Available online at www.sciencedirect.com
SCIENCE DIRECT
Talanta 68 (2005) 40–46
Talanta
www.elsevier.com/locate/talanta

A new antibody immobilization strategy based on electrodeposition of nanometer-sized hydroxyapatite for label-free capacitive immunosensor

Liu Yang, Wanzhi Wei *, Xiaohua Gao, Jianjun Xia, Han Tao

State Key Laboratory of Chemical, Biological Sensing Technologies and Chemometrics, Hunan University, Changsha 410082, China

Received 21 October 2004; received in revised form 25 April 2005; accepted 25 April 2005
Available online 25 May 2005

Abstract

A new antibody immobilization strategy was proposed for the fabrication of a label-free capacitive immunosensor based on electrodeposition of nanometer-sized bioactive hydroxyapatite (HAP). By a procedure of constant current cathodal electrodeposition, a nano-HAP film with bioactivity was formed on a self-assembled β-mercaptoethanol monolayer-modified gold electrode. A suitable amount of chitosan was added into the electrodeposition solution with the aim of obtaining a strong and homogeneous HAP-coating film. After blocking with long-chain alkylthiol and then embedding antibody by coupling with divinylsulphone, the electrode was possessed of a higher initial capacitance value, which was suitable for capacitive transduction. The sensitive layer was characterized by Fourier transform infrared spectrum, scanning electron microscopy and electrochemical method. Human transferrin immunoassay was selected as the testing system. The linear response range of the sensor for transferrin was between 1 and 100 ng/mL with a detection limit of 0.15 ng/mL. After simply rinsing with subacidity solution, the regenerated sensor achieved up to 10 assay cycles without significant loss of sensitivity.

Keywords: Immobilization; Electrodeposition; Hydroxyapatite; Capacitive; Immunosensor

1. Introduction

Biosensors provide a rapid and convenient alternative for the conventional analytical approaches to monitor chemical and biochemical substances in various fields, such as clinical diagnostic, environment control, fermentation processing and food industry. In the past few years, due to their merits of low cost, small size, short response time and the possibility of using in vivo, the biosensor field continues to be at an accelerated pace [1,2].

The development of immobilization strategies for biomaterials plays an important role in the field of biosensor research [3], especially in immunoassay. The immobilization of antibodies on the transducer surfaces or sensitive layers is the key of fundamental issues in the procedure of fabrication, a reliable immunosensor. An effective immobilization method not only ensures the biomolecules intimately contacting with the transducer, but also aids in stabilizing the biological system, enhancing the operation and storage stability. Numerous methods have been applied for the immobilization of receptor biomolecules [4], e.g., adsorption [5], covalent attachment to silanes [6], embedding in polymers [7,8] or membranes [9]. A simple antibody immobilization method is the physical (non-covalent) adsorption of a target antibody onto the substrate surface. However, due to the gradual elution of adsorbed proteins during the analytical process, this might along with the loss of sensitivity and poor reproducibility of sensors. Alternatively, considerable interests were focused on the development of covalently coupling antibodies to chemically activated surfaces by chemical covalent interaction with crosslinking agents, such as glutaraldehyde (GA) [10], carbodiimide [11], and succinimide [12], etc.

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAP) is a well-documented biomaterial mainly known for its special ability to contact bone tissue and to induce bone formation. Actually, HAP itself is a part of the inorganic ingredient of natural

* Corresponding author. Tel.: +86 731 8821861; fax: +86 731 8821967.
E-mail address: weiwz2003@126.com (W. Wei).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.04.038

bone. In a number of successful clinic applications, the bioactivity and biocompatibility of HAP have been proved [13]. For instance, HAP was used as a coating of joint replacement prosthesis and dental implants [14] as well as filling of bone defects following tumor removal. HAP coated implants, were shown to increase the quality of adhesion of structural prostheses [15] and to reduce particle release from the metal [16], playing therefore a significant role in considerably diminishing the number of rejection cases. Already well-established approaches to prepare HAP film on the metallic surfaces (mainly on orthopaedic or titanium alloy) included plasma spraying [17], magnetron sputtering [18], laser ablation [19], sol-gel processes [20] and diverse electrochemical methods [21]. The electrodeposition method was performed under a relative low temperature, thus the thermal stress did not exist between substrate and the coating interface, avoiding the phase transformation and embrittlement cracking, enhancing the binding strength between substrate and coating film, has evident advantages. Recent studies [22] indicated that by adding organic substance, the negative effects of H_2 (through water reduction) could be avoided; facilitate obtaining a more homogeneous coating. Though HAP has merits mentioned above, its potential applications in the fabrication of biosensors have not been adequately exploited. The aim of this investigation was to seek a new antibody immobilization strategy by taking advantage of the bioactivity and biocompatibility of HAP.

