Using Magnetostrictive Biosensors for *Salmonella typhimurium* and *Campylobacter jejuni* Detection

by

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Abstract

Salmonella and *Campylobacter* are two of the most common genera of foodborne pathogens. Contaminated foods, water, undercooked foods or contact with infected animals could cause salmonellosis and campylobacteriosis. Magnetostrictive materials are highly sensitive to the mass loaded on the surface. This property has been used for fabricating biosensors for pathogen detection. In this study, a magnetostrictive paticle (MSP) in size of 1.0 × 0.2 × 0.25 mm or 2 × 2 × 0.25 mm was fabricated and coated with three layers of silica and 100 nm of gold. The coatings are highly stable according to the resonance frequency response in water. Anti-*Campylobacter* and anti-*Salmonella* antibodies were well immobilized on silica and gold coated sensors by covalent bonding and adsorption, respectively. The immobilization efficiencies were tested by ELISA. Scanning electron microscope (SEM) images and resonance frequencies showed that the MSP based biosensors can capture *Salmonella typhimurium* and *Camplylobacter jejuni* in water. Comparing the SEM images and the frequency data of silica and gold coated biosensors, the performances of these two biosensors were similar and both biosensors are feasible for pathogen detection with the sensitivity of 10^2 CFU/mL in foods.

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List of Abbreviations

AOAC	Association of Official Agricultural Chemists
APC	Aerobic Plate Count
APHA	American Public Health Association
APTMOS	3-Aminopropyl-trimethoxysilane
ATP	Adenosine Triphosphate
AW	Acoustic Wave
CDC	Center for Disease Control and Prevention
CFU	Colony Forming Unit
DNA	Deoxyribonucleic Acid
EDS	Energy Dispersive Spectroscopy
ELISA	Enzyme-linked Immunosorbent Assay
ERS	Economic Research Service
FPW	Flexural Plate Wave
GBS	Guillian-Barré Syndrome
IgG	Immunoglobulin G
MC	Microcantileve
MSP	Magnetostrictive Particles
NAD(P)H	Nicotinamide Adenine Dinucleotide (Phosphate) Oxidase
PCR	Polymerase Chain Reaction

PNPP	p-Nitrophenyl Phosphate
RNA	Ribonucleic Acid
SAW	Surface Acoustic Wave
SEM	Scanning Electron Microscope
SPC	Standard Plate Count
SPR	Surface Plasmon Resonance
TEOS	Tetraethoxysilane
TSA	Trypticase Soy Agar
TSB	Tryptic Soy Both
TSM	Thickness Shear Mode
UV-Vis	Ultraviolet–visible

CHAPTER 1: INTRODUCTION

1. Background

Salmonella spp. are common pathogens that cause enteric fever and gastroenteritis in humans (Miller and Pegues 2000). For most people, salmonellosis is self-limited. However, *Salmonella typhi* and *Salmonella paratyphi* cause enteric fever with a mortality rate of 10%-15% if no treatment is applied (Micheal and others 2001). *Salmonella* can be transmitted through contaminated foods, water, or by contacting from infected animals. People of all ages can be infected with *Salmonella*. To infants, older people or those with compromised immune system, *Salmonella* has a higher chance for causing disease. For patients with severe salmonellosis symptoms, intravenous fluid injections or antibiotics are needed (Benenson and Chin 1995). There are approximately 2000 *Salmonella* serotypes that can cause disease in humans. *Salmonella enteritidis*, *Salmonella typhimurium*, and *Salmonella newport* are the most common serotypes of *Salmonella* that contribute half of the salmonellosis cases in the United States annually (CDC 2012). As of June 2012, there have been 93 persons, 18 of which have been hospitalized, infected with outbreak strains of *Salmonella infantis*, *Salmonella newport*, and *Salmonella lille*. Those cases were reported from 23 states and related to live poultry (CDC, 2012). It is estimated that 1.2 million cases of salmonellosis occur annually which cause $2.3 to $3.6 billion in economic loss (Frenzen and others 1999; Buzby and Farah 2004).

Campylobacter is the most common cause of diarrhea in the United States (CDC 2010). *Campylobacter* spp. causes nausea, vomiting, diarrhea (sometimes with blood), abdominal pain and fever in humans. The sequelae of campylobacteriosis are the Guillian-Barré syndrome (GBS) (Allos 1997) and the Reiter syndrome (Peterson 1994). Similar to *Salmonella* infections, campylobacteriosis is self-limiting to most people, but to some people with severe diarrhea, intravenous fluids and antibiotics are needed. It is estimated that there are 2.4 million cases of campylobacteriosis occur annually in the United States which contribute to a $1.2 billion in economic loss (Partnership for Food Safety Education 2010).

Good detection methods are required to avoid or to reduce contaminated foods flowing into public market. There are many well established and available detection methods such as conventional, immunochemical and molecular biological methods. However, the disadvantages of those methods are either time consuming or not suitable for onsite detections. Nowadays, food industries are in urgent need of rapid, potable, and onsite detection methods to not only minimize their economic loss for recalled food, but also to protect consumers from pathogenic infections.

2. Purpose of Study

There are many methods for pathogen detection. By culturing the pathogens, traditional methods are accurate and specific. However, it takes several hours to prepare for detection and days to get the results. Polymerase chain reaction (PCR) is a widely used molecular biological method for bacteria detection in the area of food safety. With DNA and RNA being the target of this method, it is highly accurate and specific. Trained personnel is needed for performing PCR analysis, and in some foods, there are inhibitors for DNA amplification. Immunochemical methods such as ELISA make use of the interaction between antibody and antigens. There are numerous studies related to using ELISA for pathogen detections but there are also

disadvantages associated with ELISA, such as timely testing (up to one day), high bacterial populations required (Ng and others 1996), and false-positives results (Ball and others 1996; Beutin and others 1996; Pulz and others 2003).

The methods mentioned above are all now widely used. However, food industries still need rapid, onsite detection methods for detecting pathogens. Biosensor detections are rapid which may meet those requirements. There are many biosensors that have been studied for microbial detection, such as electrochemical, optical, and acoustic wave biosensors. The acoustic wave biosensors that are based on magnetostrictive material can be used for wirelessly detecting pathogens. Currently, there are researchers who are using magnetostrictive particle (MSP) biosensors for detecting pathogens. The gold coated MSP is fabricated to prevent corrosion and to enhance the sensing elements' immobilization (Fu and others 2010, Zhang 2010).

The cost of the gold coated biosensors is very high. Therefore the purpose of this study is to investigate the performance of silica coating which is inexpensive and if silica coating can be used to substitute for the gold coating.

3. Significance of Study

Biosensors based on the MSP technology are highly sensitive, easy to use, and potable. More importantly, this type of biosensor could be used wirelessly for onsite detection. The ultimate goal of this study is to fabricate a silica coated biosensor for detecting foodborne pathogens wirelessly. If this biosensor proves to be successful, it has potential to be used in the food industry. To detect the pathogen on food products before shipping is practical, since the loss from recalling could be saved if products were contaminated during processing. To achieve this goal, the use of biosensors is of good choice, especially by the use of magnetostrictive biosensors.

CHAPTER 2: REVIEW OF LITERATURE

1. *Salmonella*

According to the Centers for Disease Control and Prevention (CDC 2012), *Salmonella* spp. belong to the enterobacteriaceae and are gram-negative, rod-shaped bacilli. Enteric fever and gastroenteritis are the main symptoms of *Salmonella* infection (Miller and Pegues 2000). *Salmonella typhi* and *Salmonella paratyphi* cause enteric fever to humans. The mortality rate is 10-15% if no treatment applied (Ohl and Miller 2001). Nontyphoidal *Salmonella* species, including *Salmonella enteriditis* and *Salmonella typhimurium*, cause self-limited enteritis in humans (Ohl and Miller 2001). People of all ages can be infected by *Salmonella* and it poses a greater risk to infants, the older and immuno-compromised people. *Salmonella* can be transmitted by contaminated foods, water, or coming into contact with infected animals. There are approximately 2,000 *Salmonella* serotypes that can cause disease in humans. *Salmonella enteritidis*, *Salmonella typhimurium*, and *Salmonella newport* make up approximately half of the confirmed *Salmonella* isolates reported by public health laboratories to the National *Salmonella* Surveillance System (CDC 2012). CDC estimated that there are 1.2 million *Salmonella* infection cases each year in the United States, where about 400 cases are fatal, and a few cases are associated with chronic arthritis. Buzby and others (1996) and Frenzen and others (1999) estimated that *Salmonella* infection causes a great number in losses of work, life and medical care cost, resulting in $2.3 to $ 3.6 billion lost annually.

2. *Campylobacter*

According to the CDC, *Campylobacter* spp. are gram-negative, spiral-shaped bacteria. Currently, they are the most common causes of diarrhea in the United States (CDC 2010). Campylobacteriosis is transmitted by raw or undercooked poultry meat, unpasteurized milk, contaminated water, cross contamination from those foods, and contact with infected animals (CDC 2010). *Campylobacter* spp. commonly causes nausea, vomiting, diarrhea (sometimes bloody), abdominal pain and fever. The disease usually lasts a week (CDC 2010). Similar to *Salmonella* infection, campylobacteriosis is self-limiting to most people, but to some with severe diarrhea, intravenous fluids or antibiotics treatments are needed. If those symptoms become worse and last longer than a week, antimicrobial therapy is needed. If it is delayed, therapy may not work (Altekruse and others 1999). *Campylobacter jejuni* can cause disease with less than 500 cells in the human body, and it is estimated that 2.4 million cases of campylobacteriosis occur annually in the United States where 124 cases are deaths (CDC 2010). According to the Economic Research Service (ERS) of the USDA, camplybacteriosis causes medical cost, loss of productivity, death and GBS, which adds up to $1.2 billion in economic loss per year (Partnership for Food Safety Education 2010).

