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In situ Electrochemical-Transmission Surface Plasmon Resonance Spectroscopy for Poly(pyrrole-3-carboxylic acid) Thin-Film-Based Biosensor Applications

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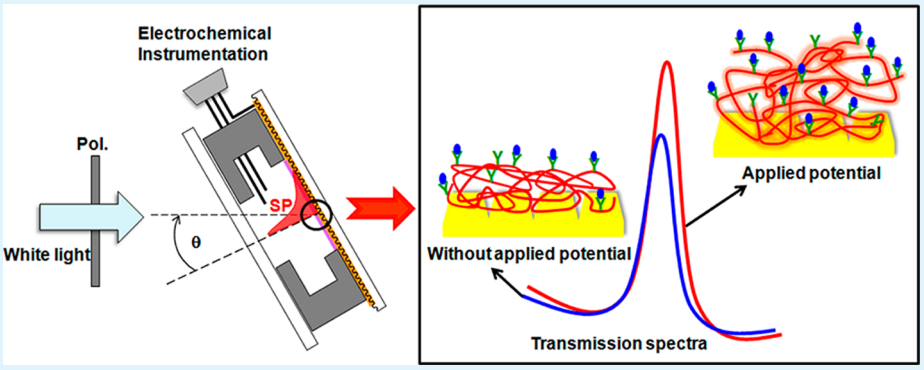
Supporting Information



ABSTRACT: In this study, we describe the combination of transmission surface plasmon resonance (TSPR) and electrochemical techniques for the application to biosensors with conducting polymers. Electropolymerization was employed to construct poly(pyrrole-3-carboxylic acid) (PP3C) film on a gold-coated grating substrate using pyrrole-3-carboxylic acid (P3C) monomer solution in 0.5 M H_2SO_4. In situ electrochemical-transmission surface plasmon resonance (EC-TSPR) measurements were carried out to study the kinetic and electroactivity properties of PP3C film. Immobilization of antihuman IgG on the activated surface and the binding process of human IgG and antihuman IgG in neutral solution could be detected in situ by EC-TSPR measurement. The surface modification steps on the PP3C layer led to an increase in intensity of the transmission peak. The performance, sensitivity, and utility of EC-TSPR spectroscopy showed obvious advantages for the detection of binding process with the simple experimental setup, and could be applied to the study of biomolecular interactions in various systems.

KEYWORDS: *electrochemical-transmission surface plasmon resonance, grating, conduction polymer, poly(pyrrole-3-carboxylic acid), biosensor, human IgG*

1. INTRODUCTION

Surface plasmon resonance (SPR) is a powerful technique to monitor interfacial reactions and thin film deposition in real time.[1−3] Recently, an optical biosensor-based technique using SPR has been explored to study the interaction of numerous biomolecules including deoxyribonucleic acid (DNA), proteins, enzyme, antibodies, and antigens.[4−7] This is because of its characteristic of being a label-free analytical method with online monitoring and high selectivity.[8] Surface plasmon waves can be excited by the attenuated total reflection (ATR) method with a prism[1] or a diffraction grating coupling configuration.[9,10] The advantages of grating-based SPR technique include the fact that it is prism-less, convenient, and propagating SPR excitation method.[11−13] More recently, several researchers have improved the sensitivity of biosensors and other immunosensor-based grating-coupled SPR methods.[14−16]

The transmission of a specific wavelength of light which can be enhanced by SPR has been discovered[17] and is termed transmission surface plasmon resonance (TSPR). The principle of detection relies on the transmission spectra of p-polarized light, which is observed when the light passes through the thin gold film coated-grating substrate under specific conditions.[18,19] The TSPR-based sensor is an attractive platform in label-free biosensor and immunosensor applications because it is applicable to simple in situ sensing systems.

