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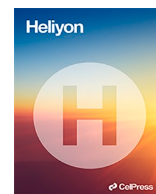

Research article

Conductometric immunosensor for specific *Escherichia coli* O157:H7 detection on chemically funcationalizaed interdigitated aptasensor

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ABSTRACT

Escherichia coli O157:H7 is a strain of *Escherichia coli* known for causing foodborne illness through the consumption of contaminated or raw food. To detect this pathogen, a conductometric immunosensor was developed using a conductometric sensing approach. The sensor was constructed on an interdigitated electrode and modified with a monoclonal anti-*Escherichia coli O157:H7* aptamer. A total of 200 electrode pairs were fabricated and modified to bind to the target molecule replica. The binding replica, acting as the bio-recognizer, was linked to the electrode surface using *3*-Aminopropyl triethoxysilane. The sensor exhibited excellent performance, detecting *Escherichia coli* O157:H7 in a short time frame and demonstrating a wide detection range of 1 fM to 1 nM. Concentrations of *Escherichia coli O157:H7* were detected within this range, with a minimum detection limit of 1 fM. This innovative sensor offers simplicity, speed, high sensitivity, selectivity, and the potential for rapid sample processing. The potential of this proposed biosensor is particularly beneficial in applications such as drug screening, environmental monitoring, and disease diagnosis, where real-time information on biomolecular interactions is crucial for timely decision-making and where cross-reactivity or interference may compromise the accuracy of the analysis.

1. Introduction

E. coli O157:H7 is a major foodborne pathogen that causes severe disease in humans globally [1]. According to the World Health

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Organisation, 220 million children suffer from diarrhoea each year, with 96 thousand dying as a result of consuming contaminated food and developing foodborne infections [2]. Foodborne outbreaks have been linked to a high morbidity rate, with 30% of the population at risk in technologically advanced countries [3]. They are expected to occur at a rate of 25,000 per 100,000 individuals worldwide, threatening human health and wealth. *E. coli O157:H7* is a pathogenic bacteria that can cause severe symptoms and potentially fatal diseases such as *hemorrhagic colitis* (HC) and *hemolytic uremic syndrome* (HUS), both of which can lead to kidney failure if not treated. It is currently spreading in both rural and urban regions, and it spreads quicker than other dangerous bacteria, making detection and treatment more challenging [4].

Several approach and devices have been proposed in the literature, however, the most effective tools are the biosensors based on interdigitated electrode (IDE), especially chemically surface modified IDE because the work on chemically functionalized interdigitated biosensors (IDE biosensors) represents a significant advancement in the field of biosensing technology [5]. While biosensors have been widely used for the detection and analysis of biomolecules, IDE biosensors offer several novel features and improvements over the state-of-the-art. The key novelty of the proposed IDE biosensor is it highly sensitive and selective nature [6]. The interdigitated electrode patterns on the biosensor surface provide a large surface area for biomolecule binding, thereby enhancing the sensitivity of detection. Additionally, the specific chemical coatings applied to the electrode surface enable selective binding of target molecules, minimizing interference from other substances present in the biological sample [7].

This combination of sensitivity and selectivity makes IDE biosensors ideal for accurate and reliable detection of biomolecules. Moreover, the novelty of IDE biosensor lies in it potential for personalized healthcare and continuous monitoring [8]. By integrating microfluidic devices, these biosensors can be used for real-time monitoring of biomarkers in bodily fluids, allowing for early detection and monitoring of diseases. This has the potential to revolutionize personalized healthcare by enabling proactive and targeted treatments. Furthermore, the proposed IDE biosensors offer advancements in the fabrication process [9]. The use of chrome masks in the photolithographic process allows for precise patterning of interdigitated electrode patterns, ensuring uniformity and repeatability in biosensor production [10]. This level of precision is crucial for achieving high sensitivity and selectivity in biosensor performance. Additionally, the integration of data analysis algorithms with IDE biosensors enhances their functionality. By analysing the signals produced by the binding of target molecules, these algorithms can provide valuable insights into the concentration and behaviour of biomolecules. This enables more accurate and detailed analysis of biological samples, leading to improved diagnostics and research outcomes. As stated earlier, the novelty of IDE biosensors lies in their highly sensitive and selective nature, their potential for personalized healthcare and continuous monitoring, advancements in the fabrication process, and the integration of data analysis algorithms [11]. These advancements have the potential to revolutionize the field of biosensing technology and open new possibilities in various domains such as healthcare, environmental monitoring, and food safety [12].

