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Thermoplastic Electrodes for Detection of *Escherichia coli*

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A new amine functionalized electrochemical immunosensor for label-free detection of *Escherichia coli* using modified thermoplastic electrodes is reported for the first time. The *E. coli*-specific antibody was immobilized on diazonium-modified TPEs. The modification process was monitored using cyclic voltammetry and electrochemical impedance spectroscopy using the hexacyanoferrate redox couple as the electrochemical probe. The linear range was from 1×10^3 to 1×10^5 CFU ml^{-1} with a linear correlation of 0.9823 and limit of detection of 27 CFU ml^{-1} for *E. coli* (DH5α strain) in phosphate buffered saline solution as a model system. The specificity of the proposed immunosensor was demonstrated by showing selective detection in the presence of different bacteria. The biosensor was successfully applied to urine samples spiked with 0.5×10^5 CFU ml^{-1} and 1×10^5 CFU ml^{-1} *E. coli* and obtained good recoveries 99% and 110%, respectively. The proposed system should be well suited for selective and sensitive detection of different pathogenic bacteria.
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Escherichia coli, a gram-negative coliform bacteria, is present naturally in the intestines of humans and animals.[1] Although most of the *E. coli* strains are harmless to humans, the consumption of pathogenic *E. coli* strains in contaminated food or water may cause cholecystitis, cholangitis, urinary tract infection, diarrhea, kidney failure, neonatal meningitis, Crohn's disease and can result in death.[2–6] Foodborne diarrheal diseases account for the deaths of 2.2 million people every year according to World Health Organization (WHO).[7] The WHO also estimates that 1.3 million children lose their lives annually because of the waterborne diarrheal diseases.[8] In addition to health problems, pathogenic *E. coli* infections lead to huge economic loss, it has been estimated by the US Centers for Disease Control that foodborne pathogens causes a total cost of US$ 78 billion each year.[9] Food and water regulatory agencies around the world have made significant efforts toward controlling food-borne infection to decrease the risks of illnesses through the contamination of food and water, including implementing good agricultural practices, good manufacturing practices, and hazard analysis and critical control point programs.[10] For instance, microbiological standard limits for water and food safety have been established by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA).[11] Therefore, the fast, sensitive and specific detection of *E. coli* is critical for clinical diagnosis as well as ensuring food and drinking water safety and protecting public health, but is currently challenging to implement.

E. coli can be detected by traditional culture-based techniques which are simple and cost-efficient. However, bacterial culture can take 2–3 d to provide results and on-site detection is not possible using this technique given the need for incubators and trained staff.[12] Techniques such as polymerase chain reaction-based methods,[13,14] DNA microarrays,[15,16] DNA sequencing,[17] enzyme-linked immunosorbent assay,[18] immunochromatographic lateral flow assay,[19] quartz crystal microbalance systems,[20] surface plasmon resonance,[21] flow cytometry[22] and chemiluminescence[23] have been employed for detecting *E. coli* contamination in food and water. However, these methods have several disadvantages in that they either require pretreatment steps, large amounts of sample, expensive instruments and reagents, trained personnel to operate the instrument, or false positives due to cross reactivity.[24] In this respect, electrochemical biosensors are quite promising among current analytical methods for detection of *E. coli* due to significant advantages including portability, fast response, ease of miniaturization and low-cost.[25–27] Moreover, it is possible to integrate electrochemical biosensors into an automated flow device to perform complex assays.[26,28,29] Among electrochemical biosensors, impedimetric biosensors measure the electron transfer at the electrode interface in the form of an impedance response. Currently, electrochemical impedance spectroscopy (EIS) is widely used with impedimetric biosensors due to its high sensitivity and ability to perform label-free detection. Antibody-based immunosensors have been the most investigated pathogen detection platform compared to other affinity-based assays including recombinant bacteriophage or lectin-based biosensors.[30–32]

Carbon electrodes have been widely used in electrochemical biosensors due to their low cost, chemical inertness, biocompatibility, and ease of fabrication.[33–35] In addition, carbon composite electrodes composed of conductive carbon and a polymer binder can be easily patterned using screen- and stencil-printing.[36] However, most composite carbon electrodes provide lower quality electrochemistry than solid electrodes such as glassy carbon and boron-doped diamond. Thermoplastic electrodes (TPEs) are a newer carbon composite type that have high conductivity, good electron transfer kinetics, reusability, and low cost.[37] In previous reports, the effects of polymer type, carbon type, and the carbon-to-plastic ratio, and the graphite types on TPE performance have been investigated.[38,39] Also, TPEs have been successfully modified using aryl diazonium salts.[40]

Here, we report a new diazonium-based label-free impedimetric biosensor for sensitive and specific detection of *E. coli* bacteria using the DH5α strain as a safe model for pathogenic *E. coli*. Using the unique properties of TPEs, the biosensor was built in a fingertight fitting to enable incorporation into a variety of flow cells. The interaction between the bioreceptor and bacteria was characterized using EIS in the presence of $Fe(CN)_6^{3-/4-}$ as the probe.

