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A reusable electrochemical immunosensor fabricated using a temperature-responsive polymer for cancer biomarker proteins

Wooyoung Hong [a], Sooyeon Lee [a], Eun Jae Kim [b], Maria Lee [b], Youngnam Cho [a,*]

[a] New Experimental Therapeutics Branch, National Cancer Center, 111 Jungbalsan-ro, Ilsandong-gu, Goyang, Gyeonggi-do 410-769, South Korea
[b] Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, South Korea

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ABSTRACT

In the present study, we describe a reusable electrochemical immunosensor for the repeated detection of cancer biomarkers using a single platform. The integration of a temperature-responsive polymer on the electrode surface enables easy manipulation of the biological sensing interface (i.e., addition of biotin, streptavidin, and antibody), thus allowing for temperature-induced regeneration and disruption of the interface architecture of the electrode surface. Using our immunosensor, we demonstrate sequential amperometric detection of three tumor markers: CA125, CEA, and PSA. Interestingly, greatly amplified signals are achieved by immersing the immunosensor in a solution of horseradish peroxidase (HRP) and antibody-labeled nanoparticles, resulting in a linear range of 0.0064 to 256 U/mL, 1 pg/mL to 100 ng/mL, and 10 pg/mL to 10 ng/mL with a detection limit of 0.007 U/mL, 0.7 pg/mL, and 0.9 pg/mL for CA125, CEA, and PSA, respectively. By alternating temperature, the immunosensor adsorbs and desorbs the biological elements without damage. Our proposed methodology can be expanded to measure other relevant biological species by repeated detection and thus has enormous potential for industrial and clinical applications.

1. Introduction

Analyzing cancer biomarkers with high precision and sensitivity enables their use in screening, diagnosing, and assessing the prognosis of cancer (Rifai et al., 2006; Rusling et al., 2010; Lie et al., 2012). Tumor markers are usually identified by conventional techniques, such as enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and mass spectrometry-based proteomics (Chester et al., 1991; Kulasingam and Diamandis, 2008; Yin et al., 2010). Particularly, immunoassays based on antigen–antibody interactions are a reliable tool for the determination of tumor biomarkers and are used in fundamental research and clinical settings (Devi et al., 2015; Huang et al., 2015). However, these techniques have limited practical use in Point-of-Care (POC) applications because they require specific instruments, large sample volumes, and laborious and time-consuming procedures. Over the past decades, many immunosensors that primarily rely on different detection techniques (i.e., electrochemical, optical, mass-sensitive, or calorimetric) have been developed for cancer screening (Owino et al., 2008; Long et al., 2009; Tseng et al., 2012). Of these, electrochemical sensors are advantageous due to their ability to measure subtle variations in electrical properties (i.e., current, voltage, or impedance) caused by the recognition of antibodies to their corresponding antigens on the electrode surface (Wan et al., 2011; Dey et al., 2012; Ge et al., 2012). In this study, we added an additional function to the electrode surface by integrating a thermo-responsive polymer, ultimately allowing switchable and reversible processes of the interface architecture. In general, fabricating reusable biosensors is challenging because of the damage to the sensing platform by the irreversible conjugation of biological elements and the difficulty to reconstruct the sensor by inconvenient and complex processes. Thus, to regenerate an immunosensing interface with high stability and reusability, we propose a novel strategy to introduce a thermo-responsive polymer, poly(N-isopropylacrylamide) (PNIPAAm), to the gold surface (P-Au) (Fig. 1, Fig. S1). PNIPAAm allows the transition between a hydrophilic and hydrophobic state in a temperature dependent manner, specifically at the threshold of the lower critical solution temperature (LCST) of 32 °C (Barhoumi et al., 2014; Nagata et al., 2015; Wong et al., 2015). To fabricate a sensitive sensing interface, biotinylated bovine serum albumin (BSA-biotin) was adsorbed to the P-Au surface as governed by the weak hydrophobic interactions at a temperature above LCST.

PNIPAAm is temperature-responsive, allowing for the repeated regeneration of the sensing interface to detect multiple tumor markers using a single platform. BSA-biotin provides flexibility by

* Corresponding author.
E-mail address: yncho@ncc.re.kr (Y. Cho).

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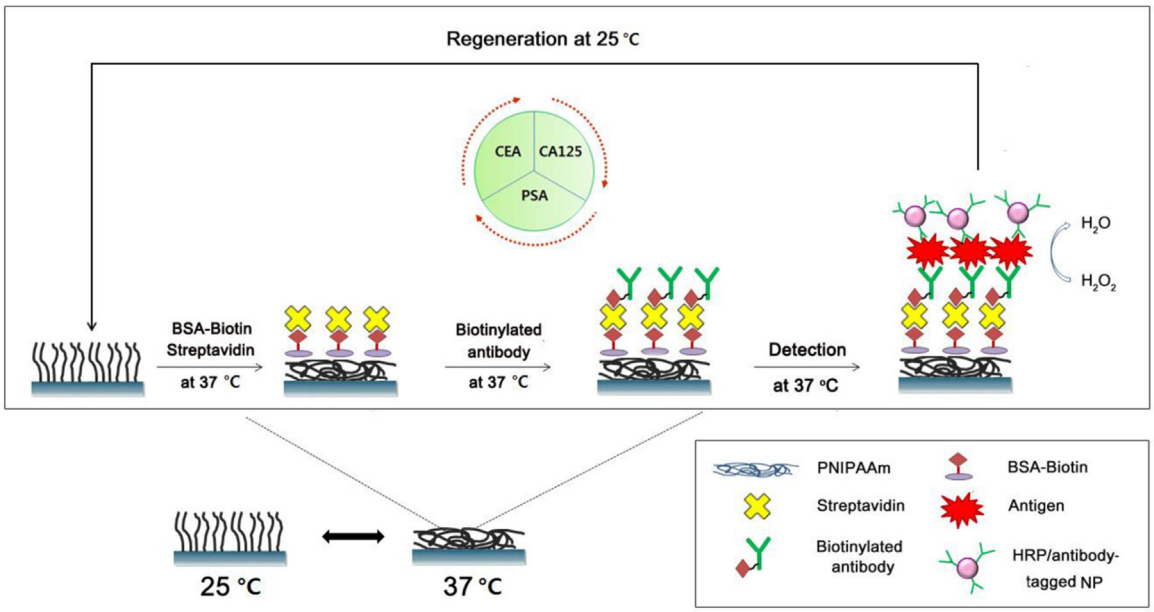

