Research Article

Magnetic Nanoparticles Immobilization and Functionalization for Biosensor Applications

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We describe an approach for *E. coli* bacteria detection using an electrochemical immunosensor. The immunosensor was based on functionalized magnetic nanoparticles immobilized onto bare gold electrode. Cyclic voltammetry and impedance spectroscopy was performed before and after magnetic nanoparticles deposition. The magnetic nanoparticles functionalized with anti-*E. coli* polyclonal antibody were used for bacteria detection. Lytic T4-phage was used to confirm the success recognition of bacteria with the developed immunosensor. The specificity of the immunosensor was tested against *Enterococcus faecium* bacteria. A limit detection of 10^3 CFU/mL *E. coli* bacteria was obtained with a good reproducibility.

1. Introduction

One of the principal characteristics of *E. coli* bacteria was their genotypes diversity responsible for diseases. These *E. coli* genotypes called pathotypes lead too much dangerous pathology which affects human and animal species. Currently, we can identify six intestinal pathotypes and two extraintestinal pathotypes agreed by the scientific community [1–3]. Among them, we can cite enterotoxigenic *E. coli* (ETEC) which is frequently an agent of human and animal diarrhea; 600 million cases of human diarrhea and 800.000 worldwide deaths principally in children under the age of five years [4] were attributed to (ETEC) infections. The EPEC infection leads to aqueous diarrhea with some associated pathologic manifestation as vomiting, fever, and dehydration [5–7]. The enteroaggregative *E. coli* (EAEC) pathology was described as watery diarrhea with the presence of abdominal spasms but no fever. It shows no invasion of blood vessels, but it is marked by persistent rashes and chronic watery diarrhea. Small outbreaks have been recorded both in industrialized countries than in developing countries [8–12].

Toward these inflectional menaces, food contamination is controlled by rigorous legislations [13], and foodborne

safety was presented as preventive solution allowing avoiding *E. coli* diseases. This is why bacterial detection and recognition are principal aims of the health strategies. Conventional methods for *E. coli* detection were based on bacterial culture and colony counting [14], this way requires 24 h to yield results and can require up to 7 days for other bacterial strains [15, 16]. However, biosensors are currently imposed as powerful analytical tools which yield together sensitivity, specificity, and real-time detection. Several successful biosensors for microbial agent were developed [17–22], among them are impedimetric biosensors [23, 24]. Magnetic nanoparticles were widely used in the biosensors conceptions [25]. They carry the advantage of increasing the ratio surface/volume, and also it is easy to immobilize to any electrode surface by applying a magnetic field. In previous works, authors were interested in exploiting the high specific interaction between bacteriophages and their target bacteria using various transducers [26]. Lytic phage can be used as a specific bioreceptor allowing bacterial recognition, where the phage-bacteria detection generates dual impedimetric behavior. The first one is corresponding to bacteria immobilization and shows impedance increase, and the second behavior is awarded to bacterial lysis which shows impedance decrease [27]. In this work, we used

T4 phages as free bioreceptor allowing *E. coli* bacteria detection as a second tool for our biosensing strategy. Functionalized magnetic nanoparticles immobilized onto a bare gold electrode were used as an immunosensor. Cyclic voltammetry and impedance spectroscopy was performed before and after magnetic nanoparticles deposition. T4-phage was used to confirm the success recognition of bacteria with the developed immunosensor. The specificity of the immunosensor was tested against nonspecific *Enterococcus faecium* bacteria.

2. Experimental Setup

2.1. Reagents and Apparatus. The used antibody is goat polyclonal IgG anti-*E. coli* (ab13627) purchased from Abcam (UK). A phosphate buffer solution (PBS) of $5 \mu\text{g mL}^{-1}$ of antibody with $\text{pH} = 7.2$ was prepared. The magnetic beads used are supplied by Sigma Aldrich (France). They are composed of iron oxide particles coated with a polymer and grafted by COOH groups, and their diameter is about 200 nm. All other materials, including 1-ethyl-3-(3-(dimethylamino)-propyl) carbodiimide (EDC) (Aldrich) and N-hydroxy succinimide (NHS) (Aldrich), were used as supplied.

The buffer solution used was phosphate-buffered saline (PBS) containing 140 mM NaCl, 2.7 mM KCl, 0.1 Mm Na_2HPO_4 , and 1.8 mM KH_2PO_4 , at pH 7.2. This solution was added by a redox couple $[\text{Fe}(\text{CN})_6]^{-4/-3}$ (5 mM) (ox/red) and used for cyclic voltammetry and impedance spectroscopy. All reagents were of analytical grade, and ultrapure water (resistance $\geq 18.2 \text{ M}\Omega \cdot \text{cm}^{-1}$) produced by a Millipore Milli-Q system was used.