3. Current Bacteria Detection Methods

3.1 Conventional Plate Count Method

The plate count method is used to detect or identify bacteria by culturing the bacteria, usually including several steps: food sampling, preparation of homogenate, culturing and recording results (Andrews and Hammack 2003).

In the sampling step, it is impractical to inspect the whole lot of food, so usually small amounts of samples are taken to determine if the food is safe to be consumed. If samples are

improperly collected or handled, the results may not represent the quality of the entire lot of the food. The sample must reflect the composition of the lot, which can be achieved by sampling adequate units from the lot statistically (Andrews and Hammack 2003). If it is a liquid, it should be shaken thoroughly before sampling and analysis. For *Salmonella* detection, 25 g of food is recommended as an analytical unit (Andrews and Hammack 2003).

After mixing the sample in a buffer by a blender, a ten-fold series of dilutions is the most common procedure followed for microbial analysis.

Standard plate count (SPC) method is used to determine the total culturable bacteria population or to identify bacteria depending on the growth media. SPC have been established by the Association of Official Agricultural Chemists (AOAC) and the American Public Health Association (APHA) (Speck 1984). Spread-plate and pour-plate are the most common methods used in SPC methods. In pour-plates microbial analysis, 1 ml of each dilution of sample is added into a petri dish followed by pouring 12-15 mL of medium at 45 ± 1 °C (Andrews and Hammack 2003). The medium should be added immediately after the sample is put into the plate and should be mixed by gently rotation of the plate. In spread-plate method, 0.1 ml of each diluted sample is added to a medium plate and spread evenly on the surface by a spreader. After culturing the microorganisms at a designated temperature and time, the results are recorded. The appropriated plates for recording the results are those that contain 30-300 colonies per plates (Koch 1994).

Traditional methods are very well established and have been used as standard methods for the detection of most bacteria. However, the SPC usually takes 24 hours or longer. For example, in *Salmonella* detection, a pre-enrichment and an enrichment are needed which takes 48 hours

(Andrews and others 2011). Besides being time consuming, conventional methods are laborious and not suitable for field detection.

3.2 Polymerase Chain Reaction (PCR)

PCR is used to amplify DNA from cells. The amplification is exponential so that after 30-40 cycles, millions of copies of target DNA can be produced from a single cell. By using specific primers, the specificity of PCR can be very high. There are three steps involved in the PCR protocol including DNA denaturation at high temperatures (~ 94 ℃); primer annealing at lower temperatures (~ 50℃); and the extension of new DNA (~ 70℃) (Principle of PCR 2010).

PCR is widely used as a rapid method for bacteria detection in the area of food safety. Bej and others (1994) used PCR to detect *Salmonella* in oysters. In Bej and others' study, *him*A gene was used as a target gene, and two primers were tested by detecting 43 strains and serotypes of *Salmonella* and 97 strains of *non-Salmonella* bacteria. Their results showed that the two primers exclusively amplified the gene from 43 strains and serotypes of *Salmonella* and not from the 97 strains of non-*Salmonella*. According to their report, their method could detect *Salmonella* contaminated oysters in 3 to 5 hours with high specificity. Bennett and others (1998) used 100 strains and serotypes of *Salmonella* and 35 *non-Salmonella* bacteria to evaluate one of the commercial PCR-based systems, BAX^{TM} system. It was reported that the BAX^{TM} system could give results within 28 hours, and those results are 95.8-98.6% in consistent with conventional detection methods. Many PCR related research have been done for detecting foodbonre pathogens, such as *Esherisheria coli* (Cannon and others 1992; Fratamico and others 1995), *Campylobacter* (Linton and others 1996, 1997), *Shigella* (Lindqvist 1999; Peng and others 2002), *Listeria monocytogenes* (Graham and others 1996; Doumith and others 2004), and etc.

The disadvantages of PCR for microbial detection in foods are (1) requiring trained personnel, (2) the existence of inhibitors to DNA amplification, such as calcium ions in milk (Bickley 1996), and (3) expensive equipment.

3.3 Immunochemical Assay

ELISA is one of the immunochemical assays which are used in the detection of antibodies, antigens, bacteria, and etc. Antibodies, antigens, enzyme-labeled antibodies, and a solid phase which has adsorption properties are involved in ELISA. The sequence of the adsorption of antigens, antibody, and enzyme-labeled antibodies are different depending on the types of ELISA. There are several types of ELISA, including direct, indirect, sandwich, and etc (Crowther 1995). In the direct ELISA, antigens or antibodies are adsorbed on the solid surface and react with enzyme-labeled antibodies or antigens (Crowther 1995). In indirect ELISA, two antibodies are used: one is the primary antibody which reacts with the immobilized antigen, and the secondary antibody is an enzyme-labeled anti-primary antibody (Crowther 1995). The sandwich ELISA is similar to the indirect ELISA. The only difference is that the secondary antibody may be the same as the primary antibody (Crowther 1995). After the enzyme-labeled antibodies are added and incubated for a certain period of time, substrate is added. After the reaction is stopped, the results can be read visually or spectroptometrically (Crowther 1995). The advantages of ELISA are that solid phases are commercially available, such as the 96-well microplate, and the results can be read visually or spectrophotometerically (Crowther 1995).

There are many researches that use ELISA for detecting and identifying foodborne pathogens. Palumbo and others (2003) used ELISA to determine the serotypes of *Listeria monocytogenes*. In their study, 101 isolates of *Listeria monocytogenes* were studied, and 89 of them were in consistent with agglutination serotyping analysis. In addition, by the ELISA

method, Palumbo and others (2003) also characterized 100 isolates of *Listeria monocytogenes* which were not studied previously. Ng and others (1996) used a monoclonal antibody T6 in their ELISA method which can be used to detect *Salmonella* at a population of 10^5 and 10^7 CFU/ml, and the excess of *E. coli* did not affect the results. In their work, 232 strains of *Salmonella* and 65 strains of *non-Salmonella* were studied, and none of the non-*Salmonella* strains tested positive. Ng and others (1996) also used their methods to detect and differentiate the serotypes of *Salmonella* in enrichment cultures of food samples including eggs, pork, and infant formula milk. The results showed 100% accuracy on 26 of *Salmonella* contaminated samples, and 99% accuracy on 101 of the non-*Salmonella* contaminated samples. ELISA can also be used with other methods, such as PCR. Gutiérrez and others (1998) developed an ELISA-PCR quantitative detection method for spoilage bacteria in refrigerated raw meat, and the results showed that the detection sensitivity was 10^2 CFU/cm^2.

Compared to conventional methods, ELISA is simpler and rapid. However, the weaknesses of ELISA method are: (1) to take 8 to 24 hours to obtain the results; (2) to have sensitivity around 10^5-10^7 CFU/ml (Ng and others 1996); and (3) to possibly show false-positive results (Ball and others 1996; Beutin and others 1996; Pulz and others 2003).

4. Biosensors Used for Bacteria Detection

In recent years, biosensors have become important analytical tools in the pharmaceutical, biotechnology, food, and other industries (Leonard and others 2003). A biosensor is an analytical device that can convert a biological stimulus into a measurable signal. Typical biosensors consist of: (1) a sensing element that can specifically interact with the target; (2) a transducer that can convert biological signals, which are generated from the interaction between targets and sensing elements, into measurable signals; and (3) an output system to record and analyze the data. Many

researchers have been devoted to developing biosensors that are sensitive, cost effective, and easy to operate for rapid detection of interested targets. Based on the types of transducers, the biosensors are mainly classified into electrochemical, optical, and acoustic wave biosensors (Grate and others 1993; Mello and Kubota 2002).

4.1 Electrochemical Biosensors

In a system where electrons are generated or consumed, the electrochemical biosensors can be used to collect the electrochemical signal. According to the types of transducers that are used to transform the signal, the electrochemical biosensors can be classified into four groups being conductimetric, impedimetric, potentiometric and amperometric sensors (Mello and Kubota 2002).

Bacteria can change the conductivity of media by metabolizing the uncharged fat or by metabolizing carbohydrates into fatty acids or organic acids (Mello and Kubota 2002). The charge increases when more fatty acids or organic acids are formed, which is directly in proportion to the growth of bacteria (Mello and Kubota 2002). Conductimetric biosensors can be used to detect the change of conductivity by two electrodes to measure the growth of bacteria (Mello and Kubota 2002). The principle of impedimetric sensors is similar to that of conductimetric sensors, but it measures the change of impedance of the media during the growth of bacteria. The advantage of impedimetric sensors is that it is more specific than conductimetric biosensors due to the use of a reference module as a control to prevent the effects caused by environmental factors such as temperature, evaporation, dissolved gases, and degradation of media (Mello and Kubota 2002). There are several impedimentric biosensors that are commercially available, including Bactometer and Malthus M 1000s (Mello and Kubota 2002). The potentiometric sensors can be used to measure the potential change that is proportional to

the biological reactions by comparing it with a reference electrode (Mello and Kubota 2002). According to the change of potential, the concentration of the substrate or antigen can be calculated (Mello and Kubota 2002). Amperometric sensors are based on the same principle, but it measures the current instead of potential (Mello and Kubota 2002).

Electrochemical biosensors haven been developed for the detection of foodborne pathogens, such as *Salmonella* (Feng 1992), *Staphylococcus aureus* (Brooks and others 1990), and *E. coli* O157:H7 (Abdel-Hamid and others 1990). Electrochemical biosensors can also be used to inspect the quality of foods. For example, the potentiometric biosensors were used to monitor the hygienic sanitary quality of foods (Taylor and others 1991; Wang 1999) and to detect the pesticides in foods (Wan and others 1999).

