Full Paper

Direct Electron Transfer of Hemoglobin Immobilized in Multiwalled Carbon Nanotubes Enhanced Grafted Collagen Matrix for Electrocatalytic Detection of Hydrogen Peroxide

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Abstract

The direct electron transfer and electrocatalytical behavior of hemoglobin (Hb) immobilized in multiwalled carbon nanotubes enhanced grafted collagen matrix (grafted collagen-MWNTs) was studied. The immobilized Hb displayed a pair of redox peaks with a formal potential of $-(0.360 \pm 0.002)$ V (vs. SCE) and a surface-controlled electrode process. The electron transfer rate constant and surface coverage of Hb was $(6.3 \pm 0.2)\ \mathrm{s}^{-1}$ and $(5.0 \pm 0.3) \times 10^{-10}$ mol/cm^2, respectively. Based on the direct electrochemistry, a novel biosensor for H_2O_2 ranging from 0.6 to 30 μM with a linear regression equation of I (μA) $= 0.026\,C + 0.029$ ($R = 0.9999$, $n = 29$) was constructed. Owing to the good biocompatibility and high enzyme loading of the matrix, the biosensor exhibited a low limit of detection of 0.13 μM at 3 σ, a time of less than 5 s to achieve 95% of the maximum steady-state response, a sensitivity of 103 mA/M cm^2 and acceptable reproducibility with a *RSD* of 2.5%. It could retain 95% of its initial response to H_2O_2 after a month. Grafted collagen-MWNTs provided a good matrix for protein immobilization and biosensors preparation.

Keywords: Biosensor, Multiwalled carbon nanotubes, Grafted collagen, Hemoglobin, Nanocomposite materials

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1. Introduction

The direct electron transfer (DET) of proteins is very important in understanding the structure–function relationship and the electron transfer mechanism of biomolecules in vivo [1]. This topic has attracted considerable attention in life science and analytical chemistry since the end of 1970s. Although the direct electrochemistry of redox proteins shows significantly irreversible characteristics due to the fact that the electroactive centers are embedded deeply in the protein structure, the direct electrochemical studies of proteins have been performed at various electrode surfaces modified with a series of functionalized molecules or matrices such as 4,4′bipyridin [2], DNA [3], metal oxides [4], clay or montmorillonite [5, 6], alkylthiol monolayer [7], polycrystalline metals [8], surfactant [9], hydrogel films [10], bilayer lipid membranes [11], molecular sieves [12], and nanoparticles including gold nanoparticles [13, 14], carbon nanotubes [15–21]. The unique properties of various nanostructures are most promising for constructing electrochemical/electron transfer environment and resistance to biodegradation due to their inherent electrical conductivity, unique structural and catalytic properties, high loading of the biocatalysts, good mechanical, thermal and chemical stability, and excellent adsorption and penetrability [22–24].

Since the discovery of carbon nanotubes (CNTs) in 1991 [25], many efforts have been devoted to employ them as electrode materials, and the resulting carbon nanotube modified electrodes have been successfully utilized in the investigation of bioelectrochemical reactions and the preparations of the biosensors [15–21, 26, 27]. However, the insolubility of CNTs in most solvents and their hydrophobicity limit their application in the design of CNT-based biosensing devices [28]. Several strategies including their covalent modification with hydrophilic groups [29], noncovalent functionalization with surfactants [30] or polymers [31] have been proposed to overcome this difficulty. This work used grafted collagen to modify multiwalled carbon nanotubes (MWNTs) for improving the hydrophilicity and biocompatibility of the immobilization matrix and studying the direct electron transfer of heme proteins. Owing to the introduction of the collagen and the presence of MWNTs in this hybrid nanocomposite, the immobilized proteins, with hemoglobin (Hb) as a model, showed high loading and fast electron transfer rate on electrode surface, and the resulting amperometric biosensor for hydrogen peroxide exhibited fast response, high sensitivity, low limit of detection, operational convenience and storage stability. All these analytical performances were better than those reported previously, including the sensitivity [27], stability [32], detection limit [26, 33]. Grafted collagen modified MWNTs or MWNTs enhanced Grafted collagen (grafted collagen-MWNTs) provided a good matrix for protein immobilization, direct electron transfer and reagentless biosensors preparation.