Experimental

Reagents and materials.—All reagents were of analytical grade and used as received without further purification. 1-ethyl-3-(3-

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dimethylaminopropyl)-carbodiimide (EDC), MES hydrate (2-morpholino ethanesulfonic acid hydrate), potassium ferricyanide (III), potassium hexacyanoferrate (II) trihydrate, and p-toluenesulfonic acid monohydrate were purchased from Sigma (St.Louis, MO, USA). N-hydroxysuccinimide (NHS) was purchased from Fluka (Buchs, Switzerland). Phosphate buffered saline packs (PBS) was purchased from Thermo Scientific (U.S.A). Bovine serum albumin (BSA) was purchased from MP Biomedicals (CA, USA). Sodium nitrite, analytical grade isopropanol, methylene chloride, and sulfuric acid (H_2SO_4), were purchased from Fisher Scientific (U.S.A). 4-Nitroaniline was purchased from Chem-Impex International (IL, U.S.A). Polyclonal anti-*E. coli* antibody—Azide free (ab48416) antibody was obtained from Abcam (USA). Urine sample (pH 6.8) was purchased from Lee BioSolutions (Missouri, U.S.A). Conductive silver paint was obtained from SPI Supplies (Pennsylvania, U.S.A.). Copper wire, epoxy glue, and sand paper (600- and 1200-grit) were purchased from local stores. A 0.1 M PBS containing 14 mM KH_2PO_4, 87 mM Na_2HPO_4, 2.7 mM KCl, and 137 mM NaCl was used as a washing buffer at pH 7.4. A $[Fe(CN)_6]^{3-/4-}$ solution containing 2.5 mM $K_3Fe(CN)_6$, 2.5 mM $K_4Fe(CN)_6$, and 0.1 M PBS (the supporting electrolyte) was used as the redox probe. All aqueous solutions were prepared with double distilled water using a Milli-Q water system ($\rho \geqslant 18.2$ MΩ·cm).

Electrode fabrication.—The TPE was fabricated according to the procedure reported previously.[37,38,40] Poly(methyl methacrylate) (PMMA) (1/8 inch thick sheet) and polycaprolactone (PCL, ThermoMorph) are used as the TPE binders. First, small centimeter-sized PMMA pieces (Optix, Plaskolite, U.S.A) were massed and placed in a vial, then mixed with methylene chloride in a ratio of ~10 ml solvent to 1 g of PCL and 0.5 g PMMA. Then, 3 g of MG1599 graphite (NovoCarbon, U.S.A) were combined in this mixture and solvent was allowed to evaporate overnight. The dried carbon composite was heat pressed using hydraulic laboratory press (Carver Inc., U.S.A, $\boldsymbol{T} > \boldsymbol{T_g}$ 121 °C and $\boldsymbol{P} \approx 50$ psi). It was then possible to mold the electrode material into fingertight fittings with 4 mm diameter (Cole Parmer, U.S.A). A copper wire (18 gauge, Cbazy) was attached to one side of electrode using conductive silver paint and sealed with epoxy glue. The fabricated TPEs were freshly sanded and polished with 600 and 1200 grit paper before testing. The fabrication of TPEs is shown Scheme 1.

Immunosensor construction.—A one-step synthesis and grafting method was applied according to the previously described procedure.[41] Firstly, electrodes were pretreated in 0.1 M H_2SO_4 at –1.5 V vs Ag/AgCl reference electrode for 30 s. Then, 0.2 mmol 4-nitroaniline was added in a mixture containing 0.6 mmol p-toluenesulfonic acid and 75 μl water. After 0.5 mmol $NaNO_2$ was added, this mixture was ground for 5 min. The reaction paste was incubated on TPEs for 5 min at room temperature. Electrodes were rinsed with water and 0.1 M H_2SO_4, and sonicated in isopropanol for 10 min. Finally, grafted nitro groups were reduced electrochemically in 0.1 M KNO_3 at –1 V for 30 s.

To prepare the sensor, anti-*E. coli* antibody was covalently linked on the electrode surface using EDC/NHS as crosslinking agents via amide bonds formed between the amine groups functionalized on the TPE and the carboxylic groups present on the antibody.[42] A crosslinking ratio of 4:1 EDC and NHS in a 100 mM MES buffer at pH 5.5 was used to activate the carboxylic groups of antibodies for 30 min. Next, 25 μg ml^{-1} activated *anti-E. coli* was immobilized on electrode surface at room temperature for 1 h and rinsed with PBS to remove unbounded antibody. After this step, the electrode was incubated with 10 mg ml^{-1} BSA in PBS for 1 h to block non-specific binding sites. Finally, the biosensor was rinsed with PBS to remove excess BSA. The developed electrode is presented in Scheme 2.

Culture of bacterial cells.—*E. coli* (DH5α strain -Thermofisher Scientific) and *Lactobacillus acidophilus* (Moro Strain—ATCC 4356) were cultivated in Luria broth (Gibco) or Lactobacillius MRS Broth (ATCC), respectively, by shaking at 170 rpm for 16 h at 37 °C. Bacterial cultures were centrifuged at 4000 rpm for 5 min (25 °C) and washed with PBS (0.1 M, pH 7.4) three times. The cell pellet was suspended in 1 ml of PBS and used as the bacterial stock solution. To determine *E. coli* and *L. acidophilus* concentration, the number of colony-forming units (CFU) of bacteria concentration in the stock solution was determined by the standard plate count method using Luria broth agar (Gibco) or Lactobacillius MRS Broth agar (ATCC) respectively.[43] *Lactobacillus acidophilus* was used as non-target bacteria and stored at –80 °C with 10% dimethyl sulfoxide.

