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ORIGINAL PAPER

Titanium dioxide–mediated resistive nanobiosensor for *E. coli* O157:H7

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Abstract

A titanium dioxide nanoparticle (TiO_2 NP)–mediated resistive biosensor is described for the determination of DNA fragments of *Escherichia coli* O157:H7 (*E. coli* O157:H7). The sol-gel method was used to synthesize the TiO_2 NP, and microlithography was applied to fabricate the interdigitated sensor electrodes. Conventional *E. coli* DNA detections are facing difficulties in long-preparation-and-detection-time (more than 3 days). Hence, electronic biosensor was introduced by measuring the current-voltage (*I–V*) DNA probe without amplification of DNA fragments. The detection scheme is based on the interaction between the electron flow on the sensor and the introduction of negative charges from DNA probe and target DNA. The biosensor has a sensitivity of 1.67×10^{13} Ω/M and a wide analytical range. The limit detection is down to 1×10^{-11} M of DNA. The sensor possesses outstanding repeatability and reproducibility and is cabable to detect DNA within 15 min in a minute-volume sample (1 μL).

Keywords Titanium dioxide nanoparticles · Poultry sample detection · Interdigitated electrode · Microlithography · Electrical detection

Introduction

In the current trends, biosensor is playing a pivotal role in the front of diagnosis for human well-being. Further, designing a high-performance sensor has also been focused much due to its wide range of potentials [1–9]. Among the biosensors, electrical biosensor provides the most effective, comprehensive, and rapid detection towards the biomolecules [10–12]. In addition, the results come out through the electrical reading in label-free manner are facilitating the easier interpretation. According to Xu et al. biosensors are in trend for detecting *E. coli* O157:H7, can be categorized into (i) optical, (ii) electrochemical, (iii) mass-sensitive, (iv) calorimetric, and others [13]. Among them, electrochemical biosensors have been tremendously studied. Electrochemical biosensor is a device that transforming the electrochemical information into an analytical signal. It is composed of a chemical recognition system and a physical transducer. The chemical recognition system can be considered as a complex set-up, which involves chemical reagents. Moreover, the system needs three electrodes which are reference, counter, and working electrodes [14]. The use of electrochemistry requires large volume of chemicals and analytes [15]. Recently, biosensor technology has been introduced with the use of electronic-biosensors that are able to detecting DNA with changes in output current, resistance, and conductance [2, 11, 16–21]. In addition, electronic biosensor can be categorized as a YES/NO device. The existence of DNA can be read by the electrical reading as low as femtomolar (10^{-15}) M [16, 17]. The working principle of electronic biosensor is shorter than

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the electrochemical, where dropping-analyte-technique has been used. This biosensor integrates the biological elements with the transducer to produce an electrical signal. Basically, output current is measured after each process that acts as a reference measurement (reference electrode) towards the next process. The main highlights of the electrical biosensor are small analyte-volume consumption (~ 1 μL), straightforward detection, and rapid in detection time.

On the other hand, the choices of target bring-out the importance of its applications. There are wide ranges of targets that have been demonstrated to be successful with biosensors [7, 22, 23]. Among different targets, detection of pathogens is the mandatory events and has been implemented with several sensors, which include optical, electrical, and electrochemical sensors [24, 25]. The performance of most of the pathogen detecting sensors covers monitoring the disease causing agents. However, detecting foodborne pathogen is limited, especially with the electrical-based sensors. *Escherichia coli* (*E. coli*) is one of the foodborne pathogen that urgently needs the ultra-fast and direct detection technique due to the limitations with the current technologies that normally needs more than 2 days. It is due to the necessity of cultured samples to obtain the clear-cut results. In addition, expertise is required to interpret the obtained results especially with the coloring and culture-based methods. In the current study, we have demonstrated an electronic resistive biosensor for a direct detection of *E. coli* O157:H7 DNA in real samples that eliminate amplification of DNA using polymerase chain reaction (PCR). Foodborne pathogenic detection in real sample using the resistive biosensor–coupled TiO_2-NP without involving PCR is not explored in the past. Herein, we report using less volume consumption, the potential of TiO_2-NP transducer for the detection of real sample, having *E. coli* O157:H7 DNA species at an extremely low concentration. Without involving any complex wet chemistry process was followed on this transducer, and the output result is simply tunable by electrical reading, which clearly shows the genuine detection. To demonstrate these appealing characteristics, the analytical performance on the TiO_2-NP-based resistive transducer is discussed in detail.

