EVALUATION OF A MICROWIRE SENSOR FUNCTIONALIZED FOR RAPID DETECTION OF \textit{ESCHERICHIA COLI} CELLS FROM LIQUID FOODS

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI‘I AT MĀNOA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

BIOLOGICAL ENGINEERING

DECEMBER 2011

By

Lin Lu

Thesis Committee:

Soojin Jun, Chairperson
Yong Li
Wei-Wen Winston Su

Keywords: Foodborne pathogen, \textit{Escherichia coli}, microwire, dielectrophoresis, bioaffinity, electrochemical impedance spectroscopy
ACKNOWLEDGEMENT

First, I would like to express my most sincere thanks, respect, and appreciation to my advisor Dr. Soojin Jun, for the continuous support of my research, and for his patient, motivation, enthusiasm and immense knowledge.

Also, many thanks to Dr. Yong Li, Dr. Wei-Wen Winston Su, Dr. Daniel Jenkins and Dr. Yong Soo Kim for technical assistance and guidance; to Dr. Samir K Khanal for all the help on my graduate study; to my fellows from the Food Engineering Lab for their assistance, advice and stimulating discussion; to the kind and helpful staff of the University of Hawai‘i HNFAS Department and MBBE Department; to my friends in Hawaii, who have showered me with love, support and patience. Thanks also to United States Department of Agriculture (USDA) Hatch, HAW00260-H for partly funding this research.

Last but not least, I dedicate this work to my parents, for supporting and encouraging me spiritually throughout my life.
ABSTRACT

Nowadays, outbreaks of foodborne illness linked to pathogenic bacteria such as *Escherichia coli* have attracted increasing public attention. Traditional culture-based methods for pathogens identification are time-consuming and labor-intensive, raising the need for fast and sensitive detection techniques. Rapid detection method for *E. coli* performed by a functionalized microwire sensor was developed and evaluated in this work. A gold-tungsten microwire with a diameter of 25 µm was immobilized with anti-*E. coli*-antibodies on the surface and used to capture *E. coli* bacterial cells from cells suspension with dielectrophoretic force generated by an alternating current (AC) electric field at 20 Vpp and 3 MHz. Both fluorescence microscopy and electrochemical techniques were employed to see the amount of cells captured on the wire. Field emission scanning electron microscopy was used to visualize the cells. The detection limit was found to be about 5 CFU/ml with only target bacterial cells captured on the wire. The developed sensor demonstrated relative high sensitivity and specificity with fast detection rate, which shows a strong potential for the application in food industry.
# TABLE OF CONTENTS

Acknowledgements ........................................................................................................... ii

Abstract ........................................................................................................................... iii

List of Figures ..................................................................................................................... vi

List of Abbreviations and Symbols .................................................................................... viii

Chapter 1: Introduction & Literature Review

  Introduction ..................................................................................................................... 1
  Literature review .......................................................................................................... 1
    Foodborne disease outbreaks ..................................................................................... 1
    Conventional methods for foodborne pathogens detection .................................. 2
    Innovative biosensors for foodborne pathogens detection .................................. 3
    Dielectrophoresis (DEP) for cells sorting ............................................................... 5
    Electrochemical impedance spectroscopy ............................................................... 7
  Conclusion .................................................................................................................... 9
  References ................................................................................................................... 11

Chapter 2: Evaluation of a Microwire Sensor Functionalized for Detection of *Escherichia coli* Cells from Liquid Foods

  Abstract ....................................................................................................................... 15
  Introduction .................................................................................................................. 16
  Materials and Methods ............................................................................................... 18
    Experimental set-up .................................................................................................. 18
    Sample preparation .................................................................................................. 20
    Wire functionalization .............................................................................................. 20
    Sensing performance and bacterial cells quantification ........................................ 22
    FESEM visualization and validation ....................................................................... 22
  Results and Discussion ............................................................................................... 22
Chapter 3: Electrochemical Impedance Spectroscopic Technique with a Functionalized Microwire Sensor for Rapid Detection of Foodborne Pathogens

Abstract .................................................................................................................. 35

Introduction ............................................................................................................. 36

Materials and Methods .......................................................................................... 39

  Materials ............................................................................................................... 39

  Instrumentation ................................................................................................... 39

  Bacterial cultivation ............................................................................................. 40

  Preparation of functionalized microwires ............................................................ 41

  *E. coli* cells detection .......................................................................................... 42

  Data analysis ........................................................................................................ 42

Results and Discussion ............................................................................................ 43

Conclusion ................................................................................................................ 46

References ............................................................................................................... 48
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Schematic of bio-components and transducers used in biosensors construction</td>
<td>4</td>
</tr>
<tr>
<td>1.2</td>
<td>Motion of particles driven by dielectrophoretic force</td>
<td>6</td>
</tr>
<tr>
<td>1.3</td>
<td>Diagram of the system for electrochemical impedance spectroscopic measurement</td>
<td>8</td>
</tr>
<tr>
<td>1.4</td>
<td>Scheme of electrochemical impedance measurement system with a three-electrode configuration</td>
<td>9</td>
</tr>
<tr>
<td>2.1</td>
<td>Experimental set-up of the microwire sensor</td>
<td>19</td>
</tr>
<tr>
<td>2.2</td>
<td>Sensing Mechanism of the functionalized microwire sensor</td>
<td>21</td>
</tr>
<tr>
<td>2.3</td>
<td>FESEM images of E. coli K-12 cells and polystyrene beads captured on a plain wire at cell-bead ratio 5:5 (v/v) with AC applied</td>
<td>23</td>
</tr>
<tr>
<td>2.4</td>
<td>FESEM images of E. coli K-12 cells and polystyrene beads captured at cell-bead ratio 9:1 (v/v) without AC on (a) plain wire and (b) functionalized wire</td>
<td>24</td>
</tr>
<tr>
<td>2.5</td>
<td>FESEM images of E. coli K-12 cells and polystyrene beads captured at cell-bead ratio 9:1 (v/v) with AC at 3 MHz and 20 Vpp on (a) plain wire (b) functionalized wire</td>
<td>27</td>
</tr>
<tr>
<td>2.6</td>
<td>FESEM images of E. coli K-12 cells and polystyrene beads captured with AC at 3 MHz and 20 Vpp on functionalized wires at cell-bead ratio (v/v) of (a) 8:2 (b) 2: 8</td>
<td>28</td>
</tr>
<tr>
<td>2.7</td>
<td>Effects of DEP force and immobilization on FI values of E. coli K-12 cells captured on the wire from TSB at 1x10³ CFU/mL</td>
<td>29</td>
</tr>
<tr>
<td>2.8</td>
<td>FI values of E. coli K-12 cells with different concentrations captured from orange juice and TSB</td>
<td>30</td>
</tr>
</tbody>
</table>
3.1. (a) Randles equivalent circuit for Ruan’s impedance biosensor (2002) (b) A

typical Nyquist plot of an electrochemical cell in presence of redox
couple .............................................................. ........................................... 39

