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A gold nanoparticles/sol–gel composite architecture for encapsulation of immunoconjugate for reagentless electrochemical immunoassay

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Abstract

A highly hydrophilic, non-toxic and conductive colloidal gold nanoparticle/titania sol–gel composite membrane with a low contact angle was prepared on a glassy carbon electrode via a vapor deposition method. With human chorionic gonadotrophin (hCG) as a model antigen and encapsulation of horseradish peroxidase-labeled hCG antibody (HRP-*anti*-hCG) in the composite architecture, this membrane could be used for reagentless electrochemical immunoassay. It displayed a porous and homogeneous composite architecture without the aggregation of the immobilized protein molecules. The presence of gold nanoparticles provided a congenial microenvironment for adsorbed biomolecules and decreased the electron transfer impedance, leading to a direct electrochemical behavior of the immobilized HRP. The formation of immunoconjugate by a simple one-step immunoreaction between hCG in sample solution and the immobilized HRP-*anti*-hCG introduced a barrier of direct electrical communication between the immobilized HRP and the electrode surface. Under optimal conditions, the hCG analyte could be determined in two linear ranges from 0.5 to 5.0 mIU/mL and 5.0 to 30 mIU/mL with a relatively low detection limit of 0.3 mIU/mL at 3σ. The hCG immunosensor exhibited good precision, high sensitivity, acceptable stability, accuracy and reproducibility. This composite membrane could be used efficiently for the entrapment of different biomarkers and clinical applications.

Keywords: Composite; Gold nanoparticles; Immunosensor; Sol–gel; Hydrophilicity

1. Introduction

The immunoassays combining the specific antigen–antibody (Ag–Ab) recognition for analytical purposes have been successfully applied to many fields including food industry [1], environmental protection [2] and clinical control [3–5]. Electrochemical immunoassays and immunosensors are drawing more attention in a wide range of uses, especially for determination of clinically important substances [4–6], due to their advantages such as simple pretreatment procedure, fast analytical time, precise and sensitive current measurements, and inexpensive and miniaturizable instrumentation [7].

In the development of electrochemical immunosensing strategies, stability or activity of the immobilized biocomponents and signal amplification of the immunoconjugates are two key factors. The latter has been achieved by using enzymes to label immunocomponents on transducer surface [8,9]. A lot of enzyme labels like horseradish peroxidase (HRP) [6,10,11], alkaline phosphatase [8], laccase [9] and glucose oxidase [12] have been used to produce the electrochemically active species for amperometric immunoassay along with some substrates added in the test solutions including hydroquinone [6], catechol [10], *o*-aminophenol [11], naphthyl phosphate [13], *p*-aminophenol phosphate [8], ferrocene [9] and glucose [12]. These amperometric immunosensors have been successfully applied to the measurement of progesterone in cow's milk [13], creatinine [14] and bacteria such as *Escherichia coli* and *Salmonella* [15]. In order to avoid the requirement of

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substrate addition, a reagentless immunosensor has been developed by the utilization of detecting the direct electrical communication between redox sites of labeled enzyme and sensing surface [16]. Nevertheless, such direct electron transfer is not easily achieved with most immobilized enzymes. Furthermore, the conventional immunosensing based on solid supports has been limited due to the lack of a simple protein immobilization approach that might avoid the denaturation of the bound redox enzymes.

Colloidal gold nanoparticles have been extensively used as an immobilized matrix for retaining the bioactivity of macromolecules such as proteins [17], enzymes [18] and antibodies [19] and promoting the direct electron transfer of the immobilized proteins [20]. They have also been employed for synthesis of functional materials [21] and electrochemical sensing [22] by combining with sol–gel (SG) technique to form a composite structure. In order to resolve two problems mentioned above, this work proposed a novel strategy to construct the reagentless immunosensor by encapsulating immunocomponent-adsorbed gold nanoparticles in titania SG matrix. This composite architecture formed via a vapor deposition method, which was developed in our laboratory [23], showed an efficient scaffold of nano-scale dimension with an orderly structural organization of a large variety of nanoparticles. It not only provided a very hydrophilic interface for retaining the bioactivity and improving the stability of the immobilized enzyme labels and immunocomponents, but also promoted the electrical communication between redox sites of enzyme labels and sensing surface for direct electrochemical immunoassay of protein or antigen analytes. A novel immunosensor for human chorionic gonadotrophin (hCG) was thus been constructed based on this promising approach.

hCG, a 37 kDa glycoprotein hormone, is an important diagnostic marker of pregnancy and one of the most important carbohydrate tumor markers. In earlier study several electrochemical immunosensors for hCG have been proposed [8,24]. A separation-free sandwich-type enzyme immunoassay based on the immunosensor could detect hCG down to 2.5 mIU/mL [8]. Recently, various immunoassay kits and strategies for hCG measurements have been reported [6,25–28]. By utilization of SG and nanotechnology, the immunosensor proposed in this work showed good sensitivity and stability, and could be prepared in mass-production. Compared with some other reports, the novel detection strategy exhibited an acceptable accuracy and appeared to be simple, practical, convenient and significant in clinic diagnoses.

