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Immunobiosensor Chips for Detection of *Escherichia coli* O157:H7 Using Electrochemical Impedance Spectroscopy

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Impedance biosensor chips were developed for detection of *Escherichia coli* O157:H7 based on the surface immobilization of affinity-purified antibodies onto indium tin oxide (ITO) electrode chips. The immobilization of antibodies onto ITO chips was carried out using an epoxysilane monolayer to serve as a template for chemical anchoring of antibodies. The surface characteristics of chips before and after the binding reaction between the antibodies and antigens were characterized by atomic force microscopy (AFM). The patterns of the epoxysilanes monolayer, antibodies, and *E. coli* cells were clearly observed from the AFM images. Alkaline phosphatase as the labeled enzyme to anti-*E. coli* O157:H7 antibody was used to amplify the binding reaction of antibody−antigen on the chips. The biocatalyzed precipitation of 5-bromo-4-chloro-3-indolyl phosphate by alkaline phosphatase on the chips in pH 10 PBS buffer containing 0.1 M $MgCl_2$ increased the electron-transfer resistance for a redox probe of $Fe(CN)_6^{3-/4-}$ at the electrode−solution interface or the electrode resistance itself. Electrochemical impedance spectroscopy and cyclic voltammetric method were employed to follow the stepwise assembly of the systems and the electronic transduction for the detection of *E. coli*. The biosensor could detect the target bacteria with a detection limit of 6×10^3 cells/mL. A linear response in the electron-transfer resistance for the concentration of *E. coli* cells was found between 6×10^4 and 6×10^7 cells/mL.

Immunobiosensors, in which antibodies are immobilized onto electrodes, optical fibers, or semiconductor chips, have attracted great interest in recent years because of their promising applications in various areas. An immunobiosensor consists of two processes, a molecular recognition process, for sensing the specific antigen−antibody binding reaction at the surface of receptor, and the signal-transfer process, for responding to changes in an optical, spectroscopic, electrochemical, or electrical parameter of the receptor caused by the specific binding. Electrochemical detection of the antigen−antibody reaction and antibody-labeled enzymatic reactions has been investigated. The changes in capacity at the electrode−electrolyte interface as a result of the formation of antigen−antibody complexes were studied to develop a series of capacitive affinity sensors.[1−3] Amperometric detection of antigen−antibody interactions in a sandwich assay was accomplished by determining the quantity of enzyme-conjugated antibodies or metal-conjugated antibodies at the electrode surface.[4−6] A quartz crystal microbalance was used as the electronic transfer means for the transduction of antigen−antibody binding phenomena to detect various antigens such as *Salmonella*,[7] cholera toxin,[8] *Chiamydia trachomatis*,[9] and bacteriophage,[10] Surface plasmon resonance transduction of the formation of antigen−antibody complexes was reported as an alternative means for immunosensor devices.[11] Electrochemical impedance was used to monitor the binding of antigen−antibody.[12]

Signal amplification of the antigen−antibody binding event is a major challenge in the development of immunosensors. Enzyme amplification is one of the commonly used signal amplification systems in immunoassay. Enzyme labels convert their substrates into enzymatic reaction products that can be readily detected, either by their optical properties such as optical absorption,[13] fluorescence,[14] and chemiluminescence[15] or by their electrochemical properties.[16,17] Precipitation of an insoluble product on a

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horseradish peroxidase (HRP)-modified monolayer electrode and HRP−glucose oxidase bienzyme-layered electrode has been used in development of new electrochemical biosensors to detect hydrogen peroxide and glucose.[18] The insoluble precipitation on a conductive electrode surface is to alter the interfacial electron-transfer feature of the redox probe in the solution at the electrode. The interfacial electron-transfer features of a redox probe such as capacitance and resistance can be sensitively monitored by electrochemical impedance spectroscopy. The charge-transfer resistance of a redox probe could be increased as a result of formation of antigen−antibody complexes or precipitation of an insoluble on a conductive electrode surface.

In this study, the anti-*Escherichia coli* O157:H7 antibodies were immobilized on an indium tin oxide (ITO) chip. Formation of an antibody monolayer and the antigen−antibody complexes on the chip was characterized by atomic force microscopy (AFM), cyclic voltammetry, and Faradaic impedance spectroscopy. The signal amplification was accomplished through probing Faradaic electron impedance of the $[Fe(CN)_6]^{3-/4-}$ redox pair after biocatalytic precipitation of products on the chip. The substrate of alkaline phosphatase, 5-bromo-4-chloro-3-indolyl phosphate, was transformed to enzymatic reaction products, 5-bromo-4-chloro-3-indolylphenol, which precipitated on the surface of chips.