3.2. (a) Experimental Set-up for electrochemical impedance spectroscopic measurements

(b) Three-electrode electrochemical cell............................................................... 41

3.3. Schematic diagram of microwire functionalization and bioaffinity reactions

of antibodies-antigens ......................................................................................... 44

3.4. Impedance spectra (a) and CV plots (b) for functionalized microwires with

E. coli binding on surfaces ................................................................................... 44

3.5. Changes of electron transfer resistance with E. coli captured on the electrode

surface at different concentrations from $10^3$ to $10^8$ CFU/ ml ......................... 45

3.6. Specificity of functionalized microwire sensor towards E. coli bacteria.......... 46
# LIST OF ABBREVIATIONS AND SYMBOLS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli</strong></td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td><em>Salmonella Typhimurium</em></td>
</tr>
<tr>
<td>AC</td>
<td>Alternating current</td>
</tr>
<tr>
<td>DC</td>
<td>Direct current</td>
</tr>
<tr>
<td>FESEM</td>
<td>Field emission scanning electron microscopy</td>
</tr>
<tr>
<td>DEP</td>
<td>Dielectrophoresis</td>
</tr>
<tr>
<td>EIS</td>
<td>Electrochemical impedance spectroscopy</td>
</tr>
<tr>
<td>TSB</td>
<td>Tryptic soy broth</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>PEI</td>
<td>Polyethyleneimine</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
</tr>
<tr>
<td>WE</td>
<td>Working electrode</td>
</tr>
<tr>
<td>RE</td>
<td>Reference electrode</td>
</tr>
<tr>
<td>CE</td>
<td>Counter electrode</td>
</tr>
<tr>
<td>FI</td>
<td>Fluorescence intensity</td>
</tr>
<tr>
<td>FRA</td>
<td>Frequency response analyzer</td>
</tr>
<tr>
<td>r</td>
<td>Radius (m)</td>
</tr>
<tr>
<td>$\Delta R_{et}$</td>
<td>Electron transfer resistance changes ((\Omega))</td>
</tr>
<tr>
<td>F</td>
<td>Dielectrophoretic force</td>
</tr>
<tr>
<td>V</td>
<td>Voltage (V)</td>
</tr>
<tr>
<td>$V_{pp}$</td>
<td>Peak-to peak voltage (V)</td>
</tr>
<tr>
<td>f</td>
<td>Frequency (Hz)</td>
</tr>
<tr>
<td>A</td>
<td>Current (A)</td>
</tr>
<tr>
<td>$F_{CM}$</td>
<td>Clausius-Mossotti factor</td>
</tr>
<tr>
<td>$\varepsilon_m^*$</td>
<td>Complex permittivity of medium</td>
</tr>
<tr>
<td>$\varepsilon_p^*$</td>
<td>Complex permittivity of particles</td>
</tr>
</tbody>
</table>
CHAPTER 1
INTRODUCTION & LITERATURE REVIEW

Introduction

An overview of various physiochemical techniques for bacteria detection was presented in this chapter. Nowadays, frequent outbreaks of foodborne diseases linked to pathogenic bacteria such as *Escherichia coli* and *Salmonella* are attracting increasing public attention. Traditional culture-based methods for pathogens identification lacked effectiveness due to the time-consuming and labor-intensive operations, making it necessary to develop new detection techniques with rapid and sensitive features. In this thesis, a functionalized microwire biosensor developed upon dielectrophoresis and antibody-antigen interaction for *E. coli* detection is demonstrated and the performance is evaluated by both fluorescence microscopy and electrochemical techniques. The purpose of this chapter is to provide a brief review of the development and application of both traditional methods and innovative biosensors used for foodborne pathogens detection in the food industry, with special attention paid to those using dielectrophoresis and electrochemical impedance techniques. The current and future trends of biosensors application in food industry are also discussed.

Literature Review

Foodborne disease outbreaks

Nowadays, an increasing number of bacteria species have been identified as hazardous water- or foodborne pathogen which can cause severe human illness with a
high morality rate (Ivnitski, Abdel-Hamid, Atanasov, & Wilkins, 1999). *Campylobacter jejuni*, *Salmonella*, *Clostridium botulinum*, *E. coli* O157:H7 and *Listeria monocytogenes* are the recognized top five foodborne pathogens that have caused numerous foodborne illnesses and greatly threatened the public health (Bhunia, 2008). It is reported that bacterial foodborne illness accounted for as much as 91% of the total foodborne outbreaks each year in the USA (Ivnitski *et al*, 1999). As estimated by the Centers for Disease Control and Prevention (CDC) in 2011, approximately 1 in 6 Americans (or 48 million people) is sick, 128,000 are hospitalized, and 3,000 die of foodborne diseases yearly. Fruits and vegetables could be contaminated by animal and human sources during various periods, such as growth, harvest, transportation, packaging and handling. People are infected by foodborne pathogens via consumption of under-cooked or uncooked products such as ground beef, cheese, ice cream, spinach and lettuce (Altekruse *et al*, 2006; Bhunia, A. K., 2007; CDC, 2006, 2010 & 2011; Doyle and Erickson, 2006; Lynch *et al*, 2006). Thus, effective detection of pathogenic bacteria during food processing and prior to consumption has gained increasing concern.

**Conventional methods for foodborne pathogens detection**

Conventional pathogens detection depends on enumeration, isolation and identification of bacteria on specific media, providing both quantitative and qualitative characterization such as number, nature and morphology of the microbes under investigation (Doyle, 2001). Such methods have demonstrated advantages of sensitive, inexpensive and simple operations. However, since the entire procedure of a traditional method generally includes pre-enrichment, selective enrichment, plating, incubation,
biochemical screening and serological determination, several days are required to complete the test and obtain the final results, which make the detection time-consuming and labor-intensive (Ivnitski et al, 1999; Vunrcrzant & Plustoesser, 1987). Additionally, microbiological analysis of food samples, especially for certain species of pathogenic bacteria are still a great challenge due to the following reasons: heterogeneity of food materials, nonuniform distribution of bacteria in foods, and interference of non-toxic indigenous microbes with the bacteria of interest (Doyle, 2001). Therefore, classic culture-based bacteria detection is not able to satisfy the need for rapid sensing of pathogens in modern food industry. To conquer these constrains, fast and accurate testing techniques as replacement for conventional detection methods are in urgent demand for food quality assurance.

Innovative biosensors for foodborne pathogens detection

In recent few decades, bacterial cells sensing techniques are developing rapidly, establishing a variety of biosensing approaches for pathogenic microbes detection (Baldrich & Garcia-aljaro, 2010). A biosensor is an analytical instrument composed of a biological recognition element and a chemical or physical transducer (Mello & Kubota, 2002). Figure 1.1 shows the general construction of a biosensor. The choice of a recognition element and corresponding transducer towards a certain analyte depends on the properties of the sample to be analyzed, as well as the type of physical magnitude to be measured. In detail, an optical transducer deals with absorption or emission of electromagnetic radiation, a thermal transducer is related to temperature change or heat release, and a piezoelectric transducer deals with mass or micro-viscosity alterations of
wave propagation, while an electrochemical transducer is in charge of electron tunneling, ion mobility and diffusion of charged species (Mello & Kubota, 2002).