Experimental section

Materials and reagents

A p-type silicon wafer (1–10 Ω cm) with 4-in. diameter and < 1 0 0 > orientation was used in this work. Buffered oxide etch (BOE) and aluminum etchant were purchased from J.T. Baker, Fisherscientific (New Hampshire, USA, https://www.fishersci.co.uk/gb/en/brands/IPF8MGDA/jt-baker.html). A negative resist (NP ma-N 1405) and resist developer (RD6) were purchased from Futurrex, Inc. (Franklin, USA, https://futurrex.com/en). Ethanol, acetone, titanium (IV) isopropoxide (TTIP) 97%, glacial acetic acid, titanium (IV) butoxide (TiB), and 3-aminopropyl triethoxysilane (APTES) 99% were obtained from Sigma-Aldrich (M) Sdn Bhd (Petaling Jaya, Malaysia, https://www.sigmaaldrich.com/malaysia.html). All DNA oligonucleotides of *E. coli* O157:H7 were purchased from AIT Biotech (Singapore, http://www.aitbiotech.com/). A 30-mer COOH-terminated probe (5′-COOH-AACGCCGATACCATTACTTATACCGCGACG-3′) was synthesized as a probe. Six carbon (C6) spacer was used to stabilize the whole molecule. For the target DNA detection, 30-mer positive control target DNA (5′-CGTCGCGGTATAAGTAATGGTATCGGCGTT-3′), 30-mer negative control target DNA (5′-GCAGCGCCATATTCATTACCATAGCCGCAA-3′), and another 30-mer single mismatch target DNA (5′-CGTCGCGGTATAACTAATGGTATCGGCGTT-3′) were utilized.

Apparatus

TiO_2 solution and photoresist were spin-coated using spin coater WS-650MZ-23NPP; Laurell Technologies Corporation (North Wales, USA, http://www.laurell.com/). DNA was extracted from the poultry samples using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany, https://www.qiagen.com/us/). Vortex mixer SA8 was purchased from Fisher scientific (Stuart™, New Hampshire, USA, fishersci.fi/fi/en/home.html). myFuge™ microcentrifuge and 12 Mini Centrifuge were purchased from Bechmark Scientific (New Jersey, USA, http://www.benchmarkscientific.com/). ThermoMixer® was purchased from Eppendorf (Selangor, Malaysia, https://www.eppendorf.com/MY-en/). MSH-30D Hotplate Stirrer was purchased from WiseStir ® (Wertheim, Germany, https://www.witeg.de/). Keithley 6487-picoammeter and Autolab software were purchased from Mouser Electronics (Penang, Malaysia, https://my.mouser.com/) that used to measure the electrical performance of the sensor.

Development of titanium dioxide–mediated resistive nanobiosensor

The device was fabricated on a p-type < 1 0 0 > silicon wafer. The fabrication process was initiated with surface cleaning to remove the native oxide layer. The wafer was immersed in BOE for 15 min at room temperature and rinsed with distilled water (DIW). It was immediately blow-dried using N_2 gun to prevent regrowing the native oxide. Next, the wafer was used as a substrate to fabricate interdigitated electrodes (IDEs) [26]. Negative photoresist NP ma-N 1405 was used for the fabrication of IDEs using the photolithography process. A complete process of the IDEs fabrication was reported in our previous study [27]. After this process, TiO_2 solutions were prepared using the sol-gel method.

Two TiO_2 solutions (20.2 mL) were prepared by varying the TiO_2 precursors, (i) TTIP and (ii) TiB. Each precursor was mixed with ethanol (as a solvent) and glacial acetic acid (as a

stabilizer) at 1:9:0.1 ratio [28]. The mixed solution was vigorously stirred using magnetic stirrer and heated at 75 °C for 1 h on hot plate (WiseStir ® & WiseTherm, Germany). The solutions were left to age for 24 h at room temperature.

