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Rapid and sensitive magnetoelastic biosensors for the detection of *Salmonella typhimurium* in a mixed microbial population

R. Guntupalli [a], R.S. Lakshmanan [a], J. Hu [a], T.S. Huang [b], J.M. Barbaree [c], V. Vodyanoy [d], B.A. Chin [a,*]

[a] *Materials Research and Education Center, Auburn University, Auburn, AL-36849, USA*
[b] *Department of Nutrition and Food Science, Auburn University, Auburn, AL-36849, USA*
[c] *Department of Biological Sciences, Auburn University, Auburn, AL-36849, USA*
[d] *Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL-36849, USA*

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Abstract

In this article, we report the results of an investigation into the performance of a wireless, magnetoelastic biosensor designed to selectively detect *Salmonella typhimurium* in a mixed microbial population. The Langmuir–Blodgett (LB) monolayer technique was employed for antibody (specific to *Salmonella* sp.) immobilization on rectangular shaped strip magnetoelastic sensors ($2 \times 0.4 \times 0.015$ mm). Bacterial binding to the antibody on the sensor surface changes the resonance parameters, and these changes were quantified as a shift in the sensor's resonance frequency. Response of the sensors to increasing concentrations (5×10^1 to 5×10^8 cfu/ml) of *S. typhimurium* in a mixture of extraneous foodborne pathogens (*Escherichia coli* O157:H7 and *Listeria monocytogenes*) was studied. A detection limit of 5×10^3 cfu/ml and a sensitivity of 139 Hz/decade were observed for the $2 \times 0.4 \times 0.015$ mm sensors. Binding kinetics studies have shown that the dissociation constant (K_d) and the binding valencies for water samples spiked with *S. typhimurium* was 435 cfu/ml and 2.33 respectively. The presence of extraneous microorganisms in the mixture did not produce an appreciable change in the biosensor's dose response behavior.

Keywords: Biosensor; Magnetoelastic; Polyclonal antibodies; *Salmonella typhimurium*; Specificity

1. Introduction

A number of foodborne illness outbreaks have occurred where the source of contamination has been traced to *S. typhimurium*. *S. typhimurium* contamination can occur in a wide range of food products (Jones et al., 1995; CDC, 2005, 2006) and its use as a potential bioterrorist weapon (Khan et al., 2001) illustrates the need for rapid and specific diagnostic methods for the detection of food borne pathogens. Illnesses caused by *S. typhimurium* can range from diarrhea, abdominal cramps, fever, vomiting and sometimes life-threatening conditions in people with a compromised immune system. The conventional methods such as culture and colony counting (Leoni and Legnani, 2001) and polymerase chain reaction (PCR) (Bej et al., 1991; Levi et al., 2003) have proven to be reliable, sensitive and specific to the target pathogens. But the sampling procedures, preparation methods (such as sample enrichment and amplification) are time consuming, laborious and impractical for in-situ real-time measurements and in vivo biological experiments.

A plethora of biosensor technologies based on surface plasmon resonance (SPR) (Oh et al., 2004; Taylor et al., 2005), piezoelectric biosensors (Ye et al., 1997; Pathirana et al., 2000), and electrochemical biosensors (Brewster et al., 1996; Muhammad-Tahir and Alocilja, 2003) are being developed for rapid and specific detection of foodborne pathogens. These sensor technologies offer sensitivity and faster response times, but

* Corresponding author. Tel.: +1 334 844 3322; fax: +1 334 844 3400.
E-mail address: bchin@eng.auburn.edu (B.A. Chin).

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often involve complex micro fluidics to pump the sample over a detector element or array. In addition, each detector or each element of the array requires wires to power the sensor and to measure outputs. This leads to multiple wires and eventually to complex multiplexing to manage signals from the array. Also, current commercial biosensor technologies suffer from high cost and miniaturization problems. These drawbacks have largely prohibited their use in real time applications such as detection in conducting liquids, in containers that are either sealed or opaque, and for in-vivo biological experiments. This research is focused on development of an alternate sensor technology (Magnetoelastic Sensors) that overcome many of the above problems encountered in remote and real-time monitoring of food borne pathogens.

Magnetoelastic materials are amorphous ferromagnetic alloys comprised of iron, nickel, molybdenum and boron. A magnetoelastic sensor oscillates at a fundamental frequency in the presence of a time varying magnetic field. These oscillations generate a magnetic flux around the sensor that can be detected by a non-contacting pickup coil. Magnetoelastic sensors are therefore remote and wireless devices that are being investigated for potential applications such as remote monitoring of food borne pathogens (Chuanmin Ruan et al., 2003; Guntupalli et al., 2007) and other sensing applications (Bouropoulos et al., 2005; Shankar et al., 2005; Ong et al., 2006; Wu et al., 2006). The aim of the present study was to develop a highly specific, biosensor technology based upon antibody immobilized magnetoelastic biosensors. The specific detection potential of this biosensor technology was evaluated by detecting *S. typhimurium* in a mixture of different bacterial species including *Escherichia coli* O157:H7 (here onwards referred to as *E. coli*) and *Listeria monocytogenes* (*L. monocytogenes*).

