**Dual-responsive competitive immunosensor for sensitive
detection of tumor marker on g-CN /rGO conjugation**

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Abstract

A new dual mode competitive immunosensing platform for sensitive determination of α -fetoprotein (AFP) combined photoelectrochemical and electrochemical methods was designed on graphite carbon nitride and reduced graphene oxide (g-CN/rGO) conjugate support. To construct such versatile support, polyamidoamine dendrimer (PAAD) decorated graphene oxide (GO) as substrate was reduced via potentiostatic technology, and the prepared rGO-PAAD nanocomposite was utilized as matrix to immobilize g-CN and capture antigen, and as electron transporter to mediate electron transfer for enhancement of photoelectrochemical and electrochemical signals. And then, horseradish peroxidase labeled antibody was competitively captured onto sensing interface with free antigen in incubated solution. Photoelectrochemical signal originated from g-CN, an excellent photoactive material with large photo-induced electric response, and was amplified by rGO-PAAD nanocomposite. The resultant concentration positively related linear calibration range was from 1 pg/mL to 40 ng/mL with ultralow detection limit of 1 pg/mL. On the other hand, the enzyme-induced electrocatalytical signal amplification was produced by using hydroquinone as reactive substrate and horseradish peroxidase as reactive enzyme in existence of H_2O_2 , which displayed a wide dynamic linear range from 0.1 ng/mL to 160 ng/mL with low detection limit of 0.1 ng/mL. Additionally, the satisfying selectivity, good reproducibility and high consistency of dual-signal outputs of this designed dual-responsive immunosensor demonstrated the promising application in developing sensitive and accurate immunosensor for clinical test and disease diagnosis.

Keywords: dual-responsive immunosensor, photoelectrochemical response, electrochemical signal, enzyme-induced signal amplification, tumor marker

1. Introduction

Highly sensitive and reliable analysis of disease-related biomarkers is currently an important subject of disease diagnostics and therapeutic analysis, such as disease early diagnosis, disease monitoring and highly reliable prediction, which is extremely significant not only for patient survival, but also for saving time in successful prognosis of the diseases[1, 2]. With medicine development, various markers associated with the cancer expression were discovered. Usually, the content of tumor biomarkers in biological samples is very low, for example, the average levels of α -fetoprotein (AFP) are lower than 25 ng/mL in human serum. Therefore, the demands of the sensitive detection methods are increasing in the clinic diagnosis and prediction of original carcinoma. Immunosensing analysis based upon specific biomolecular recognition is a powerful analytical tool for biomarker determination and widely applied in quantitative evaluation. To date, a series of immunosensing strategies was presented to determinate the biomarkers via various methods, such as enzyme-linked immunosorbent assay[3], colorimetric[4], chemiluminiscent[5], fluorescence immunoassay[6] and others[7-9]. These single-channel detection methods did bring certain values for the biomarker analysis to some extent, while, it might limit the utilization of the designed sensing interface and be affected by detection conditions limit, windage of instrument and hard operation process[10].

To enhance density and diversity of information, and then increase cost-efficiency and accuracy of result, recently, many efforts focused on the design of multiplexing analytical platform with multiple signal outputs. For example, Peng's group[11]

reported a single molecular sensor by recording dual mode imaging for cellular viscosity; Yang and his colleagues[12] performed track of tumor-cell-specific drug delivery by using fluorescence and label-free SERS techniques. These reports exposed that dual mode sensing strategies could not only provide more information than one sensing pattern and improve the accuracy and precision of conclusion, but also offer comparison between various tactics and lay a foundation for a better sensing proposal. Unfortunately, the dual-readout research based on electrical channel was also underdeveloped, which could supply easier and more effective approach for practical application in detection of targets, owing to its low cost, easy-miniaturized instrument. Here, the electrochemical and photoelectrochemical dual-responsive immunosensor based on graphite-like carbon nitride/reduced graphene oxide (g-CN/rGO) conjugation was designed as a self-calibration system for sensitive and accurate detection of tumor marker. Among various analytical techniques, photoelectrochemical and electrochemical immunosensor have attracted intensive research interests owing to their significant applications in clinical diagnosis and monitor with the promising advantages such as high sensitivity, simplicity, rapidity, easy miniaturization and cost-efficiency. More inspiring, the combination strategy of photoelectrochemical and electrochemical dual-signal outputs exhibited a wider dynamic linear response range for analyte than single electrochemical or photoelectrochemical sensor alone. In addition, the dual-signal outputs possessed a fascinating self-calibration ability, which could give a more accurate detection result.

