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An ultrasensitive and selective electrochemical biosensor for *Escherichia coli* based on bacteria-imprinted polydopamine and a magnetic nanocomposite modifier

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Abstract

This study developed an electrochemical biosensor utilizing a polydopamine film for the highly selective, ultrasensitive, and efficient determination of *E. coli*. A nanocomposite containing a magnetic graphene oxide-ionic liquid-palladium (MGO-IL-Pd) was synthesized and used as a chemical modifier to create a modified glassy carbon electrode (MGCE). Subsequently, dopamine was deposited on the GCE as a monomer during the electropolymerization process to prepare a bacteria imprinted polymer (BIP). After removing the *E. coli* as a template, the MGCE was employed for detecting the bacteria. The electrochemical performance of the BIP-based sensor was studied through square wave voltammetry (SWV) and cyclic voltammetry (CV). When *E. coli* was introduced, a significant current shift was observed, indicating bacterial entry into the BIPs' cavities. The biosensor successfully detected *E. coli* over a wide concentration range of 5.0 to 1.0×10^7 CFU/mL, with a low detection limit of 1.5 CFU/mL. The biosensor's performance was compared to a non-bacteria imprinted polymer (NBIP) control, revealing a significant synergistic enhancement in detection signal due to the selective BIP cavities. Under optimum conditions, the sensor can be effectively applied to determine *E. coli* in human urine and serum samples with high precision and excellent recovery percentages.

Keywords: Electrochemical biosensor, *Escherichia coli* detection, Bacteria-imprinted polymer.

1. Introduction:

Food safety issues arising from contamination by foodborne pathogens have become a major global concern. Annually, foodborne illnesses result in more than 400,000 deaths worldwide [1]. Prominent pathogens contributing to these illnesses comprise *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Salmonella*, and *Listeria monocytogenes* (*L. monocytogenes*) [2,3]. Among the diarrheal pathogenic bacteria, contamination of food and water by *E. coli* is pervasive [4]. This bacterium naturally inhabits the intestines of warm-blooded animals and is a key indicator of fecal contamination in drinking and recreational water. Consequently, developing rapid detection methods for *E. coli* is vital for public health and safety [5]. Traditional *E. coli* detection methods like quantitative polymerase chain reaction (qPCR), enzyme-linked immunosorbent assays (ELISAs), mass microscopy, flow cytometry, and immunoassays require costly lab equipment, complex preparation, and trained professionals [6,7]. While culture-based methods remain the gold standard for pathogen detection, they are notoriously time-consuming, often requiring several days for conclusive results, which is a

critical drawback during outbreak investigations [8]. Alternative techniques like qPCR and ELISA offer improved speed but rely on sophisticated instrumentation, specialized reagents, and skilled personnel, making them less suitable for rapid, on-site testing [6, 7]. Consequently, there is a pressing need for the development of analytical platforms that combine rapidity, high sensitivity, and operational simplicity for effective food and water quality monitoring [9]. Electrochemical sensors are gaining attention for detecting foodborne pathogens due to their affordability, miniaturization, and simplicity [10].

Electrochemical sensors use electrodes with biological receptors like aptamers, antibodies, and enzymes to selectively bind pathogens, causing detectable changes in electrical properties [11].

The performance of electrochemical sensors is profoundly influenced by the modifier type used on the working electrode. Various modifiers, including imprinted polymers [12-14], magnetic nanoparticles [15], nanoclusters [16], quantum dots [17,18], and metal-organic frameworks [19,20], are extensively employed to increase the active surface area, enhance conductivity, and facilitate electron transfer, thereby boosting sensitivity [21,22]. Among these, graphene oxide (GO) provides an excellent modifier due to its large specific surface area and conductivity. When functionalized with magnetic nanoparticles (e.g., Fe_3O_4), it enhances the surface area as well as electron transfer kinetic [15, 23]. Moreover, as advanced modifiers, molecularly imprinted polymers (MIPs) were also used to enhance the electrode surfaces

in sensors, improving selectivity for the analyte detection [13,14, 24].

However, to achieve both high sensitivity and selectivity, the combination of nanocomposite modifiers with MIPs presents a promising strategy. On the other hands, various metal nanoparticles, including gold [25], copper [26], palladium [27], and silver nanoparticles [28], are also used to coat surfaces and enhance electrochemical signals, boosting sensor sensitivity. However, the incorporation of ionic liquids (ILs) can improve the dispersion of nanomaterials and create a favorable ionic microenvironment, while noble metal nanoparticles like palladium (Pd) significantly enhance electrocatalytic activity and signal amplification [27, 29]. Therefore, designing a ternary nanocomposite that synergistically combines these components-such as magnetic graphene oxide, an ionic liquid, and palladium nanoparticles (MGO-IL-Pd)- presents a promising strategy for developing highly sensitive sensing platforms.

To effectively integrate such nanocomposite modifiers with a selective MIP recognition element on the electrode surface, a reliable and controllable deposition method is required [30]. Electropolymerization rapidly synthesizes uniform polymer films on electrodes, ideal for insulating monomers like dopamine, which oxidizes to form polydopamine (PDA) [31, 32]. PDA's adhesion, biocompatibility, and biomolecule attachment make it a versatile linker in sensing [33]. Researchers utilize electropolymerization to fabricate controllable PDA films for sensor applications. For example, Chen et al.

developed a PDA-based biosensor for *E. coli* detection [34], while Roushani et al. fabricated a new MIP for selective and sensitive *asulam* detection [31].

In this study, a novel electrochemical biosensor was developed for the ultrasensitive and selective detection of *E. coli* by integrating a bacteria-imprinted polymer (BIP) film with a designed magnetic graphene oxide-ionic liquid-palladium (MGO-IL-Pd) nanocomposite. The MGO-IL-Pd nanocomposite was first synthesized and characterized using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), vibrating sample magnetization (VSM), thermal analysis, and X-ray diffraction (XRD). Then, the polydopamine matrix was formed via electropolymerization in the presence of *E. coli*, creating highly selective recognition sites following template removal. It is hypothesized that the synergistic effect between the high-affinity BIP cavities and the enhanced electrocatalytic properties of the nanocomposite overcomes the sensitivity limitations of previous BIP sensors.

2. Experimental:

2.1. Materials and reagents

All chemicals used in this study, iron (III) chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 99%), tetramethoxysilane (TMOS, 99%), including iron (II) chloride ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ 99%), (3-aminopropyl) triethoxysilane (APTES, 98%), graphite powder, benzaldehydes (97–99%), palladium acetate (98%), dimethylsulfoxide

(DMSO), ethanol, and toluene, were sourced from Fluka, Merck, and Sigma-Aldrich Companies. Dopamine and $[\text{Fe}(\text{CN})_6]^{3-/4-}$ were utilized as received, without any additional purification, while all other reagents were sourced from Merck Company. Three bacterial strains, *E. coli*, *S. aureus*, and *Staphylococcus epidermidis* (*S. epidermidis*), and one fungal strain, *Candida albicans* (*C. albicans*), were used in this study.

2.2. Instruments and measurements

Scanning electron microscopy (SEM) images were obtained using an ISP-Korea microscope to analyze the surface morphology of all electrodes. The particle morphology was examined with a SIGMA VP field-emission scanning electron microscope (FE-SEM) from Germany. Energy-dispersive X-ray spectroscopy (EDS) was performed using a SAMX EDS instrument from France. Fourier transform infrared (FT-IR) spectroscopy was conducted with a Bruker-Vector 22 spectrometer, also from Germany. X-ray diffraction (XRD) analysis was performed using a Bruker D8 ADVANCE diffractometer. Thermal gravimetric analysis (TGA) was conducted with a Netzsch PC/PG apparatus from Germany. Lastly, the magnetic properties were evaluated using a vibrating sample magnetometer (VSM) manufactured by the Meghnatis Daghigh Kavir Company in Iran.