Electrochemical measurements.—Conductivity measurements of fabricated TPEs were carried out using a two-point probe (Fluke 187 multimeter, accuracy of 0.01 Ω) placed on opposing faces of the finger tight fitting TPEs. A CHI 660 potentiostat (Shanghai CH Instruments Co., China) was used to investigate the electrochemical properties of the electrodes using cyclic voltammetry (CV) and EIS. The impedance spectra were recorded within a frequency range of 10^1–10^5 Hz using a perturbation amplitude of 0.2 V. The values of charge-transfer resistance (R_{ct}) were obtained by fitting the Nyquist plots to an equivalent circuit. A scan rate of 100 mV s^{-1} was applied over a –0.8 V +0.8 V range. The electrochemical cell included a modified TPE (4 mm inner diameter) as the working electrode, a 1:2 PCL:carbon TPE as the counter electrode, and silver/silver chloride (Ag/AgCl) as the reference electrode. Electrochemical measurements were carried out using $K_3Fe(CN)_6/K_4Fe(CN)_6$ (5 mM, 1:1) containing sterile 0.1 M PBS (pH 7.4). All experiments were carried out at room temperature. The electron diffusion coefficient was calculated using the Randles-Sêvcîk equation (Eq. 1):[44]

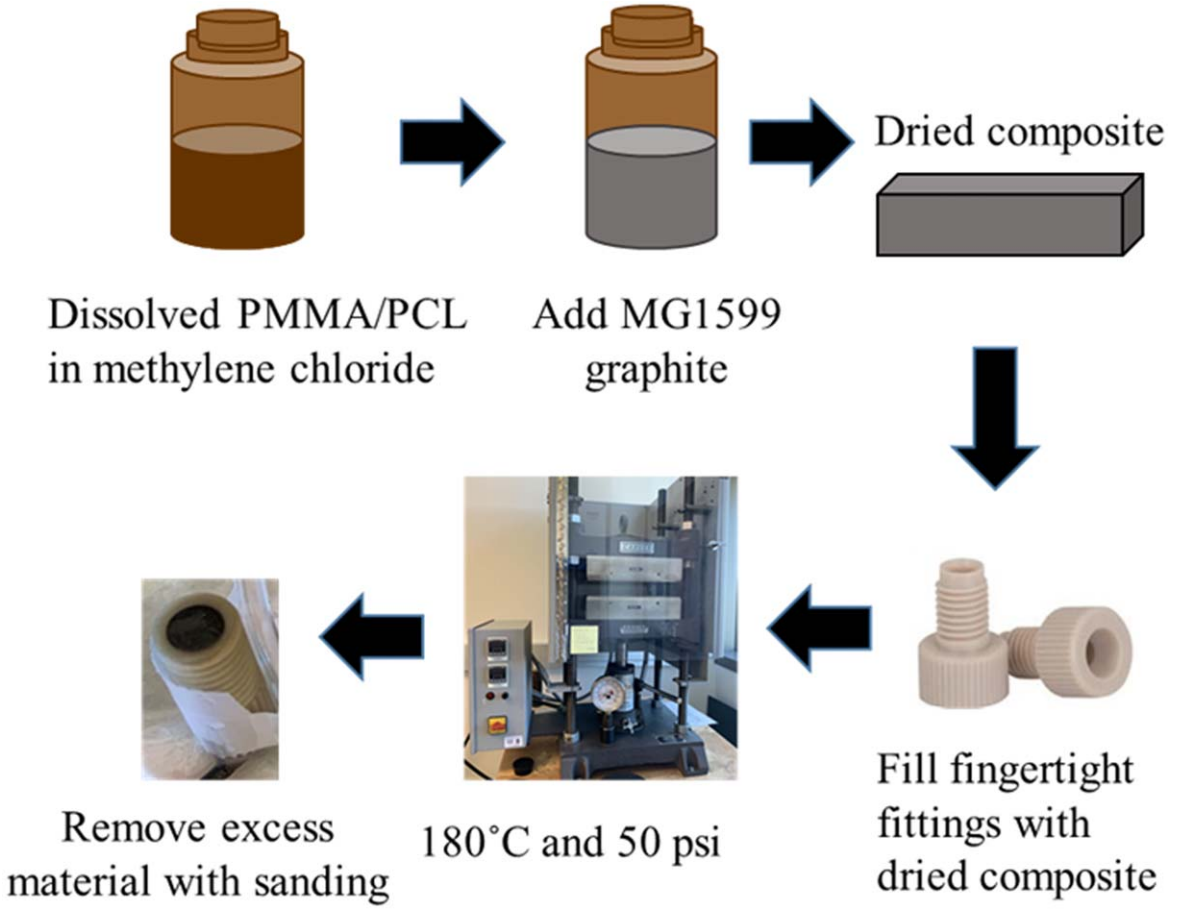
$$\boldsymbol{i_p} = 2.69 \times 10^5 \boldsymbol{A D^{1/2} n^{3/2} v^{1/2} C} \qquad [1]$$

where i_p is the peak current (A); n is the number of electrons transferred in the redox event; A is the electroactive area of the working electrode (cm^2); D is the diffusion coefficient (cm^2 s^{-1}), which for ferricyanide is estimated to be 2.2×10^{-6} cm^2 s^{-1};[45] C is the concentration of ferricyanide (mol cm^{-3}); and v is the scan rate (V s^{-1}). The sample is exposed to the circular electrolyte with surface area 0.74 cm^2.