However, it is difficult to construct a dual-responsive sensor with good performance in photoelectrochemical and electrochemical determination. Usually, the signal probe of photoelectrochemical or electrochemical immunosensor is indispensable to introduce into the sensing architecture, respectively. On the other

hand, immunosensor always suffers from the challenge of signal amplification for high sensitive detection of analyte. Among various methods, enzyme labeling was usually considered to be an efficient strategy to realize the highly sensitive detection for targets, not only due to the fact that enzyme could play the part of transducer to switch biomolecular recognition event into measurable electrical or photic signals but also ascribe to inherent catalytic activity to amplify signal[13]. Additionally, in comparison with nanoparticle labels, enzyme labels had higher specificity owing to the intrinsic substrate selective nature. Here, hydroquinone (HQ) was added into analytical solution as a mediator to transfer the electron between horseradish peroxidase (HRP) and H_2O_2 to introduce electrochemical signal. On the other hand, to output the photoelectrochemical signal, g-CN, a π -conjugated polymeric semiconductor nanosheet, was introduced as a metal-free, inexpensive and nontoxic photosensitizer. It is of particular interest that this anisotropic prototypical two-dimensional (2D) polymer has a small direct band gap of 2.7 eV, enabling charge generation, separation and diversion the sensing interface upon light excitation[14]. Moreover, 2D nanosheet nature of g-CN is also favorable for photoelectrochemistry, because the anisotropic structure can shorten the perpendicular migration distance of charge carriers from bulk to surface and promote charge transport along the in-plane direction[15]. Thus, g-CN was widely explored as photosensitive material in various fields, such as photocatalysis[16], solar cell[17] and photochemical sensor[16]. However, the photo-to-electric conversion efficiency of g-CN was limited by the high recombination rate of photoexcited electron-hole pairs, leading to lower photo-activity which became a bottleneck in its extensive application. Therefore, many methods have been used to solve above problems[18], such as chemical doping with foreign elements, constructing a **heterojunction** composite with another semiconductor and

preparing novel nanostructure complex. Thereinto, combination with carbon-based nanomaterial can effectively facilitate the separation of photogenerated carriers in the mechanism of traditional electron-hole transfer. Reduced graphene oxide (rGO), a unique planar structure with delocalized electrons from the conjugated sp^2 -bonded carbon network, exhibit the merits of large theoretical specific surface area, high inherent electron mobility and good conductivity, which makes it an excellent candidate as the electron acceptor and transporter because of the capable of promoting light absorption, charge transfer and electrical conductivity. In addition, π - π stacking interaction between g-CN and rGO not only improved the both dispersion of g-CN and rGO, but also facilitated the transfer of carriers. On the other hand, the g-CN/rGO nanohybrid provided a promising electrode matrix for immobilization of antigen/antibody, which paid a foundation for the enzyme-induced electrochemical immunosensor. Thus, g-CN/rGO conjugate was utilized to construct dual-responsive competitive immunosensor with excellent electrochemical and photoelectrochemical responses for sensitive detection of tumor marker.

Here, a sensitive photoelectrochemical and electrochemical dual-responsive immunosensor based on g-CN/rGO conjugate was developed to improve its analytical accuracy of tumor marker. As shown in Scheme 1, the photoelectrochemical response produced from g-CN and was amplified by the electron transporter (rGO-PAAD) which could be as electron donor (PAAD with abundant amido groups) and rapid transfer photogenerated charge (rGO with good electric conductivity), then resulting in decreasing recombination of photo-induced electron-hole pairs. On the other hand, HRP facilitated electrochemical oxidation HQ in existence of H_2O_2 and induced electrochemical signal amplification largely. Moreover, this dual-responsive competitive immunosensor achieve sensitive determination for AFP with ultralow

detection limit of 1 pg/mL and quite wide total linear range from 1 pg/mL to 40 ng/mL after designed amplification strategy. Additionally, the testing concentration of sample in dual-mode zone could calibrate each other roughly to improve the accuracy of gained conclusion. This work was considered to be a preliminary step for the exploration of dual-responsive immunosensor, which was promising strategy for clinical application in disease diagnosis.

