Int. J. Electrochem. Sci., 6 **(2011)** 2845 - 2858

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

One Renewable and Magnetic Electrochemiluminescence Immunosenor Based on Tris(2,2'-bipyridine) ruthenium(II) Modified Magnetic Composite Nanoparticles Labeled Anti-AFP

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Received: 12 May 2011 / *Accepted:* 15 June 2011 / *Published:* 1 July 2011

A renewable and magnetic electrochemiluminescence (ECL) immunosensor for alpha-fetoprotein (AFP) was fabricated by using RuL-MWNTs (Tris(2,2'-bipyridine) ruthenium(II) complex immobilized on multiwall carbon nanotubes through π-π stacking function between the carbon aromatic systems) doped Au (RuL-MWNTs@Au) composite as labels. Polyelectrolyte poly(dimethyldiallylammonium chloride) (PDDA) was employed to covered on the surfaces of RuL-MWNTs which made it have positive electric charge and can absorb negative colloid Au nanoparticles to fabricate RuL-MWNTs@Au through electric attraction. The primary antibody, anti-AFP was first immobilized on the Fe_3O_4(core)/gold(shell) nanoparticles (Fe_3O_4@Au, GMP) as probes. Then the antigen and the RuL-MWNTs@Au composite nanoparticles labeled $Ru(bpy)_3^{2+}$-conjuged secondary antibody (RuL-MWNTs@Au~RuLAb2) were combined successively to form RuL-MWNTs@Au~RuLAb2/AFP/anti-AFP-GMP sandwich-type immunocomplexes through the specific interaction. The resulting magnetic sandwich immune complexes can be absorbed on the planar screen printed carbon electrode (SPCE) due to the external magnetic field added on the bottom of it. The magnetic sandwich complexes can be easily washed away from the surface of SPCE when the magnetic field was removed which made the immunosensor reusable. The prepared RuL-MWNTs@Au composite nanoparticles own large surface area, good biocompatibility and highly effective ECL properties. The morphologies of the RuL-MWNTs@Au and its antibody labeled composite nanoparticles were characterized by transmission electronic microscope (TEM). The sensor performed high sensitivity and wide liner for detection AFP in the range of 0.01-50 $ng \cdot mL^{-1}$ and the limit detection was 3 $pg \cdot mL^{-1}$ (defined as S/N=3). Furthermore the sandwich immunocomplexes can be performed the rapid separation from the unbound secondary antibody quickly by magnetic field. So the immunosensor can simultaneously realize separation, enrichment and determination, with high sensitivity, which would be valuable for clinical immunoassay for AFP in human serum.

Keywords: Tris(2,2'-bipyridine) ruthenium(II)-MWNTs@Au, Magnetic probes, Alpha-fetoprotein, Sandwich-type immunoreactions, Screen printed carbon electrode, Electrochemiluminescence

1. INTRODUCTION

Tumor markers (TMs) used in oncological diagnosis are useful in detection and determination of clinical stages of cancer and radicalism of operative procedure and in monitoring of therapy [1-2]. The development of highly sensitive measurement systems for detecting trace levels of alpha-fetoprotein (AFP) in serums is very important for the early diagnosis of liver cancer [2]. Compared with conventional immunoassays such as enzyme-linked immunosorbent assay (ELISA), electrochemiluminescence (ECL) immunoassayhas attracted considerable interest in wide range for its intrinsic advantages such as high sensitivity, low cost and fast analysis which have been applied in many fields, such as environmental pollutant determination, pharmaceutical analysis, and immunoassay because of its high sensitivity and selectivity, simple instrumentation and low cost [3-6]. Among various ECL systems, Tris(2,2'-bipyridine) ruthenium(II) complex based ECL gained more attention owing to its superior properties of high sensitivity and good stability under moderate conditions in aqueous solution [7-12]. In recent years, with the development of nanoscience and nanotechnology, a variety of nanoparticles, such as silica carbon nanotube, carbon sphere and gold nanoparticles, have been applied as the labels to immobilize RuL in nanoparticle-based amplification platforms which can dramatically enhance the signal intensity of ECL immunosensor and lead to ultrasensitive bioassays [13-17]. Yuan and co-workers have proposed a novelly sensitive ECL immunoassay with AFP antibody labeled RuL-SiO_2@Au as probes [18]. However it required complex modification procedures on the electrode. Furthermore the sensor can not be renewable after the immune products formed on electrode.

However, it is still a critical demand on simple, rapid, sensitive, and low-cost detection technologies for the earlier and sensitive profiling of TMs, especially in the point-of-care applications. Despite many advances in the field, it is still a challenge to explore and exploit new schemes and strategies for improvement of the sensitivity and simplicity of ECL immunosensor. The enhancement of labeled RuL capacity on nanoparticles' surface is very important to develop feasible immobilization methods to amplify the ECL signals. Recently, as one kind of carbon nanostructure, carbon nanotube (CNT) has stimulated a vast amount of research due to its fascinating properties. CNT can work as a promoter to increase the surface area and improve the electron transfer at the electrode interface. It can also be used as carriers to load a large amount of ECL labels and biomolecules and thus afford substantial ECL signal amplification and the enhancement of performances of the biosensors. So it's fit for the matrix to lable the TMs antibody [19-21]. Many ruthenium complexes with polypyridyl ligands possess rich photochemical and photophysical properties, some of them have been fabricated on the CNTs surface by covalent attachment or non-covalent π-π-stacking interactions approaches. For example, $[Ru(bpy)_2(dcbpy)]^{2+}$ or $[Ru(bpy)_3]^{2+}$ was interconnected to the multi-walled CNTs (MWCNTs) for monitoring ammonia in atmosphere or increasing their photoconductivity [22, 23]. In addition, poly(diallyldimethylammonium chloride) (PDDA) or CNTs-PDDA has been used in biosensing owing to the good film-forming ability and susceptibility to chemical modifications [24, 25]. Conducting polymer has been shown to be a superior matrix for fluorescence detection based immunosensors [26]. Gold nanoparticles (Au NPs) have gained attention in the last years due to the unique structural, electronic, optical, and catalytic properties which have made them become very

