Biosensors and Bioelectronics 26 (2011) 3590–3595

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Electrochemical immunosensors for cancer biomarker with signal amplification based on ferrocene functionalized iron oxide nanoparticles

He Li[a,b,c,*], Qin Wei[b], Jing He[b], Ting Li[d], Yanfang Zhao[b], Yanyan Cai[b], Bin Du[b], Zhiyong Qian[e,**], Minghui Yang[b,**]

[a] School of Medical and Life Sciences, University of Jinan, Jinan 250022, PR China
[b] School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, PR China
[c] Shandong Academy of Medical Sciences, Jinan 250062, China
[d] Department of Surgery, The Second Xiang-ya Hospital, Central South University, Changsha 410011, China
[e] China State Key Lab of Biotherapy and Cancer Center, West China Hospital, West China Medical School, Sichuan University, Chengdu 610041, PR China

ARTICLE INFO

Article history:
Received 26 October 2010
Received in revised form 17 January 2011
Accepted 4 February 2011
Available online 16 February 2011

Keywords:
Iron oxide
Graphene
Ferrocene
Nitrodopamine
Prostate specific antigen

ABSTRACT

Ultrasensitive sandwich type electrochemical immunosensors for the detection of cancer biomarker prostate specific antigen (PSA) is described which uses graphene sheet (GS) sensor platform and ferrocene functionalized iron oxide (Fe_3O_4) as label. To fabricate the labels, dopamine (DA) was first anchored onto Fe_3O_4 surface followed by conjugating ferrocene monocarboxylic acid (FC) and secondary-antibody (Ab_2) onto Fe_3O_4 through the amino groups of DA (DA-Fe_3O_4-FC-Ab_2). The great amount of DA molecules anchored onto Fe_3O_4 surface increased the immobilization of FC and Ab_2 onto the Fe_3O_4 nanoparticle, which in turn increased the sensitivity of the immunosensor. GS used as biosensor platform increased the surface area to capture a great amount of primary antibodies (Ab_1) and the good conductivity of GS enhanced the detection sensitivity to FC. Using the redox current of FC as signal, the immunosensor displays high sensitivity, wide linear range (0.01–40 ng/mL), low detection limit (2 pg/mL), good reproducibility and stability. In addition, this method could be extended to the immobilization of other interesting materials (fluorescence dyes) onto Fe_3O_4 for preparing various kinds of labels to meet the different requirements in immunoassays.

1. Introduction

Sandwich type immunosensor is one of the major analytical techniques for sensitive and selective detection of proteins and has found wide applications in clinical diagnosis, biomedical research, food quality control and environmental monitoring (Liu and Ju, 2005; Yuan et al., 2001; Yang et al., 2008; Zhang et al., 2007). It is employed a sandwich format in which analytes are captured and detected by excess immobilized primary antibody (Ab_1) and labeled signal antibody (Ab_2) (respectively) providing a "direct" relationship between analyte amount and signal intensity. The increasing demand for detection of ultralow amount of analytes is pushing the enhancement of detection sensitivity by selecting different signal amplification strategy. To date, different kinds of multifunctional nanomaterials have been investigated as labels for signal amplification. For example, the loading of a great amount of electroactive species, such as quantum dot onto nanoparticle and direct use of the electrochemical response of quantum dot as signal (Xiang et al., 2010); the immobilization of enzymes, such as horseradish peroxidase (HRP) onto nanoparticles and use the catalytic current of enzyme to its substrate as signal (Yang et al., 2010; Tang and Ren, 2008). In general, the increased loading of signal species onto nanoparticles is crucial for high sensitivity immunosensors.

Ferrocene, due to its high redox activity, has been widely used as mediator for enzyme based biosensors and as label for DNA and protein detection (Kandimalla et al., 2006; Wei et al., 2010). For example, Luo et al. (2008) reported the utilization of ferrocene-labeled probe for electrochemical detection of DNA sequences. Wiswanathan et al. (2009) reported ferrocene encapsulated liposomes as label for the detection of carcinoembryonic antigen (CEA). After the release of ferrocene from the liposomes, the electrochemical response is in proportion to CEA concentration. In another study, Zhang et al. (2008) directly conjugated ferrocene carboxylic acid onto secondary antibody and achieved high sensitive detection of human IgG.

