Electrical DNA Biosensor Using Aluminium Interdigitated Electrode for Salmonella Detection

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

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 there are many alternative methods that have been discovered and developed for the rapid detection of foodborne pathogens that can cause food poisoning. Unfortunately, majority of them still requires improvement in sensitivity and selectivity issues to be of any practical use daily. In this research, biosensors was prepared from 5 µm gap Aluminium interdigitated electrode (Al IDE) to detect Salmonella enterica typhi (S. typhi). The IDE sensors in the biosensor field is extremely interest in these days due to the high number of finger electrodes as comb structure which can gain high sensitivity through electrical measurements. S. typhi is a serious food borne pathogen, makes typhoid disease which causes many deaths annually in worldwide. Functionalization steps of the Al IDE to create biosensor was based on silanization by APTES, immobilization with carboxylic functionalized S. typhi ssDNA probes and blocking agent with tween-20 were the major functionalization steps. The functionalized steps were electrically characterized using current voltage measurements. The selectivity measurement was performed with specific target was identified electrically using complementary, non-complementary and single base mismatch ssDNA target.

1. Introduction

Foodborne outbreaks have been reported with significant morbidity worldwide and pose risk towards human population [1-2]. Most commonly, the outbreaks take place due to the ingestion of pathogenic bacteria like Salmonella [3-4]. Salmonella enterica typhi (S. typhi) is one of the most commonly reported serotypes which causes typhoid fever, affects roughly 17 million people annually and causing nearly 600,000 deaths. These serotypes which can be transferred from animal-to-human or from human-to-human [5]. Therefore, generation of the right sensing approach for Salmonella will pave the way for easier detection and curing.

Most of the conventional method for Salmonella detection still lack of sensitivity [6]. Apart from that, it can only be detected after the bacteria separated all over the body. Due to that, electrical based biosensor technology with nano amperes range will help healthcare and food industry to get early and accurate detection [6-8]. Commercial application of biosensors are given significant impact on the medical and food analysis to overcome the drawbacks in conventional methods [9-11]. Nowadays, biosensor researches are widely concentrated, numerous research and excellent reviews have been
published [12-21]. Moreover, the high sensitivity, selectivity, repeatability and real-time in field detection are the main advantages of biosensors [22-27].

In this research, electrical based Al IDE on silicon substrate is fabricated. The fabricated Al IDE is physically characterized using a low power microscope (LPM) and high power microscope (HPM). The functionalization steps as biosensor are, silanization using APTES, immobilization using carboxylic ssDNA probes and blocking the non-immobilized area using tween-20. I-V characteristic were performed for selectivity detection by hybridizing with synthetic ssDNA samples as complementary, non-complementary and single base mismatch targets by using Keithley 2450, Kickstart software and probe station.

2. Material and methods
2.1 Chemicals, Reagents and Instruments
Ethanol (C2H5OH), Sodium Hydroxide (NaOH), (3-Aminopropyl) Triethoxysilane (APTES) (C9H23NO3Si), Deionized Water (DI) was obtained from Sigma Aldrich, USA. All of the other chemicals were analytical reagent grade and purchased commercially. Deionized Distilled Water (DDI – water) was used throughout this experiment. The 30-base synthetic oligonucleotides were purchased from AIT Biotech Pte. Ltd (Singapore). IDE mask was designed using the aid of AutoCAD Software for the detection of ssDNA which an extremely small scale in size before being transferred to commercial chrome mask. The design and mask then sent to Silterra (M) Sdn Bhd to fabricate the Al IDE. Al IDE surface was checked using Low Power Microscopy (LPM) and High Power Microscopy (HPM) for surface characterization. The measurements for I-V characteristics were carried out by using Keithley 2450, Kickstart software and probe station.