attractive materials for biosensor systems and bioassays [27]. If the RuL labeled antibody instead of pure antibody is employed to be labeled on ruthenium complex nanoparticles as probes, the relevant ECL immunosensor based on the probes can greatly enhance the detection sensitivity for more RuL as signal enhancement molecular are modified on the electrode. Taking into consideration the above advantages, the dual amplification RuL-CNTs@Au~RuL-Ab2 composites can become promising candidates for fabricating the novel ECL biosensors.

Conventional electrodes including glassy carbon electrode (GCE), Au electrode, magnetic carbon paste electrode (MCPE) and so on, have been used for preparing biosensor, but most of them involve some disadvantages such as relatively expensive cost, bulk mass, the difficulty of regeneration once the electrode is damaged and the time-consuming preparation process of MCPE. However, amperometric biosensors fabricated with screen printed carbon electrodes (SPCEs) have the advantages of integration of electrodes, simple manipulations, low cost and low consumption of sample which can be used for one step determination then discarded [28, 29]. Recently, magnetic nanoparticles have also gained increasing interest and have been widely applied in immunoassays [30, 31] due to their biocompatibility, superparamagnetism and good electron conductivity [32], which can simplify the process of protein immobilization and separation [33]. The magnetic nanoprobes strategy developed recently has proven to be a highly sensitive technique for detecting human tumor cells, and is especially well suited to separate and in the meantime detect low-concentrations of proteins [34, 35]. Besides using magnetic core/shell Fe_3O_4@Au [gold magnetic particle (GMP)] nanoparticles as a matrix to immobilize bimolecular has aroused great interests in recent years [36, 37]. They have both magnetic properties, owing to core Fe_3O_4, and biocompatibility, owing to shell Au, and they can be used to fix antibody and easily achieve magnetic separation. Therefore, the GMP composite nanoparticles can be used not only to immobilize AFP antibody (anti-AFP) but also to prepare "magnetic carbon nanotube" probe. More importantly, the magnetic probes can be modified and removed from its surface by magnetic field added on the flat bottom of SPCEs. All these steps can make the electrode's surface renewable and simplified the electrode's modification steps. The screen-printed (thick-film) technology, in which screen-printed carbon electrodes (SPCE) are used, is widely used for the mass production of disposable electrochemical sensors because the commercial carbon inks used for fabricating them are very cheap [38].

In this paper, we have developed a novel and simple ECL immu-nosensor based on the highly intense ECL of RuL-MCNTs@Au coupled with excellent biocompatibility and stability of GMPs labeled first antibody (anti-AFP~GMP). The formed architecture could provide an effective matrix for antibody immobilization with good stability and bioactivity. Subsequently, a multilabel-antibody functionalized RuL-MWNTs@Au composite nanoprobe was obtained and used as labels for the construction of a novel sandwich ECL immunosensor. Due to the high RuL loading capacity on MWNTs@Au bioconjugates label and the upstanding electric conductivity of MWNTs, the electrochemiluminescence response of the fabricated immunosensor was greatly enhanced and achieved the sensitive detection of AFP. More important the magnetic sandwich complexes can be easily washed away from the surface of SPCE when the magnetic field was removed which made the immunosensor reusable. Therefore, a sensitive sandwich-type ECL immunosensor based on the RuL-MWNTs@Au~RL-Ab2/AFP/anti-AFP~GMP sandwich immunocomplexes was constructed

successfully. The experimental results indicated that it exhibited good performance for detection of AFP with a wide linear range and a low detection limit.

2. EXPERIMENTAL PART

2.1. Chemicals and materials

Alpha-fetoprotein antibody (Anti-AFP, 12 $mg \cdot L^{-1}$) was from Biocell Company (Zhengzhou, China). Elecsys AFP Kits were from Roche Diagnostics GmbH. Tris(2,2'-bipyridyl)ruthenium(II) chloride Hex hydrate-($Ru(bpy)_3Cl_2 \cdot 6H_2O$) was from Strem Chemicals. Tripropylamine (TPA) was from Tokyo Kasei Kogyo Co, Ltd. Gold chloride ($HAuCl_4$) and BSA (96~99%) were bought from sinophram chemical reagent Co.Ltd (Shanghai, China). PDDA (MW: 100,000–200,000 $g \cdot mol^{-1}$, in 20% aqueous solution) was purchased from Sigma Co. Ltd. MCNTs were purchased from Shenzhen Nanotech Port Co. Ltd. Gold nanoparticles (Au-NPs, ~16 nm in diameter) were prepared according to the procedures reported by Enüstün et al [39]. Phosphate buffered solution (PBS, pH 7.4) was prepared using 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4 and 0.1 M KCl. Blocking buffer solution consisted of a PBS with 3% (w/v) BSA and 0.05% (v/v) Tween 20. Washing buffer solution consisted of a PBS with 0.1 M NaCl and 0.05% (v/v) Tween 20 (PBST).

All other chemicals were of analytical grade and all solutions were prepared with doubly distilled water.

