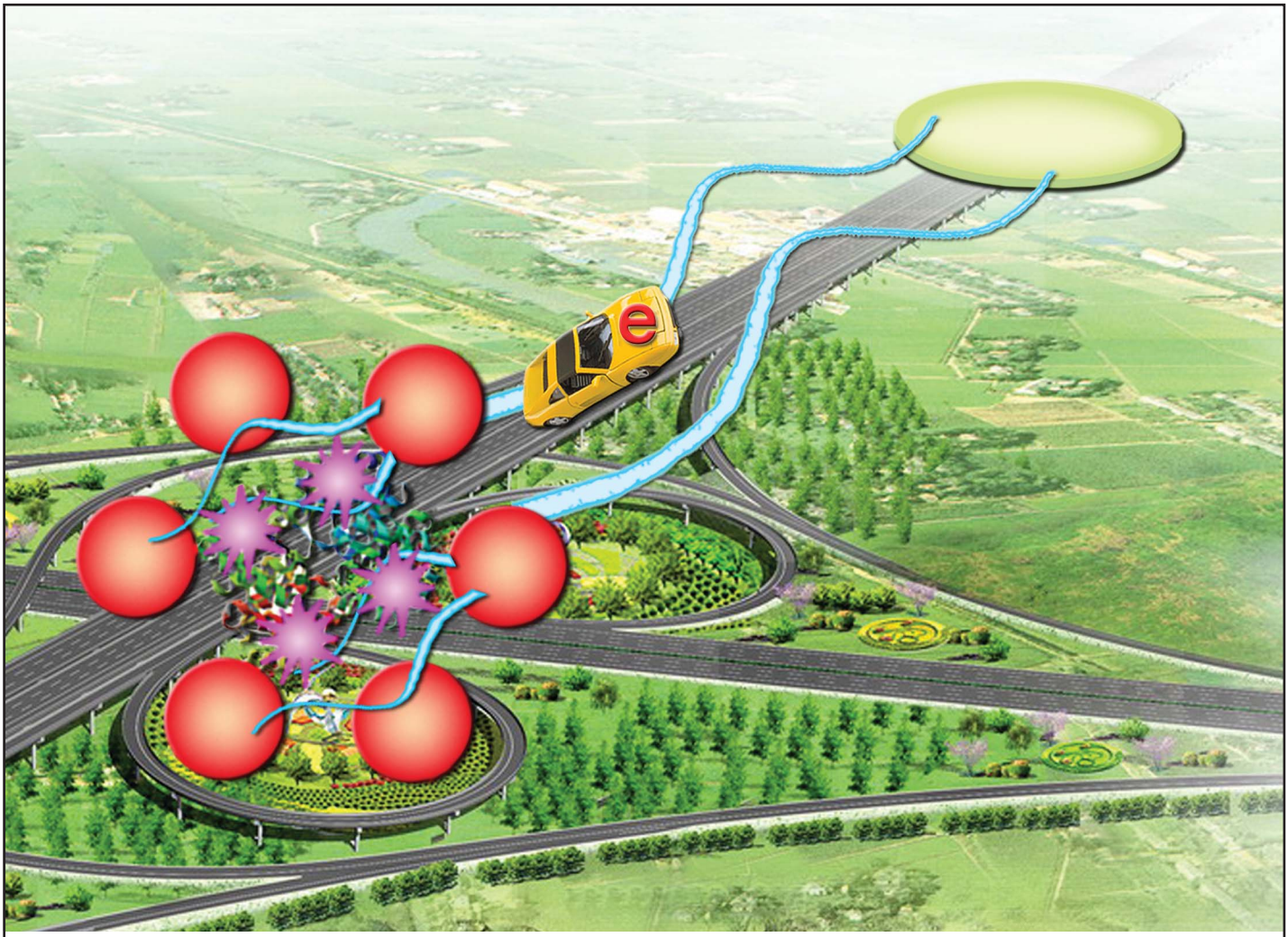

Highlighting research results from the State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, P. R. China.