2. Experimental

2.1. Materials and Reagents

Hb was obtained from Sigma and used without further purification. 5.0 mg/mL of Hb solution was stored at 4 °C as stock solution. H_2O_2 (30% W/V solution) was purchased from Shanghai Jinlu Chemical Engineering Ltd. Co (China). Other reagents were of analytical reagent grade. 0.1 M phosphate buffer solutions (PBS) with different pH values were prepared by mixing the stock standard solutions of Na_2HPO_4 and NaH_2PO_4 and adjusting the pH with 0.1 M H_3PO_4 or NaOH. All solutions were prepared with twice-distilled water.

2.2. Preparation of Grafted Collagen-MWNTs

MWNTs (purity: 92.5 – 97.1%) was obtained through catalytic chemical vapor deposition (CCVD), and subsequent thermal heat treatment at 2800 °C for 30 min in an argon atmosphere. The functionalization of the MWNTs was performed by immersing the obtained MWNTs into 1 M hydrochloric acid for 3 hours and 2.6 M nitric acid for 4 hours, respectively. The obtained products were dispersed in ethanol for further experiment.

Grafted collagen was prepared according to the literatures [34]. Preparation of grafted collagen – MWNTs nanocomposite was obtained by mixing functionalized MWNTs and the grafted collagen at a ratio of 1:49 (wt) and completely dispersing the mixture in ethanol by vigorous stirring for 4 hours followed by ultrasonic vibration for 4 – 6 hours. After filtrated with the vacuumized filtration the final product was washed thoroughly with absolute ethanol and evaporated in room temperature to remove impurities and solvents.

2.3. Apparatus and Measurements

UV-Vis absorbance spectroscopy was obtained using a UV-Vis-3100-Nir Recording Spectrophotometer (Shimadzu, Japan). Fourier transform infrared (FT-IR) spectra were recorded on a Vector 22 FT-IR spectrometer (Bruker). For morphological analysis, the sample films were prepared in the same way as those for voltammetric measurements on different slides cleaned with nitric acid and the mixture of $H_2SO_4:H_2O_2$ (1:1). After coated with Au film to improve the conductivity, these films were examined under a scanning electron microscope (SEM, LEO 1530 VP, Germany) at 5.00 kV.

Electrochemical measurements were performed on a CHI 730A electrochemical analyzer (CHI Co., China) at (20 ± 2) °C with a conventional three-electrode system with the modified graphite electrode (GE) as working electrode, a platinum wire as auxiliary electrode, and a saturated calomel electrode (SCE) as reference against which all potentials were measured. The amperometric experiments were carried out by applying a potential of -400 mV on a stirred cell. The biosensor response was measured as the difference between total and residual currents. All experimental solutions were deoxygenated by bubbling highly pure nitrogen for 15 min and maintained under nitrogen atmosphere during measurements.

2.4. Preparation of the Hb/Grafted Collagen-MWNTs/DMSO Modified Electrode

The substrate graphite electrodes (GE, 5.6 mm in diameter) were polished before each experiment with 1.0, 0.3 and 0.05 μm α-alumina slurry (Buehler) respectively, rinsed thoroughly with doubly distilled water between each polishing step, then sonicated in 1:1 nitric acid, acetone and doubly distilled water successively and allowed to dry at room temperature. To improve the dispersion of grafted collagen-MWNTs, dimethyl sulfoxide (DMSO) was used to prepare grafted collagen-MWNTs suspension (4.0 mg in 1.0 mL DMSO). 5 μL of grafted collagen-MWNTs suspension (without Hb) was cast on GE surface to obtain the grafted collagen-MWNTs/DMSO/GE; 10 μL of 5.0 mg/mL Hb solution and 5 μL water were cast on GE surface to obtain the Hb/GE; 10 μL of 5.0 mg/mL Hb and 5 μL aqueous suspension of grafted collagen-MWNTs were cast on GE surface to obtain the Hb/grafted collagen-MWNTs/GE; 10 μL of 5.0 mg/mL Hb and 5 μL DMSO solution were cast on GE surface to obtain the Hb/DMSO/GE; 10 μL of 5.0 mg/mL Hb solution and 5 μL of grafted collagen-MWNTs suspension were cast on GE surface to obtain the Hb/grafted collagen-MWNTs/DMSO/GE. The contents of Hb for above four modified electrodes were the same. A small bottle was fit tightly over the electrode for 2 h to ensure the slow evaporation of water and the formation of more uniform film. The films were then dried and aged overnight in a sealed flask at room temperature. Prior to electrochemical experiments, the electrodes were rinsed thoroughly with doubly distilled water and kept in 0.1 M pH 7.0 PBS at 4 °C in a refrigerator when not in use.