2.2. Instrumentations and Apparatus

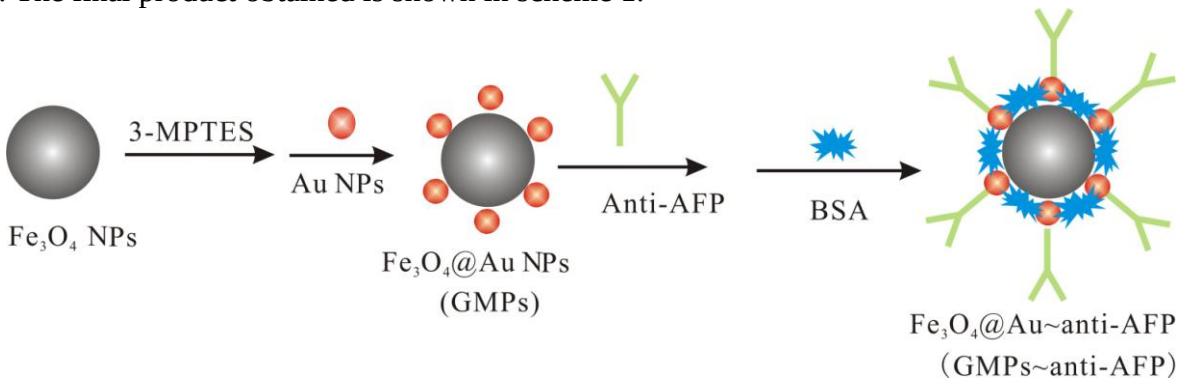
ECL experiments were carried out using a MPI-B model electrochemiluminescence analyzer (Xi'an Remax Electronic Science &Technology Co. Ltd., Xi'an, China) with the voltage of the photomultiplier tube being set at 800 V. A three-electrode system was used, which consists of a screen printed carbon working electrode (SPCE, DropSens Corporation, Spain), a carbon auxiliary electrode and an Ag/AgCl reference electrode. A H600 transmission electron microscope (Hitachi, Japan) was employed to characterize the nanoparticles.

2.3. Preparation of GMP~ anti-AFP

The Fe_3O_4@Au nanoparticles (GMPs) were obtained by absorbing gold nanoparticles onto the surface of 3-MPTES-coated Fe_3O_4 nanoparticles according to the literature with modification [40]. Experimentally, Fe_3O_4 (200 mg) in 90 mL 50% ethanol in a 250 mL flask was mixed with 3-MPTES (300 μL) and ammonium hydroxide (500 μL) under vigorous stirring. The reaction mixture was heated to 60 °C and kept for 6 h under N_2 atmosphere. The resulting precipitate was separated by magnetic decantation and washed three times with double-deionized water and ethanol respectively to give 3-MPTES-coated Fe_3O_4 nanoparticles. Following that, the pre-synthesized gold nanoparticles were added to the 3-MPTES-coated Fe_3O_4, and shaken slightly for 4 h at room temperature to make the gold

nanoparticles assemble on the surface of the formed nanoparticles. After magnetic separation, the obtained GMPs were rinsed and suspended in 10 mL double deionized water.

1.0 mL GMPs suspension was initially adjusted to pH 8.2 using Na_2CO_3, and then 1.0 mL of the original anti-AFP was added into the mixture and incubated for 24 h at 4 °C with slightly stirring. After magnetic separation, the obtained GMP~anti-AFP bioconjugates were incubated with 3.0% BSA for 1 h to block the unreacted and nonspecific sites. The synthesized GMP~anti-AFP bioconjugations were stored in 2 mL of pH 7.4 PBS at 4 °C when not in use. This procedure was repeated for three times. The final product obtained is shown in scheme 1.



Scheme.1. Schematic illustration of the preparing procedures of GMPs and GMP~anti-AFP probes

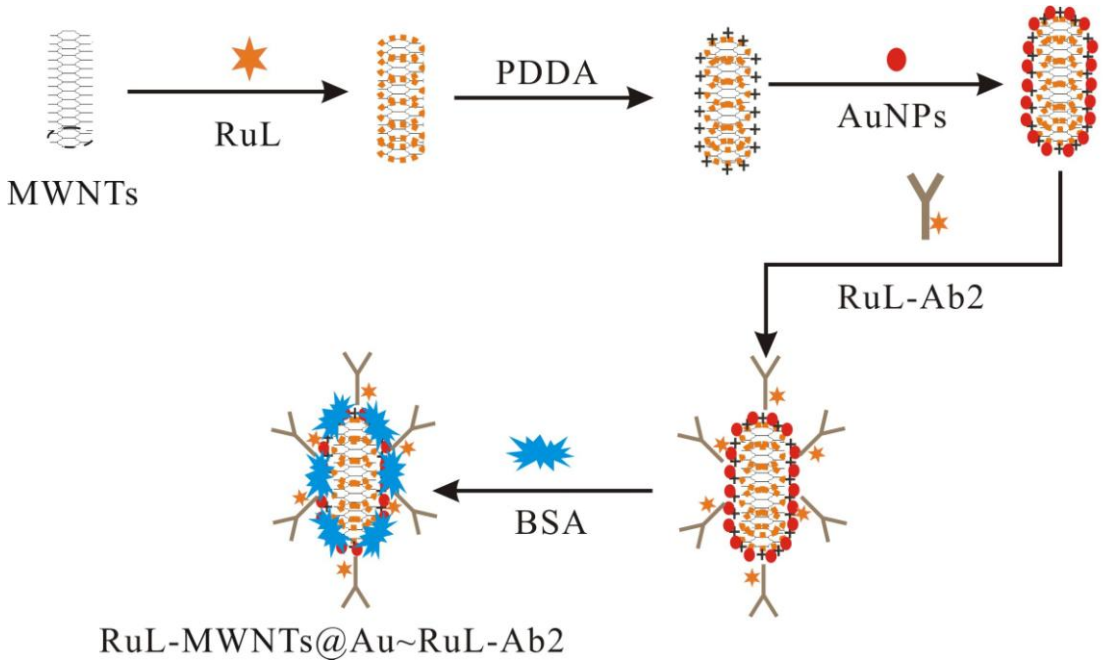
2.4. Preparation of RuL-MWNTs@Au~RuL-Ab2

