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A novel immunosensor for detection of beta-galactoside alpha-2, 6-sialyltransferase in serum based on gold nanoparticles loaded on Prussian blue-based hybrid nanocomposite film

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ABSTRACT

In this paper, we presented a novel electrochemical immunosensor using gold nanoparticles (AuNPs) loaded on the Prussian blue (PB)-based hybrid nanocomposite film that sensitively detects β-galactoside α-2, 6-sialyltransferase (ST6Gal-I). The immunosensor was fabricated based on a Prussian blue-based (PB) hybrid nanocomposite, consisting of graphene oxide (GO), multi-walled carbon nanotubes (MWCNTs), PTA (a derivative of 3, 4, 9, 10-perylenetetracarboxylicdianhydride, PTC-NH_2) and chitosan (CS). With the assistance of the abundance of amino groups from CS and PTA, AuNPs were densely adsorbed onto the surface of the nanocomposite, providing a large available surface area for the immobilization of abundant anti-ST6Gal-I. As measured by cyclic voltammogram (CV) and differential pulse voltammogram (DPV), PB served as an electroactive and biocompatible redox probe in achieving electrochemical signal. Under the optimal detection conditions, the immunosensor exhibited a good linear response to ST6Gal-I in the range from 0.01 to 250 ng mL^{-1} and a lower detection limit of 3 pg mL^{-1} (S/N = 3). Furthermore, the method was successfully applied to analyze ST6Gal-I from human serum samples and yielded satisfactory results. In conclusion, our prepared immunosensor presented a high sensitivity and excellent selectivity to ST6Gal-I, indicating its potential in future clinical and experimental analysis.

1. Introduction

Beta-galactoside alpha-2, 6-sialyltransferase (ST6Gal-I) is a member of sialyltransferases family, locates in the Golgi and trans-Golgi network and mediates the attachment of sialic acid in the α-2, 6-linkage to the N-acetyllactosaminic chain of glycoproteins and glycolipids [1]. A soluble form of ST6Gal-I also presents in systemic circulation [2,3]. Soluble ST6Gal- I is liberated from its membrane anchor by proteolytic action of the β-site APP-cleaving enzyme 1(BACE1) [4–6], which subsequently released proteolytically untethered ST6Gal- I into systemic circulation. Despite a lack of understanding for the physiological functions of the circulatory sialyltransferases, there have been numerous reports strongly correlating serum ST6Gal-I with pathologic conditions, such as cancer [7]. ST6Gal-I acts as a critical regulator of tumor cell survival by inhibiting a multiplicity of cell death pathways. In this vein, ST6Gal-I not only acts as a potential sensitive marker for diagnosis and prognosis of disease, but also has potential as a rational target for cancer therapy strategies. However, due to a lack of effective proposals in place that can accurately measure the levels of ST6Gal-I in serum, its potential remains mostly untapped.

Various techniques are currently used to analyze ST6Gal-I including immunohistochemistry (IHC) [8], western blot (WB) [9], PCR [10] and enzyme-linked immunosorbent assay (ELISA) [11]. However, there are limitations that associated with all of these approaches. Namely, they can be costly, laborious, semi-quantitative, requiring skillful operator or suffering from the drawbacks of limited sensitivity and narrow linear range [12]. Hence, it is necessary to explore a sensitive and accurate quantification approach for the detection of ST6Gal-I in research applications. In recent years, electrochemical immunosensors have shown their potential applications in clinical, biochemical, environmental and food quality analysis, owing to their advantages of relatively high sensitivity, convenience, low cost, simple instrument and the potential with in situ analysis [13–16]. Given these advances, we

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sought to fabricate a novel and effective immunosensor as a promising tool to sensitively and accurately determine ST6Gal-I levels.

Nanocomposites with electrochemical mediators (electroactive nanocomposites) have stimulated great interest in recent years in the fabrication of biosensors. Prussian blue (PB, ferric hexacyanoferrate), is known as "artificial peroxidase" due to its high activity toward the reduction of H_2O_2 and it has been used as a classical electrochemical redox mediator for the construction of the oxidase-based biosensors [17]. Recently, PB-based nanocomposites have been widely applied to detect H_2O_2 [18], glucose [19], organophosphorous pesticides [20] and a range of biomarkers [21,22]. Graphene oxide (GO) has potential applications in designing biosensors and nanocomposites due to unique physicochemical properties [23]. GO also could be used as an efficient nanocarrier of nanoparticles for high loading ratio in the field of electrochemical immunoassay [23–25]. GO-PB nanocomposite has been proved to be a promising sensing platform for the development of the electrochemical biosensors [26]. However, GO-PB is a relatively poor conductivity and instability nanocomposite, which limits its application in a biosensing field. To overcome the poor stability of GO-PB nanocomposite, a stable membrane material is of great importance. PTC-NH_2 (PTA) is a well-known porous matrix for biological substances and nanomaterial absorption and an excellent candidate for the construction of immunosensors [27,28]. Moreover, it has been previously used to successfully prevent the leakage of PB and improve the stability of PB-based biosensor [29]. On the other hand, in order to meet the demand for sensitive detection of analytes, efforts have been attempted to add nanomaterials into the electrode modified materials. Multiwalled carbon nanotubes (MWCNTs) have extensively been used to construct high sensitivity biosensors owing to its excellent electrical conductivity, high surface-to-volume ratio and great chemical stability [30,31]. Chitosan (CS), an excellent dispersing agent of MWCNTs, can improve the stability of MWCNTs-GO-PB nanocomposite. Meanwhile, abundant amino groups of CS can be utilized to adsorb metal nanoparticles, which were further used for immobilization of antibody [32,33]. Therefore, we tried to incorporate PTA and CS-MWCNTs into the GO-PB nanocomposite, enhancing the conductivity and stability of the GO-PB nanocomposite. Herein, our prepared the hybrid nanocomposite, denoted CS-MWCNTs-GO-PB-PTA, with large surface active area, high catalytic activity and good stability, which could be employed as a super sensing platform for the detection of ST6Gal-I.

