanotubes. Well-dispersed and dense AuNPs were loaded onto hollow PANI nanotubes by the binding force between -SH and AuNPs. As shown



Fig. 3. TEM images of (a, b) the PAN/PANI nanofibers, (c, d) hollow PANI nanotubes and (e, f) PANI nanotubes/Au hybrid nanostructures; (inset in (e)) selected area electron diffraction (SAED) pattern images of the AuNPs; (inset in (f)) HRTEM images of the small size AuNPs.

in the inset of Fig. 2f, EDS spectrum revealed the presence of C, O and Au on the PANI nanotubes/Au hybrid nanostructures.

TEM measurements of PAN/PANI nanofibers. hollow PANI nanotubes and PANI nanotubes/Au hybrid nanostructures are shown in Fig. 3. Images in Fig. 3a and b clearly show that the PAN core is fully enclosed by conducting PANI layer. The average thickness of the PANI coating shell was determined to be approximately 120 nm (Fig. 3c). As shown in Fig. 3c and d, the hollow PANI nanotubes have a nearly identical diameter to that of the PAN/PANI nanofibers and display a typical hollow nanotubular structure. As shown in Fig. 3e and f, PANI nanotubes/Au hybrid nanostructures with a well-defined nanotubular structure were successfully fabricated with well-dispersed AuNPs of small size loaded onto surface. The SAED pattern of the PANI nanotube/Au hybrid nanostructures is shown in the inset of Fig. 3e and reveals polycrystal rings indexed to the (200), (220), (111) and (311) planes of face-centered cubic (fcc) gold, illustrating the polycrystallinity of the AuNPs [24,25].

As shown in Fig. 4, an obvious transformation of the size and morphology of AuNPs on the PANI nanotubes/Au hybrid nanostructures occurs upon an increase in HAuCl₄ concentration, as demonstrated by SEM and TEM images. At a low concentration of HAuCl₄ (0.1 mM), the rough PANI nanotubes were covered by AuNPs with diameter of 1.18 ± 0.3 nm (Fig. 4a, e and i). As the amount of HAuCl₄ was increased, the density of the AuNPs increased sharply throughout the PANI nanotubes (Fig. 4b and c). When the concentration of HAuCl₄ was increased to 0.5 mM, the PANI nanotubes were completely enclosed by AuNPs with diameter of 15.6 ± 3.16 nm (Fig. 41) and continuous PANI nanotubes/Au hybrid nanostructures were obtained (Fig. 4d).

In addition, TEM was used to explore the evolution in morphology as a function of HAuCl₄ concentration. As shown in Fig. 4e, at the concentration of 0.1 mM, individual AuNPs with diameter of 1.18 ± 0.3 nm can be seen on the PANI nanotubes (inset of Fig. 4e). As concentration increased further from 0.25 mM to 0.4 mM, the diameter of AuNPs loaded on the surface of PANI nanotubes gradually increased from 4.46 ± 0.73 nm to 12.7 ± 1.76 nm (Fig. 4j and k). When the concentration of HAuCl₄ was increased to 0.5 mM, AuNPs with diameter of 15.6 ± 3.16 nm (Fig. 4] aggregated (Fig. 4h). HRTEM images of the AuNPs reveal distinct



Fig. 4. SEM (a-d) and TEM (e-h) images of PANI nanotubes/Au hybrid nanostructures with different concentrations of HAuCl₄ (0.1 mM, 0.25 mM, 0.4 mM and 0.5 mM, respectively); (i-l) the corresponding particle size distribution of AuNPs; HRTEM images (inset in (f)) of the small size AuNPs.



Fig. 5. STEM analysis of the PANI nanotubes/Au hybrid nanostructures. HAADF-STEM images, high resolution element mappings taken on the PANI nanotubes/Au hybrid nanostructures.

lattice fringes with d spacings of 0.23 nm, which correspond to the (111) lattice planes of fcc gold [26,27].

A HAADF-STEM image of the PANI nanotubes/Au hybrid nanostructures is shown in Fig. 5. STEM-EDX mapping of Au and S indicated the AuNPs were successfully loaded on the surface of PANI nanotubes. In the mapping of N (Fig. 5b), the densely signal area is assigned to the PANI structure.

Typical FTIR spectra of pure PAN nanofibers, hollow PANI nanotubes, and PANI nanotubes/Au hybrid nanostructures are shown in Fig. 6a. In comparison to pure PAN, characteristic peaks of hollow PANI nanotubes appear at 1586, 1496, 1301 and 1136 cm⁻¹, and are attributed to the stretching vibration of the C=C of benzenoid and quinoid rings, the C–N stretching vibration with aromatic conjugation, and the characteristic of A = NH⁺-B, where A and B denote quinoid ring and benzene ring, respectively [28–30]. These observations demonstrate that the PAN nanofibers have been removed completely by DMAc and shown the formation of PANI nanotubes. In addition, PANI nanotubes/Au hybrid nanostructures exhibit a higher intensity ratio of 1586/1496 cm⁻¹ compared with pure hollow PANI, and contain more quinoid rings, which correspond to the oxidation–reduction reaction occurred between PANI and HAuCl₄ [31,32].

