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## Photoelectrochemical Immunosensor for Detection of Carcinoembryonic Antigen Based on 2D TiO<sub>2</sub> Nanosheets and Carboxylated Graphitic Carbon Nitride

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Carcinoembryonic antigen (CEA) was used as the model, an ultrasensitive label-free photoelectrochemical immunosensor was developed using 2D TiO<sub>2</sub> nanosheets and carboxylated graphitic carbon nitride (g-C<sub>3</sub>N<sub>4</sub>) as photoactive materials and ascorbic acid as an efficient electron donor. 2D TiO<sub>2</sub> nanosheets was synthesized by surfactant self-assembly method and proved to have higher photoelectrochemical signals than TiO<sub>2</sub> nanoparticles. Firstly, carboxylated g-C<sub>3</sub>N<sub>4</sub> could be attached to 2D TiO<sub>2</sub> nanosheets through the bond formed between carboxyl group of carboxylated g-C<sub>3</sub>N<sub>4</sub> and TiO<sub>2</sub>. And the photocurrent of g-C<sub>3</sub>N<sub>4</sub>/TiO<sub>2</sub> drastically enhances compared to carboxylated g-C<sub>3</sub>N<sub>4</sub> and TiO<sub>2</sub>. Then, antibody of CEA was bonded to TiO<sub>2</sub> through the dentate bond formed between carboxyl group of anti-CEA and TiO<sub>2</sub>, leading to the decrease of the photocurrents. As proven by PEC experiments and electrochemical impedance spectroscopy (EIS) analysis, the fabrication process of the immunosensor is successful. Under the optimal conditions, the intensity decreased linearly with CEA concentration in the range of 0.01–10 ng/mL. The detection limit is 2.1 pg/mL. The work provides an effective method for the detection of tumor markers and can be extended for the application in food safety and environmental monitoring analysis.

Photoelectrochemical (PEC) sensors are fabricated on photoactive electrodes which can convert photoirradiation to electrical signal. Therefore, photoactive materials are crucial for the performance of the PEC sensors. Many photoactive materials are metal-contained semiconductors, such as TiO<sub>2</sub>, CdSe, CdTe, ZnO, ZnS, etc<sup>1–9</sup>. Among them, titanium dioxide is one of the most commonly employed materials because of its nontoxicity, low-cost and brilliant photochemical and chemical stability. Nevertheless, due to a wide energy band gap of 3.0 eV, TiO<sub>2</sub> can only absorb the ultraviolet light (<387 nm)<sup>10</sup>. So applications of pure TiO<sub>2</sub> are also limited. Another class of photoactive materials is metal-free polymeric semiconductor. Graphitic carbon nitride (g-C<sub>3</sub>N<sub>4</sub>), a moderate energy bandgap of 2.7 eV, has paid more and more attentions because it is very stable in acid or alkaline electrolytes<sup>11</sup>. However, applications of pure g-C<sub>3</sub>N<sub>4</sub> are also limited largely because of its low quantum efficiency and high electron–hole recombination rate. As mentioned above, researchers have therefore made great efforts to expand the application of pure TiO<sub>2</sub> and pure g-C<sub>3</sub>N<sub>4</sub> by various methods, such as coupling it with other materials, nanostructuring and doping<sup>12–13</sup>. Thus, owing to the proper band level between g-C<sub>3</sub>N<sub>4</sub> and TiO<sub>2</sub>, g-C<sub>3</sub>N<sub>4</sub> and TiO<sub>2</sub> are combined together leading to the easy separation of the photo-generated electron and hole. Although there are some references about the combination of g-C<sub>3</sub>N<sub>4</sub> and TiO<sub>2</sub>, the morphology of TiO<sub>2</sub> is mainly focused on nanorod<sup>10,14</sup>, nanoparticles<sup>11</sup> and nanotube<sup>15–17</sup>.