Conducting polymers in the area of biosensors have been attracting considerable attention.[20−24] Biosensors based on conducting polymers have been used to detect biomolecules

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such as glucose, hormones, neurotransmitters, antibodies, and antigens.[25−30] Immunosensors are biosensors based on specific antigen−antibody interactions in which the transducer directly or indirectly detects immunochemical reactions.[31,32] In particular, polypyrroles (PPy) and their derivatives containing the carboxylic group have been the most promising conducting polymers studied for immunosensor applications.[33−35] This is because of the possibility of grafting biologically active molecules through a covalent bond with carboxylic functionalities. Also, they have received considerable attention because of their advantages of good stability and high immobilization density.[35,36]

In recent years, a combination of SPR and electrochemical measurement has been reported for the study of conducting polymer,[37−39] and has been adapted for monitoring the interaction between biomolecules and electropolymerized conjugated polymer films in several groups.[26,35,40−43] However, a study using TSPR spectroscopy combined with an EC method for in situ investigation of the optical and electrochemical properties of conducting polymer films and the biomolecular interactions has not been reported. To our knowledge, this is the first study on biosensors using in situ EC-TSPR technique to simultaneously measure optical and electrical responses with conducting polymers. We demonstrated that the sensitivity and utility of a TSPR technique and the possibility to control the morphology, optical properties, and stability of polymer film by an electrochemically controlled method are key issues regarding possible applications of this new technique.

In this study, we report the EC-TSPR biosensors for the detection of human IgG based on a poly(pyrrole-3-carboxylic acid) (PP3C) film. The EC-TSPR technique is a promising candidate to generate an efficient tool to monitor the optical and electrical properties of conducting polymer films on metal supports, particularly for the detection of biomolecular interactions. This is because of its high affinity and inherent advantages over other techniques (e.g., simple instrumentation and a simple platform based upon an inexpensive and commercially available diffraction grating). PP3C is a particularly promising material for immunosensor applications due to its unique optical, electrical, and structural properties. A electrochemical immunosensor could have an electrochemically controlled surface morphology to enhance the sensitivity of the sensing signal. Herein, the PP3C film was fabricated by electropolymerization of a P3C monomer on a gold-coated transparent grating substrate. In situ EC-TSPR spectroscopy was performed to study the kinetic properties during electropolymerization of the deposited PP3C film and the electroactivity of PP3C film in neutral phosphate-buffered saline (PBS) solution. The EC-TSPR immunosensor-based PP3C film was constructed using monoclonal antihuman IgG produced in mice as a model analysis. The real-time TSPR response during the construction of the PP3C-based immunosensor and the binding process of antihuman/human IgG with various concentrations of human IgG were monitored in situ by EC-TSPR measurement. Moreover, to investigate the electrochemically enhanced immunosensor sensitivity, we measured the TSPR responses at several constantly applied potentials compared with the open-circuit condition.

2. MATERIAL AND METHODS

2.1. Materials. P3C monomer, PBS tablets, monoclonal antihuman IgG (Fab-specific) produced in mice, and reagent-grade human IgG serum were obtained from Sigma-Aldrich (St Louis, MO, USA) and used as received.

2.2. Instruments. In situ EC-TSPR measurement was obtained using a TSPR setup combined with a three-electrode electrochemical cell (Figure 1a). The TSPR setup was carried out in a collinear