2. Materials and methods

2.1. Material

hCG enzyme-linked immunosorbent assay (ELISA) kits were purchased from BioCheck, Inc. (Foster city, USA). The ELISA kits consisted of a series of hCG reference standard solutions containing 0, 5, 20, 50, 150 and 300 mIU/mL, an enzyme conjugate reagent of HRP-labeled hCG monoclonal antibody (HRP-*anti*-hCG) from mouse and a hCG zero buffer for in vitro diagnostic incubation. Bovine serum albumin (BSA) was the product of Sigma (St. Louis, MO USA). In the measuring system, 0.1 M pH 7.1 phosphate buffer solution (PBS) was used as the electrolyte. The dilute solution of hCG standard or sample solution was 0.1 M pH 7.1 PBS containing 0.04% BSA and 1.0 mM ethylenediamine tetraacetic acid. All other reagents were of analytical grade. All solutions were made up with deionized water of 18 MΩ purified from a Milli-Q purification system.

2.2. Preparation of hCG immunosensors

Glassy carbon electrodes (5 mm in diameter) were successively polished with 0.3 and 0.05 μm alumina slurry (Beuhler) to a mirror and rinsed thoroughly with twice-distilled water after each polishing step. After sonicleaning successively in 1:1 nitric acid, acetone and twice-distilled water, the electrodes were rinsed with twice-distilled water and allowed to dry at room temperature.

Colloidal gold solution was prepared by adding 0.5 mL 1% trisodium citrate solution to 50 mL boiling solution of 0.01% $HAuCl_4$ (Aldrich, USA), which was maintained at boiling point for 15 min and stirred for another 15 min after removing the heating source. The TEM image of the obtained colloidal gold nanoparticles indicated a homogeneous-dimensional distribution of these nanoparticles (Fig. 1a). After mixing colloidal gold solution with HRP-*anti*-hCG (1:1, *V*/*V*), a solution of HRP-*anti*-hCG-adsorbed gold nanoparticles (HRP-*anti*-hCG/nano-Au) was obtained. The TEM image did not display obvious aggregation of the adsorbed HRP-*anti*-hCG molecules and change in diameter and distribution of gold nanoparticles upon the adsorption (Fig. 1b).

After dropping 10 μL of HRP-*anti*-hCG/nano-Au solution on GCE surface, the electrode was suspended vertically above titanium isopropoxide in a sealed conical flask and kept at a temperature of 25 °C for 4 h to form a HRP-*anti*-HCG/nano-Au encapsulated titania SG membrane (HRP-*anti*-HCG/nano-Au-SG) [23]. After rinsing thoroughly with twice-distilled water, the obtained immunosensor for hCG was refrigerated at 4 °C prior to electrochemical measurements.

2.3. Electrochemical immunoassay

The one-step immunoreaction was accomplished on the immunosensor surface. Firstly 5 μL hCG standard or sample solution was diluted with 40 μL dilute solution and 40 μL hCG zero buffer to a total volume of 85 μl in an incubation cup. Then the immunosensor was incubated in the solution at 30 °C for 40 min and washed carefully with twice-distilled water to obtain an immobilized immunoconjugate layer, named as hCG/HRP-*anti*-hCG/nano-Au-SG/GCE. The amount of the immobilized immunocomplex depended on the concentration of hCG in standard or sample solution.

The detection solution was 1.0 mM pH 7.1 PBS, which was purged thoroughly with highly pure nitrogen for 10 min and maintained in nitrogen atmosphere at 20 ± 0.5 °C. The differential pulse voltammetric (DPV) measurements were performed from −100 to −700 mV with the pulse amplitude of 50 mV and the pulse width of 50 ms.

2.4. Apparatus

Transmission electron micrograph (TEM) images were recorded on a JEOL-JEM 200CX transmission electron microscope, using an accelerating voltage of 200 kV. The samples used for TEM observation were prepared by placing a drop of sample onto a copper grid coated with a layer of amorphous carbon. Scanning electron micrographs (SEM) of the membranes were obtained on glass slices with a Hitachi X-650 scanning electron microscope (Hitachi Lid, Tokyo, Japan) at an acceleration voltage of 25 kV.