To further confirm the presence of AuNPs and analyze the crystal phase structures, the hollow PANI nanotubes and PANI

nanotubes/Au hybrid nanostructures were characterized by XRD. As shown in Fig. 6b, diffraction peaks at $2\theta = 15.3^{\circ}$ are the characteristic repeating units, while $2\theta = 25.9^{\circ}$ is attributed to the periodicity parallel and perpendicular to hollow PANI nanotubes [33–36]. The XRD pattern of PANI nanotubes/Au hybrid nanostructures shows the presence of four additional peaks at 38.01°, 43.96°, 64.50° and 77.42° representing Bragg reflection from (111), (200), (220) and (311) planes of AuNPs respectively, indicating the existence of AuNPs in the PANI nanotubes/Au hybrid nanostructures [24,27]. Moreover, the size of AuNPs was calculated using Scherrer equation and the diameter was determined as about 14.7 nm.

In order to verify the formation of zero-valent gold, XPS measurements were conducted on the hollow PANI nanotubes and PANI nanotubes/Au hybrid nanostructures and results are shown in Fig. 7. As shown in more detail in Fig. 7a, Au 4f peaks at binding energies of 87.6 eV and 83.9 eV are observed. These peaks can be attributed to the binding energies of Au $4f_{7/2}$ and Au $4f_{5/2}$ doublets, respectively [37]. Furthermore, the two S 2p spin–orbit coupled doublets binding energy located at 162.8 eV and 163.8 eV (Fig. 7b) correspond to the binding energy of S $2p_{3/2}$ and $2p_{1/2}$ and indicate the presence of thiolate species on PANI nanotubes. As shown in Fig. 7c, the N 1s core-level spectrum of PANI (after dedoping with NH₃·H₂O) was detected at binding



Fig. 6. (a) FTIR spectra of pure PAN nanofibers, hollow PANI nanotubes, PANI nanotubes/Au hybrid nanostructures and (b) XRD patterns of the PANI nanotubes and PANI nanotubes/Au hybrid nanostructures.



Fig. 7. (a) Au 4f and (b) S 2p XPS spectra of the PANI nanotubes/Au hybrid nanostructures; (c) N 1s XPS spectra of PANI nanotubes (after dedoping by NH₃·H₂O) and (d) the PANI nanotubes/Au hybrid nanostructures.

energies of 397.3 eV and 399.5 eV, which belong to the quinonoid imine (=N—) and benzenoid amine (-NH–), respectively [38]. For the redox reaction between PANI and HAuCl₄, the relative intensity of the quinonoid imine (=N—) is stronger than the benzenoid amine (-NH-) in PANI nanotubes/Au hybrid nanostructures, in agreement with FTIR spectra.

As discussed previously, nearly homogeneously distributed PANI nanotubes/Au hybrid nanostructures were fabricated via a simple and novel route. The nanostructures were then used as biosensor substrate materials for H_2O_2 detection in conjuction with

HRP. The electrocatalysis of the sensors in H_2O_2 solutions (10 mM) with different modified electrodes in PBS solution (pH 6.8) is shown in Fig. 8. Cyclic voltammograms (CVs) of PANI and PANI nanotubes/Au hybrid nanostructures modified GCE with and without H_2O_2 in the presence of hydroquinone (HQ) are shown in Fig. 8A. In Fig. 8A, curve "a" and curve "b" belong to the CVs of PANI and PANI nanotubes/Au hybrid nanostructures, respectively, without H_2O_2 in 5 mM HQ. Many weak redox peaks can be observed. With the addition of H_2O_2 , the PANI/GCE nanoelectrodes exhibit a reduction in current (curve c in Fig. 8A). For PANI



Fig. 8. (A) CVs of pure PANI (a) and PANI nanotubes/Au hybrid nanostructures functionalized GCE with 5.0 mM HQ in 0.1 M PBS (pH 6.8) with (c, d) or without 10 mM H_2O_2 (a, b), and (B) CVs of PANI/Au/GCE in 10 mM H_2O_2 with different scan rates, and (C) amperometric response of the fabricated PANI nanotubes/Au hybrid nanostructures biosensor to successive addition of H_2O_2 into the stirred PBS solution containing 1.0 mM HQ, the applied potential was -0.25 V vs. Ag/AgCl, and (D) calibration curve and linear fitting curve between the current and the H_2O_2 concentration.



Fig. 9. (A) Cyclic voltammograms of 5.0 mM HQ in PBS (PH = 6.8) at different concentrations of AuNPs in the presence of 10 mM H₂O₂; 50 mV s⁻¹: (a) 0.1 mM, (b) 0.25 mM, (c) 0.4 mM and (d) 0.5 mM, and (B) The CVs cycles of HRP/PANI nanotubes/Au hybrid/GCE in the presence of 10 mM H₂O₂ in the same conditions at the scan rate of 100 mV s⁻¹.