Title: Fabrication of PEDOT nanowhiskers for electrical connection of the hemoglobin active center for H_2O_2 electrochemical biosensing

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Fabrication of PEDOT nanowhiskers for electrical connection of the hemoglobin active center for H_2O_2 electrochemical biosensing†

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Poly(3,4-ethylenedioxythiophene) (PEDOT) nanowhiskers were fabricated in a [bmim][BF_4] ionic liquid. The minimal size of the nanowhiskers is only 0.2 nm, and the size matches the thickness of a PEDOT single-molecular chain. They were successfully used as the electron transfer channels between the active center of hemoglobin (Hb) and an underlying electrode. The direct electron transfer process between Hb and the underlying electrode was realized without any electron mediator. Compared to that of a gold nanoparticle (AuNP)–Hb composite electrode, the catalytic current of a PEDOT nanowhiskers–AuNPs–Hb composite electrode for detecting H_2O_2 is increased 7 fold. A novel model of a H_2O_2 biosensor based on the PEDOT nanowhiskers–AuNPs–Hb composites was fabricated. The detection limit was estimated to be 0.6 μM at a signal/noise (S/N) ratio of 3, and the linear range of H_2O_2 concentration was from 1 μM to 1100 μM. Three constructed models of the biosensors showed a good stability, and all of them retained nearly 90% of their initial signals for 1 mM H_2O_2 when they were stored at 4 °C after 60 days. H_2O_2 concentration in contact lens nursing liquid was measured by the biosensor, and the results were in good agreement with the values provided by the supplier. It is shown that the PEDOT nanowhiskers can provide a new opportunity for the design of sensitive biosensors with long-term stability.

1 Introduction

Nanostructures of one-dimensional (1D) conducting polymers have attracted great attention due to their unique electronic, optical and chemical properties.[1] Poly(3,4-ethylenedioxythiophene) (PEDOT), a conducting polymer, has been recognized as one of the most promising candidates for applications in electrochemical devices due to its remarkable conductivity and stability.[1b,2] The past decade has witnessed rapidly increasing interest in the controlled fabrication of 1D PEDOT nanowires,[1,2d,2h,3] since 1D PEDOT nanostructures exhibit better conductivity than PEDOT films based on the enhanced electron transfer in the 1D nanoconfined structures.[3a] PEDOT nanowires have been applied in various fields, such as energy storage,[2d,3c,d] chemical sensors[3e] and electrochemical devices.[3f] Recently, PEDOT nanowires have also been used to construct versatile biomolecular sensing platforms. For example, PEDOT nanowires can electrically connect an enzyme's active center with an electrode, thus permitting observation of electron transfer in the enzyme to enable label-free protein detection.[1b] The diameters of the reported PEDOT nanowires are generally larger than 10 nm.[1a,3a,e,f] Further minimization of the PEDOT nanowires' diameter to the sub-nm size regime, where each nanowire becomes a molecular bridge that may promote electron transfer between an enzyme's active center and an electrode more efficiently, remains a major challenge.

Herein, we fabricated PEDOT nanowhiskers in a [bmim]-[BF_4] ionic liquid. The minimal diameter of a single nanowhisker was only 0.2 nm, which matched the size of a PEDOT single molecular chain. These single molecular chains served as efficient bridges between the active center of hemoglobin (Hb) and an underlying electrode. The direct electron transfer between Hb and the underlying electrode was obviously observed. Due to more active centers of Hb for the reduction of H_2O_2, compared to that of the other AuNPs–Hb composite electrodes, the catalytic current of the PEDOT nanowhiskers–AuNPs–Hb composite electrode increased greatly. A novel H_2O_2 biosensor model based on the PEDOT nanowhiskers–AuNPs–Hb composites was fabricated, and successfully applied to measuring the H_2O_2 concentration in contact lens nursing liquid.

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2 Experimental

2.1 Chemicals

3,4-Ethylenedioxythiophene (EDOT) (>97%) was purchased from Bayer AG, Hb (from bovine blood, H2625) was ordered from Sigma, [bmim][BF_4] (>99%, the viscosity of the ionic liquid is 233 cP at 20 °C and the content of H_2O is less than 1%) was bought from the Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, $HAuCl_4 \cdot 4H_2O$ was purchased from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China), H_2O_2 (30%) was from the Nanjing reagent Co. Ltd. (Nanjing, China). All the other reagents were analytical-grade and used without further treatment. All aqueous solutions were prepared using ultrapure water (18 MΩ) from a Milli-Q system (Millipore).

2.2 Material characterization

Zeta potential analysis was tested with a Zetasizer Nano-Z Zeta Potential Analyzer (Malvern). The FT-IR spectra of the samples were performed on a Bruker Vector 22 spectrophotometer. UV-Vis absorption spectra were recorded using a Shimadzu spectrophotometer (UV-3600). Atomic force microscope (AFM) images were obtained in tapping mode using an Agilent 5500. The electrochemical impedance experiment was performed by an AUTO LAB instrument (AUT 72782). Electrochemical measurements were performed on a CHI 660B workstation with a traditional three-electrode system, with a Pt wire electrode as the counter electrode, a saturated calomel electrode (SCE) as the reference electrode, and the unmodified and modified glassy carbon (GC) electrodes (d = 3 mm) as the working electrodes.