The MCNTs were directly purified by ultrasonic techniques according to report in 0.5 M HCl for 4 h, then were thoroughly washed with water to get neutral state, and finally dried at 50 ºC overnight. The mixture containing 2.5 mg mL^{-1} RuL and 1 mg mL^{-1} MCNTs were mixed round for 2 h. Then, 5.0 mg RuL-MCNTs were dispersed into 30 mL aqueous solution containing 1 wt% PDDA and 0.5 M NaCl, and stirred for 3 h, residual PDDA polymer was removed by centrifugation. Finally, the PDDA coated MCNTs-RuL was mixed 9.0 mL colloidal Au NPs and sonication for 30 min. The obtained RuL-MWNTs@Au composites were washed with water and dried in vacuum oven. Then, 2.0 mg pH-8.2 RuL-MWNTs@Au composites were added to excess RuL-Ab2 by incubation at 4 ºC for 24 h, and then separated by centrifugation., washed with pH 7.4 PBS for three times. Following that, the RuL-MWNTs@Au~RuL-Ab2 was treated with 3% BSA at 35 ºC for 1 h to block the nonspecific sites. After that, the mixture was centrifuged and washed with PBS, dispersed in PBS (pH 7.4) to obtain the RuL-MWNTs@Au~RuL-Ab2 bionanocomposite and then stored at 4 °C for further use. The procedure was shown in Scheme 2.

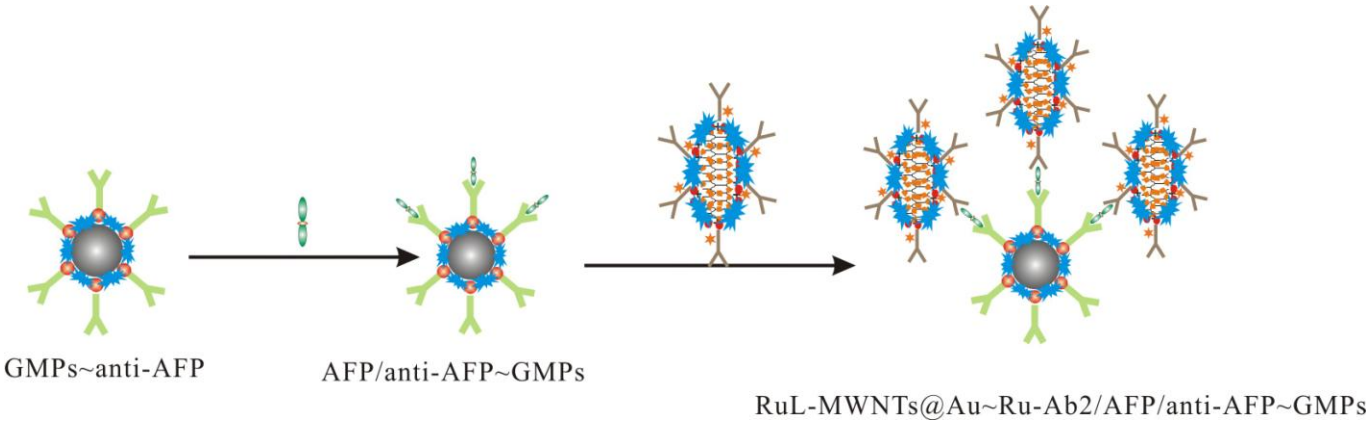
2.5. Preparation of the magnetic sandwich-type immunocomplexes

The schematic graph of the fabrication process was shown in Scheme 3. The immunocomplexes were prepared as follows: a mixture of 50 μL Fe_3O_4@ Au, 20 μL different concentrations of AFP and 50 μL RuL-MWNTs@Au~RuL-Ab2 was prepared and placed for 20 min at

room temperature. After that, the RuL-MWNTs@Au~RuL-Ab2/AFP/anti-AFP~GMP sandwich-type immunocomplexes were obtained by magnet, washed with PBST solution three times, dispersed in 50 μL PBS (pH 7.4) and stored at 4 °C for ECL tests.