Therefore, the need for an effective, quick, specific, less expensive, but reliable approach to detect *E. coli O157:H7* in food items across the production and distribution chain is urgent. Traditional approaches have low sensitivity and produce a broad range of findings for certain viruses and illnesses, reducing efficiency and making it difficult to discover infected organisms [13]. To address this, an effective, quick, specific, less expensive, but reliable approach is needed. Hence, interdigitated Electrodes (IDE) are a novel approach for identifying *E. coli O157:H7 DNA* [14]. They can be assembled into compact lab-on-a-chip devices capable of assessing samples at milliampere (mA) to picoampere (nA) currents. The pico-ampere range of current-volt biosensing technology enables high-performance analysis of biological samples [15]. By using IDE, *E. coli O157:H7 DNA* sequences can be identified with extreme sensitivity, selectivity, and speed. This work developed a novel Al-IDE pattern to design a DNA sensor with excellent sensitivity, selectivity, and speed. It can detect *E. coli* O157:H7 at low fM levels and assess infection at low bacterial counts [16]. Photolithography and the metal-oxide-semiconductor approach were used to design Al-IDE electrodes on a silicon substrate. Al is the strongest conductive and low-cost material used in IDE, but other materials such as Cu, Pt, and Au are still used. Al-oxide has a wide range of properties, including thermal tolerance, chemical resistance, biocompatibility, hardness, and a large surface area. Ultra-sensitive biosensing systems based on Al have shown promise in recent years due to nanotechnology innovation [17].

Moreover, the detection of *E. coli O157:H7* based on Al-IDE electrodes allows for the identification of harmful virus and bacteria species, which could enhance food screening [18,19]. Chemically functionalized interdigitated biosensors have emerged as a powerful tool in the field of biosensing. These biosensors consist of interdigitated electrode structures functionalized with specific chemical coatings, enabling the detection and analysis of various biomolecules [20–22]. Thus, the objective of this study is to develop a highly sensitive, selective, and reliable platform for the detection and quantification of *Escherichia coli O157:H7*. The enhancement of sensitivity via modifying the interdigitated electrode surface with suitable chemical coatings for selectively capturing target *Escherichia coli O157:H7* will allow tailoring the device with specific chemical coatings that exhibit high affinity towards the target biomolecule, reducing interference from non-specific binding.

2. Material and method

The analytical grade chemicals utilised in this research included ethanol (*C2H5OH), 3-aminopropyl triethoxysilane (APTES)*, tween-20, and were all purchased from Sigma Aldrich in the United States. AIT Biotech Pte. Ltd. (Singapore) synthesised all 30-mer oligonucleotides *ssDNA* utilised in the analysis. These oligonucleotides were obtained from the GenBank database (Access number CP044143.1) with the sequences of the E. *coli O157:H7* gene. They are, probe *[5'-(COOH)–C6-AACGCCGATACCATTACTTATACCGCG ACG–3'], target complementary [5′-CGTCGCGGTATAAGTAATGGTATCGGCGTT–3'], non-complementary [5′-GCAGCGCCATATTCATTACCATAGCCGCAA–3'] and single-base mismatch [5′-CGTCGCGGTATAACTAATGGTATCGGCGTT–3'] sequences*. DNA oligomers (100 M) were preserved in the freezer at −20 °C for future use. AutoCAD was used to construct the Al-IDE mask. From Silterra (M) Sdn Bhd the chrome mask was purchased (in Malaysia). The Al-IDE was then physically characterised

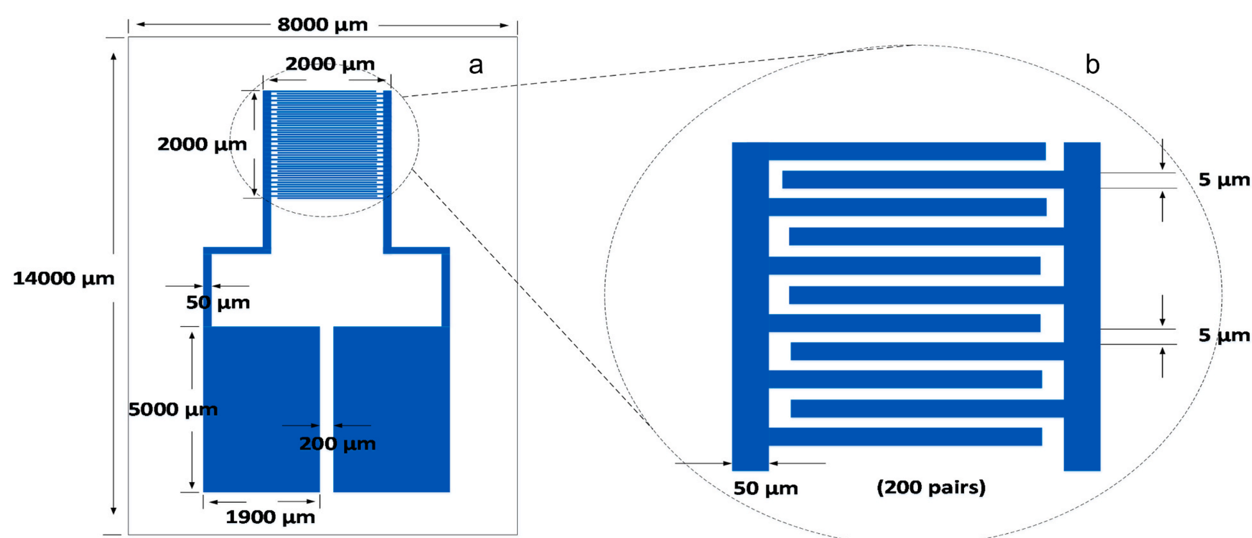

Fig. 1. The designs of the IDE chrome: Chrome masks are commonly used in the design and fabrication of biosensors to create interdigitated electrode (IDE) patterns. These patterns are essential for the functioning of biosensors by providing a platform for the detection and analysis of biomolecules. The chrome mask is used as a template to transfer the desired pattern onto the biosensor's surface. The (Figure (a) IDE type 1, (b) IDE type 2) show the IDE pattern design pattern and chrome mask).

