# DEVELOPMENT OF SHEAR HORIZONTAL SURFACE ACOUSTIC WAVE WITH SILICON DIOXIDE NANOPARTICLES WAVEGUIDE SENSOR FOR *ESCHERICHIA COLI* O157:H7 DETECTION

by

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## TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AFM | Atomic force microscope |
| ANFIS | Adaptive neuro-fuzzy inference system |
| ANN | Artificial neural network |
| ANOVA | Analysis of variance |
| APCVD | Atmospheric pressure chemical vapor deposition |
| APMS | 3-aldehydepropyltrimethoxysilane |
| APTES | (3-Aminopropyl)triethoxysilane |
| BAW | Bulk acoustic wave |
| cfu | Colony-forming unit |
| COM | Coupling-of-modes |
| CVD | Chemical vapor deposition |
| DNA | Deoxyribonucleic acid |
| DOE | Design of experiments |
| EAEC | Enteroaggregative *E. coli* |
| *E.coli* | *Escherichia coli* |
| EDX | X-ray Spectrometry |
| EHEC | Enterohemorrhagic *E. coli* |
| EIEC | Enteroinvasive *E. coli* |
| ELISA | enzyme-linked immunosorbent assay |
| EPEC | Enteropathogenic *E. coli* |
| ETEC | Enterotoxigenic *E. coli* |
| FBAR | Film bulk acoustic resonator |
| FEM | Finite element method |
| FESEM | Field emission scanning electron microscope |
| FPW | Flexural plate wave |

| | |
|---|---|
| FTIR | Fourier transform infrared spectroscopy |
| G6PDH | Glucose-6-phosphate dehydrogenase |
| He | Helium |
| HPM | High-power microscopy |
| IANFIS | Improved adaptive neuro-fuzzy inference system |
| IC | Integrated circuit |
| IDT | Interdigital transducer |
| LPCVD | low pressure chemical vapor deposition |
| LiNbO3 | Lithium niobate |
| LW | Love wave |
| MPTMS | 3-mercaptopropyltrimethoxysilane |
| $N_2O$ | Nitrous oxide |
| $O_2$ | Oxygen gas |
| OFAT | One factor at a time |
| OH | Hydroxyl group |
| PBS | Phosphate buffer saline |
| PCR | Polymerase chain reaction |
| PECVD | Plasma enhanced chemical |
| PMMA | Polymethylmethacrylate |
| PNA | Peptide nucleic acid |
| PSA | Prostate specific antigens |
| PVD | Physical vapor deposition |
| QCM | Quartz crystal microbalance |
| RF | Radio frequency |
| RSAW | “Rayleigh-type” surface acoustic waves |
| RSD | Relative standard deviation |
| RSM | Response surface methodology |

| | |
|---|---|
| SAW | Surface acoustic wave |
| SEM | Scanning electron microscope |
| SH-APM | Shear horizontal acoustic plate mode |
| SHSAW | Shear horizontal surface acoustic wave |
| $SiO_2$ | Silicon oxide |
| $SiH_4$ | Silane |
| SPR | Surface plasmon resonance |
| SSBW | Surface skimming bulk wave |
| ssDNA | Single-strain DNA |
| STW | Surface transverse wave |
| TCF | Temperature coefficient of resonant frequency |
| TNT | 2,4,6-trinitrotoluene |
| TSM | Thickness shear mode |
| UV | Ultraviolet |
| XRD | X-ray diffraction |