The fabrication of sensor was further processed by developing TiO_2 thin film on the surface of IDEs. Hence, each TiO_2 solution was separately spin-coated on the IDEs at a spin rate of 2000 rpm for 30 s. The optimization of IDE fabrication was discussed previously [27]. The TiO_2 films were dried on a hot plate with 175 °C for 10 min. The spin-coating and drying processes were repeated twice in order to obtain a 60-nm thickness. Finally, the TiO_2 film was annealed at 400 °C. The annealing temperature was discussed previously [29]. The overall process for the preparation of TiO_2 solution and the fabrication of IDEs device were summarized in Fig. 1a.

Characterization of titanium dioxide–mediated resistive nanobiosensor

The characterizations on TiO_2-NPs thin films were performed to study the surface morphology of the thin films using atomic force microscope (AFM) (SPA400; Seiko Instruments Inc.) and field-emission electron microscope (FESEM) (HITACHI SU8020). The electrical measurements of the thin films were done using Keithley 6487-picoammeter. There are two needles on probe station with positive and negative potential

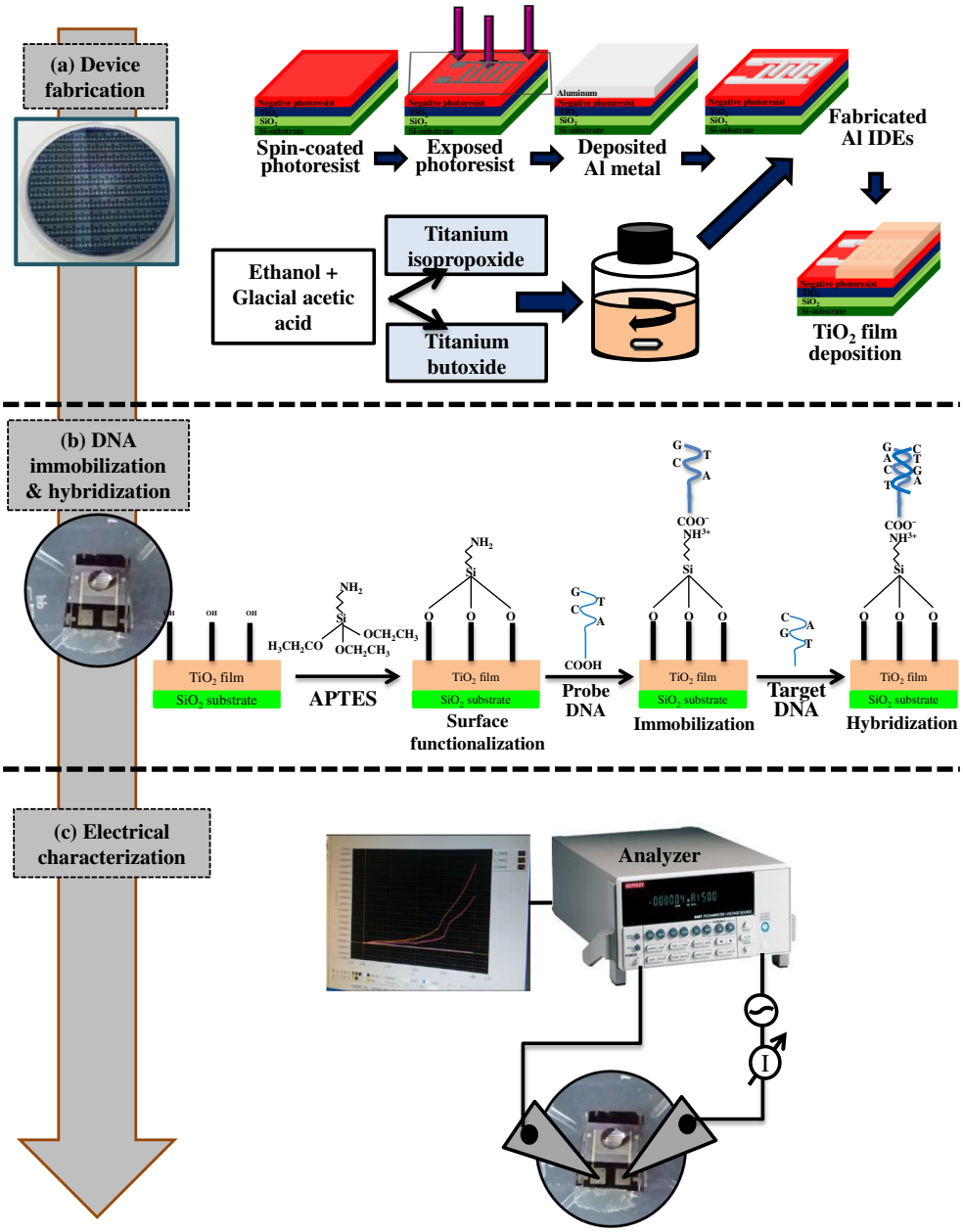

Fig. 1 **a** Fabrication of titanium dioxide–mediated resistive nanobiosensor; **b** Process to introduce oligonucleotide on the surface of inorganic TiO_2; **c** Electrical measurement using Keithley 6487 Picoammeter analyzer

supply. The probes were biased on the pads of the IDEs (Fig. 1c). The applied sweep potential was from 0 to 1 V, and the resistance and conductance graphs were plotted based on the potential of 0.5 V. The electrical measurement was applied at the end of each process with the same value of sweep voltage.

Surface functionalization on TiO_2-NPs for DNA detection

Functionalization process is crucially important for inorganic material (TiO_2-NPs) to have contact with organic sample (*E. coli* 0157:H7 DNA). Silanization is one of the surface-functionalization processes, which avail the self-assembly of triethoxysilane group of APTES on the hydroxyl group of TiO_2-NPs. A 2% APTES was diluted with ethanolic solution at 25 °C. TiO_2 surface was chemically modified by dripping 1.0 μL of 2% APTES and kept for 2 h in moist condition (> 80%). A covalent bond of –Si–O–Ti– is formed between the APTES mixture and the TiO_2 surface (Fig. 1b). The sample was rinsed with deionized DIW (dDIW) to remove the excess APTES and treated for 20 min at 50 °C in order to strengthen the bonding. Finally, the sensor was ready for electrical measurement.