3. Results and Discussion

3.1. Spectroscopic and SEM Characteristics

All systems containing Hb displayed a maximum absorption at 405 nm (curves a, b, c, and d in Fig. 1), while no absorption of DMSO and grafted collagen-MWNTs/DMSO was observed (curves f and e). Obviously, the absorption peak was attributed to the Soret band of Hb. No shift of the Soret band

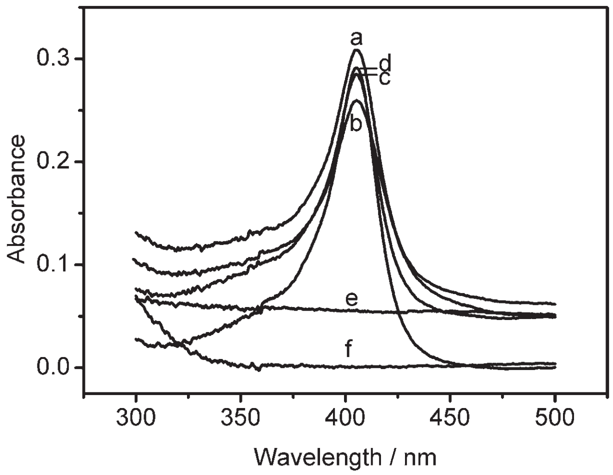

Fig. 1. UV-vis spectra of Hb/grafted collagen-MWNTs in DMSO (a), Hb in DMSO (b), Hb in water (c), Hb/grafted collagen-MWNTs in water (d), grafted collagen-MWNTs in DMSO (e), and DMSO (f).

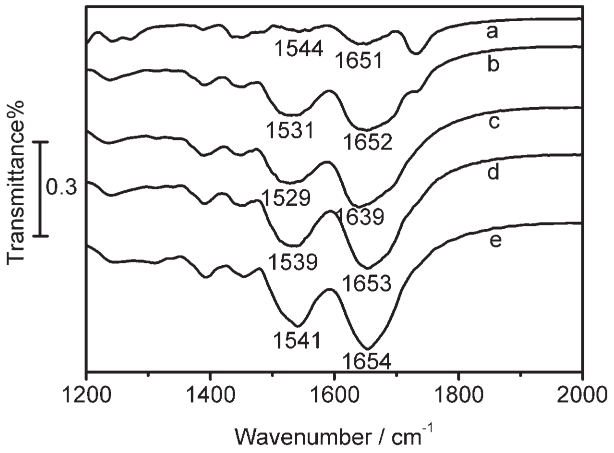

Fig. 2. FT-IR spectra of grafted collagen-MWNTs (a), Hb/grafted collagen-MWNTs /DMSO (b), Hb/DMSO (c), Hb/grafted collagen-MWNTs (d), and Hb (e) films.

upon mixing of Hb with grafted collagen-MWNTs or DMSO was observable. Thus grafted collagen-MWNTs did not change the fundamental microenvironment of Hb. The Hb mixed in these systems retained its natural secondary structure.

The FT-IR spectra of Hb, Hb/grafted collagen-MWNTs, Hb/DMSO and Hb/grafted collagen-MWNTs/DMSO films showed the interaction between grafted collagen- MWNTs nanocomposites and Hb. The FT-IR spectrum of grafted collagen-MWNTs showed the amide I and amide II infrared absorbance of collagen at 1651 and 1544 cm^{-1} (curve a in Fig. 2), while they located at 1653, 1539 cm^{-1} in Hb/grafted collagen-MWNTs film (curve d), which were close to 1654 and 1541 cm^{-1} obtained for Hb itself (curve e). The slight shift resulted from the interaction between Hb and grafted collagen-MWNTs. The presence of DMSO resulted in greater shifts in the peak positions of amide I (1639 cm^{-1}) and amide II (1529 cm^{-1}) infrared absorbance of Hb (curve c). But the shifts became smaller in presence of grafted collagen-MWNTs hybrid nanocomposites (curve b) with the peak positions of 1652 and 1531 cm^{-1} for amide I and amide II infrared absorbance of Hb, indicating the nanocomposites improved greatly the microenvironment for retaining the natural structure of the immobilized Hb.