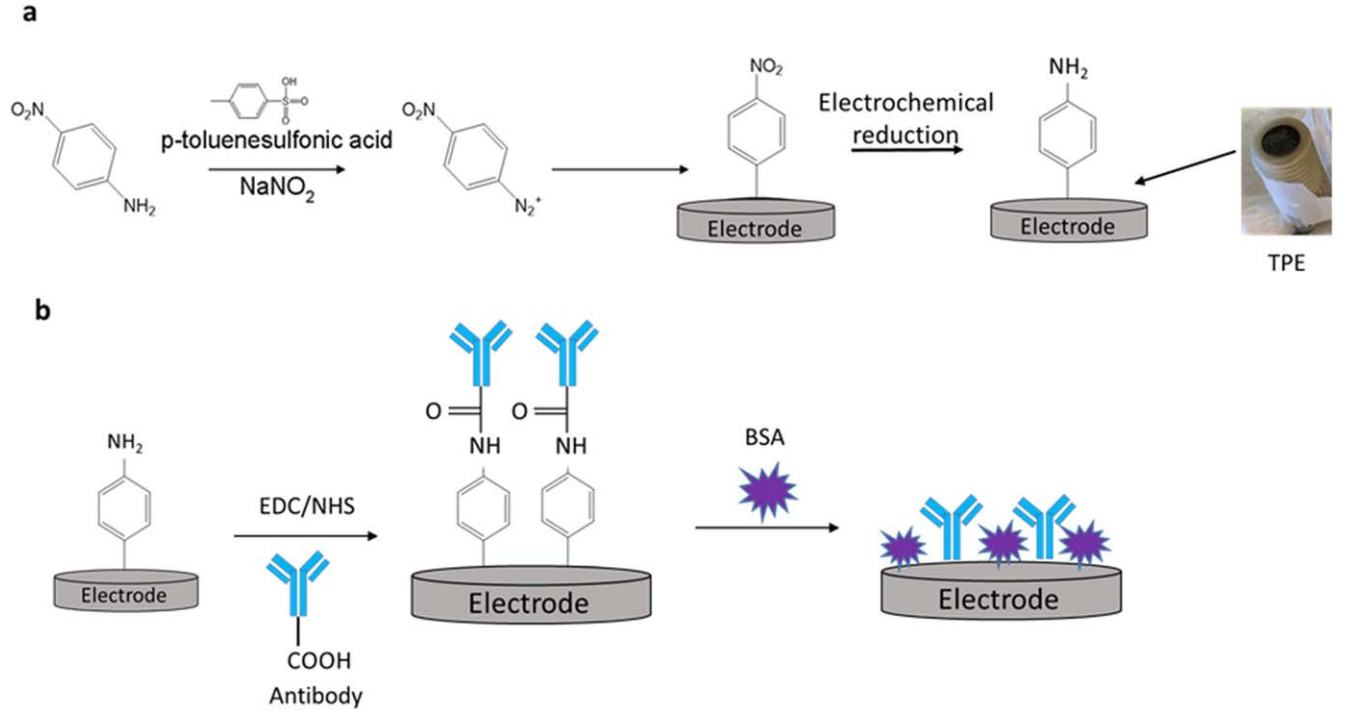
Bacterial cell immobilization was achieved by incubation of PBS solution containing different bacteria concentrations or spiked urine (50 μl) containing *E. coli* on anti-*E. coli* antibody functionalized electrodes for 30 min. Then, the immunosensors were rinsed with PBS to remove excess cells and air-dried. Finally, the electrochemical response to the 5 mM $[Fe(CN)_6]^{3-/4-}$ probe in 0.1 M PBS was recorded using EIS. Increasing concentrations of *E. coli* DH5α from 10^1 to 10^8 CFU ml^{-1} were incubated with the immunosensor surface for 30 min to attain a calibration curve.

Results and Discussion

Electrochemical characterization of the immunosensor.—Prior to antibody immobilization, the presence of the diazonium functional group on the electrode surface was confirmed in 100 mM H_2SO_4 by CV with electrochemical parameters +0.8 V to –0.8 V and back with 100 mV s^{-1} scan rate (Fig. 1). Since antibody conjugation on the electrode surface is a critical step in immunosensor construction, a wide range of antibody concentrations were evaluated. The immunosensors were incubated with varying concentrations of antibody for 1 h and tested by CV. A significant decrease in current was observed with increasing anti-*E. coli* antibody concentration as shown in Fig. 2A. After 25 μg ml^{-1} of anti-*E. coli* antibody was incubated on the electrode surface, the anodic current at 0.3 V decreased from 85 μA (at 10 μg ml^{-1} antibody) to 75 μA. Then, the same concentration of *E. coli* (10^4 CFU ml^{-1}) was incubated on the electrode surface for 30 min and immunosensors were tested by EIS. The percentage change in R_{ct} values of the



Scheme 1. Schematic display of the electrode fabrication process.



