Microchimica Acta
https://doi.org/10.1007/s00604-018-2749-y

ORIGINAL PAPER

Voltammetric determination of the *Escherichia coli* DNA using a screen-printed carbon electrode modified with polyaniline and gold nanoparticles

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Received: 20 November 2017 / Accepted: 24 February 2018

Abstract
The authors describe an electrochemical assay for fast detection of *Escherichia coli (E. coli)*. It is based on a dual signal amplification strategy and the use of a screen-printed carbon electrode (SPCE) whose surface was modified with a polyaniline (PANI) film and gold nanoparticles (AuNPs) *via* cyclic voltammetry (CV). In the next step, avidin was covalently immobilized on the PANI/AuNP composite on the SPCE surface. Subsequently, the biotinylated DNA capture probe was immobilized onto the PANI/AuNP/avidin-modified SPCE by biotin-avidin interaction. Then, DNA of *E.coli*, digoxigenin-labeled DNA detector probe and anti-digoxigenin-labeled horseradish peroxidase (HRP) were placed on the electrode. 3,3′,5,5′-Tetramethylbenzidine (TMB) and H_2O_2 solution were added and the CV electrochemical signal was generated at a potential of −0.1 V (vs. Ag/AgCl) and a scan rate 50 $mV.s^{-1}$. The assay can detect 4×10^6 to 4 CFU of *E. coli* without DNA amplification. The biosensor is highly specific over other pathogens including *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis*, *Staphylococcus haemolyticus* and *Pseudomonas aeruginosa*. It can be concluded that this genosensor has an excellent potential for rapid and accurate diagnosis of *E.coli* inflicted infections.

Keywords Infectious diseases · Cyclic voltammetry · Screen-printed carbon electrode · Capture probe · Digoxigenin-labeled detector probe

Introduction

E. coli is a member of the bacterial family of *Enterobacteriaceae* [1]. It is a most common intestinal microorganism of humans and warm-blooded animals [1]. Some strains of this bacterium have the pathogenic potential and can cause a wide range of infections from mild to life-threatening conditions, such as traveler's diarrhea, infant diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, infections of the urinary tract (UTIs), and septicemia [2, 3]. UTIs are the second highest prevalent infections and constitute nearly 25% of total bacterial infections [4]. *E.coli* is the most common gram-negative bacterium causing 80–90% of community-acquired UTIs and 50% of hospital-associated UTIs [5, 6]. There are different tests for uropathogenic bacterial detection in clinical samples such as non-culturing and culturing methods, enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) and isothermal microcalorimetry (IMC) [7]. These procedures are time-consuming, expensive and require specialist equipment and well-trained personnel. Therefore, development of a methodology that provides easy preparation of sample and rapid recognition of bacteria still remain desirable and challenging. Biosensors are an ideal candidate for the screening of infectious diseases [8]. In biosensor technology, the miniaturization is one of the important advantages, which reduces sample and reagent volumes and makes

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00604-018-2749-y) contains supplementary material, which is available to authorized users.

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Published online: 12 March 2018

the sensor more economical [9]. A typical biosensor is composed of bio-component and transducer. Binding of the analyte to the bio-component leads to the production of a determinable signal that is recognized by the transducer [6]. Based on the type of transducer, biosensors are generally classified as optical, thermal, mechanical, bioluminescent, electrical and electrochemical [6].

Electrochemical biosensors have attracted considerable attention for its intrinsic advantages, such as simplicity, high compatibility, impressive sensitivity, portability, inexpensive, and offer a fast response [10]. Furthermore, the electrochemical biosensors can tolerate matrix effects of physiological samples and provide direct detection of bacteria in samples [11]. The association of considerable features of electrochemical techniques with high-selective nature of nucleic acids has led to design biosensors for detection of specific nucleic acid sequences. The nucleic acid electrochemical biosensing devices can be effectively used in the diagnosis of bacterial/viral infections and contaminations in the various fields such as biomedical, pharmaceutical, and environmental [10]. In recent years, a series of signal amplification strategies have been applied to improve the sensitivity, signal-to-noise (S/N) ratio and low detection limit of electrochemical methods [12]. Polyaniline (PANI) is a semi-flexible conductive polymer (CP) that provides a large specific surface area, rapid electron transfer dynamics, and excellent electrochemical activity [13]. Further, it can act as a suitable matrix for immobilization of bio-components on the surface electrode [13].

Among various nanomaterials, AuNPs are widely used in sensing fabrication due to their biocompatibility, optical, electronic and electrochemical properties [14].

The simultaneous use of PANI and AuNPs can improve the large surface area for biomolecules immobilization, high conductivity, and the capacity to provide a natural environment for biomolecules [15, 16]. An electrochemical DNA biosensor was designed based on a dual signal amplified strategy using PANI/AuNP composite as a biosensor platform and HRP enzyme as a label for the specific and rapid detection of *E. coli*. The method is highly sensitive, as evinced by its ability to detect up to 4 CFU in a sample. Therefore, in future this method to be successfully used in the clinical laboratories.