4.2 Optical Biosensors

Optical biosensors can be used to measure UV-Vis absorption, fluorescence, phosphorescence, reflectance, scattering, and etc (Mello and Kubota 2002). There are several types of optical biosensors, such as luminescent and surface plasmon resonance (SPR) biosensors.

Luminescent biosensors measures ATP, NAD(P)H, or H_2O_2, and this type of biosensor uses luciferase from bacteria, such as *Vibrio fischeri* and *Vibrio harveyi* or other chemiluminescent substances together with oxidases or reductases (Mello and Kubota 2002). There are many studies on luminescent biosensors. Blum and others (1998) immobilized bioluminescence enzymes on a fiber-optic probe to detect ATP and NADH. The results showed that the biosensors immobilized with firefly luciferase could measure the concentration of ATP ranged from 2.8×10^{-10} to 1.4×10^{-6} M. In addition, if bacterial luciferase and oxidoreductase from *Vibrio fischeri* were immobilized together with the firefly luciferase, the biosensor could measure NADH with a

concentration range from 3×10^{-10} M to 3×10^{-6} M. Latif and others (1998) developed a luminescent biosensor with a sensitivity of 6×10^{-7} M to glucose and 2.5×10^{-8} M to H_2O_2.

According to Homola and others (1999), SPR biosensors have the potential to be used for food safety and environmental analysis because they are simple to use, no molecule labeling is needed, and it is able to analyze raw samples without purification. In SPR biosensors, the energy of light photons is transferred to the electrons in a metal and the excited electrons on the surface of the metal are called plasmon. If there are any chemical changes occurring in the field of the plasmon, resonance of plasmon will be changed which can be identified by the shift of the angle of incidence light. From the shift of the angle, the target can be identified (Mello and Kubota 2002).

SPR is used in laboratories for food safety analysis. Koubová and others (2001) used antibody immobilized SPR biosensors to detect the *Salmonella enteritidis* and *Listeria monocytogenes*, which showed a detection sensitivity of 10^6 CFU/mL. In the work of Taylor and others (2006), an eight channel SPR bisoensor was used which could detect *Escherichia coli* O157:H7, *Salmonella choleraesuis* serotype Typhimurium, *Listeria monocytogenes,* and *Campylobacter jejuni* at the same time. The detection limits raged from 10^3 to10^5 CFU/ml (Taylor and others 2006). Oh and others (2005) immobilized protein G on a biosensor to detect *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Legionella pneumophila*, and *Yersinia enterocolitica* in a contaminated environment.

4.3 Acoustic Wave (AW) Biosensors

AW biosensors are resonators whose resonance frequency will decrease if there is mass loaded on the sensors. This basic principle is applied to all AW biosensors. The mass sensitivity and quality merit factor (Q value) are the most important parameters to characterize AW

biosensors, which determine the sensitivity of AW biosensors. High sensitivity to mass and Q value are favorable to fabricate a sensitive AW biosensor (Mehta and others 2001). Piezoelectric materials are commonly used in acoustic devices, such as thickness shear mode (TSM) resonator, surface acoustic wave (SAW) sensors, flexural plate wave (FPW) devices and microcantilevers (MC) (Grate and others 1993). In our study, the magnetostrictive particle (MSP) which belongs to AW sensors was used. Compared to other AW biosensors, the advantages of MSP biosensors are wireless, freestanding, high sensitivity to mass and high Q value. MSP used in this study is made from Metglas® 2826MB which is one of the magnetostrictive materials. In an AC magnetic field, the MSP sensors will vibrate and the vibration of the sensor along its length direction and frequency (f_n) follows the equation:

$$f_n = \frac{n}{2l} v \qquad n=1, 2, 3\ldots$$

The acoustic velocity (v) of the magnetostrictive material is a constant and is determined by the elastic properties of magnetostrictive materials. The "l" is the length of MSP (Liang and others 2007). Since magnetic field is used to induce vibration of MSP, MSP biosensors can be used wirelessly. Theoretically, the sensitivity (S_m) of MSP biosensors follows the equation:

$$S_m = -\frac{\Delta f}{\Delta m} \cong \frac{fn}{2M} \qquad n=1, 2, 3\ldots$$

The Δm, M, and f_n are the mass load, the initial mass of MSP, and the frequency, respectively (Li and Cheng 2010). Smaller size MSP has better sensitivities than bigger sensors. Typically, the magnetostrictive materials are iron nickel-based alloy, so in order to enhance its stability and sensing elements immobilization, a layer of copper (Li and others 2010) or gold is often coated on the surface of magnetostrictive sensors (Fu and others 2010, Zhang 2010).

Using magnetostrictive biosensors to detect pathogens in foods has gained more attention in recent years. Fu and others (2010) detected *E. coli* in water using magnetostrictive biosensors,

which showed a detection limit of 10^5 CFU/ml. Li and others (2010) detected the *Salmonella typhimurium* on the surface of contaminated tomatoes using magnetostrictive biosensors and the results showed that the MSP sensors had a detection limit of 10^2 CFU/ml. Guntupalli and others (2007) used magnetostrictive biosensors to detect *Salmonella typhimurium* in a mixture of *Escherichia coli* O157:H7 and *Listeria monocytogenes* with a detection limit of 10^3 CFU/ml. Park and others (2012) compared the magnetostrictive biosensor with quantitative real time PCR in the detection of *Salmonella typhimurium* on the surface of tomatoes and the results showed that the magnetostrictive biosensors were competitive with Q-PCR (Park and others 2012).

Antibodies and bacteriophages are often used as a sensing element of MSP biosensors for foodborne pathogen detection. Antibodies are a group of glycoproteins which are also called immunoglobulin (Crowther 1995). There are five types of immunoglobulins in mammals: immunoglobulin G (IgG), A (IgA), M (IgM), D (IgD), and E (IgE) (Crowther 1995). All immunoglobulins consist of a basic unit of two light chains and two heavy chains linked by disulfide bonds (Crowther 1995). The N-terminals of both the heavy chains and light chains containing antigen-binding sites, and within the antigen-binding sites, the heterogeneity of amino acid sequences makes antibodies specific to antigens (Crowther 1995). Previously described ELISA methods are well-established antibody-based microbial detection methods. In the area of biosensor detection, antibodies are often used as sensing elements (Koubovaá and others 2001; Grogan and others 2002; Guntupalli and others 2007; Fu and others 2010). Bacteriophages are viruses whose hosts are bacteria. Bacteriophage usually contains a protein coat which encloses its DNA or RNA. Since bacteriophages exclusively infect bacteria (Kutter and Sulankvelidze 2004), they can be used as sensing elements and make MSP biosensors detect specific targets. The sheaths of bacteriophages are more tolerant to heat, to lower or to higher pH than antibodies

(Olofsson and others 2001). Currently, a great number of bacteriophage-based biosensors have been studied (Lakshmanan and others 2007; Nanduri and others 2007; Li and others 2010; Chin and others 2011; Park and others 2012).

CHAPTER 3: STUDY OF THE FEASIBILITY USING MANETOSTRICTIVE BIOSENSORS FOR PATHOGEN DETECTION

1. Materials and Methods

1.1 Materials

The following materials that were used in this study included: metglasTM 2826 ribbon (Iron Nickel-based) obtained from Metglas®, Inc. (Conway, SC), acetone (Fisher Scientific, Swanee, GA), glycerol (Ameresco Inc., Solon, OH), ethanol (Pharmco-Aaper, Philadelphia, PA), methanol (EMD, Darmstadt, Germany), tetraethoxysilane (TEOS) (Strem Chemicals, Newburyport, MA), acetic acid (Pharmco-Aaper, Philadelphia, PA), 3-aminopropyl-trimethoxysilane (APTMOS) (Acros, Pittsburgh, PA), pyridine (Acros, Pittsburgh, PA), NaOH (Fisher Scientific, Swanee, GA), glutaraldehyde (Electron Microscopy Sciences, Hatfield, PA), sodium cyanoborohydride (Acros, Pittsburgh, PA), sodium azide (Acros, Pittsburgh, PA), sodium phosphate dibasic (Fisher Scientific, Swanee, GA), sodium phosphate monobasic (Fisher Scientific, Swanee, GA), sodium chloride (Fisher Scientific, Swanee, GA), potassium chloride (Fisher Scientific, Swanee, GA), p-nitrophenyl phosphate (PNPP) (Pierce, Rockford, IL). diethanolamine (Fisher Scientific, Swanee, GA), magnesium Chloride (Fisher Scientific, Swanee, GA), acetate anhydride (Acros, Pittsburgh, PA), trypticase soy agar (TSA) (BD, Sparks, MD), tryptic soy both (TSB) (BD, Sparks, MD), anti-*Salmonella* rabbit IgG (1 mg/mL, purified from rabbit blood), anti-*Campylobacter* rabbit IgG (1 mg/mL, purified from rabbit blood), alkaline

phosphatase conjugated anti-rabbit IgG (Sigma-Aldrich, St. Louis, MO), hunt enrichment broth (HEB) (Thermo Scientific, Oxoid, England), Whirl Pack Bagò (VWR, Batavia, IL), modified campylobacter charcoal differential agar (MCCDA) (Thermo Scientific, Oxoid, England).

1.2 Preparation of Magnetostrictive Sensors

A sensor platform based on magnetostrictive particles (MSP) was made of MetglasTM 2826 ribbon which is an amorphous magnetostrictive alloy with a large magnetostriction (12 ppm) and high magnetostrictive coupling effect. Sensors with sizes of 2.0 × 2.0 × 0.025 mm and 1.0 × 0.2 × 0.025 mm were prepared by micro wafer dicing saw (Micro Automation, Rochester,NY). After cutting, the MSPs were annealed in a vacuum oven around 220 ^{o}C under -30 inch Hg vacuum for 2 h. The surface of the sensor was ultrasonically cleaned in acetone for 30 min and dried with nitrogen gas. The sensors were then ready to use.