Contact angle measurements were performed at 20 °C with a sessile drop method (Model 100-0, Rame-Hart Inc, USA). Reported contact

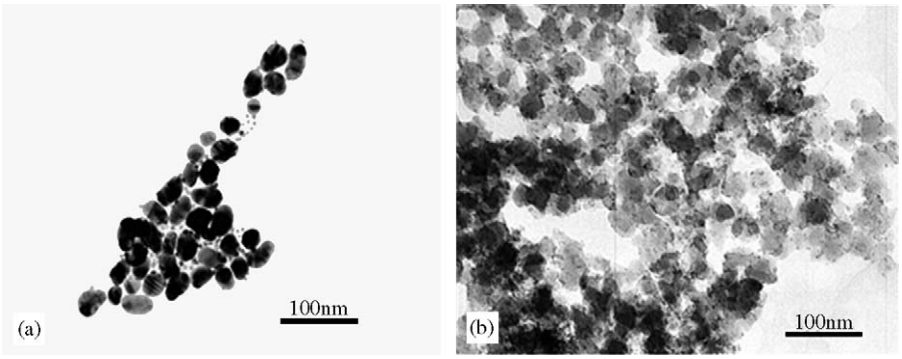

Fig. 1. Transmission electron micrographs of (a) nano-Au, and (b) HRP-*anti*-hCG/nano-Au particles.

angles were averages of measurements on three samples. The Access™ analyzer (Beckman Coulter, Inc., Chaska, USA) was used to detect hCG antigen in human serum by an automated chemiluminescent immunoassay according to the instruction and assay procedure in the operator's manual.

Faradic impedance measurements were performed on a PGSTAT30/FRA2 system (Autolab, The Netherlands) in 0.1 M pH 7.1 PBS containing 0.1 mM $Fe(CN)_6^{3-/4-}$, using a bias potential of 0.235 V and an alternating current voltage of 5 mV in the frequency range of $0.01–1 \times 10^6$ Hz. Voltammetric measurements were performed on a BAS-100B electrochemical analyzer (Bioanalytical Systems Inc., USA) with a conventional three-electrode system comprising platinum wire as auxiliary electrode, saturated calomel electrode (SCE) as reference electrode and a modified glassy carbon electrodes (GCE) as working electrode.

3. Results

3.1. Electrochemical behaviors of modified electrodes

No cyclic voltammetric peak was observed at both bare GCE and HRP-*anti*-hCG/SG/GCE in the working potential range in 0.1 M pH 7.1 PBS (curves a and b in Fig. 2). The HRP-*anti*-hCG/nano-Au-SG/GCE showed a stable and well-defined redox peaks at −0.308 and −0.454 V (vs. SCE) at 50 mV/s, respectively (curve c in Fig. 2), while no peak was observed at nano-Au-SG/GCE.

After HRP-*anti*-hCG/nano-Au-SG/GCE was incubated for 40 min in 85 μL incubation solution prepared with 5 μL standard solution without presence of hCG, the background current greatly decreased with a slightly positive shift of the cathodic peak potential, while the cathodic peak current decreased by only 3.6% (curve d in Fig. 2). Further incubation in an incubation solution containing 0.29 mIU/mL hCG, however, led to a decrease of the cathodic peak current by 67.2% (curve e in Fig. 2).

3.2. Effect of pH of detection solution on voltammetric response

Fig. 3 shows the strong dependence of both cathodic peak current and formal potential obtained from the cyclic voltammograms of HRP-*anti*-hCG/nano-Au-SG/GCE at 50 mV/s on solution pH. The optimal response occurred in the pH range of 6.0–7.1 with a maximum value at pH 7.1. Consequently, pH 7.1 PBS was selected for electrochemical immunoassay. Furthermore, the plot of formal potential versus pH showed a slope of -65.3 ± 0.94 mV/pH in the pH range of 5.3–8.2, which was close to the theoretical value of −57.6 mV/pH at 18 °C for a reversible one-proton coupled single electron transfer process.

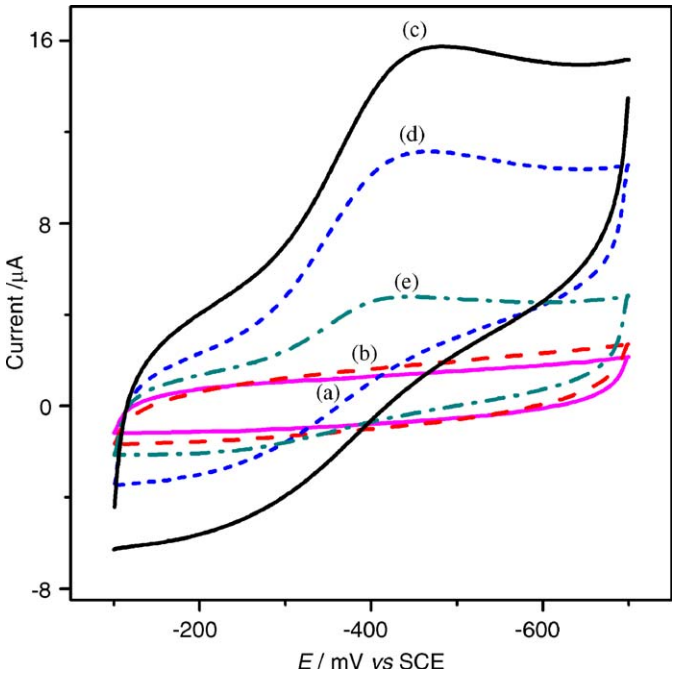

Fig. 2. Cyclic voltammograms of different electrodes in pH 7.1 PBS at 50 mV/s: (a) bare GCE, (b) HRP-anti-hCG/SG/GCE, (c) HRP-anti-hCG/nano-Au-SG/GCE, (d, e) (c) after incubated in 85 μL incubation solution containing 5 μL of 0 (d) and 5.0 (e) mIU/mL hCG at 30 °C for 40 min.

