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Fabrication of an electrochemical immunosensor with self-assembled peptide nanotubes

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Abstract

Fabrication and notably improved performance of composite electrodes based on the peptide nanotubes is described. Peptide nanotubes were constructed by self-assembly of cyclic peptides consisted of alternated D- and L-amino acid residues. The self-assembled peptide nanotubes were deposited onto the screen-printed carbon paste electrode prepared by a sol–gel method. The antibodies against *Escherichia coli* O157:H7 were reacted by themselves onto the peptide nanotubes. The binding of peptide nanotubes and antibodies was confirmed by fluorescence microscopy. The *E. coli* O157:H7 cells were attached onto the antibody-modified electrode using the antigen-antibody interaction. Direct measurement with cyclic voltammetry (CV) in the presence of $[Fe(CN)_6]^{3-/4-}$ as a redox probe showed that the immobilization of antibodies and the binding of *E. coli* cells to the peptide nanotube modified electrode increased the electron-transfer resistance.

Keywords: Peptide nanotube; *Escherichia coli* O157:H7; Electrochemical immmunosensor; Cyclic voltammetry; Sol–gel method

1. Introduction

Nanomaterials, such as nanotubes, nanowires, nanoparticles, and nanocrystals provide a key direction for the controlled fabrication of nanoscopic devices [1–6]. Since the carbon nanotubes (CNTs) were discovered [7], nanotubes exhibit several technologically important characteristics among the nanomaterials. The unique electronic, chemical, and mechanical properties of CNTs make them extremely attractive for electrochemical sensors [8]. While those have many advantages in sensing applications, there are still problems to be overcome. CNTs are often produced by expensive techniques like chemical vapor deposition (CVD); such instruments usually need large-scale water consumption and higher production costs [9].

Peptide nanotubes (PNTs) are fascinating structures because they have the scope for numerous chemical modifications and allow the use of biological systems specificity [10]. Ghadiri and co-workers [11,12] introduced the PNTs from self-assembly by cyclic peptide consisting D- and L-amino acids. Matsui et al. [13] also demonstrated another method to fabricate the PNTs using self-assembled bolaamphiphile. Those tubular structures are assembled via intermolecular hydrogen bonds between carboxylic acid groups and amide groups. PNTs have similar characteristics compared with CNTs such as small size and high aspect ratio. While CNTs can be modified with organic and inorganic materials, PNTs can be modified easier since they already have functional groups by themselves on their surface. In addition, PNTs can be produced under milder conditions in a smaller laboratory. The flexibility in functionality and the molecular-recognition ability of the PNTs distinguish them from other nanotubes [9].

The detection of foodborne pathogenic bacteria such as *Escherichia coli* O157:H7 is an important issue for ensuring food safety and security [14–18]. Currently, several immunosensors for detection of *E. coli* O157:H7 have been reported in literature. Lee et al. [19] fabricated a bioactive platform by immobilizing anti-*E. coli* O157:H7 antibodies by a sol–gel technique that can detect the pathogen by optically. Yang and Erf [14] demonstrated the label-free electrochemical impedance immunosensors for detection of *E. coli* O157:H7 was developed by immobilizing anti-*E. coli* antibodies onto an indium-tin oxide interdigitated

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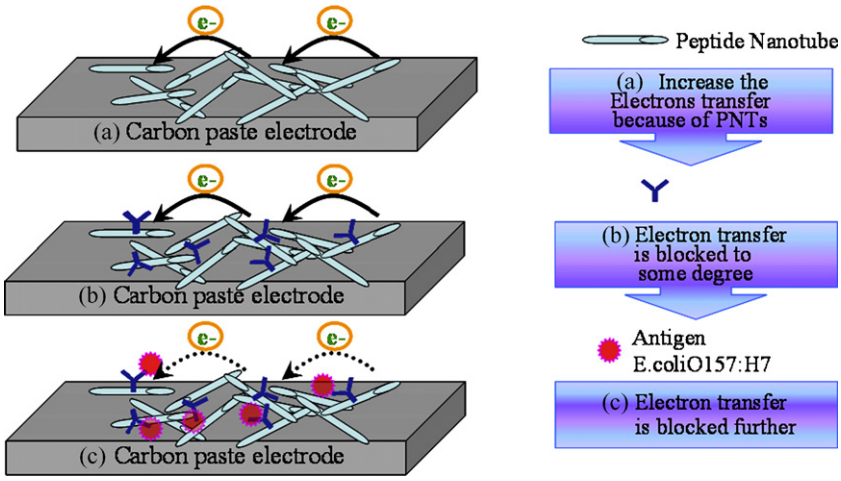