2. Experimental Section

2.1. Reagents

PAAD (generation 4), glutaraldehyde (GD), bovine serum albumin (BSA) were purchased from Sigma-Aldrich Co. (USA), J&K Scientific Ltd., Sino-American Biotechnology Co. Ltd., respectively. AFP ELISA kit was purchased from Beijing Bioss Biology Technology Ltd. (China). GO was synthesized according to Hummers' report [19], and g-CN nanosheet was prepared according to Wangs' report [20] after ultrasonic exfoliation. Serum sample was obtained from Fujian Provincial Hospital (Fuzhou, China), and others reagents were AR and bought from Sinopharm Chemical Reagent Co., Ltd. (China). Ultrapure water (UP) was from tap water purified by a Water Purifier (China) purification system. Phosphate buffer solution (PBS) with different pH value was prepared by mixing 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4 with monitoring by PHS-3C exact digital pH metre (Shanghai Leici Co. Ltd., China), which was calibrated via standard pH buffer solutions.

2.2. Apparatus

Scanning electron microscopy (SEM, Hitachi S-4800) was used to monitor the morphologies and sizes of sample. X-ray photoelectron spectroscopy (XPS) measurements were executed with an ESCALAB 250 Å 1314. All electrochemical operations, including Amperometric i-t Curve, Cyclic Voltammetry (CV), Linear

Sweep Voltammetry (LSV), Differential Pulse Voltammetry (DPV) and Open Circuit Potential-Time (OCP), were performed with a CHI 430 Electrochemical Workstation (Shanghai Chenchua Instrument Co., China). A three electrode system, a platinum wire as auxiliary electrode, Ag/AgCl reference electrode (sat. KCl) and a glassy carbon electrode (GCE, $\phi=3$ mm) as working electrode, was used to carry out all photoelectrochemical and electrochemical tests. All photoelectrochemical processes were performed with a homemade photoelectrochemical system which is similar to our previous reports [21, 22].

2.3. Preparation of dual-responsive competitive immunosensor

The schematic illustration of fabrication processes of dual-responsive competitive immunosensor was showed in Scheme 1. This dual-responsive immunosensor was fabricated on a clean glassy carbon electrode which was obtained by polishing with 3.0 μm alumina powder on chamois leather, washing with UP, 95% ethanol, UP and natural dried in air. First, 3 μL GO and PAAD mixture was deposited on GCE which was displaced below infrared lamp to dry, then cooled to room temperature to gain GO-PAAD/GCE (Fig.S1). Result modified electrode was dipped into 0.1 M PBS (pH=5) at -1.3 V to reduce GO into rGO, then rinsed with UP and dry in nature to gain rGO-PAAD/GCE. Subsequently, 3 μL g-CN suspension was dropped onto above modified electrode, which was displaced below infrared lamp to dry, and then cooled to room temperature to gain g-CN/rGO-PAAD/GCE (photoelectrode/ electrochemical sensor matrix).

A g-CN/rGO-PAAD/GCE was incubated in 40 ng/mL AFP antigen solution containing 2.5% (w/v) glutaraldehyde for 50 min to capture enough AFP molecules on the modified interface and rinsed slightly to remove residual antigen at 4 $^{\circ}\text{C}$ (Fig.S2). Then remanent active sites were blocked by 1% BSA. After gentle wash, competitive

reaction was carried out on above Ag/g-CN/rGO-PAAD/GCE by incubating in mixture of 5 μ L original HRP tagged antibody (HRP-Ab) and target solution/sample, and obtained the sensor platform (HRP-Ab/Ag/g-CN/rGO-PAAD/GCE) which was stored in refrigerator before use.

2.4. Photoelectrochemical and electrochemical determinations

Before linking HRP-Ab onto Ag/g-CN/rGO-PAAD/GCE, the photoelectrochemical response was examined in 0.1 M PBS (pH 7.4) solution with intermittent irradiation with light wavelength at 405 nm. After immobilization of HRP-Ab, the photoelectrochemical response was examined in 0.1 M PBS (pH 7.4) containing 1 mM H_2O_2 with intermittent irradiation with light wavelength at 405 nm. And electrochemical responses were executed in PBS solution containing 1 mM H_2O_2 and 1 mM HQ by DPV. Serum sample from healthy person was provided by Fujian Provincial Hospital and measured with the same procedure as standard AFP solution.