Nanometer-sized materials possess unique physical and chemical properties, which provide possibilities for improving some analytical assay methodologies. Nanoparticles greatly improved some immobilization performances of transduction layer with regard to loading density and bioactivity. Moreover, the resulting nanoparticles containing structure would be served as an extended matrix or a long “spacer” in favor of antibody immobilization with an increased immunoreactive flexibility and affinity to antigen.

In this study, a new antibody immobilization strategy was developed for fabrication of a sensitive label-free capacitive immunosensor by incorporating the bioactivity and biocompatibility of nano-HAP, capacitive transduction and covalently coupling method. Human transferrin immunoassay was selected as the testing system.

2. Experimental

2.1. Chemicals

Human transferrin, anti-human transferrin (developed in goat fractionated antiserum), β-mercaptoethanol, 1-dodecanethiol (DDT) and divinylsulphone (DS) were purchased from Sigma–Aldrich (USA). The reconstitution and storage of the antibody were according to the instructions of Sigma. Human serum albumin (HSA) was obtained from Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. Gold wire (0.2 mm in diameter, 99.99%) was purchased from Shanghai Chemical Reagents (Shanghai, China). Chitosan (deacetylation degree, DD: 86.4%; viscosity: 60 MPa s) was generously donated by Shanghai Haiqi Co. (Shanghai, China).

All other chemicals were of analytical reagent grade or better. Double-distilled water was used throughout the experiments.

0.01 M phosphate buffer saline solution (PBS) of pH 7.4 served as the background electrolyte. Probe solution was the mixture of 5 mM $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$, containing 0.01 M PBS (pH 7.4).

2.2. Apparatus

A CHI 660A electrochemical workstation (Shanghai Chenhua Apparatus Co.) with CHI software, connecting to a personal computer was used for electrochemical data collecting and electrodeposition controlling. Experiments were performed at room temperature ($20 \pm 1\,^{\circ}C$) in a conventional three-electrode system comprising a gold disk electrode (0.2 mm in diameter), a platinum wire auxiliary electrode and a saturated calomel electrode (SCE) (reference electrode). All potentials were reported with respect to the reference electrode.

Prior to use, glassware in the following experiments were immersed in 0.1 M HNO_3 solutions overnight after being cleaned, and were then rinsed with double-distilled water and dried in air.

Electrodeposition experiments were performed in a 50-mL homemade quartz glass cell with a thermostatic jacket. A magnetic stirrer (model 79-1, Shanghai Hexin Technique and Education Equipment Co., China) was used to agitate the electrodeposition solution in the reaction cell. The temperature for electrodeposition was controlled with a thermostat (model 501, Shanghai Experimental Equipments Co., China).

The hydroxyapatite samples were studied by a Fourier transform infrared spectrometer (FTIR) in model WQF-410 (Beijing second optical instrument factory, China). KBr discs were prepared with the powder of the standard HAP sample and the powder was scrapped from the coating film, respectively.

The surface morphology of modified electrodes was analyzed by a scanning electron microscope (SEM) (JEOL JSM-5600 LV).

2.3. Electrode pretreatment

Gold electrode was constructed by encapsulating a high pure gold wire (diameter 0.2 mm) in a glass tube by epoxy resin. The real surface area of the gold electrode was determined by the integration of the cathodic peak of cyclic voltammograms during the reduction of superficial AuO according to literatures [23,24].

Before each new measurements, the gold electrode was sequentially polished with 1.0, 0.3, and 0.05 μm α-Al_2O_3 power slurries on a 1200 grit Carbimet disk, rinsed thoroughly

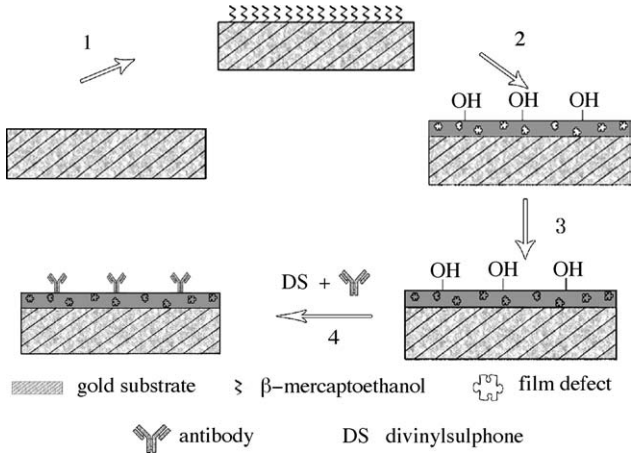

Fig. 1. Schematic illustration of the stepwise process involved in the immobilization of antibody onto the gold electrode: (1) self-assembled sublayer of β-mercaptoethanol; (2) electrodeposition of nanometer-sized HAP on the modified gold surface; (3) blocking the film defects by DDT; and (4) covalently coupling transferrin antiserum with DS on the HAP-coating film.