Table 1

Summary of the detection range and detection limit of as-prepared biosensors and the biosensors in reference.

Biosensors	Detection range (µM)	Detection limit (µM)	Refs.
HRP/PANI nanotubes/ Au hybrid nanostructures	1–605	0.25	This work
HRP/Au/PVA nanofibers	1-500	0.5	[39]
HRP/Fe ₃ O ₄ /silica	2-24	0.43	[40]
HRP/TiO ₂ /Au nanoparticles	5-400	2	[41]
Ti/TiO ₂ /Au/HRP/GCE	4-400	2	[42]
Au-SPAN/HRP/GCE	10-2000	1.6	[43]

nanotubes/Au hybrid nanostructures, the reduction current caused by HQ increased significantly (-0.25 V) accompanied with disappearance of the oxidation current peak (curve d). This observation suggests a strong reaction between H₂O₂, HRP, and HQ [39]. A series of CV curves at different scan rates ranging from 20 mV s⁻¹ to 200 mV s⁻¹ with similar shapes and increases in current density are shown in Fig. 8B and demonstrate the excellent electrochemical activity of the PANI nanotubes/Au hybrid nanostructure functionalized electrodes for detection of H₂O₂.

In response to the suitable reduction in current produced by HQ with H₂O₂, the PANI nanotubes/Au hybrid nanostructures were used as a substrate material to determine the concentration of H_2O_2 at a working potential of -0.25 V. The amperometry response of current vs. concentration of H₂O₂ is shown in Fig. 8C. A stepwise increase in the amperometric reduction current with the addition of H₂O₂ was observed. Amperometry results indicate that PANI nanotubes/Au hybrid nanostructures electrode exhibit high sensitivity and are capable of detecting $1 \ \mu M \ H_2O_2$ (inset picture of Fig. 8C). These results demonstrate that the PANI nanotubes/ Au hybrid nanostructures rapidly respond to changes in H₂O₂ concentration due to fast diffusion of H₂O₂ into the PANI nanotube structures. As shown in the Fig. 8D, the current response changes linearly with H₂O₂ concentrations with a correlation coefficient of 0.996. A detection limit of 0.25 μ M was estimated at a signalto-noise ratio of 3.

In order to analyze the relationship between the electrochemical behavior of HRP–PANI nanotubes/Au hybrid nanostructures and the density of AuNPs, cyclic voltammograms of the modified nanoelectrodes were produced with concentrations of HAuCl₄ ranging from 0.1 mM to 0.5 mM in the presence of 10.0 mM H₂O₂. As demonstrated in Fig. 9A, clearly change was observed through adjusting the concentration of HAuCl₄. As shown in Fig. 9A(a), the reduction in current caused by the addition of HQ was 80 μ A at a AuNPs concentration of 0.1 mM. Upon an increase in the concentration of AuNPs, a greater increase at reduction current to 90 μ A and 110 μ A was observed indicating that a lower concentration of AuNPs provides less efficient electron transfer in the PANI nanotubes/Au hybrid nanostructures, resulting in a less sensitive response to H₂O₂. However, when the concentration of AuNPs was increased to 0.5 mM, the current decreased to 60 µA. This phenomenon is attributed to the decrease in the number of active sites on the surface of PANI nanotubes/Au hybrid nanostructures caused by the agglomeration of AuNPs. Considering the excellent reduction current (110 μ A) caused by HQ in the presence of H₂O₂, the HRP-PANI nanotubes/Au hybrid nanostructures of 0.4 mM HAuCl₄ were selected as the optimized substrate material to determine the concentration of H₂O₂. Fig. 9B shows the CVs cycles of HRP/PANI nanotubes/Au hybrid/GCE in the presence of 10 mM H_2O_2 in the same conditions at the scan rate of 100 mV s⁻¹. As shown in the figure, the CVs were almost the same and didn't shown distinct change. Consequently, we could draw the conclusion that the HRP/PANI nanotubes/Au hybrid nanostructures/GCE revealed good reproducibility in the H₂O₂ detection.

To further demonstrate the advantage of the PANI nanotubes/ Au hybrid nanostructures compared to the previously reported biosensor, a comparison of detection range and detection limit of different H_2O_2 detection material was summarized in Table 1. As shown in the table, it can be found that the present PANI nanotubes/Au hybrid nanostructures exhibit optimum detection rang and detection limit, demonstrating potential efficient electrode material for detection toward low concentration H_2O_2 .

4. Conclusion

PANI nanotubes/Au hybrid nanostructures were successfully obtained through a novel process using PAN nanofibers as a template, $HClO_4$ as the dopant, and TA as stabilizer. The resulting structures readily facilitate ion diffusion and are able to more efficiently detect the electron signals. HRP biosensors were used in conjunction with the PANI nanotubes and provided a highly sensitive and accurate platform for the detection of H_2O_2 with a detection limit of 0.25 μ M.

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