Signal-on electrochemiluminescent immunosensor based on poly(amidoamine) dendrimer functionalized carbon nanodots amplification for ultrasensitive detection of α -fetoprotein

Shupeí Zhang,^a Lele Zang,^b Xiaozhen Zhang,^c Hong Dai,^{a*} Guifang Xu,^a Qingrong

Zhang,^a Caiping Yang,^b Yanyu Lin^a

^a College of Chemistry and Chemical Engineering, Fujian Normal University, Fuzhou, Fujian, 350108, China

^b Department of Gynaecological Oncology, Fujian provincial Cancer Hospital, Fuzhou, Fujian 350002, China

^c Nuclear Medicine Department, The Affiliated People's Hospital of Fujian University of Traditional Chinese Medicine, Fuzhou, Fujian 350002, China

Abstract

An ultrasensitive sandwich-type electrochemiluminescent biosensor based on carbonaceous based nanomaterials was fabricated for alpha-fetoprotein detection. Herein, the composite of fullerene, graphene oxide and chitosan with specific surface area, good water dispersibility, and excellent biocompatibility was first modified onto the electrode surface and then it was in situ electrochemical reduced to improve the conductivity. After the capturing antibody was immobilized onto the modified electrode surface, the detection antibody together with poly(amidoamine) dendrimers functionalized carbon nanodots as the recognizable signal tags was self-assembled onto the electrode by typical sandwich immune reaction. By this design, the abundant functional groups, good water dispersibility and good electroconductivity of poly(amidoamine) dendrimers functionalized carbon nanodots make the composite not only an immobilizing platform to carrier numerous detection antibodies, but also an promising promoter for the electrochemiluminescent signal of luminol. With the aforementioned amplify factor, the established electrochemiluminescent biosensor under the optimal conditions demonstrated a wider dynamic response from 1 fg mL^{-1} to 80 ng mL^{-1} with a low detection limit of 0.33 fg mL^{-1} . Additionally, the experimental results exhibited the immunosensor possessed excellent stability, reproducibility and selectively. Accordingly, this immunosensing strategy for alpha-fetoprotein with the assistance of poly(amidoamine) dendrimers functionalized carbon nanodots provided a promising approach in clinical disease scanning.

Keywords: electrochemiluminescent, biosensor, alpha-fetoprotein, carbon nanodots,

poly(amidoamine) dendrimers

1. Introduction

Alpha-fetoprotein (AFP), a fetal serum protein produced by the liver and the yolk sac, has been recognized as an important biomarker for hepatocellular cancer [1]. The average level of AFP is about 25 ng mL⁻¹ in healthy human serum and the increased AFP concentration can be recognized as an early indication of cancer [2]. Accordingly, developing an accurate, quick and costless quantification strategy for AFP detection is very exigent in clinical research. Until now, a series of techniques have been exploited for the determinate of AFP, such as enzyme-linked immunosorbent assay [3], fluorescent immunoassay [4], chemiluminescent immunoassay [5], electrochemiluminescent (ECL) immunoassay [6], electrochemical immunoassay [7], and surface plasmon resonance [8].

Compared with the above mentioned methods, ECL analysis is an attractive way to detect biomarkers owing to its important application in clinical diagnosis with acknowledged advantages including high sensitivity, good sensitivity, good controllability, simplicity, rapidity, and low background signal [9-11]. Among the ECL systems, luminol is one of the most extensively implicated luminescence reagents for its excellent intrinsic properties such as nontoxicity, inexpensive, and high light-emitting quantum yield [11-13], and luminol-based ECL researches have drawn great interest in biomolecule analysis. To achieve a sensitive determination for biomarkers, many efforts have been devoting to improve the ECL signal of luminol.