Fig. 1. Schematic of an electrode system: (a) carbon paste electrode, (b) peptide nanotubes-modified electrode, and (c) with antibody immobilization and with cell binding (tube: peptide nanotube, Y: anti-*E. coli* antibody, Oval: *E. coli* O157:H7 cell).

array microelectrode. In addition, the immunosensors for detection of different bacteria have exploited the surface plasmon resonance (SPR) [20] and quartz crystal microbalance (QCM) [21,22] as the transducing techniques.

The purpose of the present work is to demonstrate the fabrication of an immunosensor for detection *E. coli* O157:H7 using a carbon paste electrode immobilized with PNTs as templates. The self-assembled PNTs from cyclic peptide were deposited onto the carbon paste electrode for increase of electron transfer [23–25]. The PNTs that have functionality on their surface were modified with the anti-*E. coli* O157:H7 and then the *E. coli* O157:H7 cells attached the modified electrode using the antigen-antibody interactions. The each step was confirmed by cyclic voltammetry (CV) in the presence of $[Fe(CN)_6]^{3-/4-}$ as a redox probe, as shown in Fig. 1.

2. Experiment

2.1. Materials

All solutions were prepared in doubly distilled deionized water. Cyclic peptide (cyclo[(Gln-D-Leu)$_4$]) was obtained from jpt (Germany). Affinity purified mouse anti-*E. coli* O157:H7 were obtained from Biodesign International (USA). A 1:1 dilution was prepared with 50% glycerin solution in water before use [26]. The FITC-labeled anti-*E. coli* O157:H7 cells were obtained from Koma Biotech Inc. (Korea) to study the binding between PNTs and antibodies. Trifluoroacetic acid (TFA) was purchased from Fluka and phosphate-buffer saline (PBS; 0.01 M, pH 7.4), hexacyanoferrates (potassium ferrocyanide and potassium ferricyanide), tetraethyl orthosilicate (TEOS), ethyl alcohol, graphite (5000 mesh (3 μm)) and hydrochloric acid were purchased from Sigma-Aldrich (USA).

E. coli O157:H7 was obtained from American Type Culture Collection (USA) [27]. The pure culture of *E. coli* O157:H7 was prepared in brain heart infusion broth at 37 °C for 20 h. The culture was serially diluted with physiological saline solution. *E. coli* O157:H7 colonies were counted to determine the number of viable cells in CFU/mL (CFU: colony-forming units) by optical density (OD). The culture was then heat-killed in a boiling water bath for 15 min for further use.

2.2. Self-assembly of peptide nanotubes

Cyclic peptides were dissolved at a concentration of 1 mg/mL in neat TFA. Typically 0.5 mL of such a solution was floated in an open Eppendorf tube, which was then placed in a 50 mL conical tube, which was partially filled with water (about 30 mL). The conical tube was sealed and left undisturbed at room temperature for 2–3 days [12]. Examination of the Eppendorf tube after this time revealed a milky white suspension forming at the surface of the TFA solution.

2.3. Preparation of an electrode modified by peptide nanotubes

A sol–gel stock was prepared by mixing 4.5 mL of TEOS, 1.0 mL of ethyl alcohol, 2.7 mL of water, and 0.1 mL of 0.05 M HCl. A clear solution was obtained after 1 h of stirring. One gram of graphite powder was subsequently dispersed into the 1.4 mL sol solution with 30 min mixing. The resulting graphite paste was printed on a slide glass (0.3 cm × 1 cm) for a working electrode. The printed glasses were cured at 4 °C for 3 h. Twenty microliter of peptide nanotube solution was deposited on the surface of the working electrode and dried for 90 min at room temperature [25]. The image of scanning electron microscopy (SEM) revealed the existence of PNTs on the surface of the working electrode as shown in Fig. 2.

2.4. Antibody and antigen attachment

Antibody (0.1 mg/mL) in a solvent mixed with 10 mM PBS (pH 7.4) and 10 vol% glycerol. A PNTs modified working electrode was put in the antibody solution and shaken for 24 h at room temperature. This plate was then rinsed with PBS and deionized water (DI water) and dried under a high-purity N_2 atmosphere.