3.3. Characterization of HRP-anti-hCG/nano-Au-SG membranes

Fig. 4 shows the SEM images of different membranes. The micrograph of titania SG membrane displayed a chemically plane and clean uniform structure (Fig. 4a). This uniform open structure provided a rigid cage for loading of HRP-*anti*-hCG/nano-Au particles and good preparation reproducibility of immunosensors. The entrapment of colloidal gold nanoparticles in the SG membrane produced the aggregates of the nanoparticles

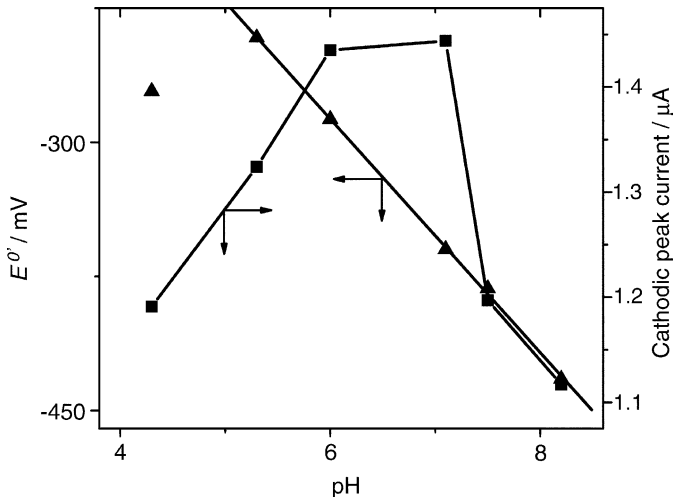

Fig. 3. Effect of pH on formal potential and dependence of cathodic peak current determined at 50 mV/s on solution pH.

(Fig. 4b). When HRP-*anti*-hCG was encapsulated in the titania SG matrix, the uniform open structure of SG could be retained, and the SEM image showed bright particles of HRP-*anti*-hCG aggregates (Figs. 4c, d). After HRP-*anti*-hCG/nano-Au was encapsulated in the SG matrix, the formed HRP-*anti*-hCG/nano-Au-SG showed a well-ordered distribution of gold nanoparticles doped in the three-dimentional framework of protein and SG membrane (Figs. 4e, f).

The contact angles of bare GCE, SG membrane and nano-Au-SG membrane surface were 76.5, 40 and $20 \pm 3^{\circ}$, respectively.

3.4. Impedance characterization of HRP-anti-hCG/nano-Au-SG/GCE

Fig. 5 shows the AC impedance spectra of $Fe(CN)_6^{3-/4-}$ at bare GCE, nano-Au-SG/GCE, HRP-*anti*-hCG/SG/GCE and HRP-*anti*-hCG/nano-Au-SG/GCE, respectively, obtained in 0.1 M pH 7.1 PBS. The data analysis of these impedance spectra gave the electron transfer resistance of $Fe(CN)_6^{3-/4-}$ to be 101 ± 3, 2850 ± 14, 6309 ± 259 and $4414 \pm 67\,\Omega$, respectively. It was clearly observed that the formation of SG layer increased remarkably the impedance, which further increased upon the entrapment of enzyme conjugate (curves a, b and c in Fig. 5). The introduction of colloidal gold nanoparticles decreased the impedance of enzyme conjugate entrapped SG layer by 30% (curves c and d in Fig. 5).

3.5. Optimization of immunoreaction conditions

The factors that influenced the immunoreaction for immunoassay of analyte included the surface character and electron transfer behavior of the immunosensors, incubation temperature and incubation time in the incubation solution. The former was related to the ratio of HRP-*anti*-hCG to colloidal gold nanoparticles used for immunosensor preparation. The effect of the volume fraction of HRP-*anti*-hCG of given concentration to colloidal gold solution on DPV peak current at a fixed total volume was shown in Fig. 6A. With the increasing amount of colloidal gold nanoparticles, the peak current increased and reached a maximum value at the volume fraction of 0.5 (1:1, *V*/*V*). More amount of the nanoparticles resulted in great decrease of the amperometric response due to the decrease of surface concentration of immobilized HRP. Thus, the immunosensor was constructed by mixing 5 μL HRP-*anti*-hCG stock solution and 5 μL colloidal Au solution.

Fig. 6B shows the effect of incubation temperature on amperometric response of obtained immunocomplex. The increase of incubation temperature was favorable to the formation of the immunocomplex that increased the barrier of electron transfer between immobilized HRP and sensing surface, thus reducing the amperometric response. The amperometric response was readily decreased until a platform response was observed at an incubation temperature of 30 °C. The incubation temperatures higher than 30 °C resulted in further decrease of the response, which was possibly due to the change in architecture of HRP-*anti*-hCG/nano-Au composite and denaturation of the immobilized HRP. At the incubation temperature of 30 °C, the amperometric response of obtained immunocomplex decreased with an increasing incubation time, and reached a platform at 40 min (Fig. 6C), indicating the saturated formation of immunocomplex in the matrix. Longer incubation time did not result in further decrease of the amperometric response. The optimal incubation conditions were at 30 °C for 40 min.