Scheme 2. Diagram for the immunosensor construction: (a) NH_2-modified TPE, (b) surface activation, antibody binding and blocking step.

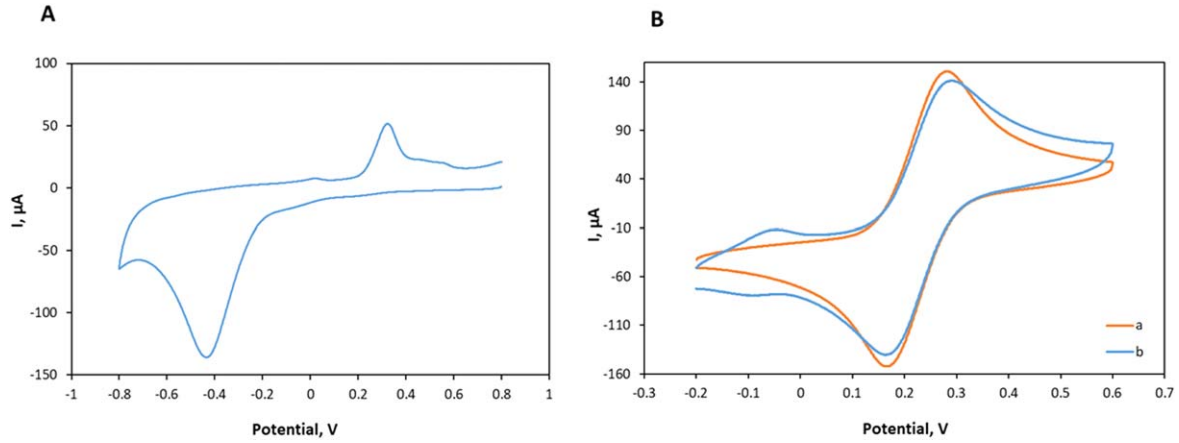


Figure 1. (A) CV response of modified TPE (4 mm inner diameter) with diazonium salt in 100 mM H_2SO_4. (B) CV response of (a) bare TPE, (b) modified TPE with diazonium salt in 5 mM $[Fe(CN)_6]^{3-/4-}$ (scan rate of CV: 100 mV s^{-1}).

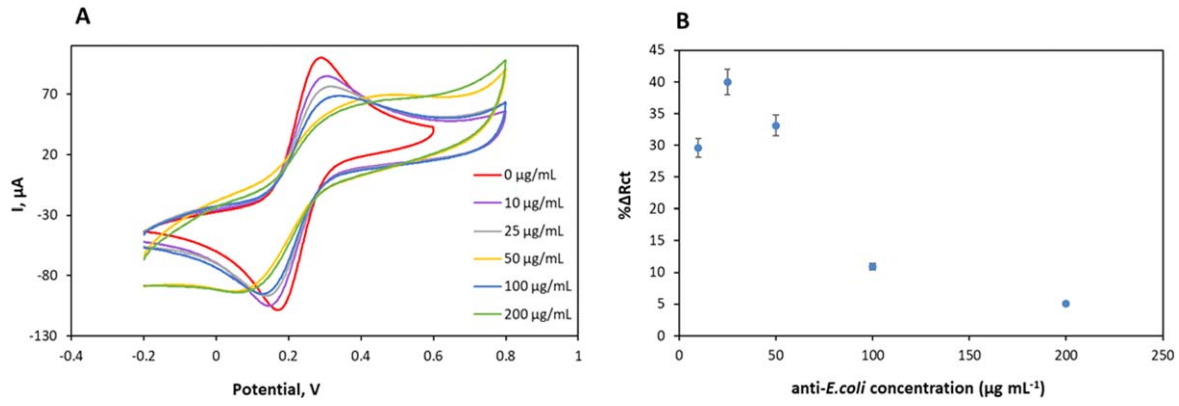


Figure 2. (A) CV of the modified electrode with different concentrations of anti-*E. coli* DH5α polyclonal antibody in 0.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ from 10 μg ml^{-1} to 200 μg ml^{-1} (scan rate of CV: 100 mV s^{-1}). (B) Optimization of antibody concentration anti-*E. coli* antibody using the EIS technique in PBS containing 5 mM $[Fe(CN)_6]^{3-/4-}$ (within a frequency range of 10^1–10^5 Hz using a perturbation amplitude of 0.2 V) (n = 3).

immunosensors is shown in Fig. 2B. The changes in R_{ct} were calculated from Nyquist plots according to a Randles equivalent circuit using the electrochemical circle fit analysis software from potentiostat. The change in R_{ct} (ΔR_{ct}) was then normalized using the following equation to account for variations in the raw R_{ct} values from electrode to electrode (Eq. 2):

$$\Delta R_{ct}(\%) = \frac{R_{ct}\,(\text{bacteria, X concentration}) - R_{ct}(\text{blank})}{R_{ct}(\text{blank})} \times 100 \quad [2]$$

The R_{ct} change initially increased with increasing antibody concentration but then decreased at higher concentrations. According to Holford et al., a high concentration of antibody may limit bioreceptor binding on the electrode surface resulting in decreased biosensor sensitivity due to steric hindrance.[46] Thus, 25 μg ml^{-1} of antibody was selected as the best concentration in the construction of the biosensor.