According to Mandal et al (2010), a cell sensing process basically involves two steps: target cells separation and concentration; identification and quantification. The evaluation of several broadly used alternatives of traditional detection methods in terms of sensitivity, specificity and time required to obtain results are performed and reported by Mandal’s group (2010): both conventional plate counting method and impedimetry could detect as low as 1 CFU/ml cells with good specificity, while take 1-3 days and 6-24 hrs to get the final results, respectively; bioluminescence and membrane filtration-direct epi-fluorescent technique (DEFT) require a very short working period of 30 min, but with high detection limit of $10^3$ to $10^4$ CFU/ml and lack of specificity; other means such as nucleic acid based assays, immunological methods and flow cytometry all have
disadvantages of high detection limit, long operation time, etc. (Skjerve, Rorvik, & Olsvik, 1990). Therefore, there is still a need to explore pathogens sensing techniques with strong strength in all aspects including running time, sensitivity, specificity, cost-effective, etc.

**Dielectrophoresis (DEP) for Cells Sorting**

DEP is the motion imparted on uncharged particles as a result of polarization induced by non-uniform electric fields (Lapizco *et al.*, 2007). This phenomenon was first introduced by Pohl in 1951 and can be used to move particles in non-uniform AC or DC electric fields. Any non-polar material will exhibit a certain degree of polarization when exposed to an electric field. Depending on the properties of the particle, electric dipoles are generated on opposing ends of the particle in response to an electric field, resulting in motions toward or away from the maximum electric field (Figure 1.2). The dielectrophoretic force acting on an isolated spherical particle can be represented as:

$$F_{DEP} = 2 \pi \varepsilon_0 \varepsilon_m r^3 \text{Re} \left[ F_{CM} \right] \nabla |E|^2$$  \hspace{1cm} (1)

where $\varepsilon_m$ is the relative permittivity of the suspending medium, $r$ is the radius of the particle, $E$ is the local electric field, and $\text{Re} \left[ F_{CM} \right]$ refers to the real part of the Clausius–Mossotti (CM) factor:

$$F_{CM} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2 \varepsilon_m^*}$$  \hspace{1cm} (2)

where $\varepsilon_p^*$ and $\varepsilon_m^*$ are the complex permittivities of the particle and the medium, respectively. Equations (1) and (2) could be used to determine the moving direction of the particles which experience the dielectrophoretic force. It is obvious that the sign of
equation (1) depends on the sign of \( \text{Re} [F_{CM}] \), which is equation (2). In equation (2), \( F_{CM} \) achieves a positive value as well as \( F_{DEP} \) when the complex permittivity of the particle is greater than that of the medium, while a negative value of both \( F_{CM} \) and \( F_{DEP} \) is obtained when the medium has a greater complex permittivity.

![Diagram of particle motion](image)

Figure 1.2 Motion of particles driven by dielectrophoretic force in a non-uniform electric field

Since the concept of DEP was proposed in as early as 1951, it was well studied and widely employed in many applications such as particle separation based on its differential polarizability (Gascoyne et al., 1997), particle collection and patterning (Rosenthal & Voldman, 2005; Velev & Kaler, 1999), and cell fusion and gene manipulation (Figeys and Pinto, 2000; Lee and Tai, 1999). According to Fernandez-Morales et al. (2008), during testing with \( E. coli \) as bioparticle model, the positive dielectrophoresis was observed for the frequency above 100 KHz, in other words, \( E. coli \) cells moved toward the maximum electric field zone and were concentrated.

**Electrochemical Impedance Spectroscopy**
Nowadays, EIS has been widely used to study the mechanisms of multi-step electrochemical reactions, investigate dielectric properties of materials, understand corrosion process and explore surface coatings (Huang et al., 2011).

The basis of EIS could be traced back to the period of 1880 to 1900, when Oliver Heaviside developed the operational calculus and first defined the terms of “impedance”, “admittance”, and “reactance” (Macdonald, 2010). According to Goldman and Macdonald (1950, 2010), impedance is only defined within Linear Systems Theory (LST). Four important constraints apply to a system defined by LST: (a) the system must be stable so that it would return to the initial state after removal of the perturbation; (b) the system must not produce any response before a perturbation is applied; (c) the output signals of the system must be demonstrated by linear (differential) equations; (d) the impedance should be finite (Macdonald, 2010). Warburg is another scientist who made exceptional contributions to the development of EIS. In 1899, Warburg developed the theory about impedance of diffusional transport of an electroactive species to an electrode surface. Epelboin and his group in Paris first propelled EIS into the forefront as a corrosion mechanism analytical tool in the 1960s. They overcame the limitation of previous “reactive bridge” techniques that can only perform impedance measurement when frequencies are above 100 Hz. Epelboin group and SOLARTRON Instruments, Ltd, have worked together to develop a “frequency response analyzer (FRA)”, which allows the impedance to be measured at 0.1 mHz (Macdonald, 2006). Expansion of the frequency range enabled diffusion process (mass transfer) occurring in an electrochemical system to be better understood in detail, and thus promoted the development of EIS. Over the last two decades, EIS has became one of the most powerful
electrochemical tools for defining reaction mechanisms, investigating corrosion processes and characterizing electrochemical systems (Huang et al., 2011). This method applies an small amplitude sinusoidal perturbation to an electrochemical system under investigation, and measures the resulting current over a wide range of frequencies, and therefore the frequency response of the system is determined, including the energy storage and dissipation properties (Murat, 2011). Figure 1.3 shows the working principle of an EIS measurement, where the input and output signals are sine wave potential and corresponding current (or other signal of interest), respectively. Overall impedance of the system could be obtained by interpreting the output plots, and the electrochemical system can be fitted with an equivalent circuit to allow further studies on different elements in the system, such as solution resistance, double layer capacitance, diffusion kinetics of the electrolyte species, etc. Figure 1.4 is the configuration of a three-electrode impedance measurement system on corrosion study. The corroding electrode located in the middle is the working electrode that under investigation. The reference electrode is used for determining the potential of the working electrode, while the counter electrode is necessary to form a closed circuit with the corroding electrode to allow the current flow.

