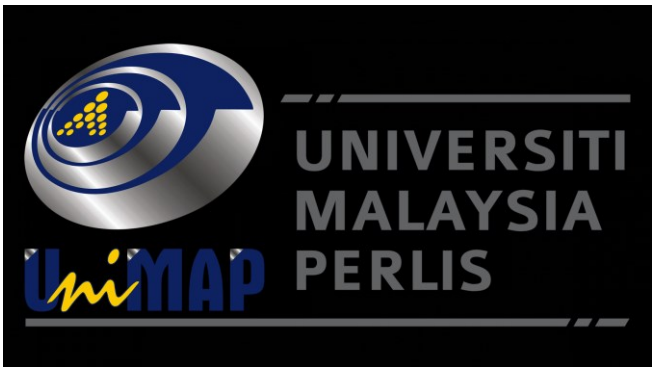

DEVELOPMENT OF SILICON ON INSULATOR BASED NANOGAP SENSOR FOR *ESCHERICHIA COLI* O157:H7 DETECTION

by

NUR HUMAIRA BINTI MD SALLEH
(1331710815)

A thesis submitted in fulfillment of the requirements for the degree of Master of Science in Nanoelectronic Engineering

Institute Of Nano Electronic Engineering
UNIVERSITI MALAYSIA PERLIS

2018

UNIVERSITI MALAYSIA PERLIS

DECLARATION OF THESIS

Author's Full Name	:	NUR HUMAIRA BINTI MD SALLEH
Title	:	DEVELOPMENT OF SILICON ON INSULATOR BASED NANOGAP SENSOR FOR *ESCHERICHIA COLI* O157:H7 DETECTION
Date of Birth	:	15 FEBRUARY 1985
Academic Session	:	2013/2017

I hereby declare that this thesis becomes the property of Universiti Malaysia Perlis (UniMAP) and to be placed at the library of UniMAP. This thesis is classified as:

☐	**CONFIDENTIAL**	(Contains confidential information under the Official Secret Act 1997)*
☐	**RESTRICTED**	(Contains restricted information as specified by the organization where research was done)*
☑	**OPEN ACCESS**	I agree that my thesis to be published as online open access (Full Text)

I, the author, give permission to reproduce this thesis in whole or in part for the purpose of research or academic exchange only (except during the period of _____ years, if so requested above)

Certified by:

SIGNATURE	**SIGNATURE OF SUPERVISOR**
850215-10-5290	PROF. DR. UDA BIN HASHIM
(NEW IC NO. /PASSPORT NO.)	**NAME OF SUPERVISOR**
Date: 18 July 2018	Date: 18 July 2018

NOTES : * If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization with the period and reasons for confidentiality or restriction. Replace thesis with dissertation (MSc by Mixed Mode) or with report (coursework)

ACKNOWLEDGMENT

All praises to Allah the Lord Almighty for giving me the opportunity and strength in completing pursue of my Master Science degree.

I wish to acknowledge the people who have helped and supported me throughout my study and their contribution towards the completion of this thesis.

First and foremost, I would like to express my deepest gratitude to Prof. Dr. Uda Hashim for giving me the chance to study and doing research in Universiti Malaysia Perlis. I truly appreciate his guidance, supervision, kindness and continuous support, as my supervisor. My immense gratitude also goes to Prof. Dr. Nor Azah Yusof for her guidance and patience as my co-supervisor. Without their help, this dissertation would not have been possible.

To INEE team, Dr. Ruslinda, Dr. Mohamad Nuzaihan, Dr. Gopi, Ir. Dr. Mohd Khairudin, Mr. Jasni and all the lecturers and staff thank you for their assistance and support during my research in the lab. To my lab mate, Nor Azizah, Fatin, Fare, Khai, Thivina, Adlyn and all, thank you very much.

My deepest appreciation and love also to my husband and family, for their continuous love, support and motivation throughout this journey.