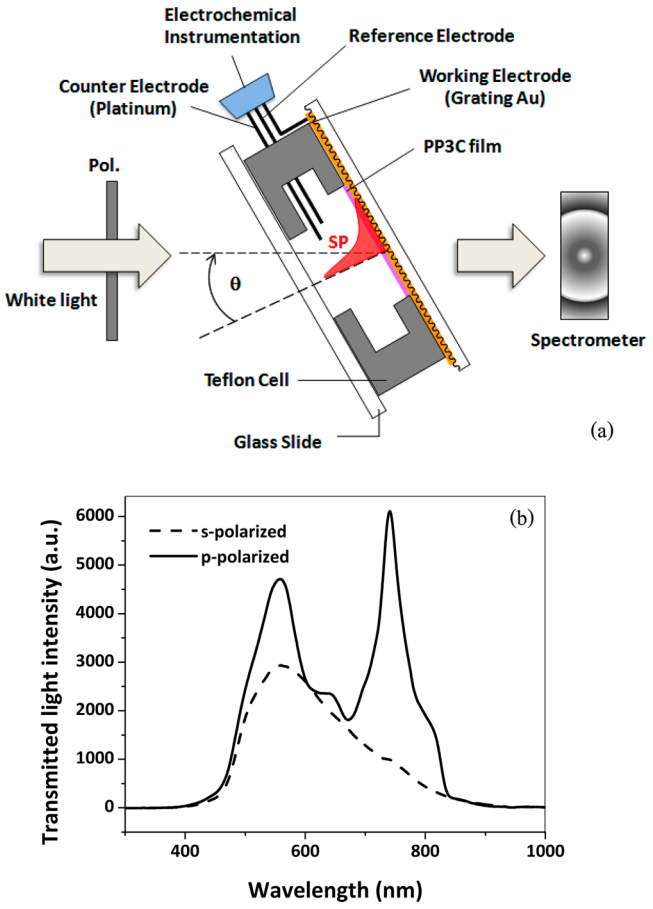

Figure 1. (a) Schematic illustration of the EC-SPR setup and (b) TSPR spectra for s-polarized and p-polarized light on a gold-coated grating substrate at an incident angle (θ) of 35°.

geometry. The white light from a halogen source (model LS1; Ocean Optics, Dunedin, FL, USA) was collimated using a convex lens with focal length of 150 mm (Newport Corporation, Irvine, CA, USA). The resulting beam was passed through a linear polarizer, which was captured using p- and s-polarized light before illuminating the grating sample through an aperture (diameter, 2 mm). The sample was mounted on a rotating sample holder for manual alignment. The transmitted light was collected by a 600-μm optical fiber and recorded with a Fiberoptic Spectrometer (SD2000; Ocean Optics). OP Wave software was used for all measurements, storage, and processing of data. For the electrochemical system, the electrochemical cell consisted of a three-electrode cell with platinum wire and silver wire used as the counter and reference electrodes, respectively. The gold-coated transparent grating substrate was used as a working electrode. All the potentials reported in this work are relative to this reference electrode. The electrochemical experiments were measured in a single-compartment three-electrode electrochemical cell with a computer-controlled potentiostat HZ-5000 model (Hokuto Denko).

2.3. Grating Construction. DVD-R (Taiyo Yuden) was used as the diffraction grating substrate in this work. Initially, the DVD-R was cut into several pieces and then grooved polycarbonate pieces were manually split from the polymer coating layer. The layer of dye on the grooved polycarbonate side was removed by soaking in concentrated nitric acid and then rinsed with ethanol and deionized water. The gold-coated grating substrate was prepared by vacuum evaporation of

approximately 3 nm Cr and 47 nm gold on the cleaned polycarbonate grating side.

2.4. Electropolymerization of Pyrrole-3-carboxylic acid. A 0.1 M P3C monomer in 0.5 M H_2SO_4 solution was used for electropolymerization of the PP3C film on the gold-coated grating substrate. The electropolymerization was performed by cyclic voltammetry from 0.0 to 1.0 V at a scan rate of 20 mV/s. The electropolymerized electrode was then rinsed with 0.5 M H_2SO_4 and deionized water. The electropolymerization process was monitored in situ by EC-TSPR measurement.

2.5. Construction of a PP3C-Based Immunosensor. The procedure for covalent immobilization for the detection of human IgG was performed after the electropolymerization of PP3C film. Briefly, PBS solution (pH 7.4) was injected to obtain the TSPR baseline. Carboxylic groups on the surface of the PP3C film were then activated with 1:1 activator solutions, 0.4 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and 0.1 M N-hydroxysuccinimide (NHS) in deionized water. The PBS solution was injected as a rinsing solution followed by the incubation of 100 μg/mL antihuman IgG for 10 min. After rinsing with PBS solution, a blocking solution, 0.2 M ethanolamine hydrochloride (EA−HCl) buffer solution, was injected to avoid nonspecific binding by deactivating the remaining unreacted NHS groups before the detection of human IgG.

2.6. Detection of Human IgG. The obtained PP3C-based immunosensors were applied at different constant potentials of −0.2, 0.3, and 0.6 V and open-circuit potentials for the detection of various concentrations of human IgG (1, 2.5, 5, and 10 μg/mL). Real-time investigation of the immobilization process and the binding process of human IgG to antihuman IgG on a PP3C-based immunosensor was then carried out.