Iron oxide based magnetic nanoparticles, due to its biocompatibility, superior magnetic properties and stability, have been found in a wide variety of biological and biomedical applications, such

* Corresponding author at: School of Medical and Life Sciences, University of Jinan, Jinan 250022, PR China.
** Corresponding author.
E-mail addresses: lihecd@gmail.com (H. Li), anderson-qian@163.com (Z. Qian), chm_yangmh@ujn.edu.cn (M. Yang).

0956-5663/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2011.02.006

as magnetic resonance imaging (MRI), drug delivery, cell labeling and biosensing (Yu et al., 2010; Chertok et al., 2010; Lee et al., 2010; Zhuo et al., 2009). However, a key requirement for these applications is the immobilization of designated molecules onto nanoparticle surface. Recently, the anchor of dopamine (DA) onto iron oxide surface and to use it as bridge for the linking of molecules onto iron oxide has gained growing interests (Shultz et al., 2007; Xu et al., 2004). It has been reported that DA forms stable, robust anchor onto the surface of iron oxide and high amount of DA molecules could be anchored. In addition, the amino groups of DA make it easy for the further conjugation of molecules. Gu et al. (2005a,b) reported the binding of ~2135 molecules of functionalized DA onto one Fe_3O_4 surface. Wang et al. studied the coating of Fe_3O_4 with DA and then COOH-terminated polyethylene glycol (PEG), and the modified Fe_3O_4 are stable in cell culture media. Based on the COOH group, they conjugated 6-hydroxy-chromone-3-carbaldehyde onto Fe_3O_4 and the results showed that on each Fe_3O_4, ~140 chromone were attached (Wang et al., 2008).

In this paper, DA functionalized Fe_3O_4 was prepared and conjugated with ferrocene carboxylic acid (FC) and secondary antibody (Ab_2). The resulting modified Fe_3O_4 (DA-Fe_3O_4-FC-Ab_2) was used as label for the fabrication of electrochemical immunosensors. Due to the great amount of FC immobilized onto Fe_3O_4, the sensitivity of the immunosensor was greatly increased, which has been illustrated through the detection of prostate specific antigen (PSA).

2. Materials and methods

2.1. Apparatus and reagents

Prostate specific antigen (PSA) and anti-PSA antibody were purchased from Dingguo Biochemical Reagents (Beijing, China). Dopamine hydrochloride and ferrocene carboxylic acid (FC) were obtained from Sigma. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were obtained from Sinopharm Chemical Reagent Co., Ltd. (China) and used as received. Phosphate buffered saline (PBS, 0.1 M containing 0.1 M NaCl, pH 7.4) was used as electrolyte for all electrochemistry measurement. All other reagents were of analytical grade and deionized water was used throughout the study.

All electrochemical measurements were performed on a CHI 760D electrochemical workstation (Shanghai CH Instruments Co., China). Transmission electron microscope (TEM) images were obtained from a Hitachi H-800 microscope (Japan). UV–vis measurements were carried out using a Lambda 35 UV/VIS Spectrometer (PerkinElmer, USA). A conventional three-electrode system was used for all electrochemical measurements: a glassy carbon electrode (GC, 3 mm in diameter) as the working electrode, an Ag/AgCl electrode as the reference electrode, and a platinum wire electrode as the counter electrode.

2.2. Preparation of dopamine functionalized Fe_3O_4 (DA-Fe_3O_4)

The 10 nm Fe_3O_4 was synthesized following the reported procedure (Xu et al., 2009). In brief, $Fe(acac)_3$ (0.706 g, 2 mmol) was dissolved in a mixture of benzyl ether (10 mL) and oleylamine (10 mL). The above mixture solution was dehydrated at 110 °C for 1 h under a flow of nitrogen, and quickly heated to 300 °C and kept at this temperature for 2 h under a blanket of nitrogen. The black-brown mixture was cooled to room temperature later. Ethanol (40 mL) was added to the mixture and the precipitate was collected by centrifugation at 8000 rpm. The obtained Fe_3O_4 was re-dispersed in hexane. The precipitated and re-dispersed process repeated three times then obtained 10 nm Fe_3O_4.