TABLE OF CONTENTS

LIST OF TABLES

NO. **PAGE**

LIST OF FIGURES

NO. **PAGE**

LIST OF ABBREVIATIONS

ALD	Atomic layer deposition
APTES	3-aminopropyltriethoxysilane
CalTech	California Institute of Technology
CFU	Colony-forming unit
CMOS	Complementary metal–oxide–semiconductor
CNT	Carbon nanotube
DC	Direct current
DI	De-ionized
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded DNA
E. coli	*Escherichia coli*
EBL	Electron-beam lithography
FEM	Field emission microscope
FESEM	Field Emission Scanning Electron Microscopy
FET	Field-effect transistor
FIB	Focus ion beam
GA	Glutaraldehyde
hCG	human Chorionic Gonadotropin
HPM	High power microscopy
HUS	hemolytic-uraemic syndrome
ICPMS	inductively coupled plasma mass spectrometry
IMRE	Institute Materials Research and Engineering
IUPAC	International Union of Pure and Applied Chemistry
KCl	Potassium Chloride
MEK	Methyl ethyl ketone
MIBK	Methyl isobutyl ketone
MIT	Massachusetts Institute of Technology
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PNA	Peptide nucleic acid

RIE	Reactive Ion Etching
SEM	Scanning electron microscope
SiO2	Silicon dioxide
SOI	Silicon on Insulator
ssDNA	Single-stranded DNA
STAN	Self-assembled monolayers templated addressable nanogaps
STEM	Scanning Transmission Electron Microscope
UVL	Ultra-violet lithography

LIST OF SYMBOLS

ε_0	Dielectric constant in vacuum
ε_r	Relative dielectric constant
ΔC	Small capacitance
Δd	Small distance
µL	Microliter
A	Area
Å	Angstrom
Al	Aluminum
Au	Gold
C	Capacitance
Cr	Chromium
d	Distance/dimension
F	Farad
Hz	Hertz
MHz	Mega hertz
mL	milliliter
mV	Millivolt
nF	nanofarad
nm	Nanometer
nM	Nanomolar
pM	Picomolar
Si	Silicon
Ti	Titanium
ε	Dielectric constant

Pembangunan Sensor Nanogap Berdasarkan Silikon Pada Penebat Untuk Pengesanan Escherichia Coli O157: H7

ABSTRAK

Kemajuan nanoteknologi yang pesat telah membantu dalam pembangunan biosensor dan aplikasinya. Penyelidikan lepas menunjukkan peranti sensor nanogap berkeupayaan mempamerkan ciri-ciri elektrikal yang paling baik dalam pengesanan sampel biomolekul. Sensor Nanogap mempunyai sepasang elektrod menghadap satu sama lain, dimana molekul yang terperangkap di antaranya dapat dikenalpasti dengan mengukur pencirian elektrik. Proses pembangunan nanogap secara konvensional memerlukan teknik tambahan yang lama dan rumit. Oleh itu, projek penyelidikan ini memberi tumpuan kepada pembangunan struktur nanogap seragam dengan beza saiz dalam skala nanometer yang mampu mengesan *Escherichia coli* O157:H7 (*E. coli* O157:H7) pada tahap kepekatan yang rendah. Pembangunan peranti ini dibahagikan kepada struktur nanogap dan struktur pad emas menggunakan kaedah litografi elektron (EBL) dan fotolitografi konvensional. Substrat silikon pada penebat (SOI) digunakan untuk membangunkan struktur nanogap dan emas digunakan untuk pad emas untuk tujuan pengambilan data. Peranti nanogap yang dibangunkan, dilaksanakan pencirian fizikalnya menggunakan Elektron Pengimbas Pancaran Medan dan Mikroskop Elektron Pengimbas. Sementara itu, prestasi peranti ini diuji dengan menilai bacaan kapasitans dan impedans pada kadar frekuensi dari 1.0 MHz ke 0.1 Hz pada suhu bilik dengan input 1.0 mV menggunakan Penganalisis Dielektrik. Peranti ini diuji dengan air ternyahion dan paras pH yang berbeza untuk mengoptimumkan sensitiviti sensor berdasarkan saiz nanogap. Sebelum pengesanan asid deoksiribonukleik (DNA) *E. coli* dilaksanakan, peranti ini diubahsuai permukaannya dengan kumpulan silana NH_2-Amine dari 3-aminopropiltrietoksilana (APTES) dan glutaraldehid adalah untuk mengikat DNA dan APTES secara kovalen. Prinsip pengesanan *E. coli* berdasarkan pada perubahan kepadatan cas selepas proses pemegunan prob dan penghibridan sasaran DNA pada permukaan yang telah diubahsuai. Keputusan menunjukkan, peranti dengan saiz nanogap 40, 80 dan 100 nm telah berjaya dibangunkan. Didapati, peranti nanogap paling kecil, 40 nm menunjukkan tahap sensitiviti dan kestabilan yang tinggi berbanding peranti nanogap yang lebih besar, 80 dan 100 nm. Projek ini berjaya menghasilkan sensor nanogap bersaiz 40 nm sebagai biosensor dalam mengesan *E. coli* O157:H7. Peranti ini mampu membezakan nilai impedans antara DNA pelengkap, bukan pelengkap dan tidak sepadan tunggal. Di samping itu, nanogap sensor ini berjaya mengesan sasaran DNA *E. coli* O157:H7 pada had kepekatan dari 10 nM sehingga 1 pM. Persamaan regresi linear adalah $C\ (\mu F) = 3\ \times 10^{-7}x + 5\ \times\ 10^{-9}$ dan pekali korelasi adalah 0.98.