2.3 Preparation of AuNPs–Hb composite and PEDOT nanowhiskers–AuNPs–Hb composite electrodes

Fig. 1 illustrates the steps involved in the fabrication of the PEDOT nanowhiskers. The whole process can be divided into two sections: for fabrication of the AuNPs–Hb composites (Fig. S2 in the ESI† shows the relationship between the AuNPs loading and the reduction peak currents of AuNPs–Hb composites electrode), firstly, a solution of negatively charged AuNPs was fabricated according to a previously published procedure[4] (zeta potential = −30 mV, pH 6.0).[5] Then, a 10 mg mL^{-1} AuNPs–Hb composites solution was prepared by adding 5 mg of positively charged Hb (isoelectric point of 7.4)[6] to the 500 μL prepared AuNPs solution (0.243 mM) followed by vortexing the mixture for 5 min. When not in use, the solution was stored at 4 °C. The strategy for constructing a H_2O_2 biosensor model: prior to use, a GC electrode was carefully polished on a polishing cloth with 1.0, 0.3, and 0.05 μm alumina powders, respectively, then sonicated successively in ethanol and ultrapure water in turn. After that, 5 μL of the AuNPs–Hb composites was drop-cast onto the GC electrode surface, which was then left to dry at 4 °C. Finally, the AuNPs–Hb composites electrode was dipped into the [bmim][BF_4] solution (without adding H_2O or further treatment) containing 0.1 M 3,4-ethylenedioxythiophene (EDOT) for 0.5 h to enable both the EDOT monomer and the [bmim][BF_4] molecules to diffuse into Hb. The polymeric reaction was maintained at 1.0 V (*vs* Ag wire pseudo-reference electrode) until the consumed charge reached ~0.05 C at 37 °C. The produced PEDOT nanowhiskers were tethered to both the GC electrode and to the Hb active center, thus wiring the enzyme to the electrode. The modified electrode was then immersed in ultrapure water to remove any excess EDOT monomer. When the electrode was not in use, it was stored at 4 °C.

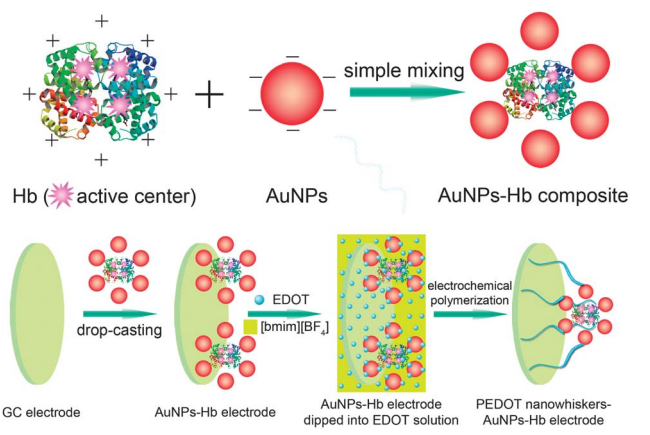

Fig. 1 Schematic illustration of the preparation of the PEDOT nanowhiskers–AuNPs–Hb composites.

2.4 Determination of H_2O_2 concentration in the contact lens nursing liquid with the biosensor model

The model of the electrochemical H_2O_2 biosensor was used to determine the level of H_2O_2 concentration in contact lens nursing liquid (AO Sept Plus, 3.5% H_2O_2 (wt %)) utilizing a standard addition method. Before the test, a calibration curve of the response current *versus* the H_2O_2 concentration was prepared by the amperometric current–time (I–t) technique. 5 mL of phosphate buffered saline (PBS) saturated with N_2 was selected as the testing solution. For the real sample, 1 μL of the contact lens nursing liquid was injected into the testing solution, and the H_2O_2 concentration in the testing solution was estimated to be about 205 μM.

3 Results and discussion

3.1 Characterization of the fabricated PEDOT nanowhiskers–AuNPs–Hb composites

UV-Vis spectra were used to determine the sizes of the AuNPs and the tertiary structure change around the heme region of Hb. Curve a in Fig. 2A shows that the characteristic absorption peak of the AuNPs was at 517 nm, which indicates that the size of the AuNPs was about 13 nm.[7] Curve b in Fig. 2A shows the Soret absorption band of native Hb in ultrapure water was at 405 nm, and curve c in Fig. 2A shows that after the formation of the AuNPs–Hb composites, the Soret band of Hb did not shift, thus indicating that the tertiary structure of Hb had not changed.[6,8]

The FT-IR spectrum of the PEDOT nanowhiskers–AuNPs–Hb composites was obtained as shown in Fig. 2B. The characteristic amide I and amide II bands of Hb provided detailed