2.2 Al IDE surface functionalization

Figure 1 shows the schematic diagram of the functionalization process of biosensor. First, the active area of the bare Al IDE surface was washed with NaOH to enhance hydroxyl on the SiO2 layer. Then, the bare Al IDE surface was functionalized with 0.1 M, 2µl of APTES. Salinization is the covering of a surface with organofunctional alkoxysilane molecules. Mineral components of Silicon dioxide surfaces can all be salinized because they contain hydroxyl groups which attack and displace the alkoxy groups on the saline thus forming a covalent –Si-O-Si- bond. The silanized sensor was incubate 60 min and DI water was used to wash the unattached APTES molecules. After that, 10 µM, 2 µl of carboxylic probe ssDNA was used for immobilization process. The immobilized sensor was incubate for 60 min and DI water was used to wash the unattached ssDNA probes from the APTES layer on the active area. The stock solutions of all oligonucleotides were prepared in autoclaved ultrapure water (> 18MΩ) to obtain a 10µM solution and kept frozen (-20°C). Then, tween-20 was used to block the non-immobilized area to avoid the attachment of non-specific targets binding. The sensor was incubate for 30 minute and DI water was used to wash the unattached tween-20 molecules. The hybridization reaction was tested with the complementary, non-complementary and mismatch target ssDNA by dropping 10 µM, 2µl to the active surface area in the biosensor surface. The hybridized sensor was incubate 30 min. After that, the surface was washed with DI water to remove non-hybridized ssDNA target from the biosensor surface.
Then electrically characterize by Keithley I-V measurement to determine and investigate the current changes after each process.

3. Result and Discussion
3.1 Surface Characterization
The fabricated Al IDE was morphologically characterized using LPM and HPM for 20x resolution power. It is significant to make sure the fabrication process is done with circumspect to ensure the measurement is not affected during the characterization process [4]. Figure 2(a) shows the LPM image surface of the well fabricated IDE which has two electrodes that connected with finger comb structured electrode surface. Large number of fingers was fabricated properly. Figure 2(b) shows the HPM image surface topography shows uniform structure without shortage and any contaminants on the surface.

![Low power Microscopic (LPM) and High power Microscopic (HPM) images](image)

Figure 2. Al IDE surface characterization using (a) Low power Microscopic (LPM) and (b) High power Microscopic (HPM) images.

3.2 Electrical Characterization
Electrical characterization was done using picoameter voltage source Keithley 2450, Kickstart software and probe station to get I-V characteristics as shown in Figure 3. The voltage between two electrodes was being prescribed from 0V to 1V. If voltage applied is higher than operating voltage, then sensor maybe damage [14],[28].

![Keithley I-V measurement](image)

In this work, electrical measurement of Al IDE’s structured is proposed and the result can be determined by electrical characterization by current voltage I-V characterization for more accurate measurement. Figure 4 shows the graph for two Al IDEs has been electrically characterized. Both Al IDEs shows the slight voltage variation at 1V. The maximum current for first and second IDEs are $2.655 \times 10^{-11}$ A and $2.747 \times 10^{-11}$ A. Due to that current range an IDE are well fabricated and processed without any shortage. In additions, these results were confirmed that Al IDE was prepared with nearly similar dimensions and parameters. The probability of the IDEs to be shorted is high during the
production process due to small finger comb dimension. So, its need to be measured repeatedly to ensure no shortage happens before proceeds with other process.

Figure 4. Graph of Current Vs Voltage for Bare Aluminum Interdigitated Electrode (Al IDE).