DNA on TiO_2-NP–mediated transducer

Immobilization is a process to introduce single-strand DNA probe onto the modified surface of TiO_2. The modified probe (5′-COOH-AACGCCGATACCATTACTTATACCGC GACG-3′) was diluted in dDIW to be 10 μM. Then, 1 μL of the probe was placed onto the sensing area. The sample was then incubated for 2 h, and the excess DNA probe was washed by rinsing the surface with dDIW. The purpose of rinsing is to remove the unbound DNA probe residues. After that, the sensor was ready for electrical measurement. Basically, when the amino group (NH_2) from APTES, reacts with the carboxyl group (COOH) from the DNA probe, it forms an electrostatic interactions and amide bond. This peptide bond is formed by releasing H_2O molecule.

The hybridization process was carried out under the similar conditions with the immobilization process. Three different controls (i) target complementary DNA (5′-CGTC GCGGTATAAGTAATGGTATCGGCGTT3-’), (ii) single mismatch DNA (5′-CGTCGCGGTATAA**C**TAAT GGTATCGGCGTT-3′), and (iii) non-complementary (porcine DNA) were used for the analyses (Fig. 1b). The target complementary DNA was diluted serially with dDIW. The purpose of performing a serial dilution test is to obtain various concentrations (10 μM to 1 pM) of target complementary DNA. The 1 μL of complementary target DNA was further dropped on the immobilized probe. The electrical measurement was performed which contributed the sensitivity test for sensing. These methods were repeated on each concentration.

After completing the hybridization of *E. coli* O157:H7 target DNA measurement, the target DNA was dehybridized (denatured) to obtain the original reading of the transducer surface that has been immobilized with the DNA probe. The de-hybridization or denaturation process was implemented by dropping 1 μL of freshly prepared 0.1 M NaOH liquid on the sensing area that has been covered with targeted *E. coli* O157:H7 DNA. This process was conducted for 5 min at room temperature. The transducer was then rinsed with dDIW, blew for drying, and ready for the next sample hybridization process.