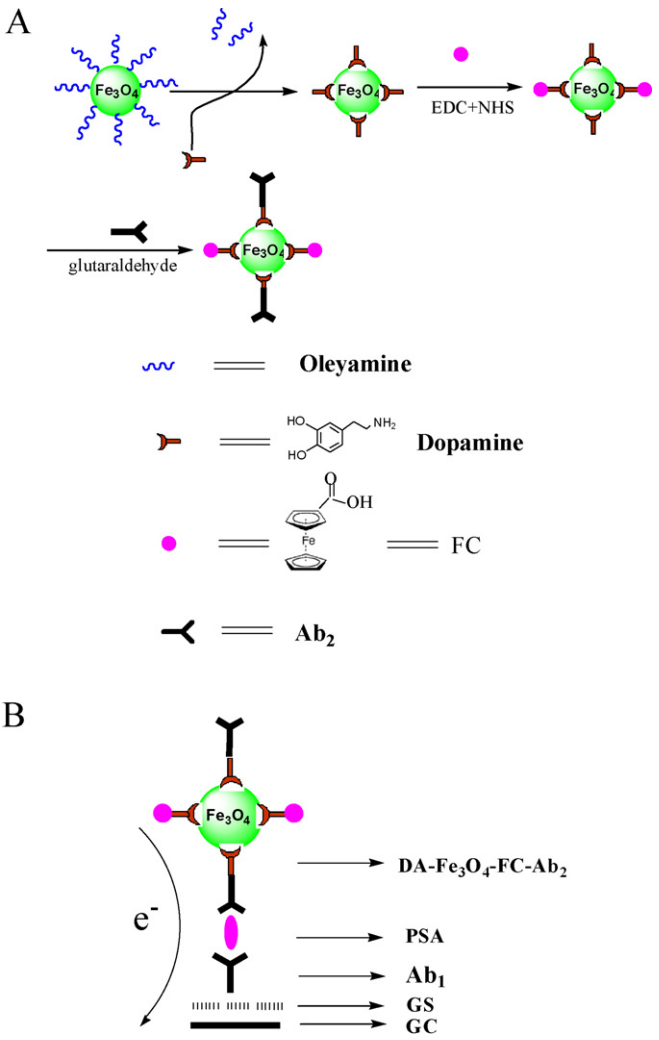

Fig. 1. Schematic representation of the preparation of the DA-Fe_3O_4-FC-Ab_2 nanoparticles (A) and immunosensor (B).

Coating of Fe_3O_4 with DA is achieved using almost the similar procedure described by Yong et al. (2009) with only slight modification. 10 mg of DA dispersed in 2 mL dimethyl sulfoxide (DMSO) was added to 4 mL of the oleylamine-capped 10 nm Fe_3O_4 (*ca.* 20 mg) dispersed in chloroform ($CHCl_3$) and incubated in an orbital shaker at 180 rpm for overnight. Then the mixture was isolated via centrifuging. The resultant DA-Fe_3O_4 was washed by DMSO then recovered by centrifugation, then dried under high vacuum at room temperature.

2.3. Synthesis of DA-Fe_3O_4-FC-Ab_2

To conjugate FC onto DA-Fe_3O_4, EDC and NHS (100 mM) were added into 10 μg/mL of FC solution and stirred for 4 h. After this, the as-prepared DA-Fe_3O_4 was added into the solution. The resulting mixture was set on an orbital shaker at 37 °C for 12 h and then centrifuged. After discarding the supernatant, the DA-Fe_3O_4-FC again were dispersed in 1 mL of phosphate buffer. Another 1 mL of 2.5% glutaraldehyde solution was added into this solution and stirred for 1 h. Then, 0.01 mg of anti-PSA Ab_2 was added into the solution. The mixture was allowed to react at room temperature under stirring for 24 h, followed by centrifuge. All the reactions were performed under N_2 atmosphere. The resulting DA-Fe_3O_4-FC-Ab_2 were washed with PBS (pH 7.4) and then re-dispersed in 1 mL of buffer and stored at 4 °C before use. Fig. 1A shows the