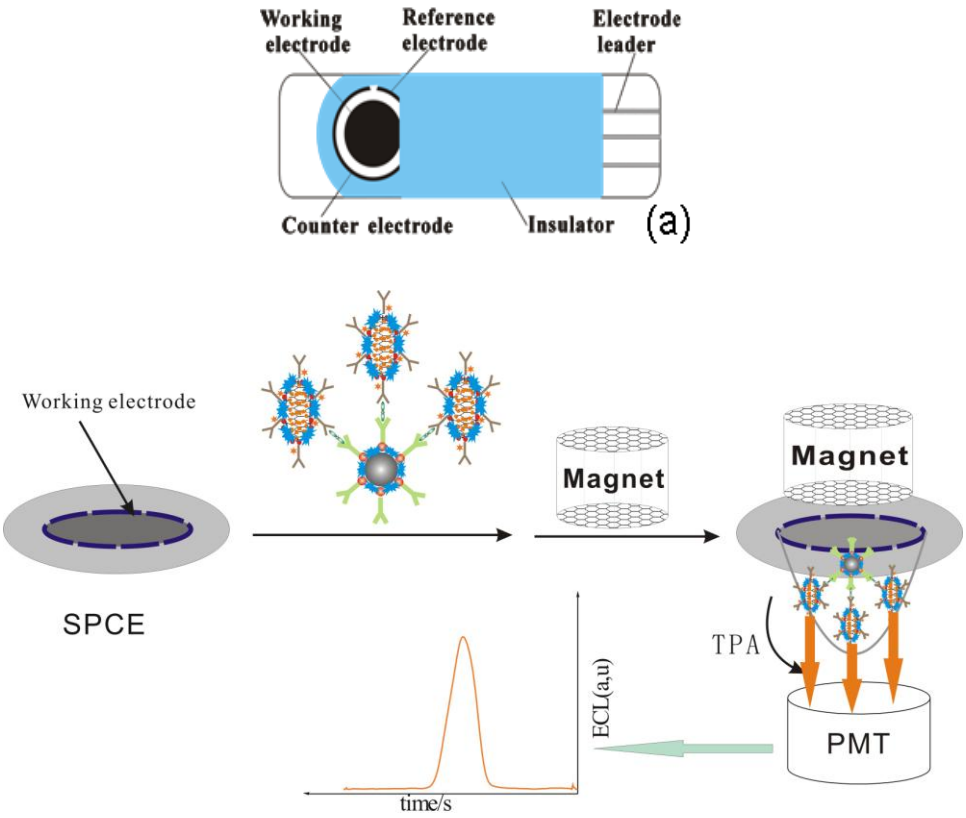
Scheme 2. Schematic illustration of the preparing procedures of RuL-MWNTs@Au ~RuL-Ab2



Scheme 3. The preparing procedures of the magnetic sandwich-type immunocomplexes.

2.6. ECL Measurements

The three-electrode system and process of ECL measurements are shown in Scheme 4. For each test, 10 μL magnetic sandwich-type immunocomplex solution prepared with different concentrations of target AFP was attached on the cleaned SPCE surface with a NdFeB permanent magnet, ECL measurements were then performed in 30 μL PBS (pH 7.4) containing 10^{-5} M TPA with a photomultiplier tube voltage of 800 V.



Scheme 4. (a) Schematic illustration of three-electrode SPCE system; (b) The process of ECL measurements

3. RESULTS AND DISCUSSION

3.1. Characterization of RuL-MWNTs@Au and GMPs~anti-AFP Nanoparticles

In this work, the core-shell GMPs were used to label anti-AFP because its high specific surface area may enhance the immobilized capacity toward anti-AFP. And RuL-MWNTs matrix loaded with Au-NPs, named RuL-MWNTs@Au, was prepared as ECL signal amplification labels and immobilization substrates for RuL labeled AFP secondary antibody (Ab2).

TEM images showed that both Fe_3O_4 and GMPs were of well spherical structure and preferable monodisperisity in size. The average diameter of Fe_3O_4 nanoparticles and core-shell GMPs were about 20 nm (Fig. 1-a) and 40 nm (Fig. 1-b), respectively. Upon deposition of gold shell to the Fe_3O_4 nanoparticles, the diameters of the particles increased by about 20 nm, demonstrating that the Au shell was about 20 nm thick. And MCNTs and RuL-MWNTs@Au membrane also were characterized using TEM. As can be seen, an obvious difference could be discerned between the microstructures of MCNTs (Fig. 1-c) and RuL-MWNTs@Au (Fig. 1-d), demonstrates that some individual Au-NPs (~20 nm diameter) and cluster-shape Au-NPs were successfully assembled on the surface of RuL-MWNTs nanoparticles.

Measurements of the surface plasmon (SP) resonance band of the nanoparticles provided an indirect piece of evidence supporting the formation of GMPs core-shell morphology. Figure 2A shows a typical set of UV-vis spectra comparing Fe_3O_4 (curve A-a) and GMPs (curves A-c). In contrast to the largely silent feature in the visible region for Fe_3O_4 particles, GMPs show a clear SP band at 532 nm. This band showed a red-shift in comparison with pure Au nanoparticles (curve A-b, 520nm). This finding suggests that Fe_3O_4 nanoparticles combined with Au nanocomposites leads to the red-shift of resonant wavelength. As shown in Figure 2B, the anti-AFP molecules being labeled onto the surface of the GMPs, two absorption peaks at 280 and 532 nm were observed (curve B-e). One peak originated in the synthesized GMPs, another derived from the absorption peak of anti-AFP proteins (curve B-d, 280 nm). On the basis of the above results, it can be concluded that GMPs~anti-AFP conjugation was successfully prepared and could be used in the electrochemiluminescence ELISA.

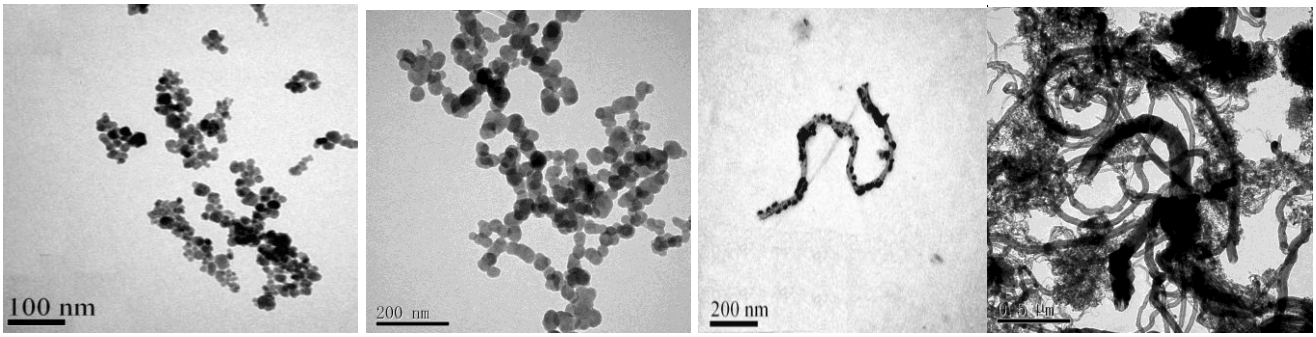


Figure.1. TEM images of (a) GMP; (b) GMP-anti AFP; (c) RuL-MWMNTs@Au; (d) RuL-MWMNTs@Au~Ru-Ab2

