

Short Communication

Oriented T4 Bacteriophage Immobilization for Recognition of *Escherichia coli* in Capacitance Method

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Received: 23 July 2016 / Accepted: 11 September 2016 / Published: 10 November 2016

One of the important aspects in biotechnology is the development of a rapid and selective approach for the identification and quantization of *Escherichia coli* (*E. coli*) bacteria. In this research, we fabricated a novel Label-free electrochemical *E. coli* biosensor based on oriented immobilization of T4 biotinylated bacteriophage as recognition receptor of *E. coli* on streptavidin immobilized on polyaniline (PANI) glassy carbon (GC) modified electrode. Labeled PANI was synthesized by chemical method and characterized by Raman and cyclic voltammetry (CVs) and field emission scanning electron microscope (FESEM). The bacteriophage/PANI/GC electrodes were applied as indicator electrodes for capacitance determination of *E. coli* bacteria. By fabricating this biosensor, *E. coli* was sensed in a linear range and detection limit of 10-98 N/mL and 10 N/mL (N stands for number of *E. coli*) respectively. The proposed biosensor shows fast response time of ~1 s and good selectivity over other bacteria. We successfully developed and implemented an *E. coli* biosensor based on oriented bacteriophage immobilization on PANI modified GC electrode. This biosensor is cost-effective, rapid, renewable and high selective for detection of *E. coli* bacteria. Also, this method can be extension to detection of other biological agents.

Keywords: *E. coli*, bacteriophage T4, capacitance, biosensor, PANI.

1. INTRODUCTION

Recently, microorganisms (viruses and bacteria) are generally found in the meal, soil, surroundings, aquatic environment and estuarine waters, and the intestinal tracts of human beings and animals [1].

Food-borne pathogenic bacteria have affected the health of living organisms. Some bacteria for example Salmonella, *E. coli*, Campylobacter, *Listeria monocytogenes*, *Bacillus cereus*, and

Staphylococcus aureus are the most important food-borne pathogen bacteria, which are caused major food-borne illness [2]. Hence, it is really important to develop methods for detecting the food-borne pathogenic bacteria in food, water and air as its critical role in public health. While, cell culture and plating are standard microbiological methods, they are costly and time consuming. And also, most of conventional methods involve complex instrumentation and may not be used on-site [3]. Thus, developing biosensors for detection of pathogens as a fast and specific technique are growing [4,5,6].

One of the dangerous human pathogen is *E. coli* [7,8]. It causes life-threatening difficulties such as bloody diarrhea and renal failure [9]. Consequently, designing and constructing a rapid and specific biosensor for the detection and quantification of *E. coli* would be very important in analysis of food, environment and medical disorders. In this regard, several rapid and expensive systems were developed. These systems include as electric DNA chips, quartz crystal microbalance, amperometric biosensor, imaging ellipsometry, opto-fluidic ring resonator sensor, and surface plasmon resonance (SPR) [10,11]. Biosensors are tools to determine levels of chemicals and microorganisms in a sample with high accuracy. They can be used in different branches of science like medicine, industry, agriculture, and etc [12].

In this work we have successfully fabricated a rapid and cost effective capacitive biosensor constructed by oriented bacteriophage modified glassy carbon (GC) electrode. Therefore, the layout of the biosensor is patterned as a two electrodes configuration. These electrodes were made by immobilization of oriented bacteriophage on polyaniline (PANI) via streptavidin on modified GC electrode (bacteriophage/streptavidin/PANI/GC). Consequently, the *E. coli* trapping by oriented bacteriophage on the PANI modified GC electrodes was reported by capacitance method. The proposed sensors show good characteristics such as; low detection limit, wide linear range, fast response time and good selectivity for *E. coli* detection.

2. MATERIALS AND METHODS

2.1. Apparatus and procedure

Raman spectroscopy was obtained by Nicolet Raman spectrometer. The morphology of the prepared bacteriophage/streptavidin/PANI/GC electrode was achieved by means of field emission scanning electron microscope (FESEM) (Hitachi S-4160, UK).

All of the capacitance evaluations were done by a LCR meter (GPS 3131B, Benchtop LCR Meter, Germany). Cyclic voltammetry (CVs) analysis were accomplished via an Autolab potentiostat/galvanostat (model 302N, Eco Chemie, Ultecht, Netherlands). The capacitance in this system was evaluated through two similar GC electrodes in 2 mm diameter (presented in Fig. 1) as a capacitor.