Specificity test

Further testing was performed for the specificity test of the sensor by varying the synthetic DNA controls: (i) complementary target DNA (1 μM), (ii) single mismatch DNA and (iii) porcine DNA. The test was carried out to prove the ability of the sensor to give the same conductivity performance with repetitive hybridization testing of samples. One microliter of single mismatch DNA was hybridized on the immobilized probe of a sensor. Then, the electrical measurement was performed. The steps were repeated for the porcine DNA. The electrical performances were observed and compared with the performance of the synthetic complementary target DNA at 1 μM concentration.

Reproducibility test

The reproducibility performance of the sensor was tested by using two synthetic DNA concentrations: (i) 1 nM and (ii) 1 pM. Lower DNA concentrations were selected as samples for this test in order to prove the device sensing ability towards these samples. The electrical performances of the synthetic target DNA were further used as a reference for real samples testing. Directly, we were able to estimate the concentration of the real samples by comparison.

Poultry sample DNA extraction and complementation analysis

The sensor was further tested with real samples. Chicken organs were used and the sample DNA was isolated from the recovered *E. coli* using QIAamp®DNA Mini purification kit and followed the instructions from Blood Mini Handbook. The extracted DNA was denatured into single stranded DNA using thermoshaker for 2 min. Then, it was quickly placed in ice for 5 min to retain the single-stranded DNA form. Without any further DNA amplification, a small volume of the extracted sample (1 μL) was then applied on the sensor. The sample was then incubated for 15 min, and the excess DNA was rinsed out from the surface using dDIW. Finally, the electrical measurement of the sample was observed.

Results and discussion

Choice of materials

TiO_2 is a metal oxide that has three phases which commonly known as brookite, anatase, and rutile [30, 31]. Among them, rutile is usually more stable than anatase at most temperatures and pressures [32, 33]. It has a wide bandgap ~3.2 eV and possesses unique electrical properties. This makes it to be an excellent candidate for DNA biosensor as the electrical reading will not be too low as it can interrupt the performance reading. In fact, the electrical reading will not be too high and prevents the DNA damaging. Furthermore, it is nontoxic, low-cost, and highly stable material in the harsh environments [34, 35]. As compared with ZnO, TiO_2 has stable active area for reusable biosensor as the structure is able to stand with multiple cleaning and drying processes (dehybridization).

Dimensions and parameters of TiO_2-NP–based resistive biosensor

In this research, two different TiO_2 thin films were synthesized via sol-gel method and spin-coating technique using TiB and TTIP as precursor, respectively. However, TiO_2 thin film with TTIP precursor had the most convincing properties. Hence, it was further selected to be used as an active area (sensing area) and fabricated as a complete resistive transducer. TiO_2-NP is an important mediator in biosensor that provides an effective surface for biomolecule immobilization [36] with desired size and growth rate. The morphological features of the TiO_2-NP information are as shown in supplementary explanation, E1, and figure supplementary, Fig. S1.

Resistive sensor for *E. coli* O157:H7 DNA

This sensor is eliminating a common step, which link the introduction between APTES and DNA. It has also been reported that glutaraldehyde is the best linker for the nucleic acid interaction. The purpose of the crosslinking is to make bonds stronger. Unfortunately, this binding can only withstand up to 14 days [37, 38]. Thus it is not suitable with sensor-device for commercialization purpose. Hence, the basic detection steps for this resistive sensor are (i) APTES silanization, (ii) DNA probe immobilization, and (iii) target DNA hybridization.