All electrochemical experiments were performed using a potentiostat/galvanostat Autolab/PGSTAT101 (Metrohm, Switzerland)

coupled with the advanced NOVA software. A standard three-electrode system was employed, consisting of a glassy carbon electrode (GCE) with a diameter of 3 mm as the working electrode, a platinum electrode as the auxiliary electrode, and an Ag/AgCl electrode as the reference electrode. Measurements were performed using cyclic voltammetry (CV) and square wave voltammetry (SWV) techniques in a solution containing 5 mmol L⁻¹ [Fe(CN)₆]^{3-/4-} and 0.1 mol L⁻¹ KCl as the redox probe. The CV experiments were conducted over a potential range of -0.3 to +0.8 V at a scan rate of 0.1 V·s⁻¹. In the SWV techniques, the potential parameters ranged from 0.0 to +0.8 V. Additionally, dopamine was electrochemically polymerized over a range of -0.5 to +0.5 V at a scan rate of 40 mV·s⁻¹. Electrochemical impedance spectroscopy (EIS) measurements were conducted using a Radstat 10 workstation (Kianshar Danesh, Iran), in a solution containing 5.0 mM [Fe(CN)₆]^{3-/4-} and 0.1 M KCl, with a frequency range of 0.05 Hz to 80 kHz and an amplitude of 10 mV.

2.3. Methods

2.3.1. Bacteria preparation

First, each bacterium's fresh colony was cultured on the culture medium. Then, it was incubated at 37°C for 24 hours. The culture concentration was adjusted to 10⁷ CFU/mL. Then, serial concentrations of 10¹ to 10⁷ bacteria were prepared and used for sensor studies [35].

2.3.2. Synthesis of MGO-IL-Pd nanocomposite

Initially, 0.25 g of graphene oxide (GO) was dispersed in 10 mL of water for 20 minutes. Then, 0.5 g of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ [36] was added, and the mixture was heated to 70 °C. During vigorous stirring for 3 hours, 250 μL of (3-aminopropyl) triethoxysilane (APTES) was added dropwise. The product, $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH/GO}$, was magnetically separated, washed with ethanol, and dried at 70 °C for 6 hours.

Next, 0.5 g of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH/GO}$ was mixed with 25 mL of toluene, and 0.25 mmol of 1-methyl-3-(3-trimethoxysilylpropyl) imidazolium chloride was added. The mixture was refluxed for 24 hours and then magnetically separated, washed, and dried to yield $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH/GO-IL}$. Finally, 0.5 g of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH/GO-IL}$ was dispersed in 20 mL of DMSO, and 0.7 mmol of $\text{Pd}(\text{OAc})_2$ was added. After stirring for 24 hours, the material was magnetically separated, washed, and dried to produce MGO-IL-Pd (Scheme 1).

Scheme. 1

2.3.3. Fabrication of the BIP-based biosensor

Initially, the GCE was polished using alumina powder and then sonicated in a water-ethanol mixture to achieve a clean surface. The bare GCE was prepared through the electropolymerization of dopamine monomer, as

described in the referenced literature [35]. The electropolymerization conditions were optimized using a phosphate buffer solution (pH 7.4) containing 0.22 mM dopamine and *E. coli* at a concentration of 10^4 CFU mL⁻¹. The mixture was degassed with nitrogen for 5 minutes to remove dissolved oxygen. Electropolymerization was performed via CV for 10 cycles, within a potential range of -0.5 to 0.5 V (vs. Ag/AgCl), at a scan rate of 0.04 V s⁻¹. After polymerization, the electrodes were thoroughly rinsed with triple-distilled water and immersed in a leaching solution (0.01 M SDS in triple-distilled water) for 15 minutes under stirring to remove the *E. coli* template. For comparative analysis, a non-bacteria imprinted polymer (NBIP) electrode was fabricated using the same procedure but without the *E. coli* template.

For the preparation of the BIP/MGCE, all conditions remained identical to those used for the BIP/MGCE, except for the addition of 0.001 g of the modifier (MGO-IL-Pd) to the solution containing the buffer and analyte. The modifier was fully dispersed before electropolymerization was carried out. This step ensured the incorporation of the MGO-IL-Pd nanocomposite into the polymer matrix, enhancing the sensor's electrochemical properties and sensitivity. This detailed fabrication process underscores the precision and robustness of the biosensor design, ensuring high performance for *E. coli* detection. Notably, employing the MGO-IL-Pd nanocomposite as a chemical modifier can enhance dopamine electropolymerization through two primary mechanisms: (1) Pd nanoparticles dispersed on MGO sheets can provide catalytic sites that may lower the oxidation overpotential and increase

reaction rates, while (2) the IL component may enhance ionic conductivity and charge transfer efficiency. Furthermore, the high surface area of MGO can promote dopamine monomer adsorption, potentially leading to higher local concentrations and faster polymerization initiation [37,38].

2.3.4. Preparation of real samples

Bacteria can be contagious within the human body, so blood serum and urine samples were analyzed to evaluate the performance of a biosensor for detecting *E. coli*. The human blood and urine samples utilized in this study were obtained as residual diagnostic specimens from Dr. Doustimotlagh Pathology Laboratory (Yasouj, Iran). All methods were carried out in accordance with relevant guidelines and regulations. The study was approved by the Dr. Doustimotlagh Pathology Laboratory (Yasouj, Iran). Informed consent was obtained from all subjects and/or their legal guardians prior to participation in the study. All samples were fully anonymized and contained no personally identifiable information.

To prepare a healthy human serum sample, we added 2% methanol (v/v) to precipitate and separate the proteins. The mixture was shaken for 30 seconds and then centrifuged for 10 minutes at 5000 rpm. The clear supernatant was collected and filtered through a 0.22 μm filter to obtain protein-free serum [39].

For the urine sample, we collected 10 mL and centrifuged it for 15 minutes at 2500 rpm. The supernatant was then passed through a 0.22 μm filter to

remove suspended particles and other interfering substances. Finally, each filtered sample was diluted with phosphate buffer at pH 7.4 and 0.1 M. The pH was adjusted, and the samples were spiked with various concentrations of *E. coli* for the biosensor improvement experiments [40].

3. Results and discussion

3.1. Characterization of MGO-IL-Pd

It is worth noting that the comparative characterization of Fe_3O_4 , graphene oxide, and MGO-IL-Pd using different techniques, including FT-IR, SEM/EDS, simultaneous thermal analysis (TGA and DTA), XRD, and VSM of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ and MGO-IL-Pd nanocomposite, was provided in our previous report [46]. However, the characterization results of MGO-IL-Pd as the final chemical modifier used in this research are presented in Fig. S1.

The crystalline structures analysis of the MGO-IL-Pd nanocomposite using XRD technique (Fig. S1e), exhibited eight well-defined peaks at 2θ values of 18.3° , 30.3° , 35.6° , 43.3° , 53.7° , 57.4° , 63° , and 74.3° , which corresponded to the (1 1 1), (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1), (4 4 0), and (5 3 3) crystallographic planes, respectively. The pattern closely matches the Fe_3O_4 standard reference (JCPDS card, file no. 19-0629) [41-43], indicating that the Fe_3O_4 nanoparticles maintained their crystalline structure during the modification process [44,45].

The FT-IR spectrum of MGO-IL-Pd nanocomposite was provided in Fig. S1. As can be seen, a broad peak is observed at 3420 cm^{-1} , which is due to the O-H bonds present on the surface of the materials. The signals located at 2830 and 2955 cm^{-1} in the MGO-IL-Pd spectrum correspond to the C-H bonds in the IL-propyl groups. Additionally, peaks at 1750 , 1635 , and 1100 cm^{-1} are associated with the carboxyl C=O, aromatic C=C, and C-O bonds in GO, respectively. Furthermore, the peaks observed at 1100 and 540 cm^{-1} can be attributed to the Si-O-Si and Fe-O bonds in the MGO-IL-Pd nanocomposite.