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Herein, two-dimensional (2D) TiO<sub>2</sub> nanosheets was prepared by forming inverse lamellar micelles of Pluronic P123 surfactant together with ethylene glycol (EG) co-surfactant in ethanol solvent. It is reported that 2D nanostructures exhibit superior catalytic, photovoltaic and electrochemical performances, due to their large surface-to-volume ratio and confined thickness on the atomic scale<sup>18</sup>. So they could have promising applications in sensors, and energy conversion and storage devices. Using carcinoembryonic antigen (CEA) as a model analyte, we developed a label-free photoelectrochemical immunosensor for detection of CEA based on 2D TiO<sub>2</sub> nanosheets and carboxylated graphitic carbon nitride. CEA, a usual tumor marker, can be used for the early detection of recurrent diseases and indicate the effect of therapy in early breast cancer and gastrointestinal cancers as well as other tumor markers<sup>19,20</sup>. Coupling carboxylated graphitic carbon nitride with 2D TiO<sub>2</sub> nanosheets can evidently extend the absorption range, increase the utilization of light energy, and promote the photocurrent intensity. Then, antibody of CEA was immobilized through the dentate bond formed between carboxyl group of anti-CEA and TiO<sub>2</sub>, leading to the decrease of the PEC signal. The decreased signal is proportional to the logarithm of CEA concentration. The CEA immunosensor exhibits high sensitivity, good selectivity and wide linear range.

## Experimental

**Materials and reagents.** Titanium isopropoxide (TTIP, 95%), ethylene glycol (EG) and ascorbic acid were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). The CEA and corresponding antibody were purchased from Beijing Dingguo Changsheng Biotechnology Co. Ltd. (China). Bovine serum albumin (BSA, 96–99%) were purchased from Sigma-Aldrich (Beijing, China). All other chemicals were of analytical grade and used without further purification. ITO glass (resistivity 10 Ω/sq) is obtained from Zhuhai Kaivo Electronic Components Co. Ltd., China.

**Apparatus.** The scanning electron microscope (SEM) images were obtained by the field emission SEM (ZEISS, Germany). Photoelectrochemical (PEC) measurements were performed on an electrochemical workstation (Zahner Zennium PP211, Germany).

**Synthesis of TiO<sub>2</sub> nanosheet.** TiO<sub>2</sub> was prepared according to the literature<sup>18,21</sup>. 1.05 g TTIP was added into 0.74 g concentrated HCl solution under vigorous stirring (solution A); and 0.2 g Pluronic P123 was dissolved in 3.0 g ethanol (solution B) under stirring for 15 min. Then, solution B was added into solution A and stirred for another 30 min. Subsequently, 2.5 mL TTIP solution with 20 mL EG was transferred into an autoclave and heated at 150 °C for 20 h. After cooled to room temperature naturally, the resulting solid powder was collected by centrifugation and washed with ethanol several times. The final products were then dried at 80 °C for 24 h.

**Synthesis of carboxylated g-C<sub>3</sub>N<sub>4</sub>.** Carboxylated g-C<sub>3</sub>N<sub>4</sub> was prepared as described previously with our reference<sup>22</sup>. In brief, 5.0 g of white melamine powder was put into a covered ceramic crucible and heated at 550 °C for 4 h in a muffle furnace. The yellow g-C<sub>3</sub>N<sub>4</sub> product was ground to powder after cooling to room temperature. Then, 1 g g-C<sub>3</sub>N<sub>4</sub> powder was placed into 100 mL 5 mol/L HNO<sub>3</sub> and refluxed for 24 h at 125 °C. After cooling to room temperature, the refluxed product was centrifuged and washed with water until pH reached 7. Finally, the resulting product was dried at 35 °C for 12 h in vacuum.

**Synthesis of carboxylated g-C<sub>3</sub>N<sub>4</sub>/TiO<sub>2</sub> solution.** In brief, 0.5 mg carboxylated g-C<sub>3</sub>N<sub>4</sub> powder was dispersed in 5 mL water by ultrasonication for over 2 h. Then, 20 mg TiO<sub>2</sub> powder was added to the above suspension and stirred for 24 h. After that, the solution was centrifuged and the products were redispersed in 5 mL of water.

