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A novel strategy for multiplexed immunoassay of tumor markers based on electrochemiluminescence coupled with cyclic voltammetry using graphene-polymer nanotags

Xiaobin Feng [a], Ning Gan [a,*], Huairong Zhang [a], Qing Yan [a], Tianhua Li [a], Yuting Cao [a], Futao Hu [b], Hongwei Yu [b], Qianli Jiang [c]

[a] State Key Laboratory Base of Novel Functional Materials and Preparation Science, Faculty of Materials Science and Chemical Engineering, Ningbo University, Ningbo 315211, PR China
[b] Faculty of Marine, Ningbo University, Ningbo 315211, PR China
[c] Department of Hematology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, PR China

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ABSTRACT

A novel immunosensing strategy was designed to simultaneous analysis of alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) by using electrochemiluminescence (ECL) coupled with cyclic voltammetry (CV) methods. In the assay, two kinds of graphene-polymer based nanotags were fabricated for tumor makers (TMs) distinction and signal amplification. The CV nanotags (rGO-H/Pt-PV) were prepared by immobilizing PowerVision doped platium nanoparticles complex (Pt-PV) on the surface of hemin modified reduced graphene oxide sheets (rGO-H) composites. Pt-PV is a kind of compact enzyme-linker linear polymer with a great number of horseradish peroxidase and was then modified with Pt NPs, which have the double catalyst abilities of the reduction of H_2O_2 at cathode potential in the presence of hemin. The ECL signal nanotags (rGO-PLL/Ru-Si@Au) were prepared by immobilizing Ru $(bpy)_3^{2+}$-silica doped gold nanoparticles composites (Ru-Si@Au) on reduced graphene oxide sheets (rGO) using poly-L-lysine (PLL) as linker and co-reactant. Moreover, the biosensor platform was prepared by co-immobilizing CEA and AFP's first antibody on gold nanoparticles modified glassy carbon electrode. Based on a sandwich-type immunoreaction, CEA and AFP, CV and ECL nanotags were conjugated on the electrode sequentially. In one-cycle voltammetric test, the CV nanotags produce a cathode current signal at −0.3 V and the ECL nanotags emit an anode luminescent signal at 1.25 V, which were respectively used for CEA and AFP detections. Under the optimum conditions, CEA and AFP could be assayed in the linear ranges of 5 pg mL^{-1} - 40 ng mL^{-1} and 3 pg mL^{-1} - 50 ng mL^{-1}, with the detection limits of 1 pg mL^{-1} and 0.5 pg mL^{-1} (at $3s_B$), respectively. More importantly, this designed method was used in real serum samples analysis and has significant potential for TMs detection in a clinical laboratory setting.

1. Introduction

Tumor markers (TMs) are kind of biochemical substances in blood or tissues, which can monitor cancer's occurrence and growth in the body [1]. Therefore, TMs detection is of crucial importance to the clinical cancer screening and early diagnosis. However, single TM immunoassay is usually not sufficient for diagnosis purpose because it is not specific to a particular tumor. Moreover, cancer diagnosis often requires us to detect multiple kinds of TMs. For instance, liver cancer diagnosed at least needs to simultaneously detect carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP). Therefore, developing multiplex immunoassays for several TMs is very necessary to improve the diagnostic efficiency and accuracy in clinical laboratories [2].

Up to now, various immunoassays include enzyme-linked immunosorbent assay [3], fluorescence immunoassay [4], chemiluminescence immunoassay [5,6], electrochemical immunoassay [7,8], electrochemiluminescence (ECL) immunoassay [9,10], quartz crystal microbalance immunoassay [11], and surface plasmon resonance immunoassay [12] have been used to realize multi-analyte determination. Among them, ECL immunoassays are considered as one of the predominant analytical techniques for TMs detection owing to its simple instrumentation, high sensitivity and ease to automate [13,14]. For example, Guo et al. have

* Corresponding author. Tel.: +86 574 87609987; fax: +86 574 87609987.
E-mail address: ganning@nbu.edu.cn (N. Gan).