Specificity detection conditions for *E. coli* O157:H7 DNA

In this research, the specificity test of the TiO_2 NP–based resistive transducer for the detection of *E. coli* O157:H7 DNA was further verified by hybridizing the similar concentration (1 μM) of complementary target DNA, single mismatched target DNA and non-complementary target DNA. Three separate devices were used to conduct this test. Figure 2a (box chart) portrays the significant differences in conductance (ΔG) values for each DNA hybridization process. Upon hybridization with fully complementary target DNA and single mismatched target DNA, the ΔG values 39.6% and 36% were obtained, respectively. However, very

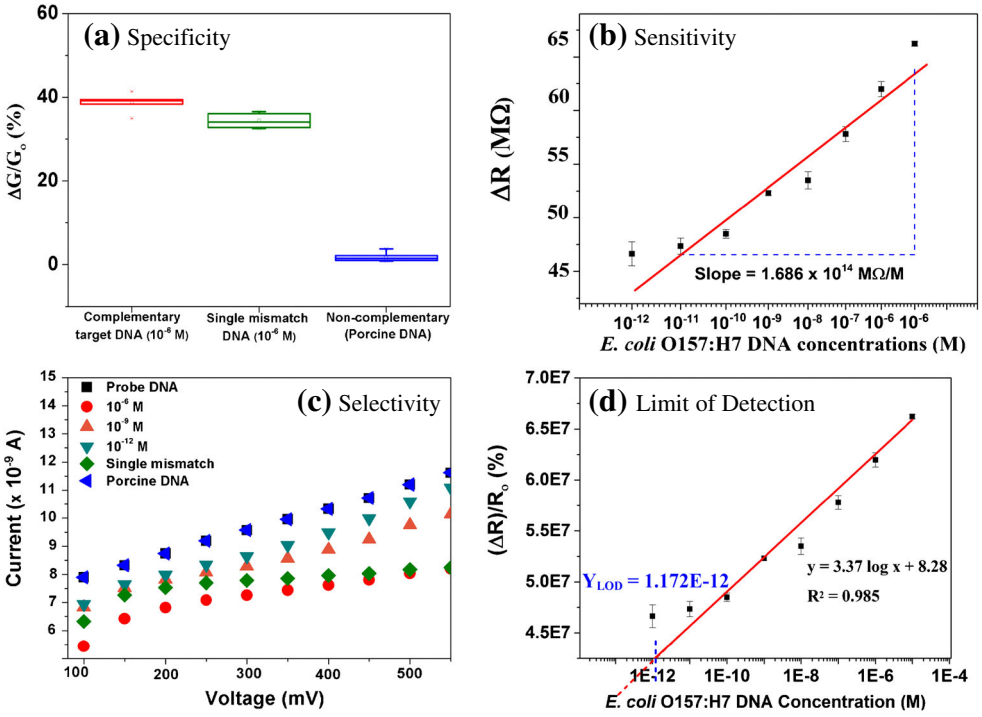

Fig. 2 Electrical measurements on TiO_2-NP–based resistive biosensor. **a** Hybridization conductance specificity of complementary *E. coli* O157:H7 DNA target, single mismatch target DNA and non-complimentary (porcine DNA) target; **b** ΔR response with different concentrations of *E. coli* O157:H7 DNA; **c** The response of the biosensor towards different *E. coli* O157:H7 DNA concentrations, porcine DNA and single mismatch DNA; **d** Calibration curve of the relative change in resistance with estimated LOD

minimal ΔG of 1.37% was recorded for the non-complementary target DNA. The magnitudes of ΔG values indicates that the sensor produces a large ΔG for the specific binding of target DNA with the immobilized probe compared with a low ΔG for improper binding with mismatched DNA target. A very low ΔG or close to no mass loading effect is recorded for non-complementary target DNA binding with the immobilized probe. This indicates that the transducer has a high specificity, which is able to discriminate among complete complementary target DNA, single mismatch target DNA, and non-complementary target DNA oligonucleotides.