# LIST OF SYMBOLS

| | |
|---|---|
| MHz | Mega hertz |
| cm | Centimeter |
| g | Gram |
| $S_m$ | Mass sensitivity |
| ρ | Substrate density |
| d | Substrate thickness |
| $K(\alpha)$ | factor depending on the Poisson ratio |
| $\lambda$ | wavelength |
| $A_0$ | Asymmetric zero-order |
| $S_0$ | Symmetric zero-order |
| $h$ | Thickness of the wave guide film |
| $h\nu$ | Photon energy |
| Hz | Hertz |
| R | Photolithography resolution |
| $K_1$ | Photolithography system constant |
| NA | Numerical aperture |
| mm | milimeter |
| [T] | Vector stress |
| [C] | Vector elastic stiffness coefficient |
| [S] | Vector strain |
| nm | nanometer |
| [E] | Vector electric field intensity |
| rpm | rounds per minute |
| [D] | Vector electrical displacement density |

| | |
|---|---|
| [Ɛ] | Vector permittivity |
| $\ddot{u}$ | Particle acceleration |
| F | Mechanical force acting on the substrate |
| $\varphi$ | Electric potential |
| u | Mechanical displacement |
| Ω | Ohm |
| °C | Degree Celsius |
| µm | micrometer |
| Å | Angstrom |
| [a] | Rotation matrx |
| [M] | Transformation matrix |
| $f_0$ | Resonant frequency |
| $v$ | Wave propagation velocity |
| $pI$ | Periodicity |
| H(f) | Transfer function |
| $K^2$ | Electromechanical coupling coefficient |
| $V_{out}$ | Output signal voltage |
| $V_{in}$ | Input signal voltage |
| $C_s$ | Capacitance for a strip pair per unit length |
| f | Frequency |
| N | Number of strip pairs |
| W | Acoustic aperture |
| L | Delay line |
| kPa | Kilo pascal |
| OH | Hydroxyl group |
| Si–O–Si | Silicon-oxygen |

| | |
|---|---|
| $f_a$ | Average shifted resonant frequency |
| $k_{11}$ | Self-coupling coefficient |
| $k_0$ | Wave vector at resonant frequency |
| $v_a$ | Average shifted velocity |
| $T_0$ | Room temperature |
| $\Delta m$ | Mass added |
| $\Delta f$ | Frequency change |
| A | Sensing area |
| $\mu$ | Shear modulus |
| $\emptyset$ | Wave phase in degrees |

**Pembangunan *Shear Horizontal Surface Acoustic Wave* Dengan *Silicon Dioxide* Nanopartikel Pandu Gelombang Pengesan Untuk Pengesanan *Escherichia Coli* O157: H7**

## ABSTRAK

*Escherichia coli* (*E.coli*) O157: H7, iaitu sejenis *strain* berbahaya di antara 225 serotipe yang unik bagi *E.coli*. Beberapa sel bakteria ini dapat menyebabkan anak-anak muda berada dalam keadaan yang serius. Terdapat lebih daripada 1 *cfu E.coli* O157: H7 dalam 25 g makanan, telah dianggap sebagai tahap yang berbahaya. Tujuan penyelidikan ini adalah untuk membangunkan pengesan nanostruktur pandu gelombang *(SHSAW)* untuk mengesan *E.coli* O157: H7. *Interdigital transducer* (IDT) adalah peranti utama dalam pengesan *SHSAW*. Ia menentukan kekerapan salunan and kepekaan pengesan. Pada umumnya, lebih tinggi kekerapan salunan, pengesan lebih peka, dimana lebar IDT mesti dibuat untuk sub mikrometer. Ini akan melibatkan proses yang rumit dan kos tinggi. Walau bagaimanapun, beberapa laporan menyatakan bahawa rekabentuk IDT seperti bilangan elektrob penghantaran dan penerimaan, aperture akustik dan panjang kelewatan talian boleh meningkatkan kepekaan pengesan SHSAW. Dengan itu, simulasi *COMSOL Multiphysics* telah digunakan dalam penyelidikan ini dan hasilnya didapati aperture akustik dan panjang kelewatan talian boleh meningkatkan kepekaan pengesan. Kajian ini diteruskan dengan pembangunan dan penilaian fabrikasi peranti SHSAW dengan menggunakan proses litografi konvensional yang ditambahbaik. Hasil kajian menunjukkan peranti dapat difabrikasi di dalam makmal dengan dimensi peranti yang tepat (kurang daripada 1%, (RSD)) dan tepat (ralat kurang daripada 4% daripada pengiraan teori) dan boleh disambung untuk eksperimen mengkaji kepekaan IDT terhadap beban mass. Dari RSM, IDT saiz nada 12 µm dengan saiz bukaan 0.72 mm dan panjang kelewatan talian 2.1mm dengan purata frekuensi resonansi 385.1607 MHz dikenal pasti sebagai parameter yang paling optimum untuk mencapai kepekaan pengesan yang maksimum. Oleh itu, parameter IDT optimum terbukti lagi oleh eksperimen yang dapat mempengaruhi kepekaan beban mass. Kepekaan peranti bersaiz nada 12 µm dipertingkatkan lagi dengan mendepositkan 130.5 nm lapisan nipis nanostruktur $SiO_2$ dengan saiz zarah kurang daripada 70 nm. Nanostruktur ini bertindak sebagai pandu gelombang serta pengubahsuaian permukaan fizikal pengesan sebelum penetapan biomolekul. Satu urutan DNA tertentu daripada *E. coli* O157: H7 yang mempunyai 22 mers diguna sebagai ssDNA dengan hujungnya terdapat kumpulan amina yang ditetapkan pada kawasan lapisan nipis melalui tindakbalas kimia [(CHO-$(CH_2)_3$-CHO) dan (APTES; $NH_2$-$(CH_2)_3$-$Si(OC_2H_5)_3$]. Penderia yang prestasi tinggi ditunjukan degan mengesan sasaran oligonucleotide tertentu dengan kepekaan 0,6439 nM / 0.1 kHz dan had pengesanan serendah 1.8 femto-molar (1.8 x 10-15 M). Prestasinya terus dinilai oleh analisis kekhususan dengan menggunakan satu urutan oligonucleotide yang satu tidak sepadan dan komplementari.