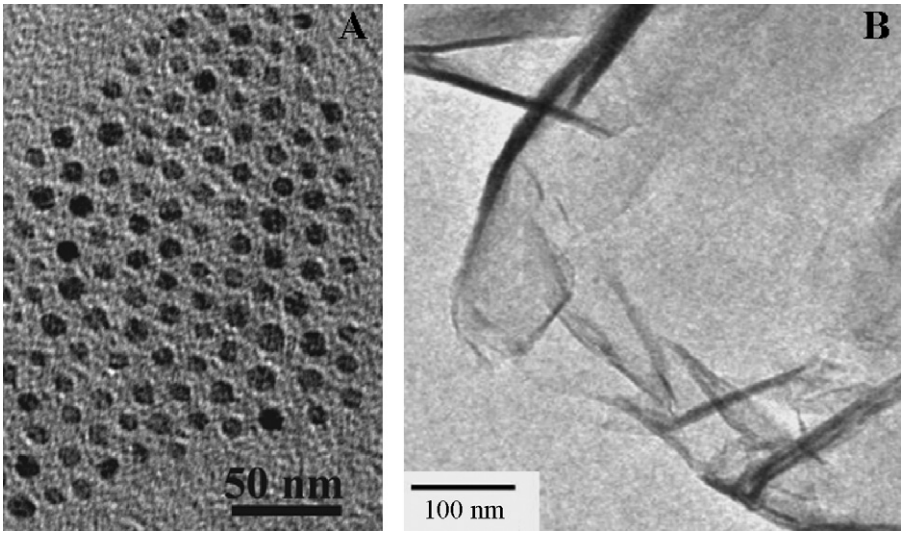

Fig. 2. TEM images of (A) DA-Fe_3O_4-FC-Ab_2 nanoparticles and (B) GS.

procedure for the preparation of the DA-Fe_3O_4-FC-Ab_2 nanoparticles.

To measure the loading content of DA onto Fe_3O_4, first, the absorption spectra of the DA solutions with a series of concentrations were measured in order to establish a calibration curve. After that, the absorption spectra of the DA solution used before and after the reaction with Fe_3O_4 were measured, and the amount of DA loaded was calculated according to the absorption difference. The loading of FC onto Fe_3O_4 was measured in a similar method.

2.4. Fabrication of the immunosensor

For the fabrication of the immunosensor, anti-PSA primary antibody (Ab_1) was immobilized onto graphene sheet (GS) surface through an amidation reaction between the carboxylic acid groups attached to graphene and the available amine groups of Ab_1. GS was prepared from graphite oxide (GO) through a thermal exfoliation method (McAllister et al., 2007). To immobilize Ab_1 onto GS, typically, into 1 mL of GS solution (2 mg/mL), EDC and NHS (100 mM) were added. The mixture was stirred for 4 h and after that, 1 mL of Ab_1 solution (100 μg/mL) was added into the mixture. After another 12 h of reaction, the GS solution was centrifuged and washed. The resulting GS–Ab_1 conjugates were stored at 4 °C in phosphate buffer solution before use.

Fig. 1B shows the fabrication procedure of the immunosensor. A glassy carbon (GC) electrode was polished repeatedly using alumina powder and then thoroughly cleaned before use. Onto the electrode, 5 μL of GS–Ab_1 buffer solution was added. After the GS–Ab_1 coated electrode was dried and washed with buffer, it was incubated in 1 wt% BSA solution for 1 h to eliminate nonspecific binding between the antigen and the electrode surface. Subsequently, PSA buffer solution with a varying concentration was added onto the electrode surface and incubated for 1 h at room temperature, and then the electrode was washed extensively to remove unbounded PSA molecules. Finally, the prepared DA-Fe_3O_4-FC-Ab_2 buffer solution was dropped onto the electrode surface and incubated for another 1 h. After washing, the electrode was cleaned and tested in the following measurements.

3. Results and discussion

3.1. Synthesis and characterization of DA-Fe_3O_4-FC-Ab_2 nanoparticles

Fe_3O_4 was synthesized with a hydrophobic oleylamine capping ligand according to a previously reported method (Xu et al., 2009). To make the Fe_3O_4 water-soluble, the oleylamine ligand is replaced by DA through a ligand exchange reaction, resulting in DA capped Fe_3O_4 (Yong et al., 2009). Previous studies suggest that catechol acts as a chelating agent, forming tight bonds with iron oxides by converting the under-coordinated cationic iron surface sites to a bulk-like lattice structure (Gu et al., 2005a,b). The carboxylic group of FC and amino group of Ab_2 can then react with the amino group of DA to form DA-Fe_3O_4-FC-Ab_2. Fig. 2A shows the TEM image of DA-Fe_3O_4-FC-Ab_2. They demonstrate a homogeneous distribution with an average size of 10 nm in diameter. Fig. 2B is the TEM image of the synthesized GS, which shows transparent paper-like structure.