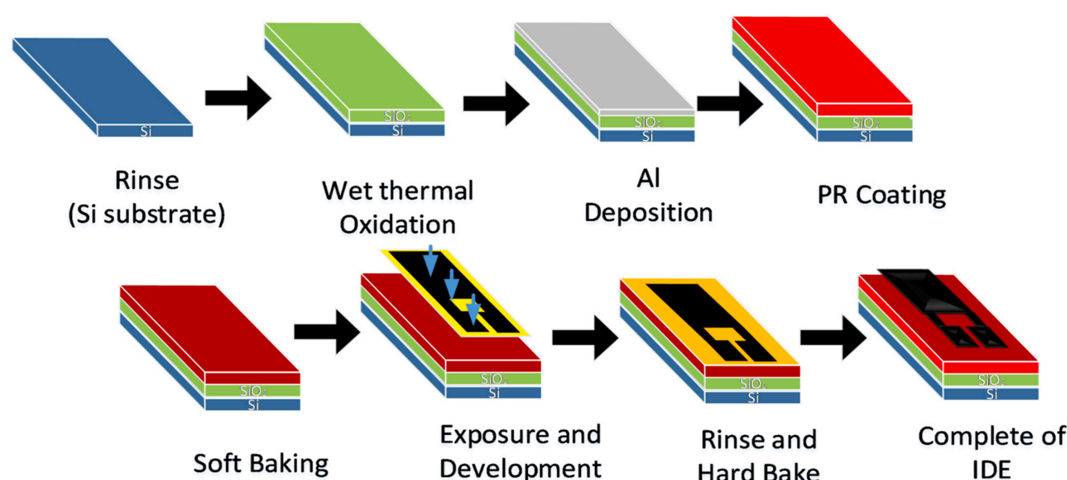

Fig. 2. Fabrication steps of AL IDE using photolithographic process: The oxidation process of silicon oxide (SiO2) involves cleaning the substrate with RCA1 and RCA 2 solutions to remove any foreign substrate. After the oxidation process, the surface is coated with aluminium by sputtering and the aluminum-deposited SiO2 sample is covered with positive photoresist (PR) and heated at 60 0C for 90 min. The soft bake technique is used to eliminate moisture from the surface and standing waves from the positive PR layer. UV light is used for 10 s to pattern transfer the IDE mask on the sample's surface. The development procedure lasts 15 s and the sample is baked at 1100 °C.

using a high-power and a low-power microscope. Al-IDE was further characterised using a scanning electron microscope and a 3D nano profiler. The Probe Station (Keithley 2450) and Kickstart apps were used to conduct current-voltage (I–V) characterization. To ensure the reproducibility of the methods used, all the design, fabrication, surface modification, detection and measurement process were repeated 5 times.

2.1. Overall design

The conductometric sensing approach was used to build a conductometric immunosensor for the detection of *Escherichia coli O157: H7*. 200 pairs of electrodes were developed and fabricated, followed by surface modification to attach to the target molecule replica. Conventional photolithography process was used to fabricate the IDE. A chrome glass wall was imprinted with a mask using a Piranha solution, thermally oxidised, coated with aluminium, hard-baked, and etched with an aluminium etching solution. The material was