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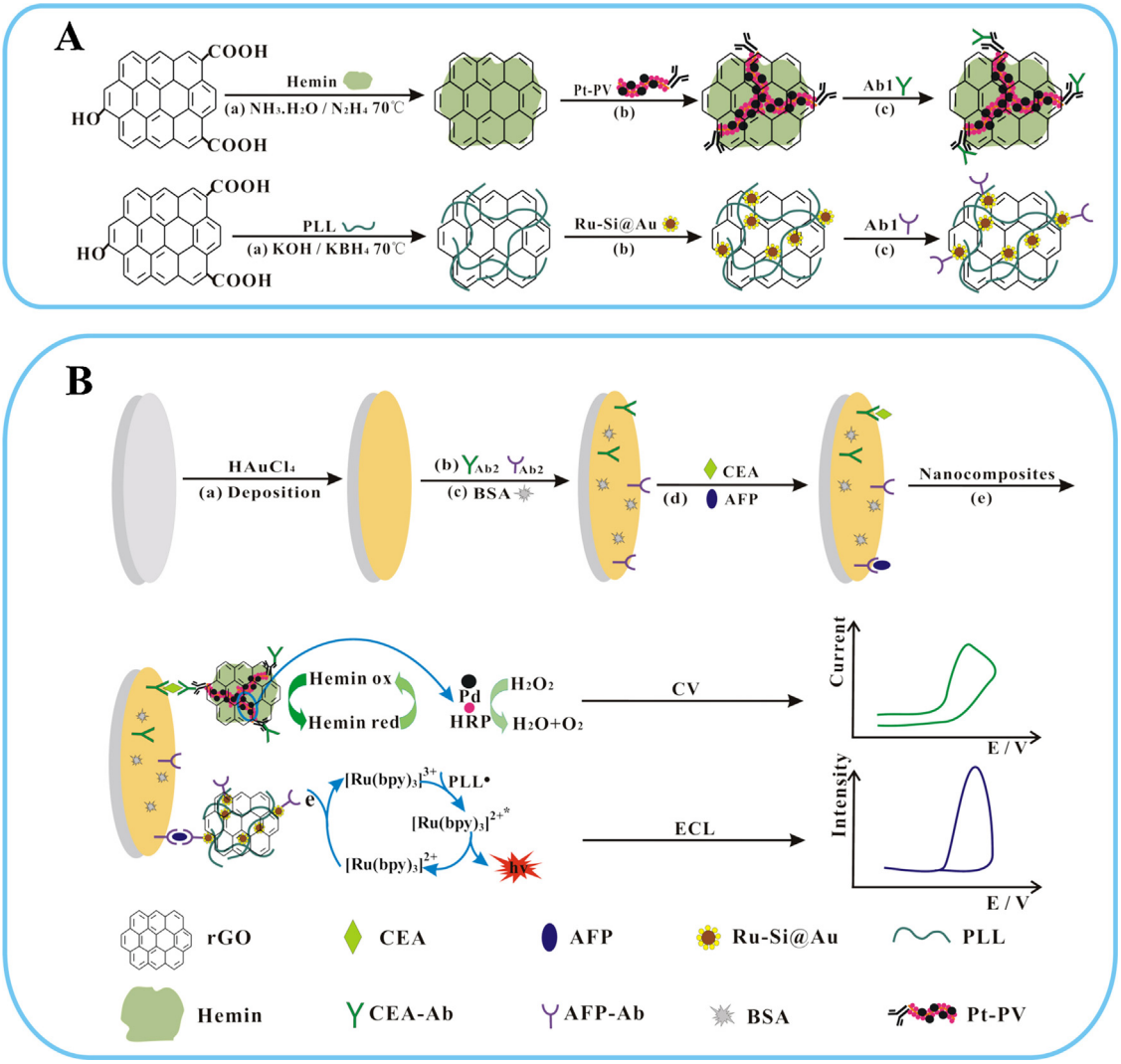
designed a novel sandwich-type ECL immunosensor for multiplexed immunoassay using multicolor quantum dots as labels with the help of two band-pass filters to detect AFP and CEA analytes with a low detection limit [15]. Unfortunately, band-pass filter can not able to completely shield the lights with other wavelength while detecting the target wavelength emitted by quantum dots, which can affect the accuracy of the assay results [16]. Therefore, it is still a great challenge to develop more facile, cheap and sensitive multiplex immunoassays for TMs detection.

It is well known that the normal ECL analyzer is assembled with a chemiluminescent analyzer and a potentiostat. However, the traditional ECL immunosensor commonly adopted the light signal to detect the targets, often ignoring the current signal [17,18]. Recently, the dual signal ECL method has been developed for analyte detection [19,20], but the requirement of luminescent reagents limited its practical applications. For instance, the two luminescent reagents not only require the large difference of excitation potential but also need to not interfere with each other. In fact, the light excitation process will also cause the change of current, which could generate a current signal. Inspired by this phenomenon, we developed a novel strategy for multiplexed immunoassay based on ECL coupled with CV methods which could be implemented by ECL analyzer at different potential. To our best knowledge, this kind of method has rarely been reported.

For the successful development of the immunoassays, it is important to create the distinguishable CV and ECL signal tags which can produce signals at different potentials. Hemin is usually employed as electron mediator for electrochemical detection, which can produce catalytic cathode current under hydrogen peroxide and HRP at pH 7.0 [21]. Tris (2,2'-bipyridyl) ruthenium(II) ($Ru(bpy)_3^{2+}$) is the most widely used ECL signal source material, which can emit luminescent signal at anode potential [22,23]. Therefore, hemin and $Ru(bpy)_3^{2+}$ could be respectively used to prepare the distinguishable CV and ECL signal tags and could be detected by one ECL analyzer. Based on the above-mentioned perspectives, we developed a novel immunosensing strategy based on ECL coupled with CV for simultaneous detection of AFP and CEA using gold nanoparticles (Au NPs) modified glassy carbon electrode (GCE) as the biosensor platform and the distinguishable signal tags as tracers. As can be seen in Scheme 1, the CV nanotags (rGO-H/Pt-PV) were prepared by immobilizing PowerVision (PV) complex doped platium nanoparticles (Pt-PV) on the surface of hemin modified reductive graphene oxide (rGO-H) composites. It could produce a dual amplified catalytic current because PV is a kind of compact enzyme-linked conjugation with a high number of horseradish peroxidase enzymes and Pt NPs possess excellent electrocatalytic activity to hydrogen peroxide [24,25]. The ECL nanotags (rGO-PLL/Ru-Si@Au) were prepared by immobilizing $Ru(bpy)_3^{2+}$-silica doped



Scheme 1. (A) Preparation procedure of CV and ECL nanotags. (B) Schematic illustration of stepwise immunosensor fabrication process and the signal generation mechanism.