3. RESULTS AND DISCUSSION

3.1. TSPR Properties on Gold-Coated Grating Substrates. The gold-coated grating substrates used in this study were prepared by vacuum evaporation of approximately 3 nm Cr and 47 nm gold on the grating DVD-R.[19] The surface morphology of gold-coated grating substrate was characterized by AFM to investigate the homogeneity of the film. The obtained results indicated a grating pitch and amplitude of gold-coated grating substrate were 670 and 136 nm, respectively. A roughness analysis revealed a smooth surface with a roughness of 7.1 nm over an area of two-dimensional views of 2 × 2 μm^2 AFM image (see the Supporting Information, SI Figure 1).

The transmission spectra measured through gold-coated grating substrates for s- and p-polarized light at an incident angle of 35° are shown in Figure 1b. The transmission spectra for s- and p-polarized light showed a broad peak at approximately 550 nm, which was related to the green transmitted color of gold films. Furthermore, an additional peak for p-polarized light transmission was observed at approximately 750 nm. The increased light transmission represented was due to the decoupling of excited surface plasmon on the gold-coated grating substrate. Moreover, to study the peak intensity at different incident angles, the sample on a rotating sample holder was rotated from 0 to 40°. The transmission spectra intensity increased as the TSPR angle was increased from 0 up to 35°, and then decreased after the TSPR angle of 40° (see the Supporting Information, SI Figure 2). Maximum transmission intensity was observed at an incident angle of 35°. Consequently, the obtained transmission spectra were evaluated by measuring the p-polarized light compared with s-polarized light at a fixed incident angle of 35° in which s-polarized light was used as a baseline.

3.2. In situ EC-TSPR Monitoring of the Electropolymerization of P3C. The conducting polymer employed in the present study was PP3C, which was fabricated by electropolymerization on a gold-coated grating substrate from a 0.1 M P3C monomer in 0.5 M H_2SO_4 solution by cyclic voltammetry with a potential range from 0.0 to 1.0 V at a scan rate of 20 mV/s. Figure 2a shows the cyclic voltammetry

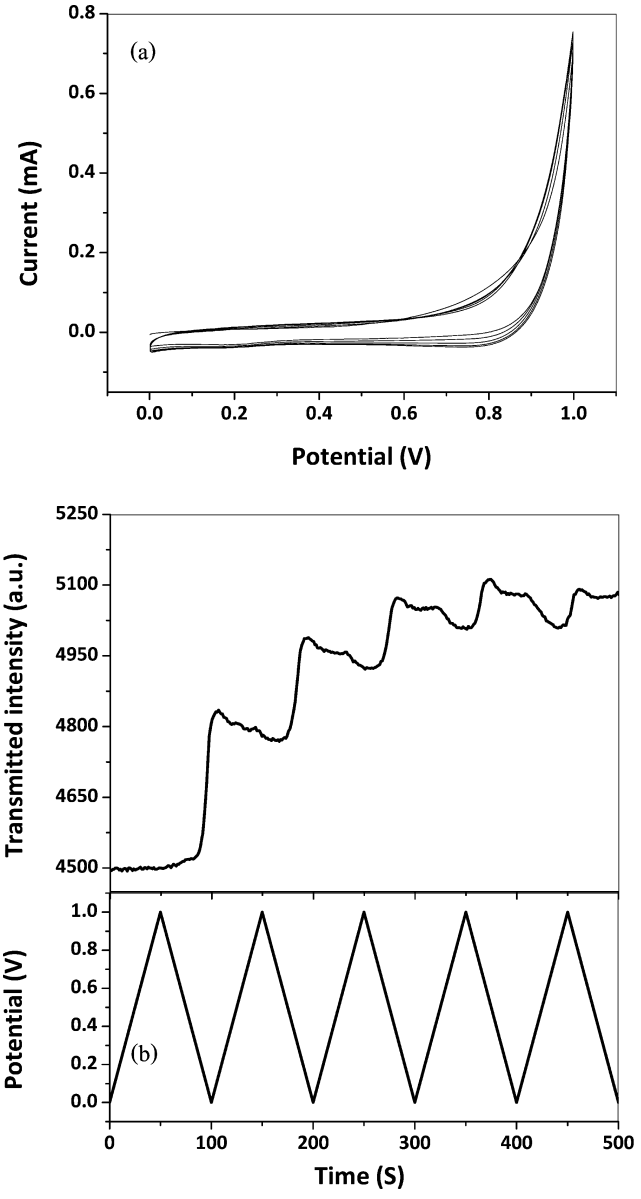

Figure 2. (a) Cyclic voltammogram of the electropolymerization of P3C and (b) the transmission kinetic data corresponding to the cyclic voltammetry scans with a potential range of 0.0 to 1.0 V as a function of time during the electropolymerization of P3C.

