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Short communication

A nanowire-based label-free immunosensor: Direct incorporation of a PSA antibody in electropolymerized polypyrrole

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ABSTRACT

We have suggested a novel method for the preparation of a label-free electrochemical immunosensor for the detection of prostate-specific antigen (PSA) as target marker for prostate cancer. Direct incorporation of PSA antibody (anti-PSA) into polypyrrole (Ppy) electropolymerized on a three-dimensional Au nanowire array has resulted in enhanced molecular interactions, ultimately leading to improved sensing performance. The electrochemical performance of the nanowire-based immunosensor array were characterized by (1) differential pulse voltammetry (DPV) to evaluate the specific recognition of PSA, (2) impedance and cyclic voltammetry to observe surface resistance and electroactivity, and (3) scanning electron microscopy (SEM) to demonstrate the three-dimensional architecture. The vertically-aligned geometric organization of Ppy provides a novel platform to improve the anti-PSA loading capacity. Overall, enhanced electrochemical performance of the proposed immunosensor has been demonstrated by its linear response over PSA concentrations ranging from 10 fg mL^{-1} to 10 ng mL^{-1} and a detection limit of 0.3 fg mL^{-1}, indicating that the strategy proposed here has great potential for clinical applications.

1. Introduction

A lot of effort has been expended to achieve significant progress in the field of immunosensors for the early detection and monitoring of cancer markers in blood. Currently, using various detection signals based on electrochemical, optical, mass, or calorimetric-sensitive mechanisms, target analytes in biological specimens can be easily detected and identified even at very low levels (Owino et al., 2008; Liu et al., 2008; Long et al., 2009; Tseng et al., 2012; Yuan et al., 2012). Among these promising approaches, electrochemical detection has attracted much interest because it allows direct, specific, and real-time monitoring (Wilson, 2005; Wan et al., 2011). Electrochemical biosensors can recognize subtle changes in the current, voltage, or electronic properties (i.e., impedance, capacitance) caused by highly specific interactions of antibodies with their corresponding antigens at the electrode surface. In addition, various nanomaterials such as gold nanoparticles, silica nanowires, carbon nanotubes, and graphene have been widely employed as novel matrices for the development of new immunoassays (Sharm et al., 2010; Qu et al., 2009; Chua et al., 2009; Wang, 2005; Yu et al., 2006, Du et al., 2010; Liu et al., 2011; Owino et al., 2008). Given its unique electrical, mechanical, and optical characteristics as well as high surface area with enhanced reaction activity, nanostructure has great potential in medical applications, particularly in diagnostic devices and point-of-care testing. However, in spite of these advances, significant challenges including complications in fabrication and subsequent functionalization with desired biomolecules as well as problems with regard to the chemical/mechanical stability of the sensor architecture remain unaddressed.

Conducting polymers such as polypyrrole (Ppy), into which various anions and cations including growth factors, anti-inflammatory drugs, ATP, glutamate, and protonated dopamine can be incorporated, can be electro-chemically deposited on electrodes for use as implantable devices or drug carriers (Abidian et al., 2006; Cui et al., 2003; Geetha et al., 2006; Kim and Martin, 2006; Pernaut and Reynolds, 2000; Wadhwa et al., 2006; Wallace and Kane-Maguire, 2002; Cho and Borgens, 2010, 2011; Kang et al., 2011). We attempted to expand this concept by fabricating a label-free electrochemical immunosensor with three-dimensional architecture with the purpose of improving stability, sensitivity, and specificity. The key strategy used in this study includes the deposition of a Ppy film on Au nanowire (NW) arrays, where anti-PSA can be simultaneously immobilized on individual Ppy NWs without additional modification steps (Fig. 1A). Advantages of using NWs as freestanding biosensing platform are (i) individual NWs can be precisely controlled and fine-tuned and (ii) they can serve as an efficient reservoir for the incorporation of high