Recently, many carbonaceous based nanomaterials with excellent electro-catalysis

activity have been widely employed as good matrices and biological labels to enhance the ECL signal in immunosensing field [14-16]. In these carbonaceous based nanomaterials, fullerene (C_{60}) is a truncated icosahedron with rich conjugated π electron structure made out of five and six member rings of sp^2 carbons [17]. The highly symmetrical structure, unique physical and chemical properties, large surface area, superconductivity of C_{60} make it play an important role in the fabrication of sensitive sensing platform [18,19]. In addition, graphene oxide (GO) is a well-known two-dimensional carbon nanomaterial and has been used in different fields due to its unique physical and chemical properties [20]. The numerous oxygen functional groups on GO make hydrophobic carbon nanomaterials easily disperse into the GO aqueous solution via strong π - π interaction [21], which facilitated the further application of C_{60} in this biosensor. Besides, the positively charged chitosan (CS) attached onto negative charge carbon materials, providing amine groups for further covalent coupling antibody [22]. Furthermore, due to the high permeability towards water, excellent membrane-forming ability, good adhesion and susceptibility to chemical modifications of CS, the introduction of CS into the carbon materials can be used to improve the adhesion and membrane-forming ability of carbon materials to glassy carbon electrode surface, playing a key role to the subsequent fabricate process of this immunosensor [23-25]. Inspired by the advantages of C_{60} , GO and CS, a biocompatible composite of $GO@C_{60}$ was prepared and then the composite was electrochemical reduced to in situ generate reduced graphene oxide (rGO) $@C_{60}$ on electrode surface to further improve the conductivity of supporting matrix because of

good conductivity of C₆₀ and rGO.

Besides, carbon nanodots (CNDs) with excellent properties including good conductivity, low cost, excellent water solubility and good biocompatibility was introduced into this system to enhance the sensitivity of this biosensor [26,27]. More importantly, the amino groups and carboxylic groups on CNDs make it forming well-dispersed aqueous colloids when dispersed in water, which make more dissolved oxygen (co-reactant) accumulate onto the surface of CNDs and significantly enhance the ECL signal of luminol [28,29]. To further improve the sensitivity of this sensor, poly(amidoamine) dendrimers (PAADs) with hundreds of amino groups in their molecular backbones was used as carriers to immobilize more CNDs through the covalent bond force [30].

Herein, rGO@C₆₀ modified electrode with outstanding conductivity was used as sensing matrix to provide large surface area for covalent binding of capture antibody (Ab₁). Covalent binding of detection antibodies (Ab₂) on the PAADs@CNDs composites as sensitive ECL bioprobes were self-assembled onto the sensing interface for AFP detection by a simple sandwich immunoassay strategy. And the ECL intensity of this immunosensor increase with the increasing of target antigen concentration because of the good electrochemical and ECL catalysis toward luminol [31]. Moreover, the ultrasensitive ECL biosensor with excellent analytical performances was successfully applied to the determination of AFP in real serum samples.

2. Experimental

2.1. Reagents

Ethanol and dichloromethane were bought from Sinopharm Chemical Reagent Co., Ltd. AFP, anti-AFP, and bovine serum albumin (BSA, 96–99%) were obtained from Biss Inc. (Beijing, China). L-Ascorbic acid (AA), glutaraldehyde (GLD), PAADs (G4) and luminol were purchased from Sigma (St. Louis, MO, USA) and used without further purification. N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and N-hydroxy succinimide (NHS) were obtained from Shanghai Medpep Co. Ltd. Other reagents were of analytical reagent grade. The phosphate buffer solution (PBS, 0.1 M, pH 8.0) as the supporting electrolyte was prepared by mixing stock solution of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 and adjusting the pH. All solutions were prepared with deionized water by using a Water purifier (China) purification system.

2.2. Instruments and measurements

The morphology and structural characterization were observed by Transmission electron microscopy (TEM, FEI F20 S-TWIN instrument). ECL intensity versus potential was detected by using a system made in our laboratory, consisting of a BPCL Ultra-Weak Chemiluminescence Analyzer controlled by a personal computer with BPCL program (Institute of Biophysics, Chinese Academy of Sciences) in conjunction with a CHI 760 electrochemical analyzer (Shanghai Chenhua Instrument Co., China). The electrochemical analyzer was used for controlling waveforms and potentials. A conventional three-electrode system was used for the electrolytic system, including a bare glassy carbon electrode (GCE) or modified GCE as the working

electrode, a platinum wire as the counter electrode and a Ag/AgCl electrode (sat. KCl) as reference electrode. A commercial 5 mL cylindroid glass cell was used as ECL cell, and it was placed directly in the front of the photomultiplier tube.