The DA-Fe_3O_4-FC-Ab_2 was characterized by cyclic voltammetry (CV) and UV–vis spectroscopy to confirm that DA, FC and Ab_2

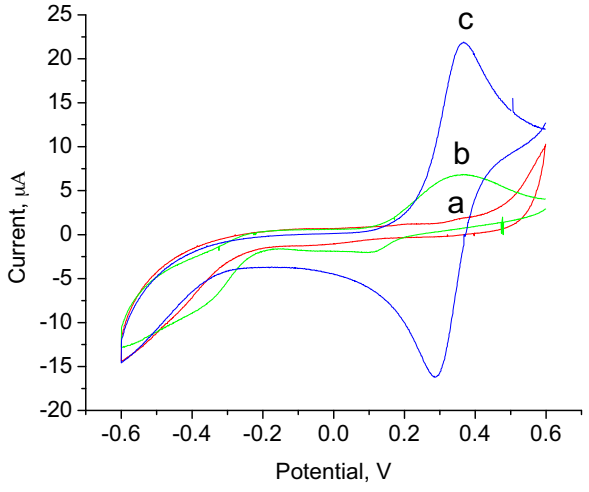

Fig. 3. Cyclic voltammograms of (a) Fe_3O_4, (b) DA-Fe_3O_4 and (c) DA-Fe_3O_4-FC modified electrode in PBS.

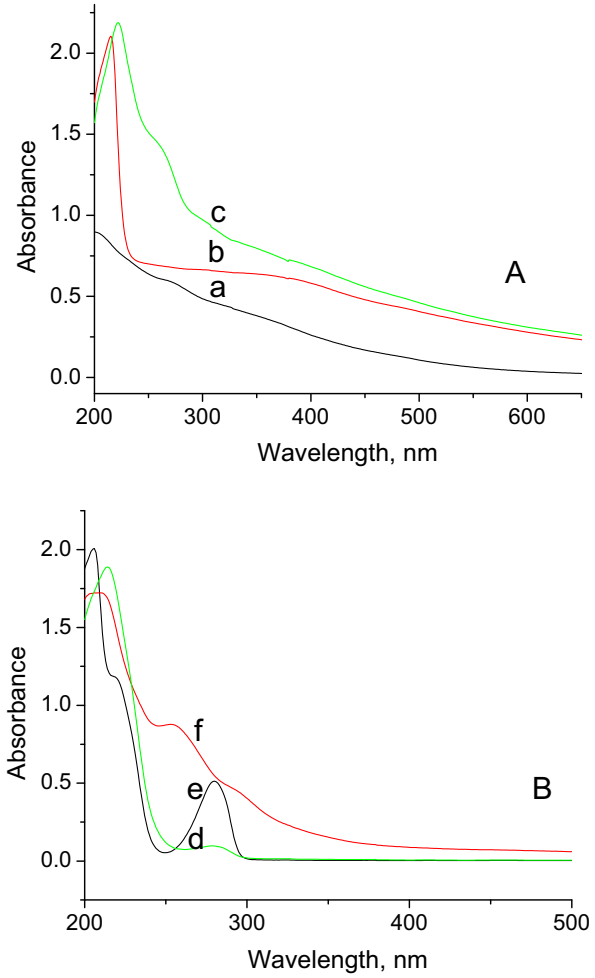

Fig. 4. UV–vis spectra of (a) Fe_3O_4, (b) DA-Fe_3O_4, (c) DA-Fe_3O_4-FC-Ab_2, (d) Ab_2, (e) DA and (f) FC.

were successfully conjugated onto Fe_3O_4. It can be seen from Fig. 3 that for Fe_3O_4 modified electrode, no current peaks were observed (curve a). For DA-Fe_3O_4 modified electrode, an oxidation peak was observed at around +0.4 V, which was due to the oxidation of DA (curve b) (Tan et al., 2010). After the conjugation of FC, the CV exhibited a pair of stable and well-defined redox peaks at +0.29 and +0.36 V (curve c). These results demonstrate the successful immobilization of DA and then FC onto Fe_3O_4, and the immobilized FC retained its redox properties.

Fig. 4 shows the UV–vis characterization of the DA-Fe_3O_4-FC-Ab_2. No absorption peaks was observed for the bare Fe_3O_4 (curve a). When DA was capped onto Fe_3O_4, a sharp absorption peak and another relatively broad peak were observed at 215 and 360 nm, respectively (curve b). Compared to the spectra of pure DA (curve e), the deviation of the DA absorption wavelength could be ascribed to the interaction of Fe_3O_4 with DA molecules. After FC and Ab_2 were conjugated onto DA-Fe_3O_4, an additional peak appeared at around 260 nm (curve c), which is due to FC (curve f). Distinct adsorption peaks from the antibody was not observed from the spectra of DA-Fe_3O_4-FC-Ab_2, which is ascribed to peak overlap among FC, DA and Ab_2 (curve d).