The SEM micrograph of grafted collagen-MWNTs film displayed that collagen molecules could strongly adsorb on the surface of MWNTs to form a uniform porous three-dimensional structure (Fig. 3a). The diameters of the grafted collagen adsorbed MWNTs were in the range of 30 to 60 nm. When Hb was cast on a glass slice, the Hb molecules aggregated together (Fig. 3b). After mixing Hb with grafted collagen- MWNTs/DMSO, a well-distributed film could be formed (Fig. 3c). In this case, the grafted collagen-MWNTs were surrounded by Hb molecules, forming stereo porous interspaces. The uniform porous structure of the grafted collagen-MWNTs film increased the homogeneous loading of protein and its affinity to the substrate, and provided good preparation reproducibility of the Hb/grafted collagen-MWNTs/DMSO modified electrodes.

3.2. Direct Electrochemistry of Hb Immobilized in Grafted Collagen-MWNTs/DMSO Matrix

The cyclic voltammogram of Hb/grafted collagen-MWNTs/DMSO/GE displayed a couple of stable and well-defined

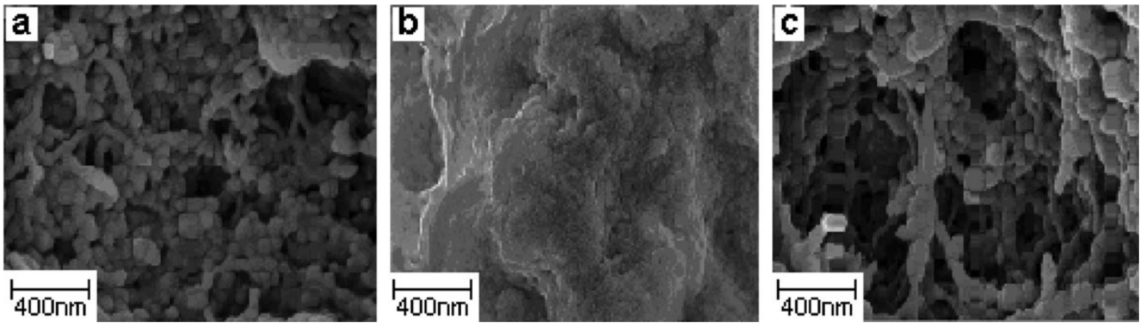

Fig. 3. Scanning electron micrographs of grafted collagen-MWNTs/DMSO (a), Hb (b), and Hb/grafted collagen-MWNTs/DMSO (c) films on glass slices.

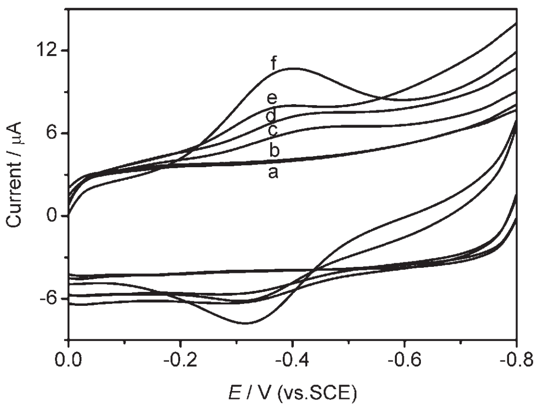

Fig. 4. Cyclic voltammograms of GE (a), grafted collagen-MWNTs/DMSO/GE (b), Hb/GE (c), Hb/grafted collagen-MWNTs/GE (d), Hb/DMSO/GE (e), and Hb/grafted collagen-MWNTs/DMSO/GE (f) in 0.1 M pH 7.0 PBS at 100 mV/s.

redox peaks at – 320 and – 399 mV at 100 mV/s, while no obvious electrochemical response was observed at both GE and grafted collagen-MWNTs/DMSO/GE (Fig. 4). Thus these peaks were attributed to the redox reaction of the electroactive center of Hb. Hb/GE, Hb/DMSO/GE, Hb/grafted collagen-MWNTs/GE exhibited only much smaller redox peaks compared those at the Hb/ grafted collagen-MWNTs/DMSO/GE. The increase of the peak currents indicated grafted collagen-MWNTs were very important for facilitating the electron exchange. From the integration of the reduction peaks of Hb/grafted collagen-MWNTs/DMSO/GE at different scan rates, an average surface coverage of Hb was calculated to be $(5.0 \pm 0.3) \times 10^{-10}$ mol/cm^2, which was much larger than those of $(5.74 \pm 0.57) \times 10^{-12}$ mol/cm^2 at Nafion-Hb-CNT [15], 3.1×10^{-11} mol/cm^2 at Hb-CNT [27], 1.5×10^{-11} mol/cm^2 at $\{TiO_2/Hb\}n$ [35] and 2.93×10^{-11} mol/cm^2 at Hb-agarose hydrogel [36], indicating a better loading of the Hb in the hybrid nanocomposite matrix.