Development Of Silicon On Insulator Based Nanogap Sensor For Escherichia Coli O157:H7 Detection

ABSTRACT

Breakthrough in nanotechnology provides a great extent in biosensor development and application. Previous studies showed that nanogap sensor device provides excellent electrical behavior in sensing biomolecules samples. Nanogap sensor is a device having a pair of electrodes facing each other, which a molecule trapped in between its will be identified by observing the electrical characterization. Conventional development process requires prolonged and tedious compulsory additional method. Thus this research project focus on developing various size of uniform nanogap structure in nanometre scales which are capable of sensing *Escherichia coli* O157:H7 (*E. coli* O157:H7) at a low concentration level. The development of the device was divided into nanogap structure and gold pad structure process using electron beam lithography (EBL) method and conventional photolithography method respectively. Silicon on insulator (SOI) substrate was used to fabricate the nanogap structure and gold was used as a gold pad for a probing purpose. The developed nanogap devices was physically characterized by Field Emission Scanning Electron Microscopy and Scanning Electron Microscope. Meanwhile, the performance of the devices was tested by evaluating the capacitance and impedance reading by sweeping a frequency from 1M Hz to 0.1 Hz at room temperature with 1.0 mV input using Dielectric Analyzer. The devices were tested with de-ionized water and different pH level to optimize the sensor sensitivity that related to the nanogap size. Prior to the detection of *E. coli* deoxyribonucleic acid (DNA), the device was surface modified with NH_2-Amine functionalized silane group using 3-aminopropyltriethoxysilane (APTES) and glutaraldehyde for DNA to be covalently bonded with the APTES modified surface. The principle of the *E. coli* detection is based on charge density changes of the DNA probe immobilization and DNA target hybridization on the modified surface. The morphological testing results shows that the developed devices were observed with 40, 80 and 100 nm nanogap size. It was found that, the device with smallest gap size, 40 nm shows the highest sensitivity and stability compared to the device with bigger gap size, 80 and 100 nm. In this project 40 nm size nanogap device was successfully developed as biosensor for *E. coli* O157: H7 detection with capability to distinguish the impedance value between complementary, non-complementary and single mismatch DNA sequences. In addition, the device was able to detect *E. coli* O157: H7 DNA target at concentration limit from 10 nM to 1 pM with linear regression equation is $C\ (\mu F) = 3\ \times 10^{-7}x + 5\ \times\ 10^{-9}$ and the correlation coefficient is 0.98.

CHAPTER 1 : BACKGROUND

1.1 Introduction

Nano-based sensor has been introduced as biosensor since decades and been continuously developed and improved with multiple methods, process and materials along with an advancement of nanotechnology (Chao, Zhu, Zhang, Wang, & Fan, 2016; Junhui, Hong, & Ruifu, 1997; Pandit, Dasgupta, Dewan, & Ahmed, 2016). In the current research work, a novel Silicon-On-Insulator (SOI) based nanogap sensor has been developed and demonstrated as a biosensor for the detection of a foodborne opportunistic pathogen, *Escherichia coli* O157:H7 (*E. coli* O157:H7). *E. coli* O157:H7 is able to release the toxic compounds, especially when they are associated with the food materials and cause the severe foodborne illness to the human.

