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Regular Article

Electrochemical immunoassay for detection of prostate specific antigen based on peptide nanotube-gold nanoparticle-polyaniline immobilized pencil graphite electrode

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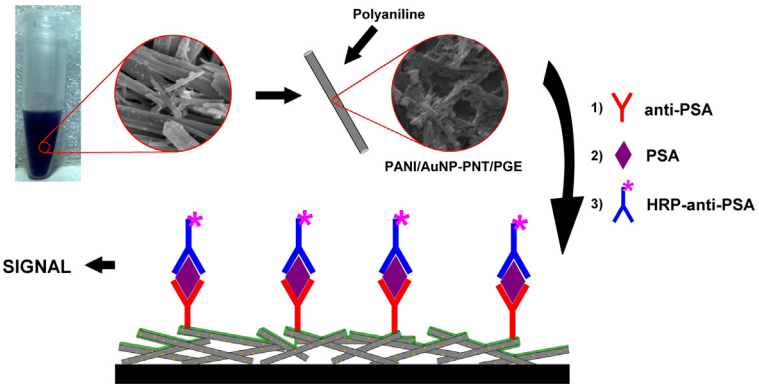
GRAPHICAL ABSTRACT



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ABSTRACT

In this work, we developed a disposable amperometric sandwich-type immunoassay to detect prostate specific antigen (PSA). A self-assembled peptide nanotube (PNT), gold nanoparticle (AuNP) and polyaniline (PANI) composite (PANI/AuNP-PNT) were used to modify a pencil graphite electrode (PGE). Anti-PSA (Ab1) was immobilized on the modified electrode (PANI/AuNP-PNT/PGE) to capture PSA. Horseradish peroxidase (HRP) labeled anti-PSA (HRP-Ab2) was used as a tracer antibody. The modified electrodes were characterized with scanning electron microscopy (SEM), thermogravimetric analysis (TGA), energy dispersive X-ray spectroscopy (EDS), transmission electron microscopy (TEM), cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS). PSA concentration in phosphate buffer (pH = 7.4) was determined with electro-catalytic reduction of H_2O_2 on the modified working electrode by using the chronoamperometric method. Limit of detection was found out to be 0.68 ng/mL in a linear range of 1–100 ng/mL with a high regression (R^2 = 0.990). To show the practicality of the modified biosensor in real matrixes, it was successfully applied for the detection of PSA in blood serum samples. The proposed method was also compared with enzyme-linked immunosorbent assay (ELISA) and compatible results were obtained. The developed immunoassay exhibited good reproducibility together with high stability and provides an efficient approach to detect PSA cost-effectively compared to traditional methods.

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1. Introduction

Prostate specific antigen (PSA) is a type of glycoprotein and is produced by the prostate. PSA is used as a biomarker for the diagnosis of prostate cancer [1,2]. The main function of the PSA enzyme is to break down the gel-forming proteins such as semenogelins I and II in the semen, thereby increasing the motility and productivity of the semen [3]. Though a certain level of PSA is found in the blood serum of a healthy person, this amount is increased in patients. Additionally, it was determined that there was a relationship between the amount of PSA and the level of the disease. PSA level is below 4 ng/mL in healthy men, while it is above 4 ng/mL in prostate cancer patients this level may increase depending on the level of cancer progression [4]. Therefore, the role of PSA plays a pioneering role in prostate cancer. Hence, the rapid, sensitive and alternative methods for the detection of PSA maintains its importance.

In the literature, some conventional methods were usually practiced for the detection of PSA such as enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), chemiluminescence immunoassay (CLIA), electro-chemiluminescence immunoassay (ECLIA), chemiluminescent microparticle immunoassay (CMIA), fluoro-immunoassay (FIA). Though these procedures are very efficient and highly accurate, the realization of these tests is laborious, time-consuming and quite costly. Therefore, biosensors which work with different principles have been developed to detect biomarkers for enabling early detection of cancer [5–7]. Among these, electrochemical biosensors stand out due to some of their superior features such as rapid and high sensitivity detection, easier, and faster and cheaper monitoring of disease progression [8–10].