with redistilled water at each step, until a mirror shiny surface appeared, and was then sonicated in ethanol and double-distilled water for 3 min to remove trace alumina and possible contamination.

To ensure a clean electrode surface, the polished electrode was dipped in a 0.5 M H_2SO_4 solution, and then potential was cycled between 0 and 1.5 V until reproducible cyclic voltammograms were obtained.

2.4. Electrode modification

The entire immobilization procedure is shown schematically in Fig. 1.

2.4.1. Self-assembling of β-mercaptoethanol

First, cleaned and surface-activated gold electrodes were immersed in a 10 mM β-mercaptoethanol–ethanol solution at 4 °C for 12 h to obtain a self-assembled monolayer, then rinsing with ethanol and dried in a N_2 stream.

2.4.2. Electrodeposition of nano-hydroxyapatite film on the electrode

The method for HAP-coating electrodeposition results from a precipitation reaction described elsewhere. The electrodeposition solution was prepared as follows. First, two initial solutions were prepared. Solution A containing 4×10^{-4} M $Ca(NO_3)_2$, 2.5×10^{-4} M $(NH_4)HPO_4$ and 0.1 M $NaNO_3$. Solution B was a 1% chitosan solution, prepared by dissolving chitosan flakes in 1% acetic acid aqueous solution. The prepared chitosan solution was colorless and stored in a refrigerator (4 °C) when not in use. Before electrodeposition experiment, appropriate volume of Solution B was added and dispersed in Solution A by stirring, and then the pH value was adjusted to 6.0 using a concentrated NaOH solution. Fifty millilitres electrodeposition solution was heated to 90 °C in the reaction cell, and then a cathodal constant current of 1–4 mA/cm^2 was applied on the working electrode for 60 min. The HAP film coated electrode was washed for few min in a stirring ethanol solution, then drying in a N_2 stream.

2.4.3. Blocking film defects

The HAP coated electrode was immersed in a 10 mM DDT ethanol solution overnight, following the electrodeposition procedure.

2.4.4. Coupling with divinylsulphone (DS)

The coupling solution containing 2% (w/w) DS was deoxidized by bubbling with highly pure N_2 for 5 min, and then the HAP film coated electrode was soaked in the deoxidized coupling solution for 30 min. After this treatment, the coupling-layer surface was rinsed with 0.01 M PBS (pH 7.4).

2.4.5. Covalently immobilization of immunoreagent

The gold electrode with coupled layer was immersed in a 0.01 M PBS solution of pH 7.4 containing 0.125 μg/mL anti-human transferrin at 4 °C for 24 h, and then washed with abundant amount of 0.01 M PBS solution (pH 7.4).

2.5. Transferrin detection

The immunosensor was mounted in a three-electrode configuration as working electrode. After the base line became stable, appropriate amount of human trasferrin in PBS solution (pH 7.4) was injected into the electrochemical cell. The background solution was 10 mL PBS of pH 7.4 and was slowly stirred. The capacitance change of the immunosensor was measured by electrochemical impedance model. Impedance-time measurements were performed at the open circuit potential with amplitude of 5 mV. The working frequency was decided by Bode plots.

3. Results and discussion

3.1. Electrodeposition of hydroxyapatite

Though the methods of HAP coating on the metallic substrates were already studied in detail, to the best of our knowledge, the approach for HAP coating on the gold substrate has never been reported. Our preliminary experiments indicated that it seems to be very hard for HAP deposition onto the gold electrode surface, and the coating film will shed off very easily. This may be interpreted that the polished gold surface and only a physical adsorption lead to the poor mechanical adhesion. As it is well known, alkane chain thiol gives a head group of sulfur with a strong preferential adsorption to the Au substrate, the bonding between them is thus strong (bond energy 44 kcal/mol) and contributes to the stability of the SAMs. Therefore, a self-assembled β-mercaptoethanol monolayer was formed on the gold surface before electrodeposition procedure with the aim to

improve the adhesion strength of the coating film. Chitosan (CHIT), a polysaccharide biopolymer, due to the presence of reactive amino and hydroxyl function groups, displays excellent film-forming ability, high water permeability, good adhesion and susceptibility for chemical modifications. For further improved quality of HAP-coating film, CHIT was introduced into the electrodeposition solution. The volume of 1% chitosan solution added in 250 mL electrodeposition solution has an optimum value of 2.5 mL. After incorporating the β-mercaptoethanol SAM and CHIT introduction, the adhesion of HAP film was greatly strengthened.