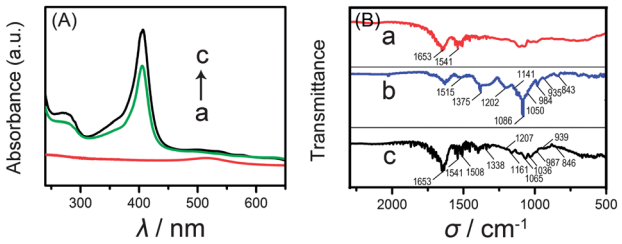

Fig. 2 (A) UV-Vis absorption spectra of (a) AuNPs, (b) native Hb and (c) AuNPs–Hb composites. (B) FT-IR spectra of (a) native Hb, (b) PEDOT nanowhiskers and (c) the PEDOT nanowhiskers–AuNPs–Hb composites.

information on the secondary structure of the polypeptide chain.[9] The amide I band (1700–1600 cm^{-1}) is attributed to the C=O stretching vibration of the peptide linkages in the backbone of Hb, while the amide II band (1620–1500 cm^{-1}) results from a combination of N–H bending and C–N stretching.[10] Curve a in Fig. 2B shows that the amide I and amide II bands of the native Hb are located at 1653 and 1541 cm^{-1}, respectively. Curve b in Fig. 2B shows the FT-IR spectrum of the PEDOT nanowhiskers. The absorption at 1515 and 1375 cm^{-1} are ascribed to C=C and C–C stretching vibrations in the quinoidal structure of the thiophene ring, respectively. The absorption at 1202, 1141, 1086 and 1050 cm^{-1} are due to the C–O–C bond stretching vibrations in the ethylene dioxy (alkylenedioxy) group. The absorption at 985, 935 and 843 cm^{-1} originate from C–S bond stretching vibrations in the thiophene ring.[1a,3a,11] Curve c in Fig. 2B displays that, after fabrication of the PEDOT nanowhiskers–AuNPs–Hb composites, the amide I and amide II spectral features are nearly the same as those of native Hb. Characteristic absorptions of the PEDOT nanowhiskers are also observed as shown in curve c in Fig. 2B. These results indicate that the secondary structure of Hb in the composites remained stable.

3.2 The morphology of AuNPs, AuNPs–Hb composites and the PEDOT nanowhiskers–AuNPs–Hb composites

AFM measurements were performed to observe the composites. Fig. 3A shows well-dispersed AuNPs on a mica substrate, with an average size of about 13 nm. Regularly distributed AuNPs–Hb composites with a diameter of about 40 nm are observed in Fig. 3B and C shows the as-prepared PEDOT nanowhiskers–AuNPs–Hb composites. The average diameter of each nanowhisker (as measured in the middle of the nanowhisker) was less than 5 nm. Fig. S1 in the ESI† further demonstrated that both hemoglobin and AuNPs were connected to the PEDOT nanowhiskers, respectively.

Fig. 4A shows the phase-flattened image of the as-prepared PEDOT nanowhiskers–AuNPs–Hb composites. Statistical results (Fig. 4A, inset) demonstrates that the minimal size of the PEDOT nanowhisker is only 0.2 nm, which is a size similar to the thickness of a PEDOT single molecular chain. Fig. 4B further reveals the profile of the PEDOT single molecular chain, where S atoms with a diameter of 0.2 nm[12] can be observed. The formation of PEDOT nanowhiskers for the electrical connection of the hemoglobin can be explained as follows: first, because the histidine structure in the Hb molecules and the [bmim][BF_4] molecules have the same imidazole structure, Hb can dissolve in [bmim][BF_4] ionic liquid by similarity considerations, namely [bmim][BF_4] molecules can also diffuse into the Hb, which enables the EDOT monomers dissolved in the [bmim][BF_4] solution to approach the Hb. Second, EDOT monomers can be slowly polymerized to form PEDOT nanowhiskers at the surface of the AuNPs–Hb composite electrode by controlling the electrochemical kinetics and decreasing mass transport of the EDOT molecules in the highly viscous [bmim][BF_4] ionic liquid electrolyte.[13] Finally, Fig. S1 in the ESI† demonstrates that both hemoglobin and AuNPs can be connected to the PEDOT nanowhiskers, respectively. When the EDOT monomers were polymerized, some PEDOT nanowhiskers grew from the hemoglobin, connecting the hemoglobin to the GC substrate electrode, whereas others grew from the AuNPs surface, reaching the GC substrate electrode. Of course, some PEDOT nanowhiskers perhaps grew from the AuNPs surface to the Hb, connecting the AuNPs and Hb. In this way, the PEDOT nanowhiskers can become electron transfer channels.

