Aluminium Interdigitated Electrode Based Biosensor for Specific ssDNA Target Listeria Detection

M. N. Afnan Uda¹*, Asral Bahari Jambek¹, U. Hashim¹, ², M. N. A. UDA³ and M. A. F. Bahrin¹

¹School of Microelectronic Engineering Group, Universiti Malaysia Perlis (UniMAP)
Putra Campus, 02600 Pauh, Perlis Malaysia
²Institute of Nano Electronic Engineering (INEE), Universiti Malaysia Perlis (UniMAP), 01000 Kangar Perlis Malaysia
³School of Bioprocess Engineering Group, Universiti Malaysia Perlis (UniMAP) 02600 Arau Perlis

Email: *nurafnan92@yahoo.com

Abstract. Nowadays interdigitated electrode (IDE) based sensor have stimulated increasing interest in the application of biosensor filed. A large number of finger electrodes as comb structure gain high sensitivity through electrical measurements. In this paper, we have investigated Listeria bacteria detection through the electrical based IDE. Listeria monocytogenes is a food borne pathogen-based bacterium that can cause dangerous disease to human, some infection may result in death. The AutoCAD software was used to design the chrome mask of IDE sensor and the fabrication process was done using conventional photolithography method. The fabricated Al IDE morphologically analyzed using a low power microscope (LPM), a high power microscope (HPM) and 3D profiler. Functionalization step of the Al IDE, silanization process was done using (3-Aminopropyl) triethoxysilane (APTES), immobilization process was done using carboxylic probe Listeria and Tween-20 as a blocking agent for nonspecific binding on the non-immobilized area of the biosensor surface. The biosensor was validated with complementary, non-complementary and single base mismatch ssDNA targets. Different concentration of complementary ssDNA target from 1 fM to 1 µM was done for the sensitivity detection.

1. Introduction
Listeria monocytogenes is a facultative intracellular pathogen that can causes listeriosis, particularly in pregnant women, new born and adult with weak immune systems. This bacterium is widespread in the environment and infections occur through ingestion of contaminated food [1],[2]. Fever, muscle aches and diarrhea are the symptoms caused by Listeria. If the infections spread to the nervous system, symptom such as headache, stiff neck, confusion, loss balance can occur [3]–[6]. Based on the International Journal of Food Microbiology reported that the prevalence of Listeria in raw chicken (60%), raw beef (50%), fresh prawns (44%) and leafy vegetables (22%). It is found that the occurrence of Listeria in imported frozen beef was 75%, 30.4% in local beef samples and 12% in fermented fish. Therefore, higher percentage of Listeria in food will give dangerous illness to people. Recently, conventional method has been introduced to detect foodborne pathogens illness. Conventional method like Polymerize Chain Reaction (PCR) has already been developed but it required expensive equipment, skilled personal and labor intensive gel-based detection that shows feeble sensitivity and specificity [7]–
Previously a lot of methods in developing biosensor for modifying sensor surface have been introduced [11]–[13]. However to detect foodborne pathogens disease there is some problem in detecting very small concentration down to fM range concentration of ssDNA target [14]–[16]. In this research, we introduce and develop a sensitive, selective and rapid biosensor using Al IDE to detect ssDNA Listeria bacteria. The major steps of functionalization as biosensors are, silanization using APTES, immobilization using carboxylic ssDNA Listeria probes and blocking the non-immobilized area using Tween-20 [17], [18]. Then, the functionalized biosensor was hybridized with complementary, non-complementary and single base mismatch ssDNA. Fabricated Al IDE is physically characterized using a low power microscope (LPM), high power microscope (HPM) and 3D profiler. The I-V characteristics were performed for every functionalization steps, selectivity, and sensitivity measurements.