3.2. FTIR and SEM characterization

The HAP-coating film on the gold electrode was confirmed by FTIR. A representative FTIR spectrum of the HAP film scraped from the electrode is shown in Fig. 2a. The multiplets located around 1000 cm^{-1} are originated by phosphate groups. The split bands, mainly at 1039 and 1092 cm^{-1}, seem to be concerned with the formation of a well-crystallized apatite. Carbonate bands were detected at 1415 cm^{-1}. The presence of hydroxyl groups in the HAP film gives rise to characteristic bands at 3570 and 631 cm^{-1}, which could be assigned to the intensity of the stretching and libration. These coincided with the standard HAP FTIR spectrum, as shown in Fig. 2b.

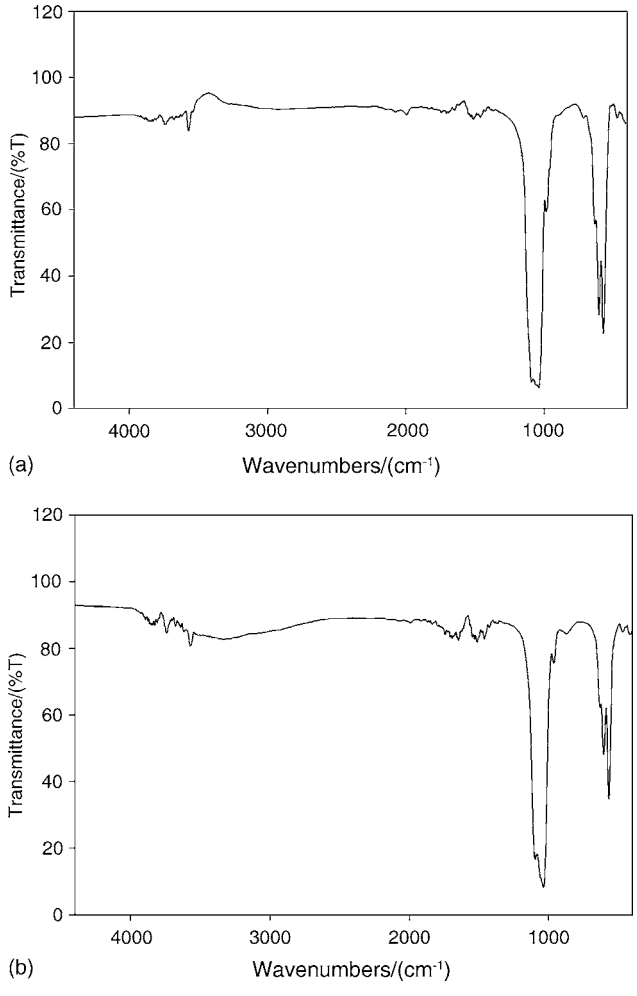

Fig. 2. FTIR spectrum of: (a) HAP sample scrapped from the gold electrode after electrodeposition; (b) standard HAP sample (resolution: 4 cm^{-1}; range: 4400–400 cm^{-1}).

SEM was used to investigate the surface morphology of the coating films. Fig. 3a is the SEM image of a bare gold electrode surface. The SEM image of the HAP-coating film obtained under a cathodal constant current density of 2 mA cm^{-2} and electrodeposition for 60 min at 90 °C is shown in Fig. 3b. Many granular HAP crystals with diameters ranging from 100 to 200 nm were observed. Experimental results indicated that the current density plays the key role for the coating quality. A bigger current density will lead to the increase in hydrogen bubbles at the solid-liquid interface. This may result in a heterogeneous and unstable coating. If a smaller current density will be applied, the compact and well-ordered HAP-coating film will obtain. The SEM image of HAP film obtained under a constant current density of 1 mA cm^{-2} is shown in Fig. 3c. As it can be seen, the HAP granules were in the nanometer scale size, homogeneously distributed throughout the electrode surface. The mean diameter was 50–100 nm, much smaller than that obtained under the current density of 2 mA cm^{-2}. The former surface structure was in favor of enhancing the bioactivity of HAP and possesses a bigger specific surface. Therefore, the current density of 1 mA cm^{-2} was adopted in the present study.