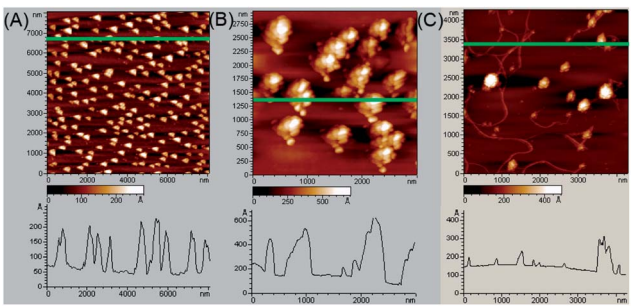

Fig. 3 AFM images of (A) AuNPs, (B) AuNPs–Hb composites, and (C) PEDOT nanowhiskers–AuNPs–Hb composites.

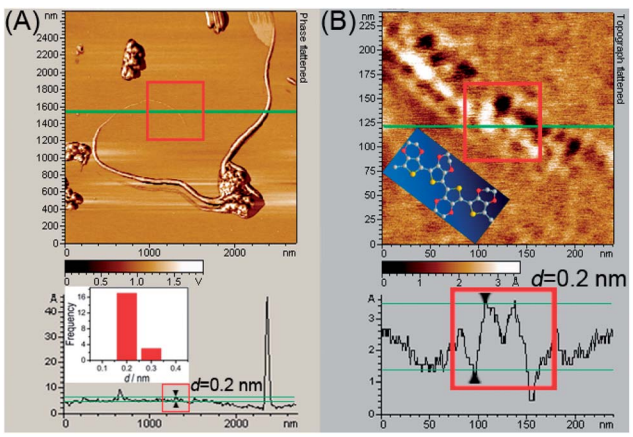

Fig. 4 (A) Phase-flattened AFM image of the PEDOT nanowhiskers–AuNPs–Hb composites; (inset) distribution of the PEDOT nanowhisker diameters as measured at the tips of the whiskers by AFM. (B) Enlargement of a PEDOT nanowhisker tip; (inset) molecular model of a PEDOT chain. The nanowhiskers' tips and the corresponding heights were marked with red panes.

3.3 Electrocatalysis of PEDOT nanowhiskers–AuNPs–Hb composite electrode in the reduction of H_2O_2

The direct electrochemistry of the AuNPs–Hb composites electrode and the PEDOT nanowhiskers–AuNPs–Hb composites electrode were studied by cyclic voltammetry. Curve a in Fig. 5 displays the cyclic voltammogram (CV) of the AuNPs–Hb composites electrode in PBS saturated with N_2. An obvious reduction peak at −0.3 V (*vs.* SCE) was observed, and the peak current was 0.32 μA. This peak was ascribed to the reduction of the iron (III)–heme to iron (II)–heme in Hb.[9] After 100 μM H_2O_2 was added into the solution, the peak current increased to 0.53 μA (curve b in Fig. 5), indicative of a typical catalytic reduction process. Compared to that of an AuNPs–Hb composites electrode, the catalytic current of a PEDOT nanowhiskers–AuNPs–Hb composites electrode should increase dramatically if the PEDOT nanowhiskers have effectively wired Hb to the electrode. The measured CVs strongly indicate that the nanowhiskers serve as electron bridges. Curve d in Fig. 5 shows that there is a marked increase in the reduction peak current for the PEDOT nanowhiskers–AuNPs–Hb electrode. The peak current (3.84 μA) nearly increases 7 times as compared with that of the AuNPs–Hb composites electrode. Because of the good capacitive properties of the PEDOT nanowires, compared to the AuNPs–Hb composites electrode, both the charge–discharge current and the current-resistance drop increased about 15 fold for the PEDOT nanowhiskers–AuNPs–Hb modified electrode, which showed that the peak potential of the PEDOT nanowhiskers–AuNPs–Hb modified electrode was more negative than that of the AuNPs–Hb modified electrode and displayed a larger resistance.

The CVs of the PEDOT nanowhiskers–AuNPs–Hb composites electrode at different scan rates were also studied. As shown in Fig. 6A, the characteristic peak potentials of Hb are not changed with the increasing scan rate. In addition, Fig. 6B displays the characteristic linear proportionality between the peak currents and the scan rates ranging from 20 to 500 mV s^{-1}. It demonstrates that the reduction process of Hb is controlled by a surface-confined process. According to the equation,

$$\Gamma = \frac{4i_pRT}{n^2F^2\upsilon A}$$

where Γ is the average apparent coverage, i_p is the peak current of Hb, R is the molar gas constant, T is the temperature, n is the electron transfer number, F is Faraday's constant, υ is the scan rate, and A is the electrode area, the Γ can be estimated to be 2.89×10^{-11} mol cm^{-2}. Based on the crystallographic dimensional structure of Hb ($6.4 \times 5.5 \times 5.0$ nm^3), and assuming one molecule with the long axis parallel to the electrode surface, the theoretical monolayer coverage of Hb is estimated to be 1.89×10^{-11} mol cm^{-2}.[13b] Therefore, Hb molecules participating in the electron transfer reaction were no more than two layers.