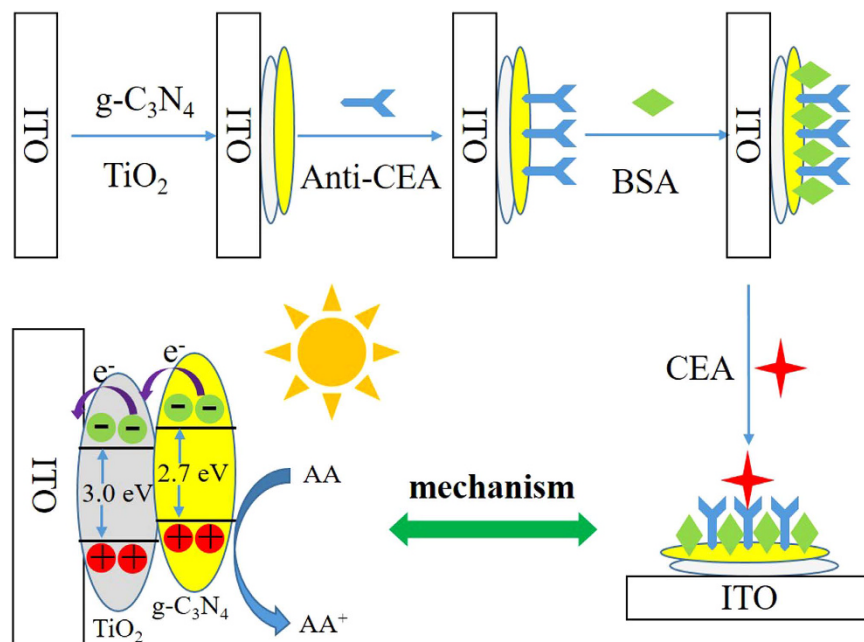
**Fabrication of PEC immunosensor.** The illustration of PEC immunosensor fabrication process is depicted in Fig. 1. ITO slices (3 × 0.5 cm<sup>2</sup>) were sonicated in acetone, ethanol and water consecutively for 30 min and dried under a N<sub>2</sub> stream. 6 μL of carboxylated g-C<sub>3</sub>N<sub>4</sub>/TiO<sub>2</sub> solution was dropped on the surface of the pre-cleaned ITO and dried after air drying, the film was sintered at 450 °C for 30 min and then cooled down to the room temperature. Then, 4 μL of 10 μg/mL anti-CEA solution was bonded onto TiO<sub>2</sub> for 1 h via the bond formed between carboxyl group of anti-CEA and TiO<sub>2</sub> at 4 °C for 1 h<sup>23,24</sup>. The unreacted active sites on the electrode surface were deactivated by 6 μL of 1% bovine serum albumin (BSA) solution for 1 h. Finally, 6 μL of CEA solutions with different concentrations were incubated for 1 h the electrode was incubated with different concentration of CEA for 1 h min at 4 °C and then washed with buffer solution to remove the excess CEA. Thus, the PEC immunosensor was fabricated completely and was ready to be used.

**PEC measurements.** Photocurrent was measured by the current–time curve experimental technique on a photoelectrochemical workstation at a bias voltage of 0.1 V with light intensity of 150 W/cm<sup>2</sup>. All experiments were carried out using a conventional three electrodes system with a modified ITO as working electrode, a Pt wire as counter electrode, and a saturated Ag/AgCl electrode as reference electrode.