Experimental section

Materials and reagents

The capture probe, detector probe sequences were designed by Liao [17], and synthesized by Fazabiotech Company (www.fazabiotech.com) that the sequences are listed in (Table S1). The capture probe was biotinylated in 5′, and the detector probe was labeled with a digoxigenin in 3′. The both oligonucleotide probes pair are complementary with species-specific region of the 16S rRNA gene of *E. coli*. Furthermore, Luria broth (LB), agar, eosin methylene blue agar, sodium hydroxide (NaOH), potassium chloride (KCl), N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide(EDC), N-hydroxysuccinimide(NHS), hexacyanoferrate(III), hexacyanoferrate(II), perchloric acid ($HClO_4$), sulfuric acid (H_2SO_4), chloroauric acid ($HAuCl_4$), aniline, 3,3′,5,5′-tetramethylbenzidine (TMB), H_2O_2, bovine serum albumin (BSA), casein, avidin, anti-digoxigenin-horseradish peroxidase (anti-dig-HRP), ultrapure water, phosphate buffer and phosphate buffer saline were purchased from Sigma Chemical Company (USA) (www.sigmaaldrich.com).

DropSens 110 SPCEs (3.4 cm length × 1.0 cm width × 0.05 cm height) were purchased from DropSens. (Spain) (www.dropsens.com/en/screen_printed_electrodes_pag). These SPCEs are a three-electrode system with a 4 mm diameter disk carbon working electrode, carbon counter electrode, and a silver reference electrode. Uropathogen isolates and clinical urine specimens were obtained from the department of medical bacteriology, Tarbiat Modares University and Dr. shoaie clinical lab, respectively.

Electrochemical experiments were performed with a potentiostat/galvanostat μ Stat 400 with a DropView software package (DropSens, Spain). (www.dropsens.com/en/potentiostats_pag.).

The scanning electron microscope images were obtained using a VEGA3-LMU (TESCAN, USA). (www.tescan.com/en-us/technology/sem/vega3**).** The Field Emission Scanning Electron Microscope (FE-SEM) imaging and the Energy Dispersive Spectroscopy (EDS) analysis were carried out by MIRA3-XMU FE-SEM (TESCAN, USA).(www.tescan.com/en-us/technology/sem/mira3). Atomic Absorption Spectroscopy (AAS) analysis was done by Atomic Absorption/flame emission spectrophotometer AA-670 (Shimazu, Japan). (www.shimadzu.eu/atomic-absorption-spectroscopy-aas).

Methods

Electrode modification

Initially, a 4 mM solution of aniline in 50 mM $HClO_4$ was prepared. Then, in order to aniline electropolymerization and PANI formation, 30 μL of the prepared solution was added to the surface of SPCE. This process was done by CV method in a potential range from −0.4 to +0.8 V and a scan rate of 50 $mV.s^{-1}$ for 10 cycles. After washing the electrode surface with 20 μL of H_2SO_4 0.5 M, 30 μL of $HAuCl_4$ solution (0.6 mM) in H_2SO_4 0.5 M was added on the PNAI-modified SPCE. Later, the AuNPs were electrodeposited on the surface of SPCE using CV method in a potential range from −0.2 to +1.2 V and a scan rate of 100 $mV.s^{-1}$ for 15 cycles. The SPCEs surface after the PANI electropolymerization and AuNPs electrodeposition were subjected to SEM analysis for studying the

morphological changes during the modification process. Also, by employing of 1 mM $[Fe(CN)_6]^{-3/-4}$ redox probe solution containing 0.1 M KCl, each step of electrode modification was examined through CV measurements in the potential range of −0.6 to +1 V and a scan rate of 0.1 $mV.s^{-1}$ [15].

The capture probe immobilization

Biotinylated capture probe was immobilized onto PANI/AuNP- modified electrode using biotin/avidin interaction. For this purpose, 1 mg of avidin was suspended in 1 mL ultrapure water. For obtaining activated avidin 10 μL of this solution was added to the 1 mL of EDC (20 mM) / NHS (40 mM) solution and incubated for 30 min at room temperature. In the next step, 10 μL of the activated avidin solution was placed onto the PANI/AuNP modified electrode for 2 h at 25 °C. Subsequently, in order to remove unbound proteins, the SPCE surface was washed 3 times with 20 μL of ultrapure water. The 4 μL of biotinylated capture probe (1 μM in phosphate buffer (1 M, pH 7.4) was dropped on the avidin-coated SPCE for 30 min [18].

Quantification of DNA of *E. coli*

Hybridization process The sensing system is based on double DNA hybridization that the target sequence was paired specifically with the capture probe and detector probe. Initially, the total DNA was extracted with G-spin™ Total DNA Extraction Mini Kit. Then, the DNA was chilled in ice to obtain denatured, single-stranded DNA. The single-stranded DNA products were stored at −20 °C to prevent degradation [19]. In next step, different concentrations of target DNA were carefully prepared. Then, 50 μL of phosphate buffer (1 M, pH 7.4) containing detector probe (0.5 μM) and BSA (2.5%) was added to each concentration of target DNA and the hybridization solution was incubated for 15 min at 66 °C to allow target-probe hybridization. Subsequently, 4 μL of the hybridization solution was added to the surface of the PANI/AuNP/avidin/capture probe-modified electrodes and incubated for 15 min at room temperature in a humidified chamber. Finally, the surface of electrode was washed 3 times using ultrapure water [16].