Electrochemical impedance spectroscopy (EIS) was used to monitor the features of the modified electrode as shown in Fig. 7. The impedance spectra of the GC electrode and the AuNPs–Hb composites electrode include a semicircle (at higher frequencies depending on the electron transfer limited process) and a linear section (at lower frequencies controlled by the diffusion limited process). The electrical equivalent circuit is shown in Fig. 7 (upper inserted), and it contains the following four elements: ohmic resistance of the electrolyte (R_s), electron transfer resistance (R_{et}), Warburg impedance (Z_w) and constant phase element (CPE).[14] The fitted results showed that at the GC electrode, R_{et} of the $[Fe(CN)_6]^{3-/4-}$ probe was about 700 Ω (curve a in Fig. 7). However, after the GC electrode was modified with the AuNPs–Hb composites, R_{et} increased to about 4500 Ω (curve b in Fig. 7), likely owing to the presence of Hb, which could have hindered electron transfer. After the PEDOT nanowhiskers were polymerized at the AuNPs–Hb composites electrode (curve c in Fig. 7), the fitted result demonstrated that R_{et} dropped to 60 Ω, indicating that the PEDOT nanowhiskers were an excellent conducting material to accelerate electron transfer. It is well known that the active redox center of Hb is deeply embedded in its protein shell, and it is difficult to realize the direct electron transfer between the iron–heme center and the electrode surface.[9] However, the extraordinary electron transport property of the PEDOT nanowhiskers can promote the electron transfer.

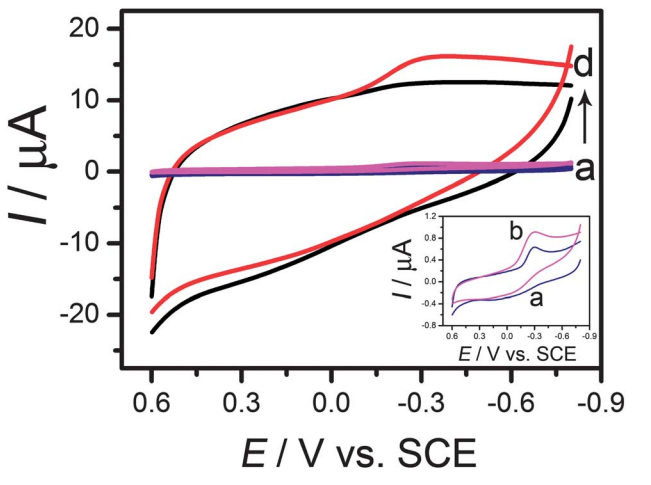

Fig. 5 CVs of the AuNPs–Hb composites electrode in 0.1 M PBS (pH 5.5) saturated with N_2 containing (a) 0 and (b) 100 μM H_2O_2, and PEDOT nanowhiskers–AuNPs–Hb composites electrode in the above mentioned solution containing (c) 0 and (d) 100 μM H_2O_2. The scan rate was 50 mV s^{-1}. Inset: magnified view of curve a and curve b.

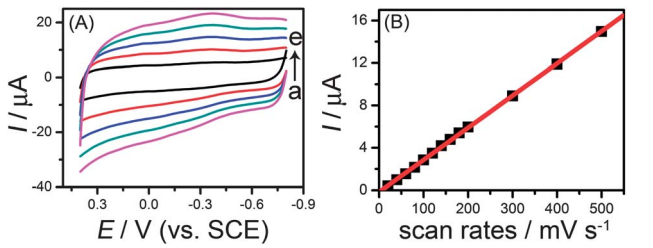

Fig. 6 (A) CVs of the PEDOT nanowhiskers–AuNPs–Hb composites electrode at different scan rates: (a) 20 mV s^{-1}, (b) 40 mV s^{-1}, (c) 60 mV s^{-1}, (d) 80 mV s^{-1}, and (e) 100 mV s^{-1}. (B) Plot of reduction peak currents *versus* various scan rates from 20 mV s^{-1} to 500 mV s^{-1}.