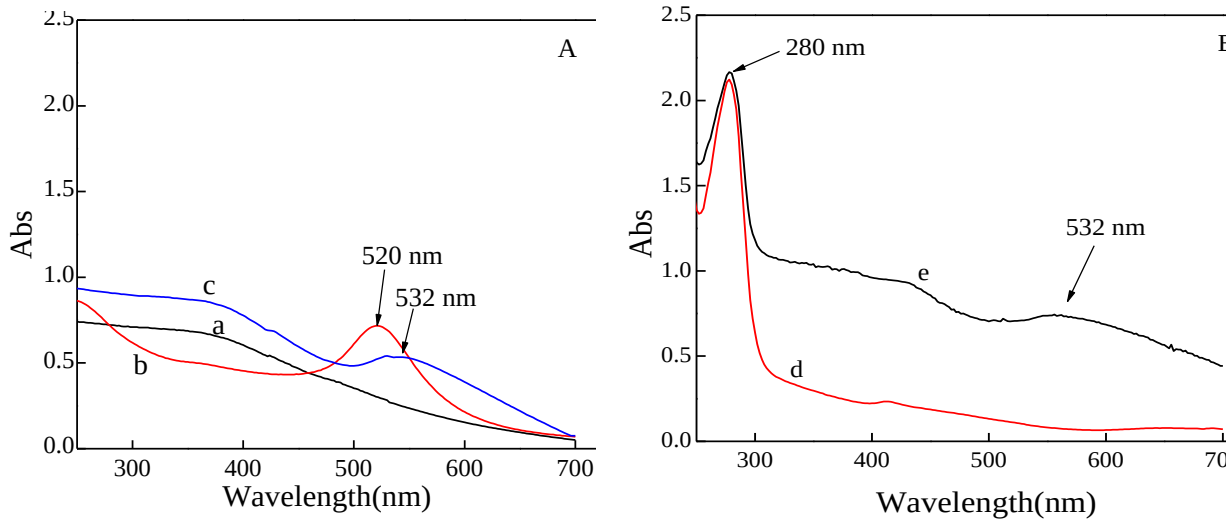


Figure.2. UV-Vis absorption spectra of (A-a) Fe_3O_4 NPs; (A-b) Au NPs; (A-c) GMPs; (B-d) anti-AFP; (B-e) GMPs~anti-AFP conjugation.

3.2. ECL characterization of RuL-MWNTs@Au~RuL-Ab2

Prior to the use of RuL-MWNTs@Au~RuL-Ab2 as labels for the preparation of ECL immunosensors, we investigated the ECL performance of RuL-MWNTs@Au~RuL-Ab2 in the presence of TPA. For sandwich-type immunosensors, the sensitivity is mainly determined by the sensitivity of the label. In this work, the signal of the ECL immunosensor was mainly from the encapsulated RuL toward TPA oxidation. Since a large number of RuL molecules were combined with WMNTs, and due to the intrinsic good electrocatalytic activity of RuL toward TPA oxidation, we hypothesized the sensitivity of the ECL immunosensor could be greatly enhanced when RuL-MWNTs@Au~RuL-Ab2 was used as recognition element of target AFP and signal amplification label. Figure 3 shows the different modified electrodes in PBS in the presence of 10^{-5} M TPA. It can be seen that only a small ECL intensity change was observed at the RuL-Ab2 modified electrode (curve a). On the contrary, a remarkable ECL intensity increase was observed for the RuL-MWNTs@Au~Ab2 modified electrode (curve b) and the largest ECL intensity response occurred at the RuL-MWNTs@Au~RuL-Ab2 modified electrode (curve c). These results indicated that a large amount of RuL molecules were combined with WMNTs and RuL labled AFP second antibody could be efficiently captured on the surface of RuL-MWNTs@Au nanoparticles.