1.3 Silica Coating

Silica coating was processed based on the method reported by Taylor and others (2000) with modification. With this method, approximately fifty 1 × 0.2 × 0.25 mm MSPs or ten 2 × 2 × 0.25 mm MSPs were placed into a 12-mL glass tube. Then 2 mL of TEOS were added, followed by 5 mL of water and 5 mL of glycerol, respectively. Then the pH was adjusted to 3.4 using 1% (v/v) acetic acid. The mixture was heated to 90 °C in a water bath until the silica was deposited on the sensors from the solution. This process required about 3 h. After the mixture was cooled to room temperature, the sensors were collected with a magnet and transferred into a 1-mL microcentrifuge tube. They were washed twice with deionised water (1 mL / time), 5 times with methanol (1 mL / time), and stored in methanol at room temperature until the next coating. The sensors were coated two more times using the same protocol. Scanning Electron Microscope and Energy Dispersive Spectrometer (SEM/EDS) were used to observe silica coating, and to measure

the composition of the sensor. The stability of the coating was tested by frequency performance in which the sensors were placed in water for 13.3 h and the frequency response of the sensor was tested by a pickup coil and a Network analyzer. If the frequency was constant, this indicated that there was no material loaded on the sensor (e.g. corrosion), or dropped from the sensor (e.g. coating peeling off).

1.4 Gold Coating

Prior to the gold deposition, a thin layer of chromium (100 nm) was sputtered on the sensor platform by using a Denton Sputtering System (Moorestown, NJ). The chromium layer was used as an adhesion layer for the gold coating. Then a 100 nm of gold layering was sputtered on the sensor to prevent corrosion and to promote the immobilization efficiency of sensing elements such as antibodies or bacteriophages. Scanning Electron Microscope and Energy Dispersive Spectrometer (SEM/EDS) were used to observe the gold coating effectiveness.

1.5 Sensor Activation and Antibody Immobilization

1.5.1 Activation of Silica Coated MSP

Before antibody immobilization, silica coated sensors were activated. The silica coated MSPs were put into 1.5-mL of centrifuge tubes, one sensor per tube. Then, 200 μL of ethanol and 100 μL of 3-aminopropyl-trimethoxysilane (APTMOS) were added into each tube and mixed thoroughly (Liao and others 2007). The tubes were placed at room temperature for 2 h for amino group immobilization onto the sensor, and then transferred to a 90 °C water bath for 10 min. The MSP in each tube was then washed with 1 mL ethanol, 1 mL water and 1 mL of 10 mM pH 9.0 pyridine-NaOH buffer in that order, respectively. After washing, the MSP was placed in 200 μL of pyridine-NaOH buffer, followed by adding 100 μL of 50% glutaraldehyde to

introduce aldehyde groups (Liao and others 2007). Pyridine-NaOH buffer and glutaradehyde were mixed well and the mixtures were held at room temperature for 2 h to introduce aldehyde groups onto the sensors. After the 2 h reaction, the MSPs were washed with water until the pH was neutral (washed twice and changed the tube for each wash). After washing, the MSPs were placed in 200 μL of a 10% acetate anhydride solution (in 95% ethanol) and held at room temperature for 30 min to block free amino groups on the sensors. The MSPs were then washed twice with PBS buffer. The activated sensors were stored at 4 °C until ready for use.

1.5.2 Immobilization of Antibody on Sensors

The antibody immobilization was performed through conjugation between antibodies and activated silica coated sensors. A solution of 2 M cyanoborohydride and 0.2 M sodium phosphate dibasic buffer were prepared one night before conjugation. Coupling buffer was prepared by combining 1 mL of 2 M cyanoborohydride with 100 mL of 0.2 M of sodium phosphate dibasic buffer.

Silica coated MSPs were transferred into 1.5-mL centrifuge tubes: each tube contained either 1 × 0.2 × 0.25 mm MSP or one 2 × 2 × 0.25 mm MSP, followed by adding 100 μl antibody (50 μg/mL) and 100 μl coupling buffer. The tubes were held at room temperature for 2 h for antibody immobilization. After being washed twice with 1 mL of PBS per time, the MSPs were transferred into new 1.5-mL centrifuge tubes with PBS and stored at 4 °C for use. The effectiveness of the antibody immobilization was tested by ELISA.

A direct adsorption method was used for antibody immobilization on gold coated sensors. For gold coated sensors, the antibodies were diluted with PBS to 25 μg/mL. Gold coated MSPs were transferred into 1.5-mL centrifuge tubes: each tube contained either 1 × 0.2 × 0.25 mm MSP or one 2 × 2 × 0.25 MSP, followed by adding 200 μl antibody. The tubes were held at room

temperature for 2 h to allow the antibodies to be adsorbed. After two washings with PBS buffer, 1 mL per time, the MSPs were transferred into new 1.5-mL centrifuge tubes with PBS and store at 4 °C. The effectiveness of antibody immobilization was tested using ELISA.

1.6 Bacteria Preparation

1.6.1 Preparation of *Salmonella typhimurium*

Salmonella typhimurium ATCC 13311 was incubated in TSB at 37.5 °C for 12 h in a shaker at 200 rpm. Then the bacteria were streaked onto TSA plate and then incubated at 37.5 °C for 12 hours. A single colony was picked and incubated in TSB at 37.5 °C for 12 h in a shaker at 200 rpm. *Salmonella typhimurium* was washed twice with PBS, through centrifugation at 4000*g* for 3 min. After washing, the bacteria were re-suspended in PBS and the $O.D._{640\ nm}$ of the bacterial suspension was measured to calculate the population with a standard curve established previously. Then, the population was adjusted to 10^8 CFU/mL for use.

1.6.2 Preparation of *Campylobacter jejuni*

One ml of a frozen *Campylobacter jejuni* was pre-enriched by adding 100 mL of Hunt Enrichment Broth (HEB), in a Whirl Pack Bag. Air was removed from the bag and a microaerophilic mixture of 5% O_2, 10% CO_2, and 75% N_2 gas was added to inflate the bag and to produce a microaerophilic environment. The bag was then sealed and incubated at 37 °C for 4 h in a shaker incubator. After 4 h, a solution of serile cefoperazone was added to yield a final concentration of 30 mg/L in the HEB culture broth. The microaerophilic atmosphere was re-established, and the bag was incubated for 20 h at 42 °C. Selective plating for *C. jejuni* was achieved using Modified Campylobacter Charcoal Differential Agar (MCCDA). MCCDA plates

were incubated at 42 °C for 24 to 48 h in a microaerophilic environment using anaerobic jars with pressure gauge valves. A culture of 10^6 CFU/mL was prepared for use.

1.7 Performance Analysis

1.7.1 Antibody Immobilization Efficiency

The efficiency of antibody immobilization was tested by ELISA. After the primary antibodies were immobilized on the sensor, the sensor was transferred to a new 1.5-mL tube and washed twice with PBS buffer. Then, 100 μL of secondary antibody were added and incubated for 1 h at room temperature. After washing the sensor twice with PBS buffer (1 mL each time), 110 μL of p-nitrophenyl phosphate (PNPP) (30 mg/10 mL) were added to react for 30 min. The PNPP was prepared by dissolving 30 mg PNPP in 10 mL of 1 0mM diethanolamine solution, pH 9.5 containing 0.5 mM $MgCl_2$. After the reaction, $O.D._{405\ nm}$ of each sample was measured.

1.7.2 Bacteria Binding

To each biosensor, 300 μl of 10^8 CFU/ml of bacteria suspension were added and the sample was held at room temperature for 1.5 h for bacteria binding. After the biosensor was air-dried and treated with osmium tetroxide (OsO_4) for 45 min. The SEM was used to observe the bacteria binding efficiency.

A pickup coil and a Network analyzer were used to measure the frequencies of the biosensors. The biosensor was placed inside of the coil, followed by adjusting the signal with a magnet outside of the coil. Once the signal was steady, the water was pumped through the coil by a peristaltic pump at a speed of 30 μl/min. The frequency of the biosensor in the water without bacteria was recorded every 5 min for 20 min. Then, a series of 10-fold dilution of

bacterial suspensions from 10^1 to 10^8 CFU/ml were pumped through the sensor at the speed of 30 µl/min. For each the suspension, the frequencies were recorded every 4 min for 1 h.

2. Results and Discussion

2.1 MSP Sensor Coating

For the gold coated sensor, a thin layer of chromium with 100 nm thickness was sputtered onto the MSP sensor and then another 100 nm gold layer was applied. The surfaces of the uncoated MSP and gold coated MSP were smooth (Figure 1-a & b). After coating with silica, the surface of MSP was rough (Figure 1-c & d) and the image showed that the rough surface consisted of small silica particles and the sizes of particles were around 100 nm. All silica and gold coated sensors were resistant to acid corrosion tested in 4 M HCl for 4 h (data not shown). According to the energy dispersive spectroscopy (EDS) analysis which is a method used to identify the element compositions of a sample, the silicon element on the sensors were approximately 7% and 13% in a one lay-layer and a 3-layer coating, respectively (Table 1). From the Network analyzer test, the result showed that the silica coating was stable and the frequency of the sensor kept steady for 800 min in water (Figure 2). The steady frequency performance of the silica coated sensor also indicated that the silica coating was very consistent, stable and not corrosive in water.

