Biosensors and Bioelectronics 23 (2008) 1832–1837

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Disposable amperometric immunosensing strips fabricated by Au nanoparticles-modified screen-printed carbon electrodes for the detection of foodborne pathogen *Escherichia coli* O157:H7

Yueh-Hui Lin[a,b,1], Sz-Hau Chen[a,1], Yao-Chen Chuang[a], Yi-Chen Lu[a], Thomas Y. Shen[b], C. Allen Chang[a,*], Chih-Sheng Lin[a,**]

[a] *Department of Biological Science and Technology, National Chiao Tung University, No. 75 Po-Ai Street, Hsinchu 30068, Taiwan*
[b] *Apex Biotechnology Corporation, Hsinchu 30078, Taiwan*

ARTICLE INFO

Article history:
Received 1 December 2007
Received in revised form 12 February 2008
Accepted 25 February 2008
Available online 14 March 2008

Keywords:
Escherichia coli O157:H7
Screen-printed carbon electrode
Immunosensor
Electrochemistry
Nanoparticles

ABSTRACT

A disposable amperometric immunosensing strip was fabricated for rapid detection of *Escherichia coli* O157:H7. The method uses an indirect sandwich enzyme-linked immunoassay with double antibodies. Screen-printed carbon electrodes (SPCEs) were framed by commercial silver and carbon inks. For electrochemical characterization the carbon electrodes were coupled with the first *E. coli* O157:H7-specific antibody, *E. coli* O157:H7 intact cells and the second *E. coli* O157:H7-specific antibody conjugated with horseradish peroxidase (HRP). Hydrogen peroxide and ferrocenedicarboxylic acid (FeDC) were used as the substrate for HRP and mediator, respectively, at a potential +300 mV *vs.* counter/reference electrode. The response current (RC) of the immunosensing strips could be amplified significantly by 13-nm diameter Au nanoparticles (AuNPs) attached to the working electrode. The results show that the combined effects of AuNPs and FeDC enhanced RC by 13.1-fold. The SPCE immunosensing strips were used to detect *E. coli* O157:H7 specifically. Concentrations of *E. coli* O157:H7 from 10^2 to 10^7 CFU/ml could be detected. The detection limit was approximately 6 CFU/strip in PBS buffer and 50 CFU/strip in milk. The SPCE modified with AuNPs and FeDC has the potential for further applications and provides the basis for incorporating the method into an integrated system for rapid pathogen detection.

1. Introduction

In the last 20 years, *Escherichia coli* O157:H7 has been an important foodborne pathogen in a variety of foods worldwide. This strain of *E. coli* is classified as an enterohemorrhagic bacterium with the ability to cause hemorrhagic colitis with symptoms such as bloody diarrhoea, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpurea (Ho et al., 2004). The monitoring of *E. coli* O157:H7 with conventional procedure (Johnson et al., 1995; Meng et al., 2001; Ho et al., 2004) could take 2–3 days. These methods are time-consuming, which delays the introduction of remedial measures. Therefore, a method for rapid detection of this pathogenic micro-organism in food and water would aid the prevention of infection, illness, and economic loss.

The field of electrochemical biosensing has grown rapidly, and biosensors for detecting antigens and nucleic acids have been reported (Bakker, 2004; Mehrvar and Abdi, 2004). Several electrochemical methods for the detection of bacterial contamination are known; for example, the amount of cells electrostatically attaching on carbon electrodes (Morisaki et al., 2000), the detection of glucose catabolism (Palenzuela et al., 2004), or the endogenous enzymatic activity of bacteria (Yemini et al., 2007). Additionally, a broad class of immunosensing techniques with improved specificity also was reported (Patel, 2002; Rasooly and Herold, 2006). Pathogens have been detected using amperometric immunoassay procedures in which the immunoreagents are immobilized on the transducer surface (Zhou et al., 2002; Chemburu et al., 2005; Rao et al., 2006). Reproducible methods of quantifying *E. coli* O157:H7 were recently studied based on an immunoassay combined with a liposome-based fluorescent-labeling method (Ho et al., 2004), a quartz crystal Au piezoelectric electrode (Su and Li, 2004), a self-assembled monolayer-based surface plasmon resonance sensor(Subramanian et al., 2006), and a fluorescent tracer-digital camera image transport system (Ligler et al., 2007). With these methods, *E. coli* O157:H7 cells could be detected at concentrations between 10^2 and 10^8

* Corresponding author. Tel.: +886 3 5131676; fax: +886 3 5729288.
** Corresponding author. Tel.: +886 3 5131338; fax: +886 3 5729288.
E-mail addresses: changca@cc.nctu.edu.tw (C.A. Chang), lincs@mail.nctu.edu.tw (C.-S. Lin).
[1] These authors contributed equally.

0956-5663/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2008.02.030

colony-forming units (CFU)/ml. However, the expense of the sensor materials or infrequent measuring instruments would limit out-of-laboratory applications for economic and fast screening.