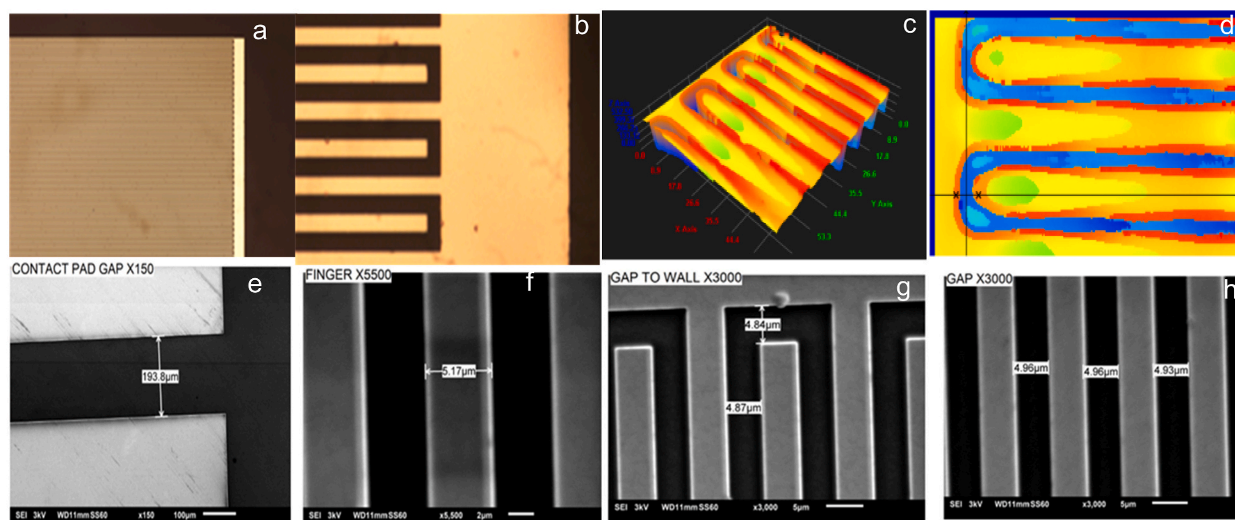

Fig. 3. Physical characterization of AL IDEs using, low, higher resolution microscopes and 3D profiler: The characterisation of AL IDE using a lower power microscope is shown in 3a. The characterization of AL IDE using a higher power microscope is shown in Fig. 3b. 3c depicts the 3D Profilometer's characterization of AL IDE. 3d depicts the characterization of AL IDE finger electrodes using a 3D Profilometer. 3e depicts the SEM characterization of two AL IDE electrodes. Fig. 3f depicts the SEM characterization of three AL IDE finger electrodes. 3g depicts the characterisation of the AL IDEs using SEM with disclosed finger electrodes and a gap size. 3 h depicts the SEM characterisation of the AL IDEs with revealed finger identical gap widths.

soaked in an aluminium etching solution before being washed with acetone. The sensor was able to detectidentify pathogens under ideal detection circumstances in a short period of time and demonstrated an excellent detection range of 1 fM to 1 nM. The sensor was simple, quick, extremely sensitive, and selective, with the potential for rapid sample processing.

2.2. Designing and fabrication of interdigitated electrode (IDE)

Chrome masks are commonly used in the design and fabrication of biosensors to create interdigitated electrode (IDE) patterns. These patterns are essential for the functioning of biosensors by providing a platform for the detection and analysis of biomolecules. The chrome mask is used as a template to transfer the desired pattern onto the biosensor's surface. The (Fig. 1 a&b) show the IDE pattern design pattern and chrome mask. This followed by the photolithography and the photolithographic process enables precise and reproducible patterning of biosensors, allowing for the creation of intricate interdigitated electrode patterns that are crucial for the functioning of these devices. It is a fundamental technique in the fabrication of biosensors and plays a vital role in achieving high sensitivity and selectivity in biosensing applications. The photolithographic process is a key step in the fabrication of biosensors using chrome masks. It involves a series of steps to transfer the pattern from the chrome mask onto the biosensor's surface, (Fig. 2).

A chrome glass wall was imprinted with the created mask and the designs of the IDE chrome masks are as shown. To eliminate undesired particles from the wafer substrate, P-type silicon substrates were cleaned using a Piranha solution, which is a mix of sulfuric acid and hydrogen peroxide. The wafer was then thermally oxidised for 1 h at 950 0C to produce a 50 m thick SiO_2 layer. The aluminium layer was subsequently coated onto the SiO_2 surface via sputtering. The design was produced using the conventional photolithography processes after the aluminium plate was deposited. The positive photoresist was coated on the aluminium plate using the spin-coating process.

The photoresist coated aluminium was baked for 1 min in a soft-bake mode at 90 °C. Following that, the IDE mask was put, and the pattern was subjected to UV light for 30 s. Using this method, the IDE pattern transferred to the wafer surface in approximately 30 s. After that, the aluminium and SiO_2 sheets were hard-baked for 1 min at 110 0C to eliminate moisture and increase adhesive strength. Because etching was the most essential fabrication stage for achieving the optimal pattern, it was done using a designed of experiment (DOE) using 3×3 factorials. Based on the enhanced results, the material was soaked for 20, 30 and 40 s in an aluminium etching solution with (10, 15, 20) mol/L HCl on 40, 50, 60 m Al thickness before being washed with acetone to remove the photoresist.

2.3. Development of the biosensor and biosensing process replication

The process of developing the biosensor in this study involves the design, surface modification detection, and measurement of biological molecules using the developed biosensor. This biosensor is designed to respond to a specific biological molecule (*Escherichia coli O157:H7*). The sensing mechanism is based on the interaction between the *Escherichia coli O157:H7* and the biosensor surface chemistry, which was introduced as a result of the surface modification, which produces a measurable signal in the form of a change in electrical All biosensing processes were replicated five times after getting biosensors designed and fabricated.