EXPERIMENTAL SECTION

Chemicals and Biochemicals. (3-Glycidoxypropyl)trimethoxysilane, 5-bromo-4-chloro-3-indolyl phosphate, disodium salt hydrate, hydrogen peroxide (30 wt %), and *N*,*N*-dimethylformamide (DFM) was purchased from Aldrich (St. Louis, MO). Ammonium hydroxide (30%) was obtained from VWR (West Chester, PA). Tris(hydroxymethyl)aminomethane hydrochloride (electrophoresis grade), $NaH_2PO_4 \cdot H_2O$, Na_2HPO_4, potassium ferrocyanide, and potassium ferricyanide were purchased from Fisher (Fair Lawn, NJ). Toluene and ethanol were obtained from EM Science (Gibbstown, NJ). Deionized water from Millipore (Milli-Q, 18.2 MΩ/cm; Millipore, Bedford, MA). Affinity-purified goat anti-*E. coli* O157:H7 antibody (1.0 mg) and alkaline phosphatase (AP)-labeled affinity-purified antibodies to *E. coli* O157:H7 (0.1 mg) were obtained from Kirkegaard & Perry Laboratories (Gaithersburg, MD) and were rehydrated with 1 mL of 50% glycerol/water solution. The dilution of AP-labeled antibody (1:10) was prepared with 50% glycerol/water solution before use.

Bacteria and Culture Plating Methods. *E. coli* O157:H7 (ATCC 43888) was obtained from American Type Culture Collection (Rockville, MD). The pure culture of *E. coli* O157:H7 was grown in brain heart infusion (BHI) broth (Remel, Lenexa, KS) at 37 °C for 20 h before use. The culture was serially diluted to 10^{-8} with physiological saline solution (PSS), and viable cell number was determined by a microbial plate count method. An *E. coli* O157:H7 culture test was performed by surface plating 0.1 mL of dilutions on MacConkey sorbitol agar (Remel). After incubation at 37 °C for 24 h, *E. coli* O157:H7 colonies on the plate were counted to determine the number of colony forming units per milliliter (cfu/mL). The culture was then heated in a 100 °C water bath for 15 min for the further use.

ITO Electrodes and Surface Modification. ITO-coated glass (CG-80IN-1515), which had a surface resistance of 30−60 Ω/cm^2 was purchased from Delta Technologies (Stillwater, MN) and was cut into 5.0 cm × 0.8 cm slides. The substrates were cleaned with acetone, ethanol, and water, respectively. Then they were immersed into 1 M HCl for 10 min. After washing thoroughly with water, they were immersed into a solution of 1:1:5 (v/v) H_2O_2/NH_4OH/H_2O for 1 h. Finally, they were rinsed with water. Before the silanization, the chips were dried under a stream of nitrogen. These chips were silanized with (3-glycidoxypropyl)trimethoxysilane in accordance with a previous report.[19] The chips were immersed into a 1% solution of (3-glycidoxypropyl)trimethoxysilane in toluene overnight at room temperature. After the coupling reaction, the modified substrates were removed from solution and rinsed several times with pure toluene and ethanol to remove the physically absorbed silanes from the surface. Then the substrates were dried under a stream of nitrogen. Prepared in this way, the surface of the substrate had many exposed active epoxy groups that react readily with protein amino groups. The anti-*E. coli* O157:H7 antibodies were introduced onto the substrate chip (8 mm × 8 mm) by dropping 5 μL of 1 mg/mL anti-*E. coli* O157:H7 antibodies on the surface and were allowed to incubate at 4 °C in a refrigerator for 24 h. The middle part of the ITO chips was coated with 5-min Epoxy to avoid contact with the solution.

The anti-*E. coli* O157:H7 antibody-modified chips were rinsed with water and incubated with 50 μL of samples two times with diluted heat-killed *E. coli* O157:H7 cells in pH 7.4 TBS solution (from 10^3 to 10^7 cells/mL) for 1 h at 37 °C. After the binding reaction between antibodies and *E. coli* antigens on the chips, 20 μL each of AP-conjugated antibodies to *E. coli* (0.01 mg/mL) was dropped on the surface of the chip, respectively. The electrode chips were washed thoroughly with water to remove nonspecifically bound AP-conjugated antibodies, which could cause a background response before measurement.

AFM Imaging. All AFM experiments were performed on a Nanoscope IIIa AFM (Digital Instruments, Santa Barbara, CA). The AFM was carried out using the tapping mode. All images are presented without any subsequent data processing. Before taking AFM, each electrode chip was washed thoroughly using water to remove nonspecific absorbed antibodies and nonspecific bound *E. coli* and dried by a stream of nitrogen. The AFM was measured in open air.