3.6. Amperometric response of immunosensor to hCG concentration

Under optimal incubation conditions, the hCG molecules in incubation solution occupied the limited binding sites of the encapsulated HRP-*anti*-hCG to form immunocomplex. The decrease percentage of DPV peak current attributed to the direct electron transfer of the immobilized HRP in detection solution increased with an increasing hCG concentration (Fig. 7A). The plot of the average decrease percentage of peak currents ($n = 5$) vs. hCG concentration in standard solution or original sample for preparation of incubation solution showed two lines in the ranges from 0.5 to 5.0 and 5.0 to 30.0 mIU/mL with the slopes of (5.38 ± 0.10) and (1.30 ± 0.06) mIU^{-1} mL and the correlation coefficients of 0.9998 and 0.9982, respectively (Fig. 7B). From the slope of 5.38 mIU^{-1} mL the detection limit was calculated to be 0.3 mIU/mL at 3σ. When the dilution fraction of 5/85 (*V*/*V*) for preparation of incubation solution was taken into account, the practical linear ranges were from 0.03 to 0.29 and 0.29 to 1.76 mIU/mL, and the detection limit in the incubation solution was 0.02 mIU/mL.

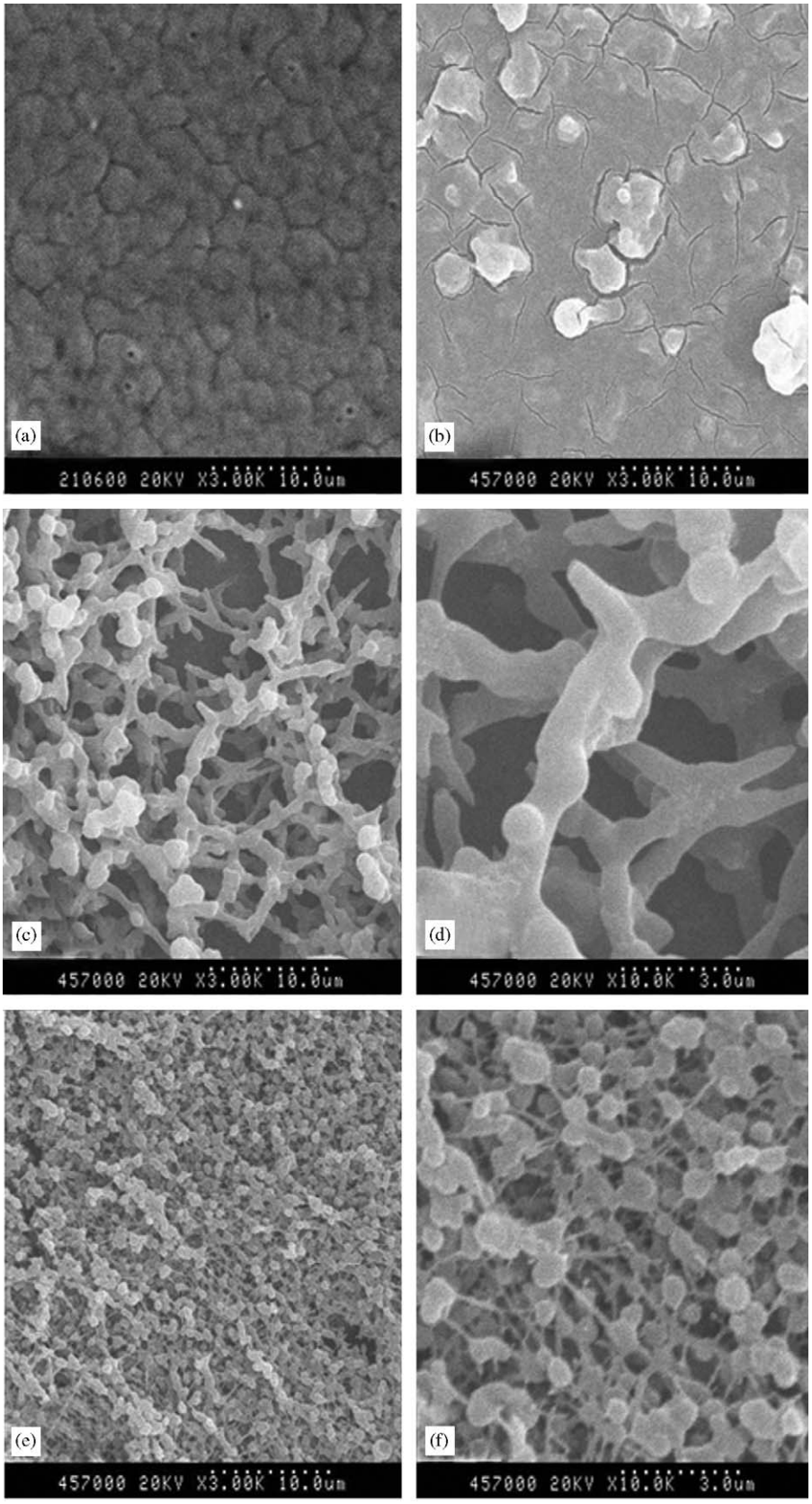

Fig. 4. Scanning electron micrographs of sol–gel (a), nano-Au-SG (b), HRP-*anti*-hCG/SG (c, d) and HRP-*anti*-hCG/nano-Au-SG (e, f) membranes at $\times 3.0$ K (a, b, c, e) and $\times 10$ K (d, f).

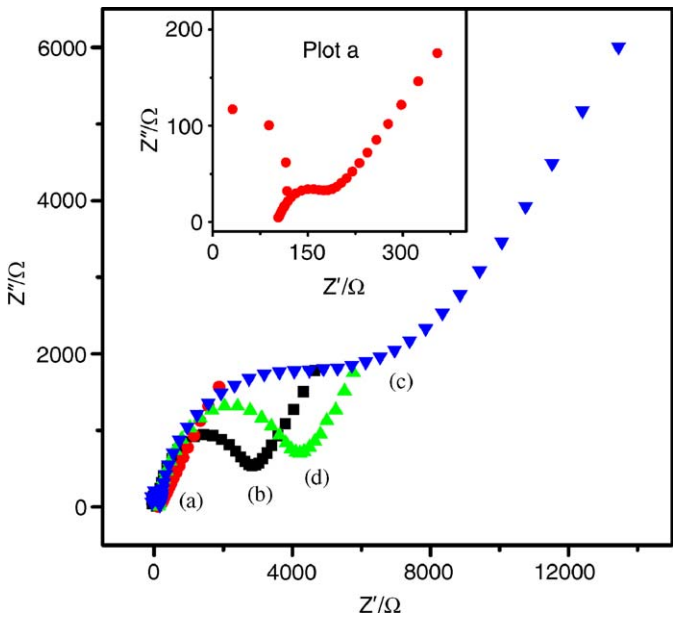