2.2. Bacterial Culture. *E. coli* cells were grown in LB broth or on LB agar plates, and *Enterococcus faecium* cells were grown in BHI broth or on BHI agar plates. High titer of bacteria suspension was prepared as follows: liquid culture mediums were inoculated by $100 \mu\text{L}$ of preculture solution and cultivated at 37°C for 8–10 h. Centrifuge the cells at 6200 rpm for 5 min, wash the cells twice, and resuspend the cells in sterile PBS. Determine the viable cells and bacterial concentration with spread-plate technique. The optical density OD of the bacterial culture has been measured for the determination of bacterial growth stationary phase.

2.3. Phage Culture. The *E. coli* K12 liquid culture mediums were inoculated at exponential phase with $500 \mu\text{L}$ of phage-T4 solution and were incubated at 37°C in a rotary shaker (200 rpm) for three hours. Chloroform was added to final concentration of 10% and kept at 4°C for 20 min before centrifugation at 4°C (19000 g). Double-layer plates of each phage dilutions were prepared to give confluent lysis of the indicator strain *E. coli* K12. This allows us to determine the phage concentration in PFU.

2.4. Gold Cleaning. Gold substrates were provided by the Neuchatel Institute (Switzerland). They were fabricated using standard silicon technologies. (100)-oriented, P-type

FIGURE 1: Cyclic voltammograms of (a) bare gold electrode; (b) bare gold electrode/magnetic beads. In presence of a 5 mM redox, probe $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ versus SCE.

($3\text{--}5 \Omega \cdot \text{cm}$) silicon wafers were thermally oxidized to grow an 800 nm-thick field oxide. Then, a 30 nm thick titanium layer and a 300 nm-thick gold top layer were deposited by evaporation under vacuum. Before modification, the gold electrodes were cleaned in acetone solution for 20 min with ultrasonic bath. After that, they were dried under a nitrogen flow and then dipped for 10 min into “piranha solution” 7 : 3 (v/v) 96% $\text{H}_2\text{SO}_4/30\% \text{H}_2\text{O}_2$. Finally, the gold substrates were rinsed 2 to 3 times with ultrapure water and immediately immersed in an ethanol solution and finally dried under nitrogen flow.

2.5. Magnetic Nanoparticles Functionalization. The stock solution of carboxylic magnetic nanoparticles was diluted in ultrapure water. A volume of $40 \mu\text{L}$ of the diluted solution of nanoparticles was injected onto a cleaned gold electrode already positioned in an electrochemical cell. A magnetic field of 300 mT was applied during all the measurements using a cylindrical magnet. The magnetic nanoparticles layer was formed on the gold surface. The immobilized nanoparticles were treated with 0.4 Mm EDC-0.1 mM NHS for 1 h to convert the terminal carboxylic groups to an active NHS ester. The polyclonal antibodies solution ($5 \mu\text{g mL}^{-1}$) was dropped on the gold surface for 1 h at room temperature. Modified electrode was finally treated by BSA solution (1%) to block the nonspecific sites.

2.6. Cyclic Voltammetry. The deposition of magnetic nanoparticles onto gold electrode was checked with cyclic voltammetry. Measurements were recorded in PBS solution with redox couple $[\text{Fe}(\text{CN})_6]^{-4/-3}$, using a scanning potential sweep from -700 mV to 700 mV with a scan rate of 100 mV/s .

FIGURE 2: Impedance spectra of (a) bare gold electrode; (b) bare gold electrode functionalized with magnetic nanoparticles.

2.7. Electrochemical Impedance Spectroscopy. Conventional electrochemical cell with a three-electrode configuration was used to measure impedance spectroscopy. Modified gold electrode was considered as working electrode (0.11 cm^2), a platinum electrode (0.54 cm^2) as the counterelectrode, and a saturated calomel electrode (SCE) as the reference electrode. The impedance spectra were recorded in a frequency range from 50 mHz to 100 kHz, and apparatus used in all electrochemical measurements is an Autolab 302N impedance analyser (Ecochemie, The Netherlands) equipped with the NOVA1.4 acquisition software.