![Electrochemical Impedance Spectroscopic Measurement](image-url)

**Figure 1.3 Principle of electrochemical impedance spectroscopic measurement**
Conclusion

Analysis of published literatures have shown that biosensors for pathogenic bacteria sensing have been developed and improved over the past a few decades. Several challenging criteria should be satisfied before a bacterial sensing method being considered useful. The extremely low infectious dose of pathogenic microbes presenting in foods or water (1 CFU/100 ml in drinking water) requires very rapid and sensitive detection (Ivnitski et al, 1999). In addition, selectivity is another important parameter for biosensors because the low numbers of pathogenic bacteria often exist along with other
non-pathogenic microorganisms in foods, water and environment. Also, a successful biosensor is expected to be inexpensive, ease in operation, portable, label free, environmental friendly, etc. However, none of the present developed sensors can fully satisfy customers’ demand. Hence, there is still a need to develop advanced biosensors by utilizing and combining several powerful techniques for optimizing the performance in food, environmental and clinic applications.
References


CHAPTER 2
EVALUATION OF A MICROWIRE SENSOR FUNCTIONALIZED FOR DETECTION OF ESCHERICHIA COLI CELLS FROM LIQUID FOODS

Abstract

The specificity of a biosensor is one of the most important parameters that have to be taken into consideration during the sensor development. The microwire sensor based on dielectrophoresis (DEP) and capillary forces developed in our previous study mainly relied on the frequency tuning for selective bacterial detection. To improve the sensing specificity besides the frequency tuning, immobilization of antibodies on a microwire was first introduced to detect *E. coli* O157:H7 in the mixture of *E. coli* cells and non-conductive polystyrene beads. However, immobilization of antibodies using conventional methods is non-compatible with the DEP driven electric field, which would challenge the surface functionalization. Therefore, this study was aimed to develop a microwire sensor based on DEP and antigen-antibody reaction for pathogen detection and evaluate the sensor’s performance to detect *E. coli* O157:H7 from juice and bacteria-bead mixture, respectively. The microwire was manipulated by an automated xyz stage and the sensing process included antibodies immobilization, bacteria detection and cells quantification. Field Emission Scanning Electron Microscope (FESEM) figures and fluorescence intensities of bacteria on the wire validated the sensing mechanism. The detection limit of the sensor was about 5 bacterial cells per microwire per sample volume when the electric field generated at 3 MHz and 20 Vpp, and only targeted *E. coli* cells were concentrated.
and captured. In developing such a sensing device for foodborne pathogens, food security could be monitored effectively, improving both food market values and public health.

**Introduction**

Food poisoning is always a great public health concern. Continual outbreaks of pathogenic microbes in food industry have made numerous people hospitalized and caused lots of panics. According to Mead *et al* (1999), approximate 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths are linked to foodborne pathogens in the United States each year. Despite the rapid development of pathogen detection techniques nowadays, foodborne illnesses raised by pathogenic bacteria are still threatening people’s health. *E. coli* as one of the most hazardous bacterial species has been recently found in various food products such as vegetables, milk products, sausages and nuts. In May 2010, intake of *E. coli* contaminated lettuce has resulted in an *E. coli* O145 outbreak among five states ("Investigation update: Multistate," 2010a). In the end of the year 2010, people were found ill after ingestion of Gouda cheese products due to the potential contamination of *E. coli* O157:H7 ("Investigation update: Multistate," 2010b). Two multi-state *E. coli* O157:H7 outbreaks linked to Lebanon bologna products and unshelled hazelnuts were reported in 2011 ("Investigation announcement: Multistate," 2011; "Investigation update: Multistate," 2011). While improvement in rapid identification after ingestion is important, it is more important to avoid infection (Shriver-Lake *et al*, 2007). As a result, in order to identify food products contaminated by *E. coli* O157:H7 prior to the national wide distribution and consumption, more efforts are needed to explore effective pathogen detection approaches.
Most existing conventional detection methods are based on microscopic, culture, biochemical, immunological and genetic techniques; and have disadvantages to varying extents, in terms of specificity, sensitivity, detection limit, detection rate, and associated cost (Zourob et al, 2008; Kim et al, 2011). Hence, a rapid, reliable, cost-effective, and environment-and worker-friendly method for detection of foodborne pathogens in food products indeed are in demand.

Dielectrophoresis (DEP) is the translational motion of neutral particles caused by polarization effects in a non-uniform electric field (Huang et al, 2008). It has been extensively utilized in separation and manipulation of bioparticles (Blanca H & Marco, 2007). In the previous work, a microwire sensor was developed to detect bacterial cells, based on DEP and capillary forces, and was proved to selectively capture E. coli cells from bacteria-spinach mixture (Kim et al, 2011). However, the developed sensor needs to improve the sensing specificity, in particular when the interfering particles size similar to E. coli cells, which leads lack of the size specificity. Immobilization of specific biorecognition elements such as antibodies on a sensor surface is believed to be an effective way to capture only the targeted particles from a mixed sample. The appropriate immobilization of antibodies on a solid surface is one of the most critical steps in the preparation of an immune-biosensor (Karyakin et al, 2000; Pilar, Manuel et al, 2008; Kyprianou et al, 2009). According to Karyakin et al (2000), the deactivation of antibodies upon immobilization was found in many cases. As indicated by Vaisocherová et al (2009), an ideal surface coating for biosensors should meet the following requirements: (1) plenty of functional groups for the attachment of targeted cells, (2) the retention of biological activities of antibodies after immobilization and (3) long-term
stability of the functionalized coating. Also, the immobilization should be compatible with an electric field in particular for the microwire sensor. In summary, the maintenance of the antibodies activity after immobilization and the consistence of the sensor performance preserved regardless of various complex food systems are of great significance to ensure the high quality of a sensor.

Few studies were found to combine immobilization and DEP force simultaneously with a microwire for pathogen detection. The objectives of this study were to (1) develop a method that could efficiently immobilize antibodies on a wire surface, (2) improve the specificity of the previous sensor and (3) evaluate the performance of the functionalized microwire sensor. A functionalized gold-tungsten wire was employed instead of a plain wire to resolve the specificity problem. The wire was functionalized with anti-\textit{E. coli} antibodies prior to use. In addition, an alternating current (AC) electric field generated at 3 MHz and 20 Vpp was applied to concentrate targeted \textit{E. coli} cells to the surrounding of a wire tip (Kim \textit{et al}, 2011). Figure 2.1 describes the sensing mechanism of the proposed method. The DEP force generated by the electric field concentrates bacterial cells from the suspension solution to the vicinity of the microwire. Then, the targeted \textit{E. coli} cells are recognized and captured by the antibodies that were pre-immobilized on the wire.

**Materials and Methods**

**Experimental set-up**

To provide an isolated sensing environment, the detection was carried out inside a transparent acrylic panel enclosure where a 18”×18” English bench plate (Edmund Optics
Inc, Barrington, NJ) and a motorized xyz stage were installed to control the motion of a 7% gold-tungsten wire in 25 µm diameter (ESPI Metals, Ashland, OR) with the probe guide panel (Figure 2.1). The precise movement of the wire was controlled using a xy plain stage (Franklin Mechanical & Control Inc., Gilroy, CA.), a z-directional slide, a single shaft stepper motor, a programmable stepping motor control and the COSMOS program (Velmex, Inc., Bloomfield, NY).