Fig. 1. Schematic illustration of the preparation of a reusable electrochemical P-Au immunosensor to detect multiple cancer biomarkers.

serving as an active site for sequential reactions, ultimately facilitating the immobilization of a biotinylated capture antibody onto the polymer-modified surface. As model analytes, we performed sequential electrochemical measurements of cancer antigen 125 (CA125), carcinoembryonic antigen (CEA), and prostate specific antigen (PSA) using our immunosensor. Additionally, we added polypyrrole nanoparticles labeled with multiple horseradish peroxidase and detection antibody (HRP/antibody-tagged NPs) to the immunosensor to significantly amplify the amperometric signals. These multilabeled nanoparticle-based bioconjugates initiate the activation of the peroxidase electrochemical cycle, particularly in the presence of a small amount of hydrogen peroxide. Thus, the current change can be measured as a result of the electro-catalytic reduction of hydrogen peroxide by applying a constant voltage (Mani et al., 2009; Munge et al., 2009; Zang et al., 2013). After measurement, the biological elements are easily released from the P-Au immunosensor by lowering the temperature to below the LCST of PNIPAAm. Interestingly, the variation in the applied temperature alters the strength of the binding affinity between BSA-biotin and PNIPAAm, ultimately resulting in the non-destructive detachment of the sensitive biological elements. The developed methodology can detect multiple cancer biomarkers using a single platform because the regeneration and removal of the sensing interface is easily controlled by temperature, thereby enabling the immunosensor to measure the electrochemical response in repeated experiments.

2. Materials and methods

2.1. Chemicals and reagents

Gold plating solution (Orotemp 24 RTU Rack) was purchased from Technic Inc (Anaheim, California, USA). Cysteamine hydrochloride, triethylamine (TEA), dichloromethane, α-bromoisobutyryl bromide (BIBB), *N*-isopropylacrylamide, pentamethyldiethylenetriamine (PMDETA), copper (I) bromide (CuBr), streptavidin, polyvinylpyrrolidone (PVP, MW= 29,000), aluminum phthalocyanine tetrasulfonate (AlPcS4), pyrrole, iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), hyaluronic acid, peroxidase from horseradish (HRP), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), hydroquinone (HQ), H_2O_2, and human serum type AB (male) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Biotinylated anti-CA125 and biotinylated anti-CEA were purchased from Biorbyt (San Francisco, California, USA). Biotinylated anti-PSA was obtained from Abcam (Cambridge, UK). CA125 antigen was purchased from R&D Systems (Minneapolis, MN, USA). CEA antigen was purchased from Abcam, and human PSA ELISA Kit (PSA antigen) was purchased from Anogen (Mississauga, Ontario, Canada). Biotinylated BSA was purchased from Thermo Scientific (Waltham, MA, USA). Milli-Q water (Millipore) was used for the preparation of all solutions.

2.2. Polymerization of PNIPAAm layers on Au surface

Electrodeposition of Au on indium tin oxide (ITO) glass electrodes was conducted in a commercial gold plating solution using a potentiostat/galvanostat (BioLogic SP-150). The Au surface was electrochemically prepared on ITO glass under cyclic voltammetry by performing 100 scans over a potential ranging from −1.1 to 0 V at a scan rate of 100 mV/s, where Ag/AgCl (3 M NaCl type), platinum wire, and ITO glass were used as reference, counter, and working electrodes, respectively. Next, the Au surface was immersed in 100% ethanol containing 2 mM cysteamine hydrochloride for 24–48 h at RT. The surface was rinsed with 100% ethanol and ultrapure water and then air-dried. The amine-thiol terminated Au surface was incubated in 3 mL of dichloromethane containing TEA (5 vol%) at RT for an additional 12–24 h. The substrate was cooled to 0 °C for 1 h, then 600 μL of initiator BIBB (CH2Cl2/TEA:BIBB (v/v)=5:1) was added dropwise to the solution. The reaction was carried out at 0 °C for 0.5 h and then left at RT for an additional 12–24 h. After cleaning with dichloromethane, acetone, and water, the resulting surface was dried under a nitrogen flow. The reaction solution consisted of NIPAAm (1.25 g, 55.23 mmol), PMDETA (140 μL, 3.35 mmol), and CuBr (32 mg, 1.12 mmol) dissolved in a 1:1 (v/v) mixture of methanol and water (5 mL). The reaction solution was sonicated for 2 min and then was added to a glass vessel with the initiator-functionalized Au. The polymerization was carried out at room temperature for 2 h. After polymerization, the obtained PNIPAAm-grafted flat Au was removed from solution, rinsed exhaustively with deionized water to remove the unreacted NIPAAm monomer and ungrafted PNIPAAm,