3. Results and Discussions

3.1. Characterization of Magnetic Nanoparticles Layer

3.1.1. Cyclic Voltammetry. Figure 1 shows the cyclic voltammetry measurement of bare gold surface before and after magnetic nanoparticles immobilization. A reversible voltammogram corresponding to a bare cleaned gold electrode can be observed. The two observed peaks correspond to the oxydoreduction potential of the used redox couple $[\text{Fe}(\text{CN})_6]^{-4/-3}$. The success immobilization of magnetic nanoparticles was confirmed with the increase of the current. This indicates that the resistance decreases due to the presence of the magnetic beads covering the electrode.

3.1.2. Electrochemical Impedance Spectroscopy. Figure 2 shows the electrochemical impedance spectra of gold bare electrode before and after the deposition of magnetic nanoparticles layer at 0 V. The obtained spectra can be modeled with an electric model shown in a previous work [19]. The diameter of semicircle corresponds to the charge transfer resistance of the electrode/electrolyte interface. The decrease of the charge transfer resistance is due to the conductivity

FIGURE 3: Impedance spectra of (a) bare gold electrode/magnetic beads/polyclonal Ab anti-*E. coli*; (b) 10^3 CFU/mL; (c) 10^4 CFU/mL; (d) 10^5 CFU/mL; (e) 10^6 CFU/mL; (f) 10^7 CFU/mL; (f=g) 10^8 CFU/mL.

FIGURE 4: Calibration curve: the variation of the absolute value of $|(\text{Log } Z - \text{Log } Z_0)/\text{Log } Z_0|$ versus bacteria concentration (CFU/mL) at 233 mHz. The specific detection was with *E. coli* and nonspecific detection with *Enterococcus faecium* bacteria.

increase at the gold-electrolyte interface after magnetic nano-particles immobilization.

3.2. Bacteria Immunodetection. The immunodetection of *E. coli* bacteria was performed in an electrochemical cell containing sterile PBS. Different volumes corresponding to different bacterial concentrations in CFU/mL were injected. Figure 3 shows the impedance spectra obtained after different bacteria concentrations injection (between 10^3 and 10^8 CFU/mL). The decrease of the charge transfer resistance

FIGURE 5: Variation of the absolute value of $|(Log Z - Log Z_0)/Log Z_0|$ after phage injection versus time at a fixed frequency of 233 mHz. *E. coli* bacteria concentration is 10^7 CFU/mL.

is due to the specific bacteria recognition. The resistance decreases gradually as the bacteria concentration increases. This decrease is different from the previous results obtained without magnetic nanoparticles [27] where we have an impedance increase. A saturation behavior was reached at a concentration of 10^7 CFU/mL.

A calibration curve was obtained by calculating the absolute value of $(Log Z - Log Z_0)/Log Z_0$ at a fixed frequency (233 mHz), where Z is the value of the impedance after bacteria binding to antibody, Z_0 is the value of impedance only with the antibody. Figure 4 shows a linear increase of the absolute value of the impedance and a beginning of saturation for higher bacteria concentrations. The detection limit of 10^3 CFU/mL was obtained with a good reproducibility. The negative test was performed by injecting several concentrations of *Enterococcus faecium* bacteria. The absence of any significant change confirms the specificity of the developed immunosensor.

3.3. T4-Phage Injection. We used lytic phage (T4-phage) as second recognition to confirm the previous signal generated by the immunosensor. For this purpose, we inject a concentration (10^7 CFU/mL) of T4 phage onto the functionalized gold electrode after *E. coli* recognition. A 50 μ L of T4 phage solution was introduced into electrochemical cell where immunosensor was already saturated with the bacteria (10^7 CFU/mL). As it is shown in Figure 5, a stable impedimetric behavior was recorded, while after 15 min, a strong decrease of the absolute value of the impedance was observed. This kinetic behavior is typical for phage-bacteria interaction [27] and confirms the immunosensing results.

4. Conclusion

In this work, we describe an approach for *E. coli* bacteria detection using immunosensor based on functionalized

magnetic nanoparticles immobilized onto a bare gold electrode. Cyclic voltammetry and impedance spectroscopy was performed before and after magnetic nanoparticles deposition. The magnetic nanoparticles functionalized with polyclonal antibody were used for *E. coli* detection. T4-phage was used to confirm the success recognition of bacteria by the developed immunosensor. The specificity of the immunosensor was tested against *Enterococcus faecium* bacteria. A limit detection of 10^{+03} CFU/mL was obtained with a good reproducibility. For future work, interdigitated microelectrodes arrays integrated in microfluidic cell will be used to perform limit detection and sensitivity.

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