A 2”×2” gold plate with a hemispheric concave (Ø= 2 mm) in the center was used as bottom electrode. A function generator (33220A, Agilent Technologies, Santa Clara, CA) was used to generate sine waveforms with different frequencies. A stereo optical microscope with 40 x magnifications was located on the top of a bench plate with microscopy holders (Edmund Optics Inc, Barrington, NJ) to monitor the sensing procedures. A fiber optic Y-shape dual light microscope illuminator (Microscope World, Carlsbad, CA) was installed as a background light source (Figure 2.1).

Figure 2.1. Experimental set-up of the microwire sensor
Sample preparation

Frozen stock cultures of *E. coli* K-12 were obtained from Food Microbiology Laboratory (University of Hawaii, Honolulu, HI). Before each experiment, the strains were transferred into tryptic soy broth (TSB; BD diagnostic systems, Franklin Lakes, NJ) at least twice to be activated by incubating at 35ºC for 24 h. The initial concentration of the *E. coli* K-12 stock culture was obtained using serial dilutions and plate counting.

To prepare bacteria-beads mixtures, 10 µL polystyrene beads reagent (Lot#: 10015, Bangs Laboratories, Inc., Fishers, IN) was added to 990 µL sterile water to make a 100-fold dilution. Hence, the number of beads presented in the diluent was determined to be 2x10^8/mL. In the meantime, *E. coli* K-12 suspension with bacterial concentration of 10^8 CFU/mL was prepared. The *E. coli* K-12 suspension was then mixed with the beads at the ratios of *E. coli* cells to beads (v/v), 1:9, 2:8, 5:5, 8:2, and 9:1, respectively.

Wire functionalization

Wires were cleaned in a sonicator, rinsed with ethanol, dried in air and mounted on the xyz stage. A polydimethylsiloxane (PDMS) coated plate was used as a substrate to hold the droplets of reagents throughout the immobilization. The sanitary wire tip was immersed into 5 µL polyethyleneimine (PEI) solution (Product# S4762, Sigma-Aldrich Co., Saint. Louis, MO) for 5 min, and then withdrawn, and left in air for 2 min. Thereafter, the wire was soaked in 5 µL streptavidin (Product# S4762, Sigma-Aldrich Co., Saint. Louis, MO) for 1 min and was pulled out. Biothynlated mouse monoclonal anti-β-galactosidase antibodies (Product# 408700, Sigma-Aldrich Co., Saint Louis, MO) were applied afterward at the same manner for 5 min. Since PEI is a cationic polyplex,
which is positive charged, the negative charged streptavidin could bind directly to PEI via electrostatic interaction. The biotinylated antibodies are linked to the streptavidin and are ready to capture bacterial cells (Figure 2.2).

![Figure 2.2. Mechanism of bacterial cells concentration and capture using the functionalized microwire sensor](image)

5 µL sample solution was placed in the hole of the gold electrode. After the functionalized wire was air-dried for 2 min, it was dipped into the sample droplet for 1 min when an AC electric field that was generated at 3 MHz and 20 Vpp was applied. The wire was withdrawn and left in air for 2 min after capturing. Finally, 5 µL fluorescein isothiocyanate (FITC) labeled polyclonal antibodies (Lot#: 20B04308, Agilent 33220A Meridian Life Science, Saco, ME) were applied for 5 min for further quantification of cells captured on the microwire surface. The wire was rinsed in water to remove nonspecific binding between the targeted cells and wire.
Sensing performance and bacterial cells quantification

Immobilization and DEP forces were selected as two important factors and single factor experiments were designed to evaluate their effects on the sensing ability of this detection technique.

Fluorescence images of the microwire with bacteria-fluorescence antibodies complex attached to the surface were obtained using an inverted charge-coupled-device (CCD) camera fluorescence microscope. Then, a 292 x 724 pixels area of the wire tip was cropped from each picture and their fluorescence intensities were measured by the Image J program (National Institutes of Health, Bethesda, MD).

FESEM visualization and validation

Field Emission Scanning Electron Microscope (FESEM) (Pacific Biosciences Research Center, University of Hawaii at Manoa, Honolulu, HI) was used to visualize and validate *E. coli* K-12 cells and polystyrene beads captured on the functionalized wire tips. Surface coating of the wires was required due to the nonconductive properties of both bacteria and beads. Wires were attached to conductive carbon tapes on aluminum stubs with tips hanging in the air. Being treated in a Hummer 6.2 sputter coater for 45 s, each wire was coated with a thin gold/palladium layer. Coated wires were then observed with a Hitachi S-4800 FESEM (Kim *et al*, 2011).

Results and Discussion

Initial concentration of the *E. coli* K-12 stock solution was determined to be $1 \times 10^9$ CFU/mL. Figure 2.3 shows the *E. coli* K-12 cells and polystyrene beads that were
captured from a cell-bead mixture using a plain wire with DEP force generated at 3 MHz and 20 Vpp. Since the mixture was made from diluents of cells and beads with the same concentration and same volume, the number of cells and beads in the sample were similar at about $10^5$. Both *E. coli* cells and beads were attached to the wire surface, which supposed to be only bacterial cells due to the specificity of the biosensor. As explained earlier in this article, this is due to limited selectivity of the plain wire with the assistance of DEP force when cells and beads have similar dimensions and dielectric properties. Therefore, immobilization of antibodies onto the wire is expected to improve the sensing specificity.

Figure 2.3. FESEM images of *E. coli* K-12 cells and polystyrene beads captured on a plain wire at cell-bead ratio 5:5 (v/v) with AC applied

Figure 2.4 illustrates the performance of a functionalized wire compared to a plain wire in the absence of DEP forces. From Figure 2.4 (a), no cell or bead was observed on the wire surface, which clearly indicates the sensing deficiency of the plain wire without the aid of DEP force. Figure 2.4 (b) shows that a large amount of bacterial cells were captured on the antibody-immobilized wire as well as a few polystyrene beads,
demonstrating a significant improvement of the sensor after being modified. However, one possible reason for unwanted beads attachment to the wire is that the beads were “non-specifically glued” to the wire by the viscous sample solution. To get a better understanding of the phenomena, the AC was applied during the sensing process and results are shown in Figure 2.5.