**Development of Shear Horizontal Surface Acoustic Wave with Silicon Dioxide Nanoparticles Waveguide Sensor for *Escherichia Coli* O157:H7 Detection**

## ABSTRACT

*Escherichia coli* O157:H7 *(E.coli* O157:H7*)*, a dangerous strain among 225 *E. coli* unique serotypes. A few cells of this bacterium are able to cause young children to be most vulnerable to serious complications. The presence of higher than 1 cfu *E .coli* O157:H7 in 25 g of food has been considered as a dangerous level. Thus, highly sensitive sensor is needed for this. The aim of this research work is to develop nanostructure waveguide shear horizontal surface acoustic wave (SHSAW) sensor for the detection of *E.coli* O157:H7. The interdigital transducer (IDT) is the heart of SHSAW sensor. It deterrmines the resonant frequency and the sensitivity of the sensor. In generally, the higher the resonant frequency, the higher sensitive the sensor will be, the width of IDT has to fabricated to sub micrometer. These involve more expensive cost and complicated methods. However, few reports mentioned IDT design parameters such number of transmission and receiving electrode fingers, electrode length or acoustic aperture and length of delay line or propagation path, can increase the SHSAW sensor sensitivity. Herein, COMSOL Multiphysics simulations were implemented for this investigation, the delay line length and aperture sizes are found that can increase the mass loading sensitivity. The research was continued by the development and evaluation of fabrication SHSAW device by using the improved conventional lithography process was conducted. The results show that the dimension of devices were precisely(less than 1%, relative standard deviation (RSD)) and accurately (less than 4% error from theoretical calculation) fabricated in laboratory for experimentally study on the effects of IDT parameters toward mass loading sensitivity. From the response surface methodology, 12 µm pitch sizes IDT with 0.72 mm aperture size, 2.1 mm delay line length and 385.1607 MHz average resonant frequency were identified as the most optimum parameters to achieve highest sensitive of devices. Thus, these optimum IDT parameters were further proven by real experiments that able to affect the mass loading sensitivity. The 12 pitch size device was further enhanced by depositing 130.5 nm thin layer of $SiO_2$ nanostructures with particle size lesser than 70 nm. The nanostructures act both as a waveguide as well as a physical surface modification of the sensor prior to biomolecular immobilization. A specific DNA sequence from *E. coli* O157:H7 having 22 mers as an amine-terminated probe ssDNA was immobilized on the thin film sensing area through chemical functionalization [(CHO-$(CH_2)_3$-CHO) and (APTES; $NH_2$-$(CH_2)_3$-$Si(OC_2H_5)_3$]. The high-performance of sensor was shown with the specific oligonucleotide target and attained the sensitivity of 0.6439 nM/ 0.1 kHz and detection limit was down to 1.8 femto-molar (1.8 x $10^{-15}$ M). Further evidence was provided by specificity analysis using single mismatched and complementary oligonucleotide sequences.

# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction

In 1885, the bacteriologist Theodor Escherich discovered the existence of *Escherichia coli (E. coli)* bacteria in the human colon (Feng et al., 2002). Today, many *E. coli* strains are known to exist in the digestive tract of humans and animals. Many of these are harmless and can act as normal microbiotas with mutual benefits for the bacteria and the host (Drasar & Hill, 1974). However, some strains that have undergone evolutionary changes which possess virulence factors to be pathogens (Lim, Yoon, & Hovde, 2010). These pathogenic *E. coli* can be divided into at least six categories based on their pathogenic mechanisms. The five most well known categories are the enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC) and enterohemorrhagic *E. coli* (EHEC) (Nataro & Kaper, 1998). The EHEC produce exotoxins known as verotoxins (also termed Shiga-like toxins) that cause several diseases, from mild diarrhea to potential fatal hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura (Goswami, Chen, Xiaoli, Eaton, & Dudley, 2015; Rahal, Kazzi, Nassar, & Matar, 2014; Wong et al., 2012).

One of the most dangerous EHEC serotypes, *E. coli* O157:H7, was first recognized in 1982 as a human pathogen associated with outbreaks of bloody diarrhea in Oregon and Michigan, U.S.A. (Wells et al., 1983). Since then, *E. coli* O157:H7 outbreaks have been reported in at least 30 countries on six continents ((Dundas et al., 2001; Michino et al., 1999; Doyle & Buchanan, 2012) with young children and the aged being most vulnerable to serious complications (Griffin & Tauxe, 1991). In Malaysia alone, there have been 62 cases of food poisoning by *E. coli* O157:H7 in 2008 and 36 cases in 2009 (Soon, Singh, & Baines, 2011). The actual number of cases, however, is likely to be higher due to a lack of foodborne disease intensive monitoring and surveillance in Malaysia (Soon, et al., 2011). Estimated of 73,480 illnesses, 2,168 hospitalizations, and 61 deaths due to the infections by *E. coli* O157:H7 annually in the United States has been reported by Centers for Disease Control and Prevention, Atlanta, Georgia, USA (Mead et al., 1999). In the U.S.A, the Center for Disease Control and Prevention in Atlanta, Georgia, estimates that there are 73 480 cases of *E. coli* O157:H7 each year in the U.S.A, resulting in 2168 hospitalizations and 61 deaths (Mead et al., 1999; C.-T. J. Lin, Jensen, & Yen, 2005), costing over US$400 million per annum (Frenzen, Drake, Angulo, & Group, 2005).

The *E. coli* O157:H7 bacteria can easily be transmitted through untreated water supply, undercooked or raw meat, milk, fruits, vegetables, food and shared use of facilities (Olsen et al., 2002; Rahal, et al., 2014; Varma et al., 2003; Wendel et al., 2009). While conventional bacterial detection methods and microbiological techniques (pre-enrichment, selective enrichment, biochemical screening and serological confirmation) can be used to detect and identify outbreaks of this bacteria, this process is labour intensive and time consuming (18-24 hours or longer) (Hobson, Tothill, & Turner, 1996; Tietjen & Fung, 1995). In addition, there are more than 1000 *E. coli* serotypes and it is very difficult to distinguish *E. coli* O157:H7 from other close serotypes (Ørskov & Ørskov, 1992). Biosensors with higher sensitivity, and rapid and accurate detection of *E. coli* O157:H7 would greatly improve food security.