2.3. Synthesis of GO, C₆₀, CNDs, GO@C₆₀, PAADs@CNDs and PAADs@CNDs based signal tags (PAADs@CNDs@Ab₂)

GO was prepared by typical Hummers' methods according to our previous work [32] and C₆₀ was synthesized according to the previous report [33]. CNDs were synthesized through one step approach according to the previous report [34]. Briefly, 2.2 g AA was dissolved in 50 mL of deionized water and 50 mL of ethanol to form a homogeneous solution. Subsequently, 50 mL of as-prepared solution was transferred into autoclave and heated at 180 °C for 4 h and then cooled to room temperature naturally. Then the dark brown solution was extracted with dichloromethane. Afterwards, the water phase solution was dialyzed by employing ester dialysis membranes for three days to remove all impurity molecules. Finally, a yellow CNDs aqueous solution was obtained.

To prepare GO@C₆₀ composite, mixing of 2 mL of 2 mg mL⁻¹ C₆₀, 2 mL of 2 mg mL⁻¹ GO and 2 mL of 0.3% CS solution under sonicated for 4 h. Afterwards, the GO@C₆₀ composite was obtained by centrifugation and redispersed in 2 mL distilled water for the further use.

The PAADs functionalized CNDs (PAADs@CNDs) were prepared as following: 40 μL of 0.2% PAADs were mixed with 50 μL of GLD (5%) under vigorously stirred for

3 h. Then, the covalent immobilization of CNDs onto PAADs surface can be achieved by virtue of the cross-linking reaction between aldehyde groups and amino groups. Finally, the PAADs@CNDs were obtained by centrifugation and redispersed in 2 mL distilled water for the further use.

The biofunctionalized signal tags (PAADs@CNDs@Ab₂) were prepared by first added 50 μL of 10 mg mL^{-1} EDC and 50 μL of 20 mg mL^{-1} NHS into 1 mL of PAADs@CNDs solution. After activating the carboxyl groups of CNDs for 2 h, 100 μL of Ab₂ was added to the above solution and reaction over night at 4 °C. Then the PAADs@CNDs@Ab₂ was collected by centrifugation and redispersed in 1 mL PBS (pH 7.4). Following that, 1.0 wt% BSA solution was added to block possible remaining active sites, and then the resulting PAADs@CNDs@Ab₂ detection bioprobe was washed with PBS (pH 7.4) and redispersed in PBS (pH 7.4), and stored at 4° C until use.

2.4. Preparation of immunosensing probe

Prior to the modification, the glassy carbon electrode was polished with 1, 0.3 and 0.05 μm alumina slurry and rinsed thoroughly with distilled water between each polishing step. Then it was washed successively with 1:1 nitric acid, acetone and distilled water in an ultrasonic bath and dried in air. Subsequently, 4 μL of the prepared GO@C₆₀ solution was carefully cast on the surface of the well-polished GCE and dried in air to obtain GO@C₆₀ modified electrode. The GO and CS electrode were obtained by dropping 4 μL of mixed solution contained 2 mg mL^{-1} GO

and 0.3% CS solution and 4 μL of 0.3% CS solution, respectively, onto the bare GCE. Afterwards, the prepared GO@C_{60} and GO electrodes were electrochemically reduced at -1.3V for 400 s in 0.1M KCl solution to be transferred to rGO@C_{60} and rGO, respectively. After that, rGO@C_{60} was soaked in 100 μL of 100 mg mL^{-1} Ab_1 solution for 1 h at 4 $^{\circ}\text{C}$. Ultimately, the excess biomolecules were removed by distilled water rinse, and the resultant electrode ($\text{rGO@C}_{60}/\text{Ab}_1$) was incubated in BSA solution (1%) for 30 min.

2.5. Immunoassay detection

The process of detection of AFP was carried out as following. The $\text{rGO@C}_{60}/\text{Ab}_1$ was firstly incubated in target AFP solution of different concentration for 90 min to obtain $\text{rGO@C}_{60}/\text{Ab}_1/\text{Ag}$. Then the result electrode was submerged into 100 μL of PAADs@CNDs@Ab_2 solution for 80 min at 4 $^{\circ}\text{C}$. After rinsing with pH 7.4 PBS to remove the unbound, the ECL response of the immunosensor was recorded in pH 8.0 PBS buffer containing $2 \times 10^{-5} \text{ mol L}^{-1}$ luminol.