Sensitivity and selectivity of *E. coli* O157:H7 DNA detection

Further tests were conducted on the device to determine the sensor's sensitivity. The resistance characteristics of this experiment at 0.5 V are shown in Fig. 2b. The device sensitivity test was performed by concentration-dependent resistance upon hybridization by the complementary target DNA. The output currents were used as an indicator towards each concentration of target DNA. The stability test for each concentration was further improved from previous study [39]. The effect of different concentrations of fully complementary target *E. coli* O157:H7, ranging from 1 pM to 10 μM were analyzed. As the target DNA concentration increased, there was a denser DNA hybridization on the surface, which consequents the increased mass loading effect. The sensitivity of the transducer is 16.69 MΩ/0.1 nM, which was calculated from the slope of the calibration curve. The current-voltage (*I*–*V*) characteristics exhibit significant differences in the measured output current with each hybridized targeted DNA (Fig. 2c). It was observed that, the decrement of target DNA concentrations (1 μM to 1 pM) were against the output current of the hybridized target DNA. This is due to the less number of targets DNA molecules, compared with the number of DNA probe molecules. The increment in output current is caused by decrease in number of negative phosphate ions originated. When the concentration of the targeted DNA was reduced to 1 nM and 1 pM, the negative ion of target DNA also decreases. Hence, the ion mobility of the electron decreases and causes the output current to decrease. Thus, the ion mobility of the electron increases when the concentration of the targeted DNA decreases.

The selectivity ability of *E. coli* O157:H7 DNA probe has been further assessed by dropping 1 μL of two target typologies: (i) single mismatch (synthetic single-strand target DNA) and (ii) porcine DNA (negative control of synthetic single-strand target DNA). The negative target DNA (1 μM single mismatch) lies near to 1 μM target DNA that shows 88% different. This is caused by the number of negative phosphate ions, which are slightly different with the positive control target DNA. Therefore, the output current is slightly different due to the single misplace (mismatch) of the DNA sequence. However, the current response towards the hybridization of porcine DNA lies exactly on the *E. coli* DNA probe (no changes). This indicated that there is no double-helix formation between the ssDNA of *E. coli* and the ssDNA of porcine.

In this electrical biosensor, limit of detection (LOD) is defined as the lowest detectable molar concentration of target DNA. It can be calculated as $Y_{lod} = Y_{blank} + 3SD_{blank}$, where Y_{blank} is mean of the blank measurement and SD_{blank} is the standard deviation of the blank measurement [11]. The LOD was calculated with 5 replicates by the standard deviation related to the response of resistive nanobiosensor towards the reduction of target DNA concentration. Figure 2d shows the extrapolated detection limit of the sensor to be 1.172×10^{-12} M (the linear range). However, the experimental plot shows the lowest detection limit is 10^{-10} M. The resistance values at lower concentration range of 10^{-11} M and 10^{-12} M were start to deviate from the extrapolated values. Hence, those data collectively demonstrated that our new device can provide a quantitatively measurement of *E. coli* O157:H7 with a detection limit down to 1×10^{-11} M using 1.0 μL volume of sample consumption in a time frame of 15 min.

Reproducibility of TiO_2-NP–based resistive biosensor

Further analysis was conducted to determine the reproducibility of the sensor in relation to 1 nM and 1 pM target DNAs

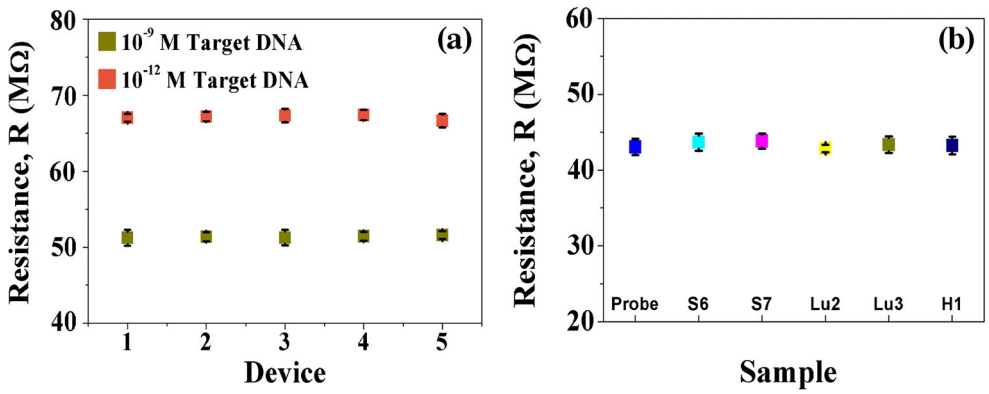

Fig. 3 TiO_2-NP–based resistive transducer for *E. coli* O157:H7 DNA; **a** Reproducibility test of TiO_2-NPs on five different transducers; and **b** Specificity test of poultry samples with the absence of *E. coli* O157:H7