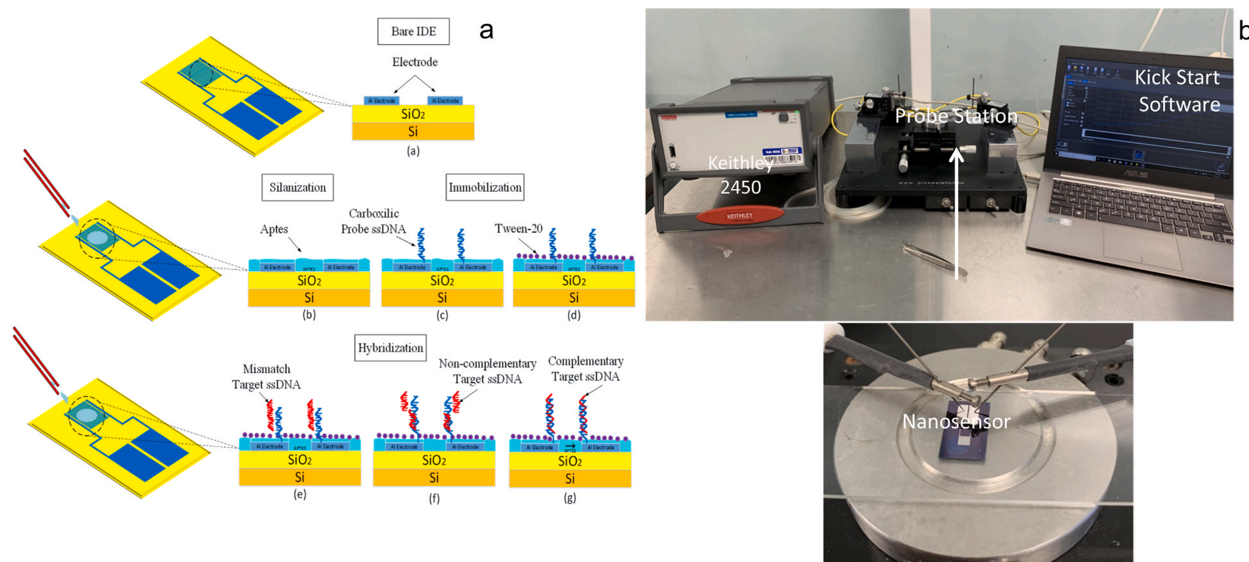

Fig. 4. Chemical Functionalization for Al-IDE Surface: The silanization method involves covering a surface with organofunctional alkoxysilane molecules (4a).Electrical Characterization using Probe Station (Keithley 2450) and Kickstart software was conducted (4b).

3. Results and discussion

Interdigitated electrodes (IDEs) are constructed from two or more interdigitated electrodes on a flexible plastic substrate with a band/gap size and two connecting tracks on a glass substrate. These IDEs have several benefits, including the flexibility to work with tiny amounts of sample and the avoidance of time-consuming solid electrode polishing. The interdigitated design frequently improves sensitivity and limits detection. They are appropriate for decentralised assays as well as the development of particular (bio)sensors. The biosensor prototype consists of a biological receptor and a transducer, as well as a signal processing device that often includes a display. The Al-IDE architecture is a structural design for a label-free, robust, and responsive biosensor device. Current voltage biosensors are among the most promising medicinal and food analysis platforms.

The majority of IDEs are current-voltage devices that may be developed based on platinum, titanium, iron, and gold, with silicon oxide and aluminium oxide being the least commonly utilised components. A novel Al-IDE pattern is being produced in the present research for DNA sequence identification. The designed mask are 8000 and 14,000 m in length and width, respectively. The distance between two fingers, finger width, finger length, distance between finger edges to electrodes, touch pad region, and active area are all 5 m, 2000 m, and 2000 m, respectively. The design was used to form 200 finger-electrode pairs in the active area (Fig. 1 a&b). Standard UV photolithography processes were used to produce the Al-IDE sensing surfaces (Fig. 2). The architecture was morphologically defined using imaging technologies prior to demonstrating the process of DNA sequence detection.

The DNA sequence of *E. coli* O157:H7 was determined dose-dependently using a range of chemical and biological changes. To validate the fabrication of intact Al-IDE surfaces, the fabricated Al-IDE surfaces were characterised using LPM, HPM, and a 3D nanoprofiler (Fig. 3a–d). Furthermore, the resulting surface morphology was confirmed by high-resolution SEM analysis, which revealed apparent gap and finger areas (Fig. 3e–h).

3.1. Morphological characterizations of AL IDEs using higher resolution microscopes

Physical characterization of AL (Aluminium) IDEs (Interdigitated Electrodes) is done using higher resolution microscopes based on High Power Microscope (HPM). These microscopes have the capability to perform high-resolution imaging, allowing for detailed analysis of the IDE structures. The HPM provides a magnified view of the IDEs, enabling observation of their morphology, size, and overall integrity. These characterization techniques are essential in evaluating the quality and performance of fabricated AL IDEs in biosensor applications. Thus, Fig. 3a shows the characterization of AL IDE using lower power microscope. Fig. 3b shows the characterization of AL IDE using higher power microscope. Fig. 3c shows the characterization of AL IDE using 3D Profilometer. Fig. 3d shows the characterization of finger electrodes of the AL IDE using3D Profilometer. Fig. 3e shows the characterization of two electrodes of the AL IDE using SEM. Fig. 3f shows the characterization of three finger electrodes of the AL IDE using SEM. Fig. 3g shows the characterization of the AL IDEs with revealed finger electrodes and a gap size using SEM. Fig. 3h shows the characterization of the AL IDEs with revealed finger equal gap sizes using SEM. The surface of Al-IDE's active region was examined at different magnifications using LPM and HPM images. Al-IDE finger electrodes embedded in the active surface layer were created with the precise required finger diameter, and the distance between the electrode fingers and the electrode was 5, 5 and 50 m, respectively. The images of the LPM and HPM indicate that the Al-IDE was appropriately made and ready to use. The image of soil topography showed a continuous