3. Results and Discussions

3.1. Characteristics of the prepared GO and C_{60}

The TEM of GO and C_{60} were performed to examine their morphologies and microstructures. As depicted in the inset of Scheme 1, a thin and irregular flake-like two-dimensional structure can be seen from the TEM image of the prepared GO. Furthermore, some of GO sheets demonstrated a crumpled microstructure, might be owing to the presence of oxygen-containing functional groups in the GO sheets, which the phenomenon was in agreement with the previous reports in the literature

[35]. In addition, the TEM image of C₆₀ showed the sphere-like nanoparticles of about 60 nm, which was in accordance with the previous reports [33].

3.2. Electrochemical behavior of various modified electrodes

Cyclic voltammetric curves (CVs) of GO (a), GCE (b), rGO (c) and rGO@C₆₀ (d) were explored in Figure 1A by using Fe(CN)₆^{3-/4-} as redox probe. A classic pair of reversible redox peaks was obtained at bare GCE (curve b). Obviously, a significant declined peak current was depicted in curve a after GO introduced onto the bare electrode, resulting from that the poor electroconductivity of GO which hindered the transfer of electrons. As expected, an enhanced current was found in curve c due to the good conductivity of electrochemical reduced GO. It is especially noteworthy that the rGO@C₆₀ (curve d) presented the bigger peak current than that of rGO, which indicating the excellent electrochemical catalysis capacity of C₆₀.

In consideration of the electrochemical response being highly sensitive to the physicochemical properties of the sensing surface, background CVs were used to investigate the rGO@C₆₀ quality in Figure 1B. The capacitance values of modified electrodes could be approximately calculated from the CVs at 0.2 V according to the equation (1) [36]:

$$C_{CV} = \frac{i}{\nu(s)} \quad (1)$$

Where C_{CV} , i , s , ν , represents the capacitance (F cm⁻²), discharge current (A), the surface area of working electrodes (cm²), and the scan rate (V s⁻¹), respectively. Apparently, from the curves in Figure 1B, rGO@C₆₀ modified electrode owned the biggest current, implying that rGO@C₆₀ owned highest capacitances, which may be

resulted from the rich conjugated π electron structure of rGO and C₆₀.

To evaluate the effective electroactive areas of different modified electrodes, the chronocoulometry and the linear relationship between charges and square roots of scan times were demonstrated in Figure 1C. The effective electroactive area could be computed according to the equation (2) [37]:

$$Q(t) = kt^{1/2} + Q_{ads} \quad (k = 2nFAc\sqrt{D/\pi}) \quad (2)$$

Where k , t , Q_{ads} , is the slope in the linear curve, the time, and Faradic charge respectively. n represents the number of transferred electrons, F represents the Faraday constant, A represents effective electroactive area, c represents the concentration of the oxidized species, D represents the diffusion coefficient. From Figure 1C, a maximal slope was clearly observed in curve d, suggesting that the rGO@C₆₀ modified electrode possessed the largest effective surface area. The electroactive area of rGO@C₆₀ was 2.34 times that of GCE and 1.1 times that of rGO, manifesting that the rGO@C₆₀ sensing matrix could provide much more sites for antibody immobilization and electron transfer.

Moreover, the influence of scan rate on oxidation peak currents at different modified electrodes was examined by CV. As depicted in Figure 1D, the oxidation peak currents linearly increased with the scan rate over the range of 30-120 mV s⁻¹, implying this electrochemical reaction process on the sensing interface is diffusion control process. Furthermore, according to the equation (3) [38]:

$$Ip = kv \quad (k = n^2F^2A\Gamma/4RT) \quad (3)$$

n , F , R , T were constant. A is the electrode area (cm²), Γ is the surface

coverage (mol cm^{-2}). Obviously, the slope of rGO@C_{60} was the maximal, which was 1.38 times that of rGO and 2.69 times that of bare GCE, indicating that the rGO@C_{60} owned the highest electrode surface coverage.

The aforementioned findings not only convincingly demonstrated the successful synthesis of the rGO@C_{60} hybrid but also strongly supported the excellent electro-catalysis properties. The mainly reason is the large surface area, rich conjugated π electron structure, superior electronic conductivity of nanocarbon materials, and the good membrane-forming ability, good adhesion of CS. Besides, the NH_2 groups in CS also provided a facility for the subsequent antibody immobilization. Accordingly, the synergistic effect of C_{60} , rGO and CS make the hybrid be a promising sensing matrix.