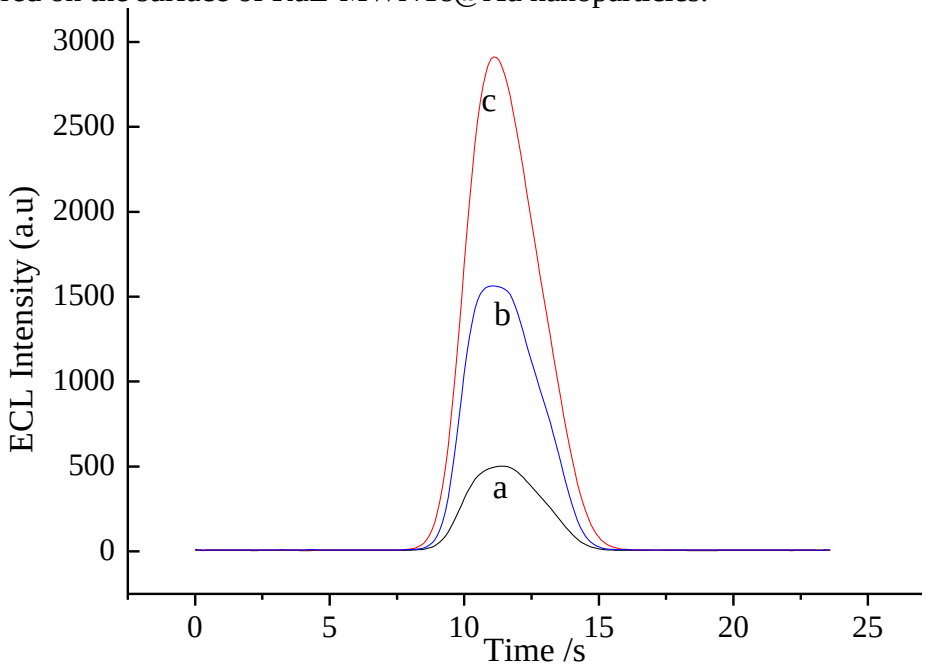


Figure.3. ECL-time curves for (a) RuL-Ab2, (b) RuL-MWNTs@Au~Ab2 and (c) RuL-MWNTs@Au~RuL-Ab2 modified electrodes in PBS (pH 7.4) containing 10^{-5} M TPA.

3.3. Performance of the ECL immunosensor

The highly sensitive label of the magnetic sandwich-type immunocomplexes was then used to construct ECL immunosensors for AFP detection. The magnetic sandwich-type immunocomplexes named RuL-MWNTs@Au~RuL-Ab2/AFP/anti-AFP~GMP were formed through antigen-antibody

interaction in the presence of different concentrations of AFP. Then they were attached on SCPEs by magnet for ECL measurements. As the amount of RuL-MWNTs@Au~RuL-Ab2/AFP/ anti-AFP~GMP sandwich-type immunocomplexes immobilized on the SCPEs was determined by the concentration of AFP, the ECL intensity (EI) of immobilized RuL in the presence of TPA could provide a sensitive output signal for AFP quantitative detection.

As shown in Figure 4, EI increased with the increasing of AFP concentration ranging from 0.01 to 50 ng·mL^{-1}. A linear relation between the logarithm of EI and the logarithm of AFP concentration was obtained (Log (ΔEI) =3.2453+0.3587 Log (c_{AFP}/ng·mL^{-1})) with a correlation coefficient R = 0.9947. The detection limit was 3 pg·mL^{-1} (3σ). Such a low detection limit is better than those of previously reported AFP immunosensors using gold nanoparticle label (0.8 ng·mL^{-1}) [41], immuno-gold silver staining signal amplification (1.0 ng·mL^{-1}) [42], and chemiluminescence microfluidic immunoassay system based on super-paramagnetic micro beads (0.23 ng·mL^{-1}) [43]. The improved sensitivity may be attributed to two aspects: 1) the high loading level of RuL molecules into the silica nanoparticles increased the sensitivity; 2) the large amount of Ab2 absorbed onto the Au-NPs surface enhanced the access chance of the antibody-antigen interaction, especially when the AFP concentration is very low.

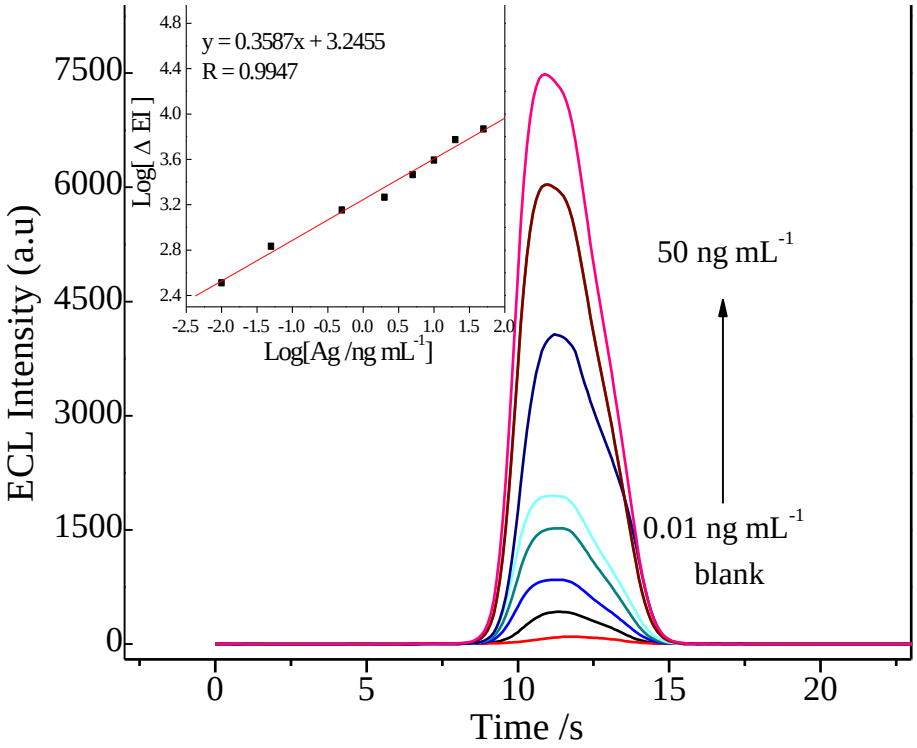


Figure.4. The schematic illustration of the ECL profiles of the immunosensor before (0.00 ng·mL^{-1}) and after (0.01 ng·mL^{-1}-50 ng·mL^{-1}) incubating in different concentration of AFP. Insert: the relationship between log of ΔECL signal towards log of different AFP concentrations

3.4 Specificity for the detection of AFP

The selectivity of the immunosensor was also tested by adding possible interfering substances in the AFP-mediated sandwich-type immunoreaction.