Table 1 Comparison of performance on electrical biosensor for *E. coli* O157:H7 detection

Method applied	Material used	Immobilized molecule	Limit of detection	References
Resistive nanobiosensor	Titanium dioxide	DNA	1 pM in 15 min	Current work
Capacitance voltage	Silicon nitride	DNA	2 nM	[40]
Electrochemical sensing	Multi-walled carbon nanotube	DNA	0.5 pmole in 20 min	[41]

(Fig. 3a). From the test, the RSD values for 1 nM and 1 pM *E. coli* O157:H7 DNA are 5.12 ± 2.04% and 4.66 ± 0.91%, respectively, which indicates this TiO_2-NP–based resistive transducer has a good reproducibility with both DNA concentrations.

Table 1 compares our research with the reported researches on *E. coli* O157:H7 biosensors. This sensor can be considered as competent biosensor with the detection limit of 1 pM; 15 min detection time towards the electrochemical sensing, which has 0.5 pM detection limit and 20 min detection time.

Poultry sample DNA-based *E. coli* detection

Fifteen samples were obtained from different chicken organs, which include the heart, liver, lung, and swab. Those samples were diagnosed by both methods (i) conventional (from a private and independent diagnostic laboratory) and (ii) TiO_2-NP-based resistive biosensor. It was found that ten samples have infected by *E. coli* except S6, S7, Lu2, Lu3, and H1. Those samples with positive *E. coli* results were further classified based on their concentrations. Figure supplementary (Fig. S2) classifies type of samples and the resistance values which are comparable with the resistance value of the DNA probe. The electrical performance of the TiO_2-NP resistive sensor with the immobilized probe was used a reference towards the electrical performance of the real samples after hybridization. From the analyses, sample S1, S3, S4, and Lu4 have been categorized to have μM concentration of *E. coli*. Whereas, S5 and Li1 were at nM and pM concentrations, respectively. However, S2, Lu1, Li2, and Li3 target samples were identified to be infected by *E. coli* with higher than 1 μM concentration. The classification of the concentrations is represented at table supplementary (Table S1).

Next, we further specified the samples on the non-infected: (i) S6, (ii) S7, (iii) Lu2, (iv) Lu3, and (v) H1 for the stability test. Figure 3b shows the comparable results were obtained with six parallel experiments, and each sample was tested for quintuplicate. The uninfected samples were compared with the DNA probe because it acts as a reference signal. From the analysis, all samples were parallel with the DNA probe with RSD values are 4.5 ± 13%, 4.6 ± 14.3%, 4.5 ± 21.3%, 4.5 ± 19.8%, and 4.5 ± 23% for S6, S7, Lu2, Lu3, and H1, respectively. Based on these results, we confirmed the samples were free from *E. coli* O157:H7 and proved the good reproducibility of the biosensor. In addition, Fig. 3b is compared with the Fig. 3b, and there are significant difference between the uninfected samples and the complementary DNA with lower concentrations. The electrical signal for the uninfected sample is ~ 44.6 MΩ. In contrast, the signals of the complementary DNA are ~ 51.0 MΩ (1 nM) and ~ 67.3 MΩ (1 pM).

Conclusion

We demonstrated TiO_2 NP–based resistive biosensor with 15 min detection of *E. coli* O157:H7 DNA. In addition, we proved the concept that this electronic biosensor has a high capability to be used commercially in *E. coli* O157:H7 DNA detection. The responsivity of this resistive biosensor with a real sample of *E. coli* O157:H7 was identified by observing the electrical detection in response to the concentrations–dependent *E. coli* O157:H7 DNA. Without amplification of DNA fragments, the resistive biosensor is able to detect the extracted DNA, as low as 10^{-10} M concentration (LOD) with high specificity, repeatability, and reproducibility. This research is useful in making a novel electrical detection of *E. coli* O157:H7 DNA. Further study should focus on the effect of reduction of probe concentration to 1 μM towards reducing the LOD.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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