3.3. The tentative mechanism for the AFP detection

As a proof-of-concept application, a sandwich-type immunosensor for AFP detection was established based on rGO@C_{60} hybrid platform in Scheme 1. In this work, Ab_1 was anchored onto the rGO@C_{60} sensing surface through covalent coupling by virtue of the GLD as a bridging linker. Based on the typical sandwich immunosensing strategy, Ab_2 labeled PAADs@CNDs as signal-on probe was connected onto the electrode surface. With the increasing of target agent concentration, more and more signal-on probe anchored onto the sensing platform, resulting in the enhancement of luminol ECL signal, thus realizing the selective detection of AFP. The possible ECL mechanism was elaborated as follows.

When the electrode was scanned from 0 V to 1.2 V, luminol (H_2L) was oxidized to an intermediate $L^{\bullet}$ (4). Afterwards, O_2 was electrochemically reduced to free radical $O_2^{\bullet-}$ (5). And then strong oxidant $O_2^{\bullet-}$ further reacted with $L^{\bullet}$ to generate the excited state $[LO_2^{2-}]^*$ (6). Finally, $[LO_2^{2-}]^*$ fell from the excited state to the ground state, aminophthalic acid (AP^{2-}) and light was detected (7). Therefore, the good water dispersibility and excellent electrical conductivity of CNDs make the surface of PAADs@CNDs gather more O_2 and expedite the transfer of electrons, which enhanced the ECL signal of luminol.

3.4. ECL immunosensor characterization and detection strategy feasibility assay

To confirm the above inferences, the immunosensor was firstly fabricated and the variation of ECL intensity during sensor fabrication was recorded. As depicted in Figure 2A, the GCE coated by rGO@C₆₀ (curve b) exhibited obviously enhanced ECL signal compared with the bare GCE (curve a), which proved the successful decoration and good electroconductivity of rGO@C₆₀. However, when Ab₁ (curve c) and Ag (curve d) were immobilized onto the electrode, the ECL intensity of luminol decreased successively, resulting from that the antigen-antibody complex acted as an inert electron and mass-transfer blocking layer. As anticipated, after incubating in PAADs@CNDs@Ab₂ solution, a dramatically improved ECL intensity was obtained (curve e), which due to the excellent ECL catalysis toward luminol. The observations

convincing demonstrated the successful fabrication of this immunosensor, which also certified the feasibility of this immunosensor for the AFP detection.

To explore the influence of carbon nanomaterials to electrochemiluminescence of luminol, the ECL intensities of different modified electrodes were examined in Figure 2B. Compared with GCE (curve a), rGO (curve b) displayed enhanced ECL signal due to the large surface area and good ECL catalysis toward luminol. Obviously, rGO@C₆₀ (curve c) showed greater ECL signal than that of rGO, owing to the better ECL performance resulted from synergistic effect of rGO and C₆₀, which the phenomenon was also vividly elaborated in Figure 2C. Besides, as depicted in Figure 2B, after bare Ab₂ was immobilized onto the sensing surface (curve d) through the immune recognition reaction, the modified electrode exhibited a very small ECL signal because the immune complex hindered the transfer of electrons. Owe to the poor conductivity of PAADs, after PAADs@Ab₂ was anchored onto the electrode (curve e), there was a negligible signal was observed. When CNDs@Ab₂ attached onto the electrode surface (curve f), the modified electrode depicted increased ECL intensity than that of bare Ab₂, owing to the good water dispersibility and excellent electrical conductivity of CNDs make the sensing surface gather more O₂ and promote the transfer of electrons. As predicted, the rGO@C₆₀/Ab₁/Ag/PAADs@CNDs@Ab₂ (cure g) demonstrated the biggest ECL signal. This phenomenon can be rational explained as the fact that PAADs could carry more CNDs which produced higher ECL intensity, which the variation of ECL signal was also vividly exhibited in Figure 2D.

3.5. Optimal conditions for the ECL biosensor

To achieve the optimal sensing performance, different experimental parameters were investigated in Figure 3 and Figure 4. As depicted in Figure 3A and 3B, the current firstly increased and then decreased with the increasing concentration of GO and C₆₀. It can be explained that the appropriate amount of carbon material can accelerate the electrons transfer. However, the thicker carbon film could block the electronic migration. Thus, 2 mg mL⁻¹ GO and 2 mg mL⁻¹ C₆₀ was selected for the following experiment.