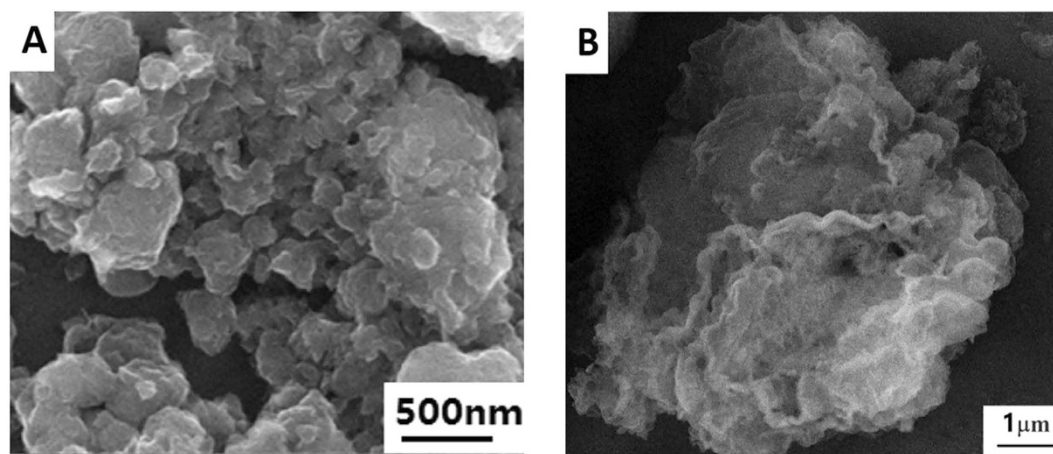
## Results and Discussion

**Characterization of TiO<sub>2</sub> nanosheet.** SEM images were used to confirm the successful synthesis of nano-materials with different morphology. Figure 2 show the SEM images of TiO<sub>2</sub> (A) and carboxylated g-C<sub>3</sub>N<sub>4</sub> (B). Obviously, TiO<sub>2</sub> exists in the form of groups of nanosheets. And the prepared carboxylated g-C<sub>3</sub>N<sub>4</sub> also has the nanosheet structure.

**Characterization of the immunosensor.** Electrochemical impedance spectroscopy (EIS), a useful tool for evaluating electron transfer resistance, was used to monitor the stepwise modification of the electrodes. The semicircle diameter corresponds to the electron transfer resistance (Ret), which reflects the restricted diffusion of



**Figure 1.** The illustration of PEC immunosensor fabrication process.

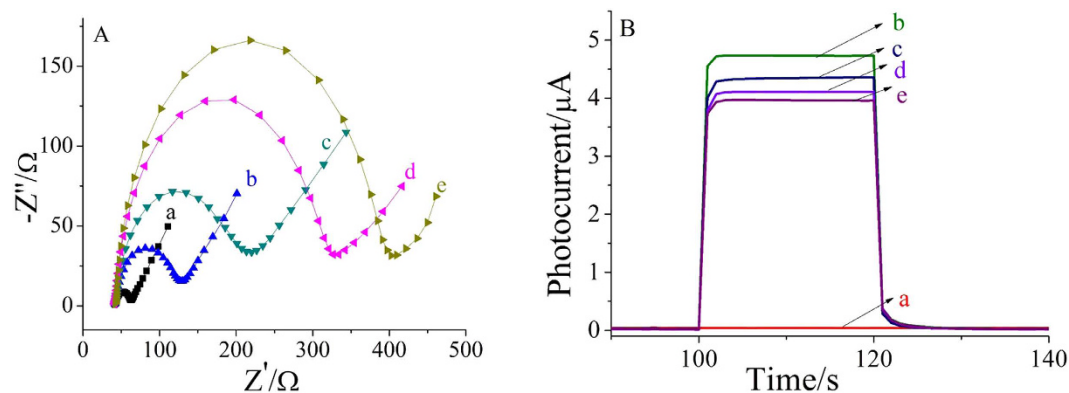


**Figure 2.** SEM of  $\text{TiO}_2$  nanosheets (A) and carboxylated  $\text{g-C}_3\text{N}_4$  (B).

the redox probe accessing the layer<sup>25</sup>. The measurements were carried out in 5.0 mmol/L  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  solution containing 0.1 mol/L KCl and the result were shown in Fig. 3A. Non-modified ITO electrode showed a small semicircle diameter (curve a), implying a low electron transfer resistance. After coating of carboxylated  $\text{g-C}_3\text{N}_4/\text{TiO}_2$  composite (curve b), semicircle diameter increases gradually because both carboxylated  $\text{g-C}_3\text{N}_4$  and  $\text{TiO}_2$  as semiconductors evidently reduced the ability of the redox probe to access the electrode surface. The sequential immobilization of CEA antibody (curve c), BSA (curve d) and CEA (curve e) led to gradual increase of the electron transfer resistance because of the insulation properties of protein.