Figure 2.4. FESEM images of *E. coli* K-12 cells and polystyrene beads captured at cell-bead ratio 9:1 (v/v) without AC on (a) plain wire and (b) functionalized wire

Figure 2.5 presents *E. coli* cells concentrated on a plain wire and a functionalized wire surfaces with the assistance of DEP forces, respectively. In Figure 2.5 (a), although numerous bacterial cells were obtained on a plain wire, which indicates the sensitivity
enhancement after applying AC, a few polystyrene beads still presented on the wire. However, this issue was easily resolved by high specific bindings between antibodies and bacterial cells as demonstrated in Figure 2.5 (b). A closer view of the functionalized wire shows no bead either on the bottom or the sidewall of the microwire. In comparison to figure 2.4 (b), this finding proves that the dielectrophoretic force generated by an AC field does significantly improve the specificity of the developed sensor. *E. coli* cells and beads both have net negative surface charge (Krishna, Powell & Borriello, 1996; Ozkan *et al*, 2002) and are both dielectrically polarizable in the presence of an AC field and thus could move due to dielectrophoretic force (Li, H, Zheng, Y., Akin, & Bashir, 2005; Wang, Vykovkal, Becker, & Gascoyne, 1998; Zhou, White, & Tilton, 2005). However, since the strength and direction of the DEP force acting on an object relies on the conditions of electric field, media in which the object suspends, and properties and size of the object, cells and beads might experience quite different DEP forces, which separate them from each other. In addition, immobilized anti-*E. coli* antibodies could recognize and seize the target to ensure only targeted *E. coli* cells are captured by the wire. Therefore, the sensing specificity was guaranteed by the functionalized wire coupled with DEP force generated at 3 MHz and 20 Vpp.

In order to better understand the dielectrophoretic behavior of *E. coli* cells and beads, results of functionalized wires in an AC field worked with cell-bead ratios of 8:2 and 2:8 were shown in figure 2.6, respectively. When cell population was larger than that of bead in the mixture, only bacterial cells were observed on the wire surface (figure 2.6(a)), which is in high agreement with figure 2.5(b). However, a small amount of beads were present on the wire along with captured bacterial cells when bead population was
four times of cell population in the sample (figure 2.6(b)). A possible explanation is that during the cells concentration process induced by DEP, the large number of polystyrene beads in the surrounding that also experience DEP force have intensely disturbed the movement of cells toward the wire, and thus limited the selectively concentration of bacterial cells.

Figure 2.7 shows the effects of wire functionalization and DEP force on the sensing efficiency in terms of FI values. Since FI value is proportional to the cell number captured on a microwire, it can be concluded that a plain wire without any treatment has the least cells on the surface. According to figure 2.7, the first column “plain wires without DEP” shows the lowest FI value among the four, which agrees with the FESEM results. Set it as the control, trials with “plain wires with DEP” and “functionalized wires without DEP” demonstrate slight FI enhancements of 6% and 11%, respectively. However, the highest FI value with a 24% increase of magnitude was achieved when wire immobilization and AC were applied simultaneously, which clearly indicates that most cells were captured on the wire.
Figure 2.5. FESEM images of *E.coli* K-12 cells and polystyrene beads captured on a functionalized wire at cell-bead ratio 9:1 (v/v) with AC at 3 MHz and 20 Vpp on (a) plain wire (b) functionalized wire
Figure 2.6. FESEM images of *E. coli* K-12 cells and polystyrene beads captured with AC at 3 MHz and 20 Vpp on functionalized wires at cell-bead ratio (v/v) of (a) 8:2 (b) 2:8
Figure 2.7. Effects of DEP force and immobilization on FI values of *E. coli* K-12 cells captured on the wire from TSB at $1 \times 10^3$ CFU/mL.

Figure 2.8 demonstrates the FI values of *E. coli* K-12 cells captured on a microwire from orange juice and TSB with cell concentrations ranging from $1 \times 10^3$ to $1 \times 10^8$ CFU/mL, respectively. For both TSB and orange juice samples, linear FI values obtained from pure buffer and orange juice at the same bacterial concentration are found to be similar within a reasonable error range. This result indicates that the juice particles have rare adverse impact on the sensor performance, and thus, can provide the promising future of the sensor being applied to other food complexes. Also, Figure 2.7 implies the lowest concentration of the bacterial suspension at which the sensor was able to function is $1 \times 10^3$ CFU/mL. Since 5 µL sample solution was used for each trial, the amount of bacterial cells in a sample at $1 \times 10^3$ CFU/mL can be estimated as: $(1 \times 10^3$ CFU/mL) x (5
μL) = 5 CFU. Therefore, based on the theory that one colony is developed from one single bacterium, the detection limit of the developed sensor is determined as 5 bacterial cells per wire per sample volume.

![Figure 2.8](image)

**Figure 2.8.** FI values of *E. coli* K-12 cells with different concentrations captured from orange juice and TSB

**Conclusion**

A functionalized microwire sensor coupled with a mechanized motion controller was designed and fabricated. A method to immobilize monoclonal anti-*E. coli* antibodies on gold-tungsten wires was developed and was proved to be transparent against surrounding electric field. An approach for pathogen detection including (1) the enrichment of bacterial cells by DEP force and (2) selective cell capture by antigen-antibody reactions was successfully evaluated. The whole immobilization and detection
procedure could be completed within 30 min with simple operations. Since the functionalized wire was used to replace the plain wire for a microwire sensor developed in our previous work, a single factor experiment was conducted and mixtures of *E. coli* cells and polystyrene beads were used to validate the performance of the modified sensor. Based on the FI measurements and FESEM images of cells and beads captured on wires, it was apparent that the functionalized microwire working with DEP force has significantly improved the sensor’s capability of detecting only target cells with a low detection limit estimated to be 5 bacterial cells per microwire per sample volume. A future study will include (a) expansion of the developed approach for complex food systems, (b) bacterial cocktail solution, and (c) a scale up and full automation of the sensing unit.
References


CHAPTER 3
ELECTROCHEMICAL IMPEDANCE SPECTROSCOPIC TECHNIQUE WITH A FUNCTIONALIZED MICROWIRE SENSOR FOR RAPID DETECTION OF FOODBORNE PATHOGENS

Abstract

Label-free biosensor based on electrochemical impedance measurement followed by dielectrophoretic force and antibody-antigen interaction was developed for detection and quantification of foodborne pathogenic bacteria. In this study, gold-tungsten wires with 25 µm in a diameter were functionalized by coating with polyethyleneimine-streptavidin-anti-\textit{E. coli} antibodies to improve the sensing specificity. The focus of this research is to evaluate the performance of the developed biosensor by monitoring the changes of electron-transfer resistance ($\Delta R_{et}$) of the microwire after the bioaffinity reaction between bacterial cells and antibodies on its surface, as an alternative quantification technique of fluorescence microscopy. Electrochemical impedance spectroscopy (EIS) were used to detect and validate the resistance changes in a conventional three-electrode system in which $[\text{Fe(CN)}_6^{3-}]/[\text{Fe(CN)}_6^{4-}]$ served as the redox probe. The impedance data demonstrated a linear relationship between the increments of $\Delta R_{et}$ and the concentrations of \textit{E. coli} suspension in the range of $10^3$ to $10^8$ CFU/mL. In addition, there were little changes of $\Delta R_{et}$ when the sensor worked with \textit{Salmonella}, which clearly evidenced the sensing specificity. Electrochemical impedance measurement was proved to be an ideal alternative of fluorescence microscopy for enumeration of captured cells.
Introduction

Rapid, sensitive and selective detection of pathogenic microorganisms from food and drinking water are of high interests nowadays, which prevents people from the risks of being infected by pathogens such as *E. coli* O 157:H7 and *S. Typhimurium*. A variety of detection techniques for this purpose based on different principles have been developed in the past few decades. According to the literature, traditional methods for pathogens detection are time-consuming, such as plate counting method and polymerase chain reaction (PCR) (Ivnitksk1 *et al.*, 1999; Zouro *et al.*, 2008). It took a few hours even days for bacteria to be isolated and grow, followed by many biochemical tests for identification. Besides the bacteria capture, the quantification and identification step is also very important to a sensing approach, offering the analytical tool to interpret the sensing results. In our previous studies, fluorescence microscopy was used for fluorescence antibody-labeled bacteria quantification, which was rapid but only provided a simple and rough enumeration without any further information.