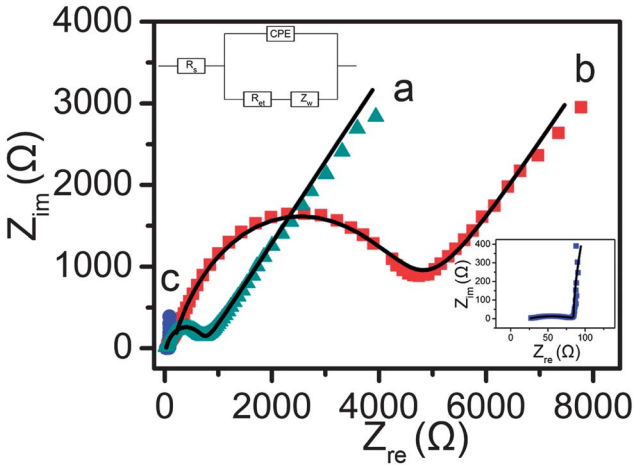

Fig. 7 The experimental (scattered line) and fitted (solid line) EIS of (a) bare GC electrode, (b) AuNPs–Hb composites electrode, and (c) PEDOT nanowhiskers–AuNPs–Hb composites electrode in 2.5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1 : 1) containing 0.5 M KNO_3. Frequency range: 10^{-2} to 10^5 Hz. Inset: magnified view of curve c and the electrical equivalent circuit applied to fit the curves.

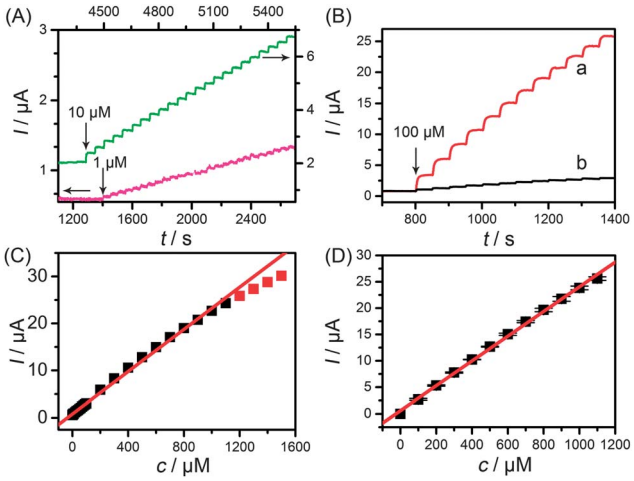

Fig. 8 (A) *I–t* response curves of the model of the biosensor with successive additions of H_2O_2 (from 1 μM to 100 μM) in 0.1 M PBS (pH 5.5). (B) *I–t* response curves of (a) the biosensor and (b) the biosensor based on AuNPs–Hb composites electrode with successive additions of H_2O_2 (from 100 μM to 1500 μM) in 0.1 M PBS (pH 5.5). (C) The relationship of the response current *versus* the H_2O_2 concentration, $R = 0.999$. (D) The calibration curve of the response currents *versus* the H_2O_2 concentrations, $R = 0.999$. Error bars = ± standard deviation. Every point was an average value of three biosensor models for independent measurements.

Thus it is expected that the PEDOT nanowhiskers can become a novel functional material for applications in electrochemistry.

3.4 Determination of H_2O_2 with the model biosensor based on the PEDOT nanowhiskers–AuNPs–Hb composites electrode

Detection of H_2O_2 is of importance as it is not only the outgrowth of some enzymes, but also an essential intermediate in clinics, pharmacies, foodstuffs, and so on.[15] Fig. 8A and curve a in Fig. 8B display the typical amperometric response of the biosensor with successive injections of H_2O_2 to a stirring PBS solution at an applied potential of −0.3 V. It was clear that the amperometric response successively increased with the addition of H_2O_2, and the steady-state current platform was reached rapidly which indicated that the biosensor possessed a quick response to the target molecule. The linear relationship of the response current *versus* the concentration of H_2O_2 is shown in Fig. 8C. The detection limit was estimated to be 0.6 μM at a signal/noise (S/N) of 3, and the linear range of H_2O_2 concentration was from 1 μM to 1100 μM. As shown in Table 1, both the linear range and the detection limit have obvious superiority in comparison with other reported results. In addition, Fig. 8B shows that the sensitivity of the model of the biosensor toward H_2O_2 (313 mA cm^{-2} M^{-1}, curve a) was 11 times greater than that of the model of the biosensor fabricated by the AuNPs–Hb composites electrode (28 mA cm^{-2} M^{-1}, curve b). This result indicates that the PEDOT nanowhiskers effectively served as bridges to wire the Hb active center to the GC electrode.