Electrochemical Measurements. Electrochemical cyclic voltammetric experiments were performed with a CV-50W voltammetric analyzer (BAS, West Lafayette, IN). Impedance measurements were performed using IM-6 impedance analyzer (BAS). A single-compartment three-electrode glass cell was used. Platinum foil and Ag|AgCl|NaCl (3.0 M) were used as a counter electrode and a reference electrode, respectively. The antibody-modified ITO, *E. coli* conjugated antibody-modified ITO, and AP-labeled antibody conjugated antigen−antibody sandwich complexes-modified ITO electrodes were used as working electrodes. Cyclic voltammetry and impedance measurements were performed in 15.0 mL of a pH 10.0 PBS containing 1.0 mL of $MgCl_2$ (0.1 M), 1.0 mL of 5-bromo-4-chloro-3-indolyl phosphate disodium salt hydrate of DFM solution (1.0×10^{-3} M), 1.0 mL of potassium hexaferricyanide (0.1 M), and 1.0 mL of potassium hexaferrocyanide (0.1 M). Impedance was measured at an open circuit potential to Ag|AgCl|NaCl (3.0 M) and an alternating potential with

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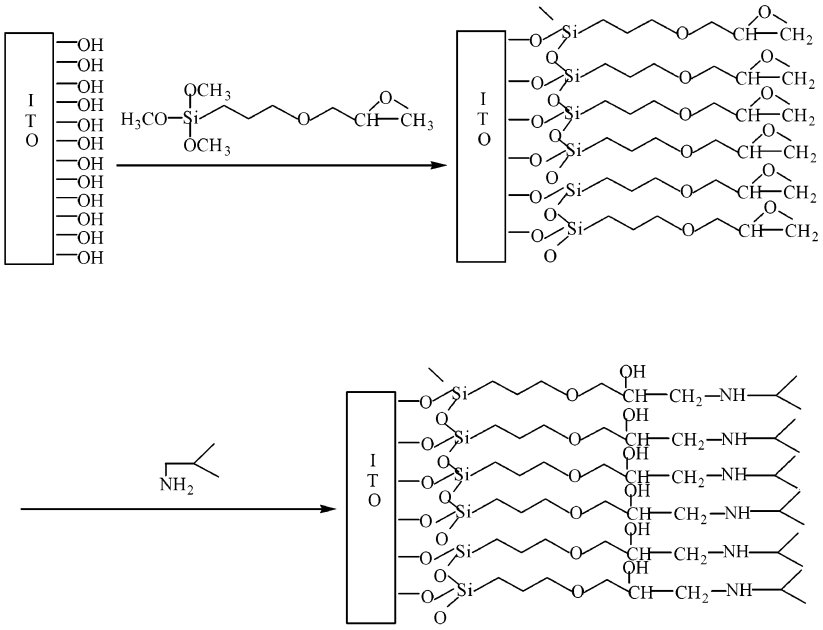
Scheme 1. Immobilization of Anti-*E. coli* O157:H7 Antibody onto an ITO Surface



amplitude of 5 mV at the frequency range from 1 Hz to 100 kHz. The impedance Z is expressed in term of a real (Z_{re}) and an imaginary (Z_{im}) component. Impedance signals were recorded after reaction between the chips and substrate solution for 5 min except when examining the effect of time on the response of the chip. All measurements were performed at room temperature.

RESULTS AND DISCUSSION

Preparation and Characterization of Biosensor Chips. Anti-*E. coli* O157:H7 antibodies were immobilized onto ITO through epoxy−amino group reaction on the ITO surface as shown in Scheme 1. Silanization of the surface of ITO using alkylsiloxane was studied by Hillebrandt and Tanaka.[20] Usually, it was necessary to clean the surface and create a high number of hydroxy groups on the surface. General preparation procedures include cleaning, surface activating, and surface modifying. In this study, ITO was cleaned with ethanol and water solution in an ultrasonic bath for 5 min, and it was found that ITO was no longer to be used as an electrode because the surface resistance of ITO was too high. Sonication must be avoided in cleaning the surface of ITO. The hydroxylating process of ITO was carried out by immersing it into 1 M HCl for 10 min. Control experiments indicated that the ITO layer on glass was decayed after immersing ITO in hot piranha solution (H_2SO_4/30% H_2O_2, 3:1 in volume). Silanization of ITO was conducted from a toluene solution with 1% epoxysilane compound as reported by Tsukruk et al.[21] Figure 1a shows the AFM image of epoxysilane films on ITO. The epoxysilane film obtained from a 1% solution was complete, smooth, and homogeneous with only a few aggregates observed on a micrometer-size area. This result is consistent with the report on expoxy-terminated self-assembled monolayers on a single-crystal silicon surface.[19] Our experiments demonstrated that the dense and homogeneous epoxysilane films could also be formed by absorption from ethanol solution with 1% silane.