CV and EIS were used to monitor the biosensor modification steps. As can be seen in Fig. 3A, CV signal decreased in the presence of $K_3[Fe(CN)_6]$ after anti-*E. coli* antibody was immobilized on the electrode surface, indicating a reduction in electron transfer between electrode and the solution (curve c). Similarly, the redox peak current of curve b further decreased with incubation of BSA, showing BSA was captured on the electrode surface (curve d). The Nyquist plots obtained from a single electrode were recorded to analyze the impedance measurements of a (a) TPE-NH_2, (b) antibody/TPE-NH_2 and (c) BSA/antibody/TPE-NH_2 modified electrode using a 5 mM $[Fe(CN)_6]^{3-/4-}$ couple (1:1) solution in PBS (pH 7.4) with representative data shown in Fig. 3B. The diameter of the semicircle (R_{ct}) increased in a stepwise fashion from bare electrode (curve a) to modification with the diazonium salt (curve b), antibody (curve c), and blocker (curve d) on the electrode surface. Thus, the construction of the immunosensor was confirmed by the increase in the electron-transfer resistance.

The response of different concentrations of E. coli and reproducibility.—Cumulative and singular incubations of *E. coli* were applied to investigate the immunosensor's response to different concentrations of analyte in this study. For cumulative incubation, the immunosensors were exposed to increasing concentrations of *E. coli* in PBS starting from the lowest concentration (10 CFU ml^{-1}) for 30 min. The biosensors were washed with PBS and EIS was recorded at concentrations of 10 CFU ml^{-1} to 10^8 CFU ml^{-1} (Fig. 4A). It was observed that impedance increased significantly with increasing bacteria concentration. A calibration curve using the average of 3 replicate electrodes is shown in Fig. 4B (line a) and gives a linear regression equation of $\%\Delta R_{ct} = 4.63 \log_{10}[E.\ coli] + 21.63$ (CFU ml^{-1}) with a correlation coefficient of 0.9715. For a singular incubation, immunosensors were incubated with a single *E. coli* concentration for 30 min. The results show that $\%\Delta R_{ct}$ values increased linearly as a function of the logarithmic bacteria concentration from 1×10^3 to 1×10^5 CFU ml^{-1} (Fig. 4B, line b). Since cumulative incubation has a drawback that some *E. coli* are already present on the electrode surface before successive incubation of

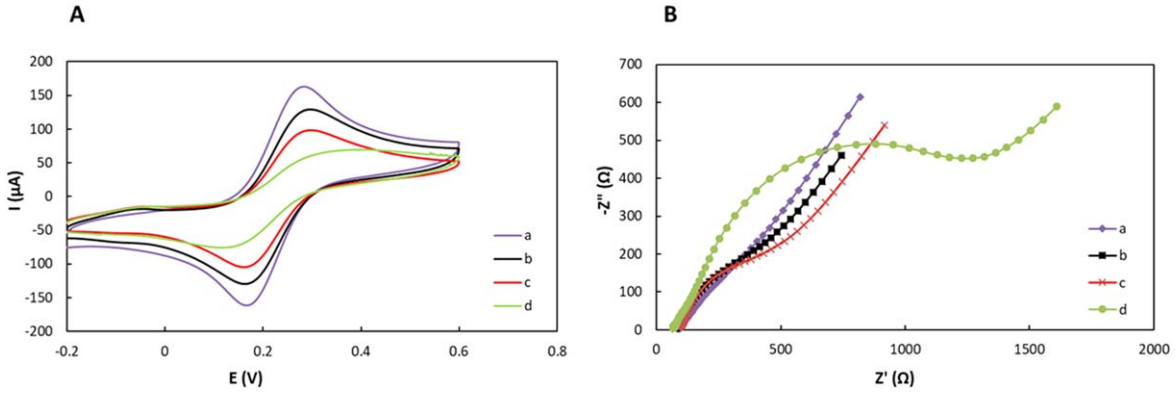


Figure 3. (A) CV studies of (a) bare TPE, (b) NH_2-TPE, (c) antibody/NH_2-TPE, (d) BSA/antibody/NH_2-TPE in PBS containing 5 mM $[Fe(CN)_6]^{3-/4-}$ (scan rate of CV: 100 mV s^{-1}) (B) EIS studies of (a) bare TPE, (b) NH_2-TPE, (c) antibody/NH_2-TPE, (d) BSA/antibody/ NH_2-TPE in PBS containing 5 mM $[Fe(CN)_6]^{3-/4-}$.