Diphenylalanine (FF) dipeptide can form a peptide nanotube (PNT) structure by self-assembly under certain conditions [11,12]. PNTs are promising materials due to their stability, ease of synthesis and biocompatibility [13–16]. Diphenylalanine based peptide nanostructures have attractive properties for many nanotechnological applications such as molecular recognition, drug release, photovoltaic cell, and energy storage [17,18]. Furthermore, the electrical conductivity of PNTs have been studied [19,20] and they have been applied in the electrochemistry field owing to their ease of synthesis and modification, ability to increase the signal/noise ratio, biocompatibility, and stability [21–24]. When PNTs are combined with other sensor materials, they can show a synergistic effect with a high selectivity and sensitivity. For instance, the greatest success of the gold nanoparticles (AuNPs) is to increase the electro-active surface area in catalytic applications [25]. Furthermore, it provides direct electron transfer, has high surface area/volume ratio and shows biocompatible properties [26]. Thus, it is used extensively in biosensor applications in the literature [27]. Conducting polymers like polyaniline (PANI), polypyrrole, poly(o-toluidine), and their composites have been extensively studied in the literature [28–32]. Especially, PANI is a highly remarkable and intensive research topic among conducting polymers. This is due to the fact that polyaniline and its derivatives, composites or co-polymers can be easily synthesized chemically or electrochemically by oxidative polymerization. Thus, PANI has been combined with various nanomaterials and nanoparticles, for improving physicochemical properties of the materials and for the construction of effective detection sensor platforms [33–39]. Considering the studies in the literature, PANI/AuNP-PNT modified sensor application was not found.

Herein, we fabricated PANI/AuNP-PNT modified PGE which was a disposable, cheap, and easy method for the chronoamperometric detection of PSA for the first time. PANI/AuNP-PNT composite enhanced the signal of PSA remarkably due to their synergistic effect, high surface area, and signal/noise ratio. Electrochemical and spectroscopic methods were carried out for revealing the effect of the modification on the electrode surface. ELISA was used as a control method and similar results were obtained with both analysis. We obtained comparable data with similar studies in the previous literature. The developed method demonstrated the adaptability of PSA detection in clinical applications instead of conventional, high cost and time-consuming process.

2. Experimental

2.1. Chemicals and reagents

Gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *n*-hydroxysuccinimide (NHS), Bovine serum albumin (BSA), hydroquinone (HQ), phosphate buffer saline (PBS) tablet (pH: 7.4, 10 mM), and aniline were purchased from Sigma-Aldrich. Pencil graphite electrode (PGE, 0.5 mm, HB) was obtained from local market (Tombow Pencil Co. Ltd., Japan). Diphenylalanine was obtained from Bachem, Switzerland. PSA antigen (Ag), anti-PSA antibody (Ab1), and secondary anti-PSA antibody (Ab2) labeled with horseradish peroxidase (HRP) were purchased from Fitzgerald, U.S.A. All aqueous solutions were prepared with Milli-Q deionized pure water with a resistivity of 18.2 MΩ cm. All reagents were analytical grade and used without further purification.

2.2. Instrumentation and measurements

To enlighten the features of the developed electrodes, scanning electron microscopy (SEM, Zeiss Evo 60), thermogravimetric analysis (TGA, Thermo Scientific), energy dispersive X-ray spectroscopy (EDS, Bruker AXS Quantax 4010), transmission electron microscopy (TEM, JEM-1400Plus) were used. All electrochemical measurements were carried out using CH Instrument Potentiostat/Galvanostat in a three-electrode configuration. The modified electrode was used as the working electrode by dipping 1 cm of the electrode in a three-electrode cell. The reference electrode was Ag/AgCl (3.0 M KCl) and the counter electrode was platinum wire. Before measurements, all solutions were deaerated with pure nitrogen gas for about 20 min.

2.3. Fabrication of the biosensor

We synthesized gold nanoparticle/peptide nanotube hybrid nanomaterial to modify pencil graphite electrodes. Firstly, 6.0 mg diphenylalanine was dissolved in 100 μL hexafluoroisopropanol and added in 0.5 mg/mL Gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$) solution in different concentrations. Then, the solution was sonicated for 10 min and then incubated at 60 °C for an hour. At the end of this period, diphenylalanine self-assembled to form nanotubes and gold nanoparticles was formed. To fabricate AuNP-PNT/PGE, pencil graphite electrode was modified by dipping into AuNP-PNT dispersion and incubated for 5, 15, and 30 min at room temperature. Afterwards, the AuNP-PNT adsorbed electrode was dried and washed with DI water. PANI thin film was synthesized electrochemically onto AuNP-PNT/PGE by cyclic voltammetry. Electro-polymerization of aniline was carried out in an electrolyte solution composed of 0.5 M H_2SO_4 and 1 mM aniline. Cyclic voltammograms (CVs) were recorded at a scan rate of 0.1 Vs^{-1} for a potential range of −0.1 to +1.1 V (vs.Ag/AgCl). Anti-PSA (Ab1) was attached to PANI/AuNP-PNT/PGE surface via 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/*n*-hydroxysuccinimide (NHS) amidization reaction described by Arya et al. [40]. To activate COOH group of Ab1, a solution was prepared containing 0.4 M EDC, 0.1 M NHS, and 10 μg/mL Ab1 in