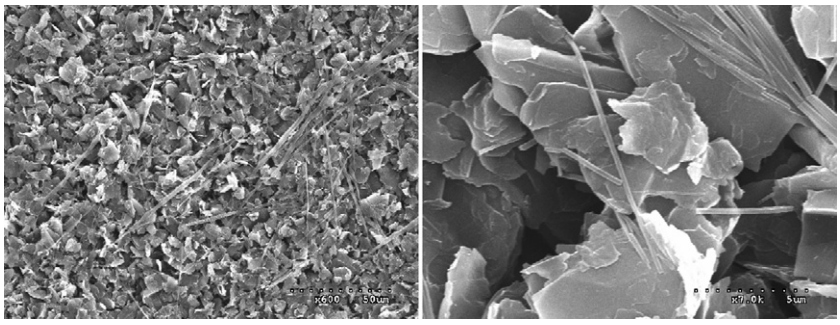

Fig. 2. SEM Image of an electrode modified with peptide nanotubes. Peptide nanotubes were shown to be deposited onto the carbon paste electrode.

The fluorescence microscope (Leica DML, Leica Microsystem, Germany) was used to the verification of the interaction between PNTs and antibody. Antigen (1.66×10^9 CFU/mL) was prepared and the antibody attached PNTs electrode was placed in the solution of antigen at room temperature for 24 h. This plate was washed with PBS and DI water and dried in the N_2 atmosphere as well. *E. coli* O157:H7 cells immobilized on the surface of PNTs were observed by scanning electron microscopy (HITACHI S-4300).

2.5. Cyclic voltammetry measurements

Cyclic voltammetry measurements were performed using a potentiostat (660a series, CH Instruments, Inc.) with a conventional electrochemical setup. The buffer solution was prepared 4 mL of 0.01 M PBS (pH 7.4) containing 10 mM $[Fe(CN)_6]^{3-/4-}$ (1:1). Ag/AgCl was used as a reference electrode and platinum as a counter electrode. Potential was scanned from −0.4 to 0.7 with a scan rate of 50 mV/s [28].

3. Results and discussion

3.1. Improvement of the electron transfer

Sensitivity, reproducibility, response time and specificity are the major concerns in the biosensor technology. The degree of amplification in a transducer is important element of sensing steps. Immunosensors take advantage of the high selectivity provided by the molecular recognition of antibodies. Because of significant differences in affinity constants, antibodies may confer an extremely high sensitivity to immunosensors. Furthermore, antibodies may be obtained in principle against an unlimited number of determinants. Immunosensors are thus characterized by high selectivity, sensitivity, and versatility [29]. Hexacyanoferrates are often used as a redox probe for the characterization of a sol–gel derived carbon paste and PNTs-modified electrode [30,31].

PNTs are exploited in many fields because of their flexibility in functionality and their molecular-recognition properties. The PNTs-modified electrode was fabricated by depositing the PNTs on the surface of carbon paste electrode to examine the properties of PNTs [25]. Fig. 3 shows the cyclic voltammograms that compared the PNTs-modified electrode to the electrode without PNTs. The carbon paste electrode has its own conducting property. The permeability of ions through a sol–gel derived carbon paste electrode is so high that a redox couple can penetrate it (Fig. 3a). With PNTs, the electron transfer of the carbon paste electrode was found to be improved significantly (Fig. 3b). The peak current of the normal electrode with PNTs is 0.659 mA. After treatment of PNTs, the peak current is shown to be increased about 30%. The results demonstrate that the presence of PNTs significantly improved the electrochemical fingerprint of the carbon paste electrode.

3.2. Immobilization of antibody on the peptide nanotubes

An antibody is a bifunctional molecule that binds an antigen at antigen-combining sites and serves as a linker of the specific antigen to immune system cells [32]. The sandwich method [19,33,34] has been used to detect the target materials such as *E. coli* O157:H7. Although this method is efficient and precise to detect, the steps to make certain forms are complicated and time-consuming. To reduce the steps and make it easier, PNTs were used to attach the antibodies. Anti-*E. coli* O157:H7 antibodies were immobilized onto the PNTs surface through amide coupling between carboxyl groups on the PNTs surface and the amine groups on antibodies. The *E. coli* antibodies are known to be covalently attached onto the amino surface and Fig. 4 clearly