The FE-SEM analysis of MGO-IL-Pd nanocomposite (Fig. S1b) revealed nanoparticles with sizes on the nanometer scale. The particle size of the nanocomposite is estimated to be in the range of 20-100 nm. The particles exhibited a uniform or irregular shape, suggesting a spherical or polygonal morphology, respectively. The overall morphology of the nanocomposite indicated that the nanoparticles were aggregated or clustered, forming larger structures. The presence of nitrogen (N), oxygen (O), carbon (C), silicon (Si), iron (Fe), and palladium (Pd) in the synthesized MGO-IL-Pd nanocomposite was confirmed by EDS analysis in our previous work [46].

The thermal stability of the MGO-IL-Pd nanocomposite was assessed through TGA analysis (Fig. S1). The TGA findings revealed three distinct weight loss stages occurring at 10 - 150°C , 152 - 225°C , and 230 - 580°C . The initial loss of weight, occurring between 10 and 150°C , corresponds to the evaporation of solvent from the nanocomposite's surface. The subsequent loss of weight,

observed from 151 to 225 °C, is due to the release of bound organic components (ionic liquid and propyl amine) from the structure of the nanocomposite. Lastly, the third loss of weight noted at temperatures exceeding 230 °C is linked to the expulsion of residual ionic liquids and the breakdown of GO components. Besides, the magnetic properties of the synthesized MGO-IL-Pd nanocomposites were studied via VSM analysis, which indicated the saturation magnetization values of 37 emu g⁻¹ (Fig. S1d).

3.2. MGO-IL-Pd/MGCE Characterization

The morphological features of the modified electrodes were examined sequentially using SEM. In Fig. 1a, it is evident that the electrode's surface is clear of any substances. Fig. 1b illustrates the presence of a uniformly distributed BIP film covering the electrode. Fig. 1c depicts the binding of the target bacteria to the polymeric surface. After rinsing the modified electrode, Fig. 1d reveals the emergence of pores on its surface. Additionally, Fig. 1e highlights the strong adhesion of bacteria to the modified electrode's surface.

Fig. 1

3.3. Electrochemical study of the designed sensor

The CV technique was employed to analyze the electrochemical characteristics of BIP/GCE, NBIP/GCE, and BIP/MGCE in solutions comprising 5 mM [Fe (CN)₆]^{3-/4-} and 0.1 M KCl (Fig. 2). In this context, the

unmodified GCE is represented (curve 2a). After the electropolymerization process, a notable decrease in peak current was observed (curve 2b), indicating successful polymerization. When the polymerized electrode was treated with a leaching solution (0.01 M SDS in triple distilled water) for 15 minutes, enhancement in the peaks for $[\text{Fe}(\text{CN})_6]^{3-/4-}$ was noted, as cavities formed on the electrode surface due to the removal of the template (curve 2c). After exposing the BIP/GCE to a solution of *E. coli* at a concentration of 10^4 CFU mL⁻¹, *E. coli* molecules were rebounded to the copolymer matrix, leading to a subsequent reduction in peak current (curve 2d). The electrochemical activity of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ after template removal on the NBIP/GCE was also studied. As illustrated in (curve 2e), the NBIP/GCE profiles exhibited no significant changes. To demonstrate that the leached BIP/MGCE effectively captures *E. coli*, a comparison was made between the imprinted and un-imprinted groups while maintaining consistent experimental conditions. The result showed no significant difference in the electrochemical behavior of the non-imprinted group, which was attributed to the lack of template bacteria and the absence of imprinting sites in the non-imprinted sensor [35]. Finally, the electrical conductivity of MGO-IL-Pd nanocomposite was assessed. As illustrated in (curve 2f), the redox current recorded for BIP/MGCE exhibited a notable increase compared to the bare electrode, suggesting that MGO-IL-Pd nanocomposite enhances electron transfer on the electrode surface, thereby strengthening the electrochemical response.

The presence of trimethoxysilylpropyl group in the IL structure may enable the covalent anchoring to the magnetic graphene oxide surface, ensuring film stability during electropolymerization and washing. Besides, the chloride counter ion provides high ionic conductivity and compatibility in aqueous media, facilitating dopamine oxidation and polymer growth.

However, when MGO-IL-Pd was applied in conjunction with *E. coli* bacteria on the electrode surface, a reduction in the current of BIP/MGCE was observed. This reduction may be due to the interactions between the negatively charged *E. coli* bacteria and the functionalized surface.

Fig. 2.

Figure 3 presents the EIS pattern bare GCE, dopamine-electropolymerized GCE, after *E. coli* removal, BIP/GCE after *E. coli* rebinding, and BIP/MGCE post *E. coli* removal. All measurements were conducted in 0.1 M KCl containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution. For the bare GCE (curve a), the impedance spectrum shows the initial baseline response. After electropolymerization, a pronounced high-frequency semicircle appears (curve b), suggesting the formation of a non-conductive layer on the electrode surface (BIP/GCE). When *E. coli* was removed from the electrode using a washing solution, the semicircle size decreased (curve c), indicating easier electron transfer due to the creation of pores roughly matching the bacterial dimensions. Upon rebinding the bacterial template in the BIP, the semicircle size increased again (curve d), confirming successful formation of the BIP

film. The electrical conductivity of the MGO-IL-Pd nanocomposite was also assessed. As shown in curve 2e, the re-removal of *E. coli* with the washing solution resulted in a sharp decrease in the semicircle size, confirming the significant increase in the redox current for the BIP/MGCE compared to the bare GCE. These findings indicate that the MGO-IL-Pd nanocomposite promotes electron transport at the electrode interface, thereby improving the electrochemical signal. This behavior aligns with the CV results and supports the conclusion that cavity structures are generated during electropolymerization in the presence of the bacterial template.

Fig. 3.

3.4. Optimization of experimental conditions

3.4.1. Accumulation time, polymerization cycles, and leaching time

Several parameters and conditions, including accumulation time, polymerization cycles, and leaching time, were optimized to improve the sensor's analytical capabilities. Various accumulation times (from 5 to 30 minutes) were evaluated for *E. coli* saturation. As shown in Fig.4a, the ΔI was computed using the formula $\Delta I = I - I_0$, where I represents the current assayed in the presence of *E. coli*, and I_0 represents the current in the absence of *E. coli* in the sensor, progressively increased with longer accumulation times, peaking at 15 minutes, indicating that the *E. coli* detection process had reached saturation; thus, 15 minutes was chosen as the optimum accumulation time. The thickness of the printed polymer layer

was analyzed to assess how varying polymerization cycles impacted sensor performance.

As depicted in Fig. 4b, the ΔI value significantly increased as the number of polymerization cycles increased from 5 to 15 cycles, stabilizing at 15 cycles. Consequently, 10 cycles were chosen for subsequent experiments. A lower number of scanning cycles results in a thinner polymer layer that is unstable and prone to damage, while excessive cycles lead to an overly thick polymer layer that hinders the removal of template molecules, resulting in insufficient site pores. The optimal leaching time is illustrated in Fig. 4c. The ΔI value increased with longer leaching times, indicating that more site pores were formed on the electrode surface as template molecules were removed. The ΔI value reached its maximum at a leaching time of 25 minutes. However, with further prolongation of the leaching time, the ΔI value began to decrease, likely due to the extended leaching time adversely affecting the polymer structure and reducing the detection capability of the bacterial site pores. Therefore, 15 minutes was chosen as the optimal leaching time.

Fig.4

3.4.2. Scan rate effect

To investigate the effect of scan rate (D) on the response of the BIP/MGCE-based sensor, several voltammograms were recorded for a typical standard solution of *E. coli* (10^4 CFU mL⁻¹) at scan rates ranging from 10 to 100 mV

s⁻¹. As depicted in Fig. 5, the voltammograms illustrate the change in current response across the applied potential range, providing insights into the electrochemical behavior of the BIP/MGCE-based sensor in the presence of *E. coli*. The results indicated a linear relationship between both the forward and backward peak currents and the D within the tested range (10 to 100 mV s⁻¹). The strong linear correlation, evidenced by a regression coefficient ($R^2 > 0.99$), indicates that the electrochemical process is predominantly adsorption-controlled [40]. In adsorption-controlled electrochemical processes, the peak current typically increases linearly with the scan rate, which is consistent with the observed results.