properties during the electropolymerization of P3C. The anodic scan began to increase at about 0.5 V, dramatically increased after 0.5 V, and then the current slightly decreased at about 0.2 V in the cathodic scan. The corresponding relationship of the transmission kinetic data with the cyclic voltammogram as a function of time during the electropolymerization of P3C is shown in Figure 2(b). From the TSPR responses, the transmission light intensity increased during doping and decreased during dedoping. This indicated formation of PP3C film deposited on the gold-coated grating substrate and the change in dielectric constants or thickness of the deposited film. Kinetic properties during electropolymerization of P3C

monomer to PP3C on the substrate was monitored in situ using EC-TSPR measurement. The results of the transmission spectra carried out before and after electropolymerization are shown in Figure 3(a). The increase in transmission intensity

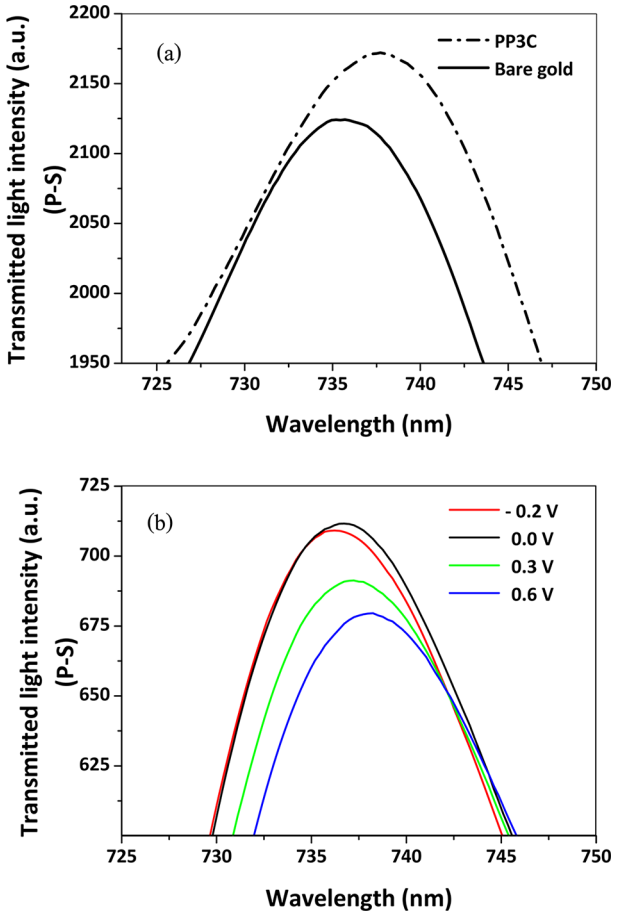

Figure 3. (a) TSPR spectra before and after the electropolymerization of P3C and (b) TSPR spectra of PP3C film in PBS solution at several constant applied potentials.

obtained after the electropolymerization was in good agreement with the formation and deposition of PP3C on the substrate, as discussed above. Therefore, the usefulness of the TSPR technique as a promising candidate allowing in situ investigation of electropolymerization properties was noted.