In our study, to realize the antibody immobilization and further enhance the sensitivity of our proposed immunosenor, we chose gold nanoparticles (AuNPs) as the interface for its high conductivity, large surface area, strong adsorption ability and favorable biocompatibility [34]. AuNPs can not only strongly interact with amino groups of CS and PTA, but also be utilized as an intermediator to efficiently immobilize antibodies, maintain its bioactivity and amplify the electrochemical signal in the fabrication of immunosensors [27,34,35].

In this work, we described the first electrochemical immunosensor for the determination of ST6Gal-I based on PB-based hybrid

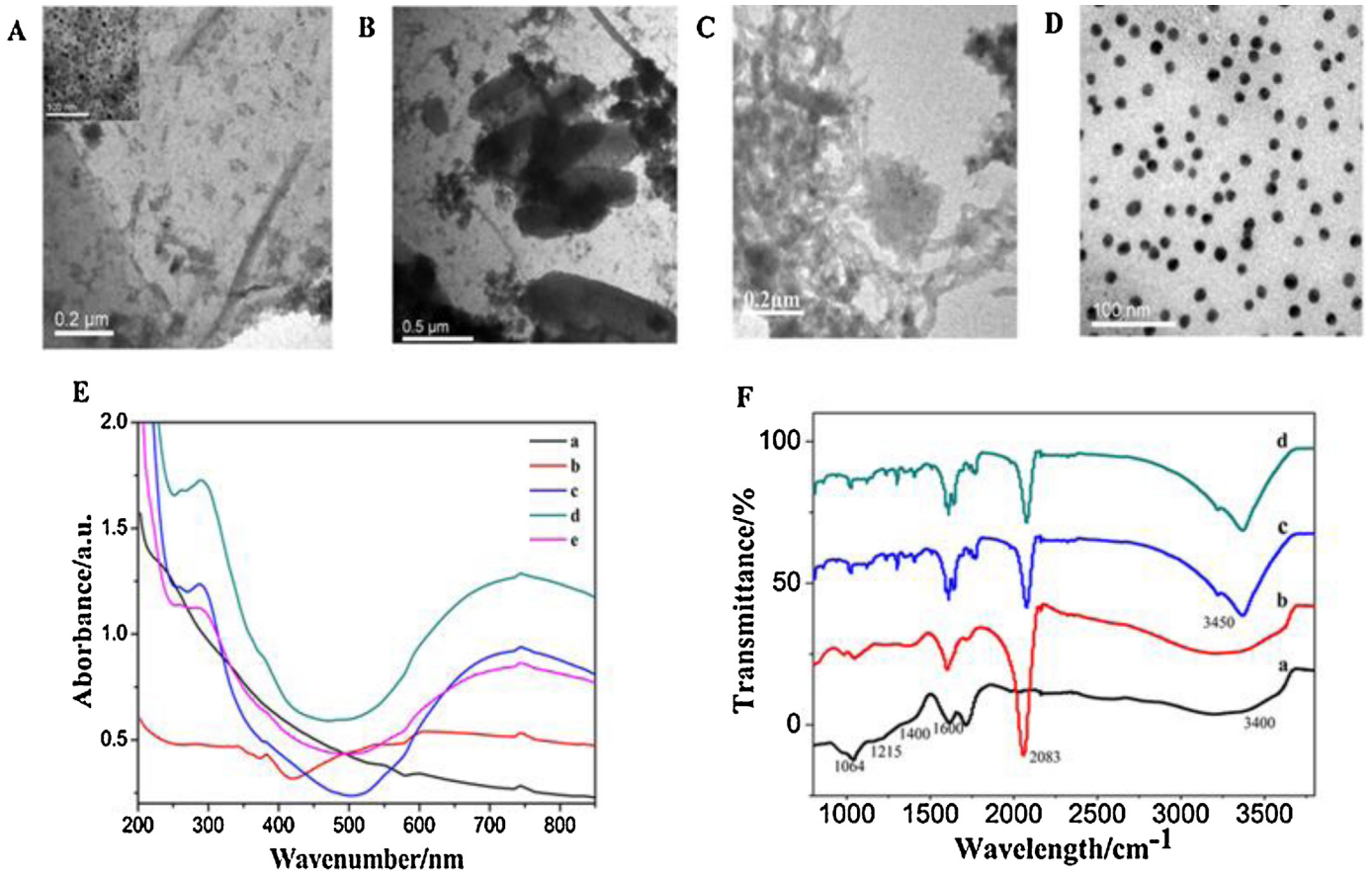

Fig. 1. (A) TEM image of GO-PB nanocomposite; (B) TEM image of GO-PB-PTA nanocomposite; (C) TEM image of CS-MWCNTs-GO-PB-PTA hybrid nanocomposite; (D) TEM image of AuNPs; (E) UV–vis absorption spectra of (a) GO, (b) PTA, (c) GO-PB nanocomposite, (d) GO-PB-PTA nanocomposite and (e) CS-MWCNTs-GO-PB-PTA hybrid nanocomposite; (F) FTIR spectra of (a) GO, (b) GO-PB nanocomposite, (c) GO-PB-PTA nanocomposite and (d) CS-MWCNTs-GO-PB-PTA hybrid nanocomposite.