Besides, the incubation time of Ab₁ and Ag were important influencing factors for the sensitivity of the ECL immunosensor. As depicted in Figure 3C, the ECL intensity at rGO@C₆₀/Ab₁ decreased rapidly from 0 min to 60 min and then tended to level off with further prolonging the incubation time, resulting from the approximate saturated Ab₁ attached onto the surface. The similar phenomenon was also observed in Figure 3D, which due to the recognition between Ab₁ and Ag reached saturated at 80 min and no further recognition with extending incubation time. Therefore, the incubation time of Ab₁ and Ag was chosen 60 min and 80 min, respectively.

In addition, the volume ratio of CNDs and PAADs in the preparation process of signal-on probe also played a crucial role in the generation of the ECL intensity, thus the ECL intensity of different volume ratio was recorded in Figure 4A. The ECL intensity increased rapidly as the CNDs:PAADs ratio increased from 100:1 to 500:1 and then decreased as it was increased from 500:1 to 700:1. The phenomenon could be explained as the inhibition effects of PAADs and promotion effects of CNDs to the

ECL signal of luminol, leading to the intensity firstly increased with the ratio increase. However, the aggregation of more CNDs inhibited the ECL response. Accordingly, 500:1 volume ratio of CNDs and PAADs was selected for the preparation of signal-on probe.

Furthermore, the volume of Ab₂ in 1 mL PAADs@CNDs solution was another important factor on ECL response signal. As shown in Figure 4B, the strongest ECL response was obtained at the volume of 100 μ L. Considering the detection sensitivity, 100 μ L of Ab₂ was chosen for the subsequent experiments.

Finally, the incubation time of signal-on bioprobe was also investigated in Figure 4C. Obviously, the biggest ECL intensity was obtained at 80 min, thus, 80 min was selected as the optimal incubation time.

3.6. Analytic performance of the biosensor

Under the optimal conditions, the typical ECL intensity-potential profile of signal-on immunosensor to the target was recorded at different AFP concentrations (Figure 5A). Furthermore, the ECL intensity linear increased with an increasing logarithm of AFP concentration in the range from 1 fg mL⁻¹ to 80 ng mL⁻¹ with a detection limit of 0.35 fg mL⁻¹ (Figure 5B), which precede the results in other reports (Table S1). The regression equation was $I = 2874.4 + 178.6 \log C_{AFP} \text{ (g mL}^{-1}\text{)}$ with a correlation coefficient of 0.989.

To explore the specificity of the proposed immunosensor for AFP detection, the fabricated immunosensor was incubated with interferences, the coexistence of AFP and different interferent and their mixture. The corresponding results in Figure 5C

demonstrated that there were no obvious influence on the ECL intensity compared with that of AFP although the existence of a large excess of interferences, implying the high specificity of the sandwich immunosensor.

In addition, the reproducibility of the ECL biosensor was evaluated by analyzing the intra-assay and inter-assay relative standard deviation (RSD) of three different electrodes fabricated under the same condition. The intra-assay RSD of the three sensors were 3.9%, 3.1% and 3.7% at 0.01 ng mL⁻¹, 1 ng mL⁻¹ and 160 ng mL⁻¹ of AFP, respectively. Similarly, the RSD on three immunosensors were 5.6%, 5.9%, and 4.9% at 0.01 ng mL⁻¹, 1 ng mL⁻¹ and 160 ng mL⁻¹ of AFP, respectively. Therefore, the developed immunosensor possessed the good reproducibility and satisfactory precision.

Moreover, the stability of the immunosensor was examined under consecutive cyclic potential scans for 11 cycles in Figure 5D. The result exhibited strong and stable ECL intensity without obvious descend, revealing the excellent chemical and structural stability. After the fabricated immunosensor stored at 4 °C for two weeks, there was no obvious change of ECL signal, indicating the good storage stability of this sensor.

To assess the applicability of the proposed immunosensor for AFP detection in real samples, five healthy human serum samples were measured by using the fabricated ECL sensor. As depicted in Table S2, the result indicated the concentrations in the samples were lower than 10.2 ng mL⁻¹, which is very accordance with the general level of AFP in healthy humans. Besides, the recovery was performed by adding the different concentration of AFP standard solution into the serum sample in Table S2.