3.3. Electrochemical characterization of sensitive layer

The capacitive transduction requires sensitive layer in both low leakage and thickness. The quality of capacitive sensor ultimately depends on the insulating property of the sensitivity of layer. Cyclic voltammetry (CV) of electroactive species is a valuable mean to probe the insulating property of a layer on the electrode. The insulatibity can be examined using CV with permeable redox couple, such as $[Fe(CN)_6]^{3-/4-}$ in the solution. To characterize the insulatibity of the electrode at each modification step, CV was performed in a 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ probe solution containing 0.1 M KCl. Curve (a) in Fig. 4 shows the cyclic voltammogram using the bare Au electrode. A pair of typical redox peaks corresponding to the redox label can be found. Curve (b) shows the CV profile using β-mercaptoethanol modified electrode. It can be found that the peak current dropped significantly in the probe solution after β-mercaptoethanol modification. The remarkable decrease in the peak current in curve (c) of Fig. 4 is obviously related with the formation of electrodeposition film, covering the electrode surface towards the redox probe to a large extent and leading to the suppression of the voltammetric response. However, the peak current can still be observed in the voltammograms.

To improve the dielectric property further, the electrode surface is supposed to be covered as completely as possible. For this purpose, a long-chain alkylthiol, DDT was used to

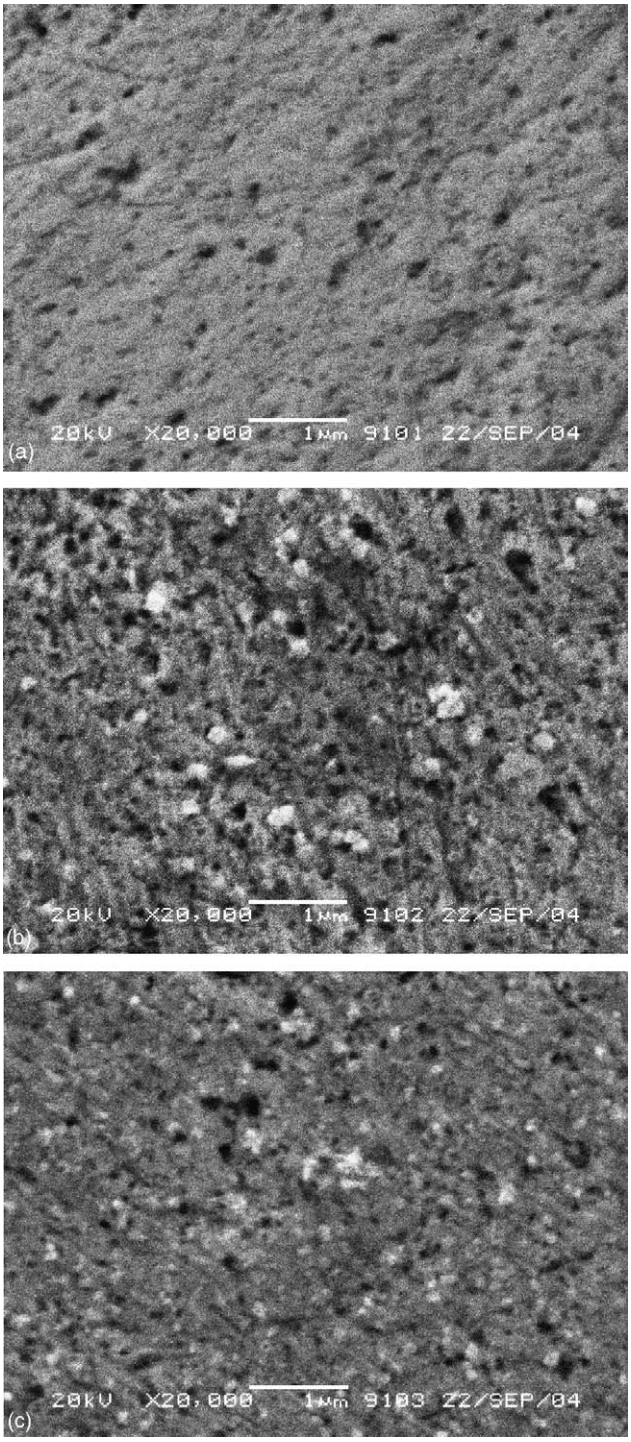

Fig. 3. SEM image of: (a) bare gold electrode surface; (b) HAP-coating film obtained under the cathodal constant current electrodeposition method with current density of 2 mA cm^{-2}; and (c) HAP-coating film obtained under the cathodal constant current electrodeposition method with current density of 1 mA cm^{-2}.

block the defects on HAP-coating film. After immersing in a 10 mM DDT ethanol solution overnight, the peak current disappeared completely, as shown in curve (d) of Fig. 4. Then, the HAP coated electrode with good insulation was prepared and qualified for capacitive transduction.

