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A Sensitive Amperometric Immunosensor Based on AuNPs-TiO_2-graphene Nanocomposites for the Detection of Carcinoembryonic Antigen

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Abstract:

A novel and sensitive sandwiched amperometric immunosensor was fabricated which mainly took advantage of AuNPs-TiO_2-graphene nanocomposites as immobilizing enzyme-labeled antibody for the detection of carcino-embryonic antigen (CEA). By strong affinity effect between TiO_2 and enediol bond of hematoxylin, nano-sized graphene oxide (GO) sheets which functionally modified by hematoxylin could effectively coat almost totally on the surface of TiO_2 spheres. Besides, TiO_2 could reduce $HAuCl_4$ solution to produce gold nanoparticles under ultraviolet light irradiation condition. Depending on their synergistic effect, a large number of secondary antibody (Ab_2) labeled Horseradish peroxidase was immobilized on the AuNPs-TiO_2-graphene nanocomposites. Primary antibody (Ab_1) was immobilized on the electrode by the means of the gold nanoparticles which was through electrochemical reduction of $HAuCl_4$ solution on the surface of bare glassy carbon electrode. Under the effect of the specific immunoreaction, on the one hand CEA was connected to Ab_1 and on the other hand it was further combined with as-prepared HRP-Ab_2 immobilized on the nanocomposites. Under the optimization of determination conditions, a linear current response to CEA was obtained in a wide range from 1 pg/mL to 200 ng/mL (R^2= 0.9927). Furthermore, the results suggest that the proposed immunosensor will be a promising alternative tool for the diagnostics application in the detection of CEA.

Key words: amperometric immunosensor, AuNPs-TiO_2-graphene nanocomposites, carcinoembryonic antigen, enzyme-labeled antibody

Introduction

Carcinoembryonic antigen (CEA) is one of the most important studied tumor markers, which exists in endoblast origin digestive system cancer [1]. Generally speaking, the average concentration of CEA is significantly lower in the colon tissues of adults, at about 2.5 ng/mL [2], and the accurate and sensitive determination of CEA in human body is very important in early diagnosis, screening disease recurrence, and patients with certain tumor-associated disease.

Preparation of sandwich immunosensor

Firstly, graphene oxide (GO) was treated by some stong oxidizing acids to form small pieces of graphene oxide [3]. Secondly, AuNPs-TiO_2-RGO nanocomposites were synthesized for immobilization of enzyme labeled antibody [4]. Lastly, CEA amperometric immunosensor was fabricated which based on HRP-Ab_2-AuNPs-TiO_2-RGO nanocomposites.

XRD spectra

The crystalline phase and structure of the as-prepared TiO_2-hematoxylin-GO and AuNPs-TiO_2-RGO were investigated by XRD. As shown in Fig. 1, characteristic peaks of TiO_2-hematoxylin-GO at 2θ=25.300, 37.774, 48.098, 53.982, 55.077, 62.617 and 75.079 were assigned to the diffraction of (101), (004), (200), (105), (211), (204) and (215) crystalline planes of anatase phase of TiO_2 (JCPDS card no. 21−1272). There was a strong diffraction peak appearing at 2θ=27.541, which was ascribed to the (110) crystalline plane of rutile phase of TiO_2 (JCPDS card no. 21−1276). This was because as-used TiO_2 was commercial P25, which included anatase and rutile phases. Besides, no diffraction peaks for other species were observed, which might be due to the low

content and relatively low diffraction intensity in the composites.

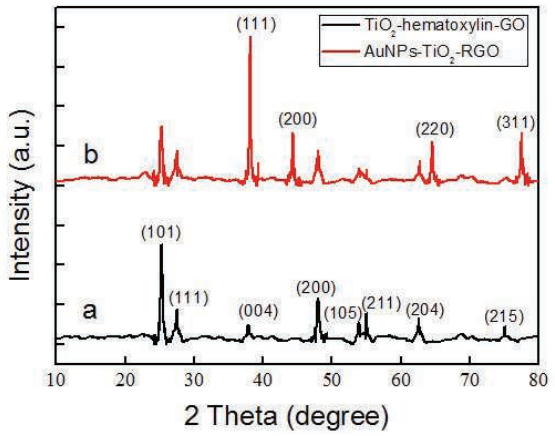

Fig. 1. XRD spectra of TiO_2-hematoxylin-GO and AuNPs-TiO_2-RGO

Electrochemical characterization

The electrochemical immunoassay depends on the electrochemical features of horseradish peroxidase (HRP), which catalyzes the oxidation of hydroquinone (HQ) and H_2O_2. With the existence of HRP and HQ, the reduction peak current of HQ would be enhanced by the corresponding increase of H_2O_2 content. The cathodic current changes before and after the addition of H_2O_2, were used as signals (shown in Fig. 2). Before the addition of H_2O_2, the electrode showed a reduction current peak of HQ (black line). After that, with the increasing of H_2O_2 concentration, reduction current of HQ was in an upward trend, indicating that the oxidation reduction reaction between HQ and H_2O_2 catalyzed by HRP was promoted. It also suggested that HRP-Ab_2 was successfully immobilized on the AuNPs-TiO_2-RGO nanocomposites.

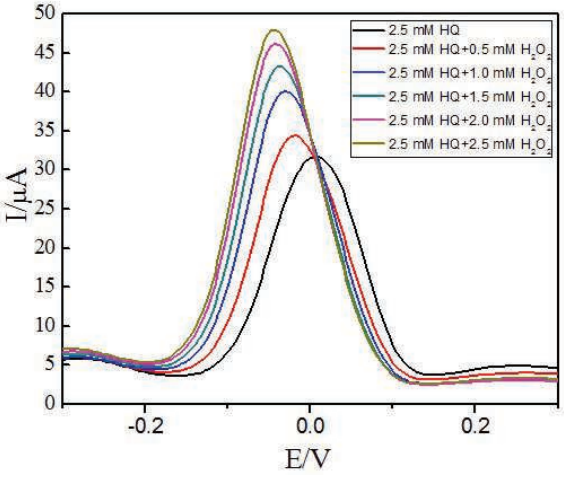

Fig. 2. DPVs of HRP-Ab_2-AuNPs-TiO_2-RGO/BSA/anti-CEA/AuNPs/GCE in 0.1 M PBS (pH 7.4) + 0.9% NaCl + 2.5 mM HQ with different concentration of H_2O_2: 0, 0.5, 1.0, 1.5, 2.0, 2.5 mM.

Conclusion

In this paper, we constructed a sandwich electrochemical immunosensor based on AuNPs-TiO_2-RGO nanocomposites immobilized by enzyme labeled antibody. The as-prepared immunosensor has potential applications in clinical diagnostics.

Acknowledgements

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