Figure 5 shows the I-V characteristics of biosensor preparation process of different steps, after salination, after immobilization and test with complementary ssDNA which is hybridization process. After silanization with APTES the current increase to nA range. APTES to provide contact between the organic and inorganic surfaces of a single-stranded DNA probe. APTES has an amine group that rich with positively charge ion. As the result, the current keep increases when drop the APTES on the bare of Al IDEs surface. The current captured at 1V shows the value which was 1.1×10⁻⁹ A. When APTES was dropped onto the IDE surface the length and area between comb fingers of an IDE is fixed then the density of an APTES become increase due to the increasing the number of hydroxide ions (OH⁻). The current value then keeps increase after ssDNA probe was immobilized on the top of APTES layer. The current captured at 1V shows the value which was 2.5×10⁻⁹ A. Thus, when applied potential difference between two electrodes then the voltage gradually increases, accumulated positive charge start to move to negative side. Then, it causes an electric field increase. The current increases due to the increasing of the electric field. The current generation mechanism can be explained using following equations.

\[
\text{Conductivity, } \sigma = \frac{l}{p} \quad (1)
\]

\[
\text{Resistivity, } R = \frac{p l}{A} \quad (2)
\]

\[
\text{Electric field, } E = \frac{k Q}{d^2} \quad (3)
\]

Finally, the 2 μl of 10 μM concentrated Salmonella ssDNA target was hybridized on the probe Salmonella. The current captured of the target at 1V is 4.3×10⁻⁹ A. The physical characteristics of ssDNA could offer substantial advantages as nucleic acid capture moieties in solid support based hybridization systems. 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.
Figure 5. I-V characteristics for functionalization steps: (1) bare IDE, (2) silanization with APTES, (3) ssDNA probe salmonella and (4) shows I-V characteristics after hybridized with complementary Salmonella target ssDNA.

Figure 6 shows the selectivity measurement that was tested for complementary, non-complementary and single base mismatches target of salmonella separately. 10μM concentrated targets and 2μl volume was used for selective measurements. Curve 3 until 6 shows the Probe Salmonella, complement, non-complementary and mismatch measurement captured at 1V are $2.5 \times 10^{-9}$ A, $4.3 \times 10^{-9}$ A, $1.7 \times 10^{-9}$ A and $2.2 \times 10^{-9}$ A respectively. As recovery, the accessibility of immobilized probes to complementary target sequences can be enhanced by treating the surface with a small molecule blocking agents. The carboxylic group rapidly displaces the weaker adsorptive contact between oligonucleotides leaving the probes was moored probe through carboxylic end group. For probe and non-complement current curve shows nearly same value. It is because non-complementary ssDNA cannot be bind with immobilized ssDNA probes. As well as they cannot bind with APTES layer because of the blocking agents cover the area which was unbounded by immobilized ssDNA. Hence, non-complementary target ssDNA thoroughly removed after washing with deionized water.

Figure 6 Selectivity measurements: (1) Bare Al IDE, (2) Silanization with APTES, (3) Immobilized ssDNA probe salmonella, (4) hybridization with complementary salmonella target ssDNA, (5) Non-complementary target ssDNA and (6) single base mismatch target ssDNA.
4. Conclusion
As conclusion, this research was totally described an approach for Salmonella target ssDNA via Al IDE based biosensor. I-V characteristic were performed for different functionalization process of the biosensor such as bare Al IDE, silanization with APTES and immobilization with ssDNA salmonella probe. Blocking step with tween-20 was important to detect target specifically. Different current variation shows for different concentration, same concentration, complementary, non-complementary and mismatch ssDNA target. Due to the point above, this method of patient diagnosis provides rapid diagnosis which allows for faster and more efficacious therapeutic intervention, thereby preventing full-blown infection and also decreasing the spread of disease.

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.