To focus on this issue, nanogap sensor was developed using a combination of high-end and a standard conventional photolithography processes, which were Electron Beam Lithography (EBL) and Ultra-Violet Lithography (UVL). The EBL process was introduced in this research to fabricate a pair of electrodes with a nanometre gap size. Meanwhile, the UVL process is a conventional method to fabricate the gold pad for the purpose of electrical characterization. For the biosensor application, *E. coli* O157:H7 detection was performed using a specific deoxyribonucleic acid (DNA) from *E. coli* O157:H7 as a target analyte placed between the nanogap electrodes by complementing with the probe DNA. The immobilized *E. coli* O157:H7 DNA probe was bind to the DNA target complementary sequence by hybridization among DNA bases (A with T and G with C). The binding process is then transduced into an electrical signal, where

the properties of dielectric change between the nanogapped electrodes during electrical characterization (Teles & Fonseca, 2008; Zhao, Ali, Brook, & Li, 2008). In this chapter, specifically discussed the problem statements, research objectives, scopes and thesis organization.

1.2 Problem Statements

Foodborne disease caused by highly virulent pathogens, *E. coli* O157:H7 is easily transmitted from the ingestion of contaminated food, water or oral contact with the contaminated surface or infected animals, that's lead to severe case hemolytic-uremic syndrome (HUS) to human especially infants, young children, pregnant women and elderly (Carl A. Batt, 2014). *E. coli* O157:H7 is sufficient to cause infection with low concentration dose compared to other strains (Arthur, Bosilevac, & Nou, 2005). So that, the quantitative measurements on *E. coli* O157:H7 is highly necessary to find and eliminate the minute contamination from the food-stuffs.

Early 20th century, conventional methods such as the culture-based and molecular methods have been applied in the detection of *E. coli.* However, most of these methods involve a culturing, screening, enrichment step, plating on the selective medium or binding of the fluorescent dye to the *E. coli* O157:H7, which may take hours to days to complete and need higher concentration for detection (Schleif, 2010; Xue, Velayudham, Johnson, & Saha, 2009). Thus, the problem is to generate a biosensor with a high-performance analysis on *E. coli* O157:H7, in a quantitative manner in order to overcome the above issues.

Several lines of the study show that nanogap devices have drawn a particular interest and opportunities in biosensing applications (Xing Chen et al., 2010). This is due to the capability of nanogap device to characterize a molecules in a nanometre size. However, the early generation of nanogaps faced difficulty in maintaining the detection stability due to lack of optimization in standardize the gap size pattern and fabrication method (Sheul, Chia, Lin, Lieh, & Tung, 2006). The electrical properties increase with decreasing the size of the gap, but the gaps must not be too small to remain accessible to the molecules and to avoid molecules posit into the distorted state (Ding, Herrmann, De Nijs, Benz, & Baumberg, 2015). In encyclopedia of nanotechnology, Nevill suggested that the nanogap size is between 1 to 100 nm as it represents the practical upper limit of the characteristic thickness of electrical double layer (J. T. Nevill, D. Malleo, 2016). Thus, a design with size of gap less than 100 nm distance for *E. coli* O157:H7 detection is focused in this study, as it is crucial.

In current studies different methods for fabricating nanogap electrodes such as electromigration (Ito, Yagi, Morihara, & Shirakashi, 2015; Motto et al., 2012), mechanically control break junctions (Muller, van Ruitenbeek, & de Jongh, 1992; Zhitenev, Meng, & Bao, 2002), controlled electrochemical platting (Morales et al., 1997) and nanoconstriction (Gehring et al., 2016) have been demonstrated. However, the methods were complex and tedious. Thus, the best suitable method to overcome the electrical double layer in samples is needed for the nanogap based biosensor generation.