In order to further confirm that the electrode was modified successfully, the stepwise fabrication process of the immunosensor was also characterized by PEC measurements, as shown in Fig. 3B. Compared with the ITO (curve a), the photocurrent response (curve b) was enhanced greatly after carboxylated  $\text{g-C}_3\text{N}_4/\text{TiO}_2$  composite was immobilized on it subsequently, suggesting that carboxylated  $\text{g-C}_3\text{N}_4/\text{TiO}_2$  have good PEC properties. With the loading of CEA antibody (curve c), BSA (curve d) and CEA (curve e) onto the modified electrode surface successively, the photocurrent intensity decreased which could be attributed to the block of biomacromolecules. Both the above results were consistent with the fact that the electrode was modified as expected.

The mechanism of electron transfers in  $\text{g-C}_3\text{N}_4/\text{TiO}_2$  PEC immunosensor in ascorbic acid (AA) electrolyte probably is that high electron–hole recombination rate in  $\text{g-C}_3\text{N}_4$  results in low PEC activity, when  $\text{g-C}_3\text{N}_4/\text{TiO}_2$  composites are exposure, the photo-generated electrons from  $\text{g-C}_3\text{N}_4$  can transfer from conduction band of  $\text{g-C}_3\text{N}_4$  to the conduction band of  $\text{TiO}_2$ . Because the conduction band and valence band edges of  $\text{g-C}_3\text{N}_4$  are higher than those of  $\text{TiO}_2$  nanosheets, the above transfer process of photo-generated carriers is easy. AA is a kind



**Figure 3.** EIS in the presence of 5.0 mmol/L  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  solution containing 0.1 mol/L KCl (A) and photocurrent–time curves in 0.1 mol/L PBS (pH = 7.0) containing 0.1 mmol/L AA with 0.1 V applied potential and 430 nm excitation light. (B) (a) ITO (b) ITO/ $\text{g-C}_3\text{N}_4/\text{TiO}_2$  composite (c) ITO/ $\text{g-C}_3\text{N}_4/\text{TiO}_2$ /Anti-CEA (d) ITO/ $\text{g-C}_3\text{N}_4/\text{TiO}_2$ /Anti-CEA/BSA (e) ITO/ $\text{g-C}_3\text{N}_4/\text{TiO}_2$ /Anti-CEA/BSA/CEA.

of excellent electron donor, which could block the recombination of photo-generated electrons and holes and meanwhile promote the electron transfer from conduction band of  $\text{TiO}_2$  nanosheets to the ITO electrode. The specific binding of CEA to its antibody blocked the electron transfer from AA to  $\text{g-C}_3\text{N}_4/\text{TiO}_2$  composite, resulting in the recombination of photo-generated holes and electrons, which could explain a decrease in photocurrent. Moreover, the photocurrent intensity decreased gradually with the increase of CEA concentration. Therefore, the quantitative detection of CEA is achieved by monitoring the photocurrent decrease after the binding of CEA.

**Optimization of experimental conditions.** As shown in Fig. 4A, no PEC signal were found for the ITO (curve a). Compared with the ITO, the PEC signal changed only a little after modified with  $\text{g-C}_3\text{N}_4$  (curve b) due to the low quantum efficiency and high electron–hole recombination rate of  $\text{g-C}_3\text{N}_4$ . Moreover,  $\text{TiO}_2$  showed obvious PEC signal, and the photocurrent intensity of  $\text{TiO}_2$  nanosheets (curve d) was 30% higher than that of  $\text{TiO}_2$  nanoparticles (curve c). It illustrates that  $\text{TiO}_2$  with different morphology has different photoelectric response performance, and nanosheet is superior to nanoparticles in this system. Although pure  $\text{g-C}_3\text{N}_4$  did not show obvious PEC signal, but when it was combined with  $\text{TiO}_2$ , the PEC signal was significantly increased (curve e), which was 12% higher than that of pure  $\text{TiO}_2$  nanosheets and much larger than the sum of the two kinds of nanomaterials, suggesting there is energy level matching between  $\text{g-C}_3\text{N}_4$  and  $\text{TiO}_2$ . This is the reason why both carboxylated  $\text{g-C}_3\text{N}_4$  and  $\text{TiO}_2$  were chosen as photoactive materials.