Due to recent developments in biosensor technology, horseradish peroxidase (HRP) are often conjugated with antigen–antibody complex (Zhou et al., 2002; Chemburu et al., 2005; Castañeda et al., 2007) on the electrodes of immunosensor as reporters which react with substrate to generate electrons to be detected. And the AuNPs-modified electrodes also can be used to amplify the detection signal, improve the electron transducer and reduce the limitation on detection in electrochemical biosensors (Daniel and Astruc, 2004; Willner et al., 2007). Here, we present an approach for the quantification of *E. coli* O157:H7 that combines amperometric detection with a disposable immunosensing strip. The system was designed for the rapid, sensitive detection of bacterial cells, such as *E. coli* O157:H7, utilizing AuNPs and ferrocenedicarboxylic acid (FeDC)-modified, disposable screen-printed carbon electrodes (SPCEs).

2. Materials and methods

2.1. Apparatus

Cyclic voltammetric and amperometric determinations were performed with a CV50W voltammetric analyzer (Bioanalytical Systems, West Lafayette, IN, USA) connected to a personal computer and a PalmSens/Personal Digital Assistant, hand-held, battery-powered instrument (Palm Instruments, BZ Houten, Netherlands) for data collection and calculation.

2.2. Reagents and solutions

G-451 carbon/graphite ink and silver-resin inks were purchased from ERCON (Wareham, MA, USA). Sodium citrate, ethanol, and H_2O_2 were obtained from Merck (Darmstadt, Germany). FeDC, chlorauric acid ($HAuCl_4$), glutaraldehyde, glycerol, glycine, sodium chloride (NaCl), sodium carbonate, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), bovine serum albumin (BSA), Triton X-100, phosphate-buffered saline (PBS) and were obtained from Sigma–Aldrich (St. Louis, MO, USA). Monoclonal anti-*E. coli* O157:H7 antibody (ab20976) and horseradish peroxidase-conjugated polyclonal anti-*E. coli* O157:H7 antibody (ab20425) were purchased from Abcam (Cambridge, UK). The buffer or solutions used in this study were prepared as follows: PBS buffer (0.1 M PBS, pH 7.2), blocking buffer (67 μM glycine in 0.1 M PBS buffer, pH 7.2), substrate solution (88 μM H_2O_2 in 0.1 M PBS buffer, pH 7.2) and FeDC solution (5 mM FeDC in 95% ethanol).

2.3. Preparation of AuNPs

The AuNPs were prepared as described by Lei et al. (2004), but with minor modifications. A volume of 2.5 ml of 38.8 mM sodium citrate was quickly added to 25 ml of boiling 1 mM $HAuCl_4$ with vigorous stirring for 10 min, giving a color change from blue to red-violet. The mixture was boiled continuously and stirred for another 10 min and then removed from the heater. Colloidal Au particles with an average diameter of 13 nm were produced. The prepared AuNPs were stored at 4 °C.

2.4. Fabrication of AuNPs-modified SPCE

An electrochemical immunosensing strip based on SPCE was made (Fig. 1, bottom) by printing the SPCEs sequentially with silver-resin inks and carbon/graphite inks, using a system consisted of automated screen-printer and oven (BUILT-IN Corp., Taichung, Taiwan) to print electrodes and dry organic solvent in inks. The SPCE consisted of a 9.8-mm^2 carbon surface working electrode and a 9.8 mm^2 carbon surface counter/reference electrode. The distance is 0.5 mm between the two electrodes. A volume of 10 μl of 2.5 mM glutaraldehyde (Hart et al., 2005), was dropped onto the working electrode area, incubated for 1 h at 4 °C and washed twice with PBS buffer. A volume of 10 μl AuNPs solution was dropped onto the glutaraldehyde-treated working electrode, incubated for 1 h at 4 °C, and then washed twice with PBS buffer.

2.5. Preparation of microbial sample

The bacterial cultures, including *E. coli* O157:H7 (ATCC 43894), *E. coli* K12 (ATCC 15153), *Listeria monocytogens* (ATCC 19114), *Salmonella choleraesuis* (ATCC 13311) and *Vibrio parahaemolyticus* (ATCC 27519), used in this study were obtained from the Bioresources Collection and Research Center (Food Industry Research and Development Institute, Hsinchu, Taiwan). The pure cultures were grown in brain/heart infusion broth (Difco Laboratories, Detroit, MI, USA) at 37 °C for 24 h before use. A conventional spread-plating method was used for bacterial counts.

For the detection using real samples, the cultured *E. coli* O157:H7 cells were inoculated into sterilized milk and subsequently treated by the method (Yamaguchi et al., 2003) for eliminating lipids and proteins. Briefly, proteinase K (0.25 mg; Promega, Madison, WI, USA) and 50 μl of 0.1% Triton X-100 were added to 100 μl samples of milk, and then incubated at 37 °C for 10 min. After incubation, 900 μl of 150 mM $NaCl_{(aq)}$ was added to the samples and the mixture was centrifuged at 12,000 × *g* for 10 min. The bacteria-containing pellets were collected and resuspended in 100 μl of 150 mM $NaCl_{(aq)}$ and used for detection of bacteria.