2. Material and methods

2.1 Chemical, Reagents and Instruments

The materials Ethanol (C2H5OH), Sodium Hydroxide (NaOH), (3-Aminopropyl) triethoxysilane (APTES) and Deionized water (DI Water) and they were obtained from Sigma Aldrich USA. All of the other chemicals were analytical reagent grade and purchased commercially. Deionized water (Di Water) was used throughout this experiment. The 30-base synthetic oligonucleotides were purchased from AIT Biotech Pvt. Ltd (Singapore). Table 1 shows the Carboxylic modified synthetic probes and target oligonucleotides sequences.

| Oligonucleotide          | Sequences                  |
|--------------------------|----------------------------|
| 30-mer probe             | 5’-(COOH) CGC GCG GCA TCC |
|                          | GCA TCA ATA ATA CCG GCC –3’|
| 30-mer complementary     | (5’- GGC CGG TAT TAT TGA  |
|                          | TGC GGA TGC CGC GCG –3’)  |
| 30-mer non-complementary | (5’- CCG GCC ATA ATA ACT  |
|                          | ACG CCT ACG GCG CGC –3’)  |
| base mismatch            | (5’- GGC CGG TAT TAT TGT  |
|                          | TGC GGA TGC CGC GCG –3’)  |

The Al IDE mask was designed using AutoCAD Software. After that, the design is transferred to a commercial chrome mask, Silterra (M) Sdn Bhd to fabricate the Al IDE. LPM, HPM, and 3D profiler were used for physical and morphological characterization of bare Al IDE. Lastly, the I-V characterization measurements were completed by using (Keithley 2450), Kickstart software and Probe Station.

2.2 Silanization, Immobilization and Hybridization

First, the active area of Al IDE was washed with NaOH to make hydroxyl layer on the SiO2 surface. After that, the bare Al IDE surface was functionalized with APTES. 2 µl APTES was dropped on the Al IDE active area. It will build an ‘active’ layer on the Al IDE. It contains hydroxyl group which attack and displace the alkoxy group on the saline and thus forming a covalent of -Si-O-Si- bond [14-15]. Then, the IDE was incubated for 1 hour inside the dry cabinet and rinsed with DI Water. After that, 2 µl of DNA probe was immobilized on the surface. Form the reaction, it will form a recognition layer of the covalent amide bond between carboxyl group [16-18]. Then, let it to dry for 1 hour inside the dry cabinet. Then, rinsed with DI water to remove unbounded probe DNA on the surface. After that, 2 µl of tween-20 was dropped on to the functionalized area and kept 15 minutes inside the dry cabinet. Remove the Tween-20 by using DI water and left for 5 minutes to dry. Tween-20 was performed as a blocking agent, to repeal unspecific ssDNA target binding on the biosensor surface. Thus, the target ssDNA unable to direct bind with APTES layer. Then, the Al IDE biosensor prepared to be hybridized. The
A functionalized IDE biosensor was validated using the hybridization process. It was accomplished using different target ssDNA Listeria. The hybridization process was done by synthetic sample of complement, non-complement and mismatch target on the active area of Al IDE’s surfaces. 2 μl of DNA targets from 1 fM to 1 µM were dropped on Al IDE biosensor to hybridize with immobilized ssDNA probes for sensitivity measurements. Then, left the hybridization process for 1 hour. After that, rinsed the Al IDE surface with DI water to remove unbounded DNA target. The I-V measurement measured nano ampere range changes through the silanization, immobilization, and hybridization process. Figure 1 shows the process preparation step of sensing surface Al IDE.

3. Result and Discussion
3.1 Surface Characterization
The physical characterization of Al IDE surface is displayed in Figure 2. Physical characterization is one of the most essential steps in making sure that there were no shortage on the Al IDE surface. A part from that, it is also to observe the material composition on Al IDE surfaces and to examine the existence of particles or squalor on top of IDE surface. It is significant to make sure the fabrication process is done with circumspect to ensure the measurement is not affected during the characterization process. All images have been taken using LPM and HPM with 5x, 10x, 20x and 50x resolution power. The surface topography shows uniform structure without shortage and any contaminants on the surface.
the grain with 532 nm and the average height is 366 nm, were confirmed by the uniformity of the surface. It is confirmed that the etching has reached the maximum developed design.

![Image](image_url)

**Figure 3.** 3D profile of the IDE surface and specific parameters.

### 3.2 Electrical Characterization for Listeria Detection

Figure 4 shows the I-V characterization was performed by using (Keithley 2450), probe station and Kickstart software with Al IDE biosensor. The voltage between the two electrodes was being prescribed from 0 V to 1 V. Electrical measurement of Al IDE’s structured is proposed and the result can be determined by electrical characterization current voltage I-V characterization for more accurate measurement. From the I-V measurement, the bare Al IDE current at 1 V was 2.24 x 10^{-11} A. I-V characteristics of biosensor preparation process of different steps, after salination, after immobilization and test with complementary ssDNA which is hybridization process.