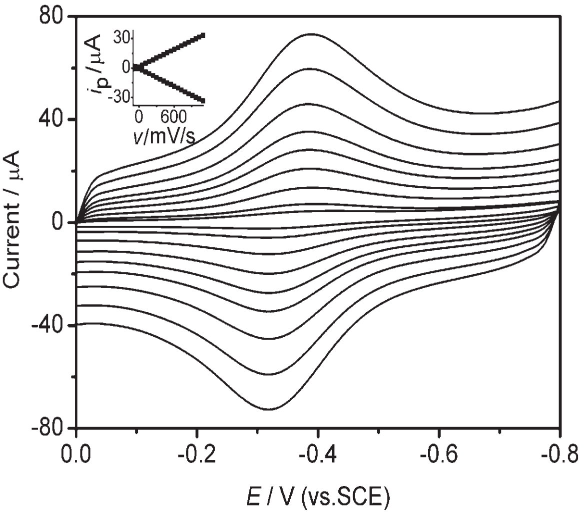

Fig. 5. Cyclic voltammograms of Hb/grafted collagen-MWNTs/DMSO/GE in 0.1 M pH 7.0 PBS at 30, 70, 150, 250, 350, 450, 600, 800, and 1000 mV/s (from lowest to highest peak current). Inset: plots of i_{pa} and i_{pc} vs. v.

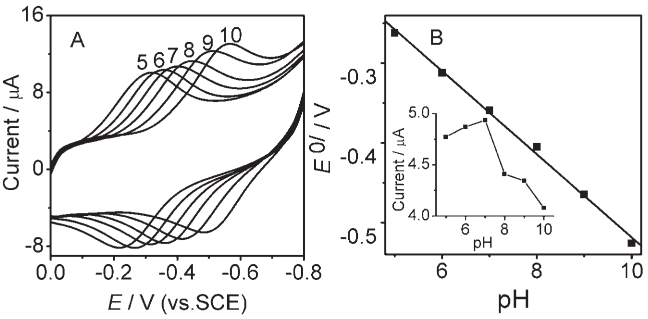

Fig. 6. Cyclic voltammograms of Hb/grafted collagen-MWNTs/DMSO/GE in 0.1 M PBS with various pH values at 100 mV/s (A) and plot of formal potential vs. pH (B). Inset in (B): effect of pH on direct electrochemical response.

The formal potential $E_{1/2}$ of the heme Fe$^{III/II}$ couple in Hb/grafted collagen- MWNTs/DMSO matrix, estimated as the midpoint of reduction and oxidation potentials, was $-(360 \pm 2)$ mV (vs. SCE) in 0.1 M pH 7.0 PBS. This value was similar to those of – 343 mV at Nafion-Hb-CNT [15], – 360 mV at Hb-CNT [27], – 348 mV at Hb-agarose hydrogel [36], suggesting that most molecules preserved their native structure after being entrapped in the grafted collagen-MWNTs matrix. The cyclic voltammogram of the Hb/grafted collagen-MWNTs/DMSO/GE showed a nearly equal height of reduction and oxidation peaks at the same scan rate (Fig. 5). With an increasing scan rate from 30 to 1000 mV/s, the anodic and cathodic peak potentials of the Hb showed shift in positive and negative directions, respectively, and the redox peak currents increased linearly (inset in Fig. 5), indicating a surface-controlled electrode process. The peak-to-peak separations of the cyclic voltammograms at scan rate from 30 to 1000 mV/s were from 54 to 79 mV. Considering the α value between 0.3 and 0.7 and the peak-to-peak separation less than 200 mV, the average electron transfer rate constant k_s was estimated to be (6.3 ± 0.2) s^{-1} according to the formula $k_s = mnFv/RT$ [37], where m is a parameter related to the peak-to-peak separation, F is the Faraday constant, R is the gas constant, T is the temperature, and n is the number of electron transfer. The k_s value was larger than those of (1.25 ± 0.25) s^{-1} at Nafion-Hb-CNT [15], 0.062 s^{-1} for Hb entrapped in carbon nanotube [26], 0.58 s^{-1} Hb entrapped in carbon nanotube [32], 0.8 s^{-1} for Hb immobilized in agarose hydrogel [36] and 0.53 s^{-1} for Hb adsorbed on nanocrystalline tin oxide [38], suggesting a reasonably fast electron transfer between the immobilized Hb and the electrode due to the presence of grafted collagen-MWNTs.