The satisfactory results indicated that this ECL immunosensor was available for detecting AFP in real serum samples.

4. Conclusion

In summary, the promising applications of CNDs for enhancing the ECL response of luminol was explored by establishing an ultrasensitive ECL immunosensor on rGO@C₆₀ support scaffold. Herein, the rGO@C₆₀ composites not only provided large surface area for the subsequent immobilization of Ab₁ but also furnished outstanding conductivity for the acceleration of electron transfer. In the case of CNDs, it employed as an efficient nanocarrier for immobilizing Ab₂ and also as a superior conducting wire for improving electron transport. More importantly, the good water dispersibility and large specific surface of CNDs make the sensing surface of this sensor gather more co-reaction reagent, oxygen molecules, which significantly enhanced the ECL signal of luminol. By virtue of a typical immunosensing strategy, the ECL signal increasing with the increase of AFP concentration, realizing the ultrasensitive determination of AFP. Furthermore, the results demonstrated the proposed ECL biosensor owned good specificity, excellent reproducibility, outstanding stability and low cost. With these promising performances, we expect the fabricated immunosensor could be expanded for the monitoring of more tumor markers.

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Scheme 1.

Scheme 1. Schematic illustration of the electrochemiluminescent immunosensor developed process. The inset were the TEM image of GO and C₆₀.

Figure 1.

Figure 1. Cyclic voltammetries (A, B), Chronocoulometries (C) and Current-v (D) of different modified electrodes in 0.1 mol L⁻¹ KCl solution in the presence (A, C, D) and absence (B) of 5 mmol L⁻¹ [Fe(CN)₆]³⁻. (a) GO, (b) GCE, (c) rGO, (d) rGO@C₆₀. The inset in C is the linear relationship between the charge and the square roots of the time.

Figure 2.

Figure 2. (A) ECL responses of different modified electrodes in 0.1 M PBS solution (pH 8.0) contained $20 \mu\text{mol L}^{-1}$ luminol. (a) GCE, (b) rGO@C₆₀, (c) rGO@C₆₀/Ab₁, (d) rGO@C₆₀/Ab₁/Ag, (e) rGO@C₆₀/Ab₁/Ag/PAADs@CNDs@Ab₂. (B) ECL response of different modified electrodes in 0.1mol L⁻¹ pH8.0 PBS solution contained $2 \times 10^{-5} \text{ mol L}^{-1}$ luminol, (a) GCE, (b) rGO, (c) rGO@C₆₀, (d) rGO@C₆₀/Ab₁/Ag/Ab₂, (e) rGO@C₆₀/Ab₁/Ag/PAADs@Ab₂, (f) rGO@C₆₀/Ab₁/Ag/CNDs@Ab₂, (g) rGO@C₆₀/Ab₁/Ag/PAADs@CNDs@Ab₂. ECL responses of (C) different electrodes and (D) rGO@C₆₀/Ab₁/Ag towards different signal probes in 0.1 M PBS (pH 8.0) solution contained $2 \times 10^{-5} \text{ mol L}^{-1}$ luminol.

Figure 3.

Figure 3. The currents on rGO@C_{60} modified electrode of different GO concentration (A) and C_{60} concentration (B). Concentration: (A) 0.3 % CS, 1 mg mL^{-1} C_{60} and (B) 0.3 % CS, 2 mg mL^{-1} GO. Effect of different incubation time of Ab_1 (C) and Ag (D) on ECL response.

Figure 4.

Figure 4. Effect of (A) the volume ratio of PAADs and CNDs, (B) volume of Ab_2 , and (C) incubation time of PAADs@CNDs@ Ab_2 on the ECL intensities.

Figure 5.

Figure 5. (A) ECL-E curves of the sensor towards different concentration of AFP. (a-i) 10^{-6} , 10^{-5} , 10^{-4} , 10^{-2} , 1, 10, 20, 40, 80 ng mL $^{-1}$. (B) The corresponding calibration curve. (C) The ECL signal responses of the sensor in the presence of various interfering antigens and their mixture. 80 ng mL $^{-1}$ AFP, and 160 ng mL $^{-1}$ CEA, BSA, hIgG, HSA, hTG, PSA. (D) The stability of the ECL sensor.