2.6. Immunosensor fabrication, recognition and sensing procedures

Each working electrode of the AuNPs–SPCE was incubated with 10 μl of the monoclonal anti-*E. coli* O157:H7 antibodies (100 ng ml^{-1}) for 15 min at 37 °C, coated with 10 μl of FeDC solution for 10 min at 42 °C, and blocked with 10 μl of blocking buffer for 60 min at 4 °C (Fig. 1, top). Between each step, each electrode was washed twice with PBS buffer. A volume of 10 μl sample was applied to the immunosensing strip on the working electrode and incubated for 15 min at 37 °C. A volume of 10 μl of HRP-conjugated polyclonal anti-*E. coli* O157:H7 antibody (100 ng ml^{-1}) was applied to the immunosensing strip and incubated for 15 min at 37 °C. The electrodes then were washed twice with PBS buffer to remove non-specific binding between antigens and antibodies. All amperometric experiments were performed in 10 μl of substrate solution applied to the electrochemical reaction area of the AuNPs/FeDC–SPCE immunosensing strip and incubated for 40 s at 25 °C. A fixed potential of +300 mV *vs.* counter/reference electrode was applied after the incubation period. Two kinds of current signals were measured in this study: (i) the response current (RC) (sum of current signals collected per 0.1 s during the following 50 s); (ii) ΔCurrent (difference in the average RC when bacteria were present and the average RC when bacteria were absent).

There were of SPCEs groups a–f, to investigate the effects of different modification for immunosensing detection of SPCEs. Groups a–f, were with the first monoclonal anti-*E. coli* O157:H7 antibody attached. Groups a and b were fabricated without AuNPs and FeDC, i.e. [AuNPs(−)/FeDC(−)]. Group c was fabricated with FeDC but without AuNPs [AuNPs(−)/FeDC(+)] and groups d and e were fabricated with AuNPs but without FeDC [AuNPs(+)/FeDC(−)]. Group f was fabricated with both AuNPs and FeDC [AuNPs(+)/FeDC(+)]. A

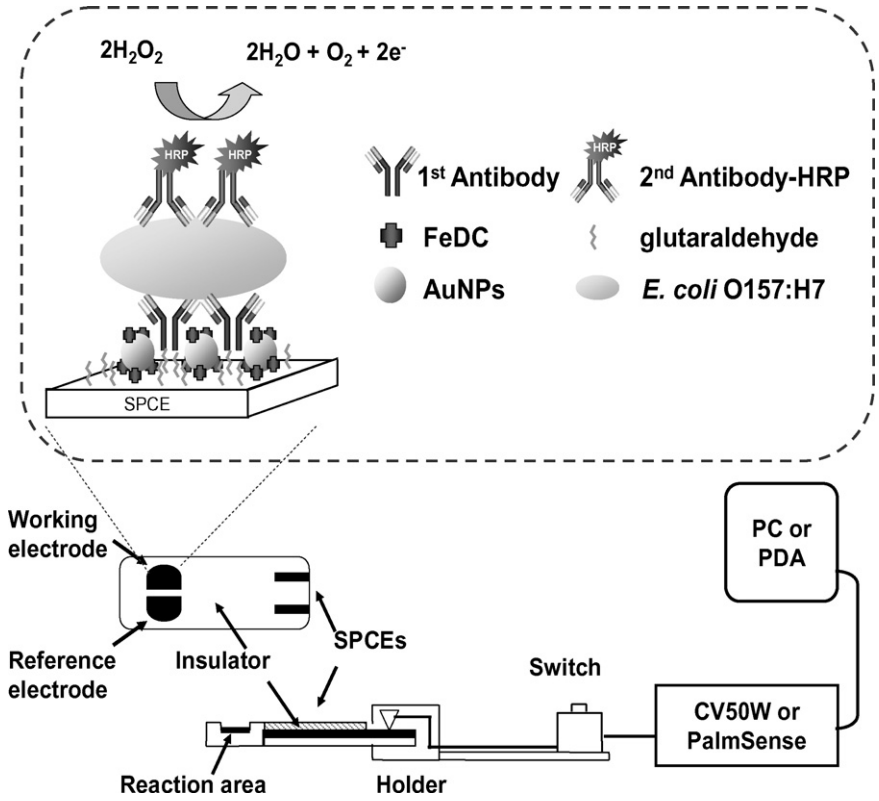

Fig. 1. A diagram of the apparatus and immunosensing processes of the AuNPs/FeDC–SPCE immunosensor system for *E. coli* O157:H7 detection. The AuNPs and FeDC were modified on the working electrode and the detection model was shown in the top panel.

volume of 10 μl sample containing the intact cells of *E. coli* O157:H7 (6.36×10^7 CFU/ml) were incubated in the immune-recognizant processes for groups b, c, e and f [*E. coli* O157:H7(+)] and the sample that contained no bacteria was used for groups a and d [*E. coli* O157:H7(−)]. Later, all groups were treated with the HRP-conjugated polyclonal anti-*E. coli* O157:H7 antibody.