When the concentration of H_2O_2 was higher than 1100 μM, a plateau was observed, which showed the characteristics of the Michaelis–Menten kinetics. The apparent Michaelis–Menten constant (K) provides an indication of the enzyme–substrate kinetics, and K is generally used to evaluate the biological activity of the immobilized enzyme. According to the electrochemical version of the Lineweaver–Burk equation, K was estimated to be 38 μM, which was smaller than most of the other reported results (see Table 1). The smaller value of K showed that the fixed Hb on the PEDOT nanowhiskers–AuNPs–Hb composites electrode obtained a greater enzymatic activity.

In order to characterize the repeatability, three biosensor models were independently fabricated in the same way for the measurement of H_2O_2. The RSD value of the results determined in the linear range from 100 to 1100 μM was less than 2.1% (see Fig. 8D).

In order to verify the reliability of the model of the biosensor, the level of H_2O_2 concentration in the contact lens nursing liquid was determined by the biosensor model. The practical clinical analysis was based on the calibration curve in Fig. 8D, and the analysis results are shown in Table 2 for six successive measurements. The mean concentration of H_2O_2 in the sample was detected to be 203.6 μM with the standard deviation (SD) of 3.3 μM, which was in good agreement with the results determined by the potassium permanganate titration method (202.9 μM) or provided by the product supplier (205.8 μM).

Table 1 Comparison of the performances of the H_2O_2 biosensor models based on different conductive wire-like nanomaterials

Materials	Linear range (μM)	Detection limit (μM)	K (μM)
This work	1–1100	0.6	38
Nafion–Hb–CNF[9]	1–70	0.1	51
Hb–bamboo-like CN_xNT[16]	5–45	1	117
Hb–MWCNT[17]	210–900	9	675
Hb–SWCNTs–CTAB[18]	23.6–134	7.87	12
Hb–CeO_2–MWCNTs CHIT[19]	5–460	0.65	—

Table 2 Determination of H_2O_2 in contact lens nursing liquid with the biosensor and the $KMnO_4$ titration method, respectively

Sample (no.)	Determined values[a] (μM)	Referenced values[b] (μM)	Referenced values[c] (μM)	SD (μM)
1	204.4	202.9	205.8	3.3
2	200.6			
3	207.8			
4	199.5			
5	206.8			
6	202.3			

[a] Values determined by the H_2O_2 biosensor. [b] Values determined by the $KMnO_4$ titration method. [c] Values provided by the product supplier.

In order to characterize the stability, three biosensor models were independently fabricated in the same way for measuring the changes of the amperometric response during storage. Fig. 9 shows the average amperometric current of the three models retained 90% of the initial signal after they were stored at 4 °C for two months, and the maximum RSD value of the reduction peak currents was 2.3%. There was a direct correlation between the stability of the model of the biosensor and the activity of hemoglobin fixed on the PEDOT nanowhiskers–AuNPs–Hb composites electrode. This remarkable stability means both the AuNPs and the PEDOT nanowhiskers might have provided a favorable microenvironment for Hb to retain its bioactivity. It demonstrates that the biosensor model is a promising candidate to determine the concentration of H_2O_2 in practical clinical analysis.

4 Conclusions

In conclusion, PEDOT nanowhiskers with a single-molecule thickness were fabricated and used as electron transfer channels between the active centers of Hb and an underlying electrode. The PEDOT nanowhiskers could serve as electron bridges, and effectively wire the Hb to the underlying electrode. The model of the biosensor based on the PEDOT nanowhiskers–AuNPs–Hb composites showed long-term stability and sensitivity to H_2O_2. The results detected in contact lens nursing liquid were consistent with the values provided by the product supplier. The PEDOT nanowhiskers–AuNPs–Hb composites can provide new opportunities for the design of high-performance H_2O_2 biosensors in practical clinical analysis.

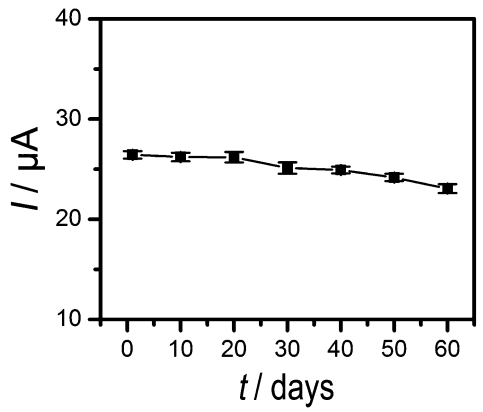

Fig. 9 The response currents of the H_2O_2 biosensor for 1 mM H_2O_2 *vs.* storage time. Error bars = ± standard deviation. Every point was an average value of three H_2O_2 biosensors for independent measurements.

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