3. Results and Discussion

3.1. Characteristic of sensing architecture materials

The typical SEM images were used to insight into the morphology and microstructure of materials for constructing immunosensor. As shown in Fig.1A, it was evident that uniformly rGO-PAAD film was covered randomly on electrode interface. The SEM image of g-CN revealed a clearly flat planar structure and good dispersion with the size range from 300 nm to 600 nm. The reduction of GO was monitored by X-ray photoelectron spectroscopy (XPS). The C1s spectrum of GO and rGO revealed that considerable oxidation degree of GO with three main components related to carbon atoms in different functional groups: the C-C bond (284.5 eV), C-O bond from epoxy and hydroxyl (286.3 eV) and C=O bond from carboxylic acid (288.2 eV). The lack of sp^2 carbon network in the GO is known to poor electron conductivity,

and reduction can enhance the sp^2 structure and produce more delocalization electron, leading to improvement of electron conductivity dramatically. Comparing with the spectrum of GO, the peaks at 286.5 and 288.8 eV decreased obviously relative to that at 284.6 eV, indicating that the GO was effectively reduced to be rGO after electrochemical process. And the XPS measurement was also taken to probe the surface composition of pure g-CN, the survey spectrum displayed the strong C1s and N1s signals with a C/N ratio of 1.34, which was very close to the idea composition of g-CN (C_3N_4 , C/N=1.33). This indicated the successful synthesis of g-CN as well as illustrated that the chemical composition and coordination of carbon and nitrogen in g-CN were retained with dispersion and ultrasonic exfoliation.

3.2. Characterizations of g-CN/rGO conjugate

The compositions and characters of materials of sensing architecture have significant effect on the sensing properties of designed sensor. Two-dimensional material conjugate, g-CN/rGO, was first introduced to improve the electron transfer ability of dual mode sensor, and its performance was evaluated by monitoring corresponding electrochemical and photoelectrochemical signals. Photoelectrochemical behaviors of g-CN/rGO conjugate were first exposed. As Fig.2(A) shown, there was hardly photocurrent response of GCE, which also indicated a **clean** GCE was obtained. While, after modification of g-CN onto electrode interface, a strong photocurrent response appeared with a little drop at the beginning moment (curve d), due to the large photoresponse but poor conductivity of the g-CN and the rapid recombination of photogenerated electron-hole pairs. It could be clearly observed that g-CN/rGO-PAAD as photoactive materials displayed much larger and more stable photoelectrochemical signal instead of g-CN, which caused by the synergistic effect of rGO with good conductivity and PAAD with abundant amide as

electron donor, leading to the effective transfer of photogenerated carriers and less recombination of photo-induced electron-hole pairs. As compare operation, the photoelectrochemical signal of g-CN/GO-PAAD was also collected, an obviously decreased photocurrent signal also illustrated the rGO-PAAD could be a good photosensitive complex and carrier transporter. Meanwhile, the small photoelectrochemical signal of this photosensitive layer, rGO-PAAD, was observed, which might because the carriers of conjugative rGO were excited with light on. The corresponding schematic diagram of the details of photo-induced charge separation was displayed in Fig.2(B). First, photoelectrochemical material (g-CN) was excited and generated excited electron-hole pair with visible light irradiation. Then, excited electron was transferred to electron transporter (rGO-PAAD) occupied matched energy level with photoelectrochemical material to separate photogenerated electron-hole pair. Finally, electron transferred to electrode and another donor contributed electron to combine with remanent hole of g-CN.

Photoelectronic conversion efficiency of as-designed g-CN/rGO conjugate was further evaluated by investigating the electron lifetime which arose from the charge recombination after cutting off the excited light. Open circuit potential (OCP) was used as testing technology to explore the recombination kinetics of photogenerated electron-hole pairs. OCP decreased with recombination of photogenerated electron-hole pairs, and slower change rate of OCP indicated less recombination speed and longer efficient electron lifetime, their corresponding relationship followed Bisquert equation [23]:

$$\tau = -\frac{k_B T}{e} \left(\frac{dE}{dt} \right)^{-1}$$

Where T is temperature, k_B is Boltzmann's constant, e is charge of a single electron, t is decay lifetime and E is the OCP at t . According to this equation, the consequential relationships between lifetimes of g-CN as well as g-CN/rGO-PAAD and OCP were displayed in Fig.2(C), which performed some interesting conclusions: first, lifetime of g-CN located in narrow potential range, revealing the photogenerated electron might rapidly recombined with hole. Second, lifetime of g-CN/rGO-PAAD delayed slowly from more than five hundreds to zero and was across wide potential scope, indicating the good conductivity of sandwiched transporter could efficiently transfer photogenerated electron, decrease recombination and lengthen electron lifetime.