2.2. Reagents

Linker biotin-NHS with MW 341.38 from Sigma, United States of America, streptavidin from Sigma, United States of America, Aniline monomer MW 93.13 from Sigma, United States of America,

LB Agar culture from Merck Germany, LB Broth culture from Merck Germany, Polyethylene glycole (PEG) 6000 from Merck, Germany, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) with MW 155.24 from Sigma, United States of America, 2-(N-morpholino) ethanesulfonic acid (MES) buffer with MW 951.24 Sigma, United States of America, Dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2) were obtained from Merck, Germany. A phosphate buffer solution (0.1 M, pH=7) was applied as supporting electrolyte. Ultrapure H_2O by a Millipore-Milli Q (18 $\text{M}\Omega$ cm, Barnstead, Dubuque, USA) system was used for all solution preparations. All the reagents were used as received, without any purification and all experiments were done at room temperature (25 $^\circ\text{C}$). *E. coli* and bacteriophage were gotten from Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

2.3. Preparation of bacteriophage/streptavidin/PANI/GC electrode

There were 3 steps for preparing bacteriophage/streptavidin/PANI/GC electrode. In the first step, biotinylated bacteriophage prepared as follow. NHS-Biotin dissolved in Dimethyl sulfoxide (DMSO) instantly in 22 mg/mL concentration. NHS-Biotin solution was added to the total volume of the bacteriophage 10%, and stirred gently, and incubated at room temperature for 4 hours. In the second step, streptavidin immobilized on PANI modified GC electrode as the following order. Firstly, 3 μL of PANI (5% W/V) in DMSO solution was dropped on GC electrode. In the other vessel, 0.7 mg of streptavidin was dispersed in MES buffer (1.5 mL, 50 mM, pH= 6.1). Then, NHS (0.5 mL, 50 mg/mL) which was prepared in MES was added to the above suspension. While stirring suspension severely, 0.3 mL of fresh EDAC solution (10 mg/mL prepared in MES buffer) was combined quickly, and the consequence mixture was stirred at room temperature for 45 minutes repeatedly. After that, 20 μL of activated streptavidin immediately dropped on the PANI/GC electrode, and set the electrode stable for 2 hours, to react slowly. Then, the streptavidin/PANI/GC electrode washed with MES buffer three times for removing the excess EDAC, NHS. In the third step, biotinylated bacteriophage, immobilized on streptavidin/PANI/GC electrode. For this purpose, 300 μL biotinylated bacteriophage solutions capped on streptavidin/PANI/GC electrode and keep away from light overnight at room temperature, then stabilized bacteriophage on streptavidin/PANI/GC electrode washed with phosphate-buffered saline (PBS) three times. In this stage oriented bacteriophage was attached on the GC electrode surface.

2.4. Procedure for *E. coli* detection on bacteriophage/streptavidin/PANI/GC electrode

The quantification of *E. coli* by this bacteriophage biosensor is based on capacitance changes. The layout of this biosensor is patterned as a two-electrode configuration as shown in Fig.1. The performance of the electrodes was investigated by measuring the capacitance change of *E. coli* bacteria which is prepared with a concentration range between 0-160 $\times 10^5$ N/mL by serial dilution. Each solution was stirred and the capacitance was recorded, as the solution became stable, and then plotted as a function of *E. coli* activity.

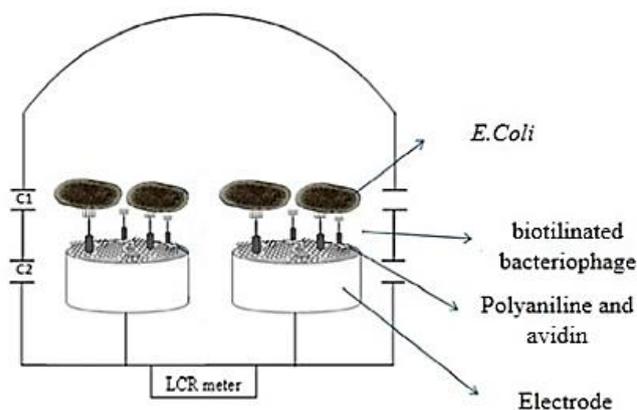


Figure 1. Capacitance measurement illustration. The interaction between bacteriophage and *E. coli* followed on the electrodes modified through streptavidin /PANI on GC electrode.

3. RESULTS AND DISCUSSION

3.1. Characterization of Streptavidin immobilized on PANI by Raman Spectroscopy

The Raman spectra recorded for PANI and streptavidin/PANI are presented in Figure 2. The Raman spectrum of PANI show bands attributed to N-H group (1500 cm^{-1}). For PANI, Raman scattering intensity at 1450 cm^{-1} , related to the C-N group. Also, streptavidin displays a number of characteristic bands, in the $300\text{-}1750\text{ cm}^{-1}$ spectral range related to amino acid groups [13]. This spectrum proves that, the streptavidin was successfully immobilized on PANI/GC electrode.