The amount of DA and FC molecules immobilized onto each Fe_3O_4 was estimated by UV–vis spectra and found out on average, ~1600 molecules of DA and ~500 molecules of FC were attached to one Fe_3O_4 surface.

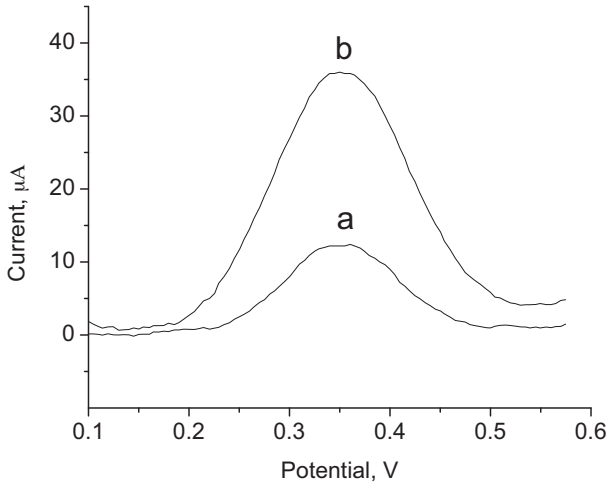

Fig. 5. Square wave voltammetry (SWV) of (a) bare GC and (b) graphene modified GC for the detection of 10 μM of FC in PBS. Pulse height, 25 mV; step height, 25 mV; frequency, 25 Hz.

3.2. Performance of the immunosensor

Since a substantial amount of FC molecules immobilized onto Fe_3O_4 has been demonstrated, we hypothesize the sensitivity of the immunosensors using this DA-Fe_3O_4-FC-Ab_2 nanoparticle as labels could be greatly increased. The signal of the immunosensor is mainly from the redox current of FC on the Fe_3O_4. In this work, Ab_1 was immobilized onto GS surface through the carboxylic group on GS surface, which was subsequently coated onto the electrode surface.

Before using DA-Fe_3O_4-FC-Ab_2 as label for the preparation of immunosensor, the performance of GS modified electrode in detecting FC was investigated. Previous reports have shown materials with good conductivity, such as carbon nanotubes and carbon nanoparticles, could enhance the sensitivity for the detection of electroactive species (Ho et al., 2009; Zhang and Gorski, 2005). Fig. 5 compares the square wave voltammograms (SWV) response of bare and GS modified electrode towards the same concentration of FC in buffer. Both electrodes give one well-defined oxidation peak at +0.3 V, however, the GS modified electrode enhanced the peak current about three times compared to that at the bare electrode. The enhanced sensitivity could be ascribed to the unique electrical properties of GS. So the use of GS as sensor platform could not only increase Ab_1 loading due to the large surface area but also could help electron transfer, increasing the FC detection sensitivity.

After the immobilization of Ab_1 onto GS and the capture of PSA, the DA-Fe_3O_4-FC-Ab_2 labels could be easily attached to the electrode surface via the specific antibody–antigen interaction to form sandwich structure. The amount of labels captured and then the redox current is in accordance with the PSA concentration. Fig. 6 shows the SWV response of the immunosensor after reacted with different concentrations of PSA. The increase of the peak current is proportional to the PSA concentration in the range of 0.01–40 ng/mL, with a satisfied detection limit of 2 pg/mL based on S/N = 3 comparing with other reported results, which is listed in Table 1 of supplementary material. The serum PSA concentration ranges from 1 to 4 ng/mL and 4 to 10 ng/mL for normal person and cancer patient, respectively, which indicate the proposed immunosensor could be used conveniently for the detection of PSA in serum samples (Lilja et al., 2008). The wide linear range and low detection limit could be ascribed to (1) the high loading level of FC onto Fe_3O_4; (2) the large surface area of graphene increased the Ab_1 loading and its good conductivity enhanced the sensitivity towards detection of FC.