The capability of nanogaps for detecting small size and quantity of biomolecules is favorable for *E. coli* O157:H7 detection. Development of nanogap based biosensor is able to enhance the sensitivity and selectivity of *E. coli* O157:H7 detection methods.

Application of nucleic acid hybridization on the nanogap electrode have been actively developing because of the specificity, speed, portability and low cost (Ch Postma, 2010; Fanget et al., 2014; Zaffino, Mir, & Samitier, 2014). Furthermore, compared with the protein-based *E. coli* detection method, DNA bound to nanogap can maintain its biological activities. Thus, these novelties are significant for the development of nanogap device for *E. coli* O157:H7 detection through DNA hybridization process.

1.3 Research Objectives

The primary aim of this research is to develop Silicon-On-Insulator nanogap device based on capacitive sensing via top-down approach for foodborne *Escherichia coli* O157:H7 (*E. coli* O157:H7) detection. This research objective for biosensor application is further accomplished through the following specific objectives:

i. To design, fabricate and characterize the nanogap device using Electron Beam and Ultra-Violet lithographic processes.

ii. To investigate the effect of the different sizes of nanogap electrodes in excitation frequency against different pH ranges.

iii. To examine the performance of the developed device for biosensing application using *E. coli* O157:H7 DNA detection quantitatively.

1.4 Research Scopes

This research study is embarked based on the following scopes:

i. To study and review the fundamental and designing Silicon-On-Insulator (SOI) nanogap based capacitive sensor, and to understand the suitable strategies for fabricating nanogap device.

ii. To design and optimize two patterns layout image of SOI nanogap device, where first pattern (Pattern 1) is chosen as the nanogap electrode structure and second pattern (Pattern 2) is chosen as a pad for the electrodes. Both patterns will be designed with the aid of the standard AutoCAD software. Pattern 1 will be transferred directly to electron beam lithography (EBL) and Pattern 2 will be transferred onto chrome glass mask.

iii. To fabricate the SOI nanogap device by combining an EBL process and a conventional photolithography process. The EBL will be the major process as the nanogap electrodes will be fabricated directly using design Pattern 1 with several sizes of gaps. It is a very important step to produce a perfect nanogap structure as small as possible in order to sense the atomic level biomolecules.

iv. To investigate and characterize the optical and physical characteristics of nanogap structures by using High-power Microscopy and Scanning Electron Microscopy.

v. To synthesize *E. coli* O157:H7 DNA probe and analyte for immobilization and hybridization processes. This preparation will be performed at MARDI under Biotechnology Department supervision.

vi. To prepare, functionalize and modify the sensing area surface between nanogap electrodes with complementary *E. coli* O157:H7 DNA to complement the probe DNA by immobilization and hybridization processes, respectively.

vii. To understand and measure the electrical characterization and testing the performance of fabricated nanogap sensor during *E. coli* O157:H7 DNA biosensing application.

1.5 Thesis Organization

This thesis is organized into five separate chapters, numbered as Chapter 1 to 5. The first chapter distinctly addressed the problem statements, primary and specific research objectives and scopes of the overall research work carried out to fulfill this thesis.

The importance of nanotechnology and existing methodologies for nanogap device fabrication, *E. coli* O157:H7 detection, and the relevant work described in the past on nanogap device as a biosensor is overviewed in the second chapter.

The third chapter focused on the research methodology, where nanogap design, fabrication and application of *E. coli* O157:H7 detection is elaborated thoroughly. This chapter explained overall process flow including preparation, characterization and optimization through morphological and electrical testing. The surface modification and functionalization processes for *E. coli* O157:H7 DNA probe immobilization and *E. coli* O157:H7 DNA target hybridization are explained in this chapter.

The fourth chapter discussed the results obtained from the morphological and electrical characterizations of the nanogap device. The capacitance result is measured using the dielectric analyzer. Studies on the potential applications of the biosensor are described with the results revealed for *E. coli* O157:H7 DNA detection. The ability of the device to discriminate DNA probe, complementary, non-complimentary, mismatched targets is assessed to justify its high-performance detection.

Finally, the fifth chapter reported the conclusion of the overall research work and propose the future directions in this field for further improvement.