Figure 1 shows a topographical AFM image of antibody layers formed after the reaction between antibodies and an active epoxy group on the ITO surface for 12 h. The antibody layer was not absolutely a monolayer, and a few aggregates of the antibodies can still be observed on the surface even though the antibody-modified surface was thoroughly rinsed (Figure 1b). To make a comparison between panels a and b of Figure 1, the distinctive difference in the topography of the surface can be observed before and after binding of antibodies onto the active surface of ITO. This feature indicated that antibody was successfully linked to the chip surface. AFM was also used to evaluate the binding of bacteria antigen to the surface-confined antibodies. A representative image of an anti-*E. coli* O157:H7 antibody array after reaction with 6.0×10^6 cells on the surface is shown in Figure 1c. Images obtained at several different locations on the surface show that the average cell number is 6/400 μm^2. The total binding cell numbers of *E. coli* O157:H7 on the area of an 8 × 8 mm surface are equal to 9.6×10^5 cells, and the binding efficiency of the immobilized anti-*E. coli* O157:H7 antibodies to antigen is ∼16% when 100 μL of 6.0×10^7 cells/mL of *E. coli* O157:H7 are dropped on the surface (two times, 50 μL of sample was used each time). There is no report concerning the binding efficiency of an immobilized anti-*E. coli* O157:H7 antibody to its antigen. Usually, a binding efficiency of an antibody to its antigen is influenced by many factors such as the affinity ability of the antibody, activity of antibody, and incubation conditions. The binding efficiency of the anti-*Salmonella* antibodies on a roughened glassy carbon surface to *Salmonella* is less than 1%.[22] Docherty et al. reported 10−15% of *Campylobacter* cells were captured by immunomagnetic particles coated with anti-*Campylobacter* antibody.[23] The results

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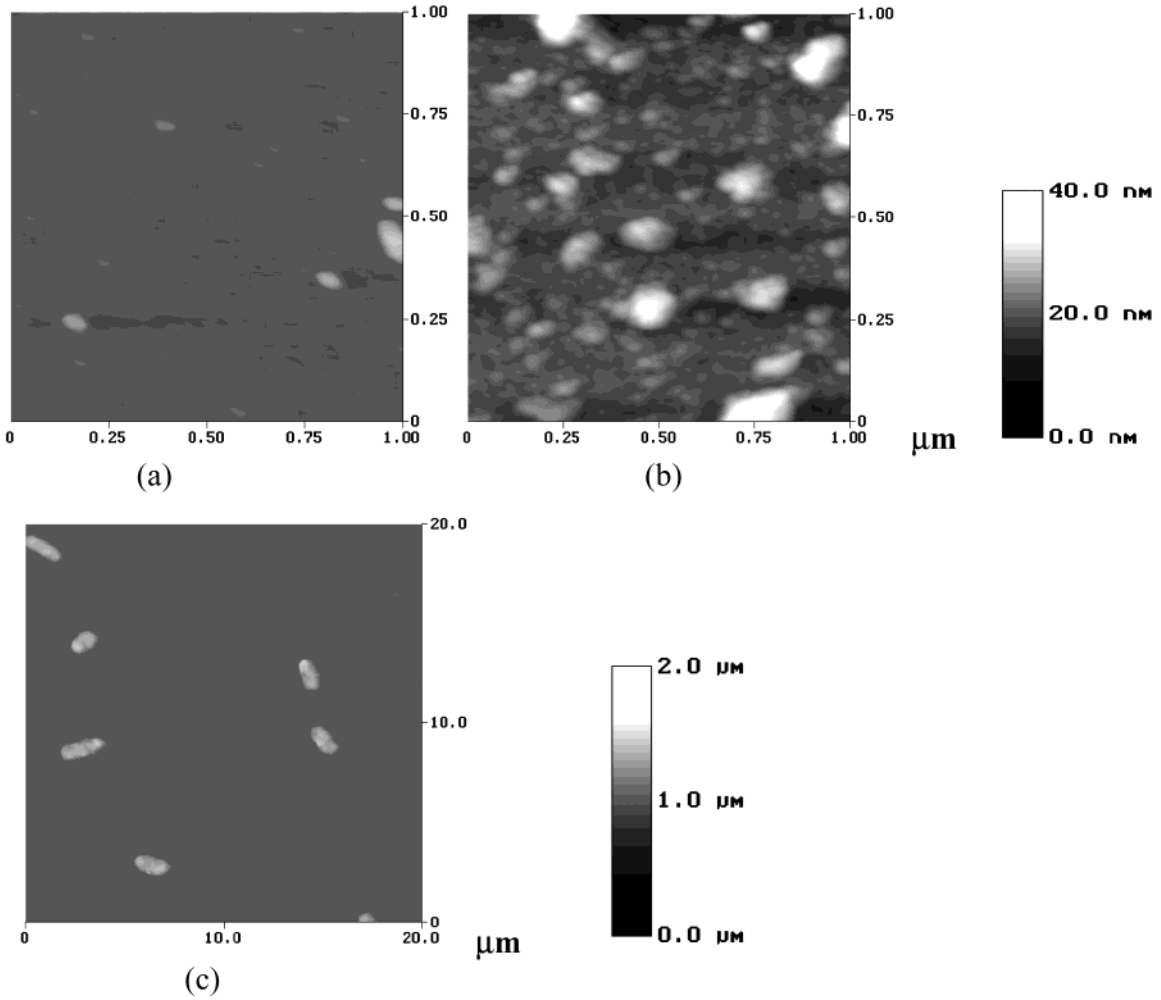
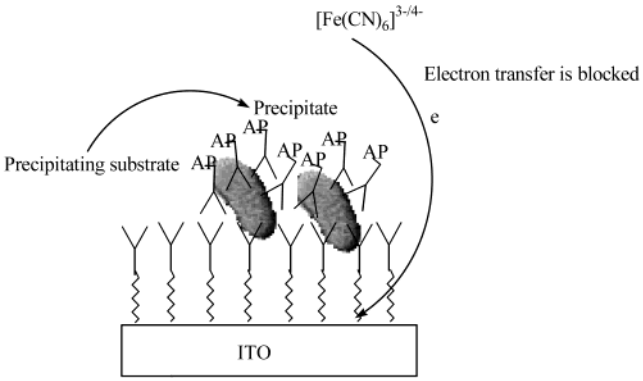