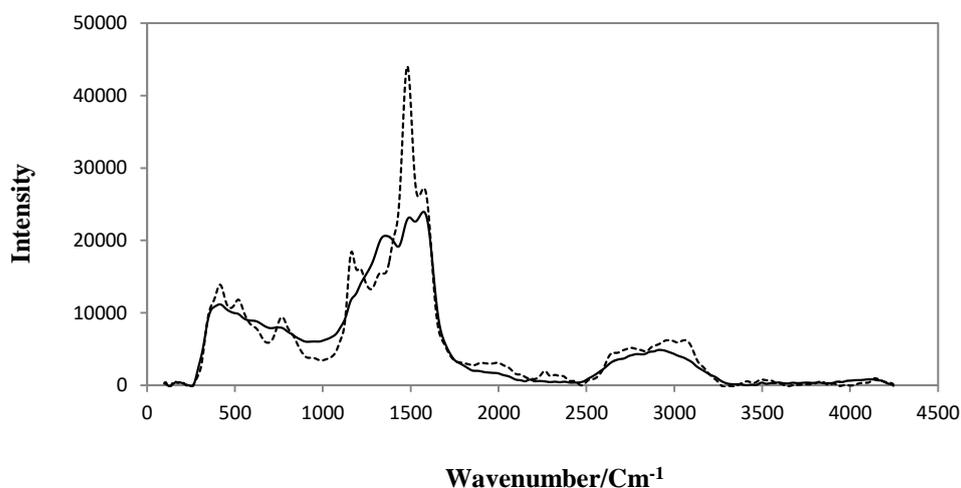


Figure 2. Raman spectra for PANI (—) and streptavidin/PANI (...).

3.2. The investigation of conductivity by Cyclic Voltammetry

Cyclic voltammograms (CVs) were used to follow the electron conductivity of the bare GC, PANI/GC and bacteriophage/streptavidin/PANI/GC electrodes (Figure. 3a, 3b and 3c respectively). In this experiment, $K_3[Fe(CN)_6]$ solution in PBS (0.1 M) is applied as redox probe. As shown in Fig. 3, the CVs of bare and modified electrodes, was seen in the potential from -0.75 to 0.5 V. The bare GC hasn't any pair redox peaks. The value of formal potential [$E^0=(E_{pc}+E_{pa})/2$] for PANI/GC and bacteriophage/streptavidin/PANI/GC modified electrodes were 0.2 and 0.15 V, respectively. This value of formal potential shows that, when the electrode surface immobilized by PANI, the current intensity was increased significantly. Since, PANI/GC electrode possess high conductivity, which could improve electron transferring activity between the electroactive species and the electrode surface in the solution[14]. Also, when the oriented biotinylated bacteriophage as an insulator, immobilized on PANI/GC electrode the current intensity was decreased.