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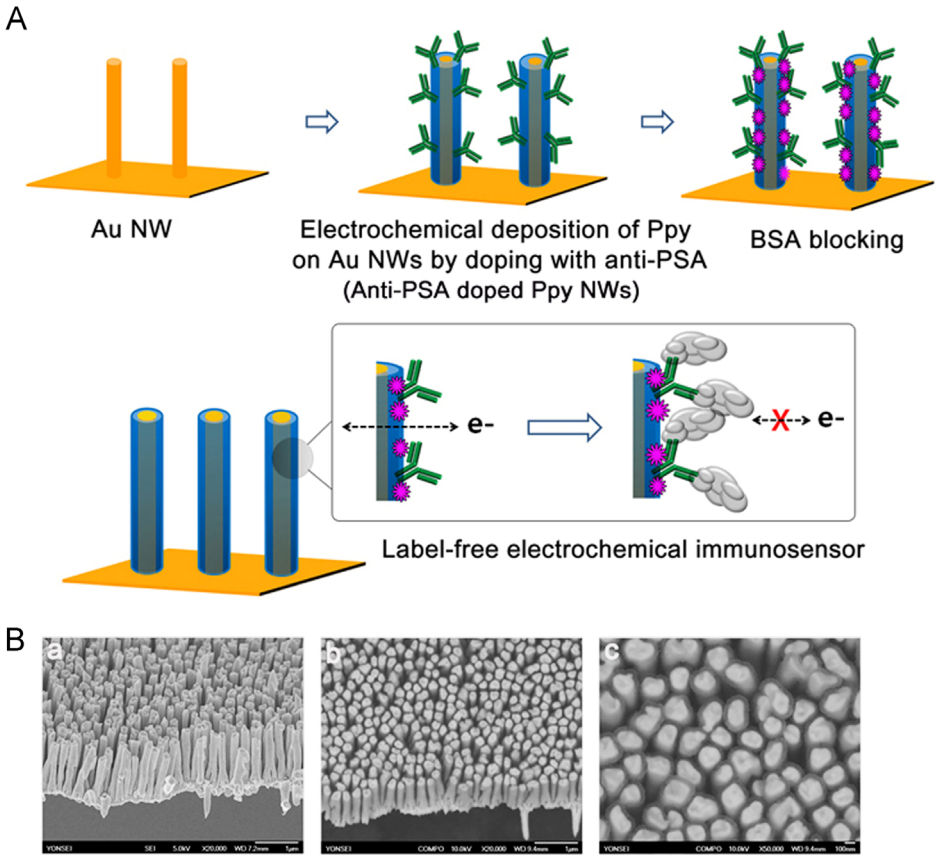

Fig. 1. (A) Schematic illustration of the preparation of BSA-immobilized, anti-PSA doped Ppy NWs (BSA/anti-PSA doped Ppy NWs) and (B) Field emission scanning electron microscope (FESEM) images of (a) free-standing uncoated Au nanowires, (b) anti-PSA doped Ppy NWs, and (c) higher magnification image of (b). FESEM revealed an evenly deposited 40-nm-thick layer of Ppy around the Au NWs.

concentrations of anti-PSA. Indeed, the large surface area and well-defined NW structure make them ideal for efficient antibody immobilization thus enhancing the loading capacity. In addition, the preferential electrostatic association between positive charges of the oxidized Ppy chains and negative carboxyl groups on the antibody affords strong immobilization while ensuring antigenic epitope conservation. As a reliable tumor marker, the concentration of PSA is directly correlated with the chance of prostate cancer. In general, PSA levels between 4.0 and 10.0 ng/mL indicate a 25–40% probability of prostate cancer; the cancer risk significantly increases to 67% if the PSA level is greater than 10 ng/mL (Gretzer and Partin, 2002; Leibovici et al., 2005). However, during the past few years, lower PSA levels (<4.0 ng/mL) have also been associated with a high incidence of prostate cancer. Hence, a detection limit in the nanogram to femtogram range is necessary.

2. Material and methods

2.1. Chemicals and reagents

Nanoporous anodic aluminum oxide (AAO) templates (Anodisc 13, 100 nm) were purchased from Whatman Corporation. Gold plating solution (Orotemp® 24 RTU Rack) was obtained from Technic Inc. Sodium hydroxide, pyrrole, poly(sodium 4-styrenesulfonate) (MW: ~70,000), potassium ferricyanide (III), potassium chloride, Tween-20, ascorbic acid, L-glutathione (reduced), immunoglobulin G from human serum, and human serum type AB (male) were obtained from Sigma-Aldrich and used as received. Mouse anti-human PSA monoclonal antibody and human PSA ELISA Kit (PSA) were from Anogen. Bovine serum albumin (BSA) was purchased from Bovogen. Milli Q water (Millipore) was used for the preparation of all solutions.

2.2. Fabrication of Au NWs and electrodeposition of anti-PSA-doped Ppy polymers

A thin film of Au (150 nm) was thermally evaporated onto one side of the AAO template with a pore diameter of 100 nm to serve as working electrode. For an electrochemical deposition experiment, an Au-backed AAO membrane was placed on a conductive 1.5×2 cm^2 indium-tin oxide surface and then inserted in a commercial Teflon electroplating cell; the open pore was exposed to the electrolyte well. Au NWs were electrochemically grown inside the pores of the AAO membrane using a commercial gold plating solution. Electrodeposition for the fabrication of Au NWs was carried out at room temperature using cyclic voltammetry by performing 100 scans over a potential ranging from -1.1 to 0 V at a scan rate of 100 mV/s. The AAO template was immediately attached to the indium-tin oxide surface using a conductive carbon paste, with the open end facing up, and subsequently dried at 60 °C overnight. The resulting membrane was dissolved in an aqueous NaOH solution (2 M) for 4 h to remove the AAO template, yielding freestanding Au NW arrays. The immobilized substrate was washed several times with water. To dope a Ppy film with anti-PSA antibody, we tested the effects of various experimental conditions on electrochemical current responses (Fig S3). After optimization of its concentration, 25 μg/mL of antibody (10 μL from a 1 mg/mL stock solution) was selected and mixed with an aqueous solution of 0.01 M pyrrole and poly(sodium 4-styrenesulfonate) (total volume: 400 μL). The resulting Ppy solution was electrochemically deposited on the vertically aligned Au NW array at an applied potential of +0.8 V (vs. Ag/AgCl) for 20 s. Conductive Ppy