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

by

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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(α)	factor depending on the Poisson ratio
λ	wavelength
A_0	Asymmetric zero-order
S_0	Symmetric zero-order
h	Thickness of the wave guide film
hv	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
φ	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
Δm	Mass added
Δf	Frequency change
A	Sensing area
μ	Shear modulus
∅	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 (1.8×10^{-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.