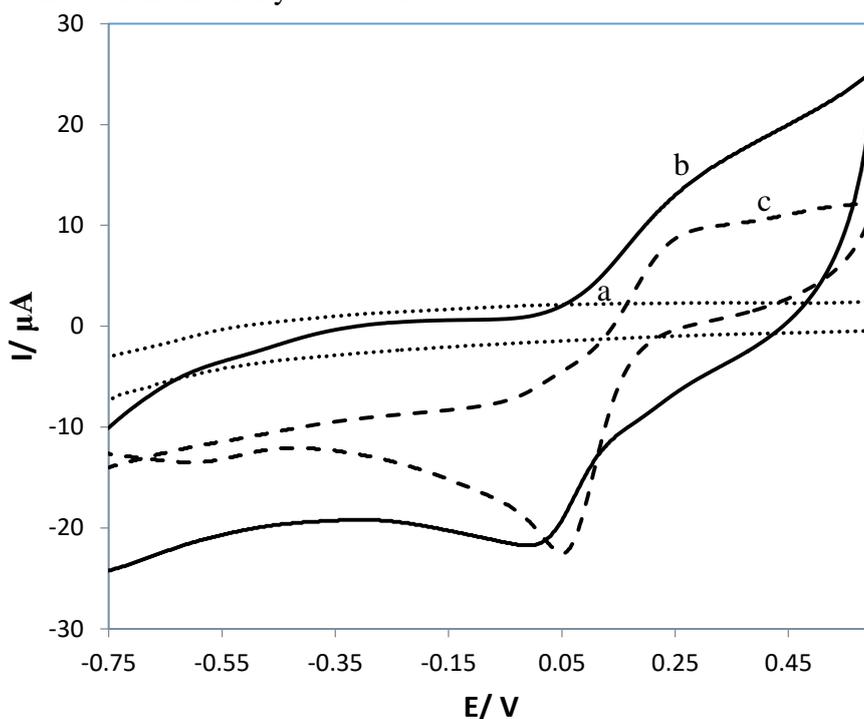


Figure 3. Comparison between CVs. GC electrode (a), streptavidin/PANI/GC electrode (b) and bacteriophage/streptavidin/PANI/GC electrode (c) CVs is recorded in PBS solution (0.1 M, pH =7.2) with scan rate of 100 mV/s at air saturated condition.

3.3. Equivalent circuit fit for biosensor

Figure 1 illustrates the correlation between each immobilized layer on the GC electrode with the capacitance intensity. The insulating self-assembled monolayer pattern on the GC electrode caused a constant capacitance and prevents any faradic processes and the capacitance got the highest level (Figure 1). According to the image illustrated in Scheme 1, two planar bacteriophage modified GC electrodes stands as two capacitor plates and the buffer between them as an insulating layer. By

developing each layer on the bacteriophage modified GC electrodes, a new capacitor value is reached by different dielectric coefficient, thickness and surface area. Therefore, by attachment of *E. coli* bacteria on the left and right bacteriophage modified GC electrodes (C_1 and C_2), the total capacitance was changed[15]. As presented in Figure 1, the total capacitance (C_t) at this level can be calculated through Eq. 1:

$$1/C_t = 1/C_1 + 1/C_2 \quad (1)$$

3.4. Calibration curve for *E. coli* biosensor

For measuring of *E. coli* the capacitance method was used. Figure 4 demonstrate the association of the change of capacitance besides *E. coli* number by bacteriophage modified GC electrodes. It is obvious that, the bare GC (without bacteriophage) fixed on electrodes do not show any response to *E. coli* concentration change. Also, oriented bacteriophage modified GC electrodes, has high sensitivity and wide concentration range, because modified electrode with bacteriophage immobilization creates a high sensitivity to area ratio for capacitance storage. In bacteriophage modified GC electrodes, *E. coli* was detected in a linear range from 3.2×10^5 to 32×10^5 N/mL by the correlation coefficient of 0.998. The detection limit (DL) of this biosensor was calculated at signal to noise ratio of 3 (S/N=3) according to the Eq.2:

$$DL = 3.3 \sigma/S \quad (2)$$

In the Eq. 2, σ stands for standard deviation of the response and S presents the slope of calibration curve [16]. According to Eq. 2, the detection limit was calculated equals to 12 N/mL. Finally, the analytical parameters of this biosensor were compared with other similar biosensors reported for detection of *E. coli* (Table 1).

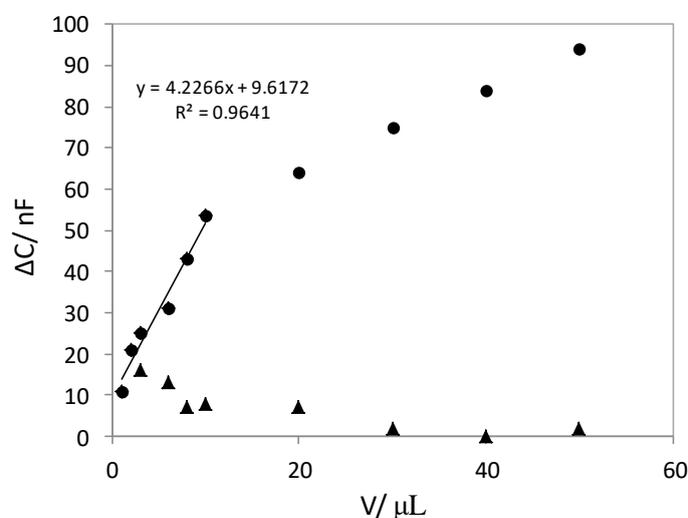


Figure 4. Comparison of the calibration curves for *E. coli* determination. Bare GC electrodes (\blacktriangle) and bacteriophage/streptavidin/PANI/GC electrode (\bullet) in PBS solution (0.1 M, pH=7.2) by capacitance method ($1\mu\text{L}=3.2 \times 10^5$).