To obtain an optimal PEC signal, pH value of substrate solution was investigated. Keeping the concentrations of CEA constant, the effect of pH on the photocurrent intensity was studied over a pH range from 5.0–8.0, as shown in Fig. 4B. The photocurrent intensity increased with the increase of pH from 5.0–7.0 and reached the maximum. After that, the photocurrent intensity decreased accordingly with pH increasing from 7.0–8.0. This suggests that the neutral condition is more advantageous to the photoelectric response of the carboxylated  $\text{g-C}_3\text{N}_4$  and  $\text{TiO}_2$  nanosheets system. Therefore, pH 7.0 was chosen as the optimal value.

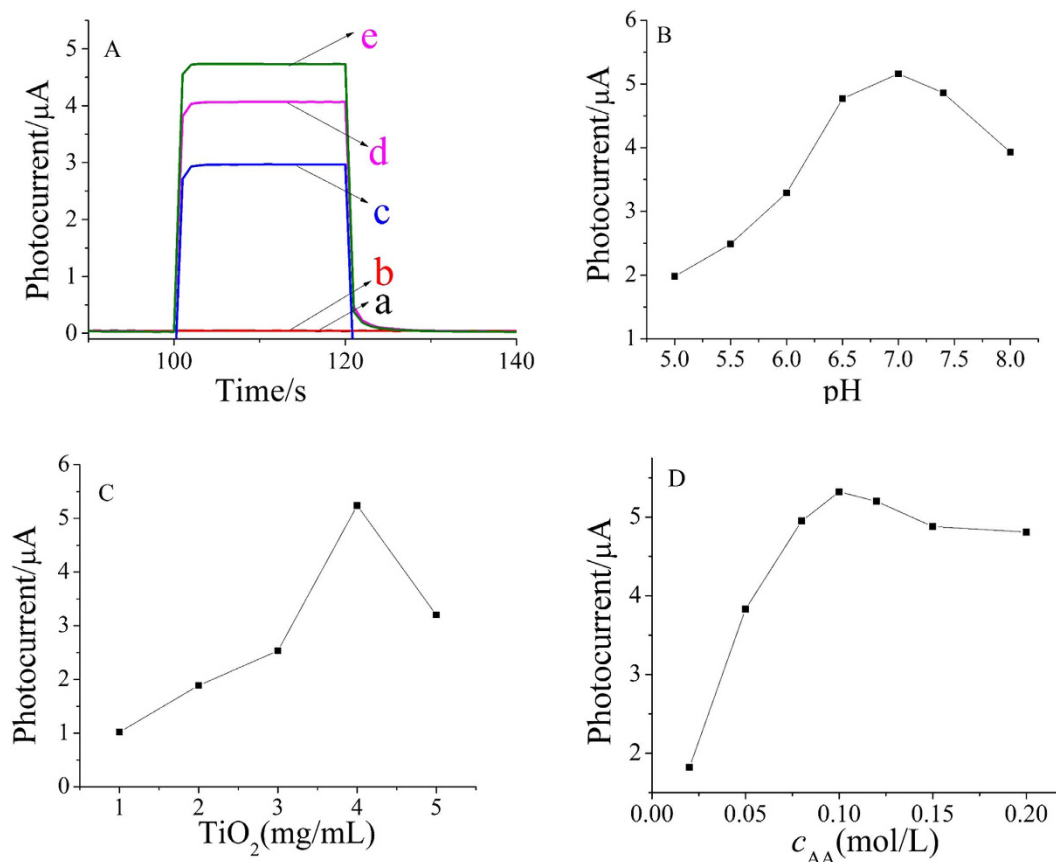
The effect of  $\text{TiO}_2$  concentration was also tested. As shown in Fig. 4C, the photocurrent intensity increased when  $\text{TiO}_2$  concentration increased from 1 to 4 mg/mL because more photoactive materials are formed and light absorption is enhanced. After that, the photocurrent intensity is decreased because thicker  $\text{C}_3\text{N}_4/\text{TiO}_2$  film could also lead to increased diffusion resistance for electron motion. Thus, 4 mg/mL  $\text{TiO}_2$  was chosen for subsequent study.