Fig. 5. AC impedance spectra of 0.1 mM $Fe(CN)_6^{3-/4-}$ in 0.1 M pH 7.1 PBS at (a) bare GCE, (b) nano-Au-SG/GCE, (c) HRP-*anti*-hCG/SG/GCE and (d) HRP-anti-hCG/nano-Au-SG/GCE.

3.7. Selectivity and analytical performance of the immunosensors

Using an incubation solution containing 10 mIU/ml carbohydrate antigen 19-9 (CA19-9) and 10 mIU/ml hCG, the selectivity of proposed hCG immunosensor was examined by detecting the amperometric responses. No difference of currents was observable in comparison with the result obtained in presence of only hCG. The increase of CA19-9 concentration to some extent did not lead to a significant current change. Furthermore, after the immunosensor was incubated in 10 mIU/ml hCG followed by rinsing with stripping buffer of pH 2.0 glycine-HCl to remove the hCG from the Ag–Ab immunocomplex, the obtained amperometric signal restored the 95% of the initial value. Thus, the immunosensor had a good selectivity to hCG as well as an acceptable regeneration efficiency.

After five serum samples from different persons were diluted with the appropriate volumes of dilution solution, the serum hCG concentrations were detected with the calibration curve of the hCG immunosensors.

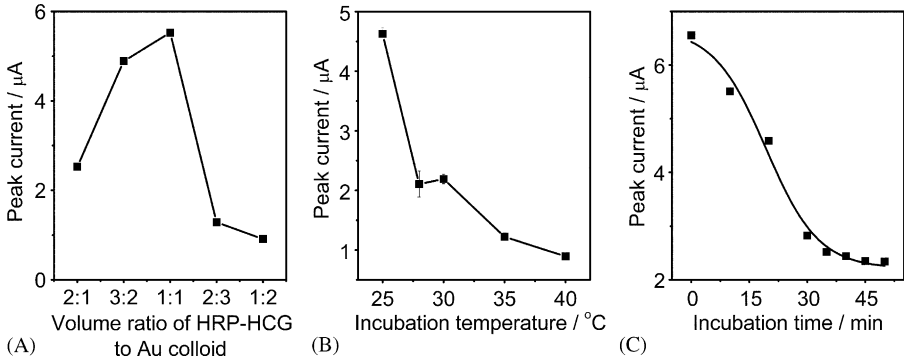

Fig. 6. Effect of volume ratio of HRP-*anti*-hCG to colloidal gold solutions for immunosensor preparation on DPV peak current (A) and dependence of DPV peak current of hCG/HRP-*anti*-hCG/nano-Au-SG/GCE in 0.1 M pH 7.1 PBS on incubation temperature for 40 min (B) and incubation time at 30 °C (C) in 85 μL incubation solution containing 5 μL 5.0 mIU/mL hCG.