2.7. Measurement procedure for plate ELISA

The plate ELISA was performed according to the indirect sandwich method (Kim et al., 2005) with slight modifications. Each well of a Maxisorb plate (Nunc, Naperville, IL, USA) was coated with a volume of 100 μl of monoclonal anti-*E. coli* O157:H7 antibody (1 μg/ml) prepared in carbonate buffer (50 mM, pH 9.6) and incubated overnight at 4 °C. After washing with PBS buffer, a volume of 200 μl of 3% BSA was added to block for 30 min at 25 °C. After washing, a volume of 100 μl of PBS buffer containing bacteria and 3% BSA was added and incubated for 30 min at 25 °C. After washing, a volume of 100 μl of HRP-conjugated polyclonal anti-*E. coli* O157:H7 antibody (200 ng ml^{-1}) was added and incubated for 30 min at 25 °C. After washing again, the enzymatic reaction was developed using ABTS as the substrate for 30 min at 25 °C. The absorbance at 450 nm was measured with an ELISA plate reader (Molecular Devices Corp., Sunnyvale, CA, USA).

2.8. Safety considerations

All bacteria were handled according to the rules appropriate for biosafety level 2 micro-organisms (no special hazard) as defined by the U.S. Centers for Disease Control and Prevention.

2.9. Data analysis

Each experiment was repeated five times using five different SPCEs to test the reproducibility of the immunosensing strips. All data with mean signals and ±standard deviation (S.D.). Differences between groups were evaluated by the two-tailed Student's *t*-test, and $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. Effect of AuNPs modification of SPCE on electrochemical characters

The results of AuNPs-modified SPCE and an *E. coli* O157:H7 cell bound to antibodies immobilized on the surface of an AuNPs-modified SPCE were confirmed by scanning electron microscope (SEM) (Supplementary Fig. S1). The size of AuNPs was very uniform and approximately 13 nm in diameter on average. There were 73.6 ± 8.1 AuNPs per 0.04 μm^2 of SPCE working surface in five random samplings from the SEM images.

In other studies, FeDC was used as the mediator in the H_2O_2–peroxidase electrochemical reaction and the biosensor operated at 150–420 mV (Wring et al., 1991; Sadeghi et al., 1997). Therefore, a non-enzymatic FeDC redox system was investigated at a scan rate of 100 mV s^{-1} and a cyclic scan range of −300 to +300 mV to verify the effects on electrochemical characteristics of the SPCE with AuNPs attached. The effects of AuNPs and FeDC modification on a typical cyclic voltammogram (CV) were evaluated using 10 μl of substrate solution. On the SPCE, modification with FeDC (line c in Fig. 2), but not AuNPs (line b in Fig. 2), the CV line could be detected in the presence of H_2O_2. Moreover, the peaks of oxidation and reduction current (i.e. CV behavior) of the SPCE treated simultaneously with FeDC and AuNPs were significantly increased (line d in Fig. 2) as using only FeDC.

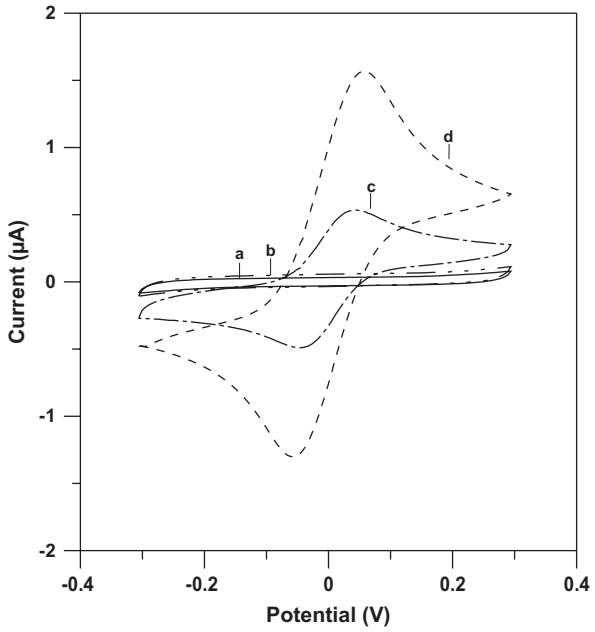

Fig. 2. Cyclic voltammograms (CV) of the SPCEs. The typical CV was obtained in 88 μM H_2O_2 in PBS buffer, which was used as a supporting electrolyte for the SPCE formed under the following conditions: (a) unmodified SPCE; (b) AuNPs-modified SPCE; (c) FeDC-modified SPCE; (d) both FeDC- and AuNPs-modified SPCE. All of the CVs were detected under the same potential step from −300 to +300 mV *vs.* counter/reference electrode. All of scan rate was 100 mV/s.