Table 1. Comparison of the parameters obtained in this work with other reported in the literature

<u>Species</u>	<u>Transduction</u>	<u>Method</u>	<u>Analytical characteristics/sample</u>	<u>Ref</u>
E.coli	EIS	Antibody/polyaniline /Au electrode	Det.range 10^2 CFU mL ⁻¹ of E. coli , detection limit of 10^7 CFU mL ⁻¹	[17]
E.coli	Amperometry	DNA/Fe ₂ O ₃ @AuNP	Det. range: up to 0.01 pM DNA target; 500 cfumL ⁻¹ ; LOD: 5 cfumL ⁻¹ /water	[18]
E.coli	DPV	Cystine/amino probe DNA	Det. range: 1×10^{-6} – 1×10^{20} M pM DNA target; 60 s hybr. time.	[19]
E.coli	Amperometry	biotin/Strept-AP as reporter.	LOD: 250 cfu μ L ⁻¹	[20]
E.coli	DPV	DNA/thiol/Au electrode	LOD: 112 cfumL ⁻¹ /milk	[21]
E. coli	Capacitance	bacteriophage/streptavidin/PANI/GC electrode	Det:10-98 N/mL and 10 N/mL (N stands for number of E. coli), fast response time of ~1 s LOD: :20 MI, aqueous solution(PBS, PH=7)	D. Ashiani1, A. H. Keihan, J. Rashidiani 1, F. Dashtestani , K. Eskandari1 .

3.5. Selectivity of the *E. coli* biosensor

The selectivity in medical diagnosis is so vital in analyzing biological samples which are within a complex matrix. In the present report, the selectivity of the designed biosensor was considered in the existence of five several gram-negative and gram-positive bacteria such as, *Klebsiellapneumoniae*, *Shigelladysenteriae*, *Vibrio cholera* and *Staphylococcus aureus* and *Salmonella typhi*. It does not significant response to oriented biotinylated bacteriophage biosensor, as showing to selectivity of system to *E. coli* bacteria detection (Figure 5).

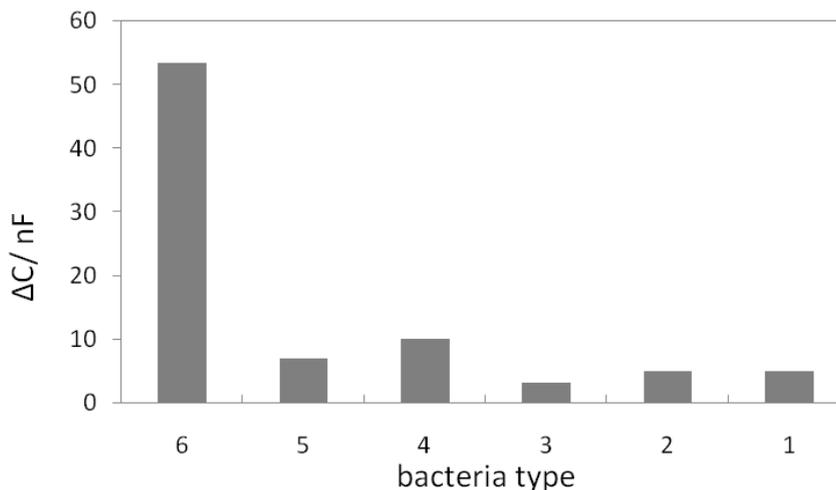


Figure 5. Comparison of several bacteria capacitance response to bacteriophage/streptavidin/PANI/GC electrode (1: Staphylococcus aureus, 2: Klebsiellapneumoniae, 3: Shigelladysenteriae, 4: Vibrio cholerae, 5: Salmonella typhi and 6: E. coli)

4. CONCLUSION

The investigation and preparation of biosensors based on *E. coli* is so essential for clinical diagnostic. Although biosensors with antibodies provide a quick and cheap method for detection of cells, spores, viruses and toxins, but they are limited in their specificity [22,23]. In addition, working with monoclonal antibodies requires the time and expertise related with the production of them [24]. Therefore, we propose inexpensive, label-free, fast, sustainable and renewable *E. coli* biosensors based on immobilization of oriented bacteriophage modified GC electrode and this electrode was used in capacitance method. Modified carbon electrode was caused to high capacitance signal by oriented bacteriophage was attached to the polyanilin surface [25]. The oriented bacteriophage electrode presents convenient linear range, good detection limit and selectivity. The fabrication of this biosensor would be suitable for detecting other pathogens like *E. coli* or so on.

ACKNOWLEDGEMENTS

Authors are grateful to Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences for providing financial support to undertake this work.

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