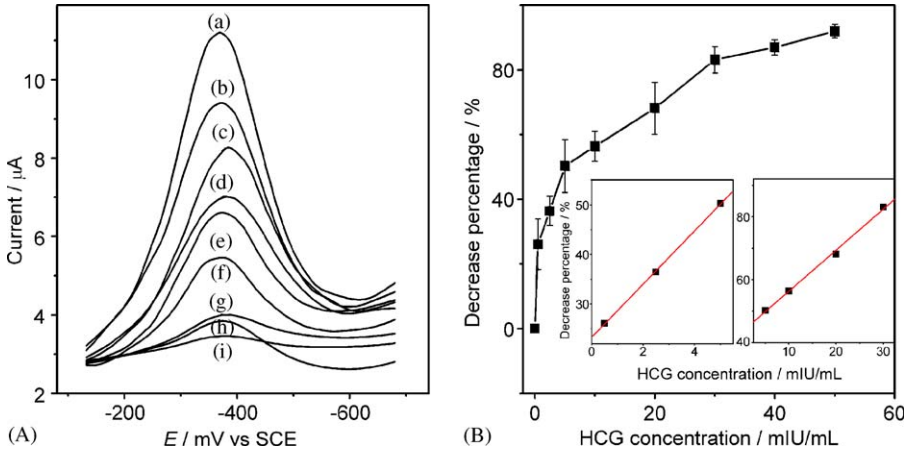

Fig. 7. DPV of hCG/HRP-*anti*-hCG/nano-Au-SG/GCE obtained by incubating immunosensor in mixture of 5 μL (a) 0, (b) 0.5, (c) 5.0, (d) 10.0, (e) 20.0, (f) 30.0, (g) 40.0 or (h) 50.0 mIU/mL hCG with 40 μL dilute solution and 40 μL hCG zero buffer at 30 °C for 40 min (A) and plot of decrease percentage of peak current vs. hCG concentration in standard solution for preparation of incubation solution (B). Insets in B show linear calibration.

Table 1
Comparison of serum hCG levels determined using two methods

Serum samples	1	2	3	4	5
This method (mIU/mL)	110.5	438.2	494.4	596	1046.8
Access^{TM} (mIU/mL)	119	430.6	490	528.7	1006
Relative deviation (%)	−7.1	1.8	0.9	12.7	4.1

In comparison, the concentrations were detected with Access^{TM} analyzer, a commonly used analyzer in clinic laboratory. The results were listed in Table 1. The relative deviations between two methods were in the range of −7.1%–12.7%.

The intra-assay precision of the immunosensor was examined by successively assaying the hCG levels of two sera for five times. The variation coefficients of the results with this method were 8.2% and 8.1% at the hCG concentrations of 5 and 20 mIU/mL in serum samples, respectively. The inter-assay precision, or the fabrication reproducibility, was estimated by determining in duplicate the hCG level in one serum sample with three immunosensors made at the same electrode independently. The inter-assay precision of this method was 4.8% at the hCG concentration of 10 mIU/mL, showing acceptable fabrication reproducibility.

The performance stability of the biosensor was examined by storage in air and in pH 7.1 PBS, separately. The biosensor lost its sensitivity rapidly if stored in air. However, when the immunosensor was stored in pH 7.1 PBS at 4 °C, it retained 90% of its initial current after a storage period of 30 days.

4. Discussion

This study was mainly focused on the development of highly hydrophilic, non-toxic and conductive composite architecture, which would be useful in biosensing and immunosensing applications. To attain that goal, a colloidal gold nanoparticles/SG composite structure was successfully constructed on a glassy carbon electrode to encapsulate HRP-labeled hCG antibody. Such composite matrix showed good hydrophilicity and non-toxicity and was effective for tunneling electrons between immobilized HRP and electrode surface and retaining the bioactivity of immobilized biomolecules.

From the recorded amperometric signal (Fig. 2), the couple of peaks were ascribed to the direct electron transfer between the redox centers of the immobilized HRP and the sensing surface. Obviously, the presence of colloidal Au particles promoted the electron transfer. The reason was that the colloidal Au could provide a congenial microenvironment similar to that of redox proteins in a native system for retaining their bioactivity [18] and allow the protein molecules more freedom in orientation [29], thus facilitated close approach of the protein to the sensing surface, reduced the insulating property of the protein shell for the direct electron transfer [20] and could be thought of as ''electron antennae'' to shuttle electrons between the electrode and the immobilized HRP [17,20,22]. At the same time the formed titania SG provided a three-dimensional homogenous matrix for the adsorption of proteins on gold nanoparticles and facilitated direct electrical communication between enzyme and electrode surface with the aid of gold nanoparticles [16,23].