Electrochemical measurements

The enzymatic reaction was used for detection of the target. So, the fixation of HRP enzyme on the modified-electrode surface is essential for electrochemical analysis. Therefore, 2 μL of solution phosphate buffer saline (1 M, pH 7.4) containing anti-digoxigenin-labeled HRP (0.5 $U.mL^{-1}$) and casein (0.5%) was dropped onto the working space of modified-SPCEs and the electrodes were incubated for 15 min at room temperature. The HRP-modified SPCEs were washed 3 times with ultrapure water that to remove the unbound enzymes. Then, the electrode was covered with 50 μL of TMB (5 mM) /H_2O_2 (0.07%) solution [16]. After 5 min incubation at room temperature, the cyclic voltammograms were subsequently obtained in a potential range from −1 to +1 V and a scan rate of 0.1 $mV.s^{-1}$ [20].

The sensor protocol for direct detection of bacteria cell was essentially the same as the target DNA test. Briefly, 1 mL of bacteria inoculated in LB medium or clean urine or 1 mL of the clinical urine sample was centrifuged at 10,000 *g* for 5 min. The supernatant was discarded, and the bacteria cells were lysed by treating with 30 μL of 1.5 M NaOH and incubation at room temperature for 10 min. In this part of the experiments, the bacterial-lysate was used as the target and hence, the other steps were carried out as described in above [16].

In addition, the stability of the PANI/AuNP/avidin/capture probe-modified SPCEs was assessed after 10 weeks of storage at 4 °C and compared to that of newly prepared electrodes. The reproducibility of the genosensor was also investigated through measurement of two concentrations in eight independent experiment runs [14].

Results and Discussion

Designing of the biosensor

A DNA electrochemical biosensor was designed for detection and quantification E.coli. With the combination of suitable properties PANI, AuNPs, the complementarity principle of nucleic acid molecules and, HRP enzyme activity, this electrochemical genosensor showed notable accuracy in evaluating different concentrations of target DNA and number of bacteria by CV analysis. Figure 1 schematizes the principal steps of the biosensing procedure. Initially, the aniline monomers were electropolymerized on the surface of SPCE. The AuNPs were subsequently electrodeposited on the surface of PANI-modified SPCE. The avidin protein was covalently fixed onto the PANI/ AuNP-modified SPCE surface. Afterwards, the biotinylated capture probe was immobilized onto the SPCE surface through biotin/avidin interaction. In next step, the working area of SPCE was covered by hybridization solution containing bacterial lysis and dig-labeled detector probe and then exposed to HRP enzyme. The final detection was carried out by CV measurements, which was based on enzymatic reaction performance and redox reactions of the H_2O_2/TMB on the SPCE surface.

Surface characterization

Polymer synthesis

Conducting polymers (CP) have unique properties, which make them suitable candidates for fabrication of