References
[1] R. D. A. A. Rajapaksha, U. Hashim, N. Z. Natasha, M. N. A. Uda, V. Thivina, C. A. N. Fernando, Proc. 2017 IEEE Reg. Symp. Micro Nanoelectron. RSM 2017, 191–194, (2017).
[2] N. Z. Natasha, R. D. A. A. Rajapaksha, M. N. A. Uda, U. Hashim, AIP Conf. Proc., 1885, (2017).
[3] R. D. A. A. Rajapaksha, M. N. A. Uda, U. Hashim, S. C. B. Gopinath, C. A. N. Fernando, IEEE Int. Conf. Semicond. Electron. Proceedings, ICSE, 2018, 93–96 (2018).
[4] M. N. A. Uda, R. D. A. A. Rajapaksha, M. N. A. Uda, U. Hashim, A. B. Jambek, AIP Conf. Proc., 2045 (2018).
[5] R. D. A. A. Rajapaksha, U. Hashim, M. N. Afnan Uda, C. A. N. Fernando, S. N. T. De Silva, Microsyst. Technol., 6, 70 (2017).
[6] R. D. A. A. Rajapaksha, U. Hashim, M. N. A. Uda, and C. A. N. Fernando, Mater. Sci. Eng. B, 10(1), 61–64 (2017).
[7] R. D. A. A. Rajapaksha, N. A. N. Azman, M. N. A. Uda, U. Hashim, S. C. B. Gopinath, C. A. N. Fernando, AIP Conf. Proc., 2045, (2018).
[8] N. A. N. Yahaya, R. D. A. A. Rajapaksha, M. N. A. Uda, U. Hashim, AIP Conf. Proc., 1885, (2017).
[9] S. C. B. Gopinath, S. Ramanathan, K. Hann Suk, M. Ee Foo, P. Anbu, M. N. A. Uda, MATEC Web Conf., 150, 06002, (2018).
[10] M. N. A. Uda et al., Adv. Mater. Res., 832, 410–414 (2014).
[11] S. C. B. Gopinath, U. Hashim, M. N. A. Uda, AIP Conf. Proc., 1808, 1–5 (2017).
[12] R. D. A. A. Rajapaksha, N. A. N. Yahaya, M. N. A. Uda, U. Hashim, AIP Conf. Proc., 2045, 1–7 (2018).
[13] S. Nadzirah, N. Azizah, U. Hashim, S. C. B. Gopinath, M. Kashif, PLoS One, 10(10), 1–15 (2015).
[14] M. Xu, R. Wang, Y. Li, “Talanta,” 162, 511–522, (2017).
[15] R. D. A. A. Rajapaksha, U. Hashim, M. N. Afnan Uda, C. A. N. Fernando, S. N. T. De Silva, Microsyst. Technol., 6, 70 (2017).
[16] P. Poltronieri, V. Mezzolla, E. Primiceri, and G. Maruccio, Foods, 3, 511–526 (2014).
[17] M. N. A. Uda et al., AIP Conf. Proc., 1808, (2017).
[18] M. N. A. Uda et al., AIP Conf. Proc., 1808, 10–14, (2017).
[19] T. Adam and U. Hashim, 2014 Fifth International Conference on Intelligent Systems, Modelling and Simulation, 1–8 (2014).
[20] M. N. Uda et al., Elsevier Inc., (2019).
[21] S. A. B. Ariffin, T. Adam, U. Hashim, S. Faridah Sfaridah, I. Zamri, and M. N. A. Uda, Adv. Mater. Res., 832, 113–117 (2013).
[22] M. G. Yanez, *Free. Semicondutor*, 1–34, (2013).
[23] U. Hashim et al., *Proc. - 2015 2nd Int. Conf. Biomed. Eng. ICoBE 2015*, 30–31, (2015).
[24] N. A. Abdul-Mutalib, A. N. Syafinaz, K. Sakai, and Y. Shirai, *Int. Food Res. J.*, 22(3), 896–901, (2015).
[25] W. P. Sharifa Ezat, D. Netty, G. Sangaran, *Malaysian J. Public Heal. Med.*, 13, 1–7, (2013).
[26] B. S. Rao and U. Hashim, *AIP Conf. Proc.*, 795, 388–392, (2013).
[27] N. A. Parmin et al., *Microchim. Acta*, 186(6), (2019).
[28] N. A. Parmin et al., *Int. J. Biol. Macromol.*, 126, 877–890, (2019).
[29] P. Intra and N. Tippayawong, “An ultra-low current meter for aerosol detection,” *C. J. Nat. Sci.*, 6(2), 313–320 (2007).