3.3. Potentiostatic Measurement in Neutral PBS Solution. The properties of a polymer film used to fabricate immunosensors are essential for sensing efficiency. One strategy is the adjustment of polymer morphology under an electrochemically controlled potential. We have previously shown that the morphology of PP3C films can be changed by changing the potential in PBS solution.[43] The morphological change can be obtained by doping/dedoping in PBS solution. Here, we measured the TSPR response of PP3C in PBS solution at constant potentials of −0.2, 0.0, 0.3, and 0.6 V and the open-circuit potential condition. Figure 3b shows the TSPR spectra of PP3C in PBS solution at several constant applied potentials. Shifts in the TSPR curve to longer wavelengths and decreases in TSPR intensity were observed with increasing constant applied potential. This behavior indicated an increase in the thickness and imaginary part of the dielectric constant of the PP3C film because of the doping effect induced by applying the potential. This is reasonable because the PP3C film shows electroactivity in neutral PBS solution owing to the self-doping effect from the functional group.[3] The polymers can be swelled as the solution is also penetrated into the film during the doping. Therefore, PP3C film can be used to control the space for the binding site in the polymer chain based on the swelling and shrinking of polymer morphology. The swelling of the film should provide more space for the effective immobilization, which plays an important role in the sensing performance in the PP3C-based immunosensor system.

3.4. Construction of a PP3C-Based Immunosensor. The electrochemically controlled TSPR technique was used to monitor the immobilization of antihuman IgG on the PP3C surface and the binding of antihuman IgG and human IgG in real time. Figure 4 shows the real-time TSPR kinetic curve

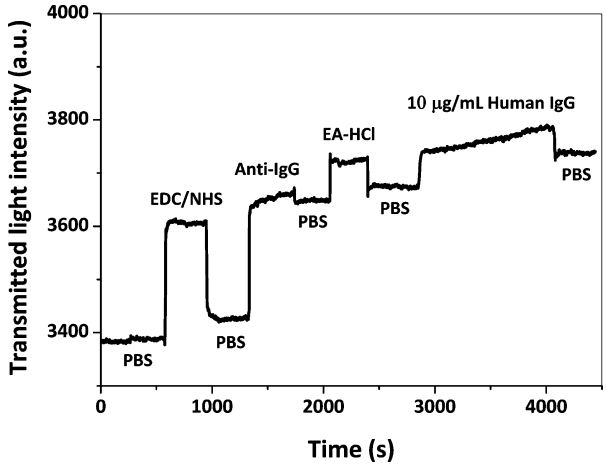

Figure 4. TSPR binding curve during the construction of a PP3C-based immunosensor for the detection of human IgG (10 μg/mL) at an open circuit.

during the construction of the PP3C-based immunosensor. The surface-modification steps led to an increase in the intensity of the transmission response. Initially, PBS solution (pH 7.4) was used to obtain the TSPR baseline. The deposited PP3C film introduced COOH groups to the substrate, which could then be activated for anti-IgG immobilization. To activate the surface COOH group, we injected a mixture of EDC/NHS solution as a coupling reagent for 10 min to promote covalent linkages on the surface of the PP3C film by forming the *N*-hydroxysuccinimide ester.[6] TSPR intensity increased during the activation process indicating the activation of carboxylic groups and formation of a stable reactive intermediate; however, the intensity decreased and became stable after rinsing with PBS solution. Amide bonding of antihuman IgG onto the activated surface was also obtained as the TSPR intensity increased again after a solution of 100 μg/mL antihuman IgG was injected. Upon rinsing by PBS solution, the TSPR intensity showed a gradual increase after the modified surface was treated with EA−HCl solution, indicating deactivation of the remaining free binding sites. After rinsing with PBS solution, a constant potential was applied for 1 min to obtain the baseline before the detection of human IgG. The TSPR response increased after the injection of human IgG, which indicated the binding process of antihuman IgG and human IgG. Finally, the TSPR signal decreased due to some dissociation of IgG after the injection of PBS solution and remained stable.

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3.5. Detection of Human IgG. The sensitivity of the PP3C-based immunosensor was compared with a standard system. Mercaptoundecanoic acid (MUA) was employed to construct an immunosensor-based self-assembled monolayer, which was attributed to be the standard system in this experiment. TSPR responses for the detection of human IgG on antihuman IgG−PP3C film in comparison with the antihuman IgG−MUA SAM system are shown in Figure 5.