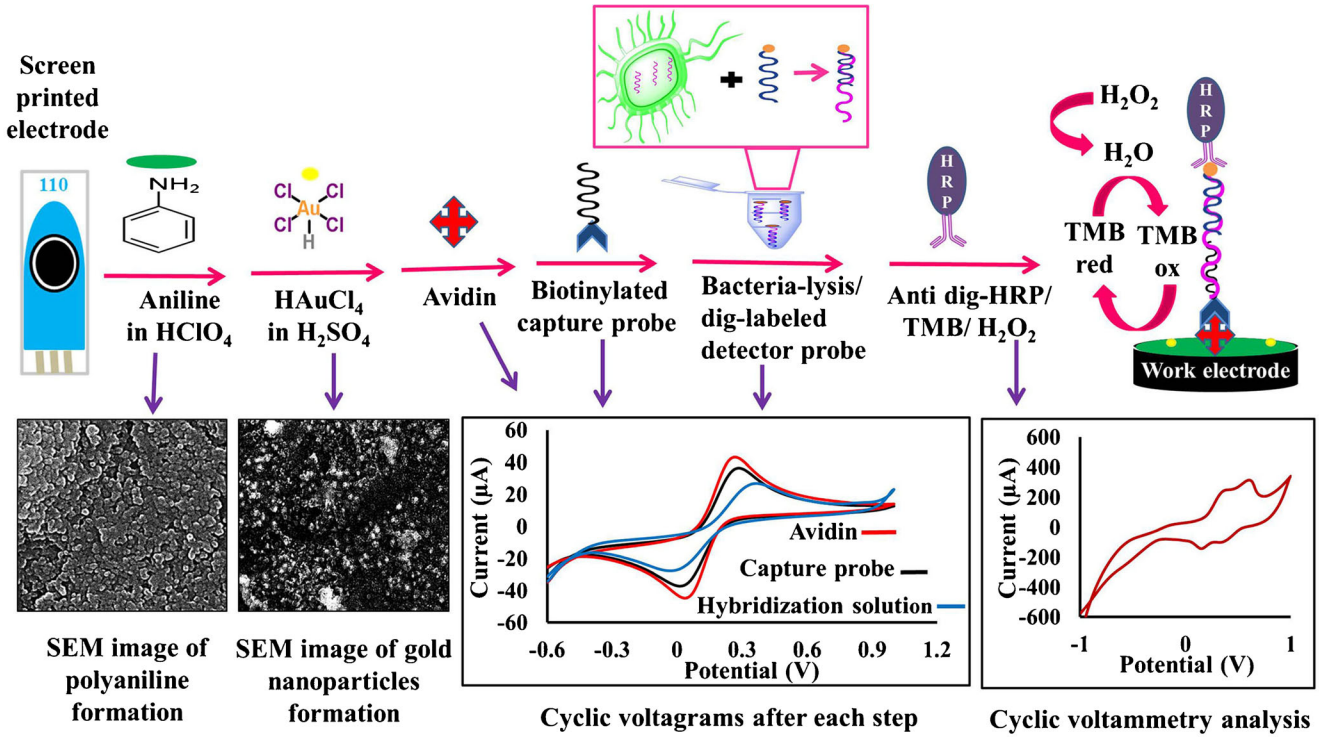

Fig. 1 Schematic presentation of the DNA electrochemical recognition strategy

electrochemical biosensors. An important feature of these materials is the presence of conjugated double bonds along the backbone of the polymer through which the free motion of electrons is possible [21]. Among different CPs, PANI has attracted much attention, particularly due to its exclusive and controllable chemical and electrical properties, high conductivity, long-term environmental stability, structural flexibility, easy synthesis and low cost [13, 22]. In spite of these advantages, the application of PANI in biosensors field is restricted because it is conductive only at acidic conditions (pH = 2.5 ~ 3.0) [15, 22]. This problem is solved through the simultaneous use of PANI with other components including gold nanoparticles, poly (acrylic acid) or polystyrene sulfonate that shift the PANI redox to a neutral pH [15].

After polymerization of aniline monomers on the surface of SPCE, a redox peak appeared in 0.2 to 0.4 V (Fig. S1a). The SEM technique was used to investigate the modification of SPCE surface after formation of PANI film. Figure 2a, b shows the SEM images of the bare electrode surface and also the PANI film formed by electropolymerization of aniline (4 mM) with 10 scans of CV. The growth of aniline monomer can be seen on the electrodes surface that has amplified from small particles to the film.

Au deposition

In the last decade, there have been significant achievements in nanotechnology. Nanomaterial-based electrochemical signal amplifications have a high potential for increasing both sensitivity and selectivity of electrochemical biosensors [23]. Owing to their exclusive physical and chemical features such as size scale, high surface-to-volume ratio, good electrocatalytic properties, and chemical stability that are interestingly useful for the progress of reliable point of care (POC) assays done in screen printed devices [24]. Due to their biocompatibility, optical and electronic properties and high conductivity,

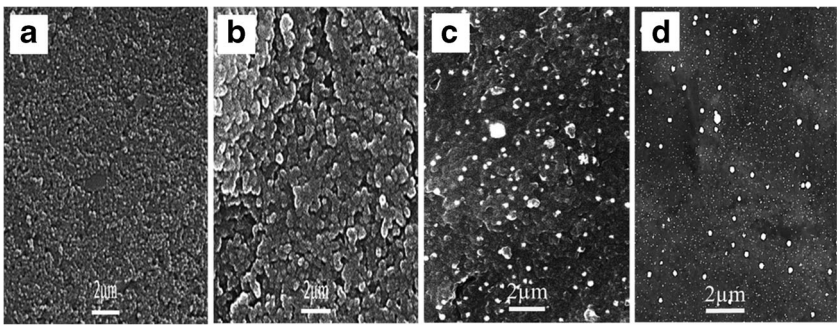

Fig. 2 SEM images of the **a** bare SPCE. **b** PANI layer prepared *via* electropolymerization with concentration 4 mM of aniline monomers. **c** PANI/AuNP-modified SPCE surface after electrodeposition of HAuCl4 solution (0.6 mM). **d** FE-SEM image of PANI/AuNP-modified SPCE surface

AuNPs are the most preferred nanomaterial for constructing electrochemical biosensors [14]. Incorporation of AuNPs into PANI has been offered as a way for increasing its electrochemical and electrocatalytic activities [22].