3.2. Amperometric characterization of E. coli O157:H7 immunosensing strip

The results of RC obtained with the amperometric immunosensing processes of groups a–f were shown in Fig. 3. Values of RC for groups a–c were similar. In contrast, value of RC for group d was higher than those in groups a–c ($P < 0.01$). Values of RC were much higher for groups e and f than for group d. Additionally, the value of RC was higher for group f than for group e. Although the surface of SPCE modified with AuNPs could elevate the electrochemical response of the background by around 3.4-fold compared to the SPCE without AuNPs modification (group d *vs.* group a), the benefit of amplification using AuNPs modification was as much as 10.7-fold increased for *E. coli* O157:H7 (group e *vs.* group b). The amplification

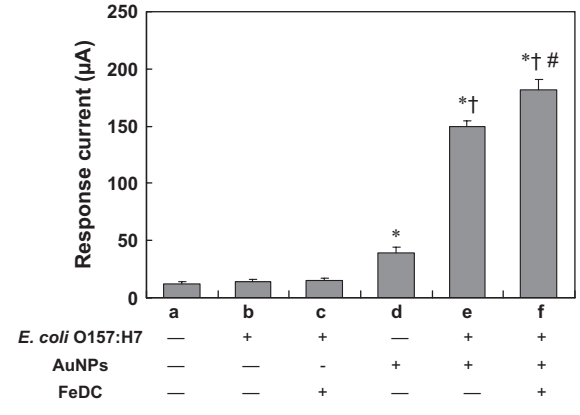

Fig. 3. Effects of AuNPs and FeDC modification on the response current in the SPCE immunosensing strips. The values of response current for the groups a–e are shown. A potential of +300 mV was applied to each working electrode (*vs.* counter/reference electrode). Each value is the mean of five independent measurements: $^{*}P < 0.01$ *vs.* groups a–c; $^{\dagger}P < 0.01$ *vs.* groups a–d; $^{\#}P < 0.01$ *vs.* groups a–e.

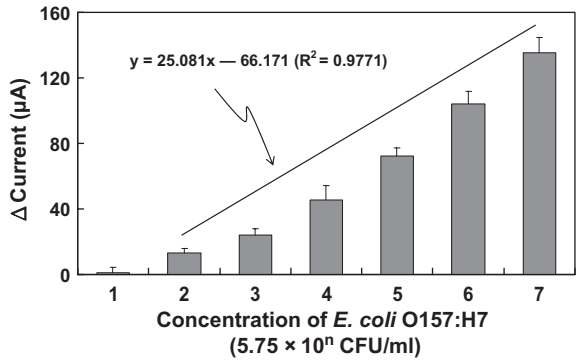

Fig. 4. The ΔCurrent due to the different concentrations of *E. coli* O157:H7 cells detected by the AuNPs/FeDC–SPCE immunosensing strips. ΔCurrent was calculated by $RC_{bacteria(+)} - RC_{bacteria(-)}$, where $RC_{bacteria(+)}$ and $RC_{bacteria(-)}$ are the mean of RC in the presence and in the absence of bacteria, respectively. Each value of RC was derived from five independent measurements. The detection limit of the immunosensing strips was approximately 6×10^2 CFU/ml, i.e., approximate 6 CFU/strip. A positive linear correlation was found between ΔCurrent and log *E. coli* O157:H7 cell concentrations from 6×10^2 to 6×10^7 CFU/ml.

benefit of both AuNPs and FeDC modification was 1.2-fold higher (group f *vs.* group e), and the benefit of FeDC modification was up to 1.1-fold higher (group c *vs.* group b). The enhancing effects of FeDC were limited in the SPCE used to detect *E. coli* O157:H7 in the immunosensing strips. Together, AuNPs and FeDC modifications could enhance the RC in the SPCE immunosensing by 13.1-fold (group f *vs.* group b) compared with unmodified electrodes.

3.3. Detection limits and specificity of E. coli O157:H7 immunosensing strips

Seven *E. coli* O157:H7 concentrations, from 5.75×10^1 to 5.75×10^7 CFU/ml were prepared by serial dilution in PBS buffer. A 10 μl sample of each bacterial suspension (i.e. approximately from 6×10^0 to 6×10^5 CFU of *E. coli* O157:H7 cells used in each detection/strip) were taken for immunosensing to evaluate the detection range by calculation of ΔCurrent. The values of ΔCurrent of the AuNPs/FeDC–SPCE immunosensing strips that were used to detect the different concentrations of *E. coli* O157:H7 cell are shown in Fig. 4. The results show that the concentration of bacteria and ΔCurrent were highly correlated. The linear relationship was well described ($R^2 = 0.9771$) by ΔCurrent (μA) = 25.081 (log CFU/ml of *E. coli* O157:H7) − 66.171. The lower limit of detection was approximately 6×10^2 CFU/ml in PBS buffer and around 6 CFU of *E. coli* O157:H7 cells per strip.

An approximately 5×10^7 CFU/ml sample of *E. coli* O157:H7, *E. coli*. K12, *L. monocytogens*, *S. choleraesuis* and *V. parahaemolyticus* cultures was used to evaluate the specificity of the AuNPs/FeDC–SPCE immunosensing strips. In the case of *E. coli* O157:H7, ΔCurrent reached 140.0(±14.7) μA shown in Fig. 5. However, the values of RC for the bacteria other than *E. coli* O157:H7 were similar to the values obtained in the absence of bacteria, indicating that there was no obvious cross-reaction of *E. coli* O157:H7-specific immunosensing strips with other bacterial species or strains.