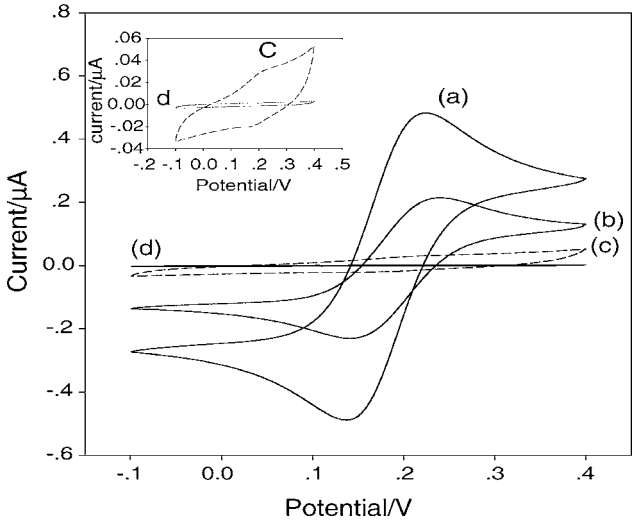

Fig. 4. Cyclic voltamogramms recorded in 5 mM $K_3[Fe(CN)_6]$/$K_4[Fe(CN)_6]$ (1:1)-mixture PBS solution of pH 7.4 containing 0.1 M KCl: (a) bare gold electrode; (b) gold electrode modified with β-mercaptoethanol; (c) β-mercaptoethanol modified electrode coated with electrodeposited nano-HAP film; and (d) further modified with 10 mM DDT ethanol solution. Scan rate: 50 mV s^{-1}. Inset is the magnification of curves (c) and (d).

3.4. Immobilization of antibody

Hydroxyl groups contained in the HAP-coating film can be chemically activated by glutaraldehyde (GA) or other crosslinking agents. A comparison investigation of the immobilization procedures reported by Si and co-workers [25,26] indicated that the stability of covalently immobilized antibodies coupling by divinylsulphone (DS) was superior to other reagents. Therefore, a 2% (w/w) DS solution was used in the present work.

The HAP-coating gold electrode treated by DS was dipped into the pH 7.4 PBS solution containing transferrin antiserum. The capacitance measurement showed that after coupling at 4 °C for 24 h, the sensor reached a stable capacitance value. The capacitance value reduced gradually along the increase in the antibody concentration at 0.125 μg/mL, and then levels off. This means that the DS activated HAP-coating film has been saturated by anti-human transferrin. Thus, the concentration of 0.125 μg/mL was selected for antibody immobilization. A newly fabricated capacitive immunosensor possessed an initial capacitance value of 3.56 ± 0.5 nF.

3.5. Capacitive detection of transferrin

The capacitive transduction is based on the double layer capacitance theory. The capacitance between two parallel plates was given by the equation below:

$$C = \frac{\varepsilon_0 \varepsilon A}{d}$$

where ε_0, ε, A and d are the vacuum dielectric constant, dielectric constant of the material between plates, plate area and distance between the plates, respectively.

Electrochemical impedance spectroscopy (EIS) is a powerful technique for the characterization of interface phenomena [27]. The promise of EIS is that with a single experimental procedure encompassing sufficiently broad range of frequencies, the influence of the governing physical and chemical phenomena may be isolated and distinguished at a given applied potential. McNeil et al. [28] discussed the fundamental principles of EIS in detail. The relationship between capacitance and impedance can be given by equation [29]:

$$C = -\frac{1}{2\pi f Z_{\text{im}}}$$

where f is the working frequency (Hz), Z_{im} is the imaginary part of impedance. The capacitance value can be directly calculated using the equation above.

According to Randles equivalent circuit model [30], the precise capacitance detection requires that the measurements must be performed in a regime that the transduction layer exhibits a near-ideal capacitor behavior, i.e., the phase angle value close to -90°.

In this study, at the frequency of 962 Hz, sensors fabricated as the procedure above demonstrated excellent capacitive properties. Consequently, the imaginary impendence factor (Z_{im}) at 962 Hz was selected as the working frequency for the capacitance detection. Because of the possible deviations in electrode surface and film thickness, the relative capacitance change ($\Delta C/C_0$), was defined to indicate the response of capacitive sensor to minimize the difference between different sensors.