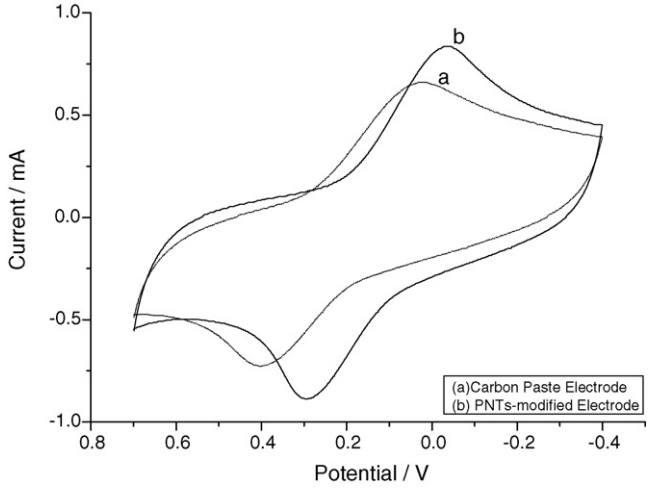

Fig. 3. Cyclic voltammogram of (a) a carbon paste electrode and (b) a peptide nanotube-modified electrode. In the presence of 10 mM of $[Fe(CN)_6]^{3-/4-}$ in 0.01 M PBS (pH 7.4), scan rate = 50 mV/s; Ag/AgCl as a reference electrode; platinum as a counter electrode.

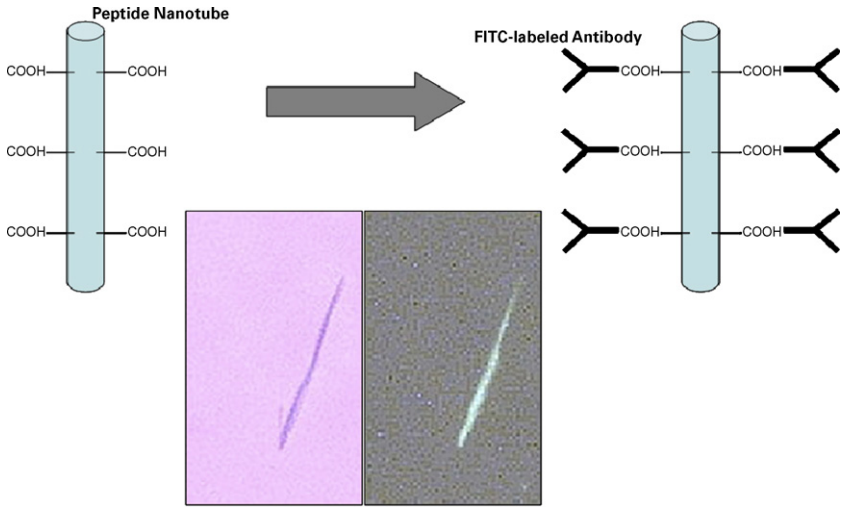

Fig. 4. Scheme of amine coupling and fluorescent microscope image of the binding peptide nanotube and antibodies.

shows immobilization of the FITC-labeled antibodies on the surface of PNTs. This result demonstrates that PNTs can be used as the templates in a biosensor because they can be attached by the target antibodies without any surface modifications.

3.3. Detection of *E. coli* O157:H7

A label-free immunosensors, in which the immune interaction between antibody–antigen can be directly monitored when none of the reaction partners is labeled, have advantages as regards speed and simplicity. An electrochemical technique is an alternative for developing biosensors for detection of *E. coli* O157:H7. Cyclic voltammetry is working in the presence of $[Fe(CN)_6]^{3-/4-}$ as a redox probe considered as an effective method for sensing the antigen–antibody interaction on electrode surfaces by probing the features of the interfacial properties such as electron-transfer of electrodes [14].