3.4. Detection of E. coli O157:H7 cells in real samples

The cultured *E. coli* O157:H7 cells were inoculated into milk at concentrations of 0, 5.03×10^2, 5.03×10^3, 5.03×10^4 and 5.03×10^5 CFU/ml. These samples were used to detect *E. coli* O157:H7 by the AuNPs/FeDC–SPCE immunosensing strips and by the plate ELISA method. A volume of 10 μl sample of each concentration was evaluated. Thus, for each sample 0, ~5×10^1, ~5×10^2,

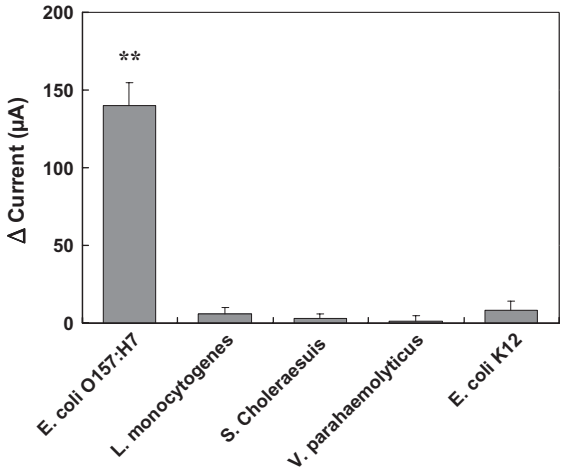

Fig. 5. Specificity of *E. coli* O157:H7 detection by the AuNPs/FeDC–SPCE immunosensing strip. The cultured bacteria, *E. coli* O157:H7, *L. monocytogens*, *S. choleraesuis*, *V. parahaemolyticus* and *E. coli* K12 cultures at a concentration around 5×10^7 CFU/ml, were applied to the detection by the immunosensing strip. Each value was derived from five independent detections: $^{**}P < 0.01$ *vs.* the groups of *L. monocytogens*, *S. choleraesuis*, *V. parahaemolyticus* and *E. coli* K12.

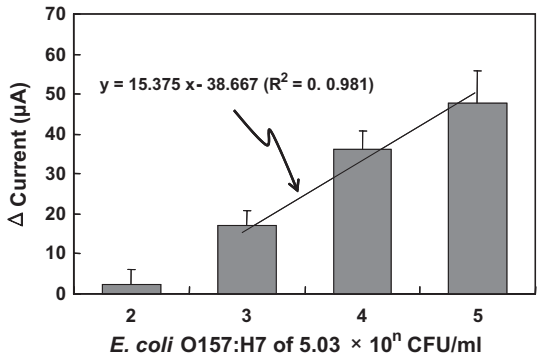

Fig. 6. Detection of *E. coli* O157:H7 in milk. Milk was inoculated with *E. coli* O157:H7 cells at concentrations of 0 (blank), 5.03×10^2 to 5.03×10^5 CFU/ml. The mean of each ΔCurrent (μA) was calculated from five independent measurements of AuNPs/FeDC–SPCE immunosensing strips. In the detection by the immunosensing strip, the values of ΔCurrent and the concentrations of *E. coli* O157:H7 from 5×10^3 to 5×10^5 CFU/ml have a positive linear correlation.

or $\sim 5 \times 10^3$ CFU of *E. coli* O157:H7 was applied to the immunosensing strip and the plate ELISA test. Using a concentration of *E. coli* O157:H7 with $>5.03 \times 10^3$ CFU/ml (i.e. approximately 50 CFU/strip), the detected values of ΔCurrent were significantly higher than background (i.e. without bacteria). The values of ΔCurrent and the concentrations of *E. coli* O157:H7 from $\sim 5 \times 10^3$ to $\sim 5 \times 10^5$ CFU/ml had a positive linear correlation shown in Fig. 6. Using the plate ELISA detection the absorbance values did not vary among samples containing 0, $\sim 5 \times 10^1$, $\sim 5 \times 10^2$, $\sim 5 \times 10^3$ CFU/ml of *E. coli* O157:H7. The absorbance value differed significantly from the background only for the milk sample with a concentration of *E. coli* O157:H7 of approximately 5×10^5 CFU/ml (i.e. 5×10^3 CFU/plate well) (data not shown).

4. Discussion

The SPCEs modified with AuNPs and FeDC were investigated in an effort to develop a rapid detection protocol for monitoring *E. coli* O157:H7 contamination in food. The recognition and sensing procedures for the *E. coli* O157:H7 immunosensing were designed without pre-incubation or pre-filtration of samples to increase or concentrate the intact cells of the pathogen. After the bacteria were recognized and bound by the first monoclonal anti-*E. coli* O157:H7 antibodies on the surface of the SPCE immunosensing strip, the HRP-conjugated polyclonal anti-*E. coli* O157:H7 antibodies were sequentially put onto the working electrode for immune recognition. Finally, HRP reacted with H_2O_2 to generate electrodes to be detected. The total analysis, including recognition, washing, and sensing processes, took ~1 h for each test. The sensitivity and specificity of the AuNPs/FeDC–SPCE immunosensing strips were examined and shown to be comparable to other conventional plate ELISA methods and biosensors.