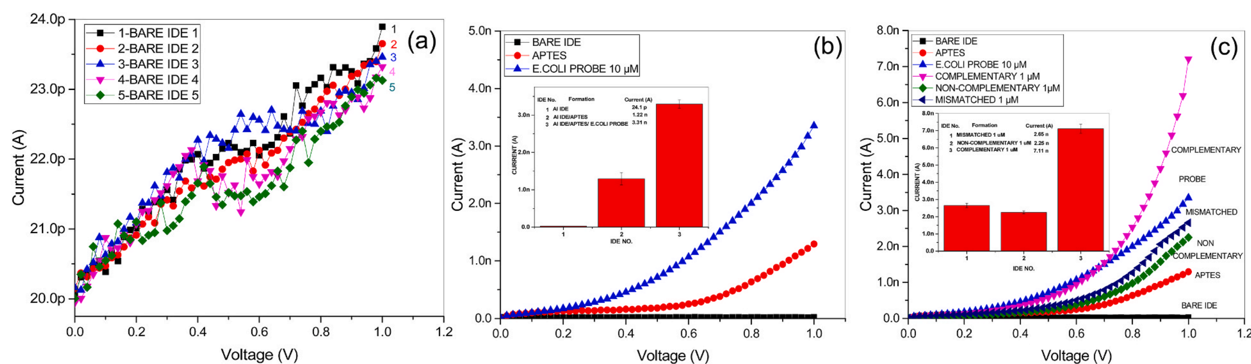

Fig. 5. Surface chemical functionalization and Selectivity Determination: Prior to functionalization with APTES, the 5 bare Al-IDEs were tested for I–V characteristics using a Probe Station (Keithley 2450) and Kickstart software (5a). 5b depicts the current-voltage characteristics of Al-DE after chemical functionalization with APTES and an *E. coli* O157: H7 probe. 5c depicts the complementary, non-complementary, and mismatched detections with current changes at 1 voltage.

structure with no shortages or contaminants.

The Al-IDE structure was determined by 3D profiler analysis, as well as the electrode size, which demonstrates that etching is at its highest level throughout development. The dimension findings in 3D mapping data were displayed with perpendicular resolution. The gap between the Al-IDE and IDE fingers was revealed, and it has been demonstrated that the IDE finger was fully developed from top to bottom. SEM was used to characterise the surface of the Al-IDE finger electrode images, demonstrating that the fingers were well-made and had smooth edges on the micro scale. SEM representations of the touch pad gap and electrode gap were 193.8 and 5.17 m, respectively. The gap to the wall and distance between the electrode fingers and the electrode were 4.84 and 4.96 m, respectively. EDX spectra were used to determine the amount of precipitation in the sensor system. Fig. 3e displayed the components Si, O, and Al as represented by Si layers, SiO2 layers, and Al finger electrodes. This showed that Al-IDE was effectively developed with no contaminations.

3.2. Chemical functionalization of the Al-IDE surface

The electrical functionalization of the Al-IDE surface can greatly improve the performance of biosensors by providing a stable and controlled environment for the immobilisation of biomolecules, such as antibodies or DNA probes. This enhanced surface functionality allows for more specific and sensitive detection of target analytes, making it a valuable technique in biosensing applications. Thus Fig. 4, show the functionalization of the Al-IDE surface based on salinization. The salinization method involves covering a surface with organofunctional alkoxysilane molecules, (Fig. 4a). As a result, it was starting to create a covalent *Si*—Si bond. APTES was most often employed in biosensor applications. Since the silane group may make powerful bonds with silicon substrates and its amine group can form covalent interactions with carboxylic groups. Firstly, the bare Al-IDE surface was cleaned with sodium hydroxide in order to improve the SiO2 layer via hydroxyl (NaOH). The active area was functionalized with 2% APTES by silanization with organofunctional alkoxysilane molecules (30% ethanol). 2 μL of APTES was applied to the active region and incubated for 1 h at 27 0C. *APTES* is an *aminosilane* that is widely employed during the salinization process to functionalize the surfaces of *alcoxysilane* molecules. To remove the unattached molecules, the coating was gently sprayed with DI water. Electrical Characterization using Probe Station (Keithley 2450) and Kickstart software was conducted (Fig. 4b).

3.3. Immobilizing E. coli O157:H7 DNA probe

For the immersive *E. coli O157:H7* investigation, 2 μL of *carboxylated* synthetic *ssDNA* probe oligonucleotide was employed after silanization. Both stock oligonucleotide solutions were resuspended in autoclaved ultra-pure water (>18 M) to produce a 10 M solution that was kept at −20 °C. After being put on the silane layer, the probe was maintained at room temperature for 1 h. The probe surface was then washed with DI water to remove any unattached DNA. The active area was then treated with 2 μL of diluted tween-20 for 15 min. The tween-20 was then rinsed off with clean DI water for 10 min. Tween-20 was used as a blocking agent to prevent unknown DNA targets from binding to the biosensor's surface.