Figure 6A shows the effect of solution pH on the direct electrochemistry of the immobilized Hb. With the increasing of solution pH from 5.0 to 10.0, the negative shift of both reduction and oxidation peak potentials was observed. In general, all changes in the peak potentials and currents with solution pH were reversible in the pH range from 5.0 to 10.0.

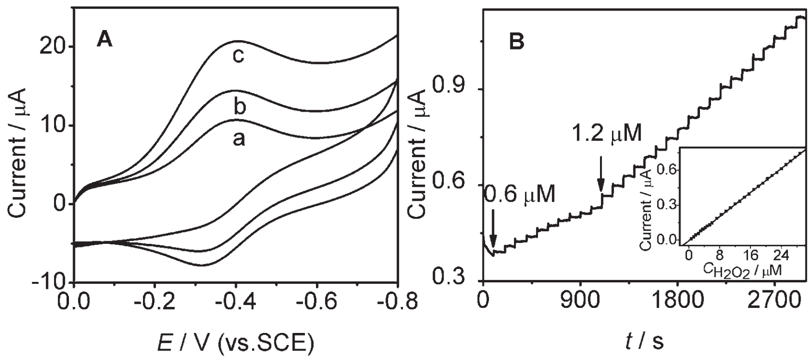

Fig. 7. Cyclic voltammograms of Hb/grafted collagen-MWNTs/DMSO/GE in 0.1 M pH 7.0 PBS containing 0 (a), 20 (b), and 60 (c) μM H_2O_2 at 100 mV/s (A) and amperometric response of the sensor in 0.1 M pH 7.0 PBS at -400 mV upon successive additions of 0.6 and 1.2 μM H_2O_2 (B). Inset: plot of amperometric response *vs.* H_2O_2 concentration.

The plot of formal potential versus pH showed a slope of -52.1 mV/pH ($R = 0.998$) (Fig. 6B), which was close to the -59.2 mV/pH unit expected for a reversible, one-electron coupled one-proton reaction process at 25 °C. The pH value also affected the peak currents of the direct electrochemistry. As shown in inset in Figure 6B the immobilized Hb showed the maximum peak current at pH 7.0.

3.3. Electrocatalysis of Hb/Grafted Collagen-MWNTs/DMSO/GE to Reduction of H_2O_2

Upon addition of H_2O_2 to 0.1 M pH 7.0 PBS, the cyclic voltammogram of the Hb/grafted collagen-MWNTs/DMSO modified electrode for the direct electron transfer of Hb changed dramatically with an increase of reduction peak current and a decrease of oxidation peak current (Fig. 7A), while the change of cyclic voltammogram of bare or grafted collagen-MWNTs/DMSO modified GE was negligible (not shown), displaying an obvious electrocatalytic behavior of the Hb to the reduction of H_2O_2. At an applied potential of -400 mV the amperometric response of the Hb/grafted collagen-MWNTs/DMSO/GE to H_2O_2 was shown in Figure 7B. Upon addition of an aliquot of H_2O_2 to the buffer solution, the reduction current increased steeply to reach a stable value. So the electrocatalytic response could be used as an efficient biosensor for H_2O_2 detection.

3.4. Analytical Performance of the Biosensor

The biosensor achieved 95% of the maximum steady-state response to H_2O_2 in less than 5 s, but in a response time of 5 s the Hb-MWNT film reached only 90% of the maximum response to H_2O_2 [27]. This demonstrated clearly that grafted collagen-MWNTs provided a well geometry capable of probing fast electrode process kinetics at relatively high steady-state current levels. So the electrocatalytic response was very fast.

The linear response range of the biosensor for H_2O_2 was 0.6–30 μM ($R = 0.9999$, $n = 29$, Inset in Fig. 7B). From the slope a limit of detection for H_2O_2 at a signal-to-noise ratio of 3 was estimated to be 0.13 μM, which was much lower than those of 9 μM for a carbon nanotube based H_2O_2 sensor with a linear range of 210 to 900 μM [26], and 1 μM for another carbon nanotube based H_2O_2 sensor with a linear range of 5 to 45 μM [33]. The sensitivity of the proposed biosensor for H_2O_2 was 103 mA/M cm^2, which was much higher than those of 1.25 mA/M cm^2 at Hb-MWNT [27] and 2.85 mA/M cm^2 at $\{TiO_2/Hb\}_{15}$ [35] due to the large specific surface area and high enzyme loading resulted from the uniform porous structure of the grafted collagen-MWNTs film.