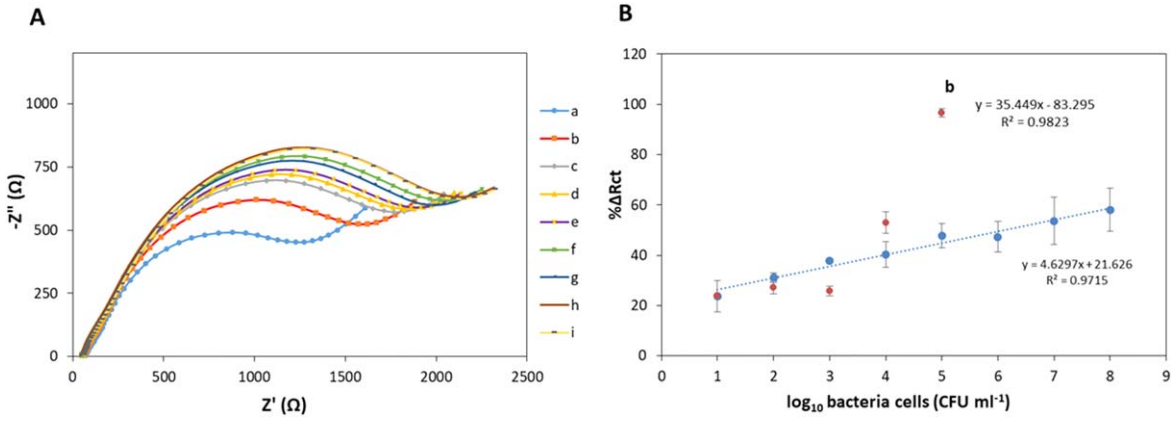


Figure 4. (A) Nyquist plots after incubation of the immunosensor with different *E. coli* DH5α concentrations in the presence of 5 mM $Fe(CN)_6^{3-/4-}$ containing PBS. (a) Without *E. coli* (b–i) represent different concentrations of *E. coli* (8.6–8.6 $\times$ 10^7 CFU ml^{-1} *E. coli* with 10 fold increase, respectively). (B) (a) Calibration curve for $\%\Delta R_{ct}$ with logarithm *E. coli* DH5α concentrations for cumulative incubation. (b) Calibration curve for $\%\Delta R_{ct}$ with logarithm *E. coli* concentrations for singular incubation. (n = 3).

analyte with higher concentration, singular incubation is preferred compared to cumulative incubation for real-time detection of *E. coli*.

Next, reproducibility of the immunosensor was investigated using multiple electrodes under optimized conditions. While the relative standard deviation (RSD) of the five electrodes made independently for 10^3 CFU ml^{-1} was found as 3.9%, RSD of 3 replicate measurements were 11% and 5.2% for cumulative incubation and singular incubation, respectively for EIS measurements. These results showed good reproducibility for the immunosensor. The limit of detection (LOD) was calculated to be 27 CFU ml^{-1} using the equation $yb+3xStd_b$, where yb is the charge transfer resistance value for the blank and Std_b is the standard deviation. Compared with previous methods (Table I), the high sensitivity and wide linear range of the biosensor can be useful in a range of applications. Thus, the proposed EIS based biosensor offers highly sensitive detection platform for bacteria.

Specificity test and human urine sample analysis.— *Lactobacillus acidophilus* was used to determine the specificity of anti-*E. coli* antibody to investigate the specificity of the proposed sensor. Mixtures of *L. acidophilus* and *E. coli* at a concentration of 10^3 CFU ml^{-1} and 10^4 CFU ml^{-1}, *E. coli* or *L. acidophilus* only at the concentration of 10^3 CFU ml^{-1} and 10^4 CFU ml^{-1}, were performed in parallel, respectively. As shown in Fig. 5, the results demonstrated that $\%\Delta R_{ct}$ value of the mixture of *L. acidophilus* and *E. coli* was close to the $\%\Delta R_{ct}$ value of only *E. coli*. It was shown that the presence of the bacteria apart from *E. coli* did not have any significant impact on the response with the increase of *L. acidophilus* and *E. coli* concentrations. According to the following equation, the degree of interference (DI) value for the interfering bacteria was calculated as

$$DI = \frac{(A - C)}{(B - C)} \times 100 \quad [3]$$

where A, B and C are the responses from (A) *L. acidophilus* mixed with *E. coli*, (B) *E. coli* and (C) the blank, respectively.[51] Thus, the presence of *L. acidophilus* revealed signal increases of 1.06% and 0.99% for 10^3 and 10^4 CFU ml^{-1} mixed solutions, respectively, demonstrating that the immunosensor is specific to *E. coli* detection.

Since sensitivity and specificity of the biosensor in a complex biological sample are critical, urine samples spiked with different concentration of *E. coli* (1×10^5 CFU ml^{-1} and 0.5×10^5 CFU ml^{-1}) were used to validate our biosensor. All immunosensors were incubated with non-spiked urine to obtain baseline impedance. Then, urine samples spiked with *E. coli* were incubated with the corresponding biosensors for 30 min. The calibration curve obtained from ΔRct (%) values (Figs. 4B–4b) were used to calculate the concentration of recovered bacteria from the sample. RSD of 4.1% and 10.9% was obtained from spiked concentration of 1×10^5 CFU ml^{-1} and 0.5×10^5 CFU ml^{-1} *E. coli* in three replicates (Fig. 5) with recoveries 99% and 110%, respectively. Thus, proposed biosensors can be applied to analyze real samples due to their high recovery.