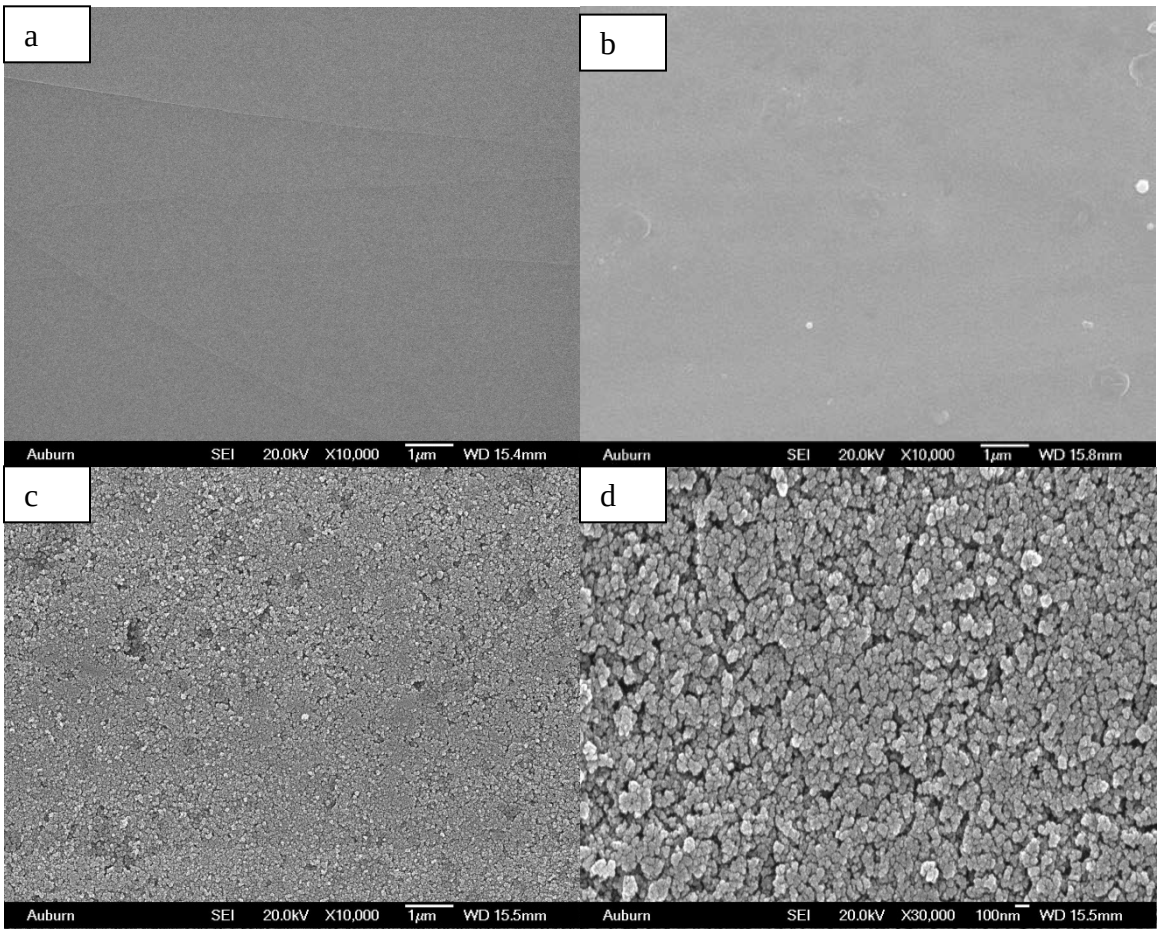

Figure 1-SEM images of MSP sensors. a: MSP; b: gold coated MSP; c: silica coated MSP; d: silica coated MSP. a, b and c: 10,000× magnification; d:30,000× magnification.

Table 1-Element composition of silica coated MSP sensors.

	Number of Coatings		
Element	1	2	3
O	10.53*	12.89	11.68
	31.76	35.80	32.52
Si	4.03	5.83	8.16
	6.93	9.22	12.95
Fe	31.86	32.07	32.63
	27.53	25.52	26.04
Ni	32.73	31.66	30.53
	26.90	23.96	23.17
Mo	6.85	6.45	6.20
	3.45	2.99	2.88
Au	14.00	11.11	10.81
	3.43	2.51	2.45

*The first row in each element is the % weight and the second row is % atom.

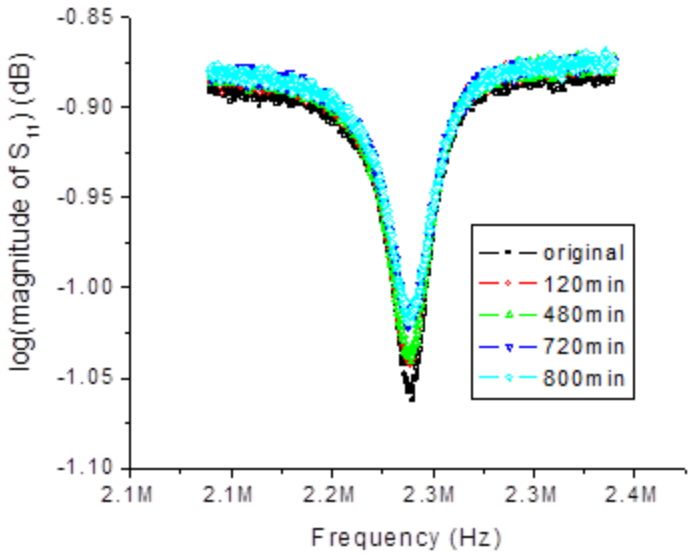

Figure 2-Frequency analysis of silica coated MSP in water for 800 min.

2.2 Antibody Immobilization Efficiency

The anti-*Campylobacter* antibody was produced from a rabbit by immunizing it with formalin inactivated *C. jejuni* cells obtained from Beijing 4A Biotech Co., Ltd (Beijing, China).

The antiserum was successfully produced and the rabbit IgGs were purified through 50% saturated ammonium sulfate precipitation and protein A affinity column. The purity of the IgG was analysed by SDS-PAGE and it was very high. The antibody also demonstrated extraordinary high reactivity to *C. jejuni*. This antibody also has very high specificity to *C. jejuni* which has low reactivity when tested against foodborne bacteria *E. coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella typhimurium* commonly found in poultry and poultry products (Figure 3). The anti-*Salmonella* rabbit antibody used in this study was produced from rabbit by our lab previously. The performance of this antibody has been characterized in Park's research (2009), and the results showed both the reactivity and specificity of the antibody to *Salmonella typhimurium* are high.

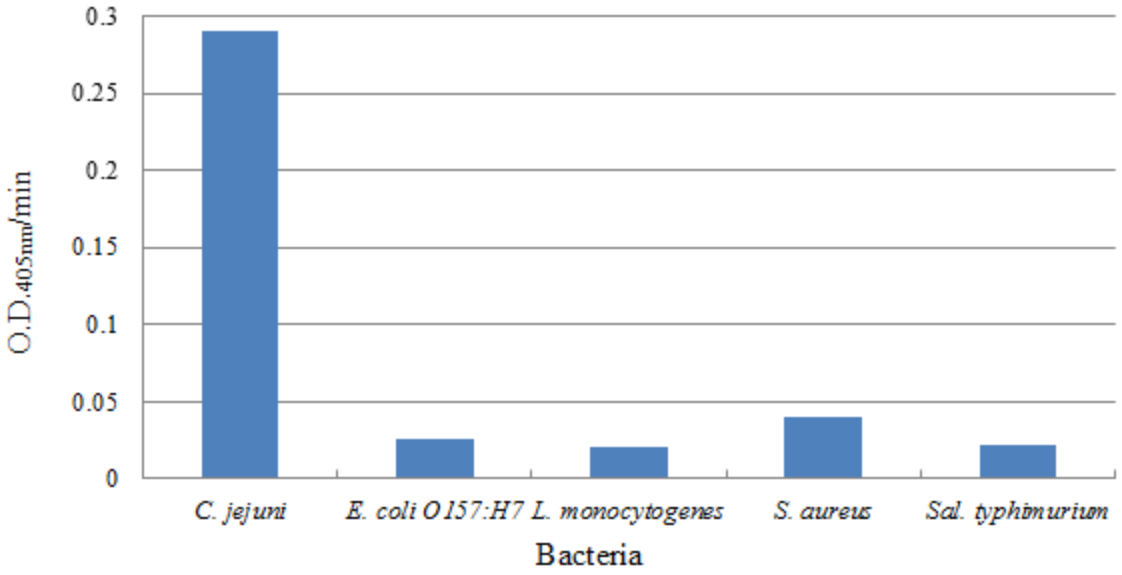

Figure 3- Specificity of anti-*Campylobacter jejuni* antibody

Antibody immobilization efficiency was measured by ELISA. The $O.D._{405\ nm}$ value was used to demonstrate the immobilization efficiency of primary antibody on sensors. The mechanisms of antibody immobilization were covalent bond conjugation and adsorption for silica and gold coated MSP sensors, respectively. Higher antibody immobilization efficiency on the sensor should have higher $O.D._{405\ nm}$ value. From the ELISA data, the O.D. values of the anti-*Salmonella* antibody immobilized silica and gold coated sensors were significantly higher than those of non-antibody coated MSP sensors which indicated the antibody was successfully immobilized on both coated MSP sensors (Table 2 and Figure 4). The immobilization efficiency on gold coated MSP sensors was slightly higher than those on silica coated sensors, but there is no significant difference. Besides these two immobilization processes, other antibody immobilization method was also applied by other researchers, such as Guntupalli and others (2007) used the Langmuir-Blodgett film technique to immobilize antibodies on gold coated mangetoelastic resonance biosensor. However, this method is tedious, time consuming and only one sensor at a time can be produced, which is not practical for commercial application. For the immobilization of anti-*Campylobacter* antibody, the ELISA data showed the similar results of those for anti-*Salmonella* antibody immobilization (Table 3 and Figure 5).