Figure 1. AFM images of an ITO chip coated with (a) monolayer of epoxysilane, (b) an epoxysilane layer modified by anti-*E. coli* O157:H7 antibodies, and (c) complexes of the antibodies and *E. coli* O157:H7 cells.

Scheme 2. Amplification of Sensing *E. coli* O157:H7 by Increasing Electron-Transfer Resistance through Biocatalytic Precipitation with Alkaline Phosphatase and Its Substrate



of this study indicated that the binding efficiency of anti-bacteria antibodies to their antigen is normally low.

Electron-Transfer Characteristics of Hexacyanoferrates on the Surface of Chips. The transduction principle for detection of bacteria antigen is shown in Scheme 2. Generally, it is based on measurements of Faradaic impedance in the presence of the redox couple of hexacyanoferrates. Hexacyanoferrates are often used as redox probes for the characterization of a self-assembled monolayer.[24,25] It is usually assumed that the electron transfer of hexacyanoferrates was blocked by formation of a highly organized hydrophobic monolayer on the electrode surface because these redox species do not penetrate the monolayers into the conductive electrode surface. Similarly, the precipitation onto the electrode surface produced by the biocatalytic reaction through the secondary antibody-labeled enzymes is expected to electrically insulate the conductive surface. The amount of precipitate formed on the electrode chips is related to the amount of enzyme-labeled antibodies at a constant reaction time with its enzymatic substrate and eventually related to the concentrations of target bacteria bound on the electrode surface through the primary antibodies. The precipitate as an electrical barrier could be reflected by the electron-transfer resistance of the redox probe of hexacyanoferrate, which can be measured using electrochemical impedance spectroscopy.

The data of electrochemical impedance measurements can be simulated with an equivalent circuit using the software provided by an IM-6 impedance analyzer (BAS). The general electronic equivalent circuit of an electrochemical cell in the presence of a

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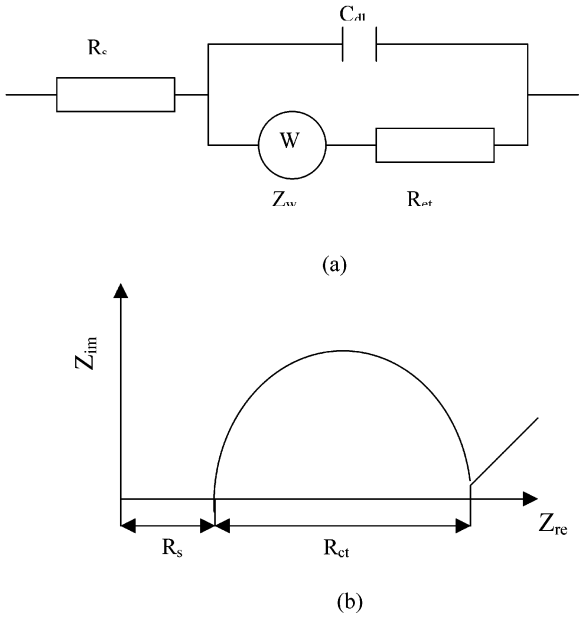
Scheme 3. General Equivalent Circuit for an Electrochemical Cell in the Presence of a Redox Probe (a) and a Typical Nyquist Plot (Z_{Im} vs Z_{Re}) of a Faradaic Impedance Spectrum (b)