In some biosensing methods, the major applications of Au materials, including AuNPs, have functions of conjugation (Castañeda et al., 2007), immobilization (Subramanian et al., 2006), reporters (Tang et al., 2007), or electrode/conductors (Radke and Alocilja, 2005). The electrodes modified with AuNPs were used for the detection of H_2O_2 with the peroxidase mechanism. In other studies, AuNPs with an average diameter of 20 nm (Xu et al., 2006) and 16.8 nm (Tangkuaram et al., 2007) were used to increase the sensitivity and stability of biosensors. In the present study, the results show that 13-nm diameter AuNPs on the SPCE improved electrochemical ability for oxidation/reduction and effectively shuttle electrons between the FeDC mediator and the SPCE. The AuNPs on our SPCE immunosensing strips increase the effective area of the working electrode and, as reported earlier, decrease, in part, the distances for the reductive-form mediator to diffuse to the stereo-electrode structures (Liang et al., 2005; Tang et al., 2007). In addition, a donor–acceptor pair model can explain the observed increase in the rate of electron transfer between redox enzymes and electrodes caused by AuNPs (Willner et al., 2007). Therefore, we supposed that the SPCE used in this study may have a large specific surface area and excellent conductivity due to the attached AuNPs. These attached AuNPs might overcome the long-range barriers to the formation of complexes of antibodies and bacterial cells that deliver an electron via peroxidase to the electrode.

There have been several reports of *E. coli*-specific biosensors; for example, a flow-through immunofiltration system (Abdel-Hamid et al., 1999), a PAPG/β-galactosidase method (Boyacı et al., 2005), and a real-time detection of DNA sequences using a circulating-flow system with a quartz crystal microbalance (Wu et al., 2007). But complex pre-culture, cell lysis, and DNA isolation, or sensitive instruments are needed for these methods to enhance specificity or sensitivity. Recently, a few *E. coli* intact cell immunosensors have been reported with electromechanical or amperometric methodology. An impedance-dependent biosensor, which was developed with an interdigitated microelectrode array, was able to detect *E. coli* O157:H7 cells specifically at concentrations of 10^4 to 10^7 CFU/ml (Radke and Alocilja, 2005). A flow-through sandwich amperometric immunoassay was utilized in which intact cells were captured by antibodies immobilized on activated carbon particles, then labeled with HRP-conjugated antibodies. The quantitative working range for *E. coli* was 10–1500 CFU/ml (Chemburu et al., 2005). In the present study, the AuNPs modification and indirect sandwich immunoassay were combined on the SPCE for immunosensing *E. coli* O157:H7 intact cells. Concentrations of *E. coli* O157:H7 in PBS buffer from 6×10^0 to 6×10^5 CFU/strip could be determined precisely using the immunosensing strips. The results indicate that the *E. coli* O157:H7-specific immunosensors fabricated with AuNPs/FeDC–SPCE were more useful for the detection of pathogen in milk than the plate ELISA method. Moreover, the estimated detection limit of the immunosensing strips was around 50 CFU of *E. coli* O157:H7 in milk. That is, the detection limit for *E. coli* O157:H7 was about 50 CFU/strip (i.e., 5×10^3 CFU/ml) in the real sample, indicating that the detection limitation should be improved when

the AuNPs/FeDC–SPCE immunosensing strips are used to detect pathogens in real samples. We propose that the protocol for bacterial cells isolated from real samples needs to be modified to increase the recovery efficiency.

The AuNPs/FeDC–SPCE immunosensing strips required a secondary labeled antibody for the amperometric detection. However, our device has the potential for commercialization because it uses a low-cost disposable electrode, it is hand-held operation, it is highly specific, and no pretreatment is needed for fast screening for *E. coli* O157:H7 contamination. The results obtained with the *E. coli* O157:H7 immunosensing strips for the detection of bacterial contamination in food are comparable to those from the plate ELISA method and other biosensing methods (Ho et al., 2004; Radke and Alocilja, 2005; Subramanian et al., 2006; Wu et al., 2007). The amperometric immunosensor for *E. coli* O157:H7 detection could be improved if the system was integrated with a lateral-flow method for rapid screening. However, the development of *E. coli* O157:H7 biosensors should continue in the pursuit of a method of detecting low levels of bacterial contamination, because a dose of *E. coli* O157:H7 as low as 10 organisms in a real sample may be harmful to humans.

5. Conclusions

Here, we describe a rapid, specific, and sensitive electrochemical technique for the detection of intact cells of *E. coli* O157:H7. The technique is based on an indirect sandwich amperometric immunoassay. The disposable AuNPs/FeDC–SPCE immunosensing strips, the key element of this detection system, were fabricated by carbon inks and modified with 13-nm diameter AuNPs and FeDC. Amperometric detection was selected because of its many benefits, including its adaptability for use in miniaturized and portable systems. The method uses a sensitive detection of HRP activity coupled with AuNPs and FeDC to amplify the amperometric effect. Interference from other bacteria was eliminated by the use of doubly specific antibodies. One of the important advantages of our AuNPs/FeDC–SPCE immunosensing strips is that approximately 50 CFU of *E. coli* O157:H7 in samples of milk can be detected in 1 h. The AuNPs/FeDC–SPCE immunosensing strip system has a potential for further applications and provides the basis for incorporating the method into an integrated system for rapid pathogen detection.