Electrodeposition of $HAuCl_4$ solution on the surface of PANI modified SPCE shows a redox peak in 0 to 0.2 V (Fig. S1b). PANI in the form of emeraldine salt is a very attractive conducting polymer in the field of biosensor, but it is just conductive in acidic solution (pH < 3) and in proton doped state, its application is still limited [22]. The presence of AuNPs with proper dispersion on the PANI film changed the PANI redox to the neutral pH that is ideal for biological interactions. Furthermore, the PANI/ AuNP composite noticeably raises the final surface area and conductivity that can improve the sensitivity of electrochemical biosensing platforms. Therefore, for the formation of AuNPs with the most suitable dispersion, 0.6 mM concentration of $HAuCl_4$ was electrodeposited on the surface of PANI-modified SPCE *via* 15 scans of CV. After electrodepositing of $HAuCl_4$, the morphological alterations occurred on the surface of PANI-modified electrodes (Fig. 2c). The appearance of shiny gold clusters, confirms the presence of AuNPs on the surface of PANI film. Also, the FE-SEM image shows that AuNPs have a proper dispersion on the surface of PANI modified electrode (Fig. 2d). Moreover, the EDS analysis clearly demonstrates the presence of the PANI and AuNPs on the modified SPCE surface (Fig. S2 and Table S2). Using (AAS) analysis, the concentration of AuNPs on the surface of PANI/AuNP-modified electrode was determined 23.7 ppb (Table S3). These results showed a reasonably good correlation with the EDX analysis.

Immobilization of avidin and biotinylated capture probe

To enhance the sensitivity of DNA biosensors, the immobilization of the probe while maintaining its innate affinity for the target DNA is an important parameter to the overall biosensor performance [15]. The adsorption, covalent bonding, microencapsulation, entrapment, and avidin-biotin interaction are techniques that used for the attachment of DNA probe on the surface of the electrode [25]. In the most of the aforementioned techniques, for immobilization of the DNA probe on the electrode surface, the chemical or electrochemical pretreatment step is requested. There are different techniques for the immobilization of avidin on the surface of electrodes in order to use biotin// avidin interaction [26]. In this genosensor, the avidin protein was covalently immobilized onto the surface PANI/AuNp modified SPCE using EDC and NHS. In this reaction, the role of chemical EDC is the convert the carboxylic group of the avidin protein and to reactivate its amine group. This form of avidin is unstable and will quickly hydrolyze. The NHS is able to produce a more stable amine reactive state that in contact with the amine group of the PANI, successfully form a crosslink bond. Its consequence was the strong stable linkage with a high surface coverage [27]. Therefore, the capture probe was tightly attached to the avidin- bound on surface modified SPCE.

Electrochemical measurement of modified electrodes and assessment of the construction procedure of the genosensor

In order to evaluate the correctness of the genosensor preparation process, CV measurements were assayed after each assembly step. Figure 3 illustrates the CV curves obtained from the bare electrode and different modified SPCEs in presence of 1 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl. As it can be seen, a typical CV curve with a couple of weak and irreversible redox peaks obtained from the bare SPCE (curve a). After electropolymerization of aniline monomer on the surface of the electrode, a couple of redox peaks with high peak currents were achieved that confirmed the formation of PANI film on the SPCE surface (curve b). The enhanced of the current intensity is indicated to excellent electrical conductivity and effective surface area of PANI film that is obviously associated with forming the electrostatic interaction between positive charge of PANI and the negative charge in $[Fe(CN)_6]^{3-/4-}$. In fact, this interaction has improved reduction/ oxidation activity of $[Fe(CN)_6]^{3-/4-}$ in comparison to the bare electrode. When AuNPs have electrodeposited on surface PNAI modified electrode, the higher peak current was observed. This increased of current was attributed to the large electroactive surface area of PANI/AuNP/SPCE and high conductivity properties of AuNPs that facilitate the electron transfer (curve c). In contrast, after coating the PANI/AuNP/SPCE with avidin protein, the peak current remarkably decreased which apparently reduces the electron transfer of $[Fe(CN)_6]^{3-/4-}$ to the surface electrode (curve d). In the next steps, we examined the effect of the biotinylated capture probe and further hybridization solution that cyclic scans were acquired by $[Fe(CN)_6]^{3-/4-}$ solution exhibited a couple of redox peaks with weak peak currents. This phenomenon is associated with the repulsion between negative $[Fe(CN)_6]^{3-/4-}$ ions and the negative phosphate groups in the structure of oligonucleotides on the electrode surface (curve e and f).

In order to calculate the active surface area was used of the Randle-Sevcik equation. Therefore, the CV of bare, PANI and PANI/AuNP modified electrodes was obtained in presence of 1 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl at scan rates 50 to 150 $mV.s.^{-1}$ The applied potentials ranged from −0.6 to 1 V. (Fig. S3) shows cyclic voltagerams of bare electrode in different scan rates. As displayed in (Fig. S3b and S4), the relationship between the cathodic peak current and the square root of the scan rate ($v^{1/2}$) is linear for bare and the modified