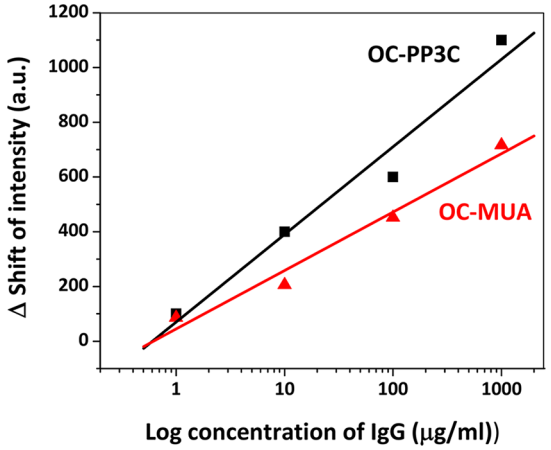

Figure 5. Plot of human IgG concentration and the shift in TSPR responses during the binding process of human IgG on antihuman IgG−PP3C film in comparison with the antihuman IgG−MUA SAM system at open circuit.

The relationship between the shift in transmission intensity and human IgG concentration on the PP3C film showed a higher sensitivity than that of the standard MUA system, and the dynamic range was from 1 to 1×10^3 μg/mL. Furthermore, the concentration of human IgG at 1 ng/mL could be still detectable with this system, although the linearity could not be obtained in this range (see the Supporting Information, SI Figure 3). On the basis of our previous study, the detection limit and dynamic range with the EC-TSPR technique is comparable or even better than that with conventional EC-SPR technique.[43]

In addition, to increase the sensitivity of the detection of human IgG by controlling the potential, we investigated the TSPR responses of the human IgG/antihuman IgG binding process by EC-TSPR measurement at different concentrations of human IgG with applied potentials of −0.2, 0.3, and 0.6 V. A plot of the shift in transmission intensity with the concentration of human IgG at different constant applied potentials is shown in Figure 6. Linearity was obtained in each applied potential with the concentrations of human IgG. This observation implied that the ability of the binding process between antihuman IgG and human IgG could be enhanced with electrochemically controlled potentials. This is because, by applying a constant potential, the electrochemically controlled PP3C film swells and opens more binding sites inside the polymer chain for the binding of antihuman IgG and human IgG, which can enhance the binding efficiency for the detection of human IgG.

4. CONCLUSIONS

The performance, sensitivity, and utility of EC-TSPR spectroscopy for investigation of the properties of PP3C film and detection of human IgG were demonstrated. This is the first report using a novel in situ EC-TSPR technique to monitor the electropolymerization and formation of PP3C film. The TSPR spectra shifted to higher wavelengths with an increase in the constant applied potential due to the swelling of PP3C films. Immobilization of antihuman IgG and its interaction with human-IgG were investigated in situ using EC-TSPR measurements. TSPR intensity increased with increasing human IgG concentration and constant applied potential on the modified surface. The binding process of the electrochemically controlled system was better than that of a standard MUA system. Enhanced binding of human IgG on electrochemically controlled PABA film could be created by morphological changes at constant applied potentials, swelling/shrinking, and open/closed spaces of the binding site inside the polymer chain. Hence, this result clearly demonstrated that an electrochemically controlled PP3C-based TSPR sensor is a sensitive tool to enhance the binding of biological molecules. We conclude that this technique could be applied to the study of biomolecular interactions in various systems, and that diagnostic tools in many formats could be developed.

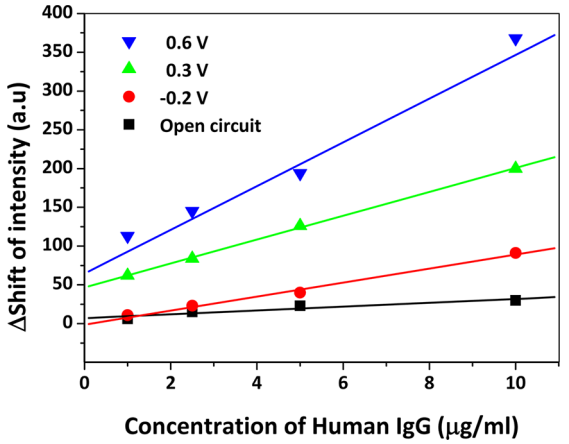

Figure 6. Plot of the shift in transmission sensitivity during the binding process with concentration of human IgG at different constant applied potentials.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

AFM image (2×2 μm^2) of gold-coated grating substrate and TSPR spectra at several angles of incidence. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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