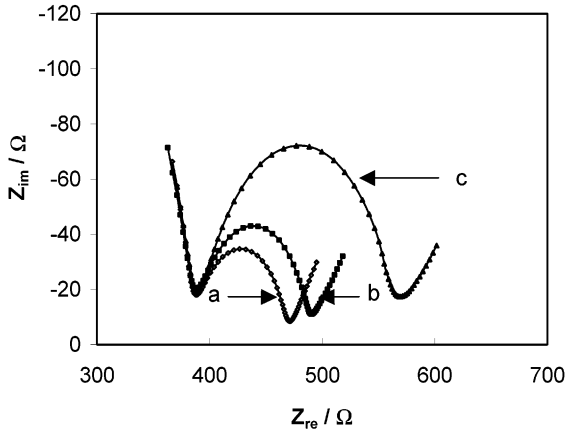


Figure 2. Nyquist diagram (Z_{im} vs Z_{re}) for the Faradaic impedance measurement of an ITO chip in pH 10 PBS containing 6.7 mM $[Fe(CN)_6]^{3-/4-}$ (1:1 mixture), $MgCl_2$, and 3 mM 5-bromo-4-chloro-3-indolyl phosphate disodium salt hydrate: (a) modified with anti-*E. coli* antibodies, (b) modified by antibody-*E. coli* complexes, *E. coli* at 6×10^5 cells/mL, (c) coated with sandwich complexes of antibodies-*E. coli*-AP-labeled antibodies. The impedance spectra were recorded within a frequency range of 1 Hz−100 kHz at the formal potential of the $[Fe(CN)_6]^{3-/4-}$ redox couple. The amplitude of the alternate voltage was 5 mV.

redox probe can be expressed in Scheme 3a (Randles model).[26] R_s, Z_w, R_{et}, and C_{dl} in Scheme 3a, represent the ohmic resistance of the electrolyte solution, the Warburg impedance resulting from the diffusion of ions from bulk electrolyte to the electrode interface, electron-transfer resistance of the redox probe, and the double-layer capacitance, respectively. The two components in the electronic equivalent circuit of an electrochemical cell, R_s and Z_w, represent bulk properties of the electrolyte solution and diffusion features of the redox probe in solution. These components are not affected by chemical transformations occurring at the electrode surface. The other two components in Scheme 3a, R_{et} and C_{dl}, depend on the dielectric and insulating features at the electrode/electrolyte. Thus, the insoluble precipitate on the electrode surface is expected to retard the interfacial electron-transfer kinetics and to increase the electron-transfer resistance. A typical shape of a Faradaic impedance spectrum presented in a Nyquist plot includes a semicircular region lying on the Z_{re} axis followed by a straight line (Scheme 3b). The semicircle portion, observed at higher frequencies, corresponds to the electron-transfer-limited process, whereas the linear part is the characteristics of the lower frequencies range and represents the diffusion-limited electron-transfter process. In case of very fast electron-transfer processes, the impedance spectrum could include only the linear part, whereas a very slow electron-transfer step results in a big semicircular region that is not accompanied by a straight line. Usually, the electron-transfer kinetics and diffusional characteristics can be extracted from the spectra. The semicircle diameter equals R_{et}. The intercept of the semicircle with Z_{re} axis at high frequencies is equal to R_s (Scheme 3b).

Figure 2 shows the Faradaic impedance spectra, presented as Nyquist plots (Z_{im} vs Z_{re}) of the anti-*E. coli* O157:H7 antibody-modified ITO electrode (curve a) and the antibody-modified ITO electrode after binding of *E. coli* cells (curve b) and after precipitate formed (5 min) through secondary AP-labeled antibodies on the surface of ITO in a pH 10.0 PBS containing $MgCl_2$, 5-bromo-4-chloro-3-indolyl phosphate disodium salt hydrate, potassium hexaferricyanide, and potassium hexaferrocyanide. The electron-transfer resistance (R_{et}, the diameter of semicircles) of the redox probe of $[Fe(CN)_6]^{3-/4-}$ on antibody-modified ITO electrode (Figure 2, curve a), on *E. coli* bound antibody-modified ITO (curve b), and the precipitate-covered ITO surface are 84, 103, and 180 Ω, respectively. It can be seen from Figure 2 that the electron-transfer resistance of $[Fe(CN)_6]^{3-/4-}$ increased by a small value after binding *E. coli* onto the antibody-modified ITO surface, but the precipitating reaction through enzyme catalytic amplification resulted in a significant change in the electron-transfer resistance. A very small increase in R_{et} after binding of antigen−antibody indicated that these redox species in solution could penetrate bacteria layers into the conductive electrode surface and could only slightly block the electron transfer between the probe and electrode. These results can be explained from the AFM image on a *E. coli* conjugated ITO surface (Figure 1c). The surface coverage of *E. coli* was less than 1.2% when 100 μL of 6.0×10^7 cells/mL *E. coli* was dropped onto the electrode surface, assuming an *E. coli* cell with a length of 1.5 μm and width of 0.5 μm. It is indicated that direct monitoring of bacteria by the antigen−antibody reaction using electrochemical impedance spectroscopy would have a very low sensitivity. Enzyme catalytic amplification through generation of precipitate on the surface is a key procedure for detection of bacteria with high sensitivity (Figure 2, curve c).