Meanwhile, as electrochemical sensing matrix, the properties of the prepared conjugate were also investigated by CV curves showed in Fig.2(D). A pair of well reversible redox peak of $K_3[Fe(CN)_6]$ was obtained on a **clean** GCE (curve b). Due to static electronic attraction between amido group of PAAD and $[Fe(CN)_6]^{3-/4-}$, more accumulation of $[Fe(CN)_6]^{3-/4-}$ on the electrode surface resulted in larger and more reversible redox peak responses of PAAD/GCE (curve c). Obviously, the electrode modified with electrochemical reduced GO-PAAD performed a dramatical amplification of redox peak current in virtue of the coordinated actions of rGO and PAAD, including static electronic attraction between PAAD and $[Fe(CN)_6]^{3-/4-}$, good conductivity, large specific surface area, absorption of rGO, so that probe molecules substantial accumulated around electrode interface and electron rapidly transferred into electrode (curve e). On the contrary, modified electrode with the same modified component without electrochemical treatment exhibited lower redox peaks and larger peak potential difference, which also proved poor conductivity of GO and had some encumbrance of electron transfer (curve a). Subsequently, with g-CN nanosheets covered on the rGO-PAAD, it could be clearly observed that the redox peak current of

probe sharply decreased (curve d), illustrating poor conductivity of semiconductor, g-CN, which was according with Zhou's report [24]. This strong impediment also indicated the stable conjugate structure formed, and impeded charge transfer between electrode and $[\text{Fe}(\text{CN})_6]^{3-/4-}$.

3.3. Construction of dual-responsive immunosensor

The properties of the dual-responsive immunosensor during the fabrication process were monitored by the photoelectrochemical and electrochemical methods. As shown in Figure 3A, the stepwise photocurrent responses of corresponding assembly were performed. Obviously, g-CN performed a much larger photoelectrochemical response (curve b) compared with bare electrode matrix (curve a), proving admirable photoactivity of g-CN. Then, its photocurrent further enhanced when the rGO-PAAD hybrid was modified onto electrode matrix into endothecium of g-CN. This increase could be attributed to the rGO-PAAD, which not only used as electron transporter layer to facilitate the photogenerated electron transfer from g-CN into electrode surface, but also acted as electron donor to further prohibit recombination of photogenerated electron-hole pair. However, the photocurrent decreased gradually when the substrate antigen and HRP-Ab were sequentially captured on the g-CN/rGO-PAAD electrode surface, which ascribed to the block of photogenerated carriers transport as well as the partial shelter of excited light intensity with the immobilization of **biomolecules**, revealing the AFP and HRP-Ab were actually immobilized onto the electrode interface. In addition, here, visible light as excited light source was another advantage to keep activity of immune objects of photoelectrochemical immunosensor.

Electrochemistry was chosen as another mode to monitor the construction processes of this immunosensor. As we could see in Fig.3(B), HQ with

electrochemical activity was easily oxidized, because of the active conjugated electron of double amido groups and benzene. Once rGO-PAADs covered on electrode interface, the current signal tempestuously amplified, which could contribute to the prominent conductivity, large specific area, absorption of rGO and π - π stacking interaction between rGO and HQ. While, due to poor conductivity of g-CN, the current response turned down with modification of g-CN. After AFP anchoring and BSA blocking, the current tended to disappear, because HQ molecules hardly went through double poor conductivity layers to contact with electrode interface for gaining enough energy to overcome energy barrier and participate in oxidation reaction. However, this oxidation reaction could arise again only if existence of catalyst which could depress energy barrier of reaction [25], so with introduction of HRP-Ab, the corresponding current signal appeared again. Moreover, active center of enzyme was maintained by cooperation of H_2O_2 and HQ[26], resulting in the enhancement of electrochemical response with the durative electro-catalytic reaction. Thereinto, potential shift could contribute to the poor conductivity of some elements in designed sensor. By tracing change of signal could achieve quantificational detection of antigen, therefore, this strategy of electrochemical sensing segment was feasible to analyze AFP.