The direct electrochemistry of the Hb/grafted collagen-MWNTs/DMSO modified GE could retain the constant values upon the continuous cyclic voltammetric sweep over the potential range from 0.00 V to -0.80 V at 100 mV/s. After it was stored in 0.1 M pH 7.0 PBS in a refrigerator at 4 °C for a week, no obvious decrease in the currents for the direct electron transfer and the response to H_2O_2 was observed. After a month, the biosensor could retain 95% of its initial response to H_2O_2 (Fig. 8), which was also more stable than the biosensor for H_2O_2 based on Hb entrapped carbon nanotube that decreased by 5% or 15% after a storage period of a week or a month at 4 °C [32] and the biosensor based on Hb immobilized in zirconia particles-chitosan matrix that retained 90% of its initial current

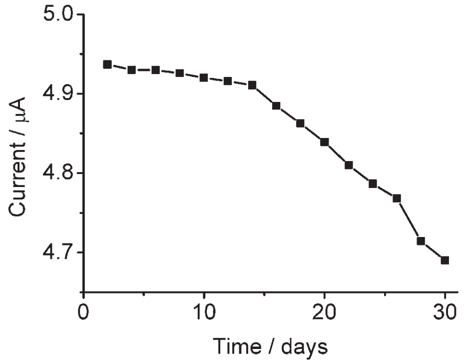

Fig. 8. Stability of the biosensor stored in 0.1 M pH 7.0 PBS at 4 °C.

response after a storage period of two weeks and only 80% of its initial current response after a storage period of one month [39]. Thus, the presence of collagen enhanced the stability of the biosensor and was very efficient for retaining the bioactivity of immobilized Hb and preventing it from leaking out of the biosensor. The fabrication of five electrodes, made independently, showed an acceptable reproducibility with the RSD of 2.5% for the current determination of 10 μM H_2O_2. When the biosensor was not in use, it was stored in 0.1 M pH 7.0 PBS at 4 °C.

4. Conclusions

Hemoglobin can be effectively immobilized in grafted collagen-MWNTs matrix due to its uniform porous structure and good biocompatibility, which provides a favorable microenvironment around the protein to retain the native structure and bioactivity of immobilized protein and results in high loading of protein. The Hb immobilized in grafted collagen-MWNTs/DMSO matrix shows a fast direct electron transfer between its electroactive center and electrode and allows a quick measurement of H_2O_2 down to 0.13 μM. The biosensor for H_2O_2 exhibits good stability, reproducibility and operational convenience. Grafted collagen-MWNTs nanocomposites would be useful for investigation of the electron-transfer properties of proteins.