Fig.5

3.6. Selectivity of the designed sensor

The BIP/MGCE sensor was investigated for the selective detection of *E. coli* in the presence of other microorganisms such as *S. epidermidis*, *S. aureus*, and *C. albicans*. As shown in Fig. 6a, the recorded current changes (ΔI) for the PDA-based BIP/MGCE sensor were significantly higher for *E. coli* than the other tested microorganisms. These current variations indicate that the BIP/MGCE sensor selectively detects *E. coli*, confirming the high selectivity of the electrochemical sensor. The minimal responses to *S. epidermidis*, *S. aureus*, and *C. albicans* further demonstrate the sensor's ability to distinguish *E. coli* from other microorganisms, making it suitable for use in complex

biological samples. This high selectivity is due to the use of the BIP, which is highly selective and designed to detect *E. coli*, making the sensor a practical and reliable tool for detecting this bacterium.

In the other experiments, the selectivity of the designed sensor toward *E. coli* compared to other microorganisms was evaluated by analyzing the effect of structurally related compounds and biologically relevant molecules, such as *S. epidermidis*, *S. aureus*, and *C. albicans*, which could potentially interfere with the detection of *E. coli*. Using the proposed technique, the sensor's response to *E. coli* was examined in the presence of these interfering species at varying concentrations (10^3 to 10^6 CFU/mL) under optimum conditions, as shown in Fig. 6b.

Fig. 6

The results in Table 1 indicate that the sensor maintains high selectivity for *E. coli* despite the presence of these interferences. The relative error percentage (RE%) and tolerance ratio (TR) were calculated to quantify the sensor's performance. For *S. aureus*, the RE% ranged from 1.16% to 11.92%, while for *S. epidermidis*, it ranged from 2.07% to 13.88%, and for *C. albicans*, it ranged from 1.85% to 12.73%. These low RE% values, along with the TR values, confirm that the sensor's detection of *E. coli* remains largely unaffected by the presence of these interfering species up to 1000-fold. This demonstrates the sensor's ability for selective detection of *E. coli* even in

complex biological environments where structurally similar microorganisms may be present.

Table 1

3.7. Figures of merit of the designed sensor

The applicability of the leached BIP/MGCE electrochemical biosensor for *E. coli* sensing was evaluated using various concentrations of *E. coli* solutions under optimum conditions. A series of *E. coli* concentrations (5.0 to 1.0×10^7 CFU/mL) were independently incubated with the prepared leached BIP/MGCE and analyzed using the SWV technique. As shown in Fig.7a, the SWV response decreased as the concentration of *E. coli* increased, which was attributed to the selective recognition of *E. coli* by the BIP, hindering electron transfer to the electrode surface. The changes in current exhibited a strong linear relationship with the *E. coli* concentration across the range of 5.0 to 1.0×10^7 CFU/mL. The calibration curve relating the SWV response to the logarithm of concentration (Fig.7b) yielded the linear regression equation $I = 122.58 - 24.96 \log C$ ($R^2 = 0.992$). The limit of detection (LOD) and limit of quantification (LOQ) were calculated using 3-fold and 10-fold of the standard deviation of the blank solution divided by the slope of the calibration curve, respectively, resulting in very low LOD (1.50 CFU/mL) and LOQ (4.95 CFU/mL) values for the present sensor.

Fig.7

The intra-day and inter-day repeatability of the biosensor was evaluated using the CV technique in a solution containing 0.1 mol/L KCl and 5.0 mmol/L of the redox probe $[\text{Fe}(\text{CN})_6]^{3-/4-}$. For intra-day and inter-day tests, the response of a typical sensor to a standard solution of *E. coli* (10^4 CFU mL^{-1}) was recorded in order during 8 hours and six days, and the RSD% of the recorded currents was calculated. The low RSD% values (1.50% and 2.69%) confirm the excellent stability and repeatability of the fabricated biosensor, as illustrated in Figure S3.

Additionally, to assess the reproducibility of the biosensor, five similar sensors were fabricated and tested in a solution containing 10^4 CFU mL^{-1} *E. coli*. The RSD of the recorded currents across the five electrodes was calculated, demonstrating high reproducibility with a very low RSD of 2.83%, as shown in Fig. S3. The results demonstrate the reliability and consistency of the biosensor in precisely detecting *E. coli*.

The reusability of the developed BIP/MGCE electrochemical biosensor for *E. coli* detection was evaluated through multiple binding/elution cycles using an aqueous solution of SDS as leaching solution (0.01 M SDS in triple-distilled water) for 15 minutes. SWV analysis demonstrated excellent sensor reusability at two concentration levels (10^2 and 10^6 CFU mL^{-1}), with RSD of 2.20% and 1.03%, respectively, across five replicate measurements.

In addition, the reusability of the designed BIP/MGCE electrochemical biosensor for *E. coli* detection was evaluated during multiple binding/elution

cycle. As obtained from the analyses using SWV technique, the fabricated sensor shows an excellent reusability at two typical concentration levels of 10^2 and 10^6 CFU mL⁻¹ *E. coli* for five replicates with low RSD of 2.20 and 1.03%, respectively.

3.8. Comparison study

Table 2 provides a comparison of this sensor's performance with other methods for *E. coli* detection [47-54]. The BIP/MGCE electrochemical biosensor demonstrates superior performance for *E. coli* detection, offering an exceptionally wide linear range (5.0 to 1.0×10^7 CFU/mL) and an ultra-low LOD (1.5 CFU/mL), outperforming methods like graphitized mesoporous carbon-modified electrodes (LOD: 50.4 CFU/mL) [47] and C₆₀-based immunosensors (LOD: 15.0 CFU/mL) [50]. The use of a BIP and MGO-IL-Pd nanocomposite enhances selectivity and applicability, making it a highly sensitive, cost-effective, and practical tool for *E. coli* detection in clinical and environmental applications.

Table 2

3.9. Real sample analysis

The biosensor's performance was evaluated for detecting *E. coli* in real samples, including human urine and serum, under optimized conditions using the SWV technique. Measurements were conducted in triplicate ($n = 3$), and the results demonstrated high accuracy and precision. According to the

results presented in Table 3, in urine samples, recovery values ranged from 98% to 103%, with relative standard deviation (RSD) values between 0.52% and 1.06%. Similarly, in serum samples, recovery values ranged from 97% to 102%, with RSD values lower than 2%. These findings highlight the biosensor's reliability and precision in a complex biological matrix.

Table 3

4. Conclusions

In this research, a novel electrochemical biosensor utilizing polydopamine (PDA) and modification with a newly developed nanocomposite (i.e., MGO-IL-Pd) was designed and successfully developed for the rapid and direct determination of *E. coli*. The biosensor demonstrates remarkable selectivity for the recognition of *E. coli* in the presence of other microorganisms. The incorporation of MGO-IL-Pd as a chemical modifier significantly enhanced the electrochemical response of the sensor. Additionally, PDA's excellent biological compatibility and strong adhesion to the electrode contributed to achieving highly sensitive electrochemical detection of *E. coli*. Compared to previously reported BIP-based electrochemical sensors, this biosensor offers distinct advantages, including an extremely low detection limit of 1.5 CFU mL⁻¹ and a remarkably short detection time. Meanwhile, the designed biosensor was applied for determination of *E. coli* content in some biological real samples, successfully with high precision and recovery percentages.

Furthermore, this method can be developed as a simple detection strategy for various bacterial species.

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Authors Contribution

The first draft of the manuscript was written and the data were interpreted by Ali Gorjizadeh Kohvade. Prof. Hamid Reza Rajabi and Prof. Mahmoud Roushani were responsible for revising the manuscript and for checking all technical and grammatical aspects. Prof. Dawood Elhamifar and Ms. Farkhondeh Dadvar assisted with the synthesis and characterization of the nanomaterials used in this study. All authors contributed equally to the conception and design of the study.

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Data Availability

Data is provided within the manuscript or supplementary information files.

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The caption of figures:

Scheme 1: Synthesis of MGO-IL-Pd nanocomposite

Fig. 1. FE-SEM images of (a) bare GCE, (b) BIP/GCE after template elution, (c) BIP/GCE before elution, (d) BIP/MGCE after elution, and (e) BIP/MGCE before elution of *E. coli*.