Table 2-ELISA test for rabbit IgG anti-*Salmonella* antibody immobilization efficiency

Sensor treatment	Sensors					Average ± STD
	1	2	3	4	5	O.D. $_{405\ nm}$
SSN[1]	0.1886[5]	0.1559	0.2714	0.1042	0.2419	0.1924±0.0667a[6]
SS[2]	0.6753	0.7276	0.7981	0.6709	0.7375	0.7219±0.0521b
GSN[3]	0.2891	0.2429	0.1574	0.2047	0.2977	0.2384±0.0587a
GS[4]	0.9620	0.8079	0.8752	0.9627	0.9608	0.9137±0.0701b

[1]SSN: silica coated sensor without rabbit IgG anti-*Salmonella* antibody immobilization (control).

[2]SS: silica coated sensor with rabbit IgG anti-*Salmonella* antibody immobilization.

[3]GSN: gold coated sensor without rabbit IgG anti-*Salmonella* antibody immobilization (control).

[4]GS: gold coated sensor with rabbit IgG anti-*Salmonella* antibody immobilization.

[5]Measured at O.D.$_{405\ nm}$.

[6]a,b: for silica or gold coating, different letters means significantly difference at $p>0.05$.

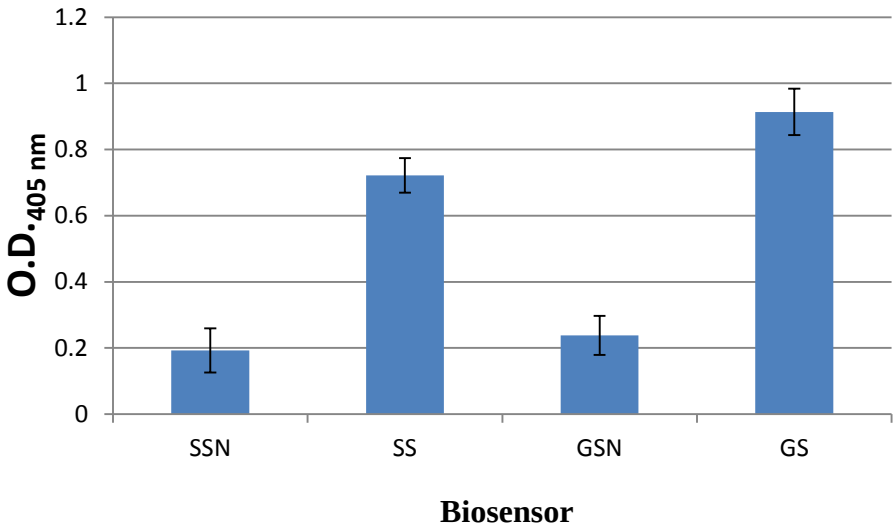

Figure 4-ELISA test for anti-*Salmonella* antibody immobilization efficiency ($O.D._{405\ nm}$, 20 min).

SSN: silica coated sensor without anti-*Salmonella* antibody immobilization (control).

SS: silica coated sensor with anti-*Salmonella* antibody immobilization.

GSN: gold coated sensor without anti-*Salmonella* antibody immobilization (control).

GS: gold coated sensor with anti-*Salmonella* antibody immobilization. Bars represent standard deviations.

Table 3-ELISA test for rabbit IgG anti-*Campylobacter* antibody immobilization efficiency

Sensor treatment	1	2	3	4	5	O.D. $_{405\ nm}$
SCN[1]	0.2891[5]	0.4452	0.2204	0.3362	0.2741	0.3130±0.0847a[6]
SC[2]	0.7919	0.6471	0.8246	0.7549	0.6885	0.7414±0.0731b
GCN[3]	0.2513	0.2567	0.2496	0.2512	0.1446	0.2307±0.0482a
GC[4]	0.7175	0.6977	0.7971	0.9246	0.9008	0.8075±0.1033b

[1]SCN: silica coated sensor without rabbit IgG anti-*Campylobacter* antibody immobilization (control).

[2]SC: silica coated sensor with rabbit IgG anti-*Campylobacter* antibody immobilization.

[3]GCN: gold coated sensor without rabbit IgG anti-*Campylobacter* antibody immobilization (control).

[4]GC: gold coated sensor with rabbit IgG anti-*Campylobacter* antibody immobilization.

[5]Measured at O.D.$_{405\ nm}$.

[6]a,b: for silica or gold coating, different letters means significantly different at $p>0.05$.

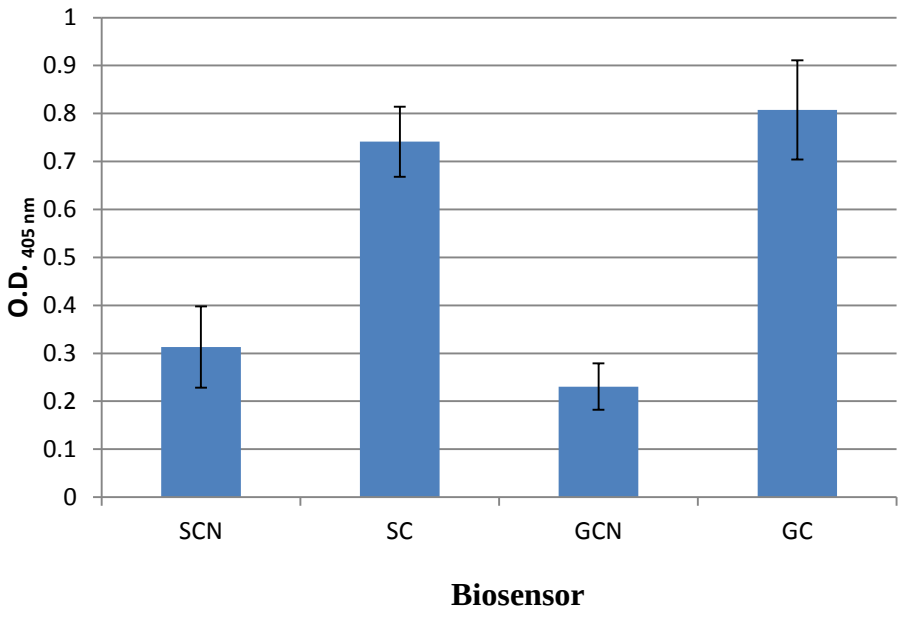

Figure 5-ELISA test for anti-*Campylobacter* antibody immobilization efficiency ($O.D._{405\ nm}$, 20 min).

SCN: silica coated sensor without anti-*Campylobacter* antibody immobilization (control).

SC: silica coated sensor with anti-*Campylobacter* antibody immobilization.

GCN: gold coated sensor without anti-*Campylobacter* antibody immobilization (control).

GC: gold coated sensor with anti-*Campylobacter* antibody immobilization. Bars represent standard deviations.

2.3 Bacteria Binding Efficiency

Bacteria Binding efficiencies of silica and gold coated MSP biosensors were tested by ELISA and confirmed by SEM and HP network analyzer (8751A) with S-parameter (87511A).

For the ELISA test, the $O.D._{405nm}$ readings on both silica and gold coated biosensors were high which indicated high bacteria binding efficiency. Although antibody immobilization efficiencies were similar between silica and gold coated MSP biosensors, both anti-*Salmonella* and anti-*Campylobacter* antibodies immobilized on silica biosensors had better binding efficiencies than those on gold coated biosensors (Figure 6 & 7). The higher bacteria binding efficiency on silica coated sensors in ELISA test is due to the stronger attachment of antibodies on sensors resulted from the covalent binding than those on the direct antibody adsorbed gold coated sensors. The ELISA involves multiple times of washing, if the bindings between antibodies and the sensors are not strong enough, the antibody will be washed off, especially after bacteria are bound to the antibodies.

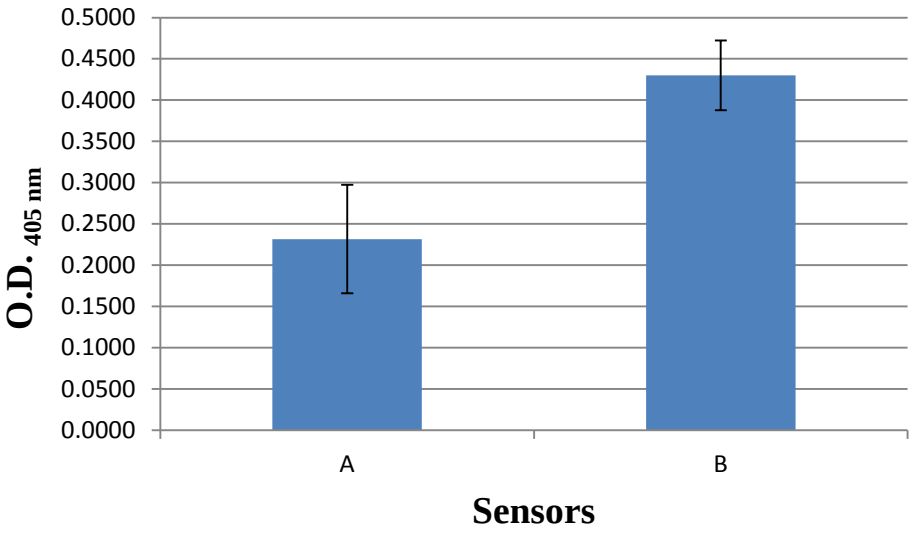

Figure 6- *Salmonella typhimurium* binding performance test of silica and gold coated MSP biosensors by ELISA. Bars represent standard deviations. A: gold coated biosensor; B: silica coated biosensor