3.4. Analysis and application of dual-responsive immunosensor

Under the optimal experimental conditions (Fig.S1 and S2), the prepared dual-responsive immunosensor was employed to assess quantificational analysis of AFP with various concentrations. As shown in Figure 4A, the photocurrent increased with increase of AFP concentration and performed a good linear relationship with the logarithm of AFP concentration from 1 pg/mL to 40 ng/mL by maintaining R^2 large than 0.995. On the other hand, the less immobilization of HRP led to typical decrease

of enzyme-induced DPV signals with increasing AFP concentration in the competitive process, its corresponding calibration plot showed a good linear relationship between DPV signal and logarithm of AFP concentration in range from 0.1 ng/mL to 160 ng/mL by maintaining R^2 large than 0.995. Corresponding limits of detection concentration were to be 1 pg/mL for photoelectrochemical and 0.1 ng/mL for electrochemical sensing, respectively. All in all, this designed dual-responsive immunosensing strategy performed much wider total linear range from 1 pg/mL to 160 ng/mL with lower detection limit of 1 pg/mL than others' reports showed in Table 1. Additionally, it was easy to found the dual mode detection zone which was located in both photoelectrochemical and electrochemical detection linear range from 0.1 ng/mL to 40 ng/mL, which could actually satisfy the detection requirement to distinguish illness or not (the general level of AFP in healthy humans was less than 25 ng/mL). Moreover, these dual-signal outputs possessed a fascinating self-calibration ability that was beneficial to eliminate false positive, which could give a more accurate detection result.

Before the practical application of designed dual-output immunosensor, the properties of this competitive immunosensor, including reproducibility, stability and selectivity, were further assessed. The plots of Figures were obtained from the three modified electrodes under the same conditions, these closely similar signals indicated the good reproducibility of designed immunosensor. Long-term storage stability of as-prepared dual-responsive immunosensor was also investigated by storing the prepared sensor at 4°C before use. Photoelectrochemical response of constructed immunosensor remained 96.7% after a week and 93.9% after two weeks, meanwhile, the electrochemical signal of constructed sensor remained 95.3% after a week and 92.1% after two weeks, which showed satisfied stability of this dual mode

immunosensor. Selectivity is an important criterion to evaluate the immunosensing properties, as nonspecific adsorption cannot be distinguished from specific absorption and usually influence the sensitivity. To confirm the dual-signal responses derived from the specific recognition, the other possible interferences were added into the testing solution. Figure 4D revealed the dual-signal outputs were hardly affected by foreign interferences, demonstrating the excellent selectivity of designed dual mode immunosensor. These preliminary results indicated that this proposed dual-responsive immunosensor was feasible to sensitively detect AFP in serum sample directly, which was promising strategy for clinical application. In order to assess the accuracy of this dual mode immunosensor in real sample measurement, standard addition method was utilized to analyze the recovery rate of concentration of AFP in serum sample. As shown in Table S1, the concentration of AFP in a serum sample from healthy people was determined to be 0.548 ng/mL by photoelectrochemical channel and 1.75 ng/mL by electrochemical channel, these results were also consistent with the clinical diagnosis (Negative). After addition of AFP standard solution with 10 ng/mL, the AFP concentrations were found to be 11.4 ng/mL by photoelectrochemical channel and 13.6 ng/mL by electrochemical channel, corresponding recovery rates were 108.5% for photoelectrochemical channel and 118.5% for electrochemical channel, respectively, implying that the designed dual-responsive competitive immunosensor could be effectively applied in the clinical detection of AFP content in serum sample.

4. Conclusions

In summary, a unique photoelectrochemical and electrochemical dual-responsive immunosensor for AFP was successfully designed on g-CN/rGO conjugate support. This versatile support was constructed by assembling g-CN sheet on the electrochemical reduced GO-PAAD composite, serving as anchor to capture antigen

and competitively recognize HRP labeled antibody. The dual-signal outputs of this immunosensor originated from g-CN with photoelectrochemical response and enzyme-induced electrochemical signal, amplifying by the rGO-PAAD composite with large surface area, good conductivity and abundant active binding sites of captured antigen. Moreover, this dual-mode competitive immunosensor performed a broad linear dynamic range, low detection limit, excellent selectivity, acceptable reproducibility and stability. Additionally, the accurate results for detection of serum sample was displayed which paved the way to promising application of this dual-responsive immunosensor in clinical analysis.