Fig. 2. Cyclic voltammograms of (a) bare GCE, (b) dopamine electropolymerization on GCE, (c) after *E. coli* removal, (d) BIP/GCE after *E. coli* rebinding, (e) NBIP/GCE after elution, and (f) BIP/MGCE after removing 10^4 CFU/mL *E. coli*. All measurements were performed in 0.1 M KCl containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at a scan rate of 0.1 V/s.

Fig. 3. Nyquist plots of (a) bare GCE, (b) dopamine-electropolymerized GCE, (c) after template (*E. coli*) removal, (d) BIP/GCE after *E. coli* rebinding, and (e) BIP/MGCE post-template elution. All measurements were performed in 0.1 M KCl containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$, with a frequency range of 0.05 Hz to 80 kHz and an amplitude of 10 mV.

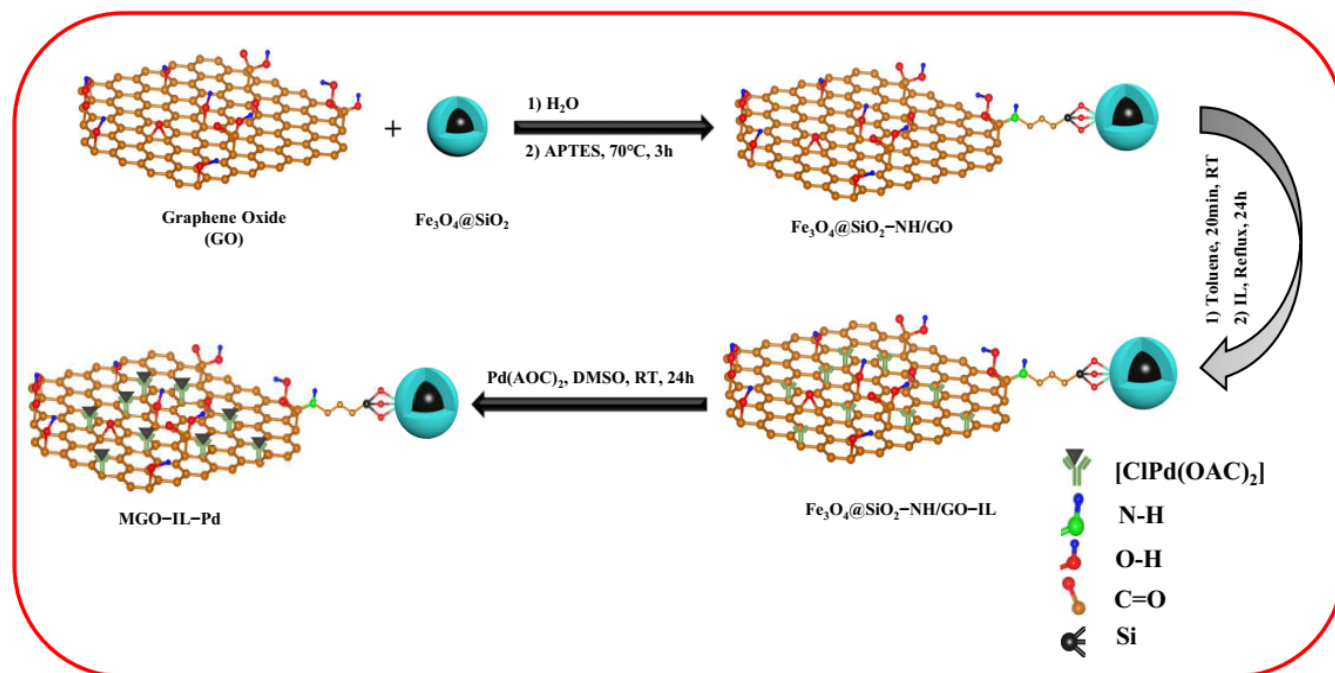
Fig. 4. Optimization of (a) accumulation time, (b) DA polymerization scan cycles, and (c) elution time using the modified electrode. Measurements were performed in 0.1 M KCl containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (SWV parameters: potential range -0.1 to +1.0 V).

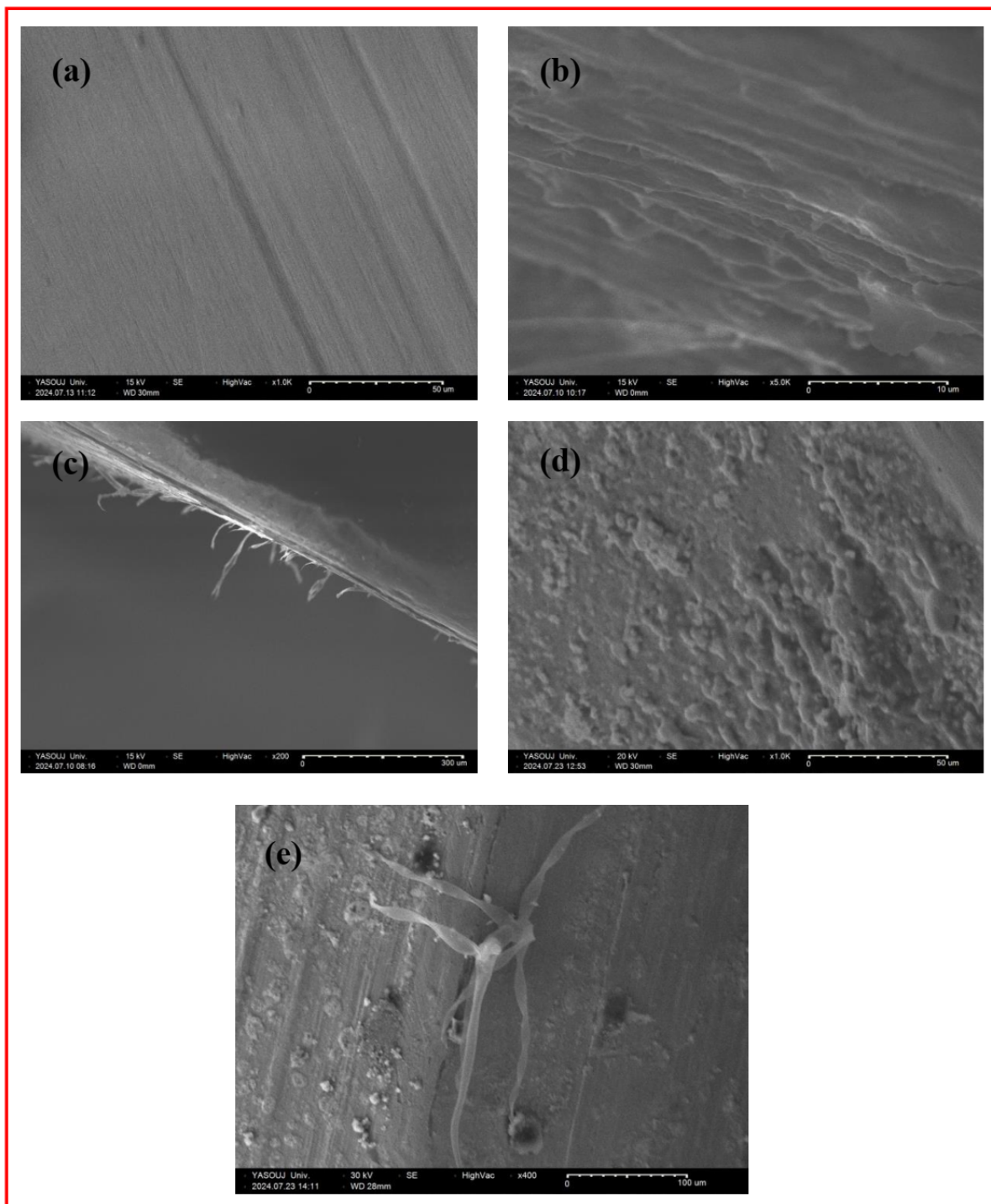
Fig. 5. (a) Cyclic voltammograms of the sensor with 10^4 CFU/mL *E. coli* at scan rates from 10-100 mV/s in 0.1 M KCl containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$, and (b) corresponding peak current versus scan rate.