The selectivity of the immunosensor was also tested by adding possible interfering substances in the AFP-mediated sandwich-type immunoreaction. Different immunocomplexes were prepared with AFP (5 ng·mL^{-1}) or AFP (5 ng·mL^{-1}) together with the following individual interferent: carcinoembryonic antigen (CEA, 5 ng·mL^{-1}), human IgG (HIgG, 1 μg·mL^{-1}), carbohydrate antigen 19-9 (CA19-9, 5 ng·mL^{-1}), human chorionic gonadotropin antigen (HCG, 5 ng·mL^{-1}), BSA (1 μg·mL^{-1}), ascorbic acid (AA, 1 μg·mL^{-1}), dopamine (DA, 1 μg·mL^{-1}) and L-lysine (LL, 1 μg·mL^{-1}). The interference degree was evaluated by comparing the ECL intensity of a mixture of AFP and interfering substance with that of AFP alone. As can be seen from Figure 5, there is no remarkable change of ECL signal was observedas compared to that of AFP only. (Figure 5), which demonstrated a good selectivity of the developed ECL immunosensor for AFP detection.

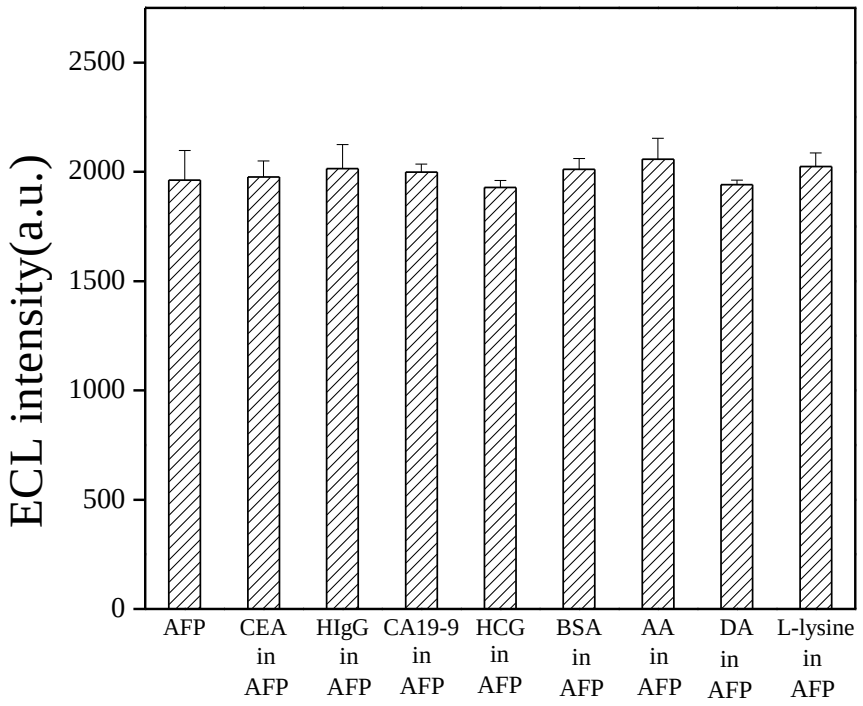


Figure.5. Selectivity analysis of the ECL immunosensor in the presence of different interferents. The concentrations of the interferents were: CEA (5 ng·mL^{-1}), HIgG (1 μg·mL^{-1}), CA19-9(5 ng·mL^{-1}), BSA (1 μg·mL^{-1}), HCG (5 ng·mL^{-1}), AA(1 μg·mL^{-1}), L-lysine (1 μg·mL^{-1}), DA (1 μg·mL^{-1}).

3.5. Determination of AFP in human serum samples

The feasibility of applying the immunosensor in clinical systems was investigated via analyzing several real clinical serum samples, and the results were then compared with the reference

values obtained by the commercial ELISA method. Table 1 shows the correlation results obtained using the proposed immunosensor and the ELISA method. The relative deviations of the proposed immunosensor ranged from1.5% to 4.7%. It obviously suggested that there was no significant difference between the results given by two methods. Therefore, the proposed sensor could be reasonably applied in the clinical determination of AFP in human serums.

Table 1. Comparison of serum AFP levels determined using two methods

Serum samples	1	2	3
Electrochemical ELISA ($ng \cdot mL^{-1}$)	4.87	10.15	19.81
ELISA ($ng \cdot mL^{-1}$)	5.11	10.06	20.12
Relative deviation (%)	4.7	0.9	1.5

4. CONCLUSIONS

In this paper, one type of RuL modified MWNTs@Au probes was successfully prepared by a simple synthetic method. The obtained RuL-MWNTs@Au composite particles could be an ideal substrate for antibody immobilization with high RuL capacity load efficiency, good stability and bioactivity. Furthermore, multilabel-antibody functionalized Fe_3O_4(core)/Au(shell) composites were also prepared and applied as labels in sandwich electrochemical immunoassay. Due to the dual signal amplification strategy of MCNTs-based particles and high RuL capacity of the probe, the electrochemical response of the fabricated immunosensor was greatly enhanced and achieved the sensitive detection of AFP. Furthermore, the magnetic sandwich-type immunocomplexes can be modified and removed from its surface by magnetic field added on the flat bottom of SPCEs, The proposed electrochemical immunosensor is large since point-of-care analyses would reduce costs, minimize sample decomposition, facilitate on-the-spot diagnosis, and alleviate patient stress. Therefore, this novel dual amplified strategy opened a new door to broaden the potential applications of early diagnosis in cancer in clinical research.

ACKNOWLEDGMENTS
The authors appreciate the support of the National Natural Science Foundation of China (no. 20805024) and Natural Science Foundation of Zhejiang Province and Ningbo (no.009A610167; Y3090068; Y4080023&Y4080017), and the Scientific Research Foundation of Graduate School of Ningbo University (YK2009045), and K.C.Wong magna fund in Ningbo University

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