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Figure captions

Scheme 1 The illustration of the fabricated processes of dual-responsive competitive immunosensor.

Fig.1 SEM graphs of rGO-PAAD (A), g-CN sheets (B). XPS curves of GO (C), rGO (D) and g-CN (E).

Fig.2 (A) Photocurrent responses of different electrodes (GCE (a), rGO-PAAD/GCE (b), g-CN/GO-PAAD/GCE (c), g-CN/GCE (d), g-CN/rGO-PAAD/GCE (e)) in 0.1 M PBS, corresponding schematic diagram of photogenerated electron transfer (B). (C) Lifetime of photogenerated electron of g-CN/GCE (a) and g-CN/rGO-PAAD/GCE (b) at different OCPs in 0.1 M PBS. (D) CV curves of different electrodes (GO-PAAD (a), GCE (b), PAAD/GCE (c), g-CN/rGO-PAAD/GCE (d), rGO-PAAD/GCE (e)) in 5 mM $K_3[Fe(CN)_6]$ containing 0.1 M KCl.

Fig.3 (A) Photocurrents obtained from different electrodes (GCE (a), g-CN/GCE (b), g-CN/rGO-PAAD/GCE (c), Ag/g-CN/rGO-PAAD/GCE (d), Ab-HRP/Ag/g-CN/rGO-PAAD/GCE (e)). (B) DPV curves produced from GCE (a), rGO-PAAD/GCE (b), g-CN/rGO-PAAD/GCE (c), Ag/g-CN/rGO-PAAD/GCE (d), Ab-HRP/Ag/g-CN/rGO-PAAD/GCE (e).

Fig.4 (A) Photocurrent density of modified electrode incubated with different concentration of AFP from 1 pg/mL to 40 ng/mL (a to h), (B) DPV curves of modified electrode incubated with different concentration of AFP from 0.1 ng/mL to 160 ng/mL (a to h), and (C) corresponding linear relationships between photocurrent densities or

electrochemical signals and nature logarithm of concentrations of AFP (n=5). The effect of different interferes (CEA: carcino-embryonic antigen; IgG: Human Immunoglobulin; G: glucose)

Table1 Comparison of analysis performance of this dual mode immuosensor for AFP with others reports.

Method	Sensing structure	Linear range (ng/mL)	LOD (ng/mL)	Ref.
EC	CS-AuNPs-Ab ₁ -Ag-Ab ₂ -CS-PB-AuNPs	0.05-100	0.03	[8]
EIT	NM-Ab-Ag-Fc	6-400	6	[27]
FIAI	PAA-FcCH ₂ OH-Ab ₁ -Ag-Ab ₂ -HRP	20-150	2	[28]
ECL	rGO-CS-AuNPs-Ab ₁ -Ag-Ab ₂ -ZnO-Ru	0.04-500	0.031	[29]
PEC	TiO ₂ -CdS-CS-Ab-Ag	0.05-50	0.04	[30]
BIE	GS-Ab ₁ -Ag-pAb-IgG-Goat	20-200	5	[31]
FM	PET-Ag-Ab ₁ -Ab ₂ -Cy3	16-50000	-	[32]
MFBS	Au-MUA-Ab ₁ -Ag-Ab ₂ - MB	0.5-10	0.001	[33]
PMB	OM-Ab ₁ -Ag-Ab ₂ -Au	0.2-1000	0.2	[34]
EC	rGO-PAADs-C ₃ N ₄ -Ag-Ab	0.1-160	0.1	This work
PEC	rGO-PAADs-C ₃ N ₄ -Ag-Ab	0.001-40	0.001	This work

EC: electrochemistry; **EIT:** electrochemical immunofiltration test; **FIAI:** flow injection amperometric immunoassay; **ECL:** electrochemiluminescence; **PEC:** photoelectrochemistry; **BIE:** biosensor based on imaging ellipsometry; **FM:** fluorescent micrographs; **MFBS:** micro-fluxgat biosensor; **PMB:** optical microfiber biosensor; **DL:** detection limit.

Scheme 1

Figure 1

Figure 2

Figure 3

Figure 4