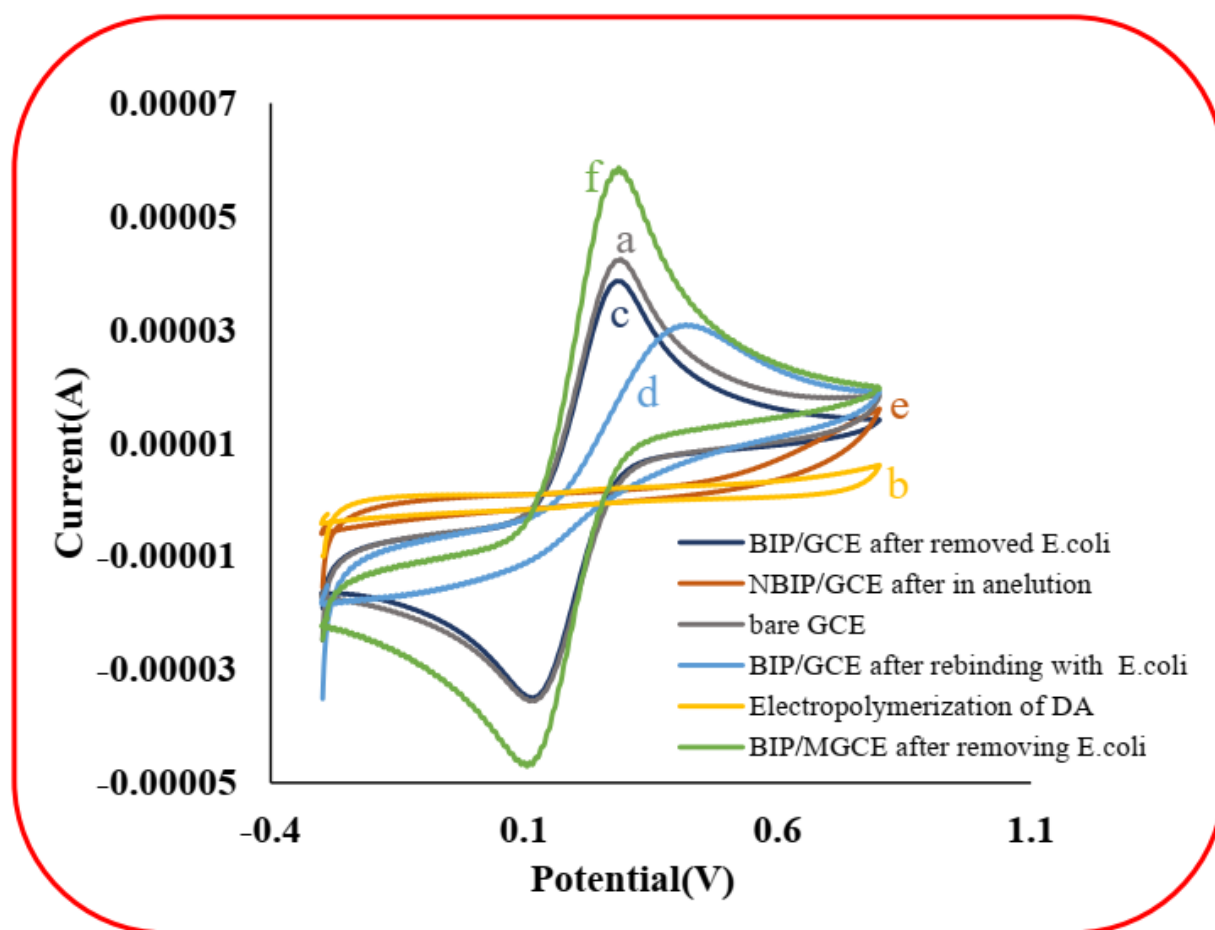
Fig. 6. (a) Selectivity of eluted BIP/MGCE toward *E. coli* under optimized conditions, and (b) voltammetric responses to interfering species ($C_1=10^3$, $C_2=10^4$, $C_3=10^5$, $C_4=10^6$ CFU/mL).

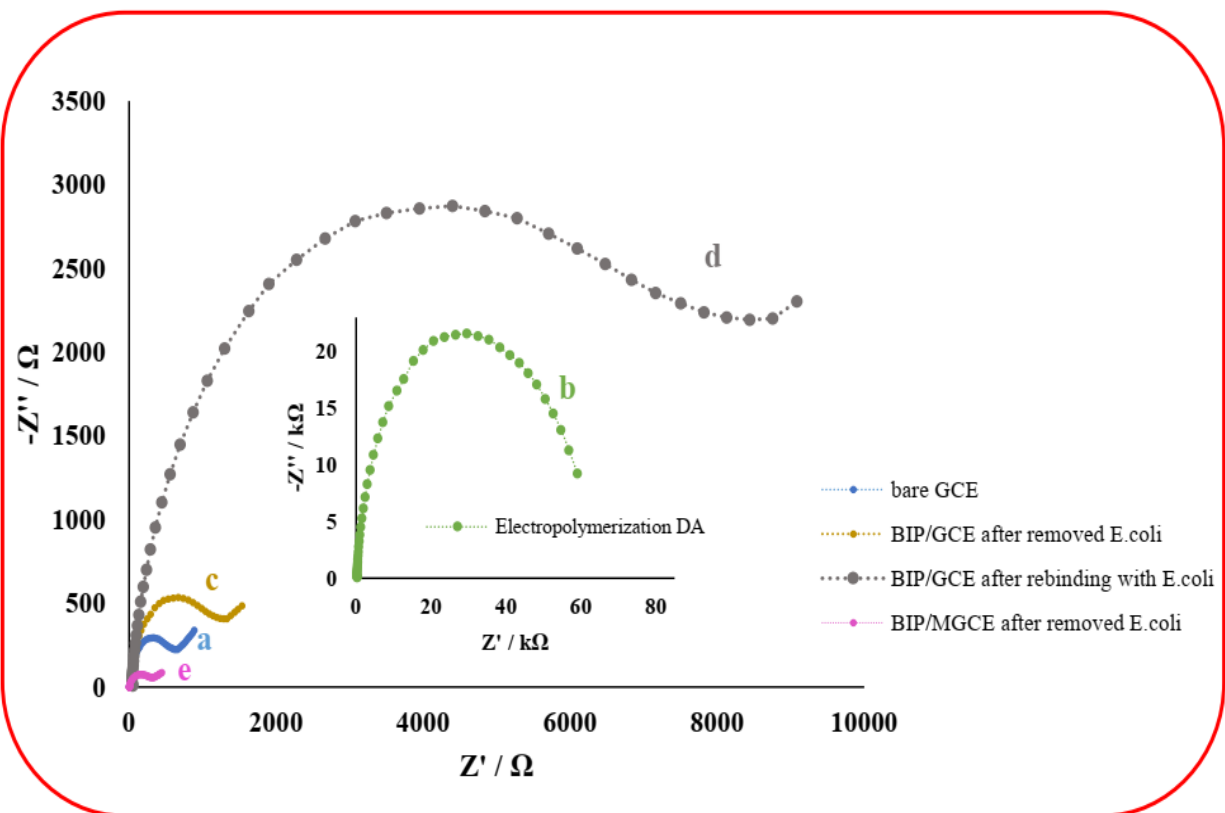
Fig. 7. (a) SWV responses of the eluted BIP/MGCE sensor to varying *E. coli* concentrations ($5-10^7$ CFU·mL⁻¹) in 0.1 M KCl containing 5 mM [Fe(CN)₆]^{3-/4-}, and (b) corresponding calibration curve plotting peak current change (ΔI) versus logarithmic *E. coli* concentration.