Due to the specific interaction of antigen-antibody, in the presence of antigen, an additional layer will be formed on the surface of immunosensor, which decreased the interfacial capacitance. The capacitive change depends on the concentration of antigen in the testing solution. By determining capacitance change versus time, the interaction between transferrin and transferrin antiserum in the electrochemical cell can be real-time monitored without additional label procedure. The real-time sequent capacitance response for transferrin detection was recorded in curve (a) of Fig. 5. As it can be seen, along with the increase of transferrin concentration in the bulk-supporting electrolyte, the capacitance change (ΔC) decreased. To some extent, the relative capacitive change ($\Delta C/C_0$) tend to be stable, the saturation was reached after an incubation time of 15–20 min. The total relative capacitive change exceeded 29%.

To verify the effect of non-specific adsorption of protein on the immunosensor, human serum albumin (HSA) that at the same concentration level of human transferrin was injected into the measuring cell. The control experiment was recorded in curve (b) of Fig. 5. The relative capacitive change observed was not more than 5%, which can be rationally interpreted as the physical adsorption of HSA. This confirmed that in the case of capacitive sensor for human transferrin detection, the specific immunoreaction between antibody and antigen is essential for the change of capacitance, not the non-specific absorption of protein to the sensor surface.

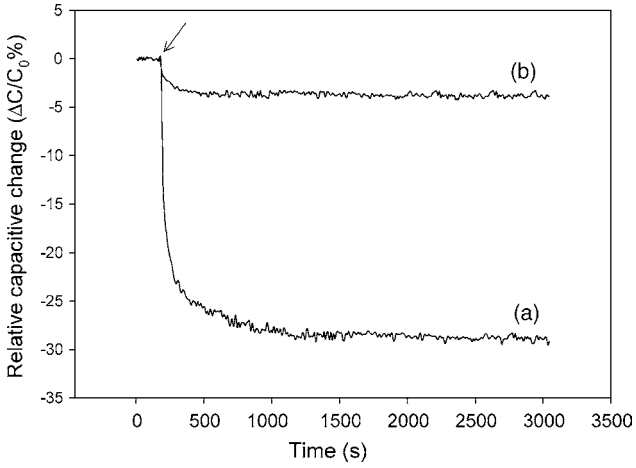

Fig. 5. Real-time monitor of the interaction between transferrin antiserum and human transferring. (a) A testing solution with transferrin concentration of 100 ng/mL. (b) Control experiment: the concentration of HSA was the same as that of human transferrin used in (a). The arrow indicates the point of samples introduction.

Samples of 1–175.0 ng/mL transferrin were added into the measuring cell separately and the relative capacitive changes were recorded when they leveled off and reached the maximum values. The relative capacitive change versus the transferrin concentration was found to give a linear relationship between 1 and 100 ng/mL. A detection limit of 0.15 ng/mL was calculated from the smallest measurable value of the capacitance variation under the optimal experimental conditions. The linear calibration graph of $\Delta C/C_0$ versus C_x can be described by the following equation:

$$\frac{\Delta C}{C_0}\,(\%) = -0.2603 C_x\,(\text{ng/mL}) - 5.8526$$

($r = 0.9957$, linear range: 1–100 ng/mL), where ΔC is the capacitance change during adsorption, C_0 the initial capacitance, and C_x the concentration of transferrin.

3.6. Activity regeneration of immunosensor

The exhausted immunosensor can be regenerated by simply washing with subacidity solution. After carefully washed in a stirring glycine-hydrochloric acid solution (pH 3.5) for 30 min, the human transferrin combined on the sensitive layer was released. The regenerated immunosensors were subjected to the background solution of PBS (pH 7.4) for capacitance measurement to confirm whether their initial capacitances were restored or not. Results indicate that the initial capacitance of the sensors can be restored to about 90% of their original values. The parallel restoring experiments showed that the activity-restored immunosensor could provide nearly response compared to the newly fabricated ones up to 10 assay cycles and without significant loss of sensitivity.

4. Conclusions

A new antibody immobilization strategy was successfully developed for the fabrication of a label-free capacitive immunosensor. Based on electrodeposition of nanometer-sized bioactive hydroxyapatite, covalently coupled with DS and capacitive transduction, the sensor was applied for human transferrin detection. The fabrication and the specificity of the immunosensor were discussed in detail. Compared with traditional immunoassays, the merit of this approach is that it achieved the real-time monitoring the antibody–antigen interaction and omitted the labeling procedure to simplify analysis process. The immobilization strategy developed in the present work can be applied for the fabrication of other specific immunosensor.