Figure 3 shows the Faradaic impedance spectra of a chip modified with enzyme-labeled antibody−bacteria−antibody complex at a different reaction time between labeled enzyme on the ITO surface and the enzymatic substrate in the solution. It can been seen from Figure 3 that the electron-transfer resistance

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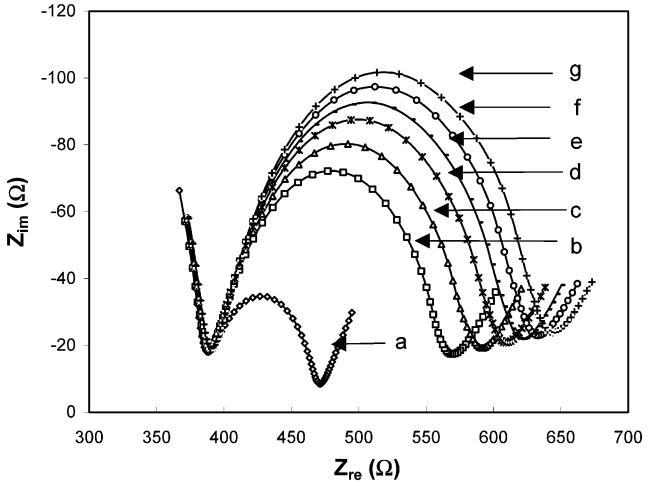

Figure 3. Nyquist diagram (Z_{im} vs Z_{re}) for the Faradaic impedance spectra of an ITO chip modified with AP-labeled antibody-*E. coli*-antibody complexes (*E. coli* at 6×10^5 cells/mL) different reaction times in pH 10 PBS containing 6.7 mM $[Fe(CN)_6]^{3-/4-}$ (1:1 mixture), $MgCl_2$, and 3 mM 5-bromo-4-chloro-3-indolyl phosphate disodium salt hydrate: (a) antibody-modified ITO chip, (b−g) represent the spectra of the reaction between an ITO chip and substrate of alkaline phophatase for 5, 10, 15, 20, 25, and 30 min, respectively.

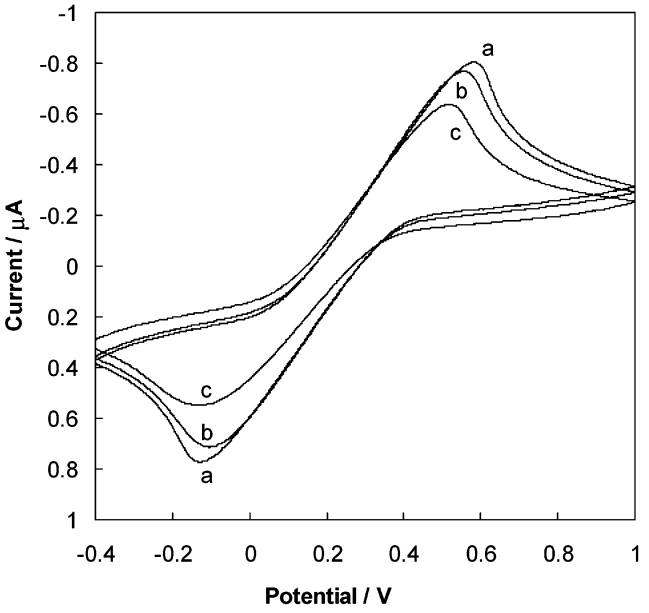

Figure 4. Cyclic voltammograms of an ITO chip modified with (a) antibodies, (b) antibody-*E. coli* complex (*E. coli* at 6×10^7 cells/mL), and (c) sandwich complex of antibodies-*E. coli*-AP-labeled antibodies in pH 10 PBS containing 6.7 mM $[Fe(CN)_6]^{3-/4-}$ (1:1 mixture), $MgCl_2$, and 3 mM 5-bromo-4-chloro-3-indolyl phosphate disodium salt hydrate.

gradually increased with increase in reaction time on the chip, indicating the amount of precipitate accumulated with time on the conductive support by a biocatalytic precipitation process through alkaline phosphatase. Large changes ($\Delta R_{et} \approx 89$ Ω) in electron-transfer resistance were observed during the first time interval (5 min), and then the changes in resistance ($\Delta R_{et} \approx 23$ Ω) became less within the same time interval, which implied that the active alkaline phosphatase is covered by precipitate and further reaction between enzyme and substrate is blocked.

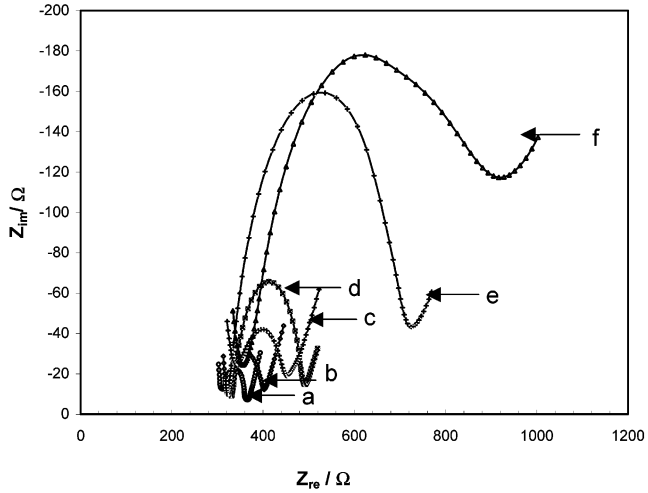

Figure 5. Faradaic impedance spectra that correspond to an antibody-modified ITO electrode reacted with different concentrations of *E. coli* O157:H7 and with the same concentrations of AP-labeled antibodies: (a−f) represent the *E. coli* O157:H7 concentrations of 0, 6×10^3, 6×10^4, 6×10^5, 6×10^6, and 6×10^7 cells/mL, respectively.