2. Materials and methods

2.1. Sensor platform

METGLAS® 2826MB alloy, obtained from Honeywell International (Conway, SC), was used as the sensor platform material. The composition is $Fe_{40}Ni_{38}Mo_4B_{18}$ and its theoretical value of the saturation magnetostriction is 12 ppm (Honeywell September, 2006). Initially, sections of the as-received, magnetoelastic ribbon material were mechanically polished using standard metallographic polishing techniques to reduce the thickness from 30 μm to 15 μm, thereby decreasing the initial mass. Following this, the individual sensor platforms ($2 \times 0.4 \times 0.015$ mm) were made using an auto-controlled, micro-dicing saw. The diced sensor platforms were then ultrasonically cleaned in methanol (100%) for 20 min to remove any organic film and other debris left by the dicing process. To improve the environmental stability and the bioactivity of the biosensors, thin layers of chromium (50 nm at 100 W DC power) and gold (100 nm at 200 W RF power) were sputtered onto the surfaces of the magnetoelastic particles using a Denton™ (Moorestown, NJ) high vacuum RF sputtering system. Here chromium acts as an interlayer between the sensor platform and gold film and gold film provides a favorable surface for the antibody immobilization. These sputtered sensors were stored at ambient temperature in a dessicator until needed.

2.2. Antibodies and bacterial cultures

Rabbit polyclonal antibody (1 mg/ml) to *Salmonella* sp. was purchased from Abcam Inc (Cambridge, MA) and immobilized onto one surface of the magnetoelastic biosensors using the Langmuir–Blodgett (LB) film technique. *S. typhimurium*, *E. coli* O157:H7, *L. monocytogenes* and *Staphylococcus aureus* were obtained from the American Type Culture Collection (Rockville, MD), confirmed for identity and propagated in the Department of Biological Sciences at Auburn University. Solutions containing individual species of bacteria and solutions containing mixed combinations of bacteria species were prepared for testing. Solutions containing individual bacteria were prepared to a uniform concentration of 5×10^8 cfu/ml. Mixed microbial cultures for the dose response measurements were prepared by co-spiking increasing concentrations of (5×10^1 to 5×10^8 cfu/ml) *S. typhimurium* with a fixed concentration of (5×10^8 cfu/ml) of multiple foodborne pathogens. All test solutions were prepared and biosensor tests conducted on the same day. Test solutions were stored at 4 °C and equilibrated to room temperature prior to biosensor testing.

2.3. Monolayer deposition

The Langmuir–Blodgett (LB) technique was used for antibody immobilization on the magnetoelastic sensors. Antibody monolayers were deposited using a LB film balance KSV 2200 LB, (KSV Chemicals, Finland). This system mainly consists of a Wilhelmy-type surface balance (Sensitivity range 0–100 mN/m), a Teflon trough of dimensions 45×15 cm^2, and a Teflon barrier (0–200 mm/min) driven by a variable speed motor. The entire LB film balance was enclosed in a laminar flow hood. To minimize variations that may arise due to vibrations, the trough is mounted on a marble table that is isolated by interposing rubber shock absorbers. The subphase temperature (20 ± 0.1 °C) control was achieved by circulating water through a quartz tube coil at the bottom of the trough. A monolayer of antibody was formed by slowly pouring 100 μl of antibody solution (1 mg/ml) onto a glass rod. The antibody was allowed to run down the wetted glass rod that was partially submerged into the subphase (deionized water). When the antibody solution reaches the air–water interface, it forms a monolayer due to surface forces (Pathirana et al., 2000). After spreading, the monolayer was allowed to equilibrate and stabilize for 10 min at 20 °C.

Monolayers were then compressed using a computer controlled compression barrier at a rate of 30 mm/min, until the pressure reached 22 ± 0.05 mN/m. The pressure was then held constant and vertical antibody film deposition was carried out at a rate of 4.5 mm/min. Multiple monolayers of antibody were obtained by successive lowering and raising the sensors through the monomolecular film deposited at a water–air interface. Seven monolayers containing antibodies were transferred onto the magnetoelastic sensor surface. Only one surface of the magnetoelastic sensor was coated with the antibody.