3.4. Hybridization of E. coli O157:H7 target DNA

The biosensor was able to hybridise with complementary, non-complementary, and mismatched *E. coli* O157:H7 targets after probe immobilisation. Only the complementary of the target ssDNA was hybridised with the ssDNA probe. 2 μL of various targets were lowered onto the various active sections and incubated for 1 h to test the selectivity of the Al-IDE biosensors. In order to improve the incubation procedure, 2 l of complementary 30-mer ssDNA targets ranging from 1 fM to 1 M were coupled individually onto the active surface and incubated for 1 h. The active region was then rinsed to remove any remaining DNA targets. The obtained current-voltage

data were utilised to create a regression curve, and the lower range of the linear curve was used to calculate the sensitivity. The detection limit was calculated using three standard deviations.

3.5. Surface chemical functionalization and Selectivity Determination

Hybridization of *E. coli* O157:H7 target DNA is a process that involves the binding of the target DNA to complementary DNA strands. Thus, Fig. 5 show the process and analysis from bare Al-IDEs until the surface functionalization were analysed based I–V characteristics prior to functionalization with APTES using a Probe Station (Keithley 2450) and Kickstart software (Fig. 5a). A voltage source ranging from 0 to 1 V was employed for the I–V characterization. The Al-IDE sensor might be destroyed if the applied voltage exceeds the appropriate voltage level. The outcome was recorded in Microsoft Excel using the Kick Start application. Furthermore, an Al-IDE biosensor requires repeatability and stability testing before performing an actual target ssDNA sample analysis. Five Al-IDE devices were used to determine electrical characteristics. The graph shows approximately comparable results throughout the voltage spectrum. At 1 V, the newest variations for five separate bare Al-IDEs are 23.9 pA, 23.6 pA, 23.4 pA, 23.3 pA, and 23.1 pA, respectively. The typical low and high currents were 0.8 pA and 23.5 pA, respectively. Because of these significant characteristics, it is clear that the Al-IDEs were correctly developed and processed. The current in the Al-IDE was projected to grow as the number of chemical compounds on the sensor's substrate grew.

In other words, the trend implies that there is more chemical/molecule present, allowing more ions to enter into Al-IDE. The current-voltage parameters of Al-DE following chemical functionalization with APTES and an *E. coli* O157: H7 probe are shown in Fig. 5b. The functionalized field created with APTES is utilised to connect the organic and inorganic surfaces for the *E. coli* O157: H7 ssDNA probe. The amine group in APTES is positively charged. As the APTES during the silanization phase was reduced on the active region of Al-IDE, the current rose to 1.2 nA, and the gap between the two electrodes acted as a sensing buffer. Surfaces of SiO2 react with hydroxide ions to form SiOH, as shown in Equation (1), and resistance varies with resistivity. The length and area between the fingers of an Al-IDE became fixed as the density of APTES on the Al-IDE surface decreased, resulting in an increase in the density of APTES owing to the amount of hydroxide ions (OH-). As seen in Equation (2), increasing conductivity leads to an increase in current range.

$$Resistivity, R = \frac{\rho \iota}{A} \tag{1}$$

$$Conductivity, \sigma = \frac{\iota}{\rho} \tag{2}$$

Because *E. coli* O157: H7 ssDNA samples serve as a bio-receptor for the specific capture *E. coli* O157: H7 aim ssDNA, a 2 μL of *E. coli* O157: H7 carboxylated probe at a concentration of 10 M was immobilised on top of the APTES sheet. The carboxylic group increases the mobility of oligonucleotides with lower adsorptive interactions. According to the curve in Fig. 5b, the current recorded at 1 V for the *E. coli* O157:H7 probe is 3.3 nA. The amount of surface charges on the APTES increases because the ssDNA is negatively charged. The accumulated positive charge flows to the negative side, raising the electric field [8]. The greater current is due to *E. coli* O157: H7 producing a stronger electric field.

Fig. 5c depicts the selectivity computation analysis of various parameters, including complementary, non-complementary, and mismatched target sequences. Fig. 5c shows the complementary, non-complementary, and mismatched detections at one voltage, the current changes were observed at: 7.1 nA, 2.25 nA, and 2.65 nA, respectively. Surface amine molecules were coupled DNA nucleotides on the AI-IDE. Dropping a small molecule blocking agent on the sensor surface and molecular linkage can increase the efficiency of conjugation [9]. Likewise, the attachment of immobilised probes to molecular sequences can be improved. The charge of the DNA from the material changed significantly after hybridization. As a result, the DNA maintains its combined charge and interacts with the positively charged APTES substrate, completing the reaction and complementing one another throughout the measurements. Although immobilisation and hybridization operations with synthetic probe targets provide a significant electrical signal, the current levels are almost identical when the probe reacts with non-complementary and mismatched targets. This is owing to the probe's failure to completely bond through the molecules under consideration.

Moreover, the APTES layer acts as a sensory connection between two electrodes in this situation. When the voltage difference between two electrodes is given and the voltage is gradually increased, the stored positive charge begins to flow to the negative side. The I–V characteristics of the biosensor following hybridization with 10 μm concentration *E. coli* O157:H7 complementary target ssDNA are shown. The total number of free positive charge carriers in the APTES layer increases as the overall negative charge of the DNA probe and target pairing increases. This sensing component affects the resistivity in the APTES layer due to the concentration of the target ssDNA collected by ssDNA probes, acting like a potentiometer, with the target ssDNA serving as a molecular gate [9,10]. As a result, the resultant current increased rapidly.