The concentration of AA as an efficient electron donor was evaluated to improve the photocurrent response of the PEC sensor. It can be seen from Fig. 4D that the photocurrent intensity reached a maximum value at 0.1 mol/L AA. Thus, 0.1 mol/L AA PBS solution (pH 7.0) was used as the buffer electrolyte for CEA detection.

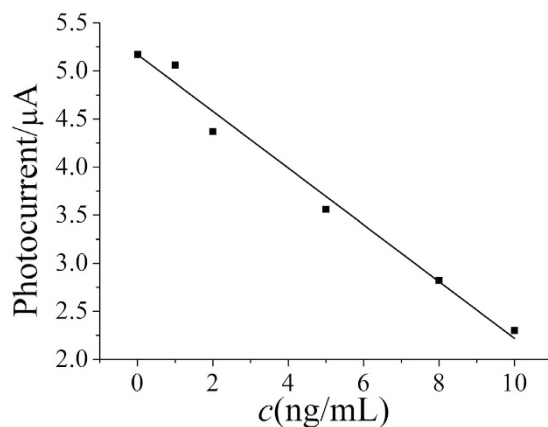
Under the optimal conditions, a series of photoelectrochemical immunosensors were incubated with different concentrations of CEA and subsequent determination was carried out. As expected, the photocurrents decreased with the increase of CEA concentration due to the biomolecular insulation properties of CEA. Moreover, it can be seen from Fig. 5 that the photocurrent intensity decreased linearly with CEA concentration in the range from 0.01–10 ng/mL with a detection limit of 2.1 pg/mL ( $S/N = 3$ ). Table 1 shows the comparison of the proposed photoelectrochemical immunosensors with other previously reported immunosensors for the detection of CEA<sup>26–30</sup>. As seen from the table, the detection limit of this work are superior to the previously reported immunosensors, indicating that the proposed PEC immunosensor has a good performance.

**Stability and Selectivity.** The stability of the designed immunosensor was also evaluated by using a prepared PEC immunosensor for the detection of 5 ng/mL CEA. The photocurrent responses were recorded under several on/off irradiation cycles for 550 s. As shown in Fig. 6A, there was only a little change of the photocurrent, indicating the developed PEC sensors have stable photocurrent response for CEA detection.

Figure 6B shows the selectivity test of the ECL immunosensor for 5 ng/mL CEA. The photocurrents were measured by mixing 5 ng/mL of CEA with 50 ng/mL prostate-specific antigen (PSA), 50 ng/mL human immune globulin G (H-IgG), 50 ng/mL BSA and 50 ng/mL glucose, respectively. The photocurrent exhibited no obvious



**Figure 4.** Photocurrent responses of different modified materials (A) effect of pH (B) the concentration of  $\text{TiO}_2$  (C) and the concentration of AA (D) on the photocurrent intensity. (a) ITO (b) ITO/ $\text{g-C}_3\text{N}_4$  (c) ITO/ $\text{TiO}_2$  nanoparticles (d) ITO/ $\text{TiO}_2$  nanosheets (e) ITO/ $\text{g-C}_3\text{N}_4$ / $\text{TiO}_2$  composite.



**Figure 5.** Calibration curve of the immunosensor for different concentrations of CEA.

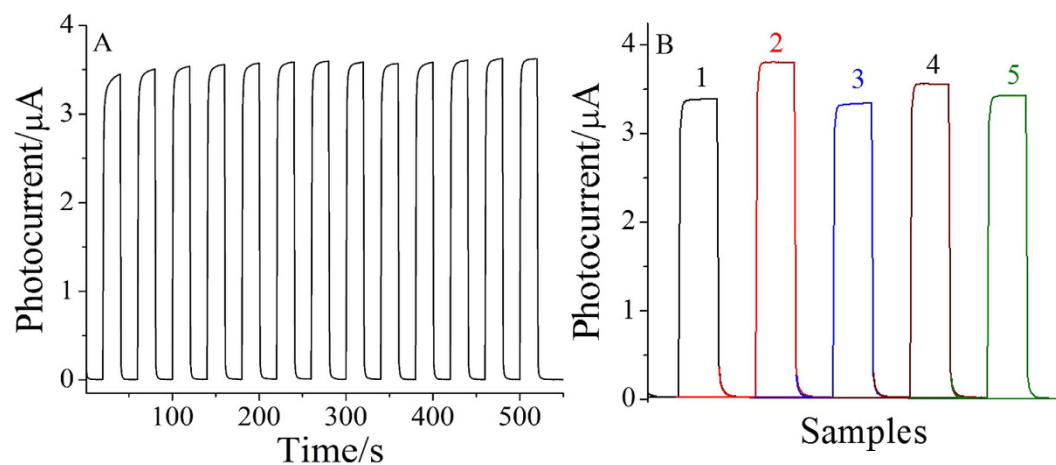
change compared with the 5 ng/mL of CEA, which indicated excellent selectivity and specificity of the PEC immunosensor for CEA.