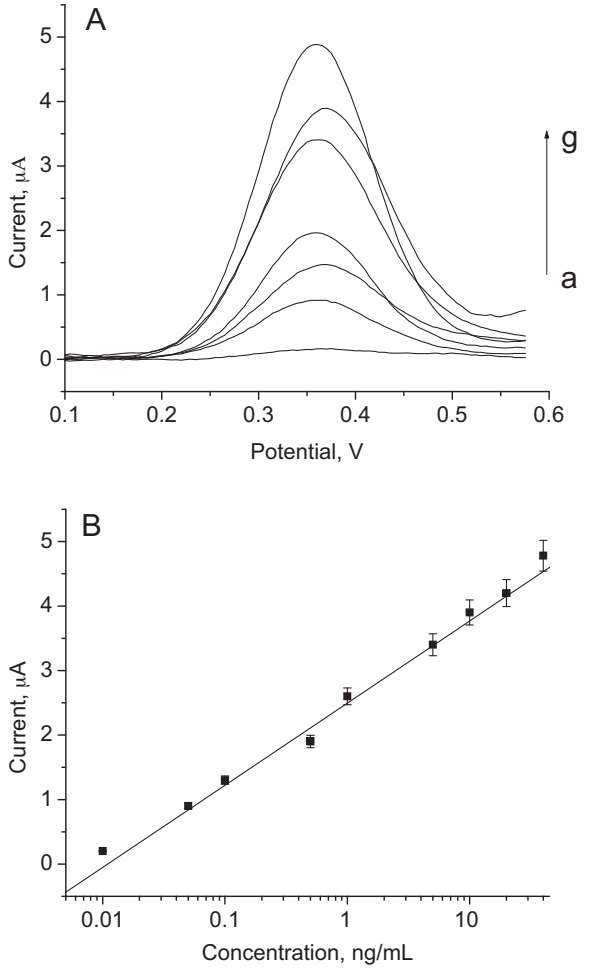

Fig. 6. (A) SWV response of the immunosensor to different concentrations of PSA, from (a) to (g): 0.01, 0.05, 0.1, 0.5, 5, 10, 40 ng/mL. (B) Calibration curve of the immunosensor to different concentrations of PSA. Error bar = RSD ($n = 5$).

To further investigate the selectivity of the immunosensor, the immunosensor was incubated in 1 ng/mL of PSA solution containing 100 ng/mL of different interfering agent, such as human IgG, α-1-fetoprotein (AFP), BSA and glucose. No remarkable change of current was observed in comparison with the result obtained in the presence of PSA only, indicating good selectivity of the proposed immunosensor.

The reproducibility of the immunosensor was evaluated. A series of five electrodes were prepared for detecting 1 ng/mL PSA and the relative standard deviation (RSD) of the five immunosensor was 6.1%. This result suggested acceptable reproducibility and precision of the proposed immunosensor.

The stability of the DA-Fe_3O_4-FC-Ab_2 was studied. When not in use, the nanoparticles were stored in buffer at 4 °C. After 1 month, the current of the immunosensor using these DA-Fe_3O_4-FC-Ab_2 nanoparticles as labels retained about 90% of its initial value. The good long-term stability could be ascribed to: (1) the good stability of DA anchored onto Fe_3O_4 surface, and (2) FC and Ab_2 were both covalently immobilized onto the DA-Fe_3O_4 surface.

3.3. Analysis of PSA in serum samples

The accuracy of the detection of PSA was investigated by recovery test in serum samples. Different concentrations of PSA were added into the normal serum and then analyzed. As shown in Table 2 of supplementary material, the percent of PSA detected by the immunosensor from these serum samples ranged from 93.5 to 110.0% of the PSA amount added to the serum samples, which is acceptable.

4. Conclusions

In this work, sensitive and selective electrochemical immunosensor for the detection of cancer biomarker has been developed based on DA functionalized Fe_3O_4. The greatly enhanced sensitivity relies upon (1) due to great amount of DA molecules anchored onto Fe_3O_4 and the available amino groups on DA, large number of FC could be easily conjugated onto Fe_3O_4; (2) the large specific surface area of GS increased Ab_1 loading and its good conductivity enhanced the detection sensitivity of FC. The resulting immunosensor possesses high sensitivity, good selectivity and reproducibility. We anticipate that the proposed immunosensor can be applied for the detection of different cancer biomarkers. In addition, this technique also has the potential to be extended to the immobilization of other interesting materials, such as fluorescence dyes and quantum dot, for the preparation of various kinds of label systems to meet the different requirements in biochemical assays.

Acknowledgements

We are grateful for the support of the Natural Science Foundation of China (No. 81000976, 21075052), National High-Tech Project of China (863-Project, 2007AA021902), the Natural Science Foundation of Shandong Province (No. ZR2010BM030, ZR2010BQ010) and the Science and Technology Research Project of Shandong Provincial Education Department (Grant No. J08LC54).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.02.006.