![Image](image_url)

**Figure 4.** Electrical Characterization measurement using (Keithley 2450), Kick start software and probe station with Al-IDE biosensor.

The current keep increasing to nA range after silanization with APTES. APTES to provide contact between the organic and inorganic surfaces of a single-stranded DNA probe. The current captured at 1V shows the value which was 1.2 x 10^{-9} A. The current value then continuous increase after ssDNA probe was immobilized on the top of APTES layer. The current captured at 1V shows the value which was 3.35 x 10^{-9} A. Finally, the 2 μl of 1 nM concentrated Listeria ssDNA target was hybridized on the probe Listeria. The current captured of the target at 1 V is 5.4 x 10^{-9} A. DNA is negatively charged and after hybridization with complementary target ssDNA, free total positive charge carrier in APTES layer is increased because of the increment of the total negative charge of DNA probe and target. This sensing component changes the resistivity in the APTES layer according
to the concentration of target ssDNA which are captured by ssDNA probes. Hybridization of synthetic target ssDNA can cause electric field increase. So current will decrease when electric field is increase. The current generation mechanism can be explained using following equations.

\[
\text{Conductivity, } \sigma = \frac{l}{p}
\]

\[
\text{Resistivity, } R = \frac{pl}{A}
\]

\[
\text{Electric field, } E = \frac{kQ}{d^2}
\]

Figure 5 shows the selectivity measurement that was tested for complementary, non-complementary and single base mismatches target of Listeria separately. 1 nM concentrated targets and 2 μl volume was used for selective measurements. Curve 3 until 6 shows the Listeria Probe, complement, non-complementary and mismatch measurement captured at 1V are 3.3×10^{-9} A, 5.4×10^{-9} A, 1.8×10^{-9} A and 2.1×10^{-9} A respectively. The accessibility of immobilized probes to complementary target sequences can be enhanced by treating the surface with a small molecule blocking agents. Moreover, carboxylic group rapidly displaces the weaker adsorptive contact between oligonucleotides leaving the probes was moored probe through carboxylic end group. While, for probe and non-complement current curve shows closely same value because non-complementary ssDNA cannot be bind with immobilized ssDNA probes. As well as they cannot bind with APTES layer due to the blocking agents cover the area which was unbounded by immobilized ssDNA. Thus, non-complementary target ssDNA thoroughly removed after washing with deionized water.

Figure 6 shows the sensitivity measurement using different concentration target Listeria. The current variations were steadily increased based on the increment the target ssDNA concentration as 1 fM, 1 pM, 1 nM, 1 µM and current value at 1 V are 3.6 x 10^{-9} A, 4.1 x 10^{-9} A, 5.4 x 10^{-9} A and 6.1 x 10^{-9} A respectively. From the graph, shows that the current will continue to increase when the concentration of target Listeria increase.
Figure 6. Graph of I-V for Al IDE with functionalized Al IDE surface and different concentration target Listeria.

4. Conclusion
In this paper, we described an approach for Listeria target ssDNA via electrical Al IDE based biosensor. The developments of biosensor based on Al IDE are very efficient. This IDE can directly detect current from very small range up to large range of current with different concentration of Listeria targets. Thus, the result shows that it is high in stability, selectivity and sensitivity in detecting complement, non-complement and mismatch target of Listeria. The sandwich layer of Al IDE which includes the salinization of APTES, immobilization of Listeria probe and the hybridization of Listeria target was also designed to overcome the selectivity problem for conventional methods like the PCR. Thus, we hope the conception of the mechanism and biosensor to be beneficial for commercial diagnostic applications.

Acknowledgement
The author would like to thank all staff members of the Institute of Nanoelectronic Engineering in Universiti Malaysia Perlis (UniMAP) for their technical advice and contributions, directly and indirectly. Collaborative Research in Science and Technology Center (CREST) is acknowledged for providing grant for this research program.

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