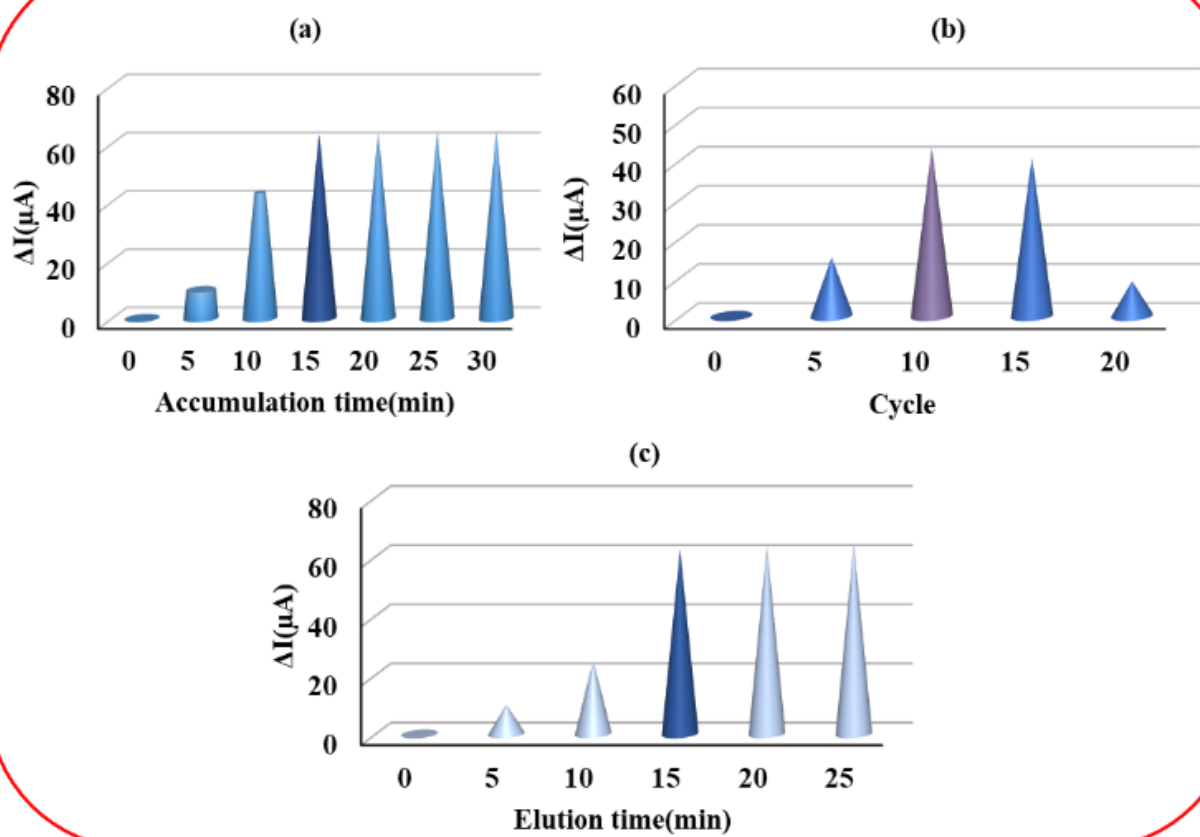
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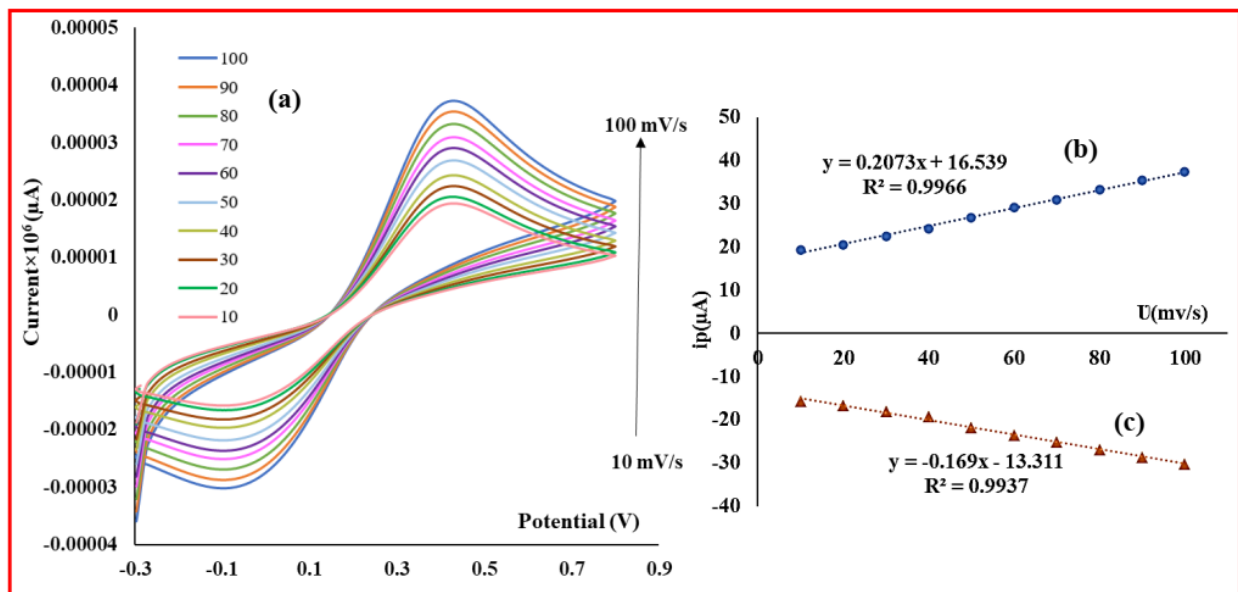
**Scheme. 1**