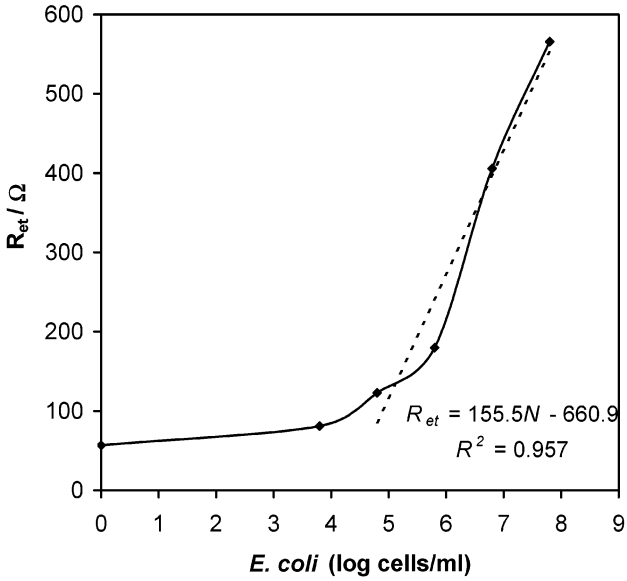

Figure 6. Concentration of *E. coli* O157:H7 (log cells/mL) in the samples versus the electron-transfer resistance on immunosensor chips.

The antigen−antibody reaction and surface precipitating reaction were also monitored by cyclic voltammetry using $[Fe(CN)_6]^{3-/4-}$ as a redox probe. Figure 4 shows the cyclic voltammograms of $[Fe(CN)_6]^{3-/4-}$ recorded for ITO electrodes modified with anti-*E. coli* antibodies (curve a), the *E. coli* conjugated anti-*E. coli* antibodies (curve b), and enzymatic catalysis precipitate (curve c). Figure 4 shows that the electron transfer between the redox probe and the electrode was slightly blocked after binding *E. coli* cells onto the antibody-modified surface of ITO. Such an electron-transfer block to the redox probe was amplified by precipitation generated on the ITO through enzymatic reaction. Figure 4 also indicates that the blocking of the interfacial electron transfer between the soluble redox probe and the electrode upon binding of protein to the ITO surface is not efficient as upon a self-assembled monolayer of octadecylsilane on ITO or self-assembled monolayer of *n*-alkanethiol on gold.[24,25]

Proteins bound on the ITO surface could only partly inhibit the electrical contact between $[Fe(CN)_6]^{3-/4-}$ and the electrode surface.

Detection of *E. coli* O157:H7. Figure 5 shows the impedance spectra obtained in pH 10.0 PBS containing $MgCl_2$, 5-bromo-4-chloro-3-indolyl phosphate disodium salt hydrate, potassium hexaferricyanide, and potassium hexaferrocyanide upon treatment of the sensing interface with different concentrations of *E. coli* cells and the same amount of AP-labeled anti-*E. coli* antibodies. The interfacial electron-transfer resistance increased with increase in the concentrations of *E. coli* cells, implying that a higher content of *E. coli* cells was linked to the interface, and generating a higher content of blocking to the electron transfer of the redox probe. It can be seen from Figure 5b that applying 100 μL of 6.0×10^3 cells/mL *E. coli* onto the antibody-modified ITO results in an increase in electron-transfer resistance of 38 Ω, indicating that 6.0×10^2 cells on the transduction surface of a chip could be detected using this immunosensor chip. A linear relationship between the electron-transfer resistance and logarithmic value of *E. coli* concentrations was found ranging from 6.0×10^4 to 6.0×10^7 cells/mL with a slope of 155.5 and a correlation coefficient of 0.957 (Figure 6). The detection limit of the immunosensor chip for detection of *E. coli* O157:H7 is comparable with several methods developed by other researchers, such as a biosensing system with a detection limit of 6×10^2 cells/mL, including immunomagnetic separation and electrochemical bienzyme electrode detection,[27] amperometric enzyme-linked immunoassay method using 1-naphthyl phosphate as an enzymatic substrate with a minimum detectable level of 4.7×10^3 cells/mL,[28] immunoligand assay/light-addressable potentiometry with a detection limit of 7.1×10^2 cells/mL,[29] and immunomagnetic separation/flow injection analysis/mediated amperometric detection with a detection limit of $\sim 10^5$ cells/mL.[30] This indicated that such an impedance method with enzymatic catalysis precipitation and antibody−antigen binding provided an amplification way to detect antibody−antigen conjugates on the chips, which resulted in better sensitivity for detection of bacteria.

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