Electrochemical impedance spectroscopy (EIS) is one of the most effective and reliable methods to analyze the properties of an electrochemical system, including double-layer capacitance, diffusion impedance, charge transfer resistance and solution resistance, etc. It has been broadly applied to areas such as corrosion, electrode kinetics, membranes, semiconductors, surface coatings, batteries, fuel cells and interfaces, etc. (Murat, 2011). The basic approach for EIS measurements is to apply small amplitude sine wave perturbation to an electrochemical system over a wide range of frequencies, and measure the responding signals (e.g. current) as a function of the frequencies.

The first electrochemical impedance based biosensor for bacterial detection was
developed in 2002 (Ruan et al., 2002). The working mechanism of the impedance biosensor can be interpreted by an equivalent circuit called Randles Cell (Figure 3.1 (a)). Although Randles Cell is only an analog rather than a real-time model of the electrochemical phenomena occurring in the system, information delivered by the circuit are valuable for people to understand the mechanisms of the electrochemical reactions. As shown in Figure 3.1 (a), the four elements of the equivalent circuit represent electrolyte solution resistance ($R_s$), double-layer capacitance ($C_{dl}$), Warburg impedance ($Z_w$) of the electrode and electron transfer resistance ($R_{et}$), respectively. Figure 3.1 (b) is a typical Nyquist plot, which is a widely used impedance plot to evaluate $R_{et}$. A Nyquist plot is generated by $Z_{im}$ (imaginary part of impedance) versus $Z_{re}$ (real part of impedance), and consists of a semicircle followed by a straight line. The semicircle part falls in the high frequency range is dominated by electron transfer process, while the tail part falls in the low frequency range is dominated by diffusion process (Yang & Bashir, 2008). When the imaginary impedance is zero, the intercept of the semicircle with the x axis represents $R_s$. The diameter of the semicircle is equal to the electron transfer resistance ($R_{et}$) of the working electrode. Several studies have reported that the attachment of bacterial cells to the electrode surface could form a blocking layer that inhibit the current flow and thus increase the resistance of the electrode (Chuanmin, Liju, & Yanbin, 2002; Escamilla-Gómez, Campuzano, Pedrero, & Pingarrón, 2009; Huang et al., 2011; Tang et al., 2004; Yang & Bashir, 2008). Therefore, changes of electrode impedance after the capture of bacterial cells are expected to be proportional to the amount of cells on the electrode surface, by which bacterial cells population could be quantitatively determined.

Among various applications, the EIS as a tool for characterizing electrodes
modified with functional coatings has been well explored by many researchers (Xiao et al., 2007; Vyas & Wang, 2008; Escamilla-Góme et al., 2009). Films coated on an electrode surface are quite heterogeneous, making the chemistry varies from point to point along the metal surface. Therefore, microelectrode such as the microwire has great advantages over conventional electrodes as a working electrode for the electrochemical impedance measurements. A conventional electrode, which has a much larger surface area, takes longer to establish a steady-state current due to the low rate of mass transport. The long time taken for the measurement may introduce problems from drift in the electrode with time. In addition, a larger area of working electrode requires a larger area of counter electrode, which increases the cost of the experiment. Moreover, the small quantity of chemicals reacted at the micro-electrode surface will not change the chemistry significantly within the measurement pool (Bai, 2006).

In this study, a microwire with a 25 µm diameter was modified by functional coatings and has been investigated for foodborne pathogen detection by electrochemical impedance measurements. The objectives of this research were to establish an electrochemical system for impedance measurement in which the microwire served as the working electrode; evaluate the performance of the microwire sensor by electrochemical impedance method as an alternative of fluorescence microscopy.
Figure 3.1 (a) Randles equivalent circuit for Ruan’s impedance biosensor (2002) (b) A typical Nyquist plot of an electrochemical cell in presence of redox couple (Yang & Bashir, 2008)

Material and Methods

Materials

7% gold-tungsten plate wire in 25µm diameter was supplied by ESPI Metals (Ashland, OR). Stock cultures of *E. coli* K-12 and *S. Typhimurium* were obtained from Food Microbiology Laboratory (University of Hawaii, Honolulu, HI). Polyethyleneimine (PEI), streptavidin and biothynlated mouse monoclonal anti-β-galactosidase antibodies were purchased from Sigma (Product# S4762, # S4762 and # 408700, Saint. Louis, MO). Electrolyte solution used for EIS and CV measurements was prepared by dissolving 5 mM K₃Fe(CN)₆ and 5 mM K₄Fe(CN)₆ in 0.1 M KCl solution (product # 244023, # P3289 and # P9541, Sigma-Aldrich Co., Saint. Louis, MO).

Instrumentation

An automated xyz stage controlled by the COSMOS program (Franklin
Mechanical & Control Inc., Gilroy, CA; Velmex, Inc., Bloomfield, NY) was applied for manipulating the motion of the wire during functionalization and bacteria deposition on wire surface.

Electrochemical tests were performed using a µ-Autolab type III potentiostatic Frequency Response Analyzer (FRA) equipped with NOVA software version 1.6 (Metrohm Autolab USA Inc., Riverview, FL). The conventional three-electrode configuration was utilized for constructing the electrochemical cell (Figure 3.2(b)). The working electrode (WE) was the microwire modified with various coatings, and a platinum wire (product # CHI 115, CH Instruments, Inc., Austin, TX) with a diameter of 0.5 mm served as the counter electrode (CE). The Ag/AgCl (3 M KCl) reference electrode (RE) was purchased from VWR (catalog # A 57194, Brisbane, CA).