References

Chertok, B., David, A.E., Yang, V.C., 2010. Biomaterials 31, 6317–6324.
Gu, H.W., Xu, K.M., Yang, Z.M., Chang, C.K., Xu, B., 2005a. Chem. Commun. 34, 4270–4272.
Gu, H.W., Yang, Z.M., Gao, J.H., Chang, C.K., Xu, B., 2005b. J. Am. Chem. Soc. 127, 34–35.
Ho, J.A., Lin, Y.C., Wang, L.S., Hwang, K.C., Chou, P.T., 2009. Anal. Chem. 81, 1340–1346.
Kandimalla, V.B., Tripathi, V.S., Ju, H.X., 2006. Biomaterials 27, 1167–1174.
Lee, E.S.M., Shuter, B., Chan, J., Chong, M.S.K., Ding, J., Teoh, S.H., Beuf, O., Briguet, A., Tam, K.C., Choolani, M., Wang, S.C., 2010. Biomaterials 31, 3296–3306.
Lilja, H., Ulmert, D., Vickers, A.J., 2008. Nat. Rev. Cancer 8, 268–278.
Liu, J.H., Ju, H.X., 2005. Biosens. Bioelectron. 20, 1461–1470.
Luo, X.T., Lee, T.M.H., Hsing, I.M., 2008. Anal. Chem. 80, 7341–7346.
McAllister, M.J., Li, J.L., Adamson, D.H., Schniepp, H.C., Abdala, A.A., Liu, J., Margarita, H.A., David, L.M., Roberto, C., Robert, K.P., Ilhan, A.A., 2007. Chem. Mater. 19, 4396–4404.
Shultz, M.D., Reveles, J.U., Khanna, S.N., Carpenter, E.E., 2007. J. Am. Chem. Soc. 129, 2482–2487.
Tang, D.P., Ren, J.J., 2008. Anal. Chem. 80, 8064–8070.
Tan, L., Zhou, K.G., Zhang, Y.H., Wang, H.X., Wang, X.D., Guo, Y.F., Zhang, H.L., 2010. Electrochem. Commun. 12, 557–560.
Wang, B.D., Xu, C.J., Xie, J., Yang, Z.Y., Sun, S.H., 2008. J. Am. Chem. Soc. 130, 14436–14437.
Wei, Z.L., Li, Z.J., Sun, X.L., Fang, Y.J., Liu, J.K., 2010. Biosens. Bioelectron. 25, 1434–1438.
Wiswanathan, S., Rani, C., Anand, A.V., Ho, J.A., 2009. Biosens. Bioelectron. 24, 1984–1989.
Xiang, Y., Zhang, Y.Y., Chang, Y., Chai, Y.Q., Wang, J., Yuan, Y., 2010. Anal. Chem. 82, 1138–1141.
Xu, Z.C., Shen, C.M., Hou, Y.L., Gao, H.J., Sun, S.H., 2009. Chem. Mater. 21, 1778–1780.
Xu, C.J., Xu, K.M., Gu, H.W., Zheng, R.K., Liu, H., Zhang, X.X., Guo, Z.H., Xu, B.J., 2004. J. Am. Chem. Soc. 126, 9938–9939.

Yang, M.H., Kostov, Y., Bruck, H.A., Rasooly, A., 2008. Anal. Chem. 80, 8532–8537.
Yang, M.H., Li, H., Javadi, A., Gong, S.Q., 2010. Biomaterials 31, 3281–3286.
Yong, K.L., Xu, C.J., Xie, J., Sun, S.H., 2009. J. Mater. Chem. 19, 6400–6406.
Yuan, J.L., Wang, G.L., Majima, K., Matsumoto, K., 2001. Anal. Chem. 73, 1869–1876.
Yu, F.Q., Zhang, L., Huang, Y.Z., Sun, K., David, A.E., Yang, V.C., 2010. Biomaterials 31, 5842–5848.
Zhang, B., Zhang, X., Yan, H.H., Xu, S.J., Tang, D.H., Fu, W.I., 2007. Biosens. Bioelectron. 23, 19–25.
Zhang, M.G., Gorski, W., 2005. J. Am. Chem. Soc. 127, 34–35.
Zhang, S.B., Zheng, F., Wu, Z.S., Shen, G.L., Yu, R.Q., 2008. Biosens. Bioelectron. 24, 129–135.
Zhuo, Y., Yuan, P.X., Yuan, R., Chai, Y.Q., Hong, C.L., 2009. Biomaterials 30, 2284–2290.