Acknowledgments

The authors thank the Apex Biotechnology Corp. for donating the SPCE. This work was supported by the MoE ATU Programs from the Department of Education, Taiwan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.02.030.

References

Abdel-Hamid, I., Ivnitski, D., Atanasov, P., Wilkins, E., 1999. Biosens. Bioelectron. 14 (3), 309–316.
Bakker, E., 2004. Anal. Chem. 76 (12), 3285–3298.
Boyacı, İ.H., Aguilar, Z.P., Hossain, M., Halsall, H.B., Seliskar, C.J., Heineman, W.R., 2005. Anal. Bioanal. Chem. 382 (5), 1234–1241.
Castañeda, M.T., Merkoci, A., Pumera, M., Alegret, S., 2007. Biosens. Bioelectron. 22 (9–10), 1961–1967.
Chemburu, S., Wilkinsa, E., Abdel-Hamidb, I., 2005. Biosens. Bioelectron. 21 (3), 491–499.
Daniel, M.C., Astruc, D., 2004. Chem. Rev. 104 (1), 293–346.
Hart, J.P., Crew, A., Crouch, E., Honeychurch, K.C., Pemberton, R.M., 2005. Anal. Lett. 37 (5), 789–830.
Ho, J.A., Hsu, H.W., Huang, M.R., 2004. Anal. Biochem. 330 (2), 342–349.
Johnson, J.L., Rose, B.E., Sharar, A.K., Ransom, G.M., Lattuada, C.P., McNamara, A.M., 1995. J. Food Prot. 58 (6), 597–603.
Kim, S.H., Park, M.K., Kim, J.Y., Chuong, P.D., Lee, Y.S., Yoon, B.S., Hwang, K.K., Lim, Y.K., 2005. J. Vet. Sci. 6 (1), 41–46.
Lei, C.X., Hu, S.Q., Gao, N., Shen, G.L., Yu, R.Q., 2004. Bioelectrochemistry 65 (1), 33–39.
Liang, R., Qiu, J., Cai, P., 2005. Anal. Chim. Acta 534 (2), 223–229.
Ligler, F.S., Sapsford, K.E., Golden, J.P., Shriver-Lake, L.C., Taitt, C.R., Dyer, M.A., Barone, M.A., Myatt, C.J., 2007. Anal. Sci. 23 (1), 5–10.
Mehrvar, M., Abdi, M., 2004. Anal. Sci. 20 (8), 1113–1126.
Meng, J.H., Feng, P., Doyle, M.P., 2001. In: Downes, F.P., Ito, K. (Eds.), Compendium of Methods for the Microbiological Examination of Foods, 4th ed. American Public Heath Association, Washington, DC, pp. 331–342.
Morisaki, H., Sugimoto, M., Shiraishi, H., 2000. Bioelectrochem. Bioenerg. 51 (1), 21–25.
Palenzuela, B., Simonet, B.M., García, R.M., Ríos, A., Valcárcel, M., 2004. Anal. Chim. Acta 524 (1–2), 167–174.
Patel, P.D., 2002. Trends Anal. Chem. 21 (2), 96–115.
Radke, S.M., Alocilja, E.C., 2005. Biosens. Bioelectron. 20 (8), 1662–1667.
Rao, V.K., Sharma, M.K., Goel, A.K., Singh, L., Sekhar, K., 2006. Anal. Sci. 22 (9), 1207–1211.
Rasooly, A., Herold, K.E., 2006. J. AOAC Int. 89 (3), 873–883.
Sadeghi, S.J., Gilardi, G., Cass, A.E.G., 1997. Biosens. Bioelectron. 12 (12), 1191–1198.
Su, X.L., Li, Y., 2004. Biosens. Bioelectron. 19 (6), 563–574.
Subramanian, A., Irudayaraj, J., Ryan, T., 2006. Biosens. Bioelectron. 21 (7), 998–1006.
Tang, D., Yuan, R., Chai, Y., 2007. Biosens. Bioelectron. 22 (6), 1116–1120.
Tangkuaram, T., Ponchio, C., Kangkasomboon, T., Katikawong, P., Veerasai, W., 2007. Biosens. Bioelectron. 22 (9–10), 2071–2078.
Willner, I., Baron, R., Willner, B., 2007. Biosens. Bioelectron. 22 (9–10), 1841–1852.
Wring, S.A., Hart, J.P., Brirch, B.J., 1991. Analyst 116 (2), 123–129.
Wu, V.C.H., Chen, S.H., Lin, C.S., 2007. Biosens. Bioelectron. 22 (12), 2967–2975.
Xu, S., Tu, G., Peng, B., Han, X., 2006. Anal. Chim. Acta 570 (2), 151–157.
Yamaguchi, N., Sasada, M., Yamanaka, M., Nasu, M., 2003. Cytometry A 54 (1), 27–35.
Yemini, M., Levi, Y., Yagil, E., Rishpon, J., 2007. Bioelectrochemistry 70 (1), 180–184.
Zhou, Y.M., Liu, G.D., Wu, Z.Y., Shen, G.L., Yu, R.Q., 2002. Anal. Sci. 18 (2), 155–159.