**Bacterial cultivation**

All the experiments involving hazardous bacteria were conducted in the biosafety level 2 laboratory. All bacterial strains were transferred into tryptic soy broth (TSB; BD diagnostic systems, Franklin Lakes, NJ) twice and incubated at 37ºC for 24 hrs prior to use. The initial concentrations of the *E. coli* K-12 and *S. Typhimurium* stock culture were obtained using serial dilutions and plate counting methods.
Figure 3.2. (a) Experimental Set-up for electrochemical impedance spectroscopic and measurement (b) three-electrode electrochemical cell

Functionalized microwires preparation

Microwires were sanitized in an ultrasonic washer, rinsed with 95% alcohol, dried
in air and mounted on the automated xyz stage. A polydimethylsiloxane (PDMS) coated plate was used as a substrate for maintaining the shape of reagents droplets throughout the immobilization process. The sanitary wire tip was immersed into 5 µL PEI for 5 min, and then dried in air for 2 min. Thereafter, the wire was soaked in 5 µL streptavidin for 1 min and then pulled out. Biothynylated anti-β-galactosidase antibodies were applied afterward in the same manner for 5 min to allow antibodies bind streptavidin. The bioeinylated antibodies were linked to the streptavidin and ready to cross-link with bacterial cells.

Detection of E. coli cells

5 µL of each E. coli K-12 cell suspension with different cell concentrations were deposited on the surface of functionalized microwires using the DEP and antibody-antigen interaction governed sensing method that developed in our previous study.

Electrochemical impedance measurements were carried out within a frequency range of $10^{-1}$ to $10^5$ Hz. The applied DC potential was 200 mV and the amplitude of the applied sinusoidal perturbation was 10 mV. 10 min interval was applied between each two measurements to ensure steady-state of the electrochemical system.

Data analysis

Experimental data were displayed by Nyquist plots. The Nyquist plots were then fitted by the build-in analytical tool “Electrical Circle Fit” in NOVA software, and both solution resistance ($R_s$) and electron transfer resistance ($R_{et}$) were collected from the fitted data. The changes of electron transfer resistance ($\Delta R_{et}$) for the redox reaction happening
at the electrode-film interface before and after bacteria binding with the antibodies were calculated as:

\[
\Delta R_{et} = R_{et} \text{(antibody-bacteria)} - R_{et} \text{(antibody)}
\]  

(1)

**Results and Discussion**

Figure 3.3 describes the configuration of the functionalized microwire proposed in this research. Elements appeared in the diagram were not drawn to real scale for visualization purpose. The first functional layer was PEI, which was capable to directly bind streptavidin via electrostatic interaction. One mole streptavidin is able to adsorb 4 moles of biotin through the well-known streptavidin-biotin interaction, making streptavidin a powerful tool to increase the biotinylated antibody loading during immobilization (Xiao *et al.*, 2007).

Figure 3.4 is the impedance spectra of the electrode with *E. coli* cells adhering to its surface with different concentrations. According to Vyas and Wang (2008), layer-by-layer buildup of thin films on a substrate is not always homogeneous and sometimes fails to fully cover the substrate surface. Since the wire was vertically soaked in electrolyte solution by 1 cm, while the functional coating on the microwire surface only presented at the wire tip in about 400 µm length, the wire with a heterogeneous surface as a working electrode has generated more complicated electrochemical spectra. The Nyquist plots could be roughly interpreted by looking at the magnitude of each semicircle, which is proportional to the impedance of the system under investigation.
Figure 3.3. Schematic diagram of microwire functionalization and bioaffinity reactions of antibodies-antigens

Figure 3.4. Impedance spectra for functionalized microwires with E. coli binding on surfaces. Operating parameters: EIS, 5 mM [Fe(CN)$_6^{3-/4-}$] (1:1) in 0.1 M KCl, 0.1 - 100,000 Hz frequency range with 0.01 V amplitude sinusoidal perturbation; E. coli concentrations: $10^3$ - $10^8$ CFU/ml
It was reported by Xiao et al (2007) that antibody-antigen complex acted as an insulating layer to resist the current flow thus affect the resulting impedance of the electrode being modified. Therefore, it was assumed that the electron transfer resistance of the microwire would increase continuously as bacterial concentration rose from $10^3$ to $10^8$ CFU/ml. The linear relationship between $\Delta R_{et}$ and *E. coli* concentration shown in figure 3.5 well validates the prediction. Regarding the slower increasing trend from $10^7$ to $10^8$ CFU/ml, one possibility is that the active sites of immobilized antibodies for binding bacteria were closed to saturation, resulting only a small impedance change. Since 5 µL bacterial suspension was used for each detection, the detection limit could be estimated as $5 \mu L \times (n \times 10^3 \text{ CFU/ml}) = 5n \text{ CFU}$, where $n$ could be 1 to 10 depending on the initial number of the bacterial stock culture.

![Figure 3.5. Changes of electron transfer resistance with *E. coli* captured on the electrode surface at different concentrations from $10^3$ to $10^8$ CFU/ml](image)

---

Figure 3.5. Changes of electron transfer resistance with *E. coli* captured on the electrode surface at different concentrations from $10^3$ to $10^8$ CFU/ml
Figure 3.6 shows the specificity of the functionalized microwire sensor towards *E. coli* when *Salmonella* was used as a control. Both *E. coli* and *Salmonella* suspensions at $10^4$ CFU/ml were employed for the test. After binding with *Salmonella*, the electron transfer resistance of the wire increased 126.5 $\Omega$ compared to that in the absence of bacteria. This may due to the slight non-specific adsorption of *Salmonella* cells onto the electrode surface. However, a much greater $R_e$ increment of 2066.5 $\Omega$ was observed with the attachment of *E. coli* cells to the wire surface, indicating high selectivity of the modified microwire sensor.

**Conclusion**

In summary, label-free rapid and selective detection of *E. coli* K-12 by a microwire sensor modified with a three-layer (PEI-streptavidin-antibodies) functional
coating was demonstrated in this chapter. The overall performance of the developed sensor was evaluated by monitoring the change in electrochemical impedance of the wire after bacterial cells deposited on its surface using electrochemical impedance measurements. A linear trend of impedance increment was obtained as *E. coli* concentration increased from $10^3$ to $10^8$ CFU/ml. EIS analysis has proved that the sensor was able to detect a few CFUs of *E. coli* cells from one milliliter of buffer. In addition, little change of $\Delta R_{et}$ was observed when the sensor worked with *Salmonella*, which clearly evidenced the sensing specificity towards *E. coli* K-12. In comparison with the bacterial quantification method used in Chapter 2, the EIS demonstrates several advantages over the use of fluorescence microscopy. According to FESEM images, bacterial cells captured on a wire were not uniformly distributed on the surface. Fluorescence intensity measurement, however, highly depends on the area selected for measurement and thus requires large amount of measurements to improve the accuracy. Also, the fluorescence intensity is based on two-dimensional pictures taken by the microscope, which is not able to include all characteristics of the wire. Electrochemical system is a three-dimensional system that allows for integrated and dynamic analysis of the microwire without any blind side. Additionally, the mechanism of bioaffinity reactions between bacterial cells and antibodies that occurred on the interface between the electrode and electrolyte solution could be better understood upon the demand by further electrochemical impedance analysis.
References