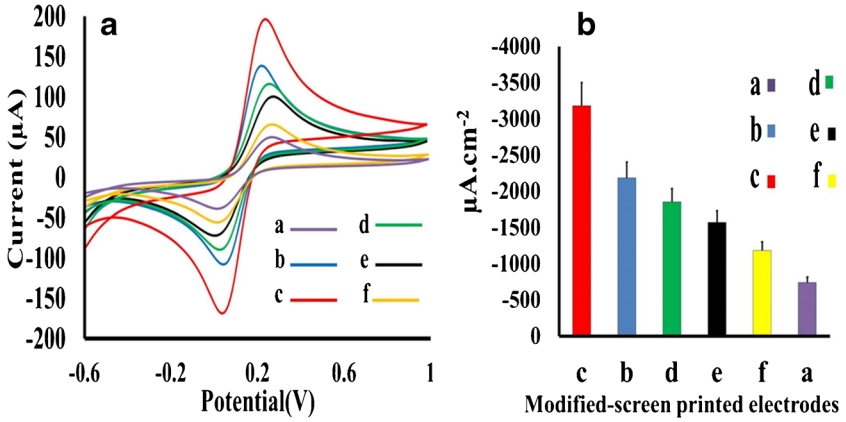

Fig. 3 **a** The cyclic voltammograms of bare electrode and different modified SPCEs. The cyclic voltammerty experiments performed in 1 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 1.0 M KCl, at a scan rate of 100 mV.s^{-1} for (a) Bare SPCE, (b) PANI/SPCE, (c) PANI/AuNP/SPCE, (d) PANI/AuNP/avidin/SPCE, (e) PANI/AuNP /avidin/capture probe/SPCE, and (f) PANI/AuNP /avidin/capture probe/hybridization solution/SPCE. **b** The current density (μA.cm^{-2}) of bare and modified-screen printed electrodes

electrodes. This confirms that a diffusional process has taken control in the reaction of $[Fe(CN)_6]^{3-/4-}$.

The active surface area of the bare SPCE calculated to be at 0.0078 cm^2, while it for PANI and PANI/AuNP-modified electrodes was obtained 0.036 cm^2 and 0.054 cm^2, respectively (Table. S4). The current value of the PANI/AuNP-modified electrode is greater than that on the PANI-modified electrode approximately 1.4 fold. The active surface area of the PANI/Au electrode is around 1.5 fold higher than that of the PANI-modified electrode, the trend of which is analogous with current. Therefore, the increase in the activity of the PANI/AuNP electrode relates to the increase in its surface roughness.

The detection mechanism is based on an enzymatic reaction. In enzyme-based biosensors, the efficiency depends on the modifications of surface electrode, type of enzyme, substrate, and the use of a mediator. Peroxidase enzymes are a class of enzymes that have a significant role in progressing the bioscience and biotechnology [28]. Among peroxidases, HRP has the most application in developing enzyme-based biosensor [28]. It is frequently used as secondary detection reagents. There are different substrates for HRP including TMB, OPD, hydroquinone, hydroxymethyl ferrocene, and osmium complex [28]. The TMB /H_2O_2 is by far the most used substrate and this undoubtedly represents the safest and most optimal choice. In this sensing system was used of CV method to investigate interaction HRP enzyme with TMB/H_2O_2. HRP electrochemically converts an inactive substrate to an electroactive product and generates electrochemical signal [19]. This means that, after dropping H_2O_2/TMB solution on the HRP-modified electrode, the HRP enzyme catalyzed the oxidation of TMB by H_2O_2 to form TMB^{2+}. The oxidized mediator is electrochemically reduced on the electrode, leading to generate a current signal. The intensity current directly related to the amount of the target DNA concentration in the sample [29].

Catalytic performance of the electrochemical genosensor

In order to evaluate the function of biosensing platform, different concentrations of target DNA (1000 to 0.001 pM) were prepared in the clean urine sample. Then, they were assayed by the double probe hybridization-based strategy, in which the biotinylated probe and digoxigenin-labeled probe were employed as the capture probe and the detector probe, respectively. The peak produced by reduction of the H_2O_2/TMB by enzymatic reaction was recorded as an electrochemical signal.

(Fig. S5a) indicates the CV peak of current density at the potential −0.1 V (vs. Ag/AgCl) that remarkably enhanced with the increase of DNA concentrations in the standard solutions. The logarithmic plot of the DNA concentration versus current was linear in the wide range from1000 pM to 0.001 pM of the target DNA (Fig. S5b). The currents of assaying different concentrations of DNA were measured in five replications, which were shown in (Table S5) with respecting RSD percentages. The presented a low RSD percentage, demonstrates the high functionality of the biosensor for detection DNA of bacteria.

The detection limit was calculated using the equation $Cm = 3sb1/m$, where $sb1$ is the standard deviation of 12 repeated CV of the blank response and m is the slope of the calibration curve. Since the standard deviation was obtained 66.4207 (sbl). Also, the slope of the calibration curve of target DNA (m) is 398.15. Therefore, the detection limit of this genosensor was exanimated to be 0.5 fM. [30]. This detection limit is considerably low and is very good for bacterial DNA recognition. A sensitivity of 398 μA. pM.$^{-1}$ Cm.$^{-2}$ was achieved for the genosensor from the slope of the current density /DNA concentration calibration curve.