Table I. Electrochemical biosensors for the label-free detection of *E. coli*.

Method	Bacteria	Immobilization on electrodes	LODs (CFU ml^{-1})	Assay time	References
Impedimetric/conductometric	*E. coli* K12	rGOP/AuNPs/Abs	1.5×10^2 in buffer	30 min	47
		SPCE/graphene/AuNPs/Abs	1.5×10^3 in buffer	Not stated	48
		ITO electrode/epoxysilane/Abs	1 in buffer	45 min	49
Potentiometric	*E. coli* surrogate CECT 675	GCE/SWCNT/aptamers	6 in milk, 26 in apple juice,	Real-time	50
Capacitive		Quartz crystal Au electrodes/SAM of MPA/Abs	10^2 in buffer, 10^3 in food samples	1 h	17
Impedimetric	*E. coli* DH5α	TPE/NH_2/Abs	27 in buffer	Real-time	This work

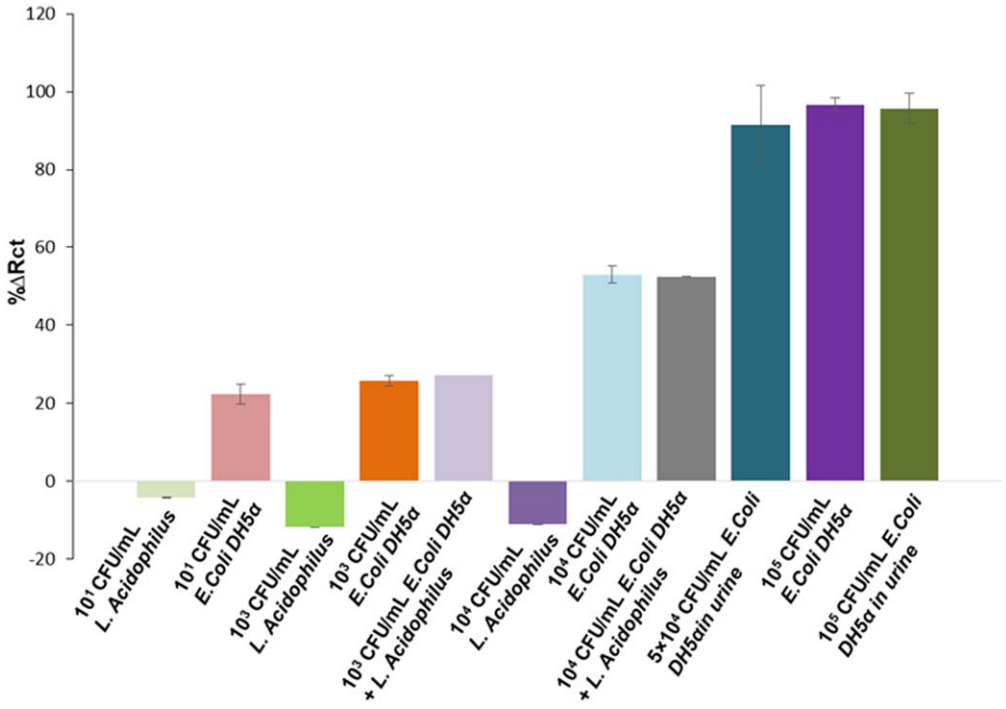


Figure 5. Bar chart depicting selectivity of anti-*E. coli*/NH_2/TPE biosensor for *E. coli* in the mixture of *E. coli* + *L. acidophilus* (n = 3).

Conclusions

Here, we report a novel label-free electrochemical biosensor to detect *E. coli* at low concentrations using diazonium-modified TPEs built into finger tight fittings. The immunosensor exhibits a wide linear range between 1×10^3 and 1×10^5 CFU ml^{-1} with a LOD of 27 CFU ml^{-1}, which is within a clinically relevant range,[49,52,53] providing detection on real samples without the need for sample pre-treatment or concentration steps. The biosensor is capable of selectively detecting single *E. coli* cells and showed good stability during measurements of electrical conductivity. Also, the sensor indicated good reproducibility (n = 5). The immunosensor demonstrated high specificity toward *E. coli* in the presence of interfering bacteria. The biosensor performance is comparable to most of the biosensors reported in literature for detection of bacteria.[26,54,55] It is noteworthy that the biosensor has advantages such as simplicity, cost effectiveness, disposability. In addition, the proposed biosensor was successfully used for detection of *E. coli* in complex human urine sample. Although these biosensors are constructed for single use, they have potential to be reused by appropriate regeneration buffer. Thus, the proposed sensing platform can be easily adapted to detect other bacteria including pathogenic bacteria in food, water safety analysis and clinical diagnosis with the use of suitable primary antibodies during the immunosensor construction. We are further investigating the potential incorporation of the biosensors with a microfluidic device for multi-detection of bacteria.

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