Acknowledgements

We are indebted to the financial support of Doctorate Fund (No. 20020532007) and Key Sci/Tech Res. Project (No. 03123) of Education Department, China.

References

[1] B. Eric, T.D. Martin, Anal. Chem. 74 (2002) 2781.
[2] B. Eric, Anal. Chem. 76 (2004) 3285.
[3] S.F. D'Souza, Appl. Biochem. Biotechnol. 96 (2001) 225.
[4] D.A. Butterfield, D. Bhattacharyya, S. Daunert, L. Bachas, J. Membr. Sci. 181 (2001) 29.
[5] D.G. Castner, B.D. Ratner, Surf. Sci. 500 (2002) 28.
[6] L.C. Shriver-Lake, W.B. Gammeter, S.S. Bang, M. Pazirandeh, Anal. Chim. Acta 470 (2002) 71.
[7] N.P. Huang, J. Vörös, S.M.D. Paul, M. Textor, N.D. Spencer, Langmuir 18 (2002) 220.
[8] S. Cosnier, Biosens. Bioelectron. 14 (1999) 443.
[9] D.A. Butterfield, J. Colvin, J.L. Liu, J.Q. Wang, L. Bachas, D. Bhattacharrya, Anal. Chim. Acta. 470 (2002) 29.
[10] Y.Q. Zhang, M.L. Tao, W.D. Shen, Y.Z. Zhou, Y. Ding, Y. Ma, W.L. Zhou, Biomaterials 25 (2004) 3751.
[11] I.S. Park, D.K. Kim, N. Adanyi, M. Varadi, N. Kim, Biosens. Bioelectron. 19 (2004) 667.
[12] A.K. Singh, A.W. Flounders, J.V. Volponi, C.S. Ashley, K. Wally, J.S. Schoeniger, Biosens. Bioelectron. 14 (1999) 703.
[13] L.L. Hench, J. Am. Ceram. Soc. 74 (1991) 1487.
[14] S. Morita, K. Furuya, K. Ishihara, N. Nakabayashi, Biomaterials 19 (1998) 1601.
[15] G.L. Lange, K.I. Donath, Biomaterials 10 (1989) 121.
[16] S.R. Sousa, M.A. Barbosa, Biomaterials 17 (1996) 397.
[17] P.L. Yu, M.S. Li, S.T. Li, Z.G. Wang, R.F. Zhu, Biomaterials 25 (2004) 4393.
[18] K. Yamashita, T. Arashi, K. Kitagaki, S. Yamada, T. Umegaki, K. Ogawa, J. Am. Ceram. Soc. 77 (1994) 2401.
[19] O. Guillot-NoeK, R.R. Gomez-San, J. Perriere, J. Hermann, V. Craciun, C. Boulmer-Leborgne, J. Appl. Phys. 80 (1996) 1803.
[20] W.J. Weng, J. Mater. Sci.-Mater. Med. 9 (1998) 159.
[21] J. Redepenning, T. Schlessinger, S. Burnhum, L. Lipiello, J. Miyano, J. Biomed. Mater. Res. 30 (1996) 287.
[22] A.M. Moursi, A.V. Winnard, P.L. Winnard, J.J. Lannutti, R.R. Seghi, Biomaterials 23 (2002) 133.
[23] Y.J. Liu, Z.H. Zhang, L.H. Nie, S.Z. Yao, Electrochim. Acta 48 (2003) 2823.
[24] G. Tremiliosi-Filho, L.H. Dall'Antonia, G. Jerkiewicz, J. Electroanal. Chem. 422 (1997) 149.
[25] Y.S. Fung, S.H. Si, D.R. Zhu, Talanta 51 (2000) 151.
[26] S.H. Si, H.F. Chen, X. Chen, J. Instrum. Anal. 21 (2002) 33 (in China).
[27] A.S. Baranski, T. Krogulec, L.J. Nelson, P. Norouzi, Anal. Chem. 70 (1998) 2895.
[28] C.J. McNeil, D. Athey, M. Ball, W.O. Ho, S. Krause, R.D. Armstrong, J.D. Wright, K. Rawson, Anal. Chem. 67 (1995) 3928.
[29] W.O. Ho, S. Krause, C.J. McNeil, J.A. Pritchard, R.D. Armstrong, D. Athey, K. Rawson, Anal. Chem. 71 (1999) 1940.
[30] H. Berney, J. West, E. Haefele, J. Alderman, W. Lane, J.K. Collins, Sens. Actuators B 68 (1–3) (2000) 100.