5. Acknowledgements

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6. References

[1] G. Victor, L. Ovadia, *J. Am. Chem. Soc.* **1993**, *115*, 2533.
[2] M. J. Eddowes, H. A. O. Hill, *J. Chem. Soc., Chem. Commun.* **1977**, *21*, 771
[3] O. Ikeda, Y. Shirota, T. Sakarai, *J. Electroanal. Chem.* **1990**, *287*, 178.
[4] I. Taniguchi, K. Watanabe, M. Tominage, F. M. Hawkridge, *J. Electroanal. Chem.* **1992**, *333,* 331.
[5] C. Lei, J. Deng, *Anal. Chem.* **1996**, *68*, 3344.
[6] S, Kroning, F. W. Scheller, U. Wollenberger, F. Lisdat, *Electroanalysis* **2004**, *16*, 253.
[7] S. Gaspar, H. Zimmermann, I. Gazaryan, E. Csoregi, W. Schuhmann, *Electroanalysis* **2001**, *12*, 284.
[8] E. E. Ferapontova, V. G. Grigorenko, A. M. Egorov, T. Borchers, T. Ruzgas, L. Gorton, *Biosens. Bioelectron.* **2001**, *16*, 147.
[9] H. Liu, X. Chen, J. Li, H. Hill, *Chin. J. Anal. Chem.* **2001**, *29*, 511.
[10] C. Lei, F. Lisdat, U. Wollenberger, F. W. Scheller, *Electroanalysis* **1999**, *11*, 274.
[11] Y. L. Zhang, S. Z. Jin, C. X. Zhang, H. X. Shen, *Electroanalysis* **2001**, *13*, 137.
[12] Z. H. Dai, S. Q. Liu, H. X. Ju, H. Y. Chen, *Biosens. Bioelectron.* **2004**, *19*, 861.
[13] S. Q. Liu, H. X. Ju, *Electroanalysis* **2003**, *15*, 1488.
[14] S. Q. Liu, D. Leech, H. X. Ju, *Anal. Lett.* **2003**, 36, 1.
[15] C. X. Cai, J. Chen, *Anal. Biochem.* **2004**, *325*, 285.
[16] L. Zhang, G. C. Zhao, X. W. Wei, Z. S. Yang, *Electroanal*ysis **2005**, *17*, 630.
[17] W. Zheng, Q. F. Li, L. Su, Y. M. Yan, J. Zhang, L. Q. Mao, *Electroanalysis* **2006**, *18*, 587.
[18] J. Q. Liu, A. Chou, W. Rahmat, M. N. Paddon-Row, J. J. Gooding, *Electroanalysis* **2005**, *17*, 138.
[19] J. H. T. Luong, S. Hrapovic, D. S. Wang, *Electroanalysis* **2005**, *17*, 47.
[20] J. H. T. Luong, S. Hrapovic, D. Wang, F. Bensebaa, B. Simard, *Electroanalysis* **2004**, *16*, 132.
[21] J. Wang, R. P. Deo, P. Poulin, M. Mangey, *J. Am. Chem. Soc.* **2003**, *125*, 14706.
[22] D. Lee, J. Lee, J. Kim, J. Kim, H. B. Na, B. Kim, C.-H. Shin, J. H. Kwak, A. Dohnalkova, J. W. Grate, T. Hyeon, H.-S. Kim, *Adv. Mater.* **2005**, *17*, 2828.
[23] O. Niwa, *Bull. Chem. Soc. Jpn.* **2005**, *78*, 555.
[24] C. E. Banks, A. Crossley, C. Salter, S. J. Wilkins, R. G. Compton, *Angew. Chem. Int. Ed.* **2006**, *45*, 2533.
[25] S. Iijima, *Nature* **1991**, *354*, 56.
[26] Y. D. Zhao, Y. H. Bi, W. D. Zhang, Q. M. Luo, *Talanta* **2005**, *65*, 489.
[27] L. Y. Zhao, H. Y. Liu, N. F. Hu, *J. Colloid Interf. Sci.* **2006**, *296*, 204.
[28] A. Salimi, R. G. Compton, R. Hallaj, *Anal. Biochem.* **2004**, *333*, 49.
[29] J. Chen, M. A. Hamon, H. Hu, Y. Chen, A. M. Rao, P. C. Eklund, R. C. Haddon, *Science* **1998**, *282*, 95.
[30] R. J. Chen, Y. Zhang, D. Wang, H. Dai, *J. Am. Chem. Soc.* **2001**, *123*, 3838.
[31] M. J. O'Connell, P. Boul, L. M. Ericson, C. Huffman, Y. Wang, E. Haroz, C. Kuper, J. Tour, K. D. Ausman, R. E. Smalley, *Chem. Phys. Lett.* **2001**, *342*, 265.
[32] L. L. Qi, C. X. Zhang, X. R. Li, *Sens. Actuators B* **2006**, *114*, 364
[33] N. Q. Jia, L. J. Wang, L. Liu, Q. Zhou, Z. Y. Jiang, *Electrochem. Commun.* **2005**, *7*, 349.
[34] Y. Cao, Y. M. Zhou, Y. Shan, H. X. Ju, X. J. Xue, Z. H. Wu, *Adv. Mater.* **2004**, *16*, 1189.
[35] H. Y. Lu, J. Yang, J. F. Rusling, N. F. Hu, *Electroanalysis* **2006**, *18,* 379.
[36] S. F. Wang, T. Chen, Z. L. Zhang, X. C. Shen, Z. X.. Lu, D. W. Pang, K. Y. Wong, *Langmuir* **2005**, *21,* 9260.
[37] E. Laviron, *J. Electroanal. Chem.* **1979**, *101*, 19.
[38] E. Topoglidis, Y. Astuti, F. Duriaux, M. Grätzel, J. R. Durrant, *Langmuir* **2003**, *19*, 6894.
[39] G. Zhao, J. J. Feng, J. J. Xu, H. Y. Chen, *Electrochem. Commun.* **2005**, *7*, 724.