Fig. 5 is a SEM image of *E. coli* O157:H7 cells immobilized on the PNTs by antigen-antibody interaction. The *E. coli* O157:H7 cells were observed to be sphere shapes that were interacted with antibodies on the PNTs surface by non-covalent bonds. Cyclic voltammetry was used to confirm the surface changes for the attachment of antibodies and antigen of the immunosensor. Fig. 6 depicts the cyclic voltammograms obtained at a PNTs electrode (curve a), after antibody immobilization (curve b) and after cell binding (1.66×10^9 CFU/mL) (curve c) in 0.01 M PBS (pH 7.4) solution containing 10 mM $[Fe(CN)_6]^{3-/4-}$ (1:1). This figure clearly shows that the electrode surface modification affects the voltammetric behavior of the redox probe. The PNTs electrode reduces the electron-transfer resistance compared with the bare carbon paste electrode that was prepared by a sol–gel method. When the PNTs electrode was immobilized with antibodies, a decrease in peak current from 0.835 to 0.659 mA (27% decrease) was observed. It was shown upon the binding of antibody immobilization to the electrode surface. The electron transfer was inhibited by a layer of antibodies. After the treatment of the antigen–antibody interaction was made, the electron-transfer of the electrode was

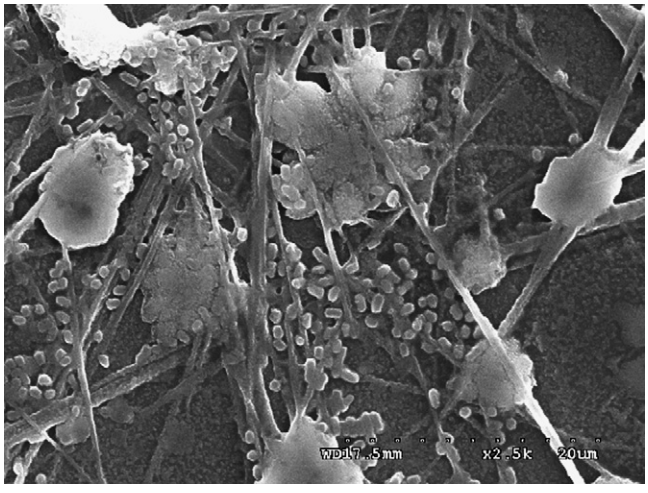

Fig. 5. SEM Image of attaching *E. coli* O157:H7 cells on the surface of peptide nanotubes by antigen–antibody interaction.

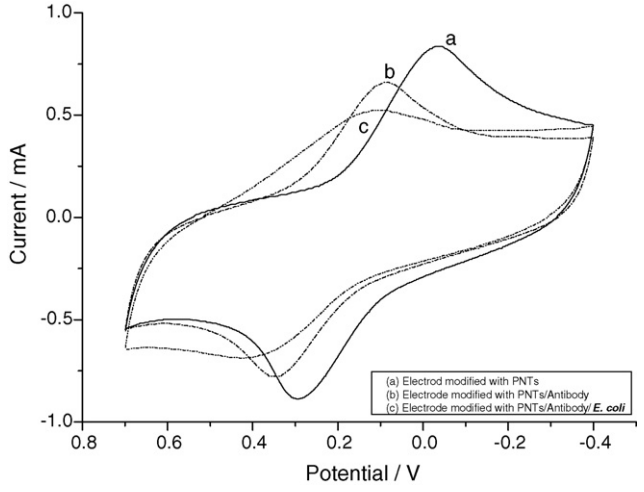

Fig. 6. Cyclic voltammogram of (a) a peptide nanotube-modified electrode, (b) with antibody immobilization and (c) with cell binding (1.66×10^9 CFU/mL). Other conditions were the same as those in Fig. 3.

measured to decrease further from 0.659 to 0.523 mA (21% decrease). Because the formation of antibody–antigen complexes create a further barrier for the electrochemical process. From those results, it can be summarized that the PNTs electrode may be applied as an electrochemical immunosensor for detection of pathogens such as *E. coli* O157:H7.

4. Conclusions

Peptide nanotube as a smart material has the functional groups on their surface that can be make a bioactive platform easier to attach biomaterials such as antibody. In the present experiments, peptide nanotubes synthesized with cyclic peptide (cyclo[(Gln-D-Leu)$_4$]) were prepared and immobilized on surface of a carbon paste electrode to amplify the transduction signal. Each electrode (PNTs, antibody and antigen) was investigated by cyclic voltammetry at the presence of hexacyanoferrates as a redox probe to detect *E. coli* O157:H7. Result of cyclic voltammetry shows that the electron-transfer resistance is systematically increased with the immobilization of antibodies and antigen onto the surface of peptide nanotubes.

An electrochemical sensor is advantageous since it offers high sensitivity, ease in fabrication, and instrumental portability, allowing both laboratory use and field sampling. Our electrochemical immunosensor with peptide nanotubes provides a convenient, low-cost, and label-free method for specific and sensitive detection of the biomolecular interactions.

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