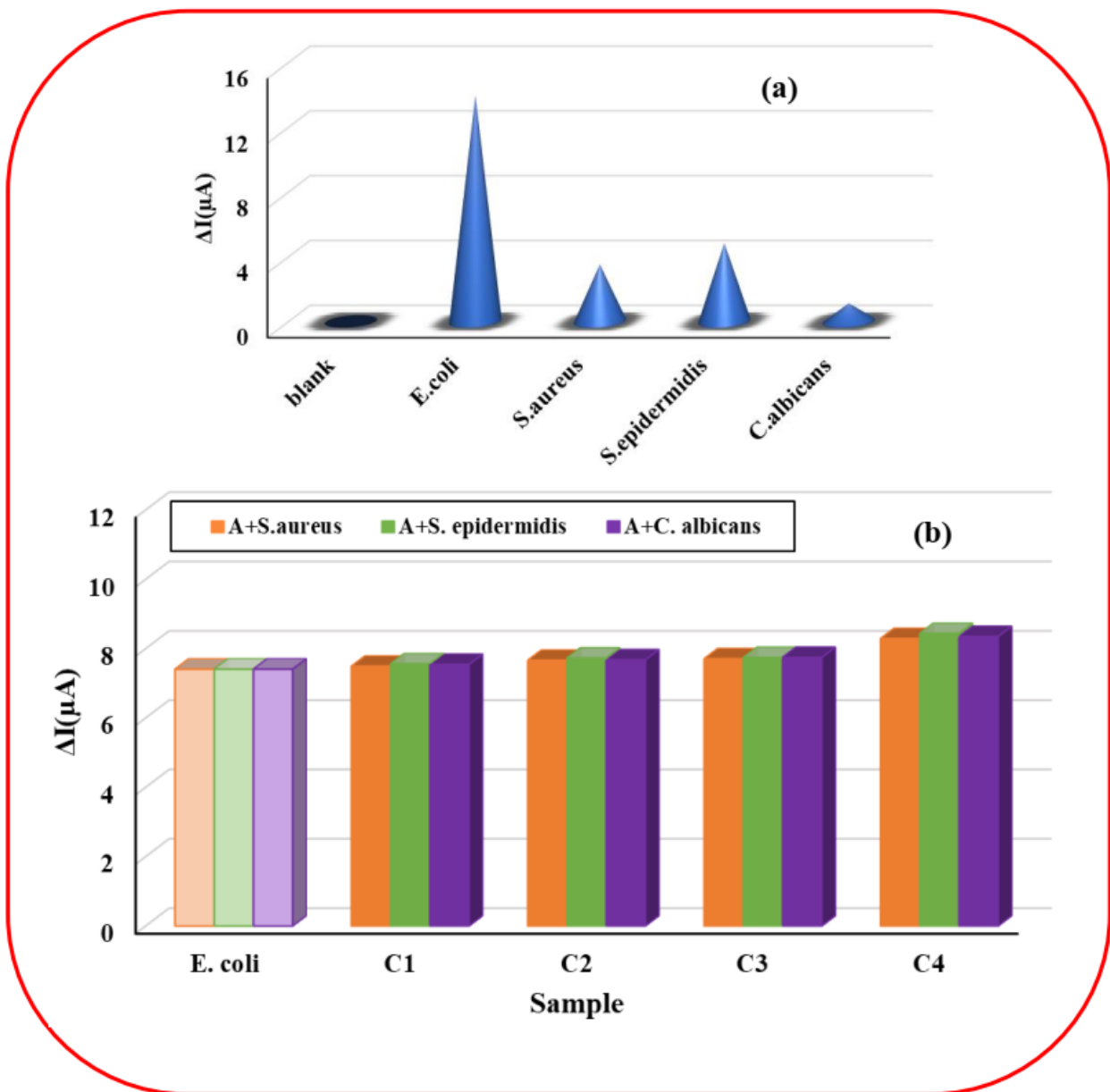
**Fig. 1**

**Fig. 2**

**Fig. 3**

**Fig.4**

**Fig.5**

**Fig. 6**

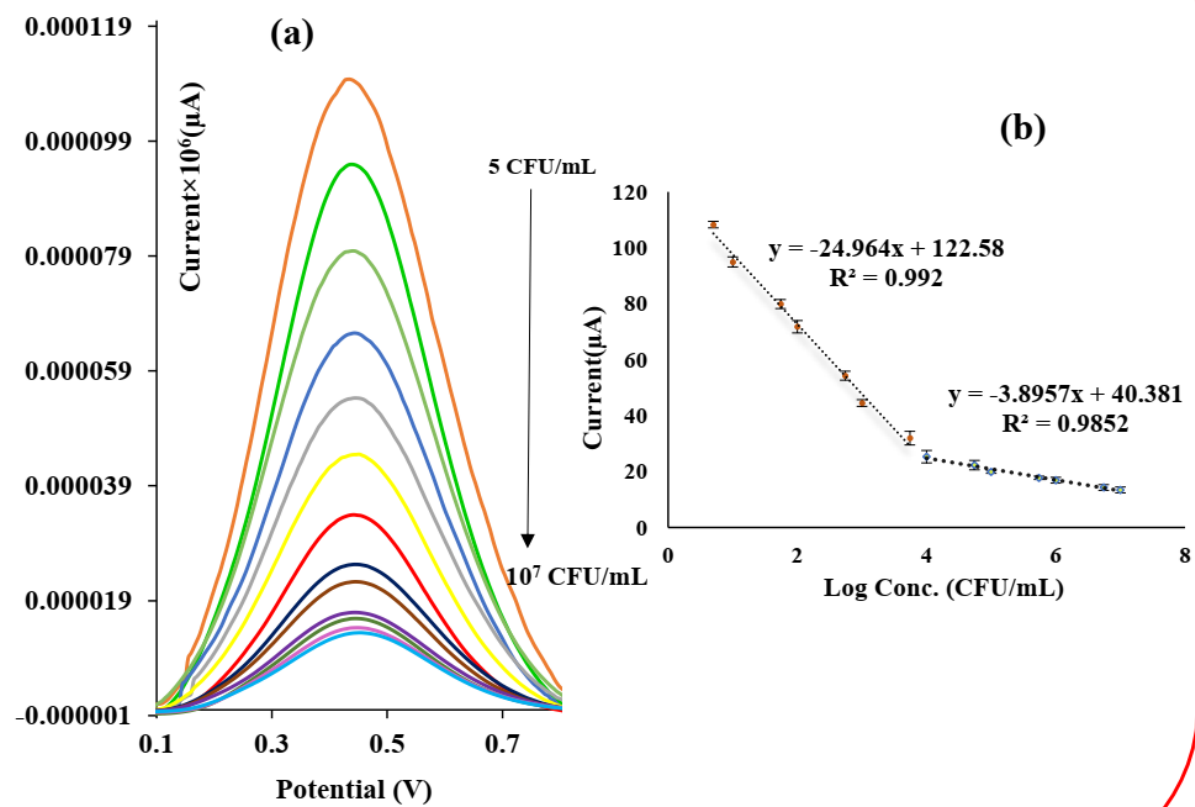
**Fig. 7**

Table 1. Interference study: Relative error (RE%) and tolerance ratio (TR) for competing species in the presence of *E. coli* (100 CFU/mL).

Sample	Conc. (CFU/mL)	RE (%)	TR = [<i>E. coli</i>]/[others]
<i>S. aureus</i>	1.0×10 ³	1.16	1000
	1.0×10 ⁴	3.45	
	1.0×10 ⁵	4.06	
	1.0×10 ⁶	11.92	
<i>S. epidermidis</i>	1.0×10 ³	2.07	
	1.0×10 ⁴	4.33	
	1.0×10 ⁵	4.53	
	1.0×10 ⁶	13.88	
<i>C. albicans</i>	1.0×10 ³	1.85	
	1.0×10 ⁴	3.65	
	1.0×10 ⁵	4.59	
	1.0×10 ⁶	12.73	

Table 2. Comparison of linear dynamic ranges and LOD values between the developed BIP/MGCE biosensor and reported *E. coli* detection sensors.

Number	Method	Linear dynamic range (CFU μ L ⁻¹)	LOD (CFU μ L ⁻¹)	Reference
1	Graphitized mesoporous carbon modified electrodes	2.5 $\times$ 10 ³ - 2.5 $\times$ 10 ⁵	50.4	[47]
2	Immunosensor for multiplexed detection of food-borne pathogens	1.0 $\times$ 10 ³ - 5.0 $\times$ 10 ⁵	4.0 $\times$ 10 ²	[48]
3	magnetic nanoparticle Immunosensor for the detection of <i>E. coli</i> O157:H7 in food specimens	4.1 $\times$ 10 ² - 4.1 $\times$ 10 ⁵	2.5 $\times$ 10 ²	[49]
4	Immunosensor for sensitive detection of <i>E. coli</i> O157:H7 using C ₆₀	3.2 $\times$ 10 ¹ - 3.2 $\times$ 10 ⁶	15.0	[50]
5	Microcontact imprinted capacitive biosensor	1.0 $\times$ 10 ² - 1.0 $\times$ 10 ⁷	70.0	[51]
6	Modification of polyacrylonitrile-derived carbon nanofibers and bacteriophages on screen-printed electrodes	1.0 $\times$ 10 ² - 1.0 $\times$ 10 ⁶	36.0	[52]
7	16S rDNA nanogap network Electrochemical biosensor	1.0 $\times$ 10 ³ - 1.0 $\times$ 10 ⁸	1.0 $\times$ 10 ²	[53]
8	Biomimetic sensing of <i>E. coli</i> at the solid-liquid interface: From surface-imprinted polymer	1.0 $\times$ 10 ³ - 1.0 $\times$ 10 ⁷	1.2 $\times$ 10 ²	[54]
9	BIP/MGCE based biosensor	5.0 - 1.0 $\times$ 10 ⁷	1.5	This work

Table 3. Analytical performance of the BIP/MGCE biosensor for *E. coli* detection in spiked biological matrices (urine and serum).

Sample	Added (CFU/mL)	Found (CFU/mL)	Recovery (%)	RSD% (n=3)
Urine	0	ND	-	-
	1.0×10^1	1.02×10^1	101.86	0.52
	1.0×10^2	9.80×10^1	98.07	0.72
	1.0×10^3	1.03×10^3	103.13	1.06
Serum	0	ND	-	-
	1.0×10^1	0.97×10^1	97.17	1.05
	1.0×10^2	9.87×10^1	98.73	1.63
	1.0×10^3	1.02×10^3	102.15	0.89

*ND: Not detected