**Serum sample analysis.** The amount of CEA was measured 5 times in human serum sample and the relative standard deviation (RSD) was calculated to obtain the precision. The accuracy was also studied through a recovery experiment using standard addition method. 1.00 ng/mL CEA standard solution was added to the corresponding samples. With the same experiments measured for five times, the average recovery was calculated. It can be found from Table 2 that the relative standard deviation is 3.1% and the recovery is 99.2%. Hence, the proposed immunosensor can be used for CEA detection with satisfied results.



Materials	Detection ranges (ng/mL)	Detection limits (ng/mL)	References
CS-CNTs-GNPs nanocomposite film	0.1–2.0	0.04	26
[Ag-Ag <sub>2</sub> O]/SiO <sub>2</sub> nanocomposite material	0.5–160	0.14	27
Thi@NPG/AuNPs	0.01–100	0.003	28
AuNP@nafion/FC@CHIT	0.01–150	0.0031	29
HRP-anti-CEA-NGGN	0.05–350	0.01	30
Carboxylated g-C <sub>3</sub> N <sub>4</sub> /TiO <sub>2</sub> nanosheets	0.01–10	0.0021	This work

**Table 1.** Comparison with other previously reported immunosensors for the detection of CEA.



**Figure 6.** The stability of the immunosensor incubated with 5 ng/mL CEA under several on/off irradiation cycles for 550 s (A) and the selectivity of the immunosensor (B). 5 ng/mL CEA (1), 5 ng/mL CEA + 50 ng/mL PSA (2), 5 ng/mL CEA + 50 ng/mL H-IgG (3), 5 ng/mL CEA + 50 ng/mL BSA (4), 5 ng/mL CEA + 50 ng/mL glucose (5).

Sample	Content of PSA (ng/mL)	Average ( $n = 5$ , ng/mL)	RSD (%)	Added (ng/mL)	Recovery value (ng/mL)	Recovery ( $n = 5$ , ng/mL)
Human serum	1.35	1.39	3.1	1.00	1.03	99.2
	1.42			1.00	0.95	
	1.45			1.00	1.01	
	1.37			1.00	0.92	
	1.36			1.00	1.05	

**Table 2.** Results for the determination of CEA in human serum sample.

## Conclusions

This work demonstrated a label-free photoelectrochemical immunosensor for detection of carcinoembryonic antigen using carboxylated g-C<sub>3</sub>N<sub>4</sub> and 2D TiO<sub>2</sub> nanosheets as photoactive materials. 2D TiO<sub>2</sub> nanosheets exhibit better photocatalytic activities than TiO<sub>2</sub> nanoparticles and g-C<sub>3</sub>N<sub>4</sub> can improve its photocatalytic performance due to the good energy level matching. Great photocatalytic activities of g-C<sub>3</sub>N<sub>4</sub>/TiO<sub>2</sub> nanosheets together with the specificity of immunoreaction made sensitive detection of CEA possible. The proposed immunosensor has excellent performance with high sensitivity, good selectivity and stability. Moreover, this strategy could be used to develop photoelectrochemical immunosensors for other targets.

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## Author Contributions

H.W. and D.W. conceived and designed the experiments. H.W., Y.W. and Y.Z. performed the experiments, analyzed the data and wrote the first draft of the manuscript. Q.W., X.R. and Q.W. contributed substantially to revisions.

## Additional Information

**Competing financial interests:** The authors declare no competing financial interests.

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