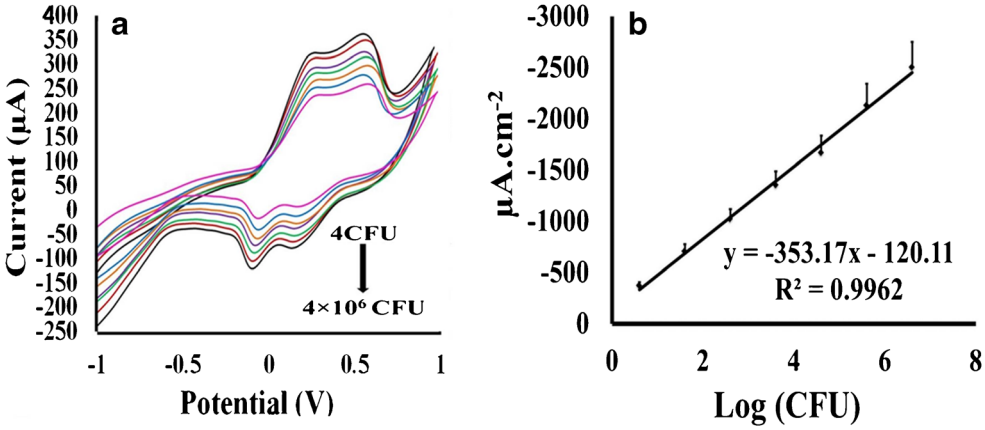

Fig. 4 **a** Cyclic voltammetry voltammograms for the electrochemical direct detection of *E.coli* upon serial dilutions of this bacteria between 4×10^6 to 4 CFU in potential range (1 to −1 V) at scan rate 50 $mV.s^{-1}$. The main electrochemical signal was created, in potential −0.1 V (vs. Ag/AgCl) **b** Calibration curve of bacterial target. Each data point is the average of five replicates

In 1997, Wang et al. reported the first electrochemical biosensor for detecting *E. coli* gene sequence [31]. This genosensor was fabricated on a screen-printed carbon electrode surface. They using Chronopotentiometric method achieved the detection limit 50 ng. mL^{-1} [31].Chen et al. have also designed a sandwich-type of DNA biosensor for sensing the specific IS6110 DNA fragment of *Mycobacterium tuberculosis* for the diagnosis of Tuberculosis. They used the PANI-reduced graphene oxide (rGO)/AuNP composite as a redox detector probe for the generation of the voltammetric signal. This genosensor showed a linear range of 0.1 pM - 10 nM and a limit of detection of 50 f. [8]. In 2015, Li and et al. constructed a sensitive DNA biosensor by employing double hybridization assay for identification *lacZ* gene sequence of *E. coli*. The biosensor demonstrated a detection limit of ~30 f. [31]. Also, Zhang and et al. By employing graphene oxide (GO)/chitosan (CS) hybrid nanocomposites fabricated a sensitive electrochemical DNA biosensor on the glassy carbon electrode surface for detection of *E.coli* O157:H7. Under the optimal hybridization conditions, electrochemical impedance spectroscopy (EIS) responses of biosensor were linear in the concentration range from 1×10^{-14} to 1×10^{-8} M. They reported the detection limit of 3.584×10^{-15} M [32].

Specificity and stability of genosensor

In order to assay the storage stability of the biosensing platform, the electrochemical performance of the stored PANI/AuNp/avidin/capture probe modified SPCEs was compared with the freshly-prepared ones over a 10-week period. The results show (Fig. S7) that the electrochemical response of biosensor absolutely preserved up to eight weeks. Later, the decrease in signal intensity of about 26% was observed. This system is highly stable, which is an important factor in commercialization of a product. Consequently, it can be concluded that DNA biosensor has a great potential for commercialization.

The *E.coli* cell detection in spiked and real samples

In the next step, the designed electrochemical DNA biosensor was used for direct detection of *E.coli* cells in the urine samples. In these experiments of bacterial lysis was used as target. Therefore, samples with different numbers of bacterial cells (10^8 to 10^2 cells) were prepared in clean urine to simulate real contamination. The dose-response curves obtained from the genosensor were presented in (Fig. 4a). It can be seen the current density at the potential −1 V (vs. Ag/AgCl) significantly enhanced with the increase in the number of bacterial cells in the standard solution. In addition, the plotted calibration curve (Fig. 4b) exhibited a good linear relationship between the reduction peak currents and the number of bacterial cells in the range of 10^8 to10^2 CFU. Given that only 4 μL of the 130 μL lysate-probe mixture (50 μL of phosphate buffer containing detector probe and, 30 μL of NaOH and 50 μL of urine), or 1/32 of the total, was applied to the sensor surface, i.e. the ability to detect as few as 4 CFU.