After the immunosensor was incubated in a solution without presence of hCG, the 3.6% decrease of cathodic peak current was attributed to the slight loss of the activity of immobilized enzyme, thus the immunosensor was stable during the incubation period. The decrease in background current was due to the non-specific adsorption of BSA in the incubation solution. After formation of the immunocomplex, which resulted in a stereo hindrance of the direct electron transfer between the immobilized HRP and sensing surface, such peak current decreased further, which was similar to that reported previously [30]. This phenomenon also indicated that some hCG molecules could readily diffuse toward the encapsulated HRP-*anti*-hCG [31], and the close contact between bound HRP and Au nanoparticles played an important role in direct electrochemistry of the HRP. The weak interaction of enzyme labeled antibody with antigen alternated the status of the close contact, led to a loss of direct electrical communication between immobilized HRP and the electrode surface, thus providing a sensing approach of antigen analyte.

In most cases, the acidity of the detection solution greatly affects the redox behavior of enzymes. The optimal amperometric response occurred at pH value of 7.1 in solution (Fig. 3) which was just the optimum pH value for living organisms. Similar results were also obtained for HRP in solution [32] and immobilized on colloidal gold modified carbon paste electrode [33], indicating that the nano-Au-SG matrix did not alter the optimal pH value for redox behavior of immobilized enzyme. The decrease in the response in high pH solutions was due to the charge change of mercapto or primary amine groups in HRP-*anti*-hCG, which weakened their electrostatic interaction with colloidal gold nanoparticles. The dependence of formal potential on solution pH indicated the electrode process involved the participation of proton for neutralizing the excess charge in electrode reaction process. Another important factor affecting the formation of immunocomplex and the amperometric signal of immobilized HRP was the surface morphology of the HRP-*anti*-hCG/nano-Au-SG membrane. From the SEM pictures (Fig. 4), the aggregation of HRP-*anti*-hCG molecules made the electron transfer between immobilized HRP and electrode surface more difficult. However, the aggregation of both protein molecules and colloidal gold nanoparticles could be improved by mixing HRP-*anti*-hCG with colloidal gold solution. The latter uniform structure allowed for free binding of hCG molecules to the encapsulated HRP-*anti*-hCG and resulted in electron-conducting pathways between the immobilized hCG and electrode surface.

Compared with the bare GCE, the presence of titania SG membrane obviously decreased the value of contact angle. Entrapping colloidal gold nanoparticles in the membrane could further decrease the contact angle. As well known, the smaller the contact angle value is, the better the surface hydrophilicity are. Colloidal gold nanoparticles greatly improved the surface hydrophilicity, thus made the immobilized enzyme conjugate more stable. Compared with other biomaterials such as poly(ethyleneglycol) monoacrylate (PEGA) grafted on polymethylmethacrylate (80.1°), PEGA on polyethylene (74.0°), PEGA on silicone (107.3°), PEGA on polyurethane (78.9°) [34], nano-Au-SG composite film showed a lower contact angle value and better hydrophilicity and compatibility with biomolecules. From the impedance measurements (Fig. 5), we could also know that the nanoparticles distributed in the SG matrix played a role similar to a conducting wire or electron-conducting tunnel, which made the direct electron transfer easier [29] and reduced the impedance of electron transfer. The impedance difference between curves b and d also showed that HRP-*anti*-hCG had been attached to the electrode surface.

The proposed detection limit was 0.3 mIU/mL at 3σ. This value was much lower than the threshold value of 14.3 mIU/mL hCG in serum considered necessary for reliable pregnancy diagnosis [35] and those of 11.2 mIU/mL [6], 0.8 mIU/mL (2.5 mIU/mL in blood) [8], 2 mIU/mL [26] and 100 mIU/mL [36] reported with heterogeneous hCG immunoassays based on electrochemical analytical principle. When compared with the results obtained from the clinically common used Access^{TM} analyzer on the same human serum samples, the two methods were in acceptable agreement. Thus, the proposed method and presented immunosensor could be used for detection of low level of hCG in practical serum sample. Higher serum hCG levels could be determined with an appropriate dilution. A long storage period of 30 days also indicated that the colloidal gold nanoparticles/SG composite structure was very efficient for retaining the bioactivity and electrical communication ability of the immobilized enzyme conjugate, and preventing the biomolecules from leaking out because of the strong interaction between colloidal gold nanoparticles and mercapto or primary amine groups in these biomolecules.

5. Conclusions

This work develops a novel nano-Au/SG composite structure for encapsulating HRP-labeled hCG antibody on glassy carbon electrode. Such non-toxic composite matrix shows good hydrophilicity and is effective for tunneling electrons between immobilized HRP and electrode surface and retaining the bioactivity of immobilized biomolecules. Thus, a reagentless immunosensor for hCG in serum samples is presented. Under optimal conditions, the immunosensor can be applied to the determination of low level of serum hCG without the requirement of separation or washing steps, when compared with those reported previously [6,26,36]. The immunosensor shows good precision and acceptable sensitivity, fabrication reproducibility and storage stability, which could be readily extended toward the measurement of other clinically important tumor markers.

Acknowledgements

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