3.6. High-performance analysis: sensitivity & limit of detection

High-performance analysis of biosensors involves evaluating their sensitivity and limit of detection. Sensitivity refers to the ability of a biosensor to detect small changes in analyte concentration accurately. It is a measure of the biosensor's responsiveness to changes in the target molecule. The limit of detection (LOD) is the lowest concentration of the analyte that can be reliably detected by the biosensor. It indicates the biosensor's ability to detect and quantify low levels of the target molecule. These parameters are crucial in

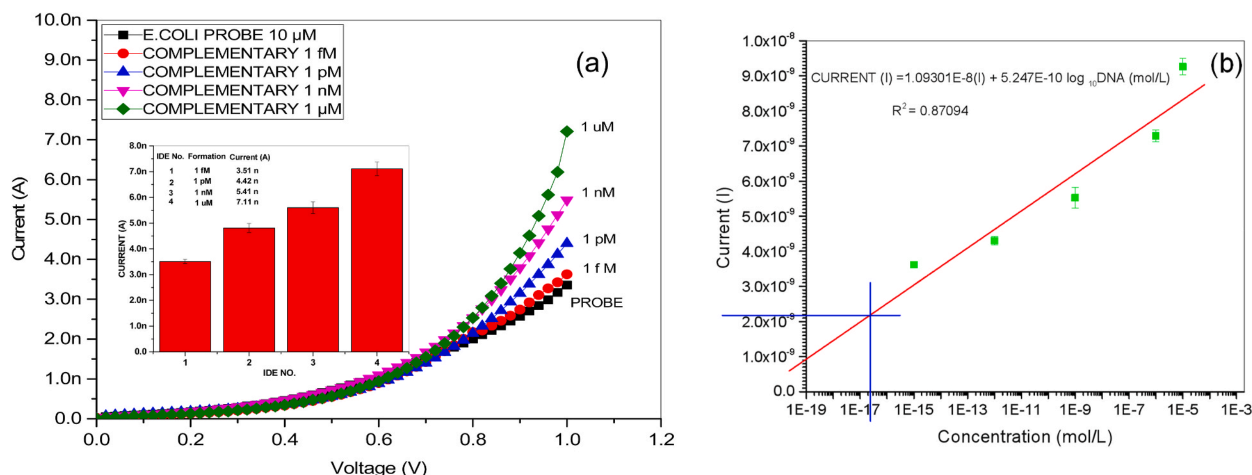

Fig. 6. Sensitive detection and linear regression analysis: Throughout this experiment, various amounts of probe target were used to assess sensitivity (6a). A linear regression curve for different target probe sequence concentrations is also shown in (6b) synuclein. The complexed regions are indicated by the structure obtained from docking analysis.

determining the effectiveness and usefulness of biosensors in various applications, such as clinical analysis, environmental monitoring, and food safety. Biosensors with high sensitivity and low LOD are desirable as they can provide accurate and reliable results even at low analyte concentrations. Thus (Fig. 6), show the results of the analysis and based on the observations, it was inferred that the existing alterations were steadily amplified as the target DNA concentration at 1V increased. Concentrations ranging from 1 fM, 1 pM, 1 nM, and increased concentrations of 1 M resulted in current values of 3.5 nA, 4.4 nA, 5.4 nA, and 7.1 nA, respectively. Fig. 6a shows that when the concentrations of target probes increase, the current becomes precisely proportional. Fig. 6b also shows a linear regression curve for varied target probe sequence concentrations. This biosensor (AI-IDE), according to the findings, is capable of detecting and measuring target concentrations at low and high levels, as well as increasing complementary target concentrations; the probe can efficiently measure complementation.

4. Conclusions

The research revealed an optimised technique for producing AL-IDE that was evaluated against *E. coli*. The results show that it is a very effective biosensor that responds to varied amounts of complementary DNA sequences from *E. coli* O157:H7. It has been demonstrated that a high-performance sensor can distinguish between non-complementary and mismatched ssDNA target sequences and has higher stability, selectivity, and sensitivity. This biosensor invention aims to solve the present limits of most sensors manufactured in a typical method with limited resources by integrating Al-IDE, APTES, and the carboxylic probe ssDNA. This Al-IDE is capable of reacting to the present extremely low quantity of *E. coli* O157: H7 DNA. Its simplicity of construction, along with its capacity to detect *E. coli* at extremely low concentrations, proves its medical and food industry relevance. This sensor, we believe, might be a game changer for the threat posed by possible new viruses. It might be used to detect not just *E. coli* but also newly developing Covid-19 viruses at the carcinoma level.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

CRediT authorship contribution statement

Muhammad Nur Afnan Uda: Resources, Conceptualization. **Alaa Kamal Yousif Dafhalla:** Writing – review & editing. **Thikra S. Dhahi:** Writing – review & editing. **Tijjani Adam:** Writing – original draft, Validation, Conceptualization. **Subash Chandra Bose Gopinath:** Writing – review & editing. **Asral Bahari ambek:** Conceptualization. **Muhammad Nur Aiman Uda:** Methodology. **Mohammed Mohammed:** Writing – review & editing. **Nor Azizah Parmin:** Conceptualization. **Nur Hulwani Ibrahim:** Methodology. **Uda Hashim:** Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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