(Table S6) exhibits the acquired RSD value in evaluating the samples with various numbers of bacterial cells in five replications. The low RSD value proves the obvious potential

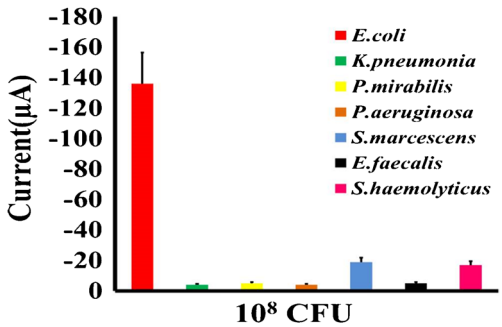

Fig. 5 The evolution the specificity performance of the electrochemical genosensor for direct detection *E.coli*. The current diagram obtained of direct recognition *E.coli* and five the prevalent uropathogenic bacteria with10^8 CFU in potential range (1 to −1 V) at scan rate 50 $mV.s^{-1}$. The electrochemical signal was generated, in potential −0.1 V (vs. Ag/AgCl)

Table 1 The comparison of the presented electrochemical genosensor with other published electrochemical biosensors for the detection of *E.coli*

Method	Brief biosensing strategy	Linear rang of detection CFU.mL^{-1}	Limit of detection CFU.mL^{-1}	Time of detection	Reference
Amperometric	The bienzyme biosensor based on the covalent immobilization of laccase and horseradish peroxidase (HRP)	1.6×10^3 to 1.0×10^7	9.7×10^2	3 h	[2]
Amperometric	Based on Fe2O3@Au core/shell nanoparticle, sandwich detection strategy and HRP enzyme	500 to 5×10^5	5	4.0 h	[3]
Chronoamperometric	Using specific antibodies labeled with (AuNPs) and magnetic beads (MBs) capture platforms in liquids suspensions	10^2 to 10^5	457 in beef and 309 in water	70–80 min	[33]
Differential pulse voltammetric (DPV)	based on target-induced aptamer displacement and signal amplification of streptavidin alkaline phosphatase(ST-AP) enzyme	2×10^2 to 2×10^7	112 in phosphate buffer saline and 305 in milk	3.5 h	[34]
(DPV)	dual signal amplification by both the sandwich hybridization structure(two biotinylated detection probes) and the ST-AP enzyme	10 to10^7	10	3.5 h	[35]
(CV)	based on a dual signal amplified strategy using PANI / AuNP composite as a biosensor platform and HRP enzyme as a label	4×10^6 to 4	4	~70 min	This work

of this genosensor for direct detection *E.coli*. The sensitivity was obtained 353 μA. CFU^{-1}. Cm.$^{-2}$

To assay the specificity of this method for *E.coli* detection, the PANI/AuNp/avidin/capture probe modified SPCEs were prepared under the similar condition. Then, bacterial lysis containing 10^8 CFU of five prevalent uropathogenic bacteria including *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis, Staphylococcus haemolyticus* and, *Pseudomonas aeruginosa* were used as target. The next steps of the test were performed as mentioned in the experimental section. No remarkable CV response was obtained from the other prevalent uropathogenic bacteria (Fig. 5). Therefore, these results confirmed that biosensor has high specificity for *E.coli*.

The mean reproducibility intra-assay CV (coefficient of variation) for 10^6 and 10^3 CFU was 3.2% and 6.3%, respectively, and the inter-assay reproducibility was 2.2% and 4.3%, respectively.

In addition, in order to investigate the applicability of our geosensor for assessing *E.coli* bacteria in real samples, clinical urine samples from 30 diagnosed *E.coli* related-UTI patients were tested. The electrochemical signals presented in (Table S7) indicate the higher numbers than 10^5 CFU of *E.coli* in urine samples of UTI patients, which is in accordance with findings of previous studies.

(Table 1) exhibits a comparative study on *E.coli* determination by different research groups, including the present work. In comparison with previous electrochemical biosensors for determining *E.coli*, the presented genosensor provide a wider linear range and lower limit of detection and shortened analysis time. According to the significant performance of this biosensor in urine as a biological sample, and without need for DNA extraction, pretreatment, or amplification. It can be concluded that the designed sensing platform can serve as an appropriate candidate for direct, sensitive and rapid quantification of *E.coli* in different samples such as urine, water or food.

Conclusion

An ultrasensitive sensing platform is fabricated based on PANI film and AuNPs for direct and pretreatment-free determination of *E. coli*. Utilizing the combination of nanomaterial-polymer, along with a specific enzyme-based detection strategy, this genosensor can be successfully used to determination as low as 4 CFU of *E.coli* cells. The genosensor has great potential for rapid identification of *E. coli* in urine and water samples. Further, by employing design of specific probes, this bio-sensing system can be used for fast and accurate screening of various pathogens. The main limitation of using the present electrochemical biosensor is need of an analysis device for obtaining the final response. Therefore, the design and construction of a portable read-out device can greatly increase the application of this biosensor.

Acknowledgements This research was supported by a grant from Tarbiat Modares University.

Compliance with ethical standards The author(s) declare that they have no competing interests.

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