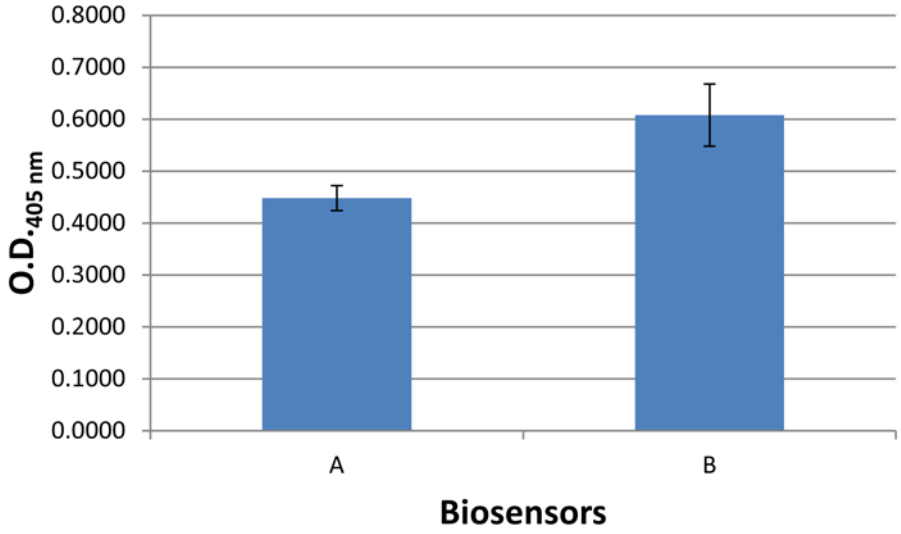

Figure 7- *Campylobacter jejuni* binding performance test of silica and gold coated MSP biosensors by ELISA. Bars represent standard deviations. A: gold coated biosensor; B: silica coated biosensor

After bacteria binding, biosensors were air dried and treated with OsO_4 for SEM observation. The anti-*Salmonella* and anti-*Campylobacter* antibodies immobilized silica and gold coated MSP biosensors showed strong bacteria binding which many bacteria were captured; while none or few bacteria were observed on the control sensors. Within the same dimension, there are about 500 and 300 *Salmonella* cells captured on silica and gold coated MSP biosensors, respectively. For bacteria binding of *Campylobacter jejuni* on antibody immobilized biosensors, within the same dimension, about 600 bacterial cells were counted on each of silica and gold coated MSP biosensors (Figures 8 and 9). The results are consistent with the previous ELISA data of bacteria binding efficiency on antibody immobilized MSP biosensors. It also agreed with the study of Guntupalli and others (2007) which showed that the antibody immobilized gold coated biosensor could capture the *Salmonella* in population of 10^5 CFU/mL or higher, and the study of Fu and others (2010) using the antibody immobilized magnetostrictive microcantilever to detect *E. coli* successfully.

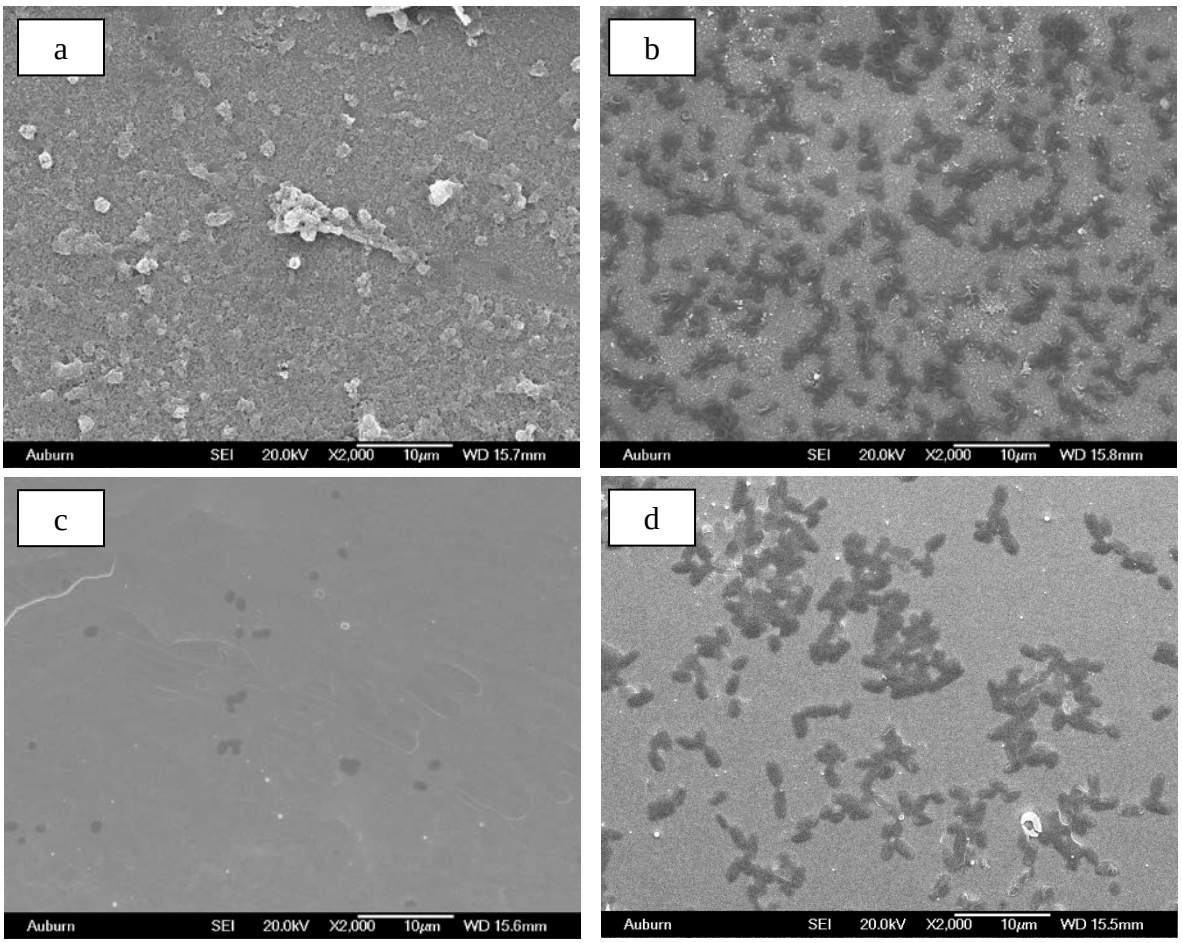

Figure 8-SEM images of *Salmonella* on biosensors. a: Silica coated MSP without anti-*Salmonella* antibody; b: silica coated MSP immobilized with anti-*Salmonella* antibody; c: gold coated MSP without anti-*Salmonella* antibody; and d: gold coated MSP immobilized with anti-*Salmonella* antibody. Images are at 2,000× magnification.

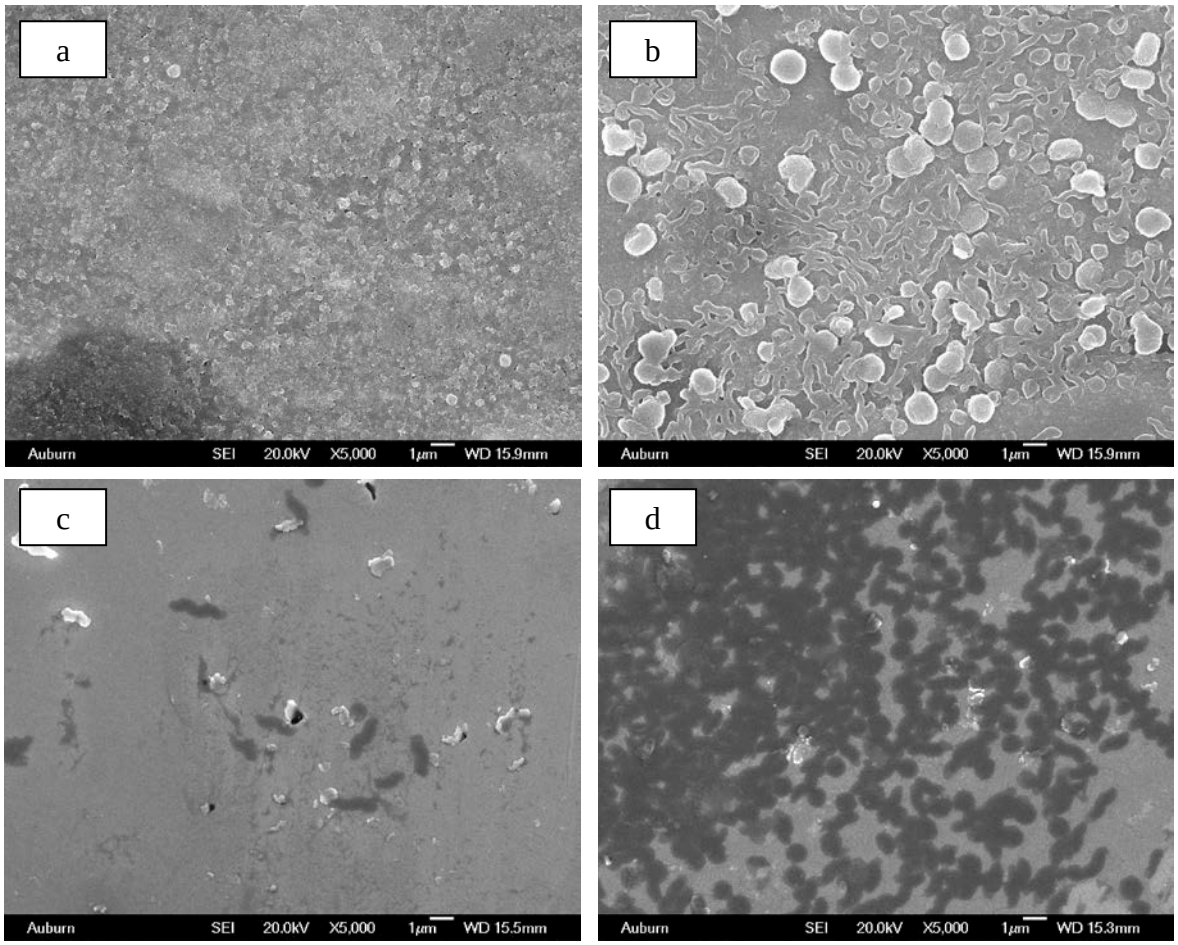

Figure 9-SEM images of *Campylobacter jejuni* on biosensors. a: Silica coated MSP without anti-*Campylobacter* antibody; b: silica coated MSP immobilized with anti-*Campylobacter* antibody; c: gold coated MSP without anti-*Campylobacter* antibody; and d: gold coated MSP immobilized with anti-*Campylobacter* antibody. Images are at 5,000× magnification

2.4 Bacterial Detection of MSP Biosensors

For bacterial detection by the MSP biosensors, a pick up coil and Network analyzer were used to measure the resonance frequency response of the sensor, and the frequencies were recorded every 5 min. With *Campylobacter jujuni* detection, 10-fold dilutions of the bacterial populations from 10^1 to 10^6 CFU/mL were used. The frequency of the silica coated MSP biosensor decreased from 2.266 to 2.256 MHz, when the bacterial population from 10^1 increased to 10^6 CFU/mL (Table 5 and Figure 10). The frequencies between the water and 10^1 CFU/mL samples were similar and showed no significance; therefore, the detection limit was around 10^2 CFU/mL. The result agreed with the study of Zhang (2010) who used gold coated MSP biosensors to detect *Listeria monocytogenes*, *Staphylococcus aureus*, and *E. coli*. The changes in the resonance frequencies to all three bacteria reported by Zhang (2010) had the same trend as our biosensor and the detection limits for the three bacteria were similar to each other around 10^2 CFU/mL, which were also close to these results. Guntupalli and others (2007) used gold coated MSP biosensors at a size of 2 mm × 0.4 mm × 15 μm to detect *Salmonella typhimurium* at a detection limit of 10^3 CFU/mL which is higher than our detection limit. However, compared to their biosensor, our sensors were smaller, 1 mm × 0.2 mm × 15 μm, and showed lower detection limits.

Table 4-Dynamic frequency of antibody immobilized silica biosensor to *Campylobacter jejuni*.

Reaction Time (min)	Bacteria population (CFU/mL)						
	water	1×10^{1}	1×10^{2}	1×10^{3}	1×10^{4}	1×10^{5}	1×10^{6}
0	2.26500						
5	2.26525	2.26488	2.26300	2.26268	2.26013	2.25975	2.25735
10	2.26488	2.26500	2.26375	2.26300	2.26125	2.26013	2.25755
15	2.26413	2.26525	2.26338	2.26338	2.26013	2.25825	2.25888
20	2.26488	2.26413	2.26300	2.26188	2.26088	2.25863	2.25775
25	2.26488	2.26488	2.26375	2.26225	2.26050	2.25825	2.25863
30	2.26413	2.26375	2.26268	2.26225	2.25975	2.25825	2.25775
35	2.26525	2.26338	2.26300	2.26263	2.26013	2.25863	2.25813
40	2.26563	2.26413	2.26263	2.26188	2.26013	2.25813	2.25735
45	2.26488	2.26488	2.26375	2.26113	2.26125	2.25900	2.25738
50	2.26413	2.26488	2.26225	2.26188	2.26013	2.25938	2.25700
55	2.26488	2.26375	2.26300	2.26113	2.26088	2.25900	2.25663
60	2.26525	2.26338	2.26375	2.26075	2.26013	2.25925	2.25738
Avg.	2.26486	2.26436	2.26316	2.26207	2.26044	2.25889	2.25765
Std.	0.00047	0.00068	0.00051	0.00079	0.00050	0.00065	0.00064

* Unit of frequency is MHz

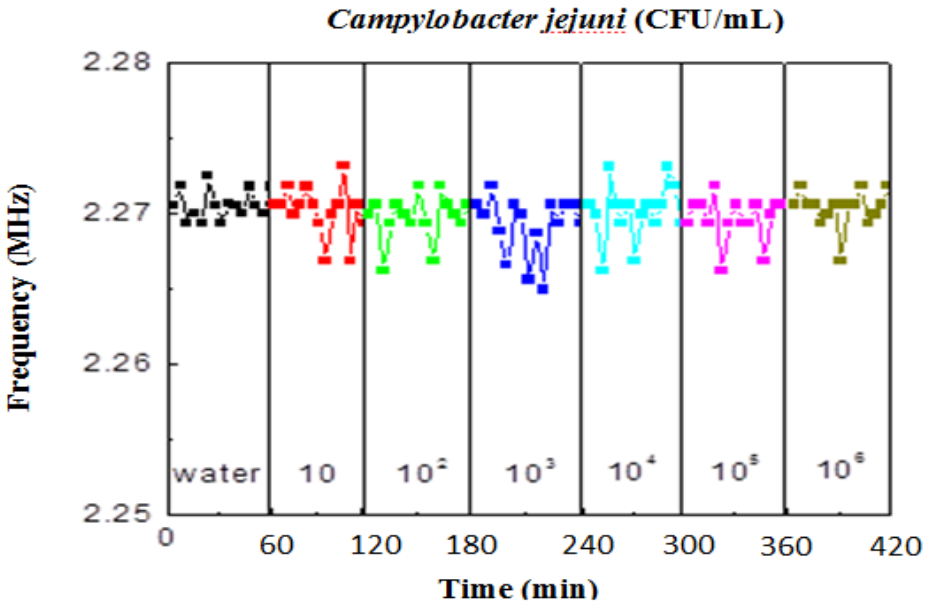

Figure 10- Dynamic response of silica coated biosensor (control sensors without antibody) for the detection of *Campylobacter jejuni* at different populations. Reaction time for each bacteria population suspension was 1h.

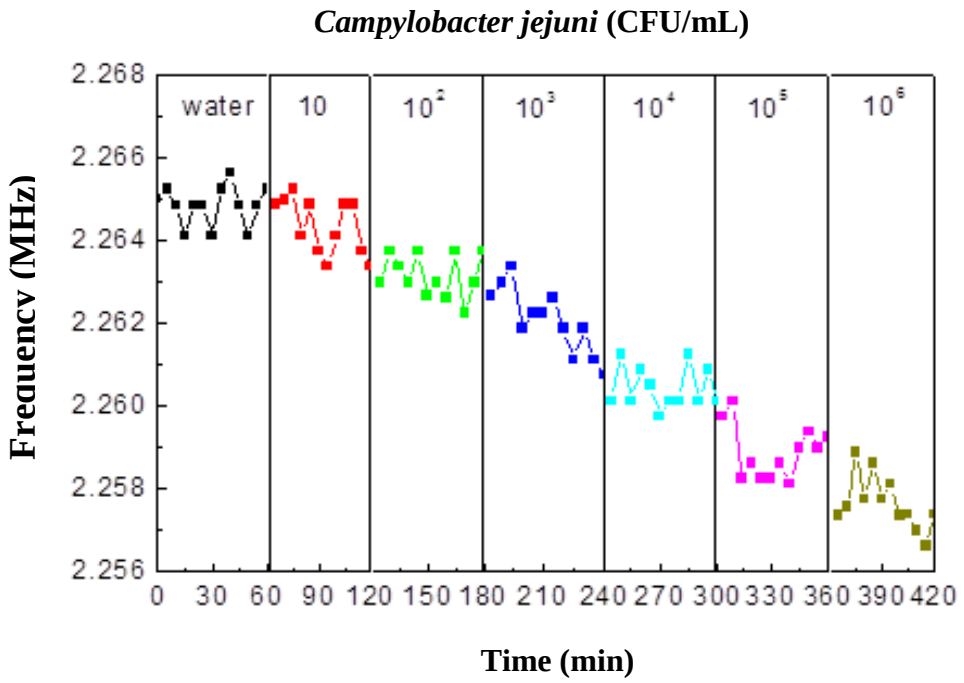

Figure 11-Dynamic response of silica coated biosensor for the detection of *Campylobacter jejuni* at different populations. Reaction time for each bacteria population suspension was 1h.

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CHAPTER 5: CONCLUSIONS

In this study, MSP sensors were made from Metglas into the sizes of 1 mm × 0.2 mm × 0.025 mm and 2 mm × 2 mm × 0.025 mm. The sensors were coated with three layers of silica or 100 nm of gold for improving the sensor performances and preventing corrosion. The sensing elements of anti-*Salmonella* and anti-*Campylobacter* antibodies were successfully immobilized on the silica and gold coated sensors through covalent bonding and direct adsorption, respectively. The antibody immobilization efficiencies on both sensors are similar to each other. From the $O.D._{405\ nm}$ values in ELISA test and the images from SEM observation, the bacteria binding was stronger on the silica coated biosensors than on the gold coated biosensors. This may be due to the antibody on covalent binding immobilization which had a stronger attachment than that on the direct adsorption immobilization during the process of bacterial detection.

The detection limits of both silica and gold coated MSP biosensors in bacterial detection were similar (around 10^2 CFU/mL). However, the silica coated MSP biosensors may have better performance in more complex food systems due to its stronger antibody attachment. The silica coated MSP biosensor is cheaper than gold coated biosensors and it doesn't need expensive sputtering equipment. Therefore, the silica coated MSP biosensors will have higher